Safety Assessment of
Ammonia and Ammonium Hydroxide as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: November 10, 2017
Panel Date: December 4-5, 2017

.

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., former CIR Senior Toxicologist.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: November 10, 2017
Subject: Draft Final Report on Ammonia and Ammonium Hydroxide

At the September 11-12, 2017 Expert Panel meeting, the Panel issued a Tentative Report with a conclusion stating that Ammonia and Ammonium Hydroxide are safe in cosmetics in the present practices of use and concentration when formulated to be non-irritating. Comments on the Tentative Report that were received from the Council (*ammoni122017pcpc1* and *ammoni122017pcpc2*) have been addressed and are attached for the Panel's review. A third comment that was received (*ammoni122017pcpc3*) that relates to suggested changes in the wording of the Discussion and the tentative conclusion. Specifically, the comment suggested that the report conclusion should address the use in hair dyes and colors separately from products applied to the skin. Please review that comment carefully, and determine whether or not you agree with the suggestions.

Also included in this package for your review are the Draft Final Report (*ammoni122017rep*), the CIR report history (*ammoni0122017hist.docx*), Flow chart (*ammoni122017flow.docx*), Literature search strategy (*ammoni122017strat.docx*), Ingredient data profile (*ammoni122017prof.docx*), 2017 FDA VCRP data (*ammoni122017FDA.docx*), and minutes from the September 11-12, 2017 Panel meeting (*ammoni122017min.docx)*.

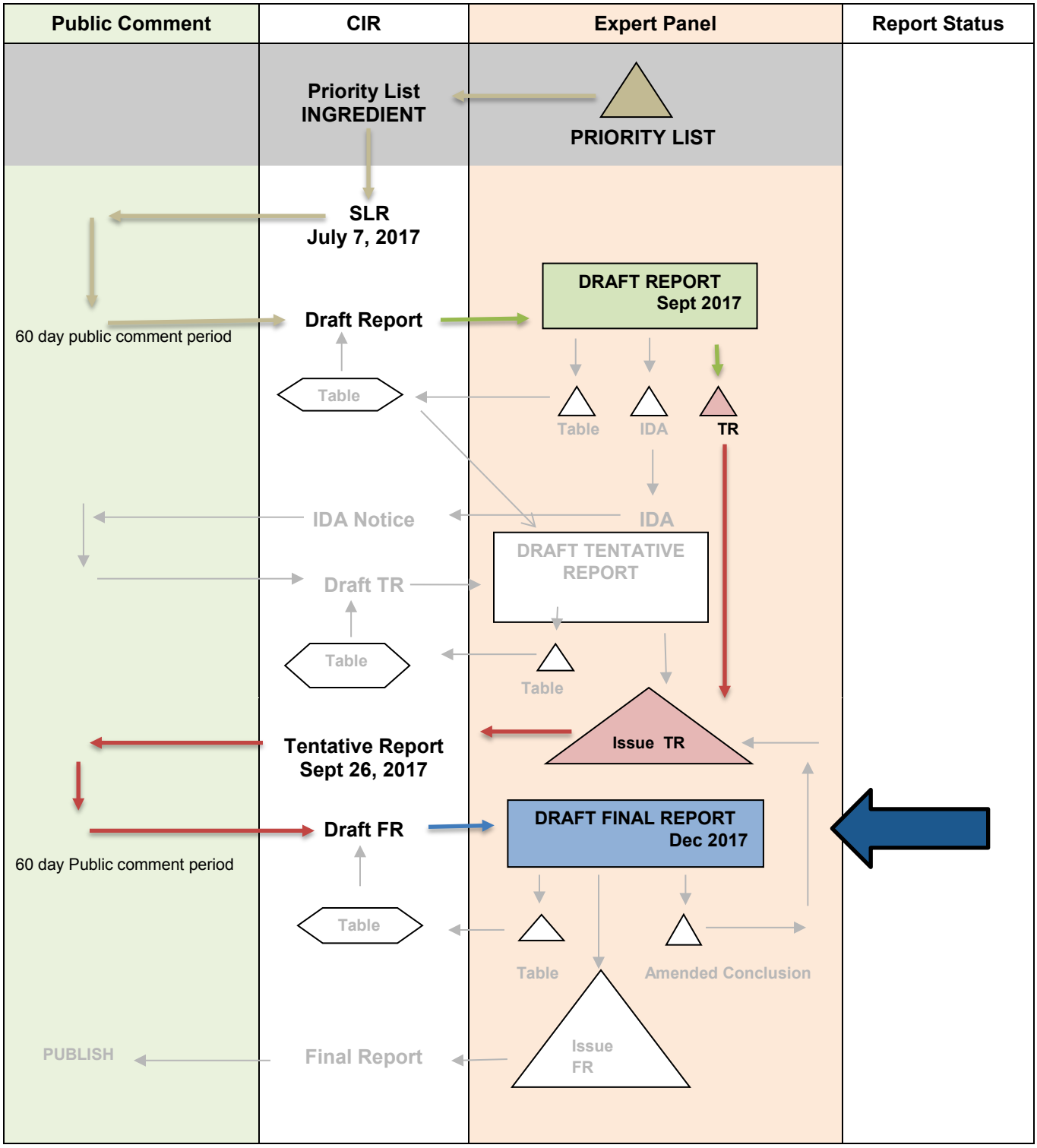
Though the evaluation of occupational safety in relation to cosmetic ingredient exposure is not within the Panel's purview, an occupational study in which hairdressers were exposed to Ammonia at concentrations ranging from 3 to 61 mg/m^3 is included (enclosed within vertical borders in the report text) for the Panel's review of exposure-related adverse effects.

After reviewing these documents and the comment relating to changes in the Panel's tentative conclusion, the Panel will determine whether or not a Final Report with the conclusion that is stated above should be issued.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Ammonium Hydroxide and Ammonia

MEETING Dec 2017



CIR History of:

Ammonia and Ammonium Hydroxide

A Scientific Literature Review (SLR) on Ammonia and Ammonium Hydroxide was issued on July 7, 2017.

Draft Report, Teams/Panel: September 11-12, 2017

The draft report contains use concentration data on Ammonia and Ammonium Hydroxide that were received from the Council.

The Panel issued a tentative report for public comment with a conclusion that Ammonia and Ammonium Hydroxide are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating.

It was noted that Ammonia and Ammonium Hydroxide, well-known skin irritants, are indistinguishable from each other in aqueous formulation. Furthermore, since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added, the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity data is reported for Ammonia or Ammonium Hydroxide, it is applicable to both (as the test articles would have had this same equilibrium).

The Panel agreed that the cosmetic ingredients Ammonium Chloride and Ammonium Sulfate, which, unlike Ammonia and Ammonium Hydroxide, would not function as pH adjusters in cosmetics, should not be counted in this safety assessment, though they agreed that the data on these other ingredients were useful as surrogates.

Draft Final Report, Teams/Panel: December 4-5, 2017

Comments of the Tentative Report that were received from Council have been addressed. The last comment that was received on November 1, 2017 relates to suggested changes in the wording of the tentative conclusion.

Ammonia and Ammmonium Hydroxide Data Profile for December 4th-5th, 2017 Panel – Wilbur Johnson

			Dermal Penetration			Nail Penetration	Penetration Enhancement	ADME					Acute Toxicity			Short-Term Toxicity	Sub-Chronic Toxicity	Chronic Toxicity	DART		Genotoxicity	Carcinogenicity	Other Relevant Studies		Dermal Irritation*	Dermal Sensitization /Phototoxicity		Ocular Irritation *		Clinical Studies	Case Reports		Epidemiology Studies
	Used in Cosmetics?	Safety Data Available?	In Vivo -Animal	In Vitro-Human	In Vivo-Human	In Vitro-Human	In Vitro-Animal	In Vitro-Human Dermal	Animal-Dermal	Animal-Oral	Animal-IV	Human-Oral	Animal-Dermal	Animal-Oral	Animal-Inhalation	Animal	Animal	Animal	In Vitro	In Vivo	In Vitro/In Vivo	In Vivo	In Vitro	In Vivo-Animal	Animal/Human/In vitro	Animal	Human	In Vitro	Animal	Human-Inhalation	Human-Dermal	Human-Inhalation	Human
Ammonia	X	X								X		X		X	X	X	X		X	X	X/X	X		X	X/X			X	X	X			X
Ammonium Hydroxide	X	X																X				X			X/X/X				X				

X = data; 0 = no data

[Ammonia and Ammonium Hydroxide (3/20/2017; Updated on 10/20/2017)]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	ECETOC
Ammonia	7664-41-7 8007-57-6	1/1	83/563	14/455	17/283	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Ammonium Hydroxide	1336-21-6	1/1	20/1064	14/1159	9/366	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

LINKS

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

FDA databases – http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm (CFR); then,
list of all databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm; then,
http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true (EAFUS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm (GRAS);
http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm (SCOGS database);
http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives (indirect food additives list);
http://www.fda.gov/Drugs/InformationOnDrugs/default.htm (drug approvals and database);
http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf (OTC ingredient list);
http://www.accessdata.fda.gov/scripts/cder/iig/ (inactive ingredients approved for drugs)

EU (European Union); check CosIng (cosmetic ingredient database) for restrictions and SCCS (Scientific Committee for Consumer Safety) opinions - http://ec.europa.eu/growth/tools-databases/cosing/
ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
OECD SIDS documents (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/
NTIS (National Technical Information Service) - http://www.ntis.gov/
NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/ (FAO);
FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
Web – perform general search; may find technical data sheets, published reports, etc
ECETOC (European Center for Ecotoxicology and Toxicology Database) - http://www.ecetoc.org/

Botanical Websites, if applicable
Dr. Duke's https://phytochem.nal.usda.gov/phytochem/search
Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
Sigma Aldrich plant profiler http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html

Fragrance Websites, if applicable
IFRA (International Fragrance Association) – http://www.ifraorg.org/

RIFM (the Research Institute for Fragrance Materials) should be contacted

Day 1 of the September 11-12, 2017 CIR Expert Panel Meeting – Dr. Belsito's Team

Ammonia and Ammonium Hydroxide

DR. BELSITO: Okay, ammonia and ammonium hydroxide. So, the big question here, back to (inaudible), does

everyone now have access to the dictionary because I did not. Bart sent me how to get access.

DR. LIEBLER: No, I've never used it.

DR. BELSITO: Yeah, well the big question here was that you know, we're using some read across materials and then whether they should be added to this report and you know, I tried to access the dictionary. I thought well it would be a no brainer if they had the same function, but if their function was different than that wouldn't be the case. And it turns out that I thought we could use materials from read across but their functions are different, so I didn't think they should be added to this report.

DR. LIEBLER: I think it's a case by case issue, depending on what the -- because it's safety data, and it depends on what the context is.

I noted several -- all the times that these other related materials are brought in. I said that I agreed to re- include the data.

DR. BELSITO: Right.

DR. LIEBLER: And I didn't consider the different use in cosmetic products as relevant, but maybe we could --

DR. BELSITO: No, no, I didn't think the different use was relevant in using them for safety of these materials, but the question was also raised, do we bring them in, do we include them in this report?

DR. SNYDER: Read across (inaudible)

DR. BELSITO: To say not only you know, ammonia and ammonium hydroxide but I think we're using ammonium chloride, and I have a few other chemicals as read across.

The question is do we bring them into this report along with some of the other ammonium compounds, and my comment to Bart well, if they have cosmetic function, that's a no brainer. If they have different functions and significantly different concentrations of use, then no, we don't do it, because that's not a no brainer.

DR. LIEBLER: Right. No, I agree with you on that, and I missed that entirely. It is in the memo.

So, I agree with using the data to determine safety of ammonia and ammonia hydroxide but if they have different uses in cosmetics the other ingredient function be brought in.

DR. BELSITO: Okay.

DR. LIEBLER: So, I agree with that.

DR. BELSITO: Okay. So, I thought these two were safe when formulated could be non-irritating. And then the only issue --

DR. SYNDER: (inaudible) systemic data?

DR. BELSITO: (overlapping conversations) they're irritants. The question and we've addressed this multiple times is obviously the exposure in hairdressers who are chronically exposed; and we decided based upon multiple other reports where we've looked at issues where chronic exposure beyond what a consumer might get, could be an issue that those were OSHA and not our prevue.

The question is, do we mention that at all in the discussion; that you know, this could be an issue for hairdressers or other people chronically exposed. Clearly there are case reports of chronic exposure causing issues, but that's not our prevue.

DR. LIEBLER: That's an appropriate place to mention it though. I think that's fine.

DR. BELSITO: I think it needs to be in the discussion, so we acknowledge that chronic exposure can be an issue, but we're not looking at you know -- we're looking at consumer exposures that were not that kind of eight-hour exposure a day would not be an issue.

DR. LIEBLER: I'm at the same place. Safe when formulated to be non-irritating. I came to the same conclusion.

DR. BELSITO: Okay. In my head, only one other comment in the paper, just to make sure that I make document. Page 21 of the PDF, under Clinical Studies, Case Reports, the second one about the male custodian. The last sentence about opacification of the optic lens. It says were described as chronic lesions that follow acute exposures. The chronic lesions that follow chronic exposures, right?

DR. LIEBLER: I have a number of edits but --

DR. BELSITO: I do to, but that was the biggest one, and I wanted to make sure that it wasn't reported that way. Okay, yeah, so I have a significant number of edits to.

DR. LIEBLER: Right.

DR. BELSITO: But I (overlapping conversations) any substantive issues? Okay, so safe as used, non-irritating.

MR. JOHNSON: Acute short-term (inaudible) inhalation toxicity starting on ammonia, while indicating toxicity to the lower respiratory tract.

DR. BELSITO: Yes, but that again, is in -- the doses for the acute are much higher than what would be even a hairdresser would be exposed to. And in the discussion Wilbur, we're going to point out that the chronic exposures you know, over several hours per day, that might be seen in an occupation such as custodial or hairdressing are not the prevue of this panel.

But we look at the safety of the ingredient as used in a cosmetic product by the consumer, and that language has been crafted into other -- I mean, I can't think right now, but we've dealt with that in other discussions, so you can use the same type of language.

MR. JOHNSON: What about the cocarcinogenicity study?

DR. LIEBLER: Are you talking about the ammonium sulfate data?

MR. JOHNSON: Yes.

DR. BELSITO: Carcinogenicity. There's no evidence of carcinogenicity on mites with ammonia. Do you mean the tumor promotion?

MR. JOHNSON: Yes.

DR. BELSITO: Okay, so that was with nitroso guanidine and.01 percent ammonia --

MR. JOHNSON: Yes.

DR. BELSITO: -- when compared to nitroso guanidine alone?

MR. JOHNSON: Yes.

DR. BELSITO: Alright, I think that's gastric irritation. I don't think that that's -- I mean any irritant will be a cocarcinogen and these are levels that would not be achieved even under chronic exposure.

I don't even know that we need to comment on that in the discussion.

DR. LIEBLER: That's fine, just leave it where it is, it's okay to report, but --

DR. BELSITO: Anything else?

MR. JOHNSON: That's been related to chronic exposure. That was relating to what types of data in the report.

DR. BELSITO: In other words you're relying on -- I thought I had given back being the chairman. We need to get back to page 21 of the PDF on the case reports Wilbur, the second paragraph about the male custodian.

MR. JOHNSON: Okay.

DR. BELSITO: The last line of that paragraph, were described as chronic lesions that followed chronic exposure.

MR. JOHNSON: Okay.

DR. SNYDER: I mean it could be called acute exposure, because they could have damaged -- an acute exposure could have resulted in permanent damage.

DR. BELSITO: It would not have resulted in bronchiectasis and fiberus obliteration of the small airways.

DR. SNYDER: Oh, I thought it was just a --

DR. BELSITO: No, the oppasocation of the lens, bronchiectasis and fiberus obliteration of small airways observed were described as chronic lesions that follow acute exposure.

MR. JOHNSON: I'll check the point of reference.

DR. LIEBLER: And there's nothing chronic about a lesion. There's chronic

exposure. A lesion is a lesion.

DR. SNYDER: Acute and chronic.

DR. LIEBLER: Lesions?

DR. SNYDER: Yes.

DR. LIEBLER: Really? okay. I stand corrected. I was going to delete a word from --

DR. SNYDER: No, no, no.

DR. LIEBLER: Alright, fine.

DR. BELSITO: Okay, anything else?

DR. LIEBLER: (inaudible)

DR. BELSITO: By turning to 26, that's a re-review. Then we're back to the admin. And then page -- Did you find it Dan?

DR. LIEBLER: Page 27.

DR. BELSITO: Okay.

DR. SNYDER: Page 27. Yes, thank you. (inaudible)

DR. BELSITO: Okay, I have no comment, so it's good to me.

DR. LIEBLER: Same.

DR. SNYDER: Same.

MR. JOHNSON: There's just one mistake in the re- review summary of the pump hairspray containing 0.63 percent

(inaudible). The inhalation exposure column is what we had.

Day 1 of the September 11-12, 2017 CIR Expert Panel Meeting – Dr. Marks' Team

<u>Ammonia and Ammonium Hydroxide</u>

DR. MARKS: Okay, next ingredient is draft report on ammonia and ammonium hydroxide. Let's see when did -- this is the first review of these ingredients and so Ron, Ron and Tom are the two ingredients, ammonia, ammonium hydroxide okay? And there was a suggestion to include ammonium chloride and ammonium sulfate too in this report after. Do all four?

DR. HILL: No.

DR. MARKS: No.

DR. HILL: Stick to the guns and keep ammonia and ammonium hydroxide, because as soon as you get into phosphate and sulfate then you have to deal with effects it might be related to those and it clouds the whole issue.

DR. EISENMANN: It's chloride.

DR. HILL: I know chloride could go, chloride could go.

DR. MARKS: And sulfate?

DR. EISENMANN: The reason why I thought sulfate is because there's data in the report on sulfate to support the other ones. So if you're going to put data in the report on it. That was the only reason why I thought maybe they should be added, because they're using the data on them to support the two ingredients. And if you're going to support -- and their end key. If you're going to put the data in the report, otherwise I wouldn't have suggest they be added, it's just because you're putting the data in the report on them so...

DR. JOHNSON: And both data were found in the ECCA report. And it's really up to the panel as to whether or not those data should remain in the safety assessment. Because those surrogate chemicals data were in that ECCA dossier.

DR. HILL: And I rather doubted that they needed to be (inaudible) seem like there was one point in there -- I'm going to remind myself where amount of ammonium toxicology was being discussed. There are a lot of -- Bart's not in here but there are a lot of -- and basically, the Henderson Hasselbach equation was done backwards because even a PH9 the vast majority of the substances (inaudible) the vast majority of the substances so you dissolve ammonia as ammonium not ammonia.

So they're a lot of things that are written backwards in here. But -- so mostly what we're actually looking at is either caustic effects which we've captured would have a higher

pH. Because assume you dissolve ammonia you've got ammonium hydroxide in the -- you don't have ammonia. There's a little bit in there you can smell it of course.

Basically, you got ammonium hydroxide unless it's gaseous or liquified ammonia. And then -- but there seem like there are one or two points where the levels of ammonium were being discussed. Where ammonium chloride could be brought in for relevance but I just -- I would agree that that ammonium chloride and ammonium sulfate could be your group. But the whole toxicology concerns are really different, it doesn't seem so.

But when you're talking about ammonia and ammonium hydroxide we're mostly concerned about irritation from caustic effects. That's how I viewed this.

DR. MARKS: So you would not add the chloride and the sulfate?

DR. HILL: No.

DR. MARKS: Ron Shank?

DR. SHANK: Well, I don't know if we spoke (inaudible) these on function. I think I was told we can't do that. But if it's just ammonia and ammonium hydroxide as a pH adjuster that's a very simple document.

DR. MARKS: Exactly.

DR. SHANK: After you throw in the solvents you have different functions. So I would prefer to keep them separate for that reason.

DR. MARKS: And that's Ron -- as a pH, yeah, that's what I have pH adjuster. Because that's going to address some of the issues from the irritation. It's absolutely corrosive, there's no sensitization data --

DR. HILL: Because you couldn't even get it.

DR. MARKS: Exactly. Because it's corrosive, you know, if we end up with a tentative report safe when formulated to be non-irritating, is that reasonable conclusion. So let's get back to chloride and sulfate, we're not going to do those, we'll just the two ingredients ammonia and ammonium hydroxide.

DR. SHANK: That's my suggestion.

DR. MARKS: Yeah. And that's both Ron's both of you said that. And then as we said pH adjuster. What needs do we have in terms of toxicologic?

SPEAKER: Any.

DR. MARKS: And then how do you like safe when formulated to be non-irritating?

SPEAKER: Don't need it.

DR. EISENMANN: The main use is in hair dyes and I'm not sure (inaudible) if saying, you know, non-irritating to be for a hair dye is may be two-part conclusion. Safe for use in a hair dye and for dermal products, safe and formulated to be non-irritating.

DR. MARKS: Why do you say with the hair dyes? Because you know as a pH adjuster it's going to be diluted down.

DR. EISENMANN: I'm not sure if that's it's only use in a hair dye, it's higher.

DR. MARKS: It's goes on the scalp.

DR. EISENMANN: I know -- well, they try not to get hair dyes --

SPEAKER: You're supposed to not, yeah.

DR. EISENMANN: So I think that's kind of would be a warning if you get the irritation on your...

DR. MARKS: I don't think we have --

DR. SLAGA: How do you dye your hair if you have no hair.

DR. MARKS: I don't think we'd have a hair dye epidemiology if we wouldn't have a hair dye boilerplate if it weren't -- that it gets on the scalp and it's absorbed. So, I'm not sure I want to split, I can leave out non-irritating when formulated to be non-irritating, I mean, it's pretty obvious it's corrosive.

DR. Hill: Here's my take on this --

DR. MARKS: (Inaudible) more focus as if somebody just looked at this and said oh, it's safe and started using it. I mean you wouldn't expect (inaudible)

DR. SHANK: Safe as a pH adjuster.

DR. SLAGA: If there's not a good (inaudible)

DR. HILL: Well, the question is are we capturing the hair dye use because I

know very well, I mean, I can smell this stuff from when my wife has used in the past. Hair dyes that had that ammonium hydroxide and it's there would be caustic -- somewhat caustic concentrations if you got a lot of that on your skin. They try not to get it on their skin when their doing it.

So that I don't know if that's pH adjuster, I mean, I guess it is adjusting to alkaline pH, is that how they refer to it in the use in hair dye?

DR. MARKS: I mean would that be handled in this --

DR. EISENMANN: Well they would have to discuss with --

DR. MARKS: Would that be handled in the discussion? Just safe and then handle the irritation.

DR. HILL: Current practices of use. (Inaudible) yeah.

DR. MARKS: And that's true the way it's being used it's safe. Okay. Tom fine, safe, Ron Shank?

DR. SHANK: Yes.

DR. MARKS: And Ron Hill and the current practices of use and it's being used as a pH adjuster. Okay.

DR. HILL: The only issue I had here is the same one I expressed earlier about what we got as far as information about the impurities is all from food kodaks or USP. And the question is does that represent the cosmetic ingredients supply. Cause otherwise we've been stonewalled.

DR. EISENMANN: For most things it's highly unlikely that there's a different standards for cosmetics. They just don't use enough of some of these things to make it --

SPEAKER: Right.

DR. EISENMANN: -- Right.

DR. HILL: So what you're thinking they're probably, well, I mean, it would be nice to have some information other than just -- I agree with you I'm almost certain you're right. But...

MR. JOHNSON: Doctor Marks, on PDF page 18 should the tumor promotion study be addressed in the discussion?

DR. MARKS: Not as if it's being used as the pH adjuster.

MR. JOHNSON: What about the acute short term and some chronic inhalation toxicity data?

DR. HILL: Well, I mean, there's a lot of ammonium related products that (inaudible) promotion in (inaudible) systems but we're only including pH adjustment we don't have to worry about that.

DR. MARKS: So let's if you want to -- do you want to put that in the discussion?

DR. SLAGA: I don't think we have to.

DR. MARKS: Any other comments, thanks Wilbur.

MR. JOHNSON: The inhalation toxicity any concerns about that?

DR. HILL: If it weren't for my knowledge that there is use in hair dyes and you can smell ammonia big time when that's happening, I wouldn't even bring this up. Because if it was strictly pH adjuster other than again adjusting to pH 10 is still adjustment to pH but...

DR. MARKS: Ron?

DR. SHANK: No, I wouldn't.

DR. MARKS: Okay.

DR. SHANK: Wouldn't include it.

MS. FIUME: Actually, I have a question about leaving out the non-irritating caveat. I understand it's because it's being used as a pH adjuster, but I don't know from the past when we've done safe as used for our readers, it's generally based on our use table and not function of use as far as I can remember. And there are --

DR. BERGFELD: We had other pH adjuster, we handled similar.

MS. FIUME: Just as safe as used but did it normally state when (inaudible) pH adjuster.

DR. BERGFELD: Well we can look it up. It might have been the discussion as well but it was on function rather than sensitization.

DR. SHANK: You want a safe say safe.
MS. FIUME: I was just curious.
DR. SHANK: And used as a pH adjuster?
MS. FIUME: Bart will balk at that but -- or just something to indicate because there are a 163 leave-on uses for ammonium hydroxide. Which would mean that's not hair colorant uses. So I just want to make sure that somehow -- and I guess it was being the discussion, just to make sure that it's communicated what we're looking at.
DR. SHANK: Definitely, in the discussion reference hearing its function as a pH adjuster.
DR. MARKS: Okay any other comments. One more ingredient to go, I believe.
DR. HILL: My only comment and whoever this is editorial is that, I don't agree with we're just using ammonia, ammonium ammonia is a surrogate for I think we do need to make sure we -- and I've marked a lot of spots in here that we are setting ammonium hydroxide because again, as soon as you put ammonia in the (inaudible) solution even at a relatively high pH it's almost all ammonium hydroxide. When you drop down to neutral pH.
First of all, there's two possibilities it's in pure water in which case you got ammonium and hydroxide but as soon as it's dissolved and it's in a formulation, the ammonium goes one and the hydroxide goes another way. It's no longer really a salt you got ammonium ion. So that's -- and that's why you can bring in things like ammonium chloride and ammonium sulfate and phosphate for certain end points here.
MR. MARKS: Next ingredient is a rear review summary of quarternium 26 looked okay.
SPEAKER: It did.
DR. MARKS: Any other comments.
SPEAKER: Nope.
DR. MARKS: Any other for the -- see you in the morning.
DR. JOHNSON: Just one Doctor (inaudible) there's a use of the ingredient in a pump hairspray at 0.63 percent. And that needs to be added to the incidental inhalation spray column and the use table and that will be done.
DR. MARKS: Great. Thank you.
DR. JOHNSON: You're welcome.
DR. MARKS: Any other comments, if not our team is adjourned. We are adjourned.

Day 2 of the September 11-12, 2017 CIR Expert Panel Meeting – Full Panel

<u>Ammonia and Ammonium Hydroxide</u>

Then, moving on to the next ingredient, which is ammonia and ammonium hydroxide, Dr. Belsito.
DR. BELSITO: Yeah. We thought these were "safe as used." There was a question raised by council as to whether some of the read-across materials that we used in our -- I'm sorry -- "when formulated to be non-irritating." We thought that we could not include the read-across materials because of different function of use in cosmetics. And so we're just staying with ammonia and ammonium hydroxide for this.
DR. BERGFELD: And that's for the pH adjusting --
DR. BELSITO: Yeah.
DR. BERGFELD: -- portion of it. Yes. Jim?
DR. MARKS: Your conclusion was "safe" or "safe when formulated to be non-irritating"?
DR. BELSITO: "Safe formulated to be non-irritating."
DR. BERGFELD: Okay.
DR. MARKS: Second.
DR. BERGFELD: Any further discussion?

DR. LIEBLER: So just to clarify, the data from a couple of the ammonium salts were included in the report based on their relevance to toxicity endpoints, but what we're saying is we're not adding those materials to the report for evaluation.

MR. JOHNSON: So the data already in the report, should they remain?

DR. LIEBLER: Yes, they're fine.

SPEAKER: I concur.

DR. BERGFELD: Any other comments? Don? Okay. Wilbur, go ahead.

MR. JOHNSON: Relating to the discussion, on PDF page 18, there are data indicating that ammonia is a tumor promoter, and I'm wondering whether or not that should be addressed in any way in the discussion.

DR. SLAGA: I don't think it's needed.

DR. BERGFELD: Okay, Tom. Tom states it's not needed. How about Paul? Tumor promoter question?

DR. KLAASSEN: I'll agree.

DR. SNYDER: That's fine.

DR. BERGFELD: Curt says, not needed. Okay.

MR. GREMILLION: Okay. Just a bit of the rationale on it. It sounds like it's not, you know, (inaudible) of it. Could someone explain the rationale for that not being (inaudible)?

DR. BERGFELD: Tom, why is it not needed?

DR. SLAGA: Come again. I didn't hear all of your --

MR. GREMILLION: I just would -- asked for a rationale why that wasn't needed.

DR. SLAGA: Well, it was certain ammonium derivatives that were used and you have to give a carcinogen to bring about tumor promotion. Okay. So without a carcinogen, you won't get anything.

MR. GREMILLION: Aren't there -- I mean, there are carcinogens everywhere, so.

DR. SLAGA: No, no, no, no, no. With the animals, a large dose of a carcinogen followed by certain ammonium compounds, okay. And it's at very large doses. This wouldn't occur here.

DR. LIEBLER: So just to be clear. I think that the term tumor promotion might not be familiar, so there's a very well-established paradigm to study the ability of chemicals to produce cancer, particularly skin cancer, but sometimes other tissues, where you give a carcinogen to the animal -- let's say to the mouse. You put it on the skin. And you might get a small yield of cancers or pre-cancerous lesions. But if you apply another type of molecule that's not itself a carcinogen, but it's able to increase the yield of cancers, those chemicals are known as tumor promoters.

And there are a number of natural products and other things that are able to do this. They all generally share the characteristic of being irritating to skin. And they stimulate the reproduction of cells, the proliferation of cells that helps to drive the cancer forward.

And so that term, "promoter," might sound, you know, to the uninitiated as something that causes cancer by itself.

SPEAKER: (Inaudible)

DR. LIEBLER: Right. So, yeah (inaudible), sorry bad product. (Laughter) That's also an inside pun.

But anyway, it's not the same thing as saying it's a carcinogen. So the promoters by themselves are not carcinogens.

DR. SLAGA: Right.

MR. GREMILLION: So is the policy, if the fact that something's a cancer promoter is irrelevant if it's not normally formulated with a known carcinogen?

DR. LIEBLER: Right.

MR. GREMILLION: Is that -- would that be?

DR. LIEBLER: Yeah.

MR. GREMILLION: Okay.

DR. BERGFELD: Okay. Any other questions? Otherwise, we call the

question. Yes, Wilbur again.

MR. JOHNSON: Yes. (Inaudible) acute, short term, and sub-chronic inhalation toxicity data in the report, you know, positive effects in those studies. Should that be addressed in the discussion?

DR. SNYDER: I think our team wanted to have that -- you mean, that would be related to occupational exposures because exposures for the consumer wouldn't be at levels of concern. So again, that would be something to be captured in the discussion about occupational use. And you know, that largely falls under an OSHA issue, but we can address and bring it to the attention of the reader in the discussion.

MR. JOHNSON: Okay.

DR. HILL: Which one was that again? I'm sorry.

MR. JOHNSON: The acute short term and sub-chronic inhalation toxicity of ammonium.

DR. BERGFELD: (Inaudible)?

DR. KATZ: Yeah, I just wanted to just go back to the question that was asked here. I think the question was what's the rationale for removing the statement about it being a promoter. I think there was an explanation of what a "promoter" is, but there wasn't answer as to what was the rationale for not wanting the language in there. I just wanted to make sure that that's what -- you asked the question, what's the rationale for not including the language for the fact that it's a promoter, but I don't know that you got an answer for what's the rationale for not including that language.

It doesn't obviously make any difference, but there was language that was mentioned that said that it's a promoter, but two people recommended removing that language. The question is, what is the rationale for removing it? And I don't know whether that rationale was actually provided that's clarifying.

DR. SLAGA: Yeah, but we're only looking at ammonium --

DR. BELSITO: It's a promoter based upon the irritating factors (inaudible) --

DR. SLAGA: Yeah, ammonium hydroxide and --

DR. KATZ: Nobody's arguing the fact that it's a promoter or not, but it's just, you know, you're saying to remove the statement and then, I think the question was, what was your scientific rationale for removing the statement and the statement being based on fact. So what is the rationale?

DR. SLAGA: That the amount that would be in the cosmetic would not (inaudible) --

DR. KATZ: -- would not make it a promoter?

DR. SLAGA: -- tumor promotion.

DR. KATZ: And you have that as evidence?

DR. SLAGA: Yeah.

DR. KATZ: Okay.

DR. HILL: The section on tumor promotion in here that's discussed is extensive nitrosating conditions. If you go look at page -- what's this page? Stop the search -- page 18 at the bottom, they're giving it with N-methyl-N'- prime-nitro-N-nitrosoguanidine, and then ammonia. And that's a very artificial set of conditions because nobody's going to be eating this MNNG. So it's specific but they're setting up nitrosating conditions and then saying it's --

DR. LIEBLER: It's already nitrosating.

(Inaudible)

DR. HILL: But then we're adding an amine, ammonia, very reactive ammonia. So that's that --

DR. SNYDER: And that's probably attributable to the gastric irritation elicited by the compound, so.

DR. HILL: And you're not suggesting taking --

DR. SLAGA: And you're giving ammonia at extremely irritating levels so that you can bring that carcinogen

(inaudible) --

DR. HILL: This is my point.

DR. KLAASSEN: But I think it's -- maybe there's something that is being

confused here is that this remains in the document and the results.

DR. HILL: Just not in the discussion.

DR. KLAASSEN: We don't bring it -- we don't think it rises to the level of importance to talk about it in the discussion.

DR. SLAGA: Right.

DR. KLAASSEN: That's kind of where we're at.

DR. SLAGA: It's still in the report.

DR. KLAASSEN: It's still in the report. We're saying that it is a promoter, etcetera. But we as a committee don't think it raises to the level that we need to talk about it again in the discussion. So that's -- if that makes sense.

DR. BERGFELD: Thank you. Wilbur?

MR. JOHNSON: Yeah. Dr. Belsito had mentioned that including information relating to the case report involving the male custodian and chronic exposure in the discussion.

DR. BELSITO: Only, again, if we wanted to bring in the issue that, you know, this is something that hair dressers might be exposed to. But again, as a pH adjuster at that level, I don't think it's an issue.

MR. JOHNSON: Okay.

DR. BELSITO: This was a custodian who was exposed to concentrated forms of the ammonium hydroxide that was irritating, so no.

MR. JOHNSON: And just one more. So the absence of sensitization data from the report.

DR. BELSITO: Ammonia's not a sensitizer.

MR. JOHNSON: So that shouldn't be mentioned at all. Okay. Thank you.

DR. BELSITO: Well, we can say that, you know, that there was -- we know there is an absence of sensitization. However, it's the (inaudible) opinion of the panel that ammonia's not a sensitizer.

DR. BERGFELD: I think that would be appropriate since it -- in keeping with what we've done before. Ron Hill?

DR. HILL: We did have some discussion yesterday about the pH adjuster function and whether that would apply to the hair dying procedures where you have a fairly high concentration of ammonium hydroxide. Because if you've been around somebody whose used one of those procedures to do the hair dying, you smell ammonia for a few minutes.

So are we capturing that? And that was, I think, a question that I asked to be sure that we did look at that in a little more detail before we got the next version of the report. Because adjusting the pH to pH 10 so you can do that chemistry in the hair dying is pH adjusting but it might not be in the way we're usually thinking about it, so that's --

DR. BERGFELD: Okay. That will be reflected in the minutes and obviously the in-house team will look at it for us. All right.

We have to call the question on this. All those in favor of a safe conclusion for the ammonia, ammonium hydroxide.

DR. BELSITO: Non-irritating.

DR. BERGFELD: Non-irritating. Okay. All those in favor, yes. So the non-irritating was

added.

(The motion passed unanimously.)

Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: November 10, 2017
Panel Date: December 4-5, 2017

.

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., former CIR Senior Toxicologist.

1620 L STREET, NW, SUITE 1200 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

ABSTRACT: The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of Ammonia and Ammonium Hydroxide, which function as pH adjusters in cosmetic products. The Panel reviewed data relevant to the safety of these ingredients and concluded that Ammonia and Ammonium Hydroxide are safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating.

INTRODUCTION

The safety of Ammonia and Ammonium Hydroxide in cosmetics is reviewed in this Cosmetic Ingredient Review (CIR) safety assessment. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), both ingredients are reported to function as pH adjusters in cosmetic products (**Table** 2).[1] Additionally, Ammonia is reported to function as an external analgesic and fragrance ingredient and Ammonium Hydroxide is reported to function as a denaturant in cosmetic products. Functioning as an external analgesic is not a cosmetic use and, therefore, the CIR Expert Panel (Panel) will not evaluate safety in relation to that use.

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

An Environmental Protection Agency (EPA) toxicological review that covers gaseous Ammonia (NH_3) and Ammonia dissolved in water (Ammonium Hydroxide, NH_4OH) was published in 2016.[2] It should be noted that portions of the EPA review are adapted from the toxicological profile for Ammonia that was developed by the Agency for Toxic Substances and Disease Registry (ATSDR). This CIR safety assessment also includes toxicity data on Ammonia/Ammonium Hydroxide that have become available since the ATSDR and EPA documents were published.

In addition to the ATSDR and EPA reports on Ammonia, an expert assessment of the effects on human health and the environment posed by Ammonia, prepared by a 14-member task group, is available.[3] This assessment was published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization.

Furthermore, in addition to the safety test data on Ammonia and Ammonium Hydroxide that are included in this safety assessment, the Panel addressed the use of chemicals for read-across, and determined that information reported for the following chemicals is appropriate for read-across: data on "ammonium ion" (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counter ion not identified) that are included in the ATSDR toxicological profile for Ammonia; diammonium phosphate (repeated dose (short-term) oral toxicity and reproductive and developmental toxicity data) that are included in the CIR Final Report on Phosphoric Acid and Its Salts and in the European Chemicals Agency (ECHA) registration dossier on Ammonia; and data on ammonium chloride (genotoxicity data [micronucleus test]) and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data) that are included also included in the ECHA dossier (Table 1).[4,5,6]

CHEMISTRY

Definition and General Characterization

Ammonia, ammonia gas, anhydrous ammonia, and liquid ammonia are terms that are often used interchangeably to refer to the ingredient, Ammonia, in either its liquid (compressed) or gaseous state.[7] Ammonia dissolved in water is referred to as aqueous ammonia, ammonia solution, and the cosmetic ingredient name, Ammonium Hydroxide. In an aqueous formulation, these two ingredients will each be comprised of at least some of the other, dependent on the effective pH of the formulation.

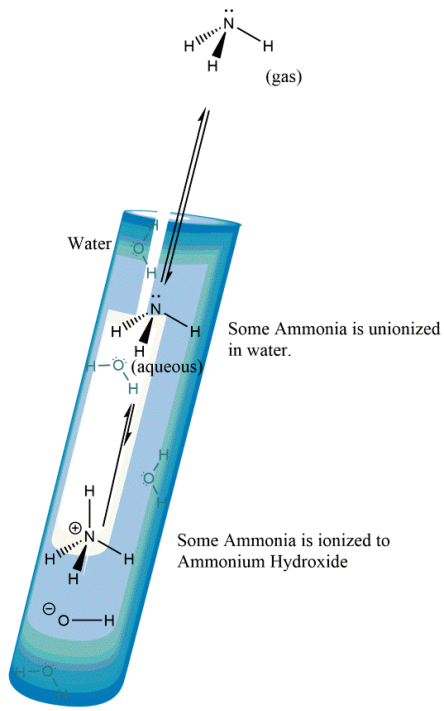


Figure 1. The aqueous relationship of Ammonia and Ammonium Hydroxide

Referring to Figure 1, some ammonia is actually ionized to ammonium ion, with hydroxide anion being formed in an equal amount (stoichiometrically) in pure water to produce Ammonium Hydroxide.

Ammonium Hydroxide is formed simply by the solution of Ammonia in water. Regardless of whether the ingredient is named Ammonia or Ammonium Hydroxide, if the formulation or test article contains water, both are present in equilibrium. At or near neutral pH, more than 99% is in the form of dissolved (i.e. molecular) Ammonia, and less than 1% is Ammonium Hydroxide. In more alkaline (i.e. higher pH) solutions, the Ammonia concentration can be significantly higher (e.g., at pH 9.25 the ratio of Ammonia to Ammonium Hydroxide is about 1:1; pK_b ~4.8 at room temperature). Accordingly, the ratio of dissolved molecular Ammonia versus the ions of Ammonium Hydroxide is dependent, in part, on the pH of the formulation. Saturation in water, at room temperature and atmospheric pressure, is approximately 34%.[8]

Since the function of external analgesic may be excluded from the purview of the CIR Expert Panel, the only function of Ammonia under review herein is pH adjuster. The term "pH" is defined as the negative $\log_{10}$ of the concentration of hydrogen ions (protons), existing as solvated forms, including hydronium ion and higher solvates in water. Above pH 7, the concentration of hydroxyl anion (OH^-) becomes greater than hydronium, increasing as pH becomes more alkaline. Accordingly, pH adjusters function in aqueous formulations and this safety assessment evaluates Ammonia and Ammonium Hydroxide in that context.

The definitions, structures, and functions in cosmetics of these ingredients are presented in **Table** 2.

Chemical and Physical Properties

Ammonia is a small nitrogenous compound with a molecular weight of 17 that is a gas at standard temperature and pressure.[9] It is a weak base that exists in hydrolytic equilibrium with Ammonium Hydroxide, as shown in Figure 1. Solvation of ammonia in water results in an equilibrium between dissolved ammonia and ammonium, the latter being formed by the abstraction of a proton from one equivalent of water molecules to produce a stoichiometric amount of hydroxyl anions (to wit, an acid-base reaction in essentially instantaneous equilibrium when in free aqueous solution). Additional chemical properties are presented in Table 3.[10,11,12,13]

Method of Manufacture

Ammonia can be formed from water gas and producer gas via the Haber-Bosch process (i.e., under high temperature and pressure, hydrogen and nitrogen are combined to produce Ammonia).[8]

Ammonium Hydroxide can be produced by passing Ammonia gas into water.[14]

Composition

According to the *Food Chemicals Codex*, Ammonium Hydroxide contains not less than 27% and not more than 30% by weight NH_3.[15] The monograph on strong Ammonia solution in the *United States Pharmacopoeia* states that this is a solution of NH_3, containing not less than 27% and not more that 31 % (w/w) NH_3.[16]

Impurities

According to the *Food Chemicals Codex*, the acceptance criteria for Ammonium Hydroxide include: lead (not more than 0.5 mg/kg), nonvolatile residue (not more than 0.02%), and readily oxidizable substances (pink color does not completely disappear within 10 minutes).[15] Similarly, according to the *United States Pharmacopoeia,* the limitations on strong Ammonia solution include: heavy metals (0.0013% limit), nonvolatile residue (not more than 5 mg of residue remains [0.05%]), and readily oxidizable substances (pink color does not completely disappear within 10 minutes).[16]

USE

Cosmetic

The safety of Ammonia and Ammonium Hydroxide is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database.[17] Use concentration data are submitted by the cosmetics industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.[18]

According to 2017 VCRP data, Ammonia is being used in 599 cosmetic products (mostly rinse-off products) and Ammonium Hydroxide is being used in 1354 cosmetic products (mostly rinse-off products) (Table 4).[17] Most of the uses of these 2 ingredients are in hair coloring products (582/599 (97.2%) uses for Ammonia and 1104/1354 (81.5%) uses for Ammonium Hydroxide). The results of a concentration of use survey provided by the Council in 2017 indicate that the highest maximum cosmetic use concentration of Ammonia is 4.6 % (in hair dyes and colors) and that the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in hair dyes and colors) (Table 4).[18] Regarding use concentrations in leave-on products, the highest maximum cosmetic use concentrations are 0.73% (Ammonia - in tonics, dressings, and other hair grooming aids) and 1.5% (Ammonium Hydroxide - in face and neck products [not spray]).

Cosmetic products containing Ammonia or Ammonium Hydroxide may be applied to the skin and hair or, incidentally, may come in contact with the eyes (e.g., at maximum use concentrations up to 0.58% (Ammonium Hydroxide in eye brow pencils) and mucous membranes (e.g., at maximum use concentrations up to 0.0012% Ammonium Hydroxide in bath soaps and detergents). Products containing Ammonia or Ammonium Hydroxide may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Ammonia (CAS No. 7664-41-7) and Ammonium Hydroxide (CAS No. 1336-21-6) are on the European Union's list of substances that cosmetics must not contain, except when subject to the following restriction: maximum concentration in ready for use preparation (6% (as NH_3)).[19] Furthermore, the following phrase appears in the wording of "conditions of use and warnings" category: above 2%: contains Ammonia.

Non-Cosmetic

Ammonia is a chemical with diverse uses, such as fertilizer and as a refrigerant.[20] Ammonia is also used in production of dyes, plastics, synthetic fibers, pesticides, explosives, refrigerants, and pharmaceuticals, and in the purification of water.[7]

Ammonium Hydroxide is affirmed as generally recognized as safe (GRAS) as a direct human food ingredient. [21CFR184.1139]. Ammonium Hydroxide must meet the specifications of the *Food Chemicals Codex* (see Impurities section), and, in accordance with these specifications, the ingredient is used in food with no limitation other than current good manufacturing practice. Concerning animals, anhydrous Ammonia is a food additive permitted in feed and drinking water, and it is used or intended for use as a source of nonprotein nitrogen in cattle feed [21CFR573.180].

In Australia, Ammonia and Ammonium Hydroxide are listed in the *Poisons Standard*, the standard for the uniform scheduling of medicines and poisons (SUSMP) in schedules 5 and 6.[21] Under schedule 5, Ammonia and Ammonium Hydroxide are permitted in preparations containing ≤ 5% Ammonia, with the following exceptions: in preparations for human internal therapeutic use; in preparations for inhalation when absorbed in an inert solid material; or in preparations containing ≤ 0.5% free Ammonia. Schedule 5 chemicals are defined as substances with a low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with simple warnings and safety directions on the label; schedule 5 chemicals are labeled with "Caution". Schedule 6 chemicals are classified as poisons with a moderate potential for harm.

Ammonia, as an intravenously-injected prescription drug, is included on the list of FDA-approved drug products.[22] Ammonia solution has been classified as an over-the-counter (OTC) drug active ingredient as a skin protectant and external analgesic, and the same is true for Ammonium Hydroxide as a skin protectant. However, FDA has determined that there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for the specified uses.[23]

TOXICOKINETIC STUDIES

Because of the equilibrium nature of these two ingredients, the studies that follow will simply recite "Ammonia" for most cases, regardless of whether Ammonia or Ammonium Hydroxide was reported.

Absorption, Distribution, Metabolism, and Excretion

Ammonia is the principal by-product of amino acid metabolism, and the liver is indicated as the central organ of Ammonia metabolism.[9] It is generated, in vivo, from the breakdown of nitrogenous substances in the gut and from the use of glutamine as a metabolic fuel in the small intestine, and is taken up by the liver where it is detoxified by conversion to urea and, to a lesser extent, glutamine.[24,25] The main source of in vivo Ammonia generation occurs in the intestines, from lysis of blood-borne urea and also from protein digestion/deamination by urease-positive bacteria and microbial deaminase.[26,27] A large amount of metabolically-generated Ammonia is absorbed into the blood and, via the portal vein, is transported to the liver where it is detoxified.[26,28,29] The normal concentration of Ammonia in the portal blood varies from 300 to 600 µM. But, in the blood leaving the liver the concentration is reduced to 20–60 µM. This confirms that the liver occupies a central position in the regulation of Ammonia levels in the organism.[30,31] According to another source, the normal range for blood serum levels is 10-40 µmol/L.[32]

The substrates from which Ammonia may be formed in the gut comprise derivatives of ingested nitrogenous material, epithelial and bacterial debris, and compounds secreted from the circulation to the mucosal cells and lumen (e.g., certain peptides, amino acids, and smaller diffusible substances such as urea).[33] Both the gut and kidneys generate substantial amounts of Ammonia from the deamidation of glutamine.[9] The glutamine-glutamate cycle in the body works in conjunction with the glucose-alanine cycle to shuttle Ammonia from peripheral to visceral organs.

Renal regulation of acid-base balance involves the formation and excretion of Ammonia to buffer hydrogen ions that are excreted in the urine. Approximately two-thirds of urinary Ammonia is derived from the amide nitrogen of glutamine, a reaction that is catalyzed by the glutaminase enzyme in renal tubular cells.[9]

The first step in the degradation of most amino acids is the removal of an α-amino residue, and an amino residue is transferred to α-ketoglutaric acid to produce glutamate.[34] Glutamate dehydrogenase converts glutamate to α- ketoglutarate and Ammonia. To prevent a toxic buildup of Ammonia, it is converted to glutamine and alanine in a number of tissues for transportation to the liver. Ammonia is then converted to urea via the urea cycle in the liver, and urea is excreted in the urine. The urea cycle, a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals.[35] In the liver, the urea cycle is essential to the conversion of excess nitrogen from Ammonia and aspartate into urea.[36] When the supply of Ammonia in mammals exceeds the capacity for its detoxification, the excretion of orotic acid in the urine increases.[35] Orotic acid (from the urea cycle) is an intermediate product in the biosynthesis of pyrimidines and purines.

There is evidence that Ammonia can cross the blood-brain barrier (BBB), mostly through ion transporters rather than by passive diffusion of gaseous Ammonia.[26,37]

Animal

Inhalation

Brain glutamine levels have been shown to increase in rats that inhaled 25 or 300 ppm Ammonia vapor for 6 hours/day for 5 days, which is likely a result of Ammonia metabolism by the astrocytic glutamate-glutamine cycle .[38,39]

Continuous exposure of rats for 24 h to concentrations up to 32 ppm Ammonia resulted in significant increase in blood Ammonia levels.[40] Exposures to 310 - 1157 ppm led to significantly increased blood concentrations of Ammonia within 8 h of exposure initiation, but blood Ammonia returned to pre-exposure values within 12 hours of continuous exposure and did not change over the remainder of the 24-hour exposure period.

Parenteral

Following the administration of [^{13}N]Ammonia to rats (via either the carotid artery or cerebrospinal fluid), most metabolized labelled nitrogen was in glutamine (amide), and little was in glutamate (plus aspartate).[41]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Acute dermal toxicity studies on Ammonia were not found in the published literature, nor were these data submitted.

Acute oral toxicity studies are presented in Table 5 and, acute inhalation studies, in Table 6.

Oral

Either serious effects (related to the pH of the solution) or no effects were reported for Ammonium Hydroxide after rats were subjected to a single oral exposure. However, when 0.3% Ammonia was administered to rats by gavage (33.3 mg/kg), gastric mucosal lesions were observed within 5 minutes. An acute oral LD_{50} of 350 mg/kg for Ammonia in rats has been reported, and the oral administration of 1 % or 3% (w/w as Ammonium Hydroxide) to rats by gavage has produced severe hemorrhagic lesions.[6,42,43,44,45]

Inhalation

In 10-minute exposure studies involving mice, LC_{50} values ranging from 8723 ppm to 10,150 ppm have been reported. In mice exposed to Ammonia (100-800 ppm) for 30 minutes, an RD_{50} (exposure concentration that produced a 50% reduction in respiratory rate) of 303 ppm was reported. The following effects were observed in mice that were exposed to Ammonia at a concentration of 21,400 ppm for 30 minutes: eye irritation, dyspnea, histopathological changes in the lungs (alveolar disruption and loss of septal continuity), coma, and death. Within the range of concentrations tested (3440 ppm to 12,940 ppm) in 1-h exposure studies involving mice**,** the following effects have been observed: hepatic lesions, congestion, and necrosis; eye irritation; dyspnea; pneumonitis and atelectasis; histopathological changes in the lung (alveolar disruption and loss of septal continuity), and, in some cases, coma and death. Additionally, LC_{50} values of 4837 ppm and 4230 ppm for Ammonia have been reported for 1-h exposures to 3600-5720 ppm and 1190-4860 ppm, respectively.[24,46,47,48,49,50,51,52]

In acute inhalation toxicity studies involving rats, LC_{50}s ranging from 7338 ppm to 45,124 ppm and RD_{50}s of 1396 ppm and 1299 ppm have been reported. Exposure durations ranged from 10 minutes (14,170-55,289 ppm) to 1-4 h (3,028-5,053 ppm). For the 10-minute exposure, LC_{50} values were ~ 22,885 ppm (males) and ~31,430 ppm (females) (at highest exposure concentration) and ~14,141 ppm (males) and ~19,769 ppm (females) (at lowest exposure concentration). For the 1-h and 4-h exposures, the LC_{50s} were ~17,633 ppm and ~7068 ppm, respectively, and corneal opacity and signs of typical upper respiratory tract irritation were observed. Signs of upper respiratory tract irritation were also associated with exposures ranging from 20 to 45 minutes, which included exposure concentrations up to 35,000 ppm. No effects were observed in rats exposed to Ammonia at a concentration of 144 ppm for 5, 15, 30, or 60 minutes. Toxic signs observed in studies in which rabbits were exposed for 1 h to Ammonia at concentrations ranging from 9800 ppm to 12,800 ppm included congestion of respiratory tract tissues, bronchiolar damage, and alveolar effects (congestion, edema, atelectasis, hemorrhage, and emphysema). At lower concentrations, there was a significant decrease in the rate of respiration (50 ppm and 100 ppm, for 2.5-3 h) and increased respiratory tract fluid output (at 3.5 ppm and 8.7 ppm, for 1 h) in rabbits. Congestion of the respiratory tract/lungs was reported in cats exposed to 1000 ppm Ammonia for 10 min and to 5200 ppm to 12,800 ppm Ammonia for 1 h . Gross pathological findings after the 10-minute exposure included varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and lung collapse.[6,24,46,53,54,55,56,57,58,59,60]

It has been noted that acute exposure data have demonstrated that injury to respiratory tissues is primarily due to Ammonia's alkaline (i.e., caustic) properties, resulting from the formation of hydroxide when Ammonia reacts with water.[2] Furthermore, Ammonia readily dissolves in the moisture on mucous membranes, forming Ammonium Hydroxide, which causes liquefactive necrosis of the tissues.

Short-Term Toxicity Studies

Animal

Dermal

Short-term dermal toxicity data on Ammonium Hydroxide or Ammonia were not found in the published literature, nor were these data submitted.

Short-term oral and inhalation toxicity studies are summarized in Table 7.

Oral

Mucosal atrophy in the stomach antrum and enlargement of the proliferative zone in the mucosa of the stomach antrum and body were observed in rats that received 0.01% Ammonium Hydroxide (in drinking water) for 8 weeks. A no-observed-adverse effect-level (NOAEL) of 250 mg/kg/day for general toxicity and a lowest-observed-adverse effect-level (LOAEL) of 750 mg/kg/day for general toxicity were reported for diammonium phosphate (identified as an appropriate read across material for short-term oral toxicity and included herein to support the overall weight of evidence) in rats dosed orally (by gavage) for 5 weeks.[6,61]

Inhalation

Rats were exposed repeatedly to Ammonia at concentrations ranging from 150 ppm (for 75 days) to 1306 ppm (for 42 days (5 days/week and 8 h/day)). The higher concentration was tolerated for 42 days in rats, and increased thickness of the nasal epithelium was observed at 150 ppm. Nasal irritation and inflammation of the upper respiratory tract were observed in rats exposed to500 ppm Ammonia for 3 weeks; reactions had cleared by week 8. When rats, rabbits, guinea pigs, monkeys, and dogs were exposed to Ammonia at a concentration of ~ 223 ppm or ~ 1105 ppm for 6 weeks (5 days/week and 8 h/day), the following effects were observed: focal pneumonitis in 1 of 3 monkeys at 223 ppm; nonspecific lung inflammation in guinea pigs and rats, but not other species at 1105 ppm; and mild to moderate dyspnea in rabbits and dogs during week 1 only at 1105 ppm. Upper respiratory effects (e.g., dyspnea and nasal lesions, irritation, and inflammation) were observed over most of the range of concentrations tested (145 ppm (5-week exposure) to 1306 ppm (42-day exposure (5 days/week and 8 h/day)) in short-term inhalation toxicity studies on Ammonia involving mice, rats, guinea pigs, pigs, rabbits or dogs. At lower Ammonia concentrations, there were no treatment-related effects in rats (at 50 or 90 ppm (continuous exposure for 50 days)) and there was no increase in the incidence of respiratory diseases in pigs exposed to Ammonia (37 ppm or ~ 14.2 ppm, inhalable dust exposure) for 5 weeks. In other studies, nearly 64% lethality was reported for rats exposed to Ammonia (653 ppm) for 25 days (continuous exposure) and 50 of 51 rats exposed to Ammonia (650 ppm) were dead by day 65 of continuous exposure. A low incidence of carcinoma of the nasal mucosa was observed in mice exposed to Ammonia (12% solution) for 8 weeks, and these results are summarized in more detail in the Carcinogenicity section. [2,6,10,24,40,46,53,62,63,64,65,66,67,68,69,70,71,72,129]

Human

Risk Assessment

A minimal risk level (MRL) of 1.7 ppm has been derived for "acute-duration" inhalation exposure (14 days or less) to Ammonia. The study involved 16 subjects exposed to Ammonia (50 ppm, 80 ppm, 110 ppm, or 140 ppm). The MRL is based on a LOAEL of 50 ppm for mild irritation to the eyes (6 subjects), nose (20 subjects), and throat (9 subjects) in humans exposed to Ammonia as a gas for 2 hours. The 1.7 ppm MRL was calculated (50 ppm ÷ 30 [uncertainty factor] = 1.7; uncertainty factor = 10 [to protect sensitive individuals] x 3 [for use of a minimal LOAEL] = 30).[73]

It should be noted that the Occupational Safety and Health Administration (OSHA) has established an 8-hour time weighted average exposure limit of 50 ppm (35 mg/m^3) for Ammonia in the workplace.[74] Exposure to Ammonia shall not exceed the 50 ppm limit in any 8-h work shift of a 40-h work week.

Subchronic Toxicity Studies

Neither subchronic dermal nor oral toxicity data on Ammonium Hydroxide or Ammonia were found in the published literature, nor were these data submitted.

Inhalation

Subchronic inhalation toxicity studies on Ammonia and Ammonium Hydroxide are summarized in Table 7.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days. The following results were reported for guinea pigs exposed to ~ 170 ppm Ammonia for 18 weeks: mild congestion of the liver, spleen, and kidneys; degenerative changes in the adrenal glands; hemosiderosis in the spleen; and cloudy swelling in proximal kidney tubules. Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks. Mild leucocytosis was noted in rats after exposure to 143 ppm, but not 43 ppm, Ammonia repeatedly (25- or 60-minute exposures every 48 h) for 3 months.[46,53, 63,75,76]

A low incidence of mortalities (13 of 515 rats and 4 of 15 guinea pigs) was observed in rats and guinea pigs exposed continuously to 671 ppm Ammonia for 90 days. However, there were no mortalities or treatment-related histopathological findings in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm Ammonia for 114 days.[53,63]

Chronic Toxicity Studies

Animal

Dermal

Chronic dermal toxicity data on Ammonium Hydroxide/Ammonia were not found in the published literature, nor were these data submitted.

Oral

Enlarged adrenal glands were observed in rabbits that received 124 mg ammonium/kg/day as (as Ammonium Hydroxide) by gavage in water for 17 months.[77]

Ammonium Sulfate (read-across for Ammonia)

Limited chronic oral toxicity data were available for Ammonia. However, ammonium sulfate was identified as an appropriate read-across surrogate. The chronic oral toxicity of ammonium sulfate was evaluated using groups of 10 Fischer 344/DuCrj rats (males and females). Ammonium sulfate was administered in the diet daily at concentrations of 0%, 0.1%, 0.6%, and 3% for 52 weeks. None of the animals died, and there were no macroscopic findings. There was a significant increase in kidney and/or liver weights in males and females of the 3% dietary group, but there were no effects on survival rate, body weights, or hematological, serum biochemical, or histopathological parameters at any concentration. Several non-neoplastic lesions, such as bile duct proliferation in the liver and focal myocarditis in the heart were observed in the 3% dietary group, but also in animals of the control group, and the difference in results was not statistically significant when the 2 groups were compared. The NOAEL for ammonium sulfate was estimated to be 0.6% in both sexes, which is equivalent to 256 and 284 mg/kg/day in males and females, respectively.[6,78] Neoplastic lesions (classified as unrelated to ammonium sulfate in the diet) reported in a carcinogenicity study in the same report are included in Table 9 .

Human

Inhalation - Risk Assessment

Chronic occupational exposure (about 12.2 years) to low levels of airborne Ammonia (9.2 ppm) had no significant effect on pulmonary function or odor sensitivity in a group of workers at a soda ash factory, compared to a control group from the same factory that was not exposed to Ammonia.[79] The ATSDR derived a chronic inhalation MRL of 0.1 ppm for Ammonia from this study.[10] An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. MRLs are based on non-cancer health effects only and are not based on a consideration of cancer effects. Derivation of the MRL is described below.

An MRL of 0.1 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to Ammonia. The MRL is based on a NOAEL of 9.2 ppm for sense of smell, prevalence of respiratory symptoms (cough, bronchitis, wheeze, dyspnea, and others), eye and throat irritation, and lung function parameters (forced vital capacity (FVC), forced expiratory volume at end of 1 second of forced expiration (FEV1), FEV1/FVC, forced expiratory flow at 50% of FVC (FEF50), and FEF at 75% of FVC (FEF75)) in humans exposed for an average of 12.2 years in a soda ash plant; no LOAEL was determined.[79] The cohort consisted of 52 workers and 35 controls (all males). The subjects were assessed on the first and last workday of their workweek. Spirometry was performed at the beginning and end of each work shift, so that each worker had four tests done. To determine the exposure levels, exposed and control workers were sampled (breathing zone air sample)

over one work shift; the average sample collection period was 8.4 hours. Air samples were collected on sulfuric acid-treated silica gel adsorption tubes (tube holder attached to the collar).

Analysis of the results showed no significant differences in the prevalence of reported symptoms, but the exposed workers reported that exposure in the plant aggravated some of their reported symptoms (cough, wheeze, nasal complaints, eye irritation, and throat discomfort). There were no significant differences in baseline lung functions between exposed and control subjects. Analysis of each worker separately showed no significant relationship between the level of Ammonia exposure and changes in lung function. Also, when the workers were divided into groups of individuals that were exposed to low (< 6.25 ppm), medium (6.25 – 12.5 ppm), and high (> 12.5 ppm) Ammonia levels, no significant association was found between reporting of symptoms, decline in baseline function, or increasing decline in function over the work shift and exposure to Ammonia. Furthermore, no association was evident between increasing years of exposure and decreasing lung function. However, the power of the indices of both level and length of exposure is low because only eight workers were in areas with relatively high Ammonia exposure. The MRL was calculated by adjusting the mean time-weighted average (TWA) exposure concentration of 9.2 ppm for continuous exposure (8/24 hours x 5/7 days) and dividing by an uncertainty factor of 10 to protect all of the sensitive individuals. A modifying factor of 3 was added for the lack of reproductive and developmental studies.[10]

Based on occupational epidemiology studies, the EPA calculated a chronic inhalation reference concentration (RfC) of 0.5 mg/m^3.[2] The critical effects in these studies were decreased lung function and respiratory symptoms.[80,79,81,82] The RfC is an estimate (with uncertainty ~ one order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental/reproductive toxicity studies are summarized in Table 8.

A relationship between the duration of exposure and the incidence of exencephaly (concentration-related increase) was observed in an in vitro study in which mouse embryos were cultured with Ammonia (38 to 300 µmol/l) for up to 93 h. In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion [read-across for Ammonia]) from gestation day 1 through day 21 of lactation, body weights of offspring were reduced by 25% (males) and 16% (females). Neither reproductive nor developmental toxicity was reported in a study in which female pigs were exposed (inhalation exposure) to ~7 ppm or ~35 ppm Ammonia from 6 weeks prior to breeding until day 30 of gestation. In an oral reproductive and developmental toxicity study on diammonium phosphate (identified as an appropriate read across material for developmental and reproductive toxicity and included herein to support the overall weight of evidence) involving rats in which embryos were treated *in vitro* and then implanted, an NOAEL of 1500 mg/kg/day (highest dose tested) was reported. The only histological findings relating to maternal toxicity were the inflammatory/degenerative changes in all treatment groups (diammonium phosphate at 250, 750, and 1500 mg/kg/day), which were considered likely to have been the result of an irritant effect.[10,6,45,83,84,85]

GENOTOXICITY STUDIES

In Vitro

Ammonia was non-genotoxic when tested at concentrations up to 25,000 ppm (with and without metabolic activation) in the following bacterial strains: *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA1538, and *Escherichia coli* strain WP2 uvr A.[6,53,45]

Ammonia was non-genotoxic to *E. coli* strain Sd-4-73 in an in vitro assay without metabolic activation.[45]

In Vivo

Blood samples from 22 workers who had been exposed to Ammonia (concentrations unknown) in a fertilizer factory were compared with samples obtained from 42 unexposed controls. Results (compared to controls) were as follows: increased frequency of chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and mitotic index, with increasing duration of exposure. Smokers had higher SCE and CA values than non-smokers and alcoholics had more CAs and SCEs than non-alcoholics. [86]

Ammonia and Ammonium Chloride (read-across for Ammonia)

An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single intraperitoneal (i.p.) doses of Ammonia (12, 25, or 50 mg/kg). The maximum number of micronucleated polychromatic erythrocytes was associated with mice that received the highest dose (50 mg/kg), and there was clear correlation of dose-yield effects. In another micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single i.p. doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride (identified as an appropriate read across material for in vivo genotoxicity and included to support the overall weight of evidence) or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h). Femoral bone marrow cells were examined for polychromatic erythrocytes, and there was no evidence of genotoxicity at the doses of ammonium chloride that were administered.[6,86]

CARCINOGENICITY STUDIES

Carcinogenicity and tumor promotion studies are summarized in Table 9.

There was no evidence of carcinogenicity in mice dosed orally with Ammonia (dissolved in water; 42 mg /kg/day; as the ammonium ion [read-across for Ammonia]) for 4 weeks. Following the oral dosing of mice (Swiss and C3H) with Ammonia (in drinking water, 193 mg/kg/day) for 2 years, there was no evidence of carcinogenicity and no effect on the spontaneous development of adenocarcinoma of the breast (associated with C3H mouse strain). Ammonium sulfate was classified as non-carcinogenic in rats in a study involving dietary concentrations up to 3% daily for 104 weeks. Neoplastic lesions were observed in this study, but were deemed not treatment-related because of the spontaneous occurrence of these lesions in the rat strain (F344/DuCrj) that was tested. After pregnant were exposed (by gavage) to ammonium ion during pregnancy and lactation, there was no evidence of lung tumors.[6,45,53,78,87,88,89,72]

Tumor Promotion

A statistically significant increase in the incidence of gastric cancer (70%) was observed in rats dosed orally with the initiator *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) and 0.01% Ammonia, when compared to dosing with MNNG alone.[90] In another study, the size, depth, and metastasis of MNNG-initiated tumors was enhanced in rats dosed orally with Ammonia (~42 mg/kg/day).[91]

OTHER RELEVANT STUDIES

Neurotoxicity

Ammonia is most toxic in the brain, and chronic hyperammonemia (metabolic disturbance) may lead to brain damage, especially in children.[9] During normal body function, approximately 10% of arterial Ammonia is extracted by the brain. Neurotoxicity is observed only when circulating levels of Ammonia are elevated. It has been reported that hyperammonemia is associated with neuronal cell loss and cerebral atrophy that lead to mental retardation and cerebral palsy in pediatric patients.[92] These toxic effects are specific to the developing brain, as neuronal damage is not observed in the brain of adult patients with hyperammonemia due to liver failure.

According to another source, many neurologic disorders are related to congenital or acquired hyperammonemia. Evidence obtained with the use of experimental hyperammonemia models suggests that acute neurotoxic effects of Ammonia are mediated by overactivation of ionotropic glutamate (GLU) receptors, mainly the *N*-methyl-D-aspartate (NMDA) receptors, and, to a lesser degree, the kainic acid (KA)/α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. [93] Results from other studies suggest that glutamine is also a mediator of Ammonia neurotoxicity.[94,95]

Toxic levels of Ammonia and alterations in pH, electrolyte disturbances, and membrane potential depolarization are thought to lead to neurological dysfunction, primarily by causing cellular swelling accompanied by brain edema and metabolic dysfunction.[26,96] Studies have suggested that Ammonia is likely to be particularly toxic to astrocytes, as they are the only cells that possess the enzyme glutamine synthetase (which is responsible for detoxifying Ammonia in the brain through condensation with glutamate).[97,98]

In in vitro studies, it has been demonstrated that acute intoxication with large doses of Ammonia leads to excessive activation of NMDA receptors.[99,100,101,102] Furthermore, excessive activation of NMDA receptors leads to neuronal degeneration and death, and is responsible for most of the neuronal damage that is found in brain ischemia.[99]

Brain Pathology

The excessive formation of Ammonia within the brains of Alzheimer's disease patients and its release into the periphery has been demonstrated.[103,104] Furthermore, a higher expression of AMP-deaminase in the brains of Alzheimer's disease patients has been observed, and this finding indicates the existence of a pathologically elevated source of Ammonia within the brain of Alzheimer's disease patients.[103,105]

Cytotoxicity

Lymphocytes separated from peripheral bovine (Holstein-Friesian cows) blood were incubated for 2 h in control medium and test medium with various concentrations of Ammonia (w/v as Ammonium Hydroxide; 0.01 mg/dl, 0.1 mg/dl, 1 mg/dl, and 10 mg/dl).[106] Viability of the lymphocytes, measured by trypan blue exclusion test, was significantly reduced after 2 h of incubation. At a concentration of 0.01 mg/ml, lymphocyte viability was significantly reduced after 24 h and 48 h of incubation. In another experiment, in which lymphocytes were preincubated with Ammonia (w/v as Ammonium Hydroxide; 10 mg/dl) and then washed and resuspended in the fresh medium with Ammonia, the number of viable cells was reduced to 51% ± 8 at 24 h, 40% ± 7 at 48 h, and to 39% ± 6 at 72 h of incubation.

Effect on Mitosis

The ability of Ammonia to affect the mitogenic response of bovine lymphocytes to phytohemagglutinin (PHA) or concanavalin A (Con A) was examined.[106] Lymphocytes from 10 Holstein-Friesian cows were incubated with various concentrations of PHA and Ammonia. Lymphocytes from 6 cows were incubated with Con A and Ammonia. Mitogenic reactivity was measured by the incorporation of methyl- ^{3}H-thymidine into the DNA of lymphocytes. Ammonia at concentrations of 0.01 mg/dl (w/v as Ammonium Hydroxide) significantly ($P < 0.01$) suppressed PHA (optimal dose = 0.5 ug/ml) stimulation of lymphocytes from only 1 animal. Other concentrations of Ammonia, at 0.1 mg/dl, 1 mg/dl, and 10 mg/dl (w/v as Ammonium Hydroxide), significantly ($P < 0.01$) reduced the response to PHA of lymphocytes from 5 cows, 9 cows, and from all animals, respectively. These concentrations significantly reduced Con A (optimal dose = 0.5 µg/ml) stimulation of lymphocytes from 1 animal, 5 animals, and all animals, respectively. A significant suppression ($P < 0.01$) of blastogenesis of lymphocytes from 1 cow by 0.01 mg/dl, 6 by 0.1 mg/dl, 14 by 1.0 mg/dl, and from 16 cows by 10.0 mg/dl was observed. The mitogenic response of lymphocytes was reduced when lymphocytes were preincubated with Ammonia for a duration as short as 1 h.

Generation of Free Radicals

Elevated concentrations of Ammonia have been shown to generate free radicals in rats and rat cell cultures,[107,108] leading to excessive production of nitric oxide (NO) by stimulating the citrulline-NO cycle.[109]

Immunological Effects

A delayed-type hypersensitivity test was used to evaluate cell-mediated immunity in groups of 8 Hartley guinea pigs.[110] The animals were vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and exposed to Ammonia (< 15 ppm, 50 ppm, or 90 ppm (6.75 µg of Ammonia per deciliter of air)) for 3 weeks. Exposure to Ammonia was followed by intradermal challenge with a purified protein derivative of tuberculin. Dermal lesion size was reduced in animals that were exposed to Ammonia at a concentration of 90 ppm (mean diameter of dermal lesion = 8.7 mm, statistically significantly different from control ($p < 0.05$)). Results were not statistically significant in the 2 other exposure groups. Also, blood and bronchial lymphocytes were harvested from guinea pigs exposed to Ammonia, and the cells were then stimulated with the mitogens PHA or Con A. Reduced T -cell proliferation was observed; however, bactericidal activity in alveolar macrophages isolated was not affected. In an *in vitro* experiment in which lymphocytes and macrophages were isolated from unexposed guinea pigs and then treated with Ammonia, reduced proliferation and bactericidal capacity were observed only at concentrations that reduced viability. These results were indicative of nonspecific effects of Ammonia-induced immunosuppression. The authors noted that the data in this study indicate that T cells may be the target of Ammonia exposure, in that specific macrophage effects were not observed.

Neurological Effects

Acute inhalation exposure to low levels of Ammonia (100 or 300 ppm) for 6 h continuously has been shown to depress free-access wheel running behavior in rodents.[111]

DERMAL IRRITATION AND SENSITZATION STUDIES

Irritation

Dermal irritation studies are summarized in Table 10.

An undiluted Ammonia solution (as 30% Ammonium Hydroxide) was classified as a corrosive material after topical application to the stratum corneum surface in reconstructed human skin cultures in vitro. At histologic examination of the

cultures, epidermal necrosis was observed. The minimum concentration of Ammonia that caused an inflammatory reaction when applied (single application) to the skin of rats and mice (6 per species) was > 25% (rats) and = 25% (mice). In a skin irritation study in which groups of 4 rats, guinea pigs, and mice were injected intradermally with Ammonia (0.01 ml), the minimum concentration that caused a positive reaction was 0.05% in rats, mice, and guinea pigs.[112] Ammonia (20% as Ammonium Hydroxide) was corrosive to the skin of rabbits. In another study involving rabbits, 12% aqueous Ammonia was corrosive to the skin, whereas 10% was not.

In clinical testing, the application of a saturated aqueous solution of Ammonia to the skin of 16 subjects resulted in blister formation and skin irritation. In a study involving 110 subjects, Ammonia (1:1 aqueous solution) was applied to the skin and minimal blistering time (MBT) served as an indicator of cutaneous irritability. The inflammatory reaction observed was considered slight, and MBT ranged from 3 to 57 minutes. Results from another study in which 50% Ammonium Hydroxide solution (0.5 ml of freshly prepared 1:1 aqueous solution of Ammonium Hydroxide) was applied to the skin indicated that the time required to produce a full blister was greatly prolonged in the aged, when compared to young adults.[6,21,45,112,113,114,115,116]

Sensitization

Skin sensitization data on Ammonia were not found in the published literature, nor were these data submitted.

OCULAR IRRITATION STUDIES

Ocular irritation studies are summarized in Table 11.

Ammonia was classified as a severe ocular irritant in the in vitro ^{51}Cr-release assay involving human corneal endothelial cell cultures. In rabbits, Ammonia (as Ammonium Hydroxide) at 1 mg was classified as an ocular irritant. At a concentration of 28.5%, Ammonia induced corneal opacity in rabbits. In a study involving groups of 6 rabbits, Ammonia caused conjunctivitis at concentrations of 1% to 10%, but not 0.3%; the 10% concentration also caused corneal opacities within 1 h of instillation. Conjunctivitis and corneal damage were also observed in a study involving 3 rabbits, whereby 3% Ammonia, 100 µl was instilled into the eyes.[10,45,117,118,119,120]

In a study involving rats, there was no evidence of ocular irritation following exposure to Ammonia at vapor concentrations ranging from 15 to 1157 ppm. However, it has been reported that Ammonia can penetrate the eye rapidly and that ocular irritation or damage can occur at airborne concentrations as low as 20 ppm.[20,24,40,121]

MUCOUS MEMBRANE IRRITATION STUDIES

The stomachs of male Sprague-Dawley rats were exposed (mounted in an ex vivo gastric chamber) to 2 ml of Ammonia (15-60 mmol/l, in saline) for 15 minutes (for microscopic study) or for 60 minutes (for macroscopic study), and exposure was followed by examination for mucosal lesions. Microscopic damage to the gastric mucosa was observed.[122]

CLINICAL STUDIES

Case Reports

A 68-yr-old male patient, employed for 18 years, was exposed frequently to anhydrous Ammonia leaks from a microfilm processor camera while on the job. He was diagnosed with interstitial lung disease and severe restrictive lung disease due to Ammonia inhalation. Marked diffuse interstitial fibrosis throughout the lung was observed.[123]

A male custodian had used Ammonia (28% Ammonium Hydroxide solution; which he dilutes in water) to clean office floors daily for 19 years.[124] He experienced regular episodes of upper airway irritation, coughing, and eye irritation when mixing the chemical in water. An evaluation of the patient revealed a negative rheumatoid factor and positive antinuclear antibody at a 1:320 dilution. The gallium lung scan was normal, but pulmonary function testing indicated a moderate restrictive defect and a formal exercise study indicated ventilator restriction upon attainment of maximum oxygen consumption. The results of a transbronchial lung biopsy with fiberoptic bronchoscopy revealed interstitial fibrosis with chronic inflammation. Granulomata were not present and cultures for tuberculosis and fungal infection were negative. A decrease in the diameter of the hypopharynx, secondary to hypertrophy of the soft tissues in the hypopharynx, was also observed. The opacification of the optic lens capsule, bronchiectasis, and fibrous obliteration of the small airways observed were described as chronic lesions that follow acute exposure to Ammonia.

Other Clinical Reports

Clinical reports relating to inhalation exposure are summarized in Table 12.

In various clinical reports, individuals were exposed to Ammonia at concentrations ranging from 25 ppm to 700 ppm. The periods of exposure ranged from 5 minutes to 6 weeks (5 days per week [2-6 h/day]). Nose, throat, and eye irritation were observed.[46,73,125,126,127,128]

EPIDEMIOLOGICAL STUDIES

Non-Cancer Endpoints

A retrospective study was performed to assess the association between petrochemical exposure and spontaneous abortion. Study participants included 2853 non-smoking women who had been pregnant at least once, 96 of whom had been exposed to Ammonia (actual exposure levels unknown). Exposure during the pre-conception period and the first trimester of pregnancy was calculated based on information on perceived Ammonia exposure. Exposure during the first, second, and third trimesters was recorded separately for each pregnancy. Data analyses did not indicate any effect on spontaneous abortion (Odds ratio: 1.2; 95% confidence interval (CI): 0.5-2.60.[6]

OCCUPATIONAL EXPOSURE

Airway Inflammation

Airway inflammation due to Ammonia exposure was evaluated in female Palestinian hairdressers in Hebron who had worked in this profession for 12 ± 7 years.[129] Study participants included 33 non-smoking hairdressers (from 13 salons total) and 35 non-smoking control subjects. The hairdressers worked in relatively small-sized salons with scarce ventilation, lacked respiratory protective equipment, and were exposed to Ammonia concentrations ranging from 3 to 61 mg/m^3 (geometric mean = 7.3 mg/m^3; geometric standard deviation = 3) . The longest and shortest durations of Ammonia measurement in a salon were 45 minutes and 305 minutes, respectively. Peak exposure to Ammonia was evaluated by obtaining the number and duration of peaks above 10.5 mg/m^3 for each measurement session in a salon. The percentage of total sampling time when Ammonia levels exceeded 10.5 mg/m^3 was very high in 3 salons (percentages of 92%, 99.6%, and 75%, respectively). Both groups completed a questionnaire relating to respiratory symptoms during the past 12 months, and lung function and exhaled nitric oxide tests were performed. Blood and sputum samples were collected from all participants. When compared to unexposed controls, hairdressers were found to have a higher level of sputum neutrophil count (i.e., sign of neutrophilic airway inflammation; absolute numbers of neutrophils per mg sputum: 376 (for hairdressers) versus 182 (for controls)). The hairdressers also had significantly elevated exhaled nitric oxide levels and blood C reactive protein levels when compared to control subjects (controlled for age and body mass index).

SUMMARY

The safety of Ammonia and Ammonium Hydroxide as used in cosmetics is reviewed in this safety assessment. According to the *Dictionary*, both ingredients function as pH adjusters in cosmetic products. Additionally, Ammonia functions as an external analgesic and fragrance ingredient and Ammonium Hydroxide functions as a denaturant in cosmetic products. Functioning as an external analgesic is not a cosmetic use and, therefore, the Panel did not evaluate safety in relation to that use in cosmetics. Additionally, the function of fragrance may be excluded from the purview of the Panel.

According to 2017 VCRP data, Ammonia is being used in 599 cosmetic products (mostly rinse-off products) and Ammonium Hydroxide is being used in 1354 cosmetic products (mostly rinse-off products). These uses of both ingredients are mostly in hair coloring products. The results of a concentration of use survey provided by the Council in 2017 indicate that the highest maximum cosmetic use concentration of Ammonia is 4.6% (in rinse off products (hair dyes and colors)) and the highest maximum cosmetic use concentration of Ammonium Hydroxide is 12.5% (in rinse off products (hair dyes and colors)). Regarding use concentrations in leave-on products, the highest maximum cosmetic use concentrations are 0.73% (Ammonia - in tonics, dressings, and other hair grooming aids) and 1.5% (Ammonium Hydroxide - in face and neck products (not spray)).

These two ingredients are indistinguishable from each other in aqueous formulation. Since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity

data is reported for Ammonia or Ammonium Hydroxide, it is applicable to both (as the test articles would have had this same equilibrium).

A large amount of metabolically-generated Ammonia is absorbed into the blood and is detoxified by the liver. The urea cycle (in liver), a cycle of biochemical reactions that produces urea from Ammonia, is the major pathway for Ammonia detoxification in terrestrial mammals. The normal range for blood serum levels has been reported as 10-40 µmol/L.

An acute oral LD_{50} of 350 mg/kg has been reported in a study involving rats dosed orally with Ammonia dissolved in water. Gastric lesions in rats have been observed after oral dosing (gavage) with 0.03% to 1% Ammonia and 1% and 3% Ammonium Hydroxide. The increase in gastric lesions observed was both concentration- and pH-dependent.

It has been noted that acute exposure data have demonstrated that injury to respiratory tissues is primarily due to Ammonia's alkaline (i.e., caustic) properties from the formation of hydroxide when it reacts with water. In acute inhalation toxicity studies, Ammonia was tested at concentrations ranging from 3.5 ppm (cats and rabbits, 1 h exposure) to 54,289 ppm (rats, 10-minute exposure). Exposure to the highest concentration resulted in hemorrhagic lungs, and increased respiratory fluid output was noted at the lowest concentration. In 10-minute exposure studies involving mice, LC_{50} values ranging from 8723 ppm to 10,150 ppm have been reported. In mice exposed to Ammonia (100 - 800 ppm) for 30 minutes, an RD_{50} of 303 ppm was reported. Within the range of concentrations tested (3440 ppm to 12,940 ppm) in 1-h exposure studies involving mice**,** the following effects have been observed: hepatic lesions, congestion, and necrosis; eye irritation; dyspnea; pneumonitis and atelectasis; histopathological changes in the lung (alveolar disruption and loss of septal continuity), and, in some cases, coma and death.

Exposure durations ranged from 10 minutes (14,170-55,289 ppm) to 1-4 h (3028-5053 ppm) in acute inhalation toxicity studies involving rats. For the 10-minute exposure, LC_{50} values were ~ 22,885 ppm (males) and ~31,430 ppm (females) (at highest exposure concentration) and ~14,141 ppm (males) and ~19,769 ppm (females) (at lowest exposure concentration). For the 1 h and 4 h exposures, the LC_{50s} were ~17,633 ppm and ~7068 ppm, respectively, and corneal opacity and signs of typical upper respiratory tract irritation were observed.

In short-term oral toxicity studies involving rats, doses of ~ 42 mg/kg/day for 8 weeks resulted in mucosal atrophy in the stomach antrum, and doses up to 1500 mg/kg/day for 35 days resulted in treatment-related changes in body weight, hematological findings, clinical biochemistry findings, and non-neoplastic histopathological findings.

Ammonia was evaluated at concentrations ranging from 0.6 ppm, to 1306 ppm in short-term inhalation toxicity studies. The results of these studies indicate histopathological changes of respiratory tissues in several animal species (lung inflammation in guinea pigs and rats; focal or interstitial pneumonitis in monkeys, dogs, rabbits, and guinea pigs; pulmonary congestion in mice; thickening of nasal epithelium in rats and pigs; nasal inflammation or lesions in rats and mice) across different dosing regimens. In general, responses in respiratory tissues increased with increasing Ammonia exposure concentration.

Fatty changes of liver plate cells were seen in rats following continuous exposure to Ammonia (642 ppm) for 90 days. Mild congestion/degenerative changes in internal organs were reported for guinea pigs exposed to ~ 170 ppm Ammonia for 18 weeks. Damaged tracheal mucosae were observed in rats exposed repeatedly to Ammonia (100 ppm) for 12 weeks. Mild leucocytosis was noted in rats after exposure to 143 ppm, but not 43 ppm of Ammonia repeatedly for 3 months (25- or 60-minute exposures every 48 h). A low incidence of mortalities (13 of 515 rats and 4 of 15 guinea pigs) was observed in mice and guinea pigs exposed continuously to 671 ppm Ammonia (reported as Ammonium Hydroxide) for 90 days. However, there were no mortalities or treatment-related histopathological findings in rats, guinea pigs, rabbits, monkeys, or dogs exposed continuously to ~57.43 ppm for 114 days.

Enlarged adrenal glands were observed in rabbits that received 124 mg /kg/day Ammonia (as Ammonium Hydroxide) by gavage in water for 17 months.

In a developmental toxicity study involving pregnant rats exposed to Ammonia in the diet (4293 mg/kg/day; as the ammonium ion [read-across for Ammonia]) from gestation day 1 through day 21 of lactation, body weights of male and female offspring were reduced. Neither reproductive nor developmental toxicity were reported in a study in which female pigs were exposed (inhalation exposure) to ~7 ppm or ~35 ppm Ammonia from 6 weeks prior to breeding until day 30 of gestation. In an oral reproductive and developmental toxicity study on diammonium phosphate (identified as an appropriate read across material for developmental and reproductive toxicity and included herein to support the overall weight of evidence) involving rats in which embryos were treated *in vitro* and then implanted, an NOAEL of 1500 mg/kg/day (highest dose tested) was reported.

In the Ames test with and without metabolic activation, Ammonia was non-genotoxic in *S. typhimurium* strains and in *E. coli* strain WP2 uvr A. Without metabolic activation, it was non-genotoxic to *E. coli* strain Sd-4-73. An increased frequency of micronuclei (compared to controls) was observed in Swiss albino mice that received single intraperitoneal doses of Ammonia (12, 25, or 50 mg/kg). The maximum number of micronucleated polychromatic erythrocytes was associated with mice that received the highest dose (50 mg/kg), and there was clear correlation of dose-yield effects. In another micronucleus test, groups of 10 (5 males, 5 females) ddY mice received single intraperitoneal (i.p.) doses of 62.5, 125, 250 and 500 mg/kg ammonium chloride (read-across for Ammonia, or i.p. doses of 31.3, 62.5, 125, and 250 mg/kg ammonium chloride (4 injections within 24 h). Ammonium chloride was not genotoxic.[6]

Increased frequencies of chromosomal aberrations, sister chromatid exchanges, and mitotic index, with increasing duration of exposure were reported for workers who had been exposed to Ammonia in a fertilizer factory.

Ammonia (whether reported as Ammonia or Ammonium Hydroxide) was not carcinogenic in Swiss and C3H mice dosed orally. A statistically significant increase in the incidence of gastric cancer (70%) was observed in rats dosed orally with MNNG and 0.01% Ammonia, when compared to dosing with MNNG alone. In another study, the size, depth, and metastasis of MNNG-initiated tumors were enhanced in rats dosed orally with Ammonia (~42 mg/kg/day). There was no evidence of a tumorigenic effect in mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg Ammonia /kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors. Ammonium sulfate was classified as non-carcinogenic in rats in a study involving dietary concentrations up to 3% daily for 104 weeks. Neoplastic lesions were observed in this study, but were deemed not treatment-related because of the spontaneous occurrence of these lesions in the rat strain (F344/DuCrj) that was tested.

It has been reported that hyperammonemia (a metabolic disturbance characterized by an excess of Ammonia in the blood) is associated with neuronal cell loss and cerebral atrophy that lead to mental retardation and cerebral palsy in pediatric patients.

At a concentration of 0.01 mg/ml Ammonia, bovine lymphocyte viability was significantly reduced after 24 h and 48 h of incubation. In another study, the mitogenic response of lymphocytes was reduced after preincubation with Ammonia.

Guinea pigs exposed to 90 ppm Ammonia for 3 weeks developed a significant decrease in the cell-mediated immune response to challenge with a derivative of tuberculin.

No overt symptoms of neurological disorders were reported in guinea pigs or monkeys that were exposed to up to 1105 ppm Ammonia for 6 weeks.

The minimum concentration of Ammonia that caused an inflammatory reaction when applied (single application) to the skin of rats and mice (6 per species) was > 25% (rats) and = 25% (mice). In a skin irritation study in which groups of 4 rats, guinea pigs, and mice were injected intradermally with Ammonia (0.01 ml), the minimum concentration that caused a positive reaction was 0.05% in rats, mice, and guinea pigs. Ammonia (reported as Ammonium Hydroxide; 20% and 12%) was corrosive to the skin of rabbits, whereas the 10% concentration was not.

The application of a saturated aqueous solution of Ammonia (reported as Ammonium Hydroxide) to the skin of 16 subjects resulted in blister formation and skin irritation. In a study involving 110 subjects, Ammonia (reported as Ammonium Hydroxide; 1:1 aqueous solution) was applied to the skin and the inflammatory reaction observed was considered slight.

In rabbits, Ammonia (1 mg of Ammonium Hydroxide) was classified as an ocular irritant and 28.5% Ammonia (reported as Ammonium Hydroxide) induced corneal opacity. Additionally, Ammonia caused conjunctivitis in rabbits at concentrations of 1% to 10% (reported as Ammonium Hydroxide), but not 0.3%.

Microscopic damage to the gastric mucosa was observed in the stomachs of male rats exposed (ex vivo) to Ammonia (up to 60 mmol/l of Ammonium Hydroxide) for 15 minutes.

In various clinical reports, ocular, nasal, and throat irritation were observed in human subjects exposed to Ammonia in the 25 ppm to 700 ppm concentration range.

A retrospective study was performed to assess the association between petrochemical exposure and spontaneous abortion. Study participants included 2853 non-smoking women who had been pregnant at least once, 96 of whom had been exposed to unknown Ammonia concentrations. Data analyses did not indicate any effect on spontaneous abortion.

Results from an occupational exposure study indicated that female Palestinian hairdressers exposed to high levels of Ammonia in the workplace had higher neutrophilic airway inflammation when compared to a non-exposed control group.

DISCUSSION

The Panel noted that Ammonia and Ammonium Hydroxide, well-known skin irritants, are indistinguishable from each other in aqueous formulation. Furthermore, since the only cosmetic function of Ammonia applicable to this safety assessment is pH adjuster (which by default means aqueous formulations only) and Ammonium Hydroxide does not exist outside of water, regardless of which ingredient is added, the final formulations will contain an equilibrium of molecular Ammonia and the ions of Ammonium Hydroxide in water. Thus, whether toxicity data are reported for Ammonia or Ammonium Hydroxide, these data are applicable to both (as the test articles would have had this same equilibrium).

The Panel addressed the use of chemicals for read-across, and determined that information reported for the following chemicals is appropriate for read-across to Ammonia and Ammonium Hydroxide: data on "ammonium ion" (reproductive and developmental toxicity, genotoxicity, and carcinogenicity data; counter ion not identified) that are included in the ATSDR toxicological profile for Ammonia; diammonium phosphate (repeated dose (short-term) oral toxicity and reproductive and developmental toxicity data) that are included in the CIR Final Report on Phosphoric Acid and Its Salts and in an ECHA registration dossier on Ammonia; and data on ammonium chloride (genotoxicity data [micronucleus test]) and ammonium sulfate (oral carcinogenicity and chronic oral toxicity data) that are included also included in the ECHA dossier. Ammonium Hydroxide, diammonium phosphate, ammonium chloride, and ammonium sulfate are all low molecular weight, inorganic ammonium salts. The Panel stated that, because the chemical and physical properties and metabolism of these salts should be essentially identical, information on diammonium phosphate, ammonium chloride, and ammonium sulfate is useful for evaluating the safety of Ammonia and Ammonium Hydroxide. The use of these chemicals for read-across is presented in Table 1.

Skin sensitization data are absent from the safety assessment. However, the Panel noted that these ingredients are corrosive, but there are no concerns relating to the sensitization potential of Ammonia or Ammonium Hydroxide. Therefore, the Panel determined that cosmetic products containing these ingredients should be formulated to be non-irritating.

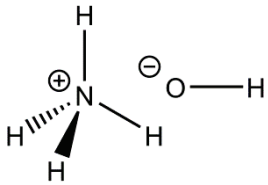
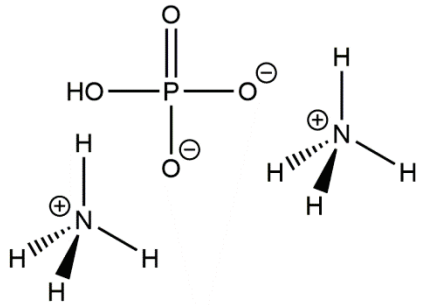
The Panel also recognized that there are reports of safety issues relating to chronic ingredient exposure experienced by hairdressers, but acknowledged that evaluation of occupational safety is not within the purview of the Panel.

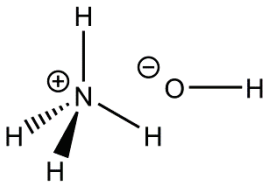
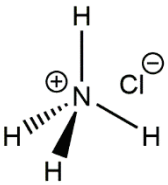
CONCLUSION

The CIR Expert Panel concluded that Ammonia and Ammonium Hydroxide are safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating.

TABLES

Table 1. Read-Across Justifications

	Target Material	Read-Across Material
Name	***Ammonia/Ammonium Hydroxide***	***diammonium phosphate***
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	7783-28-0
Structure		
read-across endpoints	• short-term toxicity – oral • reproductive & developmental	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	132.03
log K_{ow} (estimated)[12]	-4.37 – 0.28 (dissolved NH_3)	-2.85

Name	***Ammonia/Ammonium Hydroxide***	***ammonium chloride***
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	1448438-95-6; 12125-02-9
Structure		
read-across endpoints	• genotoxicity; *in vitro*	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	53.49
log K_{ow} (estimated)[12]	-4.37 – 0.28 (dissolved NH_3)	-4.37

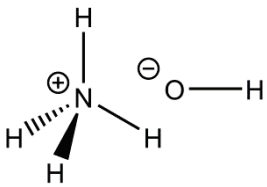
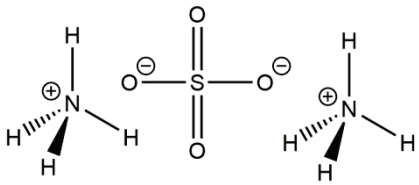
Name	***Ammonia/Ammonium Hydroxide***	***ammonium sulfate***
CAS No(s).	7664-41-7; 8007-57-6; 1336-21-6	7783-20-2
Structure		
read-across endpoints	• chronic toxicity; oral • carcinogenicity; oral	
justification	chemical properties, physical properties and metabolism are expected to be essentially identical for these two ammonium salts	
Examples:		
Formula weight (Da)	35.05	132.13
log K_{ow} (estimated)[12]	-4.37 – 0.28 (dissolved NH_3)	0.48

Table 2.Definition, Idealized Structures, and Functions of the Ingredients in this Safety Assessment.[1; CIR Staff]

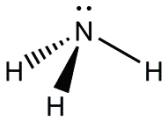
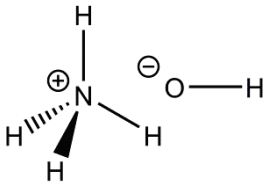
Ingredient CAS No.	Definition & Idealized Structures	Function
Ammonia 7664-41-7 8007-57-6	Ammonia is an inorganic gas that conforms to the formula:  (See also Ammonium Hydroxide)	External Analgesics; Fragrance Ingredients; pH Adjusters
Ammonium Hydroxide 1336-21-6	Ammonium Hydroxide is an inorganic base that conforms to the formula:  [In reality however, the solid, anhydrous salt does not exist. Instead, Ammonium Hydroxide is only present as an aqueous ion pair, the result of hydrolysis (not dissociation of a solid salt), in equilibrium with dissolved ammonia]	Denaturants; pH Adjusters

Table 3. Physical and Chemical Properties of Ammonia and Ammonium Hydroxide

Property	Value	Reference
Ammonia		10
physical form and/or color	Gas at room temperature; colorless	10
molecular weight (Daltons (Da))	17.03	10
water solubility (% w/w at 20°C)	33.1	10
Other solubility (%w/w at 25°C)	10 (absolute ethanol); 16 (methanol); soluble in chloroform and ether	10
density (g/L)	0.7710 (gas);	10
density (g/L at -33.5°C and 1 atm)	0.6818 (liquid); 0.7 (liquid)	10,11
vapor density (air = 1)	0.5967	10
specific gravity (g/L at 25°C)	0.747	10
melting point (°C)	-77.7	10,11
boiling point (°C)	-33.35	10,11
autoignition temperature (°C)	650	10
vapor pressure (atm at 20°C)	8.5	10
log K_{ow} (estimated)	0.23	10
Ammonium Hydroxide		
Physical form	Colorless liquid	13
density (g/L at 20°C)	0.89801(28% aqueous)	10
Formula weight (Da)	35.05	11
melting point (°C)	-58 (25% aqueous)	13
boiling point (°C)	38 (25% aqueous)	13
vapor pressure (kPa at 20°C)	48 (25% aqueous)	13
log K_{ow} (estimated)	-4.37	12

Table 4. Frequency and Concentration of Use According to Duration and Type of Exposure.[17,18]

	Ammonia		Ammonium Hydroxide	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	599	0.00002-4.6	1354	0.00028-12.5
Duration of Use				
Leave-On	7	0.00002-0.73	163	0.003-1.5
Rinse off	592	0.00015-4.6	1191	0.00028-12.5
Diluted for (bath) Use	NR	NR	NR	NR
Exposure Type				
Eye Area	1	NR	42	0.022-0.58
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation- Sprays	3***	0.73*	6*	0.29-1.3*
Incidental Inhalation- Powders	3***	0.00002-0.14**	NR	0.45-1.5**
Dermal Contact	6	0.00002-0.14	159	0.0012-1.7
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	10	0.00006-1.4	72	0.00028-3.6
Hair-Coloring	582	2.8-4.6	1104	2.5-12.5
Nail	1	0.00008-0.00075	3	0.003-1.2
Mucous Membrane	NR	NR	1	NR
Baby Products	NR	NR	NR	NR

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for Bath Product Uses.
*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.
**It is possible that these products may be powders, but it is not specified whether the reported uses are powders.
***Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 5. Acute Oral Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (0.03, 0.1, 0.3, 0.5, or 1%)	Male Wistar rats (groups of 6)	Administered by oral gavage	Minimal concentration at which gastric lesions observed was 0.03%. Over the range of concentrations administered, there was an increase in gastric juice pH and the ulcer index in an Ammonia concentration-dependent manner (mean pH: 4.38 to 8.45).[42]
Ammonia (dissolved in water)	Male Wistar rats (groups of 10)	Administered by gavage according to Organization for Economic Co-operation and Development (OECD) Guideline 401. Dosing followed by 14-day observation period	LD_{50} (calculated) = 350 mg/kg.[6,43,45]
Ammonium Hydroxide (1% or 3%)	Male Sprague-Dawley rats (groups of 4 to 8)	Administered by gavage	Severe hemorrhagic lesions produced in a concentration-related manner. The lesion scores at 1% and 3% concentrations were 26.6 ± 9.3 mm^2 and 97.7 ± 8.3 mm^2, respectively. The pH of 3% solution was 11.5. When this pH was decreased up to 7.0, by neutralizing with 0.1 N hydrochloric acid, the ulcerogenic activity of Ammonium Hydroxide was significantly mitigated at pH 10 and completely disappeared at pH 9. [44]

Table 6. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (21,400 ppm)	Mice. 30-minute exposure	Signs and symptoms included eye irritation (blinking and scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma, and death. Histopathology of the lungs of mice that died showed alveolar disruption and loss of septal continuity.[24,47]
Ammonia (8770-12,940 ppm)	Mice (groups of 20). 10-minute exposure	LC_{50} =10,150 ppm.[46,48,53]
Ammonia (8723-12,870 ppm)	Mice (groups of 20). 10-minute exposure	At 8,723 ppm, 25% of the animals died. At 12,870 ppm, 80% of the animals died. LC_{50} = 10,096 ppm.[24,48]
Ammonia (3600-5720 ppm)	Mice. 1-h exposure	Nasal and eye irritation, followed by labored breathing, in all groups. Gross examination of surviving mice showed mild congestion of the liver at the intermediate (4550 ppm) and high (5720 ppm) concentrations. LC_{50} = 4837 ppm (95% CI = 4409–5305 ppm).[24,50,53]
Ammonia (1190-4860 ppm)	ICR male mice (groups of 12). 1-h exposure	In animals that survived 14-day observation period, pathologic lesions included mild-to-moderate pneumonitis (dose-related severity), focal atelectasis in the lungs (4,860 ppm), and degenerative hepatic lesions (dose-related severity, 3,440–4,860 ppm). LC_{50} = 4,230 ppm. [24,49,53]
Ammonia (4840 ppm)	Mice. 1-h exposure	Signs and symptoms included eye irritation (blinking and scratching), dyspnea, frothing, convulsions, excitation/escape behavior, coma, and death. Histopathology of the lungs of mice that died showed alveolar disruption and loss of septal continuity.[24,51]
Ammonia (3440 ppm)	Mice (groups of 12). 1-h exposure	Liver necrosis.[49]
Ammonia (92 mg/m^3 [~132 ppm] to 1243 mg/m^3 [~1785 ppm])	SPF mice of the OF1-ICO strain. Nose-only exposure for 45 minutes	Mice appeared more susceptible to ammonia in presence of dry air (RD_{50} (exposure concentration producing a 50% decrease in respiratory rate) = 582 [407 ppm] and 732 mg/m^3 [547 ppm] in dry and wet air, respectively).[24,58]
Ammonia (100-800 ppm)	Male Swiss-Webster mice (groups of 4). 30-minute exposure	RD_{50} = 303 ppm (95% confidence limits = 188–490 ppm).[24,52,53]
Ammonia (9870 mg/m^3 air [14,170 ppm] to 37,820 mg/m^3 air [54,289 ppm])	SPF-bred Wistar rats (5 males, 5 females/group). Exposures: 20,950 to 37,820 mg/m^3 air (10 minutes); 18,290 to 23,200 mg/m^3 air (20 minutes); 12,620 to 16,840 mg/m^3 air(40 minutes), and 9870 to 13,240 mg/m^3 air (60 minutes)	LC_{50} (10-minute exposure) = 28,130 mg/m^3 air (40,300 ppm); LC_{50} (20-minute exposure) = 19,960 mg/m^3 air (28,595 ppm); LC_{50} (40-minute exposure) = 14,170 mg/m^3 air (20,300 ppm); and LC_{50} (60-minute exposure) = 11,590 mg/m^3 air (16,600 ppm). Hemorrhagic lungs in animals that died.[54]

Table 6. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (9000-35,000 ppm)	Male Sprague-Dawley rats: 4 groups of 6 (9,000 to 26,000 ppm), 1 group of 8 (30,000 ppm), and 1 group of 4 (35,000 ppm). Exposure for 20 minutes in head-only exposure system	Lung edema increased in all groups. Dose-dependent increases in ocular irritation, lacrimation, and labored breathing. LC_{50} (determined by probit analysis) = 23,672 ppm. [55]
Ammonia (9000 to 23,000 ppm)	Groups of 6 male Sprague-Dawley rats. Exposure for 20 minutes in head-only exposure system for 20 minutes	Peak inspiratory and expiratory flow decreased after exposure to 20,000 and 23,000 ppm. Weight loss, and increased total blood cell counts (white blood cells, neutrophils, and platelets) after exposure to 20,000 ppm. Morphological changes at histopathologic examination of lungs and trachea: alveolar, bronchial, and tracheal edema; epithelial necrosis, and exudate at 20,000 ppm.[56]
Ammonia (3028-14,044 ppm)	Male and female SPF-bred Wistar rats (Hsd Cpb:WU strain; 5 males, 5 females). Nose-only exposure to 9,222-14,044 ppm for 1 h and 3,028-5,053 ppm for 4 h.	Signs typical of upper respiratory tract irritation. No gross abnormalities in any organ or nasal passages were found at necropsy of surviving rats (2 weeks post-exposure). Rats that died had corneal opacity, collapsed lungs, nasal discharge, reddened larynx, and tracheal epithelial desquamation. LC_{50} (1-h exposure) = = 12,303 mg/m^3 [~17,633 ppm]). LC_{50} (4-h exposure = 4,923 mg/m^3 [~7068 ppm]).[57]
Ammonia (6210-9840 ppm)	Groups of 10 male CFE rats. 1-h exposure	Signs of eye and nasal irritation observed immediately, followed by labored breathing and gasping. Surviving animals exposed to the low concentration weighed less than controls on day 14, and gross examination showed mottling of the liver and fatty changes at the two highest concentrations. LC_{50} = 7338 ppm (95% CI = 6822–7893 ppm).[24,50,53]
Ammonia (431, 1436, and 4307 ppm)	Rats. Inhalation exposure for 5, 15, 30, or 60 minutes	Decrease in static muscular tension and other sublethal effects.[53]
Ammonia (1436, 4307, and 6814 ppm)	White rats (number not stated). Inhalation exposure for 5, 15, 30, or 60 minutes	Dyspnea, irritation of respiratory tract and eyes, cyanosis of extremities, and increased excitability.[53]
Ammonia (92 mg/m^3 [~132 ppm] to 1243 mg/m^3 [~1785 ppm])	Groups of 4 male specific pathogen free (SPF) Wistar rats of the Hsd Cpb:WU (SPF) strain. Nose-only exposure for 45 minutes	RD_{50} = 972 and 905 mg/m^3 (corresponding to ~1396 and ~1299 ppm, respectively) in rats in dry and wet air, respectively.[24,58]
Ammonia (144 ppm)	Rats (number not stated). Inhalation exposure for 5, 10, 15, 30, or 60 minutes	No effects.[53]
Ammonia (5,200-12,800 ppm)	Rabbits. 1-h exposure	Average survival: 18 h (gassed after cannulation), 33 h (gassed before cannulation). 2- to 3-fold increase in production of respiratory tract fluid. No change in water content of lungs. Increased blood hemoglobin. Increased plasma lipids.[24]

Table 6. Acute Inhalation Toxicity

Ingredient	Animals/Protocol	Results
Ammonia (10,360 ppm, average)	Rabbits. 1-h exposure	Congestion of respiratory tract tissues.[24]
Ammonia (50 ppm and 100 ppm)	16 New Zealand White rabbits. Inhalation Exposure for 2.5 h to 3 h	Significant decrease in rate of respiration.[53]
Ammonia (3.5 ppm and 8.7 ppm)	54 rabbits. Exposure for 1 h	Increased respiratory tract fluid output by 2- to 3-fold. No appreciable effect on water content of respiratory tract tissues. Transient decrease in blood hemoglobin. Lipemia also observed.[53]
Ammonia (5,200-12,800 ppm)	Cats. 1-h exposure	Average survival: 18 h (gassed after cannulation), 33 h (gassed before cannulation). 2- to 3-fold increase in production of respiratory tract fluid. No change in water content of lungs. Increased blood hemoglobin. Increased plasma lipids.[46,59]
Ammonia (10,360 ppm, average)	Cats. 1-h exposure	Congestion of respiratory tract tissues.[46,59]
Ammonia (1,000 ppm)	20 cats. 10-minute exposure	Biphasic course of respiratory pathology. Effects at 24 h post-exposure included severe dyspnea, anorexia, and dehydration; rhonchi and coarse rales evident upon auscultation. Gross pathology revealed varying degrees of congestion, hemorrhage, edema, interstitial emphysema, and collapse of the lungs at all time points. Pulmonary resistance increased throughout the study.[53,60]
Ammonia (3.5 ppm and 8.7 ppm)	18 cats. Exposure for 1 h	Increased respiratory tract fluid output by 2- to 3-fold. No appreciable effect on water content of respiratory tract tissues. Transient decrease in blood hemoglobin.[53]

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Short-term Oral Studies			
Ammonia (0.01% in drinking water)	Rats (groups of 36)	5 groups initially received tap water : group 1 (for 7 weeks and 4 days), group 2 (7 weeks), group 3 (6 weeks), group 4 (4 weeks), and group 5 (0 water before Ammonia dosing). Groups then received Ammonia at dose of ~ 42 mg/kg/day for 8 weeks.	No mucosal lesions at macroscopic or microscopic examination. Mucosal atrophy in stomach antrum and enlargement of proliferative zone in antral and body mucosa.[61]
diammonium phosphate (read-across for Ammonia,17.9% N and 46.86% P_2O_5 equivalent)	Groups of Crj: CD(SD) rats (5 males, 5 female/group)	Administered by gavage daily (doses of 0, 250, 750, and 1500 mg/kg/day, 7 days/week) for 35 days	Clinical signs were not observed, and none of the animals died. However, there were treatment-related changes in body weight, hematological findings, clinical biochemistry findings, and non-neoplastic histopathological findings. Histological examination of stomachs revealed some submucosal inflammation at all doses, but this change was not dose-dependent and was not statistically significant at the low dose. NOAEL for general toxicity of diammonium phosphate = 250 mg/kg/day; LOAEL for general toxicity of diammonium phosphate = 750 mg/kg/day.[6,53]
Short-term Inhalation Studies			
Ammonia (~1306 ppm)	Rats	5 days/week (8 h/day)	Exposure tolerated for 42 days.[63]
Ammonia (~223 ppm or ~1105 ppm)	Sprague-Dawley and Long-Evans rats (males and females, groups of 15); Male New Zealand albino rabbits (groups of 3); Princeton-derived guinea pigs (males and females, groups of 15); Male squirrel monkeys (Saimiri sciureus, groups of 3); Beagle dogs (groups of 2)	Exposure 5 days per week (8 h/day) for 6 weeks	Lung effects: Gross necropsies normal. Focal pneumonitis in 1 of 3 monkeys at 223 ppm. Nonspecific lung inflammation in guinea pigs and rats, but not in other species at 1105 ppm. Upper respiratory tract effects: mild to moderate dyspnea in rabbits and dogs exposed to 1105 ppm during week 1 only; no indication of irritation after week 1. Nasal turbinates not examined for gross or histopathologic changes[2,40,63]
Ammonia (1086 ppm)	Rats, squirrel monkeys, and guinea pigs (number per species not stated)	Inhalation exposure 5 days per week (8 h/day) for 6 weeks	No fatty changes of liver plate cells. No pathological changes in kidney.[10]
Ammonia (653 ppm)	Rats (number not stated)	Continuous inhalation exposure for 25 days	Nearly 64% lethality.[10]
Ammonia (~653 ppm)	Sprague-Dawley or Long-Evans rats (males and females, 15 to 51/group)	Inhalation exposure for 65 days	Lung effects: Focal or diffuse interstitial pneumonitis in all animals. Upper respiratory tract effects: Dyspnea and nasal irritation/discharge.[2,63]
Ammonia (650 ppm; Ct [product of concentration and exposure time (ppm-h)] = 390,000 and 1,014,000)	51 rats	Continuously for 65 days	32 of 51 rats dead by day 25 (390,000 ppm-h); 50 of 51 rats dead by day 65 (1,014,000 ppm-h).[46,63]

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (500 ppm)	27 male rats	Continuous inhalation exposure for up to 8 weeks	After 3 weeks, nasal irritation and inflammation of upper respiratory tract, but no effects observed in bronchioles and alveoli. No lesions observed at 8 weeks.[53,62]
Ammonia (250 ppm)	F344 rats (6/sex/group)	Exposure in inhalation chamber for 35 days	Increased thickness of nasal epithelium (3 to 4 times) and nasal lesions at 150 ppm.[2,64]
Ammonia (221 ppm; Ct [ppm-h] = 53,040)	Rats, guinea pigs, rabbits, squirrel monkeys, and beagle dogs (number per species not stated)	5 days per week (8 h per day) for 6 weeks	No effect.[46,63]
Ammonia (10 or 150 ppm)	Sherman rats (5/sex/group)	Inhalation exposure from bedding for 75 days	Increased thickness of nasal epithelium (3 to 4 times) and nasal lesions at 150 ppm.[2,53,64]
Ammonia (50 or 90 ppm)	Male Wistar rats (8-14 per group)	Inhalation exposure continuously for 50 days	None of the animals died and there were no treatment-related effects.[53,70]
Ammonia (12% solution)	50 maleWhite albino mice	Vapor exposure 6 days per week (15 minutes/day) for 4, 5, 6, 7, or 8 weeks	Nasal mucosa adversely affected. Histological changes progressed from weeks 4–8 from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One animal that had loss of polarity of the epithelium, hyperchromatism, and mitotic figures with an intact basement membrane also had a carcinoma *in situ* in one nostril. At week 8, one mouse had an invasive adenocarcinoma of the nasal mucosa. Histo-chemical results were also abnormal.[2,72]
Ammonia (78 ppm, 271 ppm, and 711 ppm)	Groups of 10 male Swiss mice	Exposure for 4, 9,or 14 days (6 h/day)	No clinical signs of toxicity were noted for mice exposed to ammonia. Rhinitis and pathologic lesions with metaplasia and necrosis were seen only in the respiratory epithelium of the nasal cavity of mice inhaling 711 ppm, the severity of the lesions increased with duration of exposure, ranging from moderate on day 4, severe on day 9, to very severe on day 14. No lesions were seen in the controls or in mice inhaling the 78 ppm. No effects were seen at 271 ppm, even after 9 days of exposure.[24,65]
Ammonia (303 ppm)	Groups of 16 to 24 male Swiss Webster mice	Exposure for 5 days (6 h/day)	Histopathological findings, which were confined to the respiratory epithelium of the nasal cavity, included minimal exfoliation, erosion, ulceration, and necrosis; moderate inflammatory changes; and slight squamous metaplasia.[24,66]

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (20 ppm)	Swiss albino mice (males and females, groups of 4)	Exposure for 7, 14, 21, 28, or 42 days	Lung congestion, edema, and hemorrhage observed after 42 days.[2,67]
Ammonia (170 ppm; Ct [ppm-h] =30,600 to 91,800)	12 male Guinea pigs	5 days per week (6 h per day) for 6 weeks	No histopathologic changes.[46,75]
Ammonia (50 ppm)	Guinea pigs (males and females, groups of 6)	Exposure for 42 days	Lung congestion, edema, and hemorrhage.[2,67]
Ammonia (20 ppm)	Guinea pigs (males and females, groups of 2)	Exposure for 7, 14, 21, 28, or 42 days	Lung congestion, edema, and hemorrhage after 42 days. [2,67]
Ammonia (100 ppm [average range = 20 to 203 ppm; Ct [ppm-h] =100,800) alone and with corn starch dust	Yorkshire-Landrace pigs (groups of 6)	Continuously for 6 weeks	Tracheal damage (thickened tracheal epithelium [50 to 100% increase] and goblet cells reduced) at end of week 2 in animals exposed to 100 ppm (33,600 ppm-h) without dust. Changes more prominent by week 6. Conjunctival irritation more severe in pigs exposed to ammonia and corn starch dust, persisting for 2 weeks.[2, 46,130]
Ammonia (10 ppm and 50 to 150 ppm; Ct [ppm-h] = 42,000 to 126,000)	Duroc Pigs (groups of 36)	Continuously for 5 weeks	Excessive nasal, lacrimal and mouth secretions at 50, 100, and 150 ppm; more pronounced at 100 and 150 ppm, gradually diminishing over 1-2 weeks. No histopathologic changes in nasal turbinates or lung.[6,46,71]
Ammonia (12, 61, 103, or 145 ppm)	Duroc pigs (males and females, groups of 9)	Exposure for 5 weeks	Excessive nasal, lacrimal, and mouth secretions, and increased frequency of cough at 103 and 145 ppm.[2,71]
Ammonia (5 ppm [range = 0 to 7 ppm] to 100 ppm [range = 90 to 112 ppm])	Belgian Landrace pigs (groups of 7)	Nasal lavage technique. 6-day exposure in chamber	No-observed-effect value for Ammonia-induced somatic growth inhibition < 25 ppm. Nasal irritation down to 25 ppm. Conjunctival irritation observed in 4 pigs exposed to 100 ppm. Lethargy in groups exposed to 25, 50 and 100 ppm for 2 to 3 days after placement in chamber.[68]
Ammonia (0.6, 10, 18.8, or 37 ppm)	Pigs (different breeds, groups of 24)	Inhalable dust exposure for 5 weeks	No increase in incidence of respiratory diseases.[2, 69]
Ammonia (~1.8, ~3.9, ~7.3, or ~14.2 ppm)	Pigs (different breeds, groups of 24)	Inhalable dust exposure for 5 weeks	No increase in incidence of respiratory diseases.[2, 69]
Subchronic Inhalation Studies			
Ammonia (642 ppm)	Rats (number not stated)	Continuous exposure for 90 days	Fatty changes of liver plate cells.[10]
Ammonia (43 ppm or 143 ppm)	White rats (number not stated)	Inhalation exposure for 3 months (25- or 60-minute exposures every 48 h)	Mild leukocytosis after exposure to 143 ppm. No adverse effects after exposure to 43 ppm.[53]
Ammonia (100 ppm)	Rats	Inhalation exposure 5 days per week (5 h/day) for 12 weeks	Damaged tracheal mucosae.[46,76]

Table 7. Short-Term and Subchronic Toxicity Studies

Ingredient	Animals	Protocol	Results
Ammonia (~170 ppm)	12 male guinea pigs (additional 6 were controls)	Inhalation exposure 5 days per week (6 h/day) for 18 weeks	No significant findings after 6 and 12 weeks of exposure. Results at 18 weeks were: relatively mild congestion of the liver, spleen, and kidneys; degenerative changes in adrenal glands; hemosiderosis in spleen (indicative of hepatotoxicity); and cloudy swelling in epithelium of proximal kidney tubules, with albumin precipitation in lumen.[46,75]
Ammonium Hydroxide (671 ppm)	515 rats and 15 guinea pigs	Inhalation exposure continuously for 90 days	13 rats and 4 guinea pigs died.[53]
Ammonium Hydroxide (~57.43 ppm)	15 Sprague-Dawley/ Long-Evans rats (males and females), 15 Princeton-derived guinea pigs (males and females), 3 male New Zealand albino rabbits, 3 male squirrel monkeys, and 2 purebred male beagle dogs	Inhalation exposure continuously for 114 days	No mortalities or signs of toxicity. Necropsy observations were normal and there were no treatment-related histopathological findings.[63]

Table 8. Developmental and Reproductive Toxicity Studies

Ingredient	Animals/Embryos	Protocol	Results
In Vitro/In Vivo Study			
Ammonium ion (read-across for Ammonia, 38 to 300 µmol/l)	Mouse embryos (conceived in vivo)	Embryos cultured in modified mouse tubal fluid medium (mMTF) or mMTF supplemented with 300 µmol/L ammonium ion for 48, 69, or 93 h before being transferred to pseudo-pregnant mouse dams	Examination on gestational day 15 showed apparent relationship between the duration of exposure and the incidence of exencephaly. Increased incidence of exencephaly with increased ammonium concentration (38–300 µmol/L) and decreased percentage of implantation sites with increased ammonium concentration.[84]
Oral Studies			
Ammonium ion (read-across for Ammonia)	Pregnant rats	Feeding with ammonium ion in the diet (4293 mg ammonium/kg/day) from gestation day 1 through day 21 of lactation	Growth of rats exposed during pregnancy and lactation was significantly lower that in controls until approximately day 60. Body weights of offspring reduced by 25% (males) and 16% (females).[10,85]
diammonium phosphate (read-across for Ammonia, 17.9% NH_4 and 46.86% P_2O_5 equivalent)	Groups of Crj: CD(SD) rats (5 males, 10 females [reproductive subgroup])	Administered by gavage daily (doses of 0, 250, 750, and 1500 mg/kg/day) for, at most, 28 days (males) and 53 days (females).	No treatment-related deaths and no signs of overt clinical toxicity. Body weight gain was reduced during the first week of gestation (82% of control) in females dosed with 1500 mg/kg/day, but returned to control levels for remainder of study. Mating performance and fertility were unaffected by treatment, and parental treatment had no apparent effect on the offspring to day 4 of age. NOAEL for reproductive and developmental toxicity = 1500 mg/kg/day; LOAEL not identified.[6,45]
Inhalation Study			
Ammonia (7 ppm or 35 ppm)	Groups of 40 gilts (Yorkshire x Hampshire x Chester White)	Exposure for 6 weeks (7 ppm or 35 ppm). Exposure to ~7 ppm or ~ 35 ppm from 6 weeks prior to breeding until day 30 of gestation	No statistically significant differences in ovarian or uterine weights after 6 weeks of exposure. After exposure from 6 weeks prior to breeding until day 30 of gestation, no statistically significant differences in age at puberty, number of live fetuses, fetal length, or fetus-to-corpus luteum ratio compared to pigs exposed to only about 7 ppm . No unexposed controls were included in this study.[83]

Table 9. Carcinogenicity and Tumor Promotion Studies

Ingredient	Animals	Protocol	Results
		Oral Studies	
Ammonia (dissolved in water)	Kid: CFLP mice	Development of lung tumors can be observed in Kid: CFLP mice treated intragastrically with diethyl pyrocarbonate and ammonia. The lung tumors may result from a carcinogenic substance, supposedly urethane, formed in vivo from diethyl pyrocarbonate in the presence of ammonia. Animals treated with 200 mg/kg diethyl pyrocarbonate and or 42 mg/kg Ammonia dissolved in water (8.4 mg/ml) by stomach tube twice per week for 4 weeks. Necropsy at 20 weeks after first treatment. Number of tumors counted under dissecting microscope.	No evidence of a tumorigenic effect was found in mice treated by gavage with ammonia dissolved in water alone at a dose of 42 mg Ammonia/kg/day for 4 weeks or with diethyl pyrocarbonate alone, but 9 of 16 mice treated with a combination of ammonium and pyrocarbonate developed lung tumors. [87]
Ammonium Hydroxide	Swiss and C3H mice	Exposure of mice to 193 mg ammonium/kg/day, as Ammonium Hydroxide (in drinking water), for 2 years	No carcinogenic effects, and did not affect spontaneous development of breast cancer (adenocarcinoma), which is common to C3H female mice.[45, 53,88]
Ammonium ion (read-across for Ammonia, and diethyl pyrocarbonate)	21 pregnant female mice of the Lati:CFLP strain	Exposure (by gavage) during pregnancy and lactation	No lung tumors on the surfaces of the lungs of pregnant mice.[89]
Ammonium Sulfate (read-across for Ammonia)	Groups of 100 F344/DuCrj rats (50 males and 50females per group)	Dietary concentrations of 0%, 1.5%, and 3% daily for 104 weeks	Survival rates: males - 88% (controls), 78% (1.5% group), and 76% (35 group); females - , 76% (controls), 80% (1.5% group), and 80% (3% group) gross finding: massive, nodular or focal lesions suggestive of neoplastic change neoplastic lesions (not treatment-related; occur spontaneously in rats of this strain): C-cell adenomas/adenocarcinomas in the thyroids, fibroadenomas/ adenomas/adenocarcinomas in mammary glands, adenomas/ adenocarcinomas in pituitary glands, interstitial cell tumors in testes, and endometrial stromal polyps in uteri non-neoplastic lesions: the incidence of chronic nephropathy was statistically significantly increased in low-dose males No evidence of long-term carcinogenic activity.[78]
Ammonium Sulfate (read-across for Ammonia)	Groups of 10 F344/DuCrj rats (male and female)	Dietary concentrations of 0%, 0.1%, 0.6%, and 3% for 52 weeks	Neoplastic lesions reported included malignant pheochromocytoma of the adrenal gland in males of the 3% dietary group, 2 adenomas in the anterior pituitary of females of the 3% dietary group, and uterine endometrial stromal polyp in a female control rat.[6]

Table 9. Carcinogenicity and Tumor Promotion Studies

Ingredient	Animals	Protocol	Results
		Inhalation Study	
Ammonia (12% solution)	10 male mice	Vapor exposure 6 days per week (15 minutes/day) for 4, 5, 6, 7, or 8 weeks	Histological changes progressed (weeks 4 to 8) from crowding of cells forming crypts and irregular arrangements to epithelial hyperplasia, patches of squamous metaplasia, loss of cilia, and dysplasia of the nasal epithelium. One mouse had a carcinoma *in situ* in 1 nostril. At week 8, 1 mouse with invasive adenocarcinoma of the nasal mucosa. Authors noted that prolonged exposure to Ammonia may interfere with normal protective reflexes of the respiratory nasal mucosa, resulting in the accumulation of particulate matter initiating or promoting a neoplastic process. [72]
		Tumor Promotion	
Ammonia (dissolved in water)	Rats	Rats pretreated with the initiator *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) in drinking water for 4 weeks, prior to receiving 0.01% Ammonia solution in drinking water for 24 weeks	Statistically significantly greater incidence of gastric cancer (70% of rats) and number of tumors per tumor-bearing rat (2.1) than rats that received only MNNG and tap water (31% and 1.3 tumors/rat).[53,90]
Ammonia	Rats	Rats pretreated with MNNG prior to dosing with Ammonia (~ 42 mg/kg/day)	The size, depth, and metastasis of the MNNG-initiated tumors enhanced in rats dosed with Ammonia.[91]

Table 10. Dermal Irritation Studies

Ingredient	Animals/Subjects/Cells	Protocol	Results
		Skin Irritation Studies	
<u>In Vitro Studies</u>			
Undiluted Ammonium Hydroxide (30% active material in neat substance)	Reconstructed human skin cultures	Test substance applied topically to stratum corneum surface of cultures. Skin culture damage or cytotoxicity measured as decreased 3-[4,5-dimethylthiazol-2- yl] 2,5-diphenyltetrazolium bromide (MTT) vital dye metabolism. In time-course experiments, the time (in minutes) of test material exposure eliciting a 50% reduction of MTT metabolism (i.e., t_{50} value) was calculated.	Histologic examination of the cultures indicated gradations of epidermal necrosis quantitated using a specially designed grading scale, which correlated well with the corrosivity of treatment chemicals and cytotoxicity measurements. Ammonium Hydroxide (30% active in neat substance) was classified as corrosive (t_{50} = 0.90 minutes).[113]
<u>Animal Studies</u>			
Ammonia	Wistar rats (3 males, 3 females) and ddY mice (3 males, 3 females)	Test solutions (1 ml/kg or 1 g/kg) applied once, unoccluded, to shaved skin of the back. Area of application was 3 x 4 cm for rats and 1 x 2 cm for mice. Distilled water control. Test sites observed for inflammatory reactions for 1 week after application.	Minimum concentration of Ammonia that caused a positive reaction was >25% (minimum amount = >250 mg/kg) in rats and 25% (minimum amount = 250 mg/kg) in mice.[112]

Table 10. Dermal Irritation Studies

Ingredient	Animals/Subjects/Cells	Protocol	Results
Ammonia	Wistar rats (4), Hartley guinea pigs (4), and ddY mice (4)	Injected intradermally with test solutions (0.01 ml) at 4 spots on shaved dorsal skin. Saline served as the control. The test sites were evaluated for skin irritation for up to 1 week after application.	The minimum concentration that resulted in a positive reaction was 0.05% in rats (minimum amount = 25 µg/kg), mice (minimum amount = 250 µg/kg), and guinea pigs (minimum amount = 12.5 µg/kg).[112]
Ammonium Hydroxide (10% and 20%)	Groups of 3 New Zealand Albino rabbits	Each concentration (0.5 ml) applied to the skin (2 replicates at each dose)	Results positive for skin corrosion at 20% concentration. Negative results at 10% concentration.[21,45]
Ammonium Hydroxide (10% and 12% aqueous)	Female Albino New Zealand White rabbits (groups of 3)	Each solution (0.1 ml) applied, under an occlusive patch (1" x 1"), to the skin for 4 h. There were 3 rabbits per dose, with 2 replicates per rabbit at each concentration.	The 12% solution was corrosive to the skin, but the 10% solution was not.[6]
Human Studies			
Ammonium Hydroxide (saturated aqueous solution)	16 subjects (10 men, 6 women)	Applied (via a chamber) to middle of ventral aspect of forearm	Formation of a well-defined, sub-epidermal blister (positive reaction) observed within a few minutes of chamber application; skin irritation observed in all subjects.[114]
Ammonium Hydroxide (1:1aqueous solution)	110 subjects	Test substance (0.5 ml) placed in 8 mm well drilled in acrylic plastic block (3 x 3 x 1 cm) that was strapped to the skin. Block (used to measure minimal blistering time (MBT, indicator of cutaneous irritability, defined as total exposure in well that results in a single bulla, occupying the total area of contact)).	MBT ranged from 3 to 57 minutes. Inflammatory reaction considered slight; healing was rapid and without scarring. Intensity of the dermatitis provoked by a 24-h exposure to sodium lauryl sulfate was strongly correlated with the MBT.[115]
Ammonium Hydroxide solution (50% solution)	Young adults and older adults (number not stated)	Acrylic plastic block with 14 mm well loosely strapped to skin. Well was then filled with 0.5 ml of freshly pepared 1:1 aqueous solution of Ammonium Hydroxide. Site was examined at 30-minute intervals, and blistering response was measured.	Mild discomfort during procedure. The initial response, characterized by the appearance of tiny follicular vesicles, occurred more quickly in older adults. The time required to produce a full blister was greatly prolonged in the aged.[116]

Table 11. Ocular Irritation Studies

Ingredient	Animals/Cells	Test Protocol	Results
In Vitro			
Ammonium Hydroxide	Human corneal endothelial cell cultures	^{51}Cr-release assay. Performed by loading the cells with isotope, incubating the cells with Ammonium Hydroxide, and measuring the isotope that was recovered in the medium.	Severe ocular irritant (ED_{50} = 3.9 x 10^{-3} M).[117]
Animal			
Ammonia (15, 32, 310, or 1157 ppm vapor concentrations)	Rats (Crl:COBS CD(SD) strain)	In phase 1 of study, groups of 8 rats exposed for 24 h. In phase 2, groups of 14 rats exposed for 3 or 7 days.	No clinical signs or evidence of irritation to the eyes or mucous membranes. No histologic differences in tracheal or lung sections between control and experimental groups.[24,40]
Ammonium Hydroxide	Rabbits (number not stated)	Instillation of test substance (1 mg) followed by ocular rinsing	Ocular irritant.[45]
Ammonium Hydroxide (28.5%)	Rabbits (number not stated)	Brief exposures (2 seconds). Volume instilled not stated	Corneal opacity.[10,118]
Ammonium Hydroxide (0.3%, 1%, 2.5%, and 10%)	New Zealand albino rabbits (groups of 6)	Draize test. Test substance (0.1 ml) instilled into the eye. In 1 group, eyes rinsed after instillation	Conjunctivitis (at 1% to 10%, but not at 0.3%). Ammonium Hydroxide (10%) produced pannus in 5/6 unwashed rabbit eyes and 2.5% produced pannus in 1/6 unwashed and 6/6 washed eyes. Ammonium Hydroxide at 1% produced pannus in 3/6 washed eyes. Keratoconus was produced by 10% Ammonium Hydroxide in 4/6 unwashed eyes and 2/6 washed eyes and 2.5% produced keratoconus in 2/6 unwashed eyes. Ammonium Hydroxide (10%) caused corneal opacities within 1 h of instillation.[119]
Ammonium Hydroxide (prepared with 3% Ammonia)	3 New Zealand White Albino Rabbits	Draize test. Test substance (100 µl) instilled into eye	Conjunctivitis (score = 3 at 96 h; mean maximum Draize score = 3), chemosis (score = 3 at 96 h; mean maximum score = 4), iritis (score = 1; mean maximum Draize score = 2), corneal opacity (score = 4; mean maximum Draize score = 4), and mean surface of corneal damage (70% corneal damage; mean maximum Draize value = 100%). Risk of serious damage to the eyes.[120]

Table 12. Other Clinical Reports

Ingredient	Number of Subjects	Protocol	Results
Inhalation Exposure			
Ammonia (700 ppm)	Number of subjects not available	Not available	Eye irritation.[125]
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Variable lacrimation.[125]
Ammonia (500 ppm)	Number of subjects not available	30-minute exposure	Increased blood pressure and pulse rate.[125]
Ammonia (500 pm)	Number of subjects not available	30-minute exposure	Nasal and throat irritation, increased minute volume, and cyclic pattern of hyperpnea.[125]
Ammonia (500 ppm)	7 men	30-minute exposure	Increase in ventilation minute volume of 50-250%, accompanied by cyclic increase in respiratory rate. Irritation of the nose and throat. No significant change in nitrogen or urea in blood and urine. No significant change in serum nonprotein nitrogen.[126]
Ammonia (500 ppm)	7 subjects	30-minute exposure via face mask	Ventilation minute volume increased 50 to 250% over pre-exposure values. Respiratory minute volumes fell below pre-exposure levels at termination of exposure.[46,126]
Ammonia (101 to 335 ppm)	Number of subjects not available	20-minute exposure	Decrease in exercise ventilation minute volume at 151-335 ppm, related either to a decrease in respiratory rate (at 151 ppm) or tidal volume (at 205 and 335 ppm); no significant effects at 101 ppm.[46,127]
Ammonia (50 to 140 ppm)	16 subjects	2-h exposure. Testing repeated after a 1-week interval.	110 ppm tolerable for all subjects. 140 ppm intolerable at 1 h (4 subjects) and at 2 h (4 subjects). No significant increase in vital capacity, forced expiratory volume at end of 1 second of forced expiration (FEV_1), or forced inspiratory volume inhaled at end of 1st second of forced inspiration (FIV_1). Lowest-observed-adverse-effect level (LOAEL) of 50 ppm for mild irritation to the eyes (6 subjects), nose (20 subjects), and throat (9 subjects). LOAEL divided by uncertainty factor of 30 (10 to protect sensitive individuals and 3 for the use of a minimal LOAEL)[73]
Ammonia (135 ppm)	6 subjects	5-minute exposure	Chest irritation in 1 of 6 subjects.[125]
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Nose and throat irritation.[125]
Ammonia (135 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[125]

Table 12. Other Clinical Reports

Ingredient	Number of Subjects	Protocol	Results
Ammonia (25, 50, and 100 ppm)	6 subjects	Exposure: 5 days per week (2 to 6 h per day) for 6 weeks	Mild to moderate irritation of the eyes, nose and throat: 16/54 (30%) of observations on 6 subjects in week 2; 12/90 (13%) in week 3; 2/60 (3%) in week 4; 0/78 in week 5; and 5/78 (6%) in week 6. No apparent effects on pulse, respiration rate, blood pressure, FVC, or FEV_1.[128]
Ammonia (25-100 ppm)	Not available	Exposure to varying concentrations for varying periods (2-6 h) 5 days/week for 6 weeks	Decreasing signs of irritation of the mucous membranes of the eyes, nose and throat over the 6-week observation period were reported, and there was no evidence of adverse health effects.[46,128]
Ammonia (72 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[125]
Ammonia (50 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[125]
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Eye irritation.[125]
Ammonia (50 ppm)	Number of subjects not available	120-minute exposure	Nose and throat irritation. Urge to cough.[125]
Ammonia (30 and 50 ppm)	6 subjects	10-minute exposure	Barely perceptible irritant effects (nose and eye) in 2 of 6 subjects (30 ppm). Faint to moderate irritation (nose and eye) in 5 of 6 subjects (50 ppm).[51]
Ammonia (30 ppm and 50 ppm)	6 subjects	10-minute exposure	Moderate irritation of nose and eyes at 50 ppm (4 of 6 subjects), but not at 30 ppm.[10]
Ammonia (32 ppm)	Number of subjects not available	5-minute exposure	Eye irritation with lacrimation.[125]

REFERENCES

1. Nikitakis, J. and Lange B. International Cosmetic Ingredient Dictionary and Handbook Online Version (wINCI). http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC. Last Updated 2017. Date Accessed 3-6-2017.

2. United States Environmental Protection Agency (EPA). Toxicological review of ammonia noncancer inhalation. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0422tr.pdf. Last Updated 2016.

3. World Health Organization (WHO). Ammonia - published under the joint sponsorship of the United Nations Environment Program, the International Labor Organization, and the World Health Organization. Geneva: World Health Organization, 1986.

4. Cosmetic Ingredient Review. Safety Assessment of Phosphoric Acid and Its Salts as Used in Cosmetics. Final Report. Available for review at the Cosmetic Ingredient Review, 1620 L Street, NW, Suite 1200, Washington, D.C. 20036-4702. 2016. Washiington, D.C.:

5. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia (Update). *U.S.Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Division.of Toxicology/Toxicology Information Branch., 1600.Clifton.Road.NE., E-29., Atlanta., GA 30333., USA, Sep.%2004.xix., 223p.I.* 2004;xix, 223p

6. European Chemicals Agency (ECHA). Registration, Evaluation, and Authorization of Chemicals (REACH) Dossier. Anhydrous Ammonia. https://echa.europa.eu/registration-dossier/-/registered-dossier/15557. Last Updated 2017. Date Accessed 6-8-2017.

7. Welch, A. Exposing the dangers of anhydrous ammonia. http://journals.lww.com/tnpj/Citation/2006/11000/Exposing_the_Dangers_of_Anhydrous_Ammonia.8.aspx. Last Updated 2006. Date Accessed 5-17-2017.

8. O'Neil, M. J. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 15th Edition *ed*. Cambridge, UK: Royal Society of Chemistry, 2013.

9. Souba, W. W. Review. Interorgan ammonia metabolism in health and disease: A surgeon's view. *Journal of Parenteral and Enteral Nutrition*. 1987;11(6):569-579.

10. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for ammonia. https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf. Last Updated 2004.

11. Scifinder. Chemical Abstracts Service: Columbus, OH. CAS Regsitry Numbers 7664-41-7 and 1336-21-6. Substance Identifier. http://www.cas.org/products/scifinder. Last Updated 2017. Date Accessed 6-20-2017.

12. United States Environmental Protection Agency (EPA). Estimation Programs Interface Suite™ for Microsoft® Windows, Calculations based on KOWWIN v1.68.4.10. 2017. Washington, D.C.: EPA.

13. National Center for Biotechnology Information. Pubchem Compound Database. Compound summary for CID 14923. Ammonium Hydroxide. https://pubchem.ncbi.nlm.nih.gov/compound/ammonium_hydroxide#section=Top. Last Updated 2017. Date Accessed 10-18-2017.

14. United States Food and Drug Administration (FDA). Listing of specific substances affirmed as GRAS. Ammonium hydroxide. 21 CFR 184.1139. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm. Last Updated 2016. Date Accessed 6-8-2017.

15. United States Pharmacopeial Convention. Food Chemicals Codex. Tenth *ed*. Rockville, MD: The United States Pharmacopeial Convention, 2016.

16. The United States Pharmacopoeial Convention. The United States Pharmacopeia (USP). Rockville, MD: The United States Pharmacopeial Conventioni, 2009.

17. United States Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2017. Washington, D.C.: FDA.

18. Personal Care Products Council. Concentration of use by FDA product category: Ammonia and Ammonium Hydroxide. Unpublished data submitted by the Personal Care Products Council on 2-2-2017. 2017.

19. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. http://ec.europa.eu/growth/tools-databases/cosing/. Last Updated 2017. Date Accessed 6-8-2017.

20. Bhattacharya, S. K. Hom G. G. Fernandez C. and Hom L. G. Ocular effects of exposure to industrial chemicals: Clinical management and proteomic approaches to damage assessment. *Cutaneous and Ocular Toxicology*. 2007;26(3):203-225.

21. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Human health tier II assessment for ammonia and ammonium hydroxide. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1180. Last Updated 2013. Date Accessed 6-8-2017.

22. United States Food and Drug Administration (FDA). Drugs@FDA: FDA Approved Drug Products. Ammonia. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=browseByLetter.page&productLetter=A. Last Updated 2017. Date Accessed 6-11-2017.

23. United States Food and Drug Administration (FDA). Drug products containing certain active ingredients offered over-the-counter (OTC) for certain uses. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm. Last Updated 2016. Date Accessed 6-11-2017.

24. Cavender, F. and Milner G. Exposure to ammonia. Salem, H. and Katz S. A. In: *Inhalation Toxicology*. 3rd ed. Boca Raton: CRC Press; 2015:257-293.

25. Cooper, A. J. L. Ammonia metabolism in normal and portacaval-shunted rats. *Advances in Experimental Medicine and Biology*. 1990;272:23-46.

26. Dasarathy, S. Mookerjee R. P. Rackayova V. Thrane V. R. Vairappan B. Ott P. and Rose C. F. Ammonia toxicity: from head to toe? *Metab.Brain Dis.* 2017;32(2):529-538.

27. Jones, E. A. Smallwood R. A. Craigie A. and Rosenoer V. M. The enterohepatic circulation of urea nitrogen. *Clin.Sci.* 1969;37:825-836.

28. Cooper, J. L. A. and Plum F. Biochemistry and physiology of brain ammonia. *Physiol.Rev.* 1987;67:440-519.

29. Brusilow, S. W. Koehler R. C. Traystman R. J. and Cooper A. J. L. Astrocyte glutamine synthetase: importance in hyperammonemic syndromes and potential target for therapy. *NeuroRx*. 2010;7:452-470.

30. Oja, S. S. Saransaari P. Korpi E. R. Neurotoxicity of ammonia. *Neurochem.Res.* 2017;42:713-720.

31. Walker, V. Ammonia metabolism and hyperammonemic disorders. *Adv.Clin.Chem.* 2014;67:73-150.

32. Odigwe, C. C. Khatiwada B. Holbrook C. Ekeh I. S. Uzoka C. Ikwu I. and Upadhyay B. Noncirrhotic hyperammonemia causing relapsing altered mental status. *Proc (Bayl.Univ.Med.Cent.)*. 2017;28(4):472-474.

33. Summerskill, V. H. J. and Wolpert E. Ammonia metabolism in the gut. *The American Journal of Clinical Nutrition*. 2017;23(5):633-639.

34. Katayama, K. Ammonia metabolism and hepatic encephalopathy. *Hepatology Research*. 2004;30S:S71-S78.

35. Visek, W. J. Ammonia metabolism, urea cycle capacity and their biochemical assessment. *Nutrition Reviews*. 1979;37(9):273-282.

36. Sandesh, C. S. Nagamani and Erez A. A metabolic link between the urea cycle and cancer cell proliferation. DOI: 10.1080/23723556.2015. 1127314. *Molecular & Cellular Oncology*. 2016;3(2):e1127314

37. Sorensen, M. Update on cerebral uptake of blood ammonia. *Metab.Brain Dis.* 2013;28:155-159.

38. Manninen, A. T. A. and Savolainen H. Effect of short-term ammonia inhalation on selected amino acids in rat brain. *Pharmacol.Toxicol.* 1989;64(3):244-246.

39. Manninen, A. Anttila S. and Savolainen H. Rat metabolic adaptation to ammonia inhalation. *Proc.Soc.Exp.Biol.Med.* 1988;187(3):278-281.

40. Schaerdel, A. D. White W. J. Lang C. M. et al. Localized and systemic effects of environmental ammonia in rats. *Lab Anim.Sci.* 1983;33(1):40-45.

41. Cooper, A. J. L. and Lai J. C. K. Cerebral ammonia metabolism in normal and hyperammonemic rats. *Neurochemical Pathology*. 1987;6:67-95.

42. Mori, S. Kaneko H. Mitsuma T. et al. Implications of gastric topical bioactive peptides in ammonia-induced acute gastric mucosal lesions in rats. *Scand.J.Gastroenterol*. 1998;33(4):386-393.

43. Ruden, C. and Hansson S. O. How accurate are the European Union's classifications of chemical substances. *Toxicology Letters*. 2003;144:159-172.

44. Takeuchi, K. Ohuchi T. Harada H. et al. Irritant and protective action of urea-urease ammonia in rat gastric mucosa. *Dig.Dis.Sci.* 1995;40(2):274-281.

45. Organization for Economic Co-operation and Development (OECD). Final Assessment Report. SIDS Dossier on Ammonium Hydroxide. SIDS Ammonia Zip: SIDS_Dossier_Ammonia_1336216. http://webnet.oecd.org/HPV/UI/SIDS_Details.aspx?key=d5ae737b-77d7-4d61-8687-4df45f52cace&idx=0. Last Updated 2007.

46. Legters, L. Biological effects of short, high-level exposure to gases: Ammonia. Contract No. DAMD17-79-C-9086. 1980. pp.1-87. Fort Detrick, Frederick, Maryland: U.S. Army Medical Research and Development Command.

47. Hilaldo, C. J. Casey C. J. and Furst A. Effect of ammonia on Swiss albino mice. *J.Combust.Toxicol.* 1977;4:385-388.

48. Silver, S. D. and McGrath, FP. A Comparison of Acute Toxicities of Ethylene Imine and Ammonia to Mice. *Journal of Industrial Hygiene and Toxicology*. 1948;30(1):7-9.

49. Kapeghian, J. C. Mincer H. H. Hones A. B. et al. Acute inhalation toxicity of ammonia in mice. *Bull.Environ.Contam.Toxicol.* 1982;29:371-378.

50. MacEwen, J. D. and Vernot, EH. Toxic Hazards Research Unit Annual Technical Report. *Aerospace Medical Research Laboratory, Air Force.Systems Command., Wright.-Patterson.Air Force.Base., Ohio., Report No.AMRL.-TR.-72.-62., NTIS AD755.-358., 162.pages., 37.references.* 1972;

51. MacEwen, J. D., Theodore, J, and Vernot, EH. Human Exposure to EEL Concentrations of Monomethylhydrazine. *Aerospace Medical Research Laboratory, Aerospace Division., Air Force.Systems Command., Wright.-Patterson.Air Force.Base., Ohio., Report No.AMRL.-TR.-70.-102., (Proceedings of the First.Annual Conference.on Environmental Toxicology, 1970).* 1970;(Proceedings of the First Annual Conference on Environmental Toxicology):355-363.

52. Barrow, C. S. Alarie Y. and Stock M. F. Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. *Arch.Environ.Health*. 1978;33:79-88.

53. Organization for Economic Co-operation and Development (OECD). SIDS Dossier. CAS number 76645-41-7. Ammonia, anhydrous. http://webnet.oecd.org/hpv/ui/Search.aspx. Last Updated 2007.

54. Appelman, L. M., Ten Berge, WF, and Reuzel, PG. Acute inhalation toxicity study of ammonia in rats with variable exposure periods. *Am Ind Hyg Assoc J*. 1982;43(9):662-665.

55. Perkins, M. W. Wong B. Tressler J. Coggins A. Rodriguez A. Devorak J. and Sciuto A. M. Assessment of inhaled ammonia-induced lung injury in rats. *Inhal.Toxicol.* 2016;28(2):71-79.

56. Perkins, M. W. Wong B. Tressler J. Rodriguez A. Sherman K. Andres J. Devorak J. Wilkins W. L. and Sciuto A. M. Adverse respiratory effects in rats following inhalation exposure to ammonia: respiratory dynamics and histopathology. *Inhalation Toxicology*. 2017;29(1):32-41.

57. Pauluhn, J. Acute inhalation toxicity of ammonia: Revisiting the importance of RD50 and LCT01/50 relationships for setting emergency response guideline values. *Regulatory Toxicology and Pharmacology*. 2013;66:315-325.

58. Li, W. L. and Pauluhn J. Comparative assessment of sensory irritation in rats and mice nose-only exposed to dry and humidified atmospheres. *Toxicology*. 2010;276:135-142.

59. Boyd, E. M., MacLachland, ML, and Perry, WF. Experimental Ammonia Gas Poisoning in Rabbits and Cats. *Journal of Industrial Hygiene and Toxicology*. 1944;26(1):29-34.

60. Dodd, K. T. and Gross D. R. Ammonia inhalation toxicity in cats: A study of acute and chronic respiratory dysfunction. *Arch.Environ.Health*. 1980;35:6-14.

61. Tsujii, M. Kawano S. Tsuji S. et al. Cell kinetics of mucosal atrophy in rat stomach induced by long-term administration of ammonia. *Gastroenterology*. 1993;104(3):796-801.

62. Richard, D. Bouley G. and Boudene C. Effects of continuous inhalation of ammonia in the rat and mouse (French). In: Agency for Toxic Substances and Disease Registry (ATSDR). 2004. Toxicological profile for ammonia. https://www.atsdr.cdc.gov/toxprofiles/tp126.pdf. Last Updated 2004. Date Accessed 5-23-0017.

63. Coon, R. A. Jones R. a. Jenkins L. T. Jr. and Siegel J. Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine, and ethanol. *Toxicol.Appl.Pharmacol.* 1970;16:646-655.

64. Broderson, J. R. Lindsey J. R. and Crawford J. E. The role of environmental ammonia in respiratory mycoplasmosis of rats. *Am.J.Pathol.* 1976;85:115-130.

65. Zissu, D. Histopathological Changes in the Respiratory Tract of Mice Exposed to Ten Families of Airborne Chemicals. *Journal of Applied Toxicology*. 1995;15(3):207-213.

66. Buckley, L. A. Jiang X. Z. James R. A. Morgan K. T. and Barrow C. S. Respiratory tract lesions induced by sensory irritants at the median respiratory rate decrease concentration. *Toxicol.Pharmacol.* 1984;74:417-429.

67. Anderson, D. P. Beard C. W. and Hanson R. P. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. *Avian.Dis.* 1964;8:369-379.

68. Urbain, B. and Gustin P. Prouvost J. F. and Ansay M. Quantitative assessment of aerial ammonia toxicity to the nasal mucosa by use of the nasal lavage method in pigs. *Am.J.Vet.Res.* 1994;55(9):1335-1340.

69. Done, S. H. Chennells D. J. Gresham A. C. Williamson S. Hunt B. Taylor L. L. Bland V. et al. Clinical and pathological responses of weaned pigs to atmospheric ammonia and dust. *Vet.Rec.* 2005;157:71-80.

70. Stolpe, J. and Sedlag R. Die Einzel- und Komplexwirkung von Ammoniak und Schwefelwasserstoff in der Luft auf kleine Versuchstiere (Ratten) bei unterschiedlichen Umweltbedingungen. *Ach.Exper.Vet.Med.* 1976;30:533-539.

71. Stombaugh, D. P., Teague, HS, and Roller, WL. Effects of Atmospheric Ammonia on the Pig. *Journal of Animal.Science*. 1969;20:844-847.

72. Gaafar, H. Girgis R. and Hussein M. et al. The effect of ammonia on the respiratory nasal mucosa of mice. A histological and histochemical study. *Acta Otolaryngol (Stockh)*. 1992;112(2):339-342.

73. Verberk, M. M. Effects of ammonia in volunteers. *Int.Arch.Occup.Environ.Health*. 1977;39:73-81.

74. Occupational Safety and Health Administration (OSHA). Air contaminants. 29 CFR:1910.1000. https://www.ecfr.gov/cgi-bin/text-idx?SID=c5407149c832a3a7892a2e80712a59ba&mc=true&node=se29.6.1910_11000&rgn=div8. Last Updated 2017. Date Accessed 6-21-2017.

75. Weatherby, J. H. Chronic toxicity of ammonia fumes by inhalation. *Proc.Soc.Exp.Biol.Med.* 1952;81:300-301.

76. Dalhamn, T. and Reid I. Ciliary activity and histologic observations in the trachea after exposure to ammonia and carbon particles. Davies, C. N. In: *Inhaled particles and vapors II.* Elmsford, NY: Pergamon Publishing Company; 1967:299-306.

77. Fazekas, I. G. Experimental suprarenal hypertrophy induced by ammonia. *Endokrinologie*. 1939;21:315-337.

78. Ota, Y. Hasumura M. Okamura M. et al. Chronic toxicity and carcinogenicity of dietary adminstered ammonium sulfate in F344 rats. *Fd.Chem.Toxicol.* 2006;44:17-27.

79. Holness, D. L., Purdham, JT, and Nethercott, JR. Acute and Chronic Respiratory Effects of Occupational Exposure to Ammonia. *American Industrial Hygiene Association Journal*. 1989;50(12):646-650.

80. Curtis, S. E., Anderson, CR, Simon, J, Jensen, AH, Day, DL, and Kelley, KW. Effects Of Aerial Ammonia, Hydrogen Sulfide And Swine-House Dust On Rate Of Gain And Respiratory-Tract Structure In Swine. *Journal of Animal.Science*. 1975;41(3):735-739.

81. Ballal, S. G. Ali B. A. Albafr A. A. Ahmed H. O. and Al-Hasan A. Y. **Bronchial asthma in two chemical fertilizer producing factories in eastern Saudi Arabia**. *Tuberc.Lung.Dis*. 1998;2:330-335.

82. Ali, B. A. Ahmed H. O. Ballal S. G. and Albar A. A. Pulmonary function of workers exposed to ammonia: A study in Eastern Province of Saudi Arabia. *Int.J.Occup.Environ.Health*. 2001;7:19-22.

83. Diekman, M. A. Scheidt A. B. Sutton A. L. et al. Growth and reproductive performance, during exposure to ammonia, of gilts afflicted with pneumonia and atrophic rhinitis. *Am.J.Vet.Res.* 1993;54(12):2128-2131.

84. Lane, M. and Gardner D. K. Increase in postimplantation development of cultured mouse embryo by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J.Reprod.Fertil.* 1994;102(2):305-312.

85. Minana, M. D. Marcaida G. Grisolia S. et al. Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. *J.Neuropathol.Exp.Neurol.* 1995;54(5):644-650.

86. Yadav, J. S. and Kaushik V. K. Genotoxic effect of ammonia exposure on workers in a fertilizer factory. *Indian J.Exp.Biol.* 1997;35(5):487-492.

87. Uzvolgyi, E. and Bojan F. Possible in vivo formation of a carcinogenic substance from diethyl pyrocarbonate and ammonia. *J.Cancer Res.Clin.Oncol.* 1980;(97):205-207.

88. Toth, B. Hydrazine, methylhydrazine and methylhydrazine sulfate carcinogenesis in swiss mice. Failure of ammonium hydroxide to interfere in the development of tumors. *Int.J.Cancer*. 1972;9:109-118.

89. Uzvolgyi, E. and Bojan F. In vivo formation of a carcinogenic substance from diethyl pyrocarbonate in the presence of ammonia. *Arch.Toxicol.Suppl.* 1985;8:490-493.

90. Tsujii, M. Kawano S. Tsuji S. et al. Ammonia: A possible promoter in Helicobacter pylori related gastric carcinogenesis. *Cancer Lett.* 1992;65(1):15-18.

91. Tsujii, M. Kawano S. Tsuji S. et al. Mechanism for ammonia-induced promotion of gastric carcinogenesis in rats. *Carcinogenesis*. 1995;16(3):563-566.

92. Cagnon, L. and Braissant O. Hyperammonemia-induced toxicity for the developing central nervous system. *Brain Research Reviews*. 2007;56:183-197.

93. Albrecht, J. Mini-Review. Roles of neuroactive amino acids in ammonia neurotoxicity. *Journal of Neuroscience Research*. 1998;51:133-138.

94. Albrecht, J. Zelinska M. and Norenberg. Glutamine as a mediator of ammonia neurotoxicity: A critical appraisal. *Biochemical Pharmacology*. 2010;(doi:10.1016/j.bcp.2010.07.024)

95. Cooper, A. J. Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. *Ment.Retard.Dev.Disabil.Res.Rev.* 2001;7:280-286.

96. Bosoi, C. R. Zwingmann C. Marin H. Parent-Robitaille C. Huynh J. Tremblay M. and Rose C. F. Increased brain lactate is central to the development of brain edema in rats with chronic liver disease. *J.Hepatol.* 2014;60:554-560.

97. Martinez-Hernandez, A. Bell K. P. and Norenberg. Glutamine synthetase: glial localization in brain. *Science*. 1977;195:1356-1358.

98. Hertz, L. and Zielke H. R. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci.* 2004;27:735-743.

99. Monfort, P. Montoliu C. Hermenegildo C. Munoz M. D. and Felipo V. Differential effects of acute and chronic hyperammonemia on signal transduction pathways associated with NMDA receptors. *Neurochemistry International*. 2000;37:249-253.

100. Marcaida, G. Felipo V. Hermenegildo C. Minana M. D. and Grisolia S. Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. *Federation of European Biochemical Society Letters*. 1992;296:67-68.

101. Hermenegildo, C. Marcaida G. Montoliu C. Grisolia S. Minana M. D. and Felipo V. NMDA receptor antagonists prevent acute ammonia toxicity in mice. *Neurochemical Research*. 1996;21:1237-1244.

102. Monfort, P. Kosenko E. Erceg S. Canales J. J. and Felipo V. Molecular mechanisms of acute ammonia toxicity: Role of NMDA receptors. *Neurochemistry International*. 2002;41:95-102.

103. Seiler, N. Review. Ammonia and Alzheimer's disease. *Neurochemistry International*. 2002;41:189-207.

104. Hoyer, S. Henneberg N. Knapp S. Lannert H. and Martin E. Brain glucose metabolism is controlled by amplification and desensitization of the neuronal insulin receptor. *Ann.N.Y.Acad.Sci.* 1996;777:374-379.

105. Sims, B. Powers R. E. Sabina R. L. and Theibert A. B. Elevated adenosine monophosphate deaminase activity in Alzheimer's disease brain. *Neurobiol.Aging*. 1998;19:385-391.

106. Targowski, S. P. Klucinski W. and Jaworek D. Effect of ammonia on viability and blastogenesis of bovine lymphocytes. *Veterinary Immunology and Immunopathology*. 1984;5:297-310.

107. Kosenko, E. Kaminsky Y. Kaminsky A. Valencia M. Lee L. Hermenegildo C. and Felipo V. Superoxide production and antioxidant enzymes in ammonia intoxication in rats. *Free Radic.Res.* 1997;27:637-644.

108. Murthy, C. R. Rama Rao K. V. Bai G. and Norenberg. Ammonia induced production of free radicals in primary cultures of rat astrocytes. *J.Neurosci.Res.* 2001;66:282-288.

109. Zielinska, M. Ruszkiewicz J. Hilgier W. Fresko I. and Albrecht J. Hyperammonemia increases the expression and activity of the glutamine/arginine transporter y + LAT2 in rat cerebral cortex: implications for the nitric oxide/cGMP pathway. *Neurochem.Int.* 2011;58:190-195.

110. Targowski, S. P. Klucinski W. Babiker S. et al. Effect of ammonia on in vivo and in vitro immune response. *Infect.Immun.* 1984;43(1):289-293.

111. Tepper, J. S. Weiss B. and Wood R. W. Alterations in behavior produced by inhaled ozone or ammonia. *Fundam.Appl.Toxicol.* 1985;5:1110-1118.

112. Sekizawa, J. Yasuhara K. Suyama Y. Yamanaka S. Tobe M. and Nishimura M. A simple method for screening assessment nof skin and eye irritation. *The Journal of Toxicological Sciences*. 1994;19:25-35.

113. Perkins, M. A. Osborne R. and Johnson G. R. Development of an in vitro method for skin corrosion testing. *Fundamental and Applied Toxicology*. 1996;31:9-18.

114. Hamami, I. and Marks R. Structural determinants of the response of the skin to chmcial irritants. *Contact Dermatitis*. 1988;18:71-75.

115. Frosch, P. J. and Kligman A. M. Rapid blister formation in human skin with ammonium hydroxide. *British Journal of Dermatology*. 1977;96:461-473.

116. Grove, G. L. Duncan S. and Kligman A. M. Effect of aging on the blistering of human skin with ammonium hydroxide. *British Journal of Dermatology*. 1982;107:393-400.

117. Goldberg, A. M. Product Safety Evaluation. In: *Alternative Methods in Toxicology*. Vol. 1. New York: Mary Ann Liebert, Inc.; 1983:

118. Grant, W. M. Toxicology of the eye. 2nd *ed*. Springfield, IL: Charles C. Thomas, 1974.

119. Murphy, J. C. Osterberg R. E. Seabaugh V. M. and Bierbower G. W. Ocular irritancy responses to varous pHs of acids and bases wih and without irrigation. *Toxicology*. 1982;23:281-291.

120. Jacobs, G. A. OECD eye irritation tests on 2 alkalis. *Journal of the American College of Toxicology*. 1992;11(6):727

121. United States Department of Health and Human Services. Agency for Toxic Substances and Disease Registry's medical management guidelines for ammonia. https://www.atsdr.cdc.gov/mmg/mmg.asp?id=7&tid=2. Last Updated 2014.

122. Murakami, M. Saita H. Teramura S. Dekigai H. Asagoe K. Kusaka S. and Kita T. Gastric ammonia has a potent ulcerogenic action on the rat stomach. *Gastroenterology*. 1993;105:1710-1715.

123. Brautbar, N. Wu M. and Richter E. D. Chronic ammonia inhalation and intersittial pulmonary fibrosis: A case report and review of the literature. *Archives of Environmental Health*. 2003;58(9):592-596.

124. Kollef, M. H. Chronic ammonium hydroxide exposure. *Annals of Internal Medicine*. 1987;107(1):118

125. Michaels, R. A. Emergency planning and the acute toxic potency of inhaled ammonia. *Environmental Health Perspectives*. 1999;107(8):617-627.

126. Silverman, L. Whittenberger J. L. and Muller J. Physiological response of man to ammonia in low concentrations. *J.Ind.Hyg.Toxicol.* 1949;31(2):74-78.

127. Cole, T. J. Cotes J. E. Johnson G. R. Martin H. Reed J. W. and Saunders M. J. Ventilation, cardiac frequency and pattern of breathing during exercise in men exposed to o-chlorobenzylidine malonitrile (CS) and ammonia gas in low concentrations. *J.Exp.Physiol.* 1977;64:341-351.

128. Ferguson, W. S. Koch W. C. Webster L. B. and Gould J. R. Human physiological response and adaptation to ammonia. *J.Occup.Med.* 1977;19(5):319-326.

129. Nemer, M. Sikkeland L. I. B. Kasem M. Kristensen P. Nijem K. Bjertness E. Skare O. Bakke B. Kongerud J. and Skogstad M. Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study. *Occup.Environ.Med.* 2015;72:428-434.

130. Doig, P. A. and Willoughby R. A. Response of swine to atmospheric ammonia and organic dust. *J.Am.Vet.Med.Assoc.* 1971;159(11):1353-1361.

2017 FDA VCRP Data

Ammonia

03G - Other Eye Makeup Preparations	1
05A - Hair Conditioner	2
05D - Permanent Waves	5
05F - Shampoos (non-coloring)	1
05I - Other Hair Preparations	2
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	465
06B - Hair Tints	115
06G - Hair Bleaches	2
08G - Other Manicuring Preparations	1
12A - Cleansing	2
12C - Face and Neck (exc shave)	3
Totals	**599**

Ammonium Hydroxide

03B - Eyeliner	22
03D - Eye Lotion	1
03F - Mascara	16
03G - Other Eye Makeup Preparations	3
05A - Hair Conditioner	7
05C - Hair Straighteners	13
05D - Permanent Waves	34
05F - Shampoos (non-coloring)	11
05G - Tonics, Dressings, and Other Hair Grooming Aids	2
05I - Other Hair Preparations	5
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	1075
06B - Hair Tints	1
06F - Hair Lighteners with Color	3
06G - Hair Bleaches	9
06H - Other Hair Coloring Preparation	16
07C - Foundations	1
07I - Other Makeup Preparations	1
08E - Nail Polish and Enamel	2
08G - Other Manicuring Preparations	1
10E - Other Personal Cleanliness Products	1
12A - Cleansing	19
12B - Depilatories	1
12C - Face and Neck (exc shave)	46
12D - Body and Hand (exc shave)	13
12F - Moisturizing	11
12G - Night	16
12H - Paste Masks (mud packs)	1
12I - Skin Fresheners	4
12J - Other Skin Care Preps	18

13A - Suntan Gels, Creams, and Liquids	1
Totals	**1,354**

Memorandum

TO: Bart Heldreth, Ph.D.
Interim Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 7, 2017

SUBJECT: Draft Report: Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics (draft prepared for the September 11-12, 2017 CIR Expert Panel Meeting)

Key Issues

If a CIR report is relying on data on surrogate chemicals, literature searches should also be completed on the surrogate chemicals, as there may be new information available since the surrogate chemicals were included in the reviews/dossiers.

Although the following sentence has been added to the Cosmetic Use section: "Most of the uses of these 2 ingredients are in hair coloring products", it would be helpful if the text also stated what is meant by "most" (582/599 (97.2%) uses for Ammonia; 1104/1354 (81.6%) for Ammonium Hydroxide).

Introduction, Definition and General Characterization, Summary - CIR does not review ingredients by function. The report should clearly state that "external analgesic" is not an appropriate function in a cosmetic product, but it is not necessary to state that "the function of fragrance may be excluded from the purview of the Panel" and that "the only function of Ammonia under review herein is pH adjustor."

Although the Journal may want all references placed at the end of a paragraph, it would be helpful to place the reference with the information that came from the reference. This would make it easier to find a reference if more details about a study were needed. The references in the text should be the same as those found in a table. When a primary reference is available, it is not necessary to also cite the information to a secondary source unless the secondary source has a differing opinion about the study. Some specific examples are provided under Additional Considerations.

Additional Considerations

Data Profile - The title of the table says "Mentha piperita-derived Ingredients"

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Introduction - It should be made clear that for unpublished data, secondary sources such as ECHA dossiers are used. Published primary references should be obtained and cited whenever possible.

Toxicokinetic Studies - Please correct the following sentence: "According to another source, the normal range for blood serum levels is of 10-40 μmol/L."

Acute, Oral - Although they are included at the end of the paragraph on acute oral studies, references 42, 43 and 44 are not found in Table 4. Based on the titles of these references in the reference section, they do not appear to be relevant to the acute oral section. For example, the title of reference 44 (published in 1946) is "Ethylenediamine dihydrochloride or chlor-ethamine. II. Untoward and toxic reactions" suggesting it may not even be relevant to this report. Reference 44 is not cited anywhere else in this CIR report.

Acute, Inhalation; Summary; Table 5 (reference 5, 57) - It does not make sense to report a "higher concentration" and a "lower concentration" LC_{50}. Table 5 suggests that two durations of exposure were used, 10 minutes and 60 minutes. Perhaps the higher LC_{50} is for the 10 minute exposure and the lower LC_{50} is for the 60 minute exposure?

Short-Term, Inhalation - Reference 90, included at the end of the short-term inhalation paragraph is not in Table 6 the table in which the short-term studies are summarized. The title of this reference suggests it is a carcinogenesis interference study, not a short-term toxicity study.

Chronic, Ammonium Sulfate - The primary reference for the 52-week rat dietary study of Ammonium Sulfate is reference 80. Therefore, although it may also be presented in the ECHA dossier (reference 5) it is not necessary to cite the ECHA dossier for this study.

Developmental and Reproductive Toxicity Studies - Were any parental effects observed in the rat study (rats exposed to Ammonia in the diet at 4293 mg/kg/day)?

In the rat study of diammonium phosphate, it should be made clear that the highest dose tested was 1500 mg/kg/day, a dose at which no adverse effects were observed. This study did not actually identify a LOAEL.

Genotoxicity, In Vivo - The details of the occupational study should be cited to the primary reference (88). Who identified the limitations of the study? All of the remaining references are secondary references. If the limitations of the study were only mentioned by the secondary references, it should be made clear which secondary references noted the limitations.

Carcinogenicity, Summary - As 4 weeks is generally not long enough to develop tumors, what was examined to reach the conclusion of no evidence of carcinogenicity in mice treated with 42 mg/kg/day Ammonia for 4 weeks?

Please include a reference for the 104 week study of Ammonium Sulfate.

Summary - The Summary does not make it clear that these two ingredients are primarily used in hair dyes and colors. The Summary states that these ingredient are mostly used in rinse-off products. As the VCRP indicates an overwhelming use in hair dyes and colors, please be more specific in the Summary.

Rather than the highest LC_{50}, please state the lowest LC_{50} in the 10-minute mouse exposure studies.

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Jonas, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 16, 2017

SUBJECT: Tentative Report: Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics

Key Issues

Please follow the CIR report format outline at http://www.cir-safety.org/sites/default/files/CIR%20Report%20Format%20Outline.pdf and present the human inhalation exposure studies (Table 12) in the appropriate toxicity duration sections (as was done for dermal irritation studies). Clinical is defined as: "relating to the observation and treatment of actual patients rather than theoretical or laboratory studies." The human studies in Table 12 involve volunteers exposed to Ammonia, not patients.

Chemistry, Summary and Discussion - These sections state that the only function under review is pH adjuster. What about the function of denaturant listed for Ammonium Hydroxide? With the exception of not considering functions considered as drugs in the United States, the CIR Expert Panel does not generally review safety by function.

It would be helpful if the references were placed with the information that came from the reference. This would make it easier to find a reference if more details about a study were needed. The references in the text should be the same as those found in the associated table. When a primary reference is available, it is not necessary to also cite the information to a secondary source unless the secondary source has a differing opinion about the study.

Additional Considerations

Cosmetic Use - Although the following sentence has been added to the Cosmetic Use section: "Most of the uses of these 2 ingredients are in hair coloring products.", it would be helpful if the text also stated what is meant by "most" (582/599 (97.2%) uses for Ammonia; 1104/1354 (81.6%) uses for Ammonium Hydroxide).

Non-Cosmetic Use - Either the second or third paragraph of this section should be deleted as they are redundant.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

ADME - Please correct: "is transported tohe liver where irt is detoxified."

Acute, Oral - The text of this section does not accurately represent the information in Table 5. The first sentence says: "Either no effects or no serious effects were reported for Ammonium Hydroxide in single oral exposure animal studies." Table 5 includes 3 studies, two studies reported gastric lesions that increased with increasing concentration, and the other study only reported the LD_{50} without reporting any doses. None of the studies reported "no effects".

Acute Inhalation, Summary - Stating that "LC_{50} values of $\leq$ 10,150 ppm have been reported" is not helpful. The lowest, not the highest LC_{50} value should be stated.

Acute Inhalation - The range of rat LC_{50}s should be reported separately from the range of rat RD_{50} values.

Reference 59 at the end of the rat paragraph is not in Table 6.

The acute human exposure studies should be discussed in this section.

Short-Term, Oral - It should be stated that the 0.1% concentration given to rats was in drinking water, and that the study of diammonium phosphate was a gavage study.

Short-Term, Inhalation - Please revise the following sentence: "Nasal irritation and inflammation of the upper respiratory tract were rats exposed to500 ppm Ammonia for 3 weeks; reactions had cleared by week 8."

Reference 74 (OSHA regulations) is not in Table 7, and it is not clear why it is listed as a reference at the end of the Inhalation subsection.

Subchronic, Inhalation, Table 7 - Rather than stating "A low incidence of mortalities", it should state how many died (13/515 rats and 4/15 guinea pigs). The text states "mice" while Table 7 states "rats". In addition to no mortalities, it should also be stated that there were no histopathological findings in the study that exposed rats, guinea pigs, rabbits, monkeys and dogs continuously to about 58 ppm Ammonia for 114 days.

Chronic, Inhalation - Risk Assessment - The first paragraph of this section gives the concentration that serves as the basis for the MRL as 12.5 ppm, and the duration as "about 14 years"; the second paragraph states the concentration as 9.2 ppm with an average duration of 12.2 years. Which description is correct?

Developmental and Reproductive Toxicity Studies - It should be made clear that in the *in vitro* study, only the exposure was *in vitro*. After exposure, the embryos were then transferred back into mice. Rather than stating a "NOAEL of 1500 mg/kg/day and a LOAEL of >1500 mg/kg/day", it would be clearer to state that no effects were observed at the highest tested dose, 1500 mg/kg/day.

Reference 53 listed at the end of this section is not included in Table 8.

Carcinogenicity, Table 9 - The studies of Ammonia in Swiss and C3H mice and the study of ammonium sulfate in rats are presented twice in this section. Table 9 includes a study in which pregnant mice were treated (reference 89); this study is not mentioned in the text.

Immunological Effects - What was the source of the "protein derivative"?

Neurological Effects - It is not clear why the study in this section is not presented in the Acute inhalation section.

Ocular Irritation, Table 11 - The basis of ocular irritation at 20 ppm is not clear. Does this concentration represent a concentration in air?

Other Clinical Reports (which should be in the acute inhalation section) - Reference 128 is not included in Table 12.

Summary - The second and third paragraphs are redundant (the second paragraph should be deleted).

The paragraph summarizing the short-term inhalation studies should indicate the concentrations at which adverse effects were observed, and the highest concentration at which no adverse effects were observed.

The 1 and 2 year studies of ammonium sulfate should be mentioned in the Summary.

Discussion - The first sentence in the Discussion should also state that Ammonia and Ammonium Hydroxide are respiratory and eye irritants.

Table 1 - References still need to be added to log K_{ow}

Table 2 - As indicated in the column heading, CAS numbers need to be added to the first column of Table 2 (or the heading needs to be revised).

Table 3 - More physical and chemical properties for Ammonium Hydroxide (such as vapor pressure) can be found in the PubChem summary at https://pubchem.ncbi.nlm.nih.gov/compound/ammonium_hydroxide#section=Top.

Table 6 - The results column for reference 6, 54 is not clear relative to the protocol column. The protocol column indicate that multiple exposure concentrations were used for the 10 and 60 minute exposures. The results column gives results for a higher concentration and lower concentration. The LC_{50} for the higher concentration is greater than the highest exposure concentration stated in the protocol column.

In the description of reference 55, it is not clear what is meant by "head-out exposure". Although the authors may have used this terminology, it should be made clear if this is what is generally considered "head-only" exposure. Sometimes body only exposures are completed to determine examine dermal absorption of a gas/vapor. Was this a head exposure or a body exposure?

Table 7, short-term oral - Please clarify if the doses provided for the diammonium phosphate study are for diammonium phosphate or Ammonium.

Table 7, Short-term inhalation - Please indicate hours/day, days/week for the 65 day study cited to references 2 and 63. If this is a continuous exposure study, perhaps it is the same as the study cited to reference 46 and 63 (and may also be the same as that cited to reference 10).

Please correct "con starch" in the ingredients column.

Table 8 - The species column needs to completed for the first inhalation study.

Table 9 - What strain of mice was used in reference 89? Was the lack of lung tumors observed in the treated dams or the offspring?

Table 11 - The first row under animal is not a study. The information: "Ammonia can penetrate the eye rapidly. Ocular irritation or damage can occur at [air] concentrations beginning at 20 ppm." should be in the text, but not in Table 11 because it is not a "study".

Table 12 - The studies in this table are human experimental exposure studies. They are not clinical reports. These studies should be presented in a human section under the appropriate duration of exposure as suggested by the CIR report format outline.

At what concentrations were the effects observed (references 127; 46, 127)?

The results statement "Tolerance appears to develop with repeated exposure." This does not appear to be a study and should not be presented in Table 12. To what effect does "tolerance appear to develop"?

Memorandum

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

FROM: CIR Science and Support and Hair Coloring Technical Committees of the Personal Care Products Council

DATE: November 1, 2017

SUBJECT: Tentative Report: Safety Assessment of Ammonia and Ammonium Hydroxide as Used in Cosmetics

Thank you for the opportunity to comment on the CIR tentative report on Ammonia and Ammonium Hydroxide.

We find the conclusion for Ammonia and Ammonium Hydroxide of "safe in cosmetics in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating" to be difficult to interpret for its primary reported use in hair dyes and colors. It is understood that the pH of hair dyes may make them irritating. The irritation potential of hair dyes is addressed in product directions to limit skin contact. A clearer conclusion would be safe as used in hair dyes and colors, and safe in cosmetics applied directly to the skin in the present practices of use and concentration described in the safety assessment, when formulated to be non-irritating.

Whether or not the conclusion of the report is changed, the discussion should be revised to note that to prevent irritation, package directions should always be followed when using hair dyes and colors.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org