Safety Assessment of Alkyl Betaines

as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	November 15, 2013
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The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, 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 Director is Lillian J. Gill, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer, and Bart Heldreth, Ph.D., Chemist CIR.

1101 17th Street, NW, Suite 412 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊fax 202.331.0088 ◊
cirinfo@cir-safety.org

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett
Scientific Writer/Analyst
Date: November 15, 2013
Subject: Draft Tentative Report on Alkyl Betaines

At the September 2013 CIR Expert Panel Meeting, the Panel issued an insufficient data announcement on the safety assessment of alkyl betaine ingredients. The data needs included:

(1) method of manufacturing; and
(2) impurities.

Since the announcement, we have received method of manufacturing and impurities data from a supplier of coco-betaine. This data along with basic composition data on a betaine analog from the European Chemicals Agency (ECHA) database and basic method of manufacturing data on food-grade betaine have been incorporated in the report. The comments that were received from Council prior to the September meeting have been considered. Both the data and comments are available for your review in this report's package.

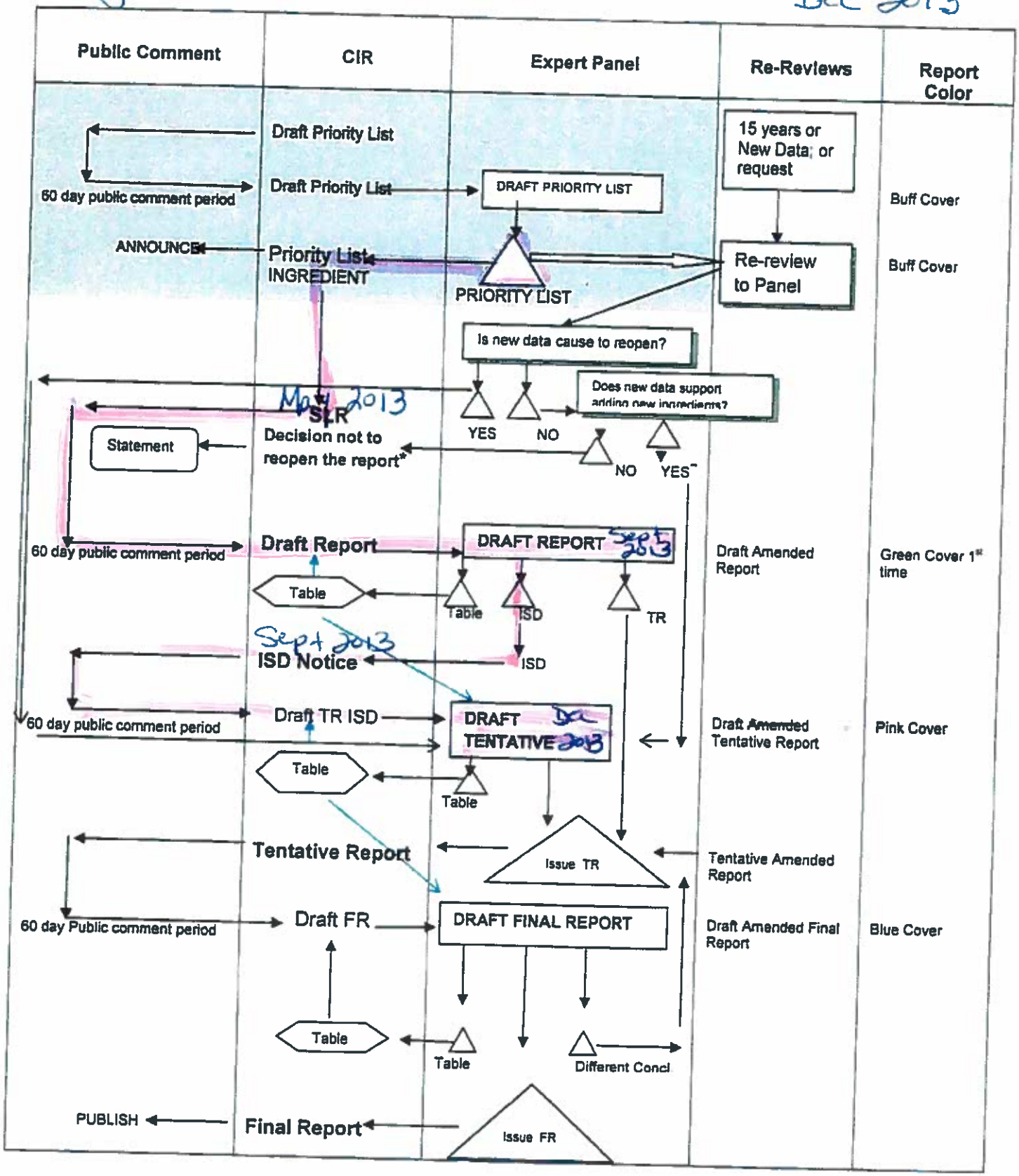
Please carefully review the draft abstract, discussion, and conclusion.

If the information now available is sufficient, the Panel should issue a Tentative Report with an appropriate discussion/conclusion. If the information is still insufficient, then a tentative conclusion of insufficient data should be issued.

Alkyl Betaines

SAFETY ASSESSMENT FLOW CHART

Dec 2013



Alkyl Betaines History

May 2013 – Scientific Literature Review announced.

September 2013 - The Panel requested additional data to support the safety of 11 alkyl betaines. The additional data needed were (1) method of manufacturing, and (2) impurities. CIR received legal permission to summarize data on the European Chemical Agency's (ECHA's) website. The Panel reviewed a table with data on a C12-C14 betaine analog.

Alkyl Betaines Data Profile – December 2013 – Writer, Christina Burnett																								
	In-Use	Composition /Impurities	Method of Mfg	Toxicokinetics	Acute Tox - Derm	Acute Tox - Oral	Acute Tox - Inhalation	Acute Tox - IV	Acute Tox - Other	Repeated Dose - Dermal	Repeated Dose - Oral	Repeated Dose - Inhalation	Repeated Dose -IV	Repeated Dose - Other	Repro/Dev Tox	Genotoxicity	Carcinogenicity/ Tumor Promoton	Dermal Irritation – Non-Human	Dermal Irritation- Human	Dermal Sens – Non-Human	Dermal Sens – Human	Ocular /Mucosal Irritation	Case Studies	Phototoxicity/ Photosensitization
Betaine	X	X	X	X		X		X			X					X	X		X	X	X	X		
Behenyl Betaine	X																							
Cetyl Betaine	X			X	X	X			X		X				X									
Coco-Betaine	X	X	X			X					X							X	X	X		X	X	
Decyl Betaine																								
Hydrogenated Tallow Betaine																								
Lauryl Betaine	X			X	X	X			X										X		X	X		
Myristyl Betaine	X																							
Oleyl Betaine	X																							
Stearyl Betaine	X																							
Tallow Betaine																								
Betaine analog C12-C14		X				X					X				X	X		X		X		X		

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

Search Strategy for Alkyl Betaines

January 2013: SCIFINDER, TOXLINE, and PUBMED search for Alkyl Betaines using INCI name and CAS #

Searches were then further filtered for adverse effects and document type.

Further limits were placed on betaine to remove efficacy studies, and on coco-betaine, to remove CAPB relation.

	TOXLINE, minus PUBMED	SCIFINDER	PUBMED
Betaine	6 (under lauryl)	5953	2468
Behenyl Betaine	0	3	1
Cetyl Betaine	6	66	0
Coco-Betaine	3	0	1
Decyl Betaine	0	21	0
Hydrogenated Tallow Betaine	0	0	0
Lauryl Betaine	2	201	1
Myristyl Betaine	0	58	0
Oleyl Betaine	1	6	0
Stearyl Betaine	0	27	0
Tallow Betaine	0	0	0

Total references ordered: 14

Search Updated October 18, 2013.

September 9-10, 2013 Panel Meeting

Belsito's Team

DR. BELSITO: So the next one is alkyl betaines. It's one that --

MS. BURNETT: According to Wikipedia it's beta because it's derived from beets so it's supposed to be pronounced beet.

DR. BELSITO: Okay. Wikipedia is always right.

MS. BURNETT: Yes.

MR. ANSELL: Yeah, we love Wikipedia.

DR. BELSITO: Wikipedia is even better. Okay. So, you know, God bless you, Christina, for having to go through these ECHA documents because, boy, they are hard to review. You click and then you have to click again and then you have to click again and then you have to read and you scroll down.

MS. BURNETT: Ask my officemates how much of a headache it was for me.

DR. BELSITO: Oh, no. I mean --

MS. BURNETT: There was language you did not want to hear from me.

DR. BELSITO: I did my own, too. I mean, this was -- I sort of thought that you bombed on this great summary. I wish you had sent this out before I blinded my eyes.

MS. BURNETT: I finished it Friday. I only did the analogs. I didn't get to the actual betaines itself because I needed to do cross because I already had data (inaudible).

DR. BELSITO: Well, I did the betaines, too, but not as well as you did the C12-15 or C12-14. You know, I think when you add all this data in we're going to find it's sufficient. The question is do we want to table it? And if so, we can do that and go to lunch. Or do we want to look at what Christina has summarized and say it's sufficient, in which case I don't think we can do that in 10 minutes, but maybe we can.

Did anyone, other than myself, try and get through all of that ECHA data?

DR. SNYDER: I scanned it.

DR. BELSITO: I thought by the time you finish it all it's pretty sufficient to say that these are safe.

DR. LIEBLER: I got this this morning.

MR. ANSELL: Well, I think that's definitive.

(Recess)

DR. BELSITO: Okay. So when we left with the fire alarm we were just discussing – and I just spoke with Pope Francis. He's agreed to start beatification for Christina for going through the ECHA documents.

DR. LIEBLER: It's a long process so don't get excited.

DR. BELSITO: But I mean, I guess I scanned them. We could go safe as used but it sounds like Dan hasn't.

DR. LIEBLER: I just read them during the lunch break.

DR. BELSITO: Okay. And then there's a whole other series of betaines, as well.

MS. BURNETT: Correct, but a lot of that data is already incorporated.

DR. BELSITO: Right.

MS. BURNETT: So I don't think there's a whole lot to add.

DR. BELSITO: Right. I mean, I'm fine safe as used or we could table to await formal incorporation of the ECHA into the documents. I'm comfortable either way. I mean, I'm comfortable going safe as used.

DR. SNYDER: The only thing is we have no impurity data.

DR. BELSITO: Okay. We have method of manufacture though, don't we?

MS. BURNETT: No.

DR. SNYDER: Nothing.

DR. LIEBLER: Can we table it to incorporate these data even though we think they'll probably be sufficient and to get impurities data or method of manufacture? Do you anticipate that those data would be gettable?

DR. BELSITO: I have got impurities, method of manufacture. We also have no UV but there are no ring structures so I didn't think we needed that. We need the aerosol boilerplate. And it looked like there was barrier disruption caused by some of these, so I suspect that we may want to put the usual --

DR. SNYDER: Irritation?

DR. BELSITO: Yeah, well, I think it would go when formulated to be nonirritating but I guess if there's no irritation you won't see barrier disruption, so then penetration wouldn't be an issue. That's a good point. Yeah, I mean, impurities and method of manufacture.

MS. BURNETT: Or you could – however you go, I can get -- since this is already in table form it's going to exist in table form in the report, so it's just a matter of carrying over. I can get (inaudible) put it in pretty fast, so you can just issue an insufficient data announcement now and not table it, and then by the time you see it again it will be complete.

MR. ANSELL: We continue to have a problem with concluding it's insufficient when the original review just doesn't have stuff that you'd like but were never asked for. So at this point, you know, we feel that if the first review is somehow deficient --

MS. BURNETT: This is the first review.

MR. ANSELL: Right. At had the first review it would be sent back to staff to search for things and ask for things that you think should be there. And not conclude that it's insufficient because the staff did or didn't conclude --

MS. BURNETT: We weren't saying concluded. Just issue an announcement asking for--

DR. BELSITO: Right. Because the data are currently insufficient.

MS. BURNETT: So that we have --

DR. BELSITO: I mean, it's not a final.

MR. ANSELL: No. It just suggests that it was deficient, and we fully support the idea that the staff should be able to go through the data and conclude what they think is important and not important. But in the first review, to conclude that it's insufficient somehow puts the onus on industry to come up with this data when, in fact, it may be there. It could have just been that the staff chose not to include it because --

DR. BELSITO: We're not saying – I mean, it's not a formal. I mean, just currently the data are not sufficient to allow for assessment of safety and what the panel is asking for, and we've always used the term insufficient, and these are our data means.

MS. GILL: And the data was requested and what came in was insufficient to make a safety decision, so the data is insufficient to draw a conclusion.

DR. BELSITO: You know, and it may actually be there in the ECHA documents. I mean, there were some tabs that I didn't bother opening. There were tabs that said impurities, I remember, and there were tabs that said method of manufacturing. And quite honestly, I didn't bother to open them. I looked more at the dermal irritation sensitization and chronic toxicity. So it may be in those documents.

MS. BURNETT: I have this website up right now.

MS. GILL: If it's -- I think if it's there and it has not had an opportunity to be included then that's different than we did not have the data and it was not submitted. So Christina, are you checking to see?

MR. ANSELL: I would prefer that in these circumstances, on the first go-through, that it be tabled to determine whether the material is available as opposed to a conclusion that the data is insufficient. It's just a recommendation.

DR. BELSITO: I mean, we're going to have to re- read the entire document anyway with the inclusion of all the material because it's going to be a huge amount of material that will have been included from the ECHA documents. So I don't have a problem with tabling it for inclusion in the ECHA documents. And when you go through them again, Christina, just check all the tabs for impurities and method of manufacturing.

MS. BURNETT: Looking at it right now. I'm crying.

DR. BELSITO: Is it there?

MS. BURNETT: If it is, it's not easily accessible. I will (inaudible) and I think my concern is going backwards. What I understand is the procedures were rewritten to cut out a step. It says when you table it you go back and see whether or not it is there when you've clearly asked for it before.

In this case, if it was there and the site -- we weren't able to get it or now that we can summarize the data, it may be linked to this particular ingredient (inaudible).

MS. GILL: And I think my concern (inaudible).

DR. LIEBLER: So between the Council and the CIR, maybe you all could work out what you'd like to call this situation because we basically are saying we can't go forward until the information is in hand. And it doesn't matter to us that much whether it's referred to as tabling the document or saying that it's insufficient. Insufficient without pejorative intent.

DR. BELSITO: Right. I think we should-- I'd like to move ahead. You know, Christina is looking at it right now. Under manufacturing, what they're doing is they're labeling for REACH and they're doing tonnage and how it's used. And also when you look at the safety tabs, what they're talking about is more like occupational exposure. You know, rinse the eyes, wash the skin with water. So I know those tabs aren't going to help us because I opened them and they're basically like a material safety data sheet.

MS. BURNETT: It's referring to some kind of -- I'm looking at manufacture for betaines. And it says process category. And there is a bunch of codes.

DR. BELSITO: Yeah.

MS. BURNETT: (Inaudible) use enclosed process. No likelihood of exposure. And it gives a couple more (inaudible). It doesn't tell you the actual chemical process, however they make it. So in this case the ECHA site is not helpful.

(Discussion off the record)

MS. BURNETT: So we're going to have to have outside help finding this because it's not -- I mean, I've looked --

DR. BELSITO: Okay.

MS. BURNETT: -- my searches have already covered everything else.

DR. BELSITO: So --

DR. LIEBLER: So the information --

DR. BELSITO: -- impurities, method of manufacturing, we need to insert the aerosol boilerplate into the cosmetic use and we have to mention that there was no photo data but we're not concerned because there are no ring structures that would likely absorb.

DR. LIEBLER: No chromophores.

DR. BELSITO: No chromophores. Just chromophores.

MS. BURNETT: Is that with a P-H?

DR. LIEBLER: Yeah, P-H. Chromophores.

MS. BURNETT: I figured it was.

DR. LIEBLER: So I expect that method of manufacture and impurities, once they are in-hand, will be unremarkable. The issue isn't that we have any suspected concern; it's we just don't have anything yet. If we do have them, I don't anticipate it to alter our course of action at all.

DR. BELSITO: Okay.

DR. SNYDER: Wheat?

DR. BELSITO: Do you want to take this now --

MS. BURNETT: Sure.

DR. BELSITO: -- because my corrections are in here.

MS. BURNETT: I'll give it a good look over and then you can help me with your handwriting.

DR. BELSITO: Yeah. No, it's mainly just misspellings.

MS. BURNETT: Okay.

DR. BELSITO: It's nothing major. Okay. So that's the alkyl betaines.

Dr. Marks' Team

DR. MARKS: Okay. Next are the alkyl betaines. There's a draft report, and, of course, this is going to -- so this is the first review, 11 ingredients, I'm sure we'll go back to the betaine as 459 uses, so a lot of uses. The lauroyl betaine has 338. Rons, Tom, the 11 ingredients okay, and then we need, what are our needs to move forward?

DR. SHANK: Well, I have for needs, we need HRIPT sensitization data on one of the alkyl betaines, lauroyl betaine is used in mud packs at 0.06 percent, and then there are several alkyl betaines that are used in cleansing preparations at 8 or higher percent.

DR. MARKS: So you wouldn't take the betaine alone with the HRIPT and say that can be read-across with the others?

DR. SHANK: Correct. And the other need I had was more data on the reproductive development toxicity studies on page 12 PDF. It says there were toxic effects in the fetuses and newborns, but I don't know what they are. I think that's important. I have to find page 12.

DR. MARKS: And let's go -- so, Ron, let me just be sure I have this right. So you want sensitization --

DR. SHANK: Well, that's me, yeah.

DR. MARKS: Yeah, that's okay. I've got sensitization data --

DR. SHANK: On at least one of the alkyl betaines.

DR. MARKS: Alkyl betaines. And then two was the reproductive developmental or just re --

DR. SHANK: Yeah, on page 12, at the very top, you get a little bit on the bottom -- well, actually, at the bottom of page 11, it says there were fetal malformations that weren't significant from the controls, that's fine. Some bone, skeletal changes.

MR. HELDRETH: I'd also like to remind of the extra data that Christina submitted today, there is a dermal sensitization there on the last page for lauroyl betaine, there may be some other ones there.

DR. SLAGA: I thought we can't use this to put it in the report, but you looked at it, right?

DR. MARKS: Well, it will go on a report in the next edition of the report, right?

DR. BERGFELD: You mean the European --

DR. SLAGA: Yeah.

DR. MARKS: Pardon? I'm sorry, Tom, repeat?

MR. HELDRETH: Legal actually cleared us to use this data. We have the summaries here, we don't actually have what the consortium has for the actual report, but we can use this.

DR. SLAGA: You can?

MR. HELDRETH: You can.

DR. HILL: That means that we have to digest it.

DR. MARKS: Let's take a couple minutes and look over this, because I really didn't. So, Ron --

DR. SHANK: I'm still looking.

DR. MARKS: Bart, it's this page to your -- well, we'll come back to the sensitization.

(Pause)

DR. MARKS: Ron, are you --

DR. SHANK: Okay, I found it, I had them marked in the wrong place, it's PDF page 11, the top. There's a dermal developmental toxicity study in rabbit, and I guess this was used as a dose finding range study, and it says near the bottom of the first paragraph that there were changes in the fetuses and newborns, but it doesn't say what they are. And this was used as a basis for an oral study in the rat, presumably, and I was wondering if the authors described the effects in fetuses or not, and I didn't have access to the journal for some reason. So if there's more information there, we should get that. And that was a dermal study, the rest of studies are oral, and they showed no problem, so, to me, that's confusing.

DR. MARKS: What study is that, can I --

DR. SHANK: Okay, yes.

DR. MARKS: Who is the lead author, I guess?

DR. SHANK: I have to find the references, now.

DR. BRESLAWEC: What page is that?

DR. MARKS: Page 11, Halyna. Because that, in the ECHA website, this summary, here, Bart, you mentioned there's a number of repro and developmental toxicity studies on this, so obviously, do you -- and then there's actually -- Ron, you probably didn't get to look at this, I just pointed out there are a number of alkyl betaines that were tested in animals -- right. And then, as Bart mentioned, this one with lauroyl in an HRIPT. Small number of volunteers but everything would indicate it's not sensitizing, so I think we could probably eliminate that insufficient data.

DR. SHANK: Okay. The reproductive toxicity studies are EPA reports, and I couldn't get ahold of those.

DR. MARKS: Okay. So that's an EPA report.

DR. SHANK: And then you say we, the sensitization data we have now?

DR. MARKS: Yes. If you look on, it would be the third sheet, third page at the top, you'll see betaine C12 to 14, or three studies there in Guinea pigs, and they were all not sensitizing. And then we comb to cocoyl betaine --

DR. SHANK: Right here.

DR. MARKS: -- that's non sensitizing. And, as Bart mentioned earlier, the lauroyl betaine in HRIPT was non sensitizing, so I think we can eliminate that.

DR. SHANK: Okay, fine. I didn't know it could --

DR. MARKS: Yeah, neither did I, I hadn't looked at that, either.

DR. SHANK: Okay, good.

DR. MARKS: So, do we want to -- I'm not sure it really would be an insufficient data notice, because we're trying to clarify the reproductive -- Tom, were you able to look through this? And, Ron, obviously, you haven't, how should we handle this? Should we take another five minutes and kind of scan these, or do you want to do that overnight, and then tomorrow in the discussion?

DR. SLAGA: I think we need to study it.

DR. MARKS: Okay.

DR. HILL: Can we at least talk about why we've got betaine lumped with all the rest of these? That was raised by somebody in industry, and I totally agreed with them. I had that in my reading it when I encountered that memo, I think this is the one. Yeah, it's page 58 right at the bottom in the memo from Halyna to, it was actually Dr. Anderson. Actually, it just says please include a rationale as to why it is appropriate to review these ingredients in one report. The only outlier seems to be betaine itself, I don't know why it's in the same report.

DR. BRESLAWEC: I think we could live with it in the same report, it's the question of to what extent you can read across between it.

DR. HILL: None, zero, zilch, none. So without this data that came from the ECHA, I was ready to say you don't have anything. That, we've got plenty of information to conclude, I just --

DR. MARKS: So, Ron, you're going back, Ron Hill, you're going back to my original question, here, the 11 ingredients, okay.

DR. HILL: Yes. If you asked that, I missed it, because I was on a tangent right at that moment.

DR. MARKS: So we're going to look over this. Rons and Tom, I had questionable, do we need more robust manufacturer and impurities.

DR. HILL: I have that as a need.

DR. MARKS: Okay. Sufficient --

DR. HILL: Method of manufacture, not impurities.

DR. MARKS: Method of -- well, I had a question with impurities. Is DMAP or amino amine found in these?

DR. HILL: No. At least I can't see how that would arise.

DR. MARKS: Okay, so that shouldn't be an issue. And the manufacturing of these DMAP or amino amine is not used in the manufacture or these betaines? Because that was the problem with cocamidopropyl betaine was these were used in the manufacture or it could be residual.

DR. HILL: If the methylation were to be done at the end, and doubt that's the way it's done, because I suspect it's reaction of dimethylglycine with an alkylating agent that has the long chain on it. I wouldn't say I'd bet my life on it, but I'd come somewhere into that. So the only issue is, if there's dimethylglycine in there, which I highly doubt for these lipophilic ones, which is, again, another why we have betaine lumped with these surfactants. And do we have any concern about making sure it's all out so we don't have nitrosation problems, or we have -- And if we had just sketchy information about the method of manufacture, which I think is going to tell us react dimethylglycine with a long chain alkylating agent. We know how to reach conclusions on a lot of that.

DR. MARKS: So method of manufacture is insufficient, correct?

DR. HILL: I would like to see it, because I don't think anybody has to collect anything, nobody has to generate any data, they just need to supply it.

DR. MARKS: Impurities, not an issue. And then the second issue is reproduction and development, that page 11, that EPA report; and then we're going to review these tonight, Tom and Ron and Ron, and if there are more things --

DR. HILL: There are only two repro tox that is listed on here, so that makes that, for you, fairly quick, and it's right at the very bottom of the last page.

DR. MARKS: That's okay, I don't want to rush it, although we do --

DR. HILL: Okay, fair enough.

DR. MARKS: -- certainly have the time now, but I got the sense from Tom that he would like to spend more time to look over these.

DR. HILL: I have written composition, here, but I have no idea why, but I suspect it was related to some of the plant source fatty acids, but I'm not all that fired up consumed about it. I think I was, what's in hydrogenated tallow and tallow, I think that was it, and coco, but that's kind of, I don't think I have to have that.

DR. MARKS: Okay. So, so I'm going to be the one who is making a motion tomorrow, this gives us plenty of time, actually, if this turns out to be the way we go. At this point, our team and Ron, Ron and Tom, does this sound reasonable, an insufficient data notice, and what we would like is method of manufacture. And then, two, clarification of the reproductive and the development issue on page 11, the EPA report. And then, after reviewing these ECHA sheets, we'll add anything more to that. Does that sound reasonable?

DR. SHANK: Yes.

DR. HILL: And, on the composition, I think the reason I didn't, in terms of moving forward, I didn't have any issue is because we have that information from, I think it's the vegetable oils report for those; it's coconut oil, hydrogenated tallow, and tallow. Maybe we could just capture that data and roll it over.

DR. MARKS: Okay. Any other comments?

DR. BERGFELD: Do you think you want, in your discussion, to make a declaration of the betaine versus the surfactants?

DR. HILL: I still think we should separate report.

DR. BERGFELD: Well, you haven't got anything in here about it.

DR. HILL: The betaine --

DR. BERGFELD: Well, you have, you don't discuss the lipophilic portion versus the surfactant portion of this group anywhere in here, I just went back to read it. You discuss each one individually, but you're talking about studies, absorption, enhancement, penetration. You don't discuss that they may act differently. I would this is the place to put it.

DR. HILL: In which place are you think --

DR. BERGFELD: In discussion.

DR. HILL: In discussion?

DR. BERGFELD: Maybe in chemistry.

DR. HILL: Yeah, up in chemistry, I think there's, I thought there was enough, but maybe I need to look at that. I'll look at it again.

DR. BERGFELD: Well, you feel very strongly about it, you mentioned it here --

DR. HILL: Just, no, what I felt strongly about was that we didn't need to have betaine itself in with these, the rest of them are all long chain behaving as surfactants, whereas betaine would not. Betaine is small, water soluble, very different from the other substances, that's what I was driving at. I would have liked to have seen that in a separate report all its own.

DR. MARKS: Okay.

DR. HILL: Because I think it just obfuscates when you mix together things that are so different and put all that data in one report. Because there's no reason to read-across, no basis for using that as read-across.

DR. MARKS: Okay. Any other comments? Wilma, any? Okay. So, tomorrow, I will move that we issue an insufficient data notice. One, we need the method of manufacture, two, we want to clarify that reproductive and the development issue from an EPA report document on page 11 of this draft safety assessment. And then we'll also, I'd huddle with the team before the meeting and ask about the review of this ECHA and whether that, if there brings anything else of concern. Or if you want to spend, do you want -- Tom, I just got the sense that five minutes isn't an adequate amount of time. I mean, we could do --

DR. SLAGA: Well, we can go over it in the morning before we start.

DR. MARKS: Okay. Sounds good. Because it will come out, obviously, in the discussion tomorrow if there are any concerns, I just don't want to do this too quickly.

DR. SLAGA: It says in our thing that we can't use this without permission, and we do have permission now?

DR. MARKS: Yes, that's what Bart said, we just can't --

DR. BERGFELD: We can use the summaries, we can't use the specifics.

DR. SLAGA: You can use the summary.

MR. HELDRETH: You can use what's in front of you, you don't even have access to the full document.

DR. MARKS: So if there are concerns raised, then we have -- okay. We'll deal with that, then, tomorrow morning. And the next one is not going to be any easier. This is the last, is this the last one, are we.

DR. SHANK: It is.

Full Panel Meeting

DR. MARKS: So this is the first time we've seen these 11 ingredients. Our team felt all 11 were okay. When looking at the data, particularly the summary sheets that we received yesterday, we felt we could move forward with a tentative report, "safe as used." And that's a motion.

DR. BERGFELD: Is there a second from the Belsito team? No? All right. Any comments to be made?

DR. BELSITO: Yeah. We want to congratulate Christina for trying to get through the ECHA site. I've also tried to get through it, and it's a series of clicking and clicking and trying to sort through lots of information. But we've not yet gone through the betaine report completely. We have gone through the C12 14 alkyl betaines. And I think the data will support safety, but we felt that we were still lacking impurities and manufacturing. And we weren't able to obtain that, at least in our brief attempt yesterday to look at the ECHA site since this is the first time that we've seen it. We haven't seen all the data that would be available on betaine itself.

We thought we would go "insufficient for impurities and manufacturing" and get all of the data from the ECHA site into a document and look at it again.

DR. BERGFELD: Dr. Marks?

DR. MARKS: So I'll ask Ron Shank and/or Tom to comment on the manufacturing and impurities.

DR. SHANK: I think we have sufficient test data to cover the safety of the use of these in cosmetics. And I don't think the method of manufacture or impurities would help me, but it might help the chemists.

DR. BERGFELD: Dan?

DR. LIEBLER: I felt that -- I didn't expect that there would be anything remarkable in that information, but we just don't have it, and we normally have it in our reports. And it seems like it's gettable information. And since the getting process has just been formally approved now for us to be able to access the rest of these data, I think we should do that.

DR. BERGFELD: Ron Hill?

DR. HILL: I rather -- I'm operating on the assumption that n-dimethylglycine is being alkylated, and that's the way these things are made. So I guess the only end result issue in my mind on the impurities, if that's true, but I'm conjecturing, and it would be nice to have that confirmed, is if there is an n-dimethylglycine, I guess is are nitrosamines a worry in that particular case?

But I also had opposition method of manufacture down here, and it was a method of manufacture question.

I still feel strongly that betaine itself should be separated from these long chain alkyl betaines because the data from one is not relevant to another. And I think toxicologically, they should be separated. I think it would make for a nice clean report on the betaine and a nice, much cleaner and much more useful in terms of read-across report if we just used then the long-chain aklyl grouping. And there was at least one opinion from industry that suggested that that's how we should've gone on this.

So I realize -- I don't think that that would demand much more work from the staff, but that's just my thinking on it. So I'll leave that up to you all.

DR. MARKS: So I have no problem in terms of "insufficient data." I mean, that's – I think we didn't think it would really change the conclusion. Neither do you. But let's get it because it is a usual part of the safety assessment. Is that fine, Ron Shank? Yeah.

DR. SHANK: Yes.

DR. BERGFELD: Since we're in discussion, Halyna?

DR. BRESLAWEC: Yeah. "Insufficient data" is, I think, generally used when you don't know if the information is out there. And I think based on your discussion, it's pretty clear that the information is there, but it has not been accessed. And so, we would recommend your considering tabling it. Obviously your decision.

DR. BERGFELD: Don?

DR. BELSITO: I'm not sure, Halyna, is you're saying the information is out there and it's in the ECHA report, we really looked and it didn't seem to be. It seemed to be a whole bunch of different regulations as to safety precautions in terms of manufacturing with all of these codes, procedural codes. It didn't give a this is reactive with this, and this, and these are the impurities. It was not straightforward like that.

And, you know, I tried going through some tabs last night. Christina was tabbing away during our meeting yesterday. I don't think that information as we would want it is there, but it may be. I mean, there are so many tabs to try and go through, and you tab on it, and there are multiple tabs below it. And that ECHA, it's very difficult to get through it.

DR. BERGFELD: Carol?

MS. EISENMANN: I saw in the composition section for the mixture was that sodium glycolate and sodium chloride were other components that had sold as 20 to 40 percent active, that they made the powder just for testing. That's what I saw under composition. And, yes, under manufacturer, you know, it's done in a closed procedure, and then mostly the focus was use in that area rather than manufacture.

DR. BELSITO: Right.

MS. EISENMANN: But there was a little bit of information on composition, in the composition section. It didn't give amounts, but it gave what I just said.

DR. BERGFELD: Ron Hill?

DR. HILL: I have to say I appreciate that there is a desire to supply that kind of information in a way that doesn't give away trade secrets, and so I appreciate that that's always true with manufacturing processes. And so, if we can get how to get the information we're looking for without having to compromise something, that would -- I'm just tossing out I appreciate that aspect of it.

DR. BERGFELD: Halyna, do you care to comment again? No? So we have no motion on the table. We have one suggestion of tabling. And, Halyna, we have another for insufficient. I'd like to hear a motion.

DR. BELSITO: I'd like to move this ahead, and hopefully we can get that information if it's not available on the ECHA website from one of the manufacturers at the next meeting, and be able to go with this as a final say rather than slowing down the process by tabling it, I mean, because there's a huge amount of information.

And, you know, it's always possible, as Ron said, that when we look at all of the studies, and we haven't seen all of the studies, you know, that we may feel that, you know, there are no concerns about impurities because at the levels they're used in these studies, we're seeing no toxic effects. And those are levels are higher than what we're using in cosmetics or whatever.

But I just don't think we've had adequate time to look at all the information, and there clearly is one piece of information that seems to be missing, and that's method of manufacturing impurities. So I'd like to go ahead with an "insufficient" and move this along.

DR. BERGFELD: And that's a motion.

DR. BELSITO: Yes.

DR. BERGFELD: Is there a second?

DR. MARKS: Second.

DR. BERGFELD: Ron Hill?

DR. HILL: Yeah, one last comment is that besides the n-dimethylglycine, which is probably in there nitrosating potential, if the reaction is done in the way I think it is, then we have long chain alkylating agents, and that would present a concern if they were there. So there's some indication as to how those are removed, if that's the way it's done, would also be helpful to us.

And that's the kind of toxicology where you might not have seen any incident in the studies, but that still could be there. And over time, long duration use of some product with such a thing present could present a hazard more subtle than --

DR. BERGFELD: So you're supporting the methods of manufacturing the request.

DR. HILL: Yes, at least get some parameters for what sorts of things we're interested in finding out about.

DR. BERGFELD: Any other discussion? No? I'm going to call the question for "insufficient data notice." Unanimous? All right. There was something put on the table about separating this group of ingredients. Any support for that?

DR. LIEBLER: Yeah. I think Ron's point about betaine having somewhat different characteristics in terms of polarity, hydrophobicity is chemically -- I agree with him on the chemistry. But I don't think it is worth separating that out and to do a separate report. And largely because there are a lot of uses for betaine itself.

And the issue is more a matter of degree rather than a totally different profile, use profile, and physiochemical -- physical chemical properties. So I think these all should stay together.

DR. BERGFELD: Thank you. Any other comments related to this?

Safety Assessment of Alkyl Betaines as Used in Cosmetics

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The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, 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 Director is Lillian J. Gill, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer, and Bart Heldreth, Ph.D., Chemist CIR.

1101 17th Street, NW, Suite 412 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊fax 202.331.0088 ◊
cirinfo@cir-safety.org

DRAFT ABSTRACT

The Cosmetic Ingredient Review Expert Panel reviewed the product use, formulation and safety data of eleven alkyl betaines, which mostly function as antistatic agents, hair and skin-conditioning agents, surfactants-cleansing agents, and viscosity increasing agents in cosmetic products. Although there are data gaps, the shared chemical core structure, similar functions and concentrations in cosmetics, and the expected similarities in physicochemical properties, enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group. The Panel concluded alkyl betaines were safe as cosmetic ingredients in the present practices of use and concentration, when formulated to be non-irritating.

INTRODUCTION

This safety assessment addresses the safety of 11 alkyl betaines as used in cosmetics. The parent compound, betaine, is a naturally occurring *N*-trimethylated amino acid, also called trimethylglycine, and can be isolated from sugar beets.[1] It is a common component in the human diet. These cosmetic ingredients mainly function as antistatic agents, hair and skin-conditioning agents, surfactants-cleansing agents, and viscosity increasing agents in cosmetic products.[2] CIR has reviewed the safety of cocamidopropyl betaine and related amidopropyl betaines as used in cosmetics.[3]

The common core chemical structure, similar functions and concentrations in cosmetics, and the predicted physicochemical properties enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group.

Toxicological data on betaine and betaine analogs (synonym: betaines, C12-14 (even numbered)-alkyldimethyl) in this safety assessment were obtained from robust summaries of data submitted to the European Chemical Agency (ECHA) by private companies as part of the REACH chemical registration process. These data are available on ECHA's website.[4,5]

CHEMISTRY

The definitions of the 11 alkyl betaines in this safety assessment can be found in Table 1, and formulas and idealized structures of these ingredients can be found in Figure 2.

The alkyl betaines are zwitterionic ingredients comprised of tertiary ammonium substituted acetate. These ingredients have a core structure of 2-(alkyldimethylammonio)acetate (i.e., *N*,*N*,*N*-trisubstituted glycine) (Figure 1).

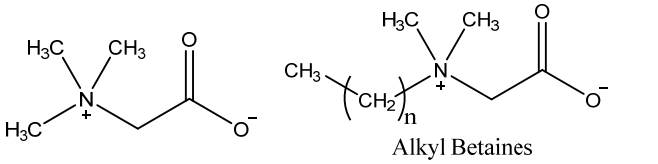

Figure 1. Betaine and the Alkyl Betaines

Therein, the "alkyl" is either methyl, as in the case of betaine itself, or an actual alkyl group ranging in length from about ten (e.g., decyl betaine) to about twenty-two (e.g., behenyl betaine) carbons. Ten to twenty-two carbons are an estimate, however, as the compositions of the ingredients derived from plant and animal sources (e.g., coco-betaine, tallow betaine, and hydrogenated tallow betaine) are variable.

The zwitterionic structures of these ingredients makes them amphoteric, a hallmark characteristic of classical surfactants. The fatty chains, found on most of these ingredients, add a lipophilic tail to these hydrophilic head groups, further imparting surfactant properties. Most of these ingredients are colorless, crystalline materials with good solubility in water and polar organic solvents.

Physical and Chemical Properties

Available chemical properties can be found in Table 2.

Method of Manufacturing

Betaine

Betaine (food-grade) may be extracted from sugar beets via liquid chromatography separation from sugar beet molasses.[6,7] It is subsequently refined and crystallized. Betaine anhydrous (as animal feed additive) is produced by reacting chloroacetic acid and sodium hydroxide with heat and stirring.[8] Trimethylamine is then added to the mixture and the resultant solutions filtered and purified. Betaine hydrochloride (as animal feed additive) follows the same synthesis pathway as betaine anhydrous except hydrochloric acid is added and the filtrate is purified.[8]

Coco-betaine

In data supplied by a manufacturer, coco-betaine is produced by reacting fatty amines from coconuts with chloroacetic acid in aqueous solution.[9]

Impurities

Betaine

Betaine (food-grade) contains very small quantities of chloride, sulfate, and heavy metals.[7] Trace analysis show very small amounts of PCB, PAH and dioxins. No pesticide traces have been detected. Betaine does not contain methanol, ethanol, or isopropanol (limits of detections were 5.0, 2.5, and 0.5 ppm, respectively).

Betaine anhydrous and betaine hydrochloride (as animal feed additives) contained < 2.0 mg/kg arsenic and < 10 mg/kg heavy metals (expressed as lead).[8] Dioxin content was < 0.50 ng/kg and PCB content was < 0.35 mg/kg. Betaine content for the anhydrous and hydrochloride forms was $\geq$ 96% and $\geq$ 93%, respectively.

Coco-betaine

According to information supplied by a manufacturer, coco-betaine is composed of approximately 31% coco-betaine, 7% sodium chloride, and 62% water.[9] There are no solvents, preservatives and other additives. The product may contain a maximum of: 100 ppm dichloroacetic acid, 100 ppm monochloroacetic acid, 0.5% free amines, 2% glycolic acid, 20 ppm heavy metals (copper, lead, tin, platinum, palladium, mercury, arsenic, cadmium, antimony, nickel, chromium, and cobalt), 2 ppm arsenic, 10 ppm iron, and < 3% volatile organic compounds.

Betaine analogs

According to information supplied to ECHA, betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) is comprised of betaine, C12-alkyldimethyl; betaine, C14-alkyldimethyl; (carboxylatomethyl) hexadecyldimethylammonium; sodium chloride; sodium glycolate; and unknown impurities.[5] Percent composition was not provided and there are no further details.

USE

Cosmetic

Table 3 presents the current product-formulation data for alkyl betaines. Betaine mainly functions as a hair conditioning agent, humectant, and skin-conditioning agent-humectant in cosmetic products.[2] The remaining alkyl betaines additionally function as antistatic agents, skin-conditioning agents-misc., surfactants-cleansing agents and foam boosters, and viscosity increasing agents. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), betaine has the most reported uses in cosmetic and personal care products, with a total of 459; the majority of the uses are in leave-on skin care preparations.[10] Lauryl betaine has the second greatest number of overall uses reported, with a total of 338; the majority of those uses are in rinse-off personal cleanliness products. The 8 ingredients with reported VCRP uses are:

- betaine
- behenyl betaine
- cetyl betaine
- coco-betaine
- lauryl betaine
- myristyl betaine
- oleyl betaine and
- stearyl betaine

Three ingredients are included in this safety assessment for which no uses have been reported to the VCRP:

- decyl betaine
- hydrogenated tallow betaine
- tallow betaine

In the Personal Care Products Council's use concentration survey, betaine had a maximum use concentration range of 0.0001% to 8.7%, with the 8.7% reported in rinse-off non-coloring hair conditioners.[11] Lauryl betaine had a maximum use concentration range of 0.015% to 8.8%, with 8.8% reported in rinse-off non-coloring hair shampoos. The Council reports that they do not have any suppliers listed for decyl betaine, hydrogenated tallow betaine, stearyl betaine, or tallow betaine.[12]

Betaine and lauryl betaine were reported to be used in hair sprays, body and hand products, non-coloring hair powders, and indoor tanning preparations that may be aerosolized or become airborne and could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays.[13-16] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[14,15]

Alkyl betaines are not restricted from use in any way under the rules governing cosmetic products in the European Union.[17]

Non Cosmetic

A biocide that contains cetyl betaine is currently being studied as a preventative treatment of human immunodeficiency virus type 1 (HIV-1) and other sexually transmitted diseases in vaginal microbicides and contraceptives.[18,19]

Betaine hydrochloride has been approved by the FDA to treat homocystinuria (by reducing homocysteine levels).[20] It is also present as an active ingredient in over-the-counter digestive aids; however, FDA has determined that there are inadequate data to establish general recognition of the safety and effectiveness of the ingredient for this specified use (21 CFR §310.545).

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Betaine

Low percutaneous permeation capability for betaine was observed in a percutaneous absorption study using Franz chambers with freshly isolated human epidermis.[4] The study followed OECD Guideline 428, but was not GLP compliant. Betaine at 5% in saline or emulsion was applied to the epidermis samples. The exposure was observed for 24 h. Approximately 0.1% of the applied dose in both vehicle types permeated through the epidermis samples.

The pharmacokinetics and acute effects on plasma total homocysteine (tHcy) of orally administered betaine (see use of betaine hydrochloride in treating homocystinuria above) was assessed in healthy human volunteers (3 men and 7 women).[21] Information on the absorption and elimination of betaine was also developed. In a double-blind crossover study, each subject ingested the betaine in doses of 1, 3, and 6 g mixed with 150 ml orange juice. The doses were ingested 7 days apart following a 12-h overnight fast. Blood samples for serum betaine concentration measurement were drawn just before receiving the betaine dose, at 20-min intervals during the first 3 h, and then at 4-, 7-, and 24-h post dosing. Urine samples were collected before dosing and during the 24-h follow-up period. Within 2 h, a dose-dependent effect on serum betaine concentration was observed. Absorption and elimination of betaine were dose dependent, with urinary excretion of betaine increasing with betaine dose. Only a very small proportion of the ingested betaine was excreted in urine, however, with 3.2%, 4.3%, and 7.4% of the 1, 3, and 6 g doses accounted for, respectively.

Cetyl Betaine and Lauryl Betaine

The absorption of radiolabeled cetyl betaine (5.4 mM) and lauryl betaine (16 mM) was determined using diffusion cells containing excised hairless mouse skin.[22] Lauryl betaine was well absorbed (approximately 50% within 24 h) while cetyl betaine partitioned into the skin but slowed transfer to the receptor phase (approximately 1.3% absorbed within 24 h). This study also examined the effects of cetyl betaine and lauryl betaine (same concentrations as used in the absorption study) on skin barrier function in hairless mouse skin in vitro. Excised skin was pretreated with each test material for 16 h. After pretreatment, the permeation of the model compound, nicotinamide, across membranes was measured and the results were compared to the flux across control membranes that were exposed to buffer alone. All surfactants decreased skin barrier function to some extent. The degree of nicotinamide penetration

enhancement was correlated with the ratio of the surfactant pretreatment concentration to the surfactant critical micelle concentration. The authors of the study suggested that solubilization of stratum corneum lipids may be an important mechanism explaining the effects observed.

The dermal uptake of cetyl betaine and lauryl betaine was measured in vivo with human skin.[23] Male volunteers received ^{14}C-radiolabeled test materials in aqueous solution on the dorsal upper arm for 30 min. The concentrations of cetyl betaine and lauryl betaine applied were 0.14, 1.0, and 5.4 mM and 16, 100, and 800 mM, respectively. The positive control was 50 mM caffeine. At the end of the exposure period, the remaining test materials were rinsed from the skin and the skin was washed. The stratum corneum at the test sites were removed with repeated tape-stripping. Dermal uptake was assessed by measuring the recovered radioactivity from the tape strips and compared to predicted penetration values. The measured uptake of cetyl betaine and lauryl betaine was 28-160 nmol/cm^2 and 2.3-19.5 nmol/cm^2, respectively. The predicted penetration values were 51-292 nmol/cm^2 for cetyl betaine and 3.7-35 nmol/cm^2 lauryl betaine. Caffeine penetrated at expected amounts. The tape stripping indicated that the radiolabel was mostly found in the outer layers of the stratum corneum.

The same study also assessed skin barrier function using the same test concentrations for both test materials.[23] Non-radiolabeled cetyl betaine and lauryl betaine were applied to the skin for 30 min. The transepidermal water loss (TEWL) was assessed. No changes in TEWL values were observed after treatment of the skin with the betaines or with saline controls.

TOXICOLOGICAL STUDIES

Acute Toxicity

Acute toxicity studies are presented in Table 4. The oral LD_{50} of betaine, cetyl betaine, lauryl betaine and a betaine analog were 11.1 g/kg, 1.62 g/kg, 0.071 g/kg, and 3 ml/kg, respectively, in rats, and 2.64 g/kg for coco-betaine (30%) in a mouse study. Also in rats, the dermal LD_{50} values were greater than 16 g/kg for cetyl betaine and 1.3 g/kg for lauryl betaine. The intravenous LD_{50} of betaine in mice has been reported to be 0.83 g/kg bodyweight. The LD_{50} values were 0.15 g/kg for cetyl betaine and 0.053 g/kg for lauryl betaine in an intraperitoneal study in rats.

Repeated Dose Toxicity

Repeated dose toxicity data are summarized in Table 5. No observable effects levels (NOEL) could not be determined for betaine due to the high tolerance of the ingredient in rats. No significant toxic effects were observed in rats that received up to 0.35 g/kg/day cetyl betaine in a 91-day oral study. In coco-betaine, the NOEL was 250 mg/kg/day and the lowest observable effect level (LOEL) was 500 mg/kg/day in a 90-day oral study in rats when tested up to 500 mg/kg/day. In a betaine analog, systemic no observable adverse effects levels (NOAEL) were 50 mg/kg bw/day and 100 mg/kg bw/day in oral rat studies that tested the material up to 300 mg/kg/day and 1000 mg/kg/day, respectively. The systemic lowest observable adverse effects levels (LOAEL) for these 2 studies were 150 mg/kg bw/day (due to increased salivation, increased urea, and non-neoplastic histopathologic changes in the kidney and bladder) and 300 mg/kg bw/day (due to decreased food consumption, body weight gain, and absolute body weight), respectively.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Reproductive and developmental toxicity studies are summarized in Table 6. Dermal reproductive and developmental toxicity studies of cetyl betaine in rabbits determined the maternal LOAEL to be 10 mg/kg/day due to decreased body weight gain and a maternal NOAEL could not be established. The developmental LOAEL was 40 mg/kg/day and the developmental NOAEL was 20 mg/kg/day. In oral reproductive and developmental toxicity studies of cetyl betaine in rats, the LOAEL for the dams was 50 mg/kg due to decreased body weight gain and a maternal NOAEL could not be calculated. The developmental LOAEL was 250 mg/kg and the developmental NOAEL was 150 mg/kg. In an oral betaine analog study, the reproductive NOEL was 150 mg/kg bw/day and the reproductive LOAEL was 300 mg/kg bw/day due to pup weight, litter size, post-implantation loss, and postnatal loss. Another oral betaine analog study determined the reproductive NOAEL to be 300 mg/kg/day when the test material was tested up to 1000 mg/kg/day.

CARCINOGENICITY

See repeated dose toxicity results in Table 5. Betaine was not carcinogenic when tested up to 5% in a 104-week oral rat study.

GENOTOXICITY

In vitro and in vivo genotoxicity studies are presented in Table 7. Betaine and a betaine analog were not genotoxic in in vitro and in vivo studies.

IRRITATION AND SENSITIZATION

Irritation and Anti-Irritation

Non-human and human irritation and anti-irritation studies are presented in Table 8. Betaine had anti-irritating effects on the skin in several efficacy studies in humans. In dermal studies, coco-betaine was not irritating in a rabbit study when tested at 16%, and was less irritating than sodium lauryl sulfate (SLS) in a human study at an unknown concentration. No dermal irritation reactions were observed in human studies of lauryl betaine at 0.1%, but were observed at concentrations of 1% and 10%. Dermal irritation results were mixed in rabbit studies of a betaine analog, with irritation observed at 30% and at an unknown concentration in 2 studies, and no irritation was observed in 2 other studies at unknown concentrations. Betaine at 10% was not an ocular irritant in rabbits, nor was a betaine analog at unknown concentrations in several rabbit studies; however, coco-betaine at 16% and 30% and lauryl betaine at 10% were ocular irritants. In human mucosal studies testing the efficacy of toothpaste, betaine did not produce adverse effects.

Sensitization

Non-human and human sensitization studies are presented in Table 9. Betaine (up to 50%), coco-betaine (up to 5%), lauryl betaine (0.1%, and a betaine analog (up to 100%) were not sensitizing in non-human and human dermal studies.

Phototoxicity

No relevant published phototoxicity studies on alkyl betaines were discovered and no unpublished data were submitted.

CLINICAL USE

Case Reports

Coco Betaine

Two cases of eczematous lesions were reported following exposure to shampoos containing coco betaine.[24] In the first case, a 44-year old woman presented with acute eczematous lesions with erythema, edema, and vesiculation on the backs and palms of her hands a few days after using a shampoo with chestnut leaf extract. Her scalp also itched and was slightly red. Previous patch tests showed positive reactions to PPD, benzocaine, wool alcohols, parabens, chinoform, perfumes, nickel sulfate, and cobalt chloride. Patch tests with the shampoo and individual components showed a ++ reaction to the shampoo in open test as is and in patch test at 2% aq., ++ reaction to parahydroxybenzoic acid esters (5% pet.), and +++ reaction to coco betaine (2% aq.). No reactions were observed to the perfume component. The dermatitis cleared when the patient changed shampoos.

In the second case, a 22-year old woman presented with red, swollen face and weeping eczematous lesions. Red, oozing and crusted acute lesions were also observed on her shoulders and scalp. The symptoms occurred after using a new shampoo. Patch tests with the shampoo and the individual components showed a +++ reaction to the shampoo in open test as is and in patch test at 2% aq., ++ reaction to coco betaine (2% aq.), and ++ reaction to sodium lauryl ether sulfate (2% aq.). The symptoms cleared when the patient changed shampoos.[24]

SUMMARY

The alkyl betaines are zwitterionic ingredients comprised of tertiary ammonium substituted acetate. These cosmetic ingredients mainly function as antistatic agents, hair conditioning agents, skin-conditioning agents, surfactants-cleansing agents, and viscosity increasing agents in cosmetic products. The common core chemical structure, similar functions and concentrations in cosmetics, and the expected bio-handling enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group.

According to information supplied to FDA's VCRP, betaine has the most reported uses in cosmetic and personal care products, with a total of 459; the majority of the uses are in leave-on skin care preparations. Lauryl

betaine has the second greatest number of overall uses reported, with a total of 338; the majority of those uses are in rinse-off personal cleanliness products. In an industry survey, betaine was reported to have a maximum use concentration range of 0.0001% to 8.7%, with the 8.7% reported in rinse-off non-coloring hair conditioner. Lauryl betaine was reported to have a maximum use concentration range of 0.015% to 8.8%, with 8.8% reported in rinse-off non-coloring hair shampoos.

Absorption and elimination of betaine in humans were dose dependent, with urinary excretion of betaine increasing with betaine dose.

Cetyl betaine and lauryl betaine were observed to decrease skin barrier function in hairless mouse skin in vitro. Cetyl betaine and lauryl betaine absorbed into mouse skin in vitro, with lauryl betaine absorbing at a faster rate. Dermal penetration rates for cetyl betaine and lauryl betaine were measured in vivo with human skin.

The oral LD_{50} of betaine, cetyl betaine, lauryl betaine and a betaine analog were 11.1 g/kg, 1.62 g/kg, 0.071 g/kg, and 3 ml/kg, respectively, in rats, and 2.64 g/kg for coco-betaine (30%) in a mouse study. Also in rats, the dermal LD_{50} values were greater than 16 g/kg for cetyl betaine and 1.3 g/kg for lauryl betaine. The intravenous LD_{50} of betaine in mice has been reported to be 0.83 g/kg bodyweight. The LD_{50} values were 0.15 g/kg for cetyl betaine and 0.053 g/kg for lauryl betaine in an intraperitoneal study in rats.

In repeated dose studies, the NOEL could not be determined for betaine due to the high tolerance of the ingredient in rats. No significant toxic effects were observed in rats that received up to 0.35 g/kg/day cetyl betaine in a 91-day oral study. In coco-betaine, the NOEL was 250 mg/kg/day and the LOEL was 500 mg/kg/day in a 90-day oral study in rats when tested up to 500 mg/kg/day. In a betaine analog, systemic NOAEL were 50 mg/kg bw/day and 100 mg/kg bw/day in oral rat studies that tested the material up to 300 mg/kg/day and 1000 mg/kg/day, respectively. The systemic LOAEL for these 2 studies were150 mg/kg bw/day (due to increased salivation, increased urea, and non-neoplastic histopathologic changes in the kidney and bladder) and 300 mg/kg bw/day (due to decreased food consumption, body weight gain, and absolute body weight), respectively.

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Betaine was not carcinogenic when tested up to 5% in a 104-week oral rat study. Betaine and a betaine analog were not genotoxic in in vitro and in vivo studies.

Betaine had anti-irritating effects on the skin in several efficacy studies in humans. In dermal studies, coco-betaine was not irritating in a rabbit study when tested at 16%, and was less irritating than SLS in a human study at an unknown concentration. No dermal irritation reactions were observed in human studies of lauryl betaine at 0.1%, but were observed at concentrations of 1% and 10%. Dermal irritation results were mixed in rabbit studies of a betaine analog, with irritation observed at 30% and at an unknown concentration in 2 studies and no irritation observed in 2 other studies at unknown concentrations. Betaine at 10% was not an ocular irritant in rabbits, nor was a betaine analog at unknown concentrations in several rabbit studies; however, coco-betaine at 16% and 30% and lauryl betaine at 10% were ocular irritants. In human mucosal studies testing the efficacy of toothpaste, betaine did not produce adverse effects.

Non-human and human sensitization studies are presented in Table 9. Betaine (up to 50%), coco-betaine (up to 5%), lauryl betaine (0.1%, and a betaine analog (up to 100%) were not sensitizing in non-human and human dermal studies.

Allergic reactions to coco-betaine have been reported in case studies.

DRAFT DISCUSSION

The Panel considered that the available data on alkyl betaines and noted the lack of systemic toxicity at high doses in single dose and repeated dose oral animal studies, no teratogenic or carcinogenic effects in animal studies, no genotoxicity in in vitro and in vivo studies, and no sensitization in multiple tests. The Panel noted that most surfactants

exhibit some irritancy, as was noted in dermal and ocular studies of coco-betaine, lauryl betaine, and a betaine analog. Thus the Panel stated that products that include these ingredients should be formulated to be non-irritating.

Although there are data gaps, the shared core chemical structure, similar functions and concentrations in cosmetics, and the expected similarities in physicochemical properties enabled grouping these ingredients and reading across the available toxicological data to support the safety assessment of each individual compound in the entire group.

The Panel noted that there were no data available on the UV absorption or phototoxicity of alkyl betaines; however, because none of the molecules that comprise these ingredients are chromophores, the Panel felt that there was no concern that these ingredients would cause adverse effects from UV exposure.

The Panel discussed the issue of incidental inhalation exposure from hairsprays, body and hand products, non-coloring hair powders, and indoor tanning preparations. There were no inhalation toxicity data available. Betaine is reportedly used at concentrations up to 3% in cosmetic products that may be aerosolized and up to 0.0001% in cosmetic products that may become airborne. The Panel believes that the sizes of a substantial majority of the particles of these ingredients, as manufactured, are larger than the respirable range and/or aggregate and agglomerate to form much larger particles in formulation. The Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. Coupled with the small actual exposure in the breathing zone, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

DRAFT CONCLUSION

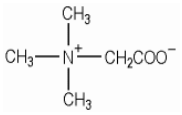
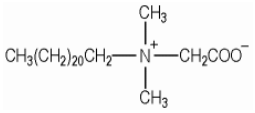
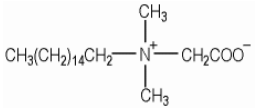
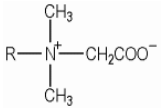
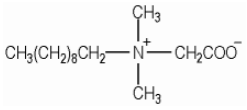
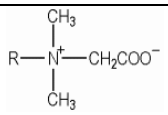
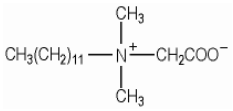
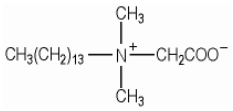
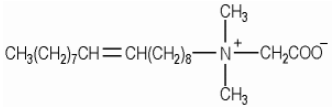
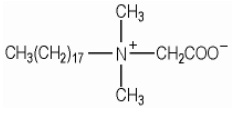
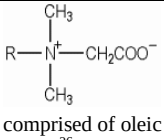
The CIR Expert Panel concluded that the eleven alkyl betaines listed below are safe in the present practices of use and concentration in cosmetics, when formulated to be non-irritating.

Behenyl Betaine
Betaine
Cetyl Betaine
Coco-Betaine
Decyl Betaine*
Hydrogenated Tallow Betaine*
Lauryl Betaine
Myristyl Betaine
Oleyl Betaine
Stearyl Betaine
Tallow Betaine*

*Not in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.

FIGURES

Figure 2. Formulas and idealized structures of the ingredients in this safety assessment.

Betaine	
Behenyl Betaine	
Cetyl Betaine	
Coco-Betaine	 where R represents the alkyl groups derived from coconut oil. (wherein coconut is primarily comprised of capric (6-10%), lauric (44-52%), myristic (13-19%), and palmitic (8-11%) acids).[25]
Decyl Betaine	
Hydrogenated Tallow Betaine	 where R represents the alkyl groups derived from hydrogenated tallow (wherein tallow is primarily comprised of oleic (37-43%), palmitic (24-32%), stearic (20-25%), myristic (3-6%), and linoleic (2-3%) acids; and hydrogenation of tallow would result in the reduction of some of the unsaturated acids to saturated acids (i.e., increased stearic acid and decreased linoleic and oleic acids)).[26]
Lauryl Betaine	
Myristyl Betaine	
Oleyl Betaine	
Stearyl Betaine	
Tallow Betaine	 where R represents the alkyl groups derived from tallow (wherein tallow is primarily comprised of oleic (37-43%), palmitic (24-32%), stearic (20-25%), myristic (3-6%), and linoleic (2-3%) acids).[26]

TABLES

Table 1. Definitions and functions of the ingredients in this safety assessment.[2] (The italicized text below represents additions made by CIR staff.)

Ingredient/CAS No.	Definition	Function
Betaine 107-43-7	Betaine is the zwitterion (inner salt) that conforms to the formula. *Betaine is the N,N,N-trimethylammonium zwitterion of glycine.*	Hair conditioning agents; humectants; skin-conditioning agents-humectants
Behenyl Betaine	Behenyl Betaine is the zwitterion (inner salt) that conforms to the formula. *Behenyl Betaine is the N-behenyl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Cetyl Betaine 693-33-4	Cetyl Betaine is the zwitterion (inner salt) that conforms to the formula. *Cetyl Betaine is the N-cetyl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Coco-Betaine 68424-94-2	Coco-Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Coco-Betaine is the N-cocyl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Decyl Betaine 2644-45-3	Decyl Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Decyl Betaine is the N-decyl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Hydrogenated Tallow Betaine	Hydrogenated Tallow Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Hydrogenated Tallow Betaine is the ammonium zwitterion of glycine, wherein nitrogen is substituted with two methyl groups and a fatty chain derived from hydrogenated tallow.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Lauryl Betaine 683-10-3	Lauryl Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Lauryl Betaine is the N-lauryl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Myristyl Betaine 2601-33-4	Myristyl Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Myristyl Betaine is the N-myristyl-N,N-dimethylammonium zwitterion of glycine.*	Abrasives; antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Oleyl Betaine 871-37-4	Oleyl Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Oleyl Betaine is the N-oleyl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Stearyl Betaine 820-66-6	Stearyl Betaine is the zwitterion (inner salt) that conforms to the formula. *Stearyl Betaine is the N-stearyl-N,N-dimethylammonium zwitterion of glycine.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous
Tallow Betaine	Tallow Betaine is the zwitterion (inner salt) that conforms generally to the formula. *Tallow Betaine is the ammonium zwitterion of glycine, wherein nitrogen is substituted with two methyl groups and a fatty chain derived from tallow.*	Antistatic agents; hair conditioning agents; skin-conditioning agents-misc.; surfactants-cleansing agents; surfactants-foam boosters; viscosity increasing agents-aqueous

Table 2. Physical and chemical properties.

	Property	Reference
Betaine		
Physical Form	Deliquescent scales or prisms	[27]
Molecular Weight	117.15	[6,27]
Melting Point	293 (decomposes)	[6]
Water Solubility g/L	160	[27]
Other Solubility g/L	55 in methanol, 8.7 in ethanol, sparingly sol in ether	[27]
Cetyl Betaine		
Vapor pressure mmHg@ 25 ^{o}C	2.4×10^{-12}	[28]
Melting Point ^{o}C	243	[28]
Boiling Point ^{o}C @ 760 mmHg	566	[28]
Water Solubility mg/L @ 25 ^{o}C	171	[28]
log K_{ow}	2.44	[28]
Lauryl Betaine		
Physical Form	Crystals or colorless needles	[27]
Molecular Weight g/mol	271.44	[27]
Melting Point ^{o}C	183-185	[27]
Water Solubility	Easily soluble in water	[27]
Other Solubility	Easily soluble in methanol, ethanol, and benzene; moderately soluble in acetone	[27]
Dissociation constants (pKa)	1.8	[27]

Table 3. Frequency and concentration of use (2013) according to duration and type of exposure for alkyl betaines.[10,11]

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Behenyl Betaine		**Betaine**		**Cetyl Betaine**	
Totals*	**4**	**8.4**	**459**	**0.0001-8.7**	**14**	**0.36-7.4**
Duration of Use						
Leave-On	1	NR	326	0.0001-8	NR	NR
Rinse-Off	3	8.4	130	0.09-8.7	14	0.36-7.4
Diluted for (Bath) Use	NR	NR	3	0.01	NR	NR
Exposure Type						
Eye Area	NR	NR	31	0.1-3	NR	NR
Incidental Ingestion	NR	NR	6	0.05-3	NR	NR
Incidental Inhalation-Spray	NR	NR	4	0.2-3[a]	NR	NR
Incidental Inhalation-Powder	NR	NR	4	0.0001[b]	NR	NR
Dermal Contact	4	8.4	379	0.01-6.5	12	0.36-7.4
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	50	0.0001-8.7	2	NR
Hair-Coloring	NR	NR	22	0.44	NR	NR
Nail	NR	NR	NR	3	NR	NR
Mucous Membrane	NR	NR	19	0.01-3	6	0.36-7.4
Baby Products	NR	NR	4	NR	NR	NR

	Coco-Betaine		**Lauryl Betaine**		**Myristyl Betaine**	
Totals*	**227**	**0.53-9.8**	**338**	**0.015-8.8**	**6**	**0.84**
Duration of Use						
Leave-On	4	1.8-2	29	0.016-1.2	NR	NR
Rinse Off	213	0.53-9.8	281	0.015-8.8	6	0.84
Diluted for (Bath) Use	10	3.1-5.1	28	1	NR	NR
Exposure Type						
Eye Area	1	NR	2	0.016	NR	NR
Incidental Ingestion	NR	2	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	1	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR
Dermal Contact	141	0.53-9.8	308	0.016-8	6	0.84
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	70	0.63-8	29	0.015-8.8	NR	NR
Hair-Coloring	16	1.5-2.3	1	0.19-3	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	87	2-5.1	262	0.75-4.2	6	0.84
Baby Products	3	NR	1	NR	NR	NR

	Oleyl Betaine		**Stearyl Betaine**	
Totals*	**5**	**23.7**	**1**	**NR**
Duration of Use				
Leave-On	NR	NR	1	NR
Rinse-Off	5	NR	NR	NR
Diluted for (Bath) Use	NR	23.7	NR	NR
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR
Dermal Contact	3	23.7	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	2	NR	1	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	1	23.7	NR	NR
Baby Products	NR	NR	NR	NR

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

NR = none reported

[a] 0.5% in an aerosol hair spray, 3% in a pump hair spray, 0.2% in a body and hand spray.

[b] 0.0001% in a powder non-coloring hair preparation.

Table 4. Acute toxicity studies in animals

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
	Oral		
betaine anhydrous, > 97% pure; doses = 5, 10, 12.5, 15, 20 g/kg bw in water	OECD Guideline 401 for acute oral toxicity in Crj:CD(SD) rats; 5 rats of each sex per dose; GLP compliant	LD_{50} (calculated) = 11.1 g/kg bw in males and females; symptoms observed in each dose group included lethargy, decreased motor activity, prone posture, ataxia, musculature - tremor, bradypnea, hyperpnea, piloerection, ungroomed appearance, hunched posture and death	[4,6]
cetyl betaine (94.9% pure) and lauryl betaine (98.9% pure) in 25% w/v solutions; doses not reported	Groups of 5 male Sprague-Dawley rats received either test material via oral gavage	LD_{50} = 1620 mg/kg for cetyl betaine and 71 mg/kg for lauryl betaine; symptoms in some animals for either test material were sluggishness, diarrhea, and lacrimation; weight gains were within normal parameters in surviving animals; gross necropsy of the animals that died during the study found the gastrointestinal tract distended with red fluid and lungs mottled and red; no significant differences in the pharmacotoxic signs or gross necropsy findings between the 2 test materials	[22]
coco-betaine tested at 30% active ingredient and at 10% in water; doses = 6670, 8350, 10000 mg/kg bw	OECD Guideline 401 for acute oral toxicity in CF-1 mice; 10 mice per dose; not GLP compliant	LD_{50} (calculated) = 2640 mg/kg bw for 30% active ingredient and 8800 mg/kg bw for 10% in water	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) in aqueous solution tested at 0, 1.6, 2.5, 3.2, 4.0, 5.0, 8.0 ml/kg bw	OECD Guideline 401 for acute oral toxicity in CFY rats; 5 rats of each sex per dose; not GLP compliant	LD_{50} (calculated) = 3 ml/kg bw in males and females; lethargy and diuresis observed at 3.2 ml/kg and greater; death preceded by ataxia and coma and occurred within 5-27 h post-dosing; total recovery in survivors 5 days post-dosing	[5]
	Dermal		
cetyl betaine (94.9% pure) and lauryl betaine (98.9% pure); doses not reported	Groups of 5 male Sprague-Dawley rats received either test material dermally on clipped trunks; test sites were occluded for 24 h, after which the test sites were wiped clean of the test materials	LD_{50} = > 16 g/kg for cetyl betaine and 1.3 g/kg for lauryl betaine; erythema, edema, desquamation, necrosis, and scab formation observed on test sites for both test materials, as was sluggishness and reddish nasal and ocular discharges; body weight gains within normal parameters; no treatment-related changes due to either test material observed at gross necropsy	[22]
	Intravenous/Intraperitoneal		
betaine; no further details provided	Intravenous acute study in mice; no further details provided	LD_{50} = 830 mg/kg bw; no further details provided	[6]
cetyl betaine (94.9% pure) and lauryl betaine (98.9% pure) in 5% and 25% w/v solutions in distilled water; doses not reported	Groups of 5 male Sprague-Dawley rats received either test material intraperitoneally	LD_{50} = 150 mg/kg for cetyl betaine and 53 mg/kg for lauryl betaine; sluggishness, diarrhea, lacrimation, and distended abdomen observed in animals that received either test material; body weight gains within normal parameters; no treatment-related changes due to either test material observed at gross necropsy	[22]

Table 5. Repeated dose toxicity in animals

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
betaine > 95% pure; doses = 0%, 1%, 2%, 5% in animal feed	OECD Guideline 407 for repeated dose 28-day oral toxicity in female Sprague-Dawley rats; number per dose not provided; GLP compliant	NOAEL > 5771 mg/kg bw/day; NOEL could not be derived due to high tolerance and reversibility of slight to moderate hepatocellular vacuolation effects in rats	[4]
betaine > 95% pure; tested up to 5% in animal feed	OECD Guideline 408 for repeated dose 90-day oral toxicity in male and female Sprague-Dawley rats; 20 rats of each sex per dose; GLP compliant	NOAEL and NOEL could not be determined due to high tolerance; slight hematology and hepatic changes that included increased liver weights, hepatocellular vacuolation, but no microscopic evidence of hepatotoxicity; no significant systemic signs of toxicity were observed in any treatment group during dosing	[4]
betaine > 99.9% pure; test up to 5% in animal feed for both chronic and carcinogenicity studies	OECD Guideline 453 for combined chronic toxicity/carcinogenicity studies in male and female Fischer 344 rats; 52-week study had 10 rats of each sex per dose; 104-week study had 25 rats of each sex per dose;	NOEL determined for up to 5%, betaine was not carcinogenic; increased live and kidney weights observed in both sexes in the 5% dose group; decrease in mean corpuscular volume and mean corpuscular hemoglobin observed; increased platelet count observed; minor effects in blood biochemistry for chronic study	[4]
cetyl betaine tested at 32% active adjusted to be delivered at doses of 0, 0.05, 0.15, 0.35 g/kg/day	91-day subchronic oral toxicity study in Sprague-Dawley rats; 10 rats of each sex per dose group; test material administered in feed; GLP compliant	All animals survived until end of treatment period; no treatment-related clinical observations; mean body weights and body weight gains significantly decreased in high dose males which was accompanied by significantly decreased total feed consumption – these observations were attributed to palatability problems of diet than toxic effects of test material; slight clinical chemistry changes observed in high dose animals; no gross or histologic alterations attributed to test material observed	[29]
coco-betaine tested at 29-33% active material in water at 0, 125, 250, and 500 mg/kg bw/day	OECD Guideline 408 for repeated dose 90-day oral toxicity in male and female Sprague-Dawley rats; 10 rats of each sex per dose; GLP compliant	NOEL = 250 mg/kg bw/day; LOEL = 500 mg/kg bw/day due to increased water consumption. Irritative effects observed in the forestomach of mid and high dose group, possibly due to gavage dosing. No adverse effects on reproductive organs.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) in water at 0, 50, 150, 300 mg/kg bw/day	OECD Guideline 422 for combined repeated dose oral toxicity study with reproduction/developmental toxicity screening test in male and female Wistar rats; 10 rats of each sex per dose; GLP compliant	NOAEL (systemic) = 50 mg/kg bw/day; LOAEL (systemic) = 150 mg/kg bw/day due to increased salivation, increased urea, and non-neoplastic histopathologic changes in the kidney and bladder; NOEL (reproduction) = 150 mg/kg bw/day; LOAEL (reproduction) = 300 mg/kg bw/day due to pup weight, litter size, post-implantation loss, and postnatal loss.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) in water at 0, 33, 100, 300, and 1000 mg/kg bw/day	OECD Guideline 422 for combined repeated dose oral toxicity study with reproduction/developmental toxicity screening test in male and female Wistar rats; 3 rats of each sex per dose; not GLP compliant	NOAEL (systemic) = 100 mg/kg bw/day; LOAEL (systemic) = 300 mg/kg bw/day due to decreased food consumption, body weight gain, and absolute body weight; no reproductive results reported due to small group sizes.	[5]

Table 6. Reproductive and developmental toxicity

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0, 50, 150, or 300 mg/kg bw/day active ingredient in water	OECD Guideline 422 for combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in male and female Wistar rats by oral gavage; 10 animals per sex per dose; males dosed 4 weeks, females dosed 7 weeks; GLP compliant	In parental animals, sedation, salivation, and irritation effects in the stomach and bladder due to the irritating nature of the test material were observed in the high dose group. Additionally, reduced weight gain and reduced absolute body weights were observed in the high dose group and in the mid dose group, but in a milder form. Reduced pup weight, liter size, and increased post-implantation and postnatal loss were observed in the high dose group and were considered secondary to maternal toxicity.	5
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0, 33, 100, 300, or 1000 mg/kg bw/day in water	OECD Guideline 422 for combined repeated dose toxicity study with the reproduction/developmental toxicity screening test in male and female Wistar rats by oral gavage; 3 animals per sex per dose; males dosed 4 weeks, females dosed 6 weeks; not GLP compliant	All parental animals in the 1000 mg/kg/day dose group died within 24 h of the first dose. At 300 mg/kg/day, no adverse effects on reproduction were observed. The NOAEL of the test substance for reproduction was 300 mg/kg/day.	5
cetyl betaine; 0, 10, 20, 40, 100, or 200 mg/kg/day in 5% isopropanol in dosages of 2 ml/kg.	Dermal development toxicity/teratogenicity study in female New Zealand White rabbits. Groups of 8 artificially inseminated rabbits received the test material for 4 hours daily on approximately days 6 through 18 of gestation. Test substance-related mortality and severe topical effects occurred in the 100 and 200 mg/kg dose groups after the eighth and sixth dosages, respectively, and administration of these dose levels was discontinued. Two additional test groups (n=8 each) of non-inseminated rabbits were added: one received a new vehicle control (not reported) and the other received 2 mg/kg/day of the test material in the new vehicle control. All animals were observed daily for signs of toxicity, skin irritation, abortion (inseminated rabbits), death, body weight and feed consumption. Rabbits that died during the study were examined for pregnancy (inseminated rabbits) and cause of death. The inseminated rabbits were killed on day 19 of gestation and the non-inseminated rabbits were killed 24 h after the 13th daily dosage was administered. Inseminated rabbits underwent a complete gross necropsy, including examination of the brain, uterus, and fetuses.	Maternal LOAEL = 10 mg/kg/day; maternal NOAEL could not be established; developmental LOAEL = 40 mg/kg/day; developmental NOAEL = 20 mg/kg/day. In the 100 and 200 mg/kg dose groups, 3 rabbits each died or were killed during the course of the study. Clinical observations in these groups and the 40 mg/kg dose group included uncoordinated movement, partial paralysis, red exudate of vaginal origin present in the cage pan, green matted fur, ataxia, and alopecia. All skin reactions, including erythema, desquamation, atonia, fissuring, eschar and exfoliation were dose-dependent. All rabbits in each dose group had a minimum of grade 1 erythema observed at least once. No rabbits in any dose group had edema. When compared to the control group, average body weight gain was inhibited in rabbits of the 2 through 200 mg/kg dose groups and was considered to be dose dependent. The severity of the effect was slight in the 2 and 10 mg/kg dose groups and marked in the 100 and 200 mg/kg dose groups. Decreased average daily feed consumption was noted in the 2 through 200 mg/kg dose groups and was also considered to be dose dependent. It was considered biologically significant in the 40 to 200 mg/kg dose groups. Pregnancy was observed in 6 or 7 of the 8 rabbits in each dose group. An increased incidence of resorptions was observed in the maternally toxic doses of 40, 100, and 200 mg/kg/day. A decrease in average litter size was observed in the 100 and 200 mg/kg dose groups. All fetuses were alive at Caesarean-sectioning, but were not examined and no further data about the fetuses are available. The results determined that doses of 0, 2, 10, and 20 mg/kg would be used in a definitive rabbit teratology study (results of this study have not been found).	28,30

Table 6. Reproductive and developmental toxicity

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
cetyl betaine; received 0, 50, 150, and 250 mg/kg /day of 30.4% active cetyl betaine in 10% ethanol (correction factor of 3.2895 was utilized to achieve proper amount of active ingredient)	Oral developmental toxicity/teratogenicity study, female Sprague-Dawley rats.[28,30] The control group received ethanol in deionized water at a volume of 5 ml/kg, which was the same amount of ethanol that the 250 mg/kg cetyl betaine dose group received. The rats received the test material daily for 10 days starting on gestation day 6. The animals were observed twice daily for signs of toxicity and body weights and feed consumption were recorded on day 0, 6, 9, 12, 16, and 20 of gestation. On gestation day 20, all surviving rats were killed and the uterus and the fetuses were examined and measured for number and location of viable and nonviable fetuses, early and late resorptions, number of total implantations and corpora lutea, fetal body weights, sex, external malformations or developmental variations, and skeletal abnormalities.	Maternal LOAEL = 50 mg/kg based on the inhibited body weight gain; maternal NOAEL could not be calculated; developmental LOAEL = 250 mg/kg; developmental NOAEL = 150 mg/kg. No mortalities observed in any of the dams in the control or treatment groups. In the 250 mg/kg dose group, clinical observations included stained and matted fur primarily on the limbs, neck, ventral thorax, and facial area, excessive salivation, respiratory rales, diarrhea, decreased activity, hypothermia, lacrimation, labored breathing, and wheezing. Similar observations were made in the 150 mg/kg dose group, with the stained and matted fur and respiratory rales the predominant signs of toxicity. Inhibition of maternal body weight gain was observed as a dose-related trend during overall gestation and the treatment periods at all dose levels. Weight loss was observed during the first treatment interval in the 150 and 250 mg/kg dose groups. Decreased feed consumption was also observed in all treated groups during the treatment period in a dose-dependent manner. Feed consumption was noted to be inhibited at 250 mg/kg during the overall gestation period, but the mean values for the 50 and 150 mg/kg dose groups were comparable to controls. In the fetuses, no significant differences between the control and treated groups were evident with respect to number of corpora lutea, total implantations, post implantation loss, viable fetuses, and fetal body weights. Fetal malformation in the treated groups was not significantly different from that of the controls. Reduced or absent ossification of the skull, sternebrae #5 and/or #6, and other sternebrae occurred more frequently in the 250 mg/kg dose group. These effects were considered to be biologically significant as they were observed in conjunction with reduced maternal body weight gains. No other developmental variations were noted.	[28,30]

Table 7. Genotoxicity

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
	In Vitro		
betaine monohydrate > 95% pure; concentrations up to 10 mg/ml with and without S9 metabolic activation	Chromosome aberration study using human lymphocytes in whole blood cultures with and without metabolic activation; GLP compliant	Not clastogenic	[4,6]
betaine monohydrate > 97% pure; concentrations plated = 8 to 5000 µg/plate with and without S-9 activation	Bacterial reverse mutation assay using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 with and without S-9 metabolic activation; GLP compliant	Not genotoxic	[4,6]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); up to 5.0 mg/plate with and without S9 metabolic activation	OECD Guideline 471 for bacterial reverse mutation (Ames) assay in *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, TA102 with and without metabolic activation; GLP compliant	Not genotoxic with or without metabolic activation.	[5]
alkyl dimethyl betaine (no further description) 30.2% active ingredient; up to 100 µg/ml with S9 and up to 75 µg/ml without S9	OECD Guideline 476 for in vitro mammalian cell gene mutation test in Chinese hamster ovary (CHO) cells – HGPRT locus with and without metabolic activation; GLP compliant	Not genotoxic with or without metabolic activation	[5]
alkyl dimethyl betaine (no further description) 30% active ingredient; up to 200µg/ml without S9 with 4 h exposure, up to 100 µg/ml without S9 with 20 h exposure, and up to 150 µg/ml with S9	OECD Guideline 473 for in vitro mammalian chromosome aberration test in CHO cells with and without metabolic activation; GLP compliant	Not genotoxic with or without metabolic activation	[5]
	In Vivo		
betaine monohydrate > 98% pure; doses = 0, 0.5, 1, or 2 g/kg in saline	OECD Guideline 474 for mammalian erythrocyte micronucleus test using male and female CD-1 mice; test material or the positive control cyclophosphamide administered by gavage; exposure periods were 24, 48, or 72 h; GLP compliant.	Not genotoxic to micronuclei in the bone marrow of mice dosed up to 2 g/kg	[4,6]

Table 8. Irritation and anti-irritation studies

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
	Dermal – Non-Human		
coco-betaine tested at 16% in solids solution	OECD Guideline 404 for acute dermal irritation/corrosion in 3 albino rabbits; occlusive on shaved and abraded skin	Mean erythema score = 0.5/4, fully reversible within 24 h; mean edema score = 0.5/4, fully reversible within 24 h. Not irritating.	5
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine) tested as 30% active material neat	OECD Guideline 404 for acute dermal irritation/corrosion in 3 New Zealand White rabbits; semiocclusive on shaved skin; GLP compliant	Very slight to slight edema between 30 min and 72 h post-dosing. Very slight to moderate erythema up to day 7. Skin was dry, rough and had fine to coarse scales with desquamation. Effects fully reversible within 14 days. Classified as irritating.	5
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 404 for acute dermal irritation/corrosion in 6 New Zealand White rabbits; occlusive on shaved skin; not GLP compliant	Mean erythema score = 1.83/4, not fully reversible within 72 h Mean edema score = 0.83/4, not fully reversible within 72 h Not irritating.	5
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 404 for acute dermal irritation/corrosion in 6 New Zealand White rabbits; occlusive on shaved skin; GLP compliant	Mean erythema score = 1.17/4, not fully reversible within 7 days Mean edema score = 0.72/4, not fully reversible within 7 days Not irritating.	5
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 404 for acute dermal irritation/corrosion in 6 New Zealand White rabbits; occlusive on shaved skin; not GLP compliant	Mean erythema score = 1.83/4, not fully reversible within 72 h Mean edema score = 1.08/4, not fully reversible within 72 h Moderate irritant.	5
	Dermal - Human		
betaine; tested up to 10%	Efficacy study of test material in reducing irritation in soap was assessed in 2 studies with healthy subjects (n=28 and n=21)	Soap containing betaine found to be less irritating than those without the test material, but not in a dose-dependent manner	1,31
betaine; tested up to 5% in several vehicles	OECD Guideline 404 for acute dermal irritation/corrosion in 26 healthy volunteers; 2 occlusive Finn patches for 2 consecutive 24 h periods; test site area = 50 mm^2; GLP compliant	Not irritating; some anti-irritancy observed with some of the vehicles	4
betaine; > 95% pure tested at 3.5% with 2% sodium lauryl sulfate (SLS) in water	20 male and 20 female test subjects with test products applied on permuated test areas on different arms inside of the crook of the elbow for 4 weeks; test areas washed and measurement of transepidermal water loss (TEWL) was measured after 4 weeks and 6 hours after the last washing; signs of irritation were observed at the end of the test and test areas were assessed for erythema, flaking, roughness, pruritus and formation of papules; not GLP compliant	Betaine found to lessen irritation effects of SLS and considered anti-irritating	4
coco-betaine in distilled water	Potential of 4 surfactants, including coco-betaine, to cause dermal irritation was assessed in a TEWL study with Finn chambers in 27 healthy volunteers; 24 h exposure	Sodium lauryl sulfate (SLS) had greatest mean TEWL (15.5 g/m^2h), followed by coco-betaine (12.6 g/m^2h), sodium laurate (10.6 g/m^2h), and polysorbate-60 (9.8 g/m^2h); no severe irritation (3+ or 4+) was observed following the exposure to 2 g/100 ml of the test substances (the mean overall scores for coco-betaine and SLS were 1.03 and 1.833, respectively); coco-betaine had less irritation potential than SLS	32
lauryl betaine at 0.1% active ingredient	Acute dermal irritation in 19 human subjects; not occluded; 30 h exposure; not GLP compliant	No reactions observed.	5
lauryl betaine at 1% and 10% active ingredient	Acute dermal irritation in 7 human subjects; occluded; 24 h exposure; not GLP compliant	10% solution had 1 strong erythema, 4 moderate, and 2 mild; 1% solution had 5 strong erythema, 1 moderate and 1 mild.	5
	Ocular– Non-Human		
betaine monohydrate > 95% pure; 10% w/v in distilled water	OECD Guideline 405 for acute ocular irritation/corrosion in albino rabbits; GLP compliant no further details were provided	Not irritating	4,6
coco-betaine tested at 16% solids with no vehicle	OECD Guideline 405 for acute ocular irritation/corrosion in 3 albino rabbits; unwashed eyes; not GLP compliant	Test material caused corneal involvement and conjunctival irritation that did not clear by day 7 post-dosing. Irritating.	5
coco-betaine tested at 30% active material with no vehicle	OECD Guideline 405 for acute ocular irritation/corrosion in 3 albino rabbits; unwashed eyes; not GLP compliant	Test material caused corneal and iris involvement and conjunctival irritation that did not clear by day 7 post-dosing. Irritating.	5

Table 8. Irritation and anti-irritation studies

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
lauryl betaine tested at 10% (v/v) solution in distilled water	OECD Guideline 405 for acute ocular irritation/corrosion in 3 New Zealand White rabbits; unwashed eyes; GLP compliant	Irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand White rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0/4; mean iris score = 0.11/2, fully reversible within 72 h; mean conjunctivae score = 0.78/4, not fully reversible within 72 h; mean chemosis score = 0.17/4, fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand White rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0.83/4, not fully reversible within 72 h; mean iris score = 0.55/2, not fully reversible within 72 h; mean conjunctivae score = 1.33/3, not fully reversible within 72 h; mean chemosis score = 0.72/4, not fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 6 New Zealand White rabbits; unwashed eyes; GLP compliant	Mean cornea score = 0.22/4, fully reversible within 72 h; mean iris score = 0.55/2, not fully reversible within 72 h; mean conjunctivae score = 1.33/3, not fully reversible within 72 h; mean chemosis score = 0.83/4, not fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 6 New Zealand White rabbits; unwashed eyes; GLP compliant	Mean cornea score = 0/4; mean iris score = 0.11/2, fully reversible within 48 h; mean conjunctivae score = 0.78/3, fully reversible within 72 h; mean chemosis score = 0.28/4, fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand White rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0.22/4, fully reversible within 72 h; mean iris score = 0.22/2, fully reversible within 72 h; mean conjunctivae score = 1.16/3, not fully reversible within 72 h; mean chemosis score = 0.33/4, not fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand White rabbits; washed and unwashed eyes; not GLP compliant	Mean cornea score = 0.61/4, not fully reversible within 72 h; mean iris score = 0.22/2, not fully reversible within 72 h; mean conjunctivae score = 1.44/3, not fully reversible within 72 h; mean chemosis score = 0.72/4, not fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 6 New Zealand White rabbits; unwashed eyes; GLP compliant	Mean cornea score = 0/4; mean iris score = 0.28/2, fully reversible within 72 h; mean conjunctivae score = 1.05/3, not fully reversible within 72 h; mean chemosis score = 0.72/4, fully reversible within 72 h. Not irritating.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine), concentration not reported	OECD Guideline 405 for acute ocular irritation/corrosion in 9 New Zealand White rabbits; washed and unwashed eyes; GLP compliant	Mean cornea score = 0.06/4, fully reversible in 48 h; mean iris score = 0.16/2, fully reversible within 48 h; mean conjunctivae score = 0.67/3, fully reversible within 72 h; mean chemosis score = 0.22/4, fully reversible within 72 h. Not irritating.	[5]
	Mucosal – Human		
betaine at 4% in a toothpaste	Study of the effects of betaine to reduce mucosal irritation in toothpastes containing SLS in 20 subjects; subjects exposed to the test materials on buccal mucosa with a test chamber kept in place for 15 min. Irritation was assessed visually and with electrical impedance for up to 45 min	Toothpaste containing 4% betaine alone did not irritate the mucosa in vivo; toothpastes that contained SLS, including those with betaine, were observed to have irritating effects on the oral mucosa.	[33]
betaine at 4% in a toothpaste	Study testing the efficacy of betaine to reduce "dry mouth" in toothpaste with SLS using 13 subjects	No adverse effects to the toothpaste containing 4% betaine	[34]

Table 9. Dermal sensitization studies.

Ingredient and Concentration/Dose	Method	Results/Conclusions	References
	Non-Human		
betaine monohydrate > 97% pure; up to 50% tested for induction and challenge	OECD Guideline 406 for skin sensitization – guinea pig maximization test in female Dunkin-Hartley guinea pigs; groups of 10 animals; GLP compliant	Not sensitizing	[4,6]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 5%, 10%, 25%, 50% (w/v) in ethanol/water (7/3, v/v), and 100%	OECD Guideline 429 for LLNA in CBA mice; 4 mice per dose; GLP compliant	Stimulation indices (SI) = 2.4 (5%), 6.2 (10%), 14.7 (25%), 19.0 (50%), and 26.0 (100%). EC3 = 5.8% w/v. Slight (at 10%) to severe (at 100%) erythema observed upon second application. Not sensitizing.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0.1% of 30% active material	Draize test in 6 male Dunkin-Hartley guinea pigs	Not sensitizing	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 5% for induction, 1% for challenge; water vehicle	OECD Guideline 406 for skin sensitization – Buehler test in 20 female Himalayan spotted guinea pigs with 10 control animals; GLP compliant	No mortalities or signs of systemic toxicity. No skin effects observed in 1st and 2nd week of induction. Discrete/patchy to moderate confluent erythema (grade 1 and 2) observed in 12/20 animals in 3rd week of induction. Not sensitizing.	[5]
betaines, C12-14-alkyldimethyl (a mixture of lauryl betaine and myristyl betaine); 0.5% (w/w) intradermal induction, 25% (w/w) epicutaneous induction, 1% (w/w) challenge	OECD Guideline 406 for skin sensitization – Magnusson and Kligman guinea pig maximization test in 10 male and 10 female Hartley guinea pigs; 5 of each sex for control; GLP compliant	Irritation reactions observed. Discrete erythema (grade 1) in 5/20 at 24 h post-challenge. Moderate erythema (grade 2) in 2/20 at 48 h post-challenge. Not sensitizing.	[5]
coco-betaine; 0.5% intradermal induction, 5% epicutaneous induction, 1% challenge	OECD Guideline 406 for skin sensitization – guinea pig maximization test in female Dunkin-Hartley guinea pigs ; 10 animals/dose; GLP compliant	Not sensitizing	[5]
	Human		
betaine at 8.7% in a fragrance-free white lotion/moisturizer; tested neat	Human repeat insult patch test (HRIPT) in102 subjects; semi-occluded patches consisted of 2 cm^2 Webril pads with 0.2 ml of the test material and were applied to the infrascapular area of the back or to the upper arm	Not sensitizing	[35]
betaine at 5% in a leave-on product	HRIPT in 51 subjects; occlusive; subjects received on their backs 0.2 ml of the test material with Parke-Davis Readi-Bandage® (approximately 0.05 ml/cm^2)	No skin irritation or allergic contact dermatitis	[36]
lauryl betaine at 0.1% active ingredient	HRIPT in 20 volunteers; occlusive; not GLP compliant	One strong reaction in volunteer after day 6 of induction and a mild reaction in another volunteer after day 7. No reactions observed immediately after challenge. Four delayed reaction observed during the next 4 days with 1 strong, 1 moderate, and 2 mild in form. Reactions were considered due to primary irritation and not to sensitization.	[5]

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35. TKL Research Inc. 2002. Human repeated insult patch study on a moisturizer lotion containing 8.7% Betaine. Unpublished data submitted by Personal Care Products Council.

36. Essex Testing Clinic, Inc. 2013. Clinical safety evaluation repeated insult patch test of a leave-on product containing 5% betaine. Unpublished data submitted by Personal Care Products Council.

2013 VCRP Raw Data

12A - Cleansing	BEHENYL BETAINE	3
12C - Face and Neck (exc shave)	BEHENYL BETAINE	1
01B - Baby Lotions, Oils, Powders, and Creams	BETAINE	4
02D - Other Bath Preparations	BETAINE	3
03D - Eye Lotion	BETAINE	13
03E - Eye Makeup Remover	BETAINE	3
03F - Mascara	BETAINE	2
03G - Other Eye Makeup Preparations	BETAINE	13
05A - Hair Conditioner	BETAINE	15
05C - Hair Straighteners	BETAINE	1
05F - Shampoos (non-coloring)	BETAINE	15
05G - Tonics, Dressings, and Other Hair Grooming Aids	BETAINE	12
05H - Wave Sets	BETAINE	1
05I - Other Hair Preparations	BETAINE	6
06B - Hair Tints	BETAINE	22
07E - Lipstick	BETAINE	6
07F - Makeup Bases	BETAINE	4
07I - Other Makeup Preparations	BETAINE	4
10A - Bath Soaps and Detergents	BETAINE	8
10E - Other Personal Cleanliness Products	BETAINE	2
11A - Aftershave Lotion	BETAINE	1
12A - Cleansing	BETAINE	46
12C - Face and Neck (exc shave)	BETAINE	90
12D - Body and Hand (exc shave)	BETAINE	12
12F - Moisturizing	BETAINE	109
12G - Night	BETAINE	10
12H - Paste Masks (mud packs)	BETAINE	17
12I - Skin Fresheners	BETAINE	10
12J - Other Skin Care Preps	BETAINE	26
13B - Indoor Tanning Preparations	BETAINE	2
13C - Other Suntan Preparations	BETAINE	2
05F - Shampoos (non-coloring)	CETYL BETAINE	2
10A - Bath Soaps and Detergents	CETYL BETAINE	3
10E - Other Personal Cleanliness Products	CETYL BETAINE	3
12A - Cleansing	CETYL BETAINE	6
01A - Baby Shampoos	COCO-BETAINE	3
02A - Bath Oils, Tablets, and Salts	COCO-BETAINE	1
02B - Bubble Baths	COCO-BETAINE	7
02D - Other Bath Preparations	COCO-BETAINE	2
03E - Eye Makeup Remover	COCO-BETAINE	1
05A - Hair Conditioner	COCO-BETAINE	1
05D - Permanent Waves	COCO-BETAINE	2
05F - Shampoos (non-coloring)	COCO-BETAINE	62
05I - Other Hair Preparations	COCO-BETAINE	2
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	COCO-BETAINE	14

06D - Hair Shampoos (coloring)	COCO-BETAINE	2
10A - Bath Soaps and Detergents	COCO-BETAINE	67
10E - Other Personal Cleanliness Products	COCO-BETAINE	10
11E - Shaving Cream	COCO-BETAINE	4
12A - Cleansing	COCO-BETAINE	47
12D - Body and Hand (exc shave)	COCO-BETAINE	1
12J - Other Skin Care Preps	COCO-BETAINE	1
01C - Other Baby Products	LAURYL BETAINE	1
02A - Bath Oils, Tablets, and Salts	LAURYL BETAINE	1
02B - Bubble Baths	LAURYL BETAINE	12
02D - Other Bath Preparations	LAURYL BETAINE	15
03D - Eye Lotion	LAURYL BETAINE	1
03E - Eye Makeup Remover	LAURYL BETAINE	1
05A - Hair Conditioner	LAURYL BETAINE	1
05F - Shampoos (non-coloring)	LAURYL BETAINE	27
05I - Other Hair Preparations	LAURYL BETAINE	1
06D - Hair Shampoos (coloring)	LAURYL BETAINE	1
10A - Bath Soaps and Detergents	LAURYL BETAINE	36
10E - Other Personal Cleanliness Products	LAURYL BETAINE	198
11E - Shaving Cream	LAURYL BETAINE	1
12A - Cleansing	LAURYL BETAINE	16
12C - Face and Neck (exc shave)	LAURYL BETAINE	13
12D - Body and Hand (exc shave)	LAURYL BETAINE	5
12F - Moisturizing	LAURYL BETAINE	1
12J - Other Skin Care Preps	LAURYL BETAINE	6
13B - Indoor Tanning Preparations	LAURYL BETAINE	1
10A - Bath Soaps and Detergents	MYRISTYL BETAINE	3
10E - Other Personal Cleanliness Products	MYRISTYL BETAINE	3
05A - Hair Conditioner	OLEYL BETAINE	1
05F - Shampoos (non-coloring)	OLEYL BETAINE	1
10E - Other Personal Cleanliness Products	OLEYL BETAINE	1
12A - Cleansing	OLEYL BETAINE	2
05G - Tonics, Dressings, and Other Hair Grooming Aids	STEARYL BETAINE	1

Personal Care Products Council
Committed to Safety, Quality & Innovation

1101 17th Street, N.W., Suite 300
Washington, D.C. 20036-4702

Memorandum

TO: Lillian Gill, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 24, 2013

SUBJECT: Product Information (including method of manufacture and impurities): Coco-Betaine

Evonik Industries. 2011. TEGO® Betain AB1214. Product data record.

Edition 1
16 December 2011
Mat. Number 210177

TEGO® Betain AB 1214

Product data record

1. General information

1.1 Manufacturer / Supplier

Evonik Industries AG
Business Line Personal Care
Goldschmidtstrasse 100
D-45127 Essen / Germany
Phone: +49 (201) 173-2524
Fax: +49 (201) 173-1828
personal-care@evonik.com
http://www.evonik.com/personal-care

1.2 Product Description

1.2.1 Raw material category Surfactant

1.2.2 Ingredients according to INCI

Coco-Betaine

1.2.3 Composition

Components	Source	Ratio
Coco-Betaine	vegetable / synthetic	approx. 31 %
Sodium chloride		approx. 7 %
Water		approx. 62 %

1.2.4 Solvents, preservatives and other additives

Ingredient	CAS No.	EINECS / EC No.	content [%]	Function
no preservatives/additives				

No components which are listed in Annex II of the Regulation (EC) No 1223/2009 and its modifications and updates are added to and are not to be expected in the above mentioned product due to the raw materials used and the production process.

2. Information on production process

General description of production process:
Conversion of fatty amine into betaine (IR spectrum conforms) by reaction with chloroacetic acid in aqueous solution.

The product is not irradiated.

Origin of vegetable starting material: cocos

GMO-Status:
The item does not contain ingredients that might have been derived from GM sources. However max 0.9 % cross-contamination is possible. Any protein or DNA is not present. Consequently the product will be PCR negative when tested.

2.1 By products

		method
1,4-Dioxane	not applicable	
Residual solvents	not applicable	
Residual monomers	not applicable	
Dichloroacetic acid	max. 100 ppm	Chromatography
Monochloroacetic acid	max. 100 ppm	Chromatography
Free amines	max. 0.5 %	Potentiometric titration
Glycolic acid	max. 2 %	Chromatography
Pesticides	meets the valid regulatory requirements for limits on agricultural pesticides	
Heavy metals (Cu, Pb, Sn, Pt, Pd, Hg, As, Cd, Sb, Ni, Cr, Co)	max. 20 ppm	AAS-ICP
As	max. 2 ppm	AAS-ICP
Fe	max. 10 ppm	AAS-ICP
Latex	not to be expected in the product due to the raw materials used and the production process	
Phthalates	Annex II forbidden use not to be expected in the product due to the raw materials used and the production process	
Glycol Ethers	Annex III restricted use not to be expected in the product due to the raw materials used and the production process	
VOC	< 3 % according to SR (Swiss Right) 814.018	

Diethylene Glycol	EU: not to be expected in the product due to the raw materials used and the production process	
	Non-EU: not to be expected in the product due to the raw materials used and the production process	

2.2 CMR (Carcinogenic, Mutagenic or Reprotoxic)

The use in cosmetic products of substances classified as CMR substances, of category 1A or 1B or 2 under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited.

Further Information:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF

Some of the CMR substances listed in Annex VI to Regulation (EC) No 1272/2008 are used as starting materials for the production of our cosmetic raw materials.

Some of the CMR substances mentioned below and listed in Annex VI to Regulation (EC) No 1272/2008 are used as starting materials for the production of our cosmetic raw materials and may require California reporting under Proposition 65 or the Safe Cosmetics Act, SB 484.

CMR substance	Starting material	max. concentration	method
Ethylene Oxide	no		
Propylene Oxide	no		
Octamethylcyclotetrasiloxane (D4)	no		
2-Ethylhexanoic Acid	no		
n-Hexane	no		
Methyl Chloride	no		
Dimethyl Sulphate	no		

2.3 "Allergens" according to the Regulation (EC) No 1223/2009

The presence of substances, the mention of which is required under the column 'Other' in Annex III, shall be indicated in the list of ingredients in addition to the terms parfum or aroma.

The cosmetic raw materials and the cosmetic actives supplied by Evonik Personal Care are manufactured without the use of perfumes and fragrances. An analytical proof for the absence in traces of the substances to be mentioned in addition to the terms parfum or aroma is not performed in cosmetic raw materials, which are chemically produced.

None of these substances have been intentionally added to our cosmetic raw materials or are formed during the manufacturing process according to our knowledge of the chemistry.

2.4 Food Ingredients listed in Annex IIIa of Commission Directive 2007/86/EC.

None of these substances have been intentionally added to our cosmetic raw materials or are formed during the manufacturing process according to our knowledge of the chemistry.

3. Microbiological status

Total Viable Count	max. 100 cfu/g
Pathogens	absent/g

4. Shelf life / storage conditions

24 months after production (unopened original packaging)

5. Regulatory Status

5.1 Customs tariff number

5.2 Regulatory status (chemical regulations)

Europe

Components	REACH status	CAS No.	EINECS / EC No.
Coco-Betaine	not pre-registered	68424-94-2	270-329-4

Other countries
(yes / no is relevant for the mixture of the above mentioned ingredients)

Country		yes / no	Remark
USA	US-MSDS		for this document the CAS Nos. 683-10-3 and 2601-33-4 are used
Australia	AICS:	yes	
China	IECSC:	yes	
Canada	DSL: NDSL:	yes	

In the following countries the relevant authorities currently do not require pre-market approval for cosmetic raw materials:

Brazil, Japan, South Korea, Philippines, USA

5.2.1 Regulatory status (cosmetic regulation)

Country		yes / no	Remark
China	SFDA:	yes	
Japan	JSQI:	yes	532143, but specifications not controlled

6. Toxicology and Ecotoxicology

Refer to summary of ecotoxicological and toxicological data

7. Certificates

none	

Personal Care Products Council
Committed to Safety, Quality & Innovation

1101 17th Street, N.W., Suite 300
Washington, D.C. 20036-4702

Memorandum

TO: Lillian Gill, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 3, 2013

SUBJECT: Cetyl Betaine

Hazelton Laboratories America, Inc. 1990. 91-Day subchronic oral toxicity study in rats with Cetyl Betaine.

Note: Although the tested ingredient was 32% active, on p. I I of the report it says the amount of test material was adjusted to 100% activity so that the doses stated (0, 0.05, 0.15, 0.35 g/kg/day) are the doses of Cetyl Betaine.

[redacted]

BIOLOGICAL SAFETY TEST SUMMARY REVIEW

Test Substance: Lonzaine 16S This is cetyl betaine (699-33-4) 32% active

Test Substance Identification Number: P2252

Contract Laboratory: Hazleton-Virginia Report #: [redacted]

Type of Study: 91-Day Subchronic Oral Toxicity [redacted]

Toxicologist: [redacted] [redacted]

Date Report Written: 6-13-90 Date Rec'd by Operations Section: 6-26-90

[ ] This report was not selected for review by the Operations Section.

This report has been reviewed and found in agreement with the Protocol and to comply with all applicable Federal Regulations:

Operations Section Monitor: [redacted]

This report has been reviewed for specific scientific content: [redacted]

This report has been reviewed or scientific quality and is summarized, with comments (if any), as follows: Where applicable, the retention limits for specimens to be returned to the P&G Archive are given.

See attached summary

Retention Limits (month & year)	
Slides:	Indef
Blocks:	15yr 12/05
Wet Tissues:	2yr 12/92
Teratology:	NA

[redacted]

[redacted]

[redacted]

91-Day Subchronic Oral Toxicity Study (rat) **Report** [redacted]

Test Material: Lonzaine 16S (P2252)

Study Design

This study was designed to evaluate the subchronic toxicity of Lonzaine 16S in Sprague-Dawley rats when administered in the diet for a period of at least 91 days. Groups of 10 rats per sex received P2252 in the diet at dose levels of 0, 0.05, 0.15, and 0.35 g/kg/day, Groups 1, 2, 3, and 4, respectively. Criteria used to evaluate compound effect included survival, clinical observations, ophthalmologic examinations, body weights, food consumption, clinical pathology, gross pathology, and microscopic pathology.

Results

All animals survived to termination. No treatment-related clinical observations were noted. Mean body weights and body weight gains were significantly decreased in the Group 4 males. Total food consumption of the Group 4 males was also significantly decreased. Mean body weights and food consumption of the remaining male groups and all female groups were comparable to groups of the same sex. Clinical laboratory findings were limited to slight clinical chemistry changes in the Group 4 animals. No gross or histologic alterations attributable to dietary exposure to P2252 were noted. Since there were no histologic or clinical pathology changes of a substantive nature, the decrease in body weights of the Group 4 male rats is most likely the result of a palatability problem with the formulated diet rather than a toxic effect measurable within the limits of the study.

HAZLETON
LABORATORIES AMERICA, INC.
1330-B PICCARD DRIVE, ROCKVILLE, MARYLAND 20850

Sponsor:

[redacted]

FINAL REPORT

Study Title:

91-Day Subchronic Oral Toxicity Study in Rats
with P2252 [redacted]

Author:

Gary M. Wolfe, Ph.D., D.A.B.T.

Study Completion Date:

June 13, 1990

Performing Laboratory:

Hazleton Laboratories America, Inc.
1330-B Piccard Drive
Rockville, Maryland 20850-4373

Laboratory Project Identification:

HLA Study No. [redacted]

[redacted]

2070/ID:445

HAZLETON
LABORATORIES AMERICA, INC.
9200-B RICCARD DRIVE, ROCKVILLE, MARYLAND 20850

HLA [redacted]

COMPLIANCE STATEMENT
91-Day Subchronic Oral Toxicity Study in Rats

To the best of my knowledge this study was conducted in compliance with the Good Laboratory Practice Regulations as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58. All deviations from the protocol and/or GLP's are listed in Appendix 10. There were no deviations from the aforementioned regulations which affected the quality or integrity of the study or the interpretation of the results in the report.

Study Director:

Gary W. Wolfe 6/13/90

GARY W. WOLFE, Ph.D., D.A.B.T. /DATE
Life Sciences Division

2070/10:445

HAZLETON
LABORATORIES AMERICA, INC.
[redacted]

HLA [redacted]

QUALITY ASSURANCE UNIT

Project Title: 91-Day Subchronic Oral Toxicity Study in Rats with P2252 [redacted]

Project No.: [redacted]

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the procedures described in the standard operating procedures of the Quality Assurance Unit and according to the general requirements of the Good Laboratory Practice Regulations, 21 CFR Part 58 as issued by the Food and Drug Administration. Findings from the inspections and the final report review were reported to management on the following dates:

Inspections/Review	Findings Reported	Inspector/Reviewer
Protocol 11/10; 12/22/87	12/22/87	J. Hochman
Inspection 1 12/18, 22/87	1/13/88	J. Hochman
Inspection 2 2/5,8,22; 3/2/88	3/9/88	J. Hochman
Final Report 7/22,27,29; 8/3,4-10/88	8/17/88	S. Hawlk

Susan Hawlk — Final Report Reviewer, Quality Assurance Unit

6/15/90 — Date Released

2070/10:445

STUDY IDENTIFICATION
91-Day Subchronic Oral Toxicity Study in Rats

HLA Study Number	[redacted]
Test Material	P2252 [redacted]
Sponsor/Study Monitor	[redacted]
Study Director	Gary W. Wolfe, Ph.D., D.A.B.T. Hazleton Laboratories America, Inc. 1330-B Piccard Drive Rockville, Maryland 20850-4373 (301) 670-9600
Study Timetable	
Initiation Date (Date Protocol was Signed)	February 17, 1987
Initiation of Dosing	December 1, 1987
Termination Date	March 3, 1988

STUDY PERSONNEL
91-Day Subchronic Oral Toxicity Study in Rats

Study Director:	Gary W. Wolfe, Ph.D., D.A.B.T.
Study Coordinator:	Cathy L. Murphy, B.S.
Veterinarian:	Joseph A. Manda, D.V.M., M.S., M.B.A.
Clinical Pathologist:	Richard D. Alsaker, D.V.M., M.S., D.A.B.T. Diplomate, American College of Veterinary Pathologists
Pathologist:	Richard H. Cardy, D.V.M., D.A.B.T. Diplomate, American College of Veterinary Pathologists
Statistician:	Ajit Thakur, Ph.D.
Analytical Chemist:	Susan Lewis, Ph.D.
Laboratory Supervisor:	Howard D. Thornett, B.S.

HAZLETON LABORATORIES AMERICA, INC.
[illegible] RICHARD DRIVE, ROCKVILLE, MARYLAND [illegible]

HLA [redacted]

CONTENTS

2070/10:445

HAZLETON
LABORATORIES AMERICA, INC.
1330-B PICCARD DRIVE, ROCKVILLE, MARYLAND 20850

RLA [redacted]

CONTENTS (Continued)

HAZLETON
LABORATORIES AMERICA, INC.
7320-E FORSYTHE DRIVE, ROCKVILLE, MARYLAND 20855

HLA [redacted]

SUMMARY

This study was designed to evaluate the subchronic toxicity of P2252 in Sprague-Dawley rats when administered in the diet for a period of at least 91 days. Groups of 10 rats per sex received P2252 in the diet at dose levels 0, 0.05, 0.15, and 0.36 g/kg/day, Groups 1, 2, 3, 4, respectively. Criteria used to evaluate compound effect included survival, clinical observations, ophthalmologic examinations, body weights, food consumption, clinical pathology, gross pathology, and microscopic pathology.

All animals survived to termination. No treatment-related clinical observations were noted. Mean body weights and body weight gains were significantly decreased in the Group 4 males. Total food consumption of the Group 4 males was also significantly decreased. Mean body weights and food consumption of the remaining male groups and all female groups were comparable to groups of the same sex. Clinical laboratory findings were limited to slight clinical chemistry changes in the Group 4 animals. No gross or histologic alterations attributable to dietary exposure to P2252 were noted. Since there were no histologic or clinical pathology changes of a substantive nature, the decrease in body weights of the Group 4 males is most likely the result of a palatability problem with the formulated diet rather than a toxic effect measurable within the limits of this study.

INTRODUCTION

The purpose of this study was to assess the toxicity of a substance when it is ingested daily over a period of at least 91 days. The in-life phase of the study was initiated on December 1, 1987 and the in-life phase was completed on March 3, 1988. The study was conducted by Hazleton Laboratories America, Inc. (HLA), at 1330-B Piccard Drive, Rockville, Maryland 20850-4373, and was conducted in accordance with FDA Good Laboratory Practice Regulations (21 CFR, Part 58).

CONTROL AND TEST MATERIAL

The test material, identified as P2252 [redacted], was received from [redacted] on December 2, 1986. The test material, a yellow gel, was stored at room temperature. The purity of the test material was designated by the Sponsor as 32% active. Information on the methods of synthesis and stability as well as data on composition or other characteristics which define the test material are on file with the sponsor.

TEST ANIMALS AND HUSBANDRY

Sixty-three male and Sixty-three female Sprague-Dawley (Crl:CD®(SD)BR) rats were received from Charles River Laboratories, Raleigh, North Carolina on November 17, 1987. Upon receipt the animals were weighed, given temporary animal numbers and acclimated for approximately two weeks prior to the initiation of the study. The rats were assigned permanent animal numbers via a computer-generated weight randomization program. At initiation of the study the rats were 43 days old.

The animals were housed individually in suspended stainless-steel cages and uniquely identified by cage card and tail tattoo. Purina Laboratory Rodent Chow® #5002 basal diet and tap water were available ad libitum. There

were no known contaminants in either the food or water that were expected to interfere with the study.

In the animal room the temperature was maintained within 72±4°F and the relative humidity within 50±20%. A controlled diurnal cycle (12 hours light/12 hours dark) was maintained.

Rats were used because they historically have been the animal of choice due to the large amount of background information on this species.

METHODS

Group Assignments and Dose Levels

Following a health status examination, 80 clinically acceptable rats (40 males and 40 females) were placed on study. A weight randomization computer program was used to select and assign the rats (body weight range at the initiation of the in-life phase for the males: 210.2 - 240.6 grams, body weight range at initiation of the in-life phase for the females: 143.7 - 170.1 grams) to the following groups:

Group	Number of Animals		Dose Level g/kg/day
	Male	Female	
1	10	10	Control (0)
2	10	10	0.05
3	10	10	0.15
4	10	10	0.35

Remaining animals which were not placed on study were removed from the study room and euthanized with carbon dioxide.

Compound Preparation and Administration

The test diets for Groups 2-4 were prepared and administered to the appropriate groups on a weekly basis. The control group (Group 1) received the basal diet only. An appropriate sized beaker was filled with feed from

[redacted]

the bulk feed. The feed was then placed into the beaker and a hole was made in the center of the feed. The beaker was then placed on an analytical balance and tared to zero. The exact amount of test material (adjusted to 100% activity) was then weighed into the beaker. This mixture was then placed into a small waring blender and mixed until a homogeneous mixture was achieved. This mixture was then placed into a twin shell blender with the remaining bulk feed and mixed for eight minutes. The feed mixture was then dispersed into jars.

The dietary route of administration was chosen since potential human exposure is by the oral route.

Analytical Chemistry

Analysis for concentration levels of P2252 were conducted for the control and test diets for Weeks 1, 2, 3, 6, 10 and 13. The Week 1 mix was analyzed for homogeneity for Groups 2 and 4 only. The analytical method is presented in Appendix 1.

Observations and Records

The rats were observed twice daily for mortality and moribundity. Cageside observations were conducted twice daily for obvious indications of toxic and pharmacologic effects. Detailed physical examinations of each animal for gross signs of toxic and pharmacologic effects were performed weekly at each weighing interval. Individual body weights, results of physical examinations, and food consumption values were recorded weekly.

Ophthalmoscopic Examinations

All animals were examined for ophthalmoscopic lesions prior to the initiation of the study and during Week 14 of the study. Ophthalmoscopic examinations were performed using an ophthalmoscope with 1% Mydriacyl to dilate the pupils.

Clinical Pathology

Clinical pathology evaluations for hematology and clinical chemistry parameters were performed on all animals at terminal sacrifice (Week 14). The following parameters wre analyzed:

Hematology

Absolute Reticulocyte Count (A RETIC)
Cell Morphology
Corrected Leukocyte Count (COR WBC)
Differential Leukocyte Count
Erythrocyte Count (RBC)
Hematocrit (HCT)
Hemoglobin (HGB)
Leukocyte Count (WBC)
Mean Corpuscular Volume (MCV)
Mean Corpuscular Hemoglobin (MCH)
Mean Corpuscular Hemoglobin Concentration (MCHC)
Platelet Count (PLATELET)
Reticulocyte Count (RETIC)

Clinical Chemistry

Albumin (ALBUMIN)
Alkaline Phosphatase (ALK P)
Blood Urea Nitrogen (BUN)
Calcium (CALCIUM)
Chloride (CHLORIDE)
Creatinine (CREAT)
Gamma Glutamyltransferase (GGT)
Glucose (GLUCOSE)
Phosphorous (IN PHOS)
Potassium (POTAS)
Sodium (SODIUM)
SGOT (AST)
SGPT (ALT)
Total Bilirubin (T BILI)
Total Protein (T PROT)

Blood samples for hematology measurements were collected from the orbital sinus while under ketamine hydrochloride anesthesia. Clinical chemistry measurements were collected from the abdominal aorta while under sodium pentobarbital anesthesia during the exsanguination procedure. A computer generated randomization program was used as a sequence for bleeding. References for the clinical pathology measurements are listed following the text portion of the report.

Sacrifice and Gross Pathology

Necropsies were performed on all fasted animals following at least 91 days of compound administration. Terminal body weights were recorded prior to necropsy. The animals were sacrificed by exsanguination under sodium pentobarbital anesthesia. The necropsies were performed by trained personnel under

the direct supervision of a board-certified pathologist. A computer-generated randomization was used as a sequence for necropsy. Each necropsy included the examination of the following:

external surfaces
all orifices
carcass
external surface of the brain
thoracic, abdominal, and pelvic cavities and their viscera
cervical tissues and organs

Organ Weights

The following organs from each animal were weighed following careful dissection and trimming to remove fat and other contiguous tissue in a uniform manner:

liver
kidneys
brain with stem
testes with epididymides
ovaries

Tissue Preservation

The following tissues from each animal were preserved in 10% buffered formalin for histopathological examination:

Adrenals (both)
Aorta (thoracic)
Bone (femur with marrow)
Brain (cerebellum, mid-brain, and cerebrum)
Cervical Lymph Node
Esophagus, trachea, and thyroid
Eyes (both)
Gluteal plus Biceps Femoris Muscle (caudal to femur)
Gross Lesions
Heart
Pituitary
Prostate/Seminal vesicles
Psoas Muscle (left)
Rectum
Salivary gland (submandibular)
Sciatic Nerve
Skin (inguinal to include mammary gland, if visible)
Small Intestine (to include duodenum, jejunum, and ileum)
Spinal Cord (thoraco-lumbar)
Spleen

Kidneys (left midsaggital, right transverse)
Large intestine (to include caecum and colon)
Larynx
Liver
Lung
Lymph Node (submandibular and ileo-cecocolic)
Ovaries
Pancreas
Stomach (to include cardiac, fundic and pyloric regions)
Te[illegible] with Epididymus
Thy[illegible] Lymph Nodes
Thymus
Tongue
Ureters
Urinary Bladder
Uterus
Vagina

Histopathology

The tissues shown above from all animals in Groups 1 and 4 and gross lesions from all animals were embedded in Paraplast®, sectioned and stained with hematoxylin and eosin, and examined microscopically.

Statistical Analyses

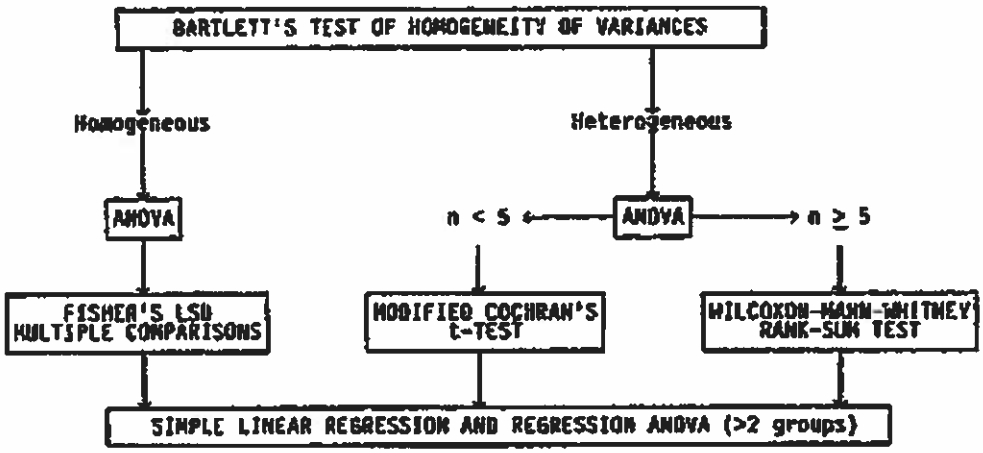
Absolute body weights (Weeks 0 and 13), body weight gain (Weeks 0-13), total food consumption (Weeks 1-13), clinical pathology data (except for cell morphology), terminal body weights, and organ weight data of the control groups were compared statistically to the data from the same sex of the treated groups. Statistical analyses were performed as diagrammed in Figure 1. The references for the statistical methods follow the text portion of this report.

Control vs. compound-treated group mean comparisons were evaluated at the 5.0% two-tailed probability level.

Statistical significance is designated throughout the text of this report by the term significance and/or as follows:

S1+ = Significantly higher than the control value; overall ANOVA not significant.
SA+ = Significantly higher than the control value; overall ANOVA significant.
S1- = Significantly lower than the control value; overall ANOVA not significant.
SA- = Significantly lower than the control value; overall ANOVA significant.

Statistical Analyses Flowchart



Significant linear regression and/or lack of fit are designated as follows:

R = Significant linear regression
L = Significant lack of fit.

For mean tables, the above indications, followed by the letters M, F or B, indicate the statistical finding was for either the males, females, or both sexes, respectively.

Specimen, Raw Data and Final Report Storage

All tissue specimens, blocks and slides, and copies of all raw data and the final report will be retained by Hazleton Laboratories. The items will be retained until the quality of the specimen has deteriorated beyond the point of meaningful evaluation or the sponsor has requested the materials to be transferred or discarded.

RESULTS AND DISCUSSION

Analytical Chemistry

Test diet analyses data and methodology are presented in Table 1 and Appendix 1, respectively.

Homogeneity determinations revealed that highest and lowest concentrations for Week 1 were within 9% of the target levels. Routine analysis revealed levels of P2252 in the diets presented to the study animals to be within ± 10% of the target levels for all week analyzed except for the Group 3 males Week 1 (within 10.6%), the Group 3 females Week 1 (within 13%) and the Group 2 females Week 2 (within 19.5%).

Survival

All animals survived to termination.

Clinical Observations

Summaries of clinical observations are presented in Table 2 and individual clinical observations are presented in Appendix 2.

In general, findings were observed sporadically at a low frequency and were of the type commonly observed in the species in this laboratory and were not considered related to treatment.

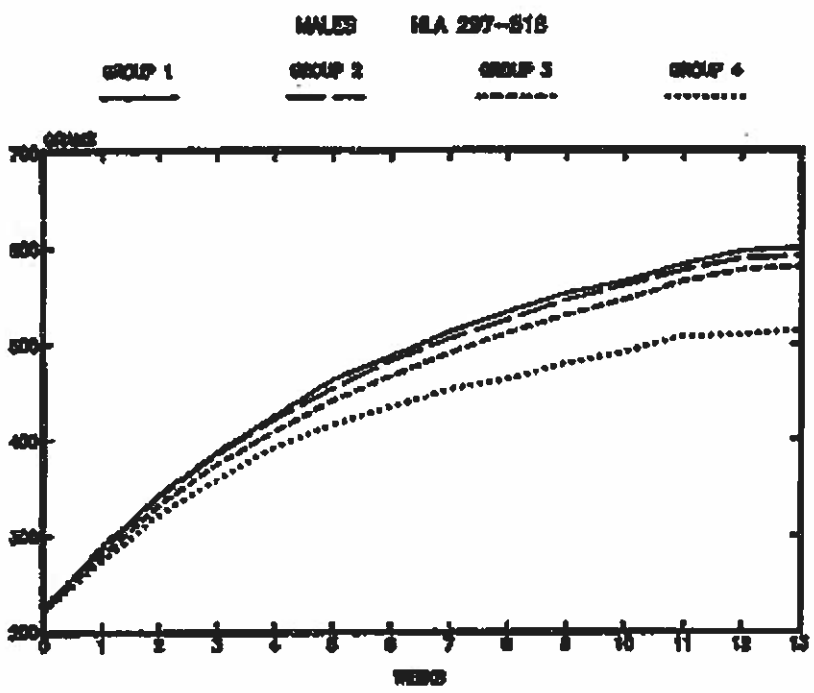
Ophthalmoscopic Examinations

All rats placed on study were found to be normal prior to the initiation of the study and during the Week 14 eye examinations.

Body Weights

Mean data (body weights and body weight change) are presented in Tables 3A and 3B, respectively. Individual absolute body weights are presented in Appendix 3. Graphic presentations of mean body weights and mean body weight gains are presented in Figures 2 and 3, respectively.

FIGURE 2 -- MEAN BODY WEIGHTS



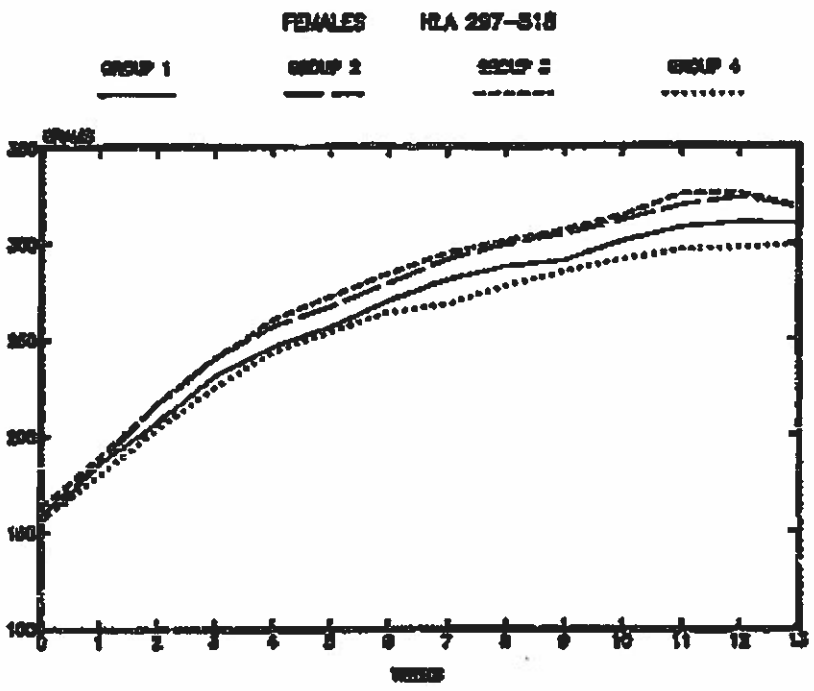
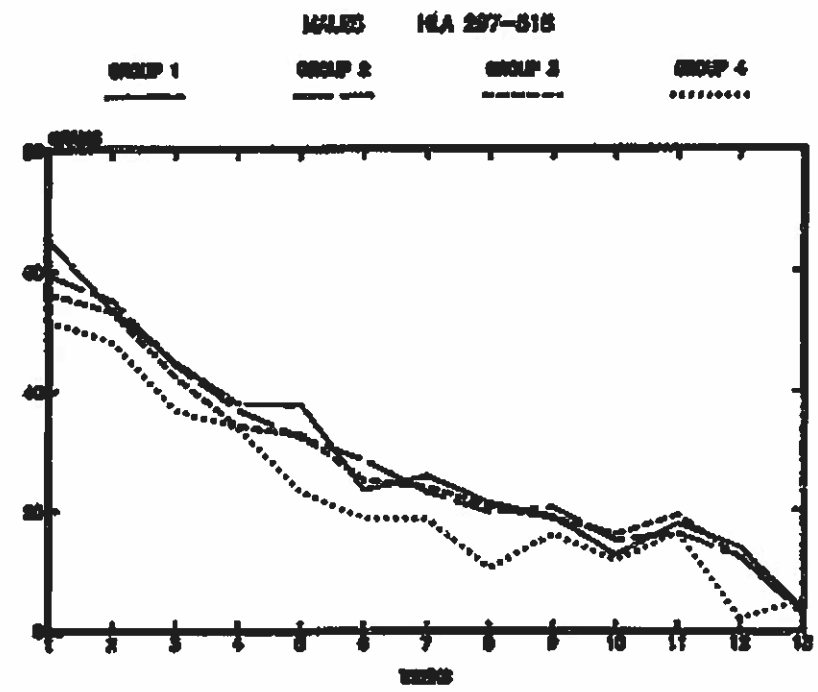
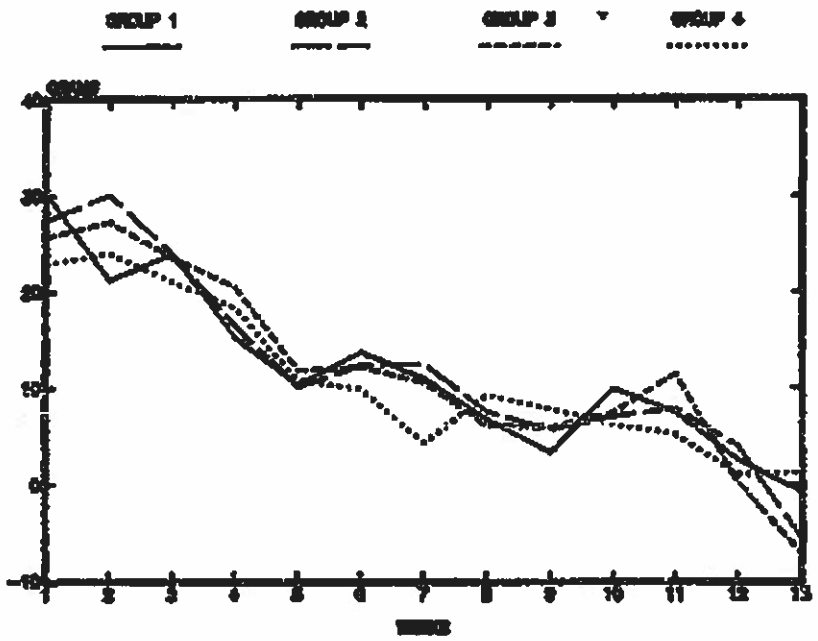


FIGURE 3 -- MEAN BODY WEIGHT GAINS





A treatment-related decrease in mean body weights and body weight gains was noted for the Group 4 males. There was a significant regression for the males in mean body weights at Week 13 and body weight gains from initiation to Week 13. These were accompanied by a significant decrease in the mean body weights and body weight gain from initiation to Week 13 for the Group 4 males. No other treatment-related findings were noted in the male groups or female groups. There was a significant lack of fit for the females at initiation and a significant increase in mean body weight for the Group 3 females. Mean body weights of the female groups were comparable at randomization and the significant increase of the mean body weight of the Group 3 females at initiation is not of biological significance and did not affect the interpretation of the resultant data.

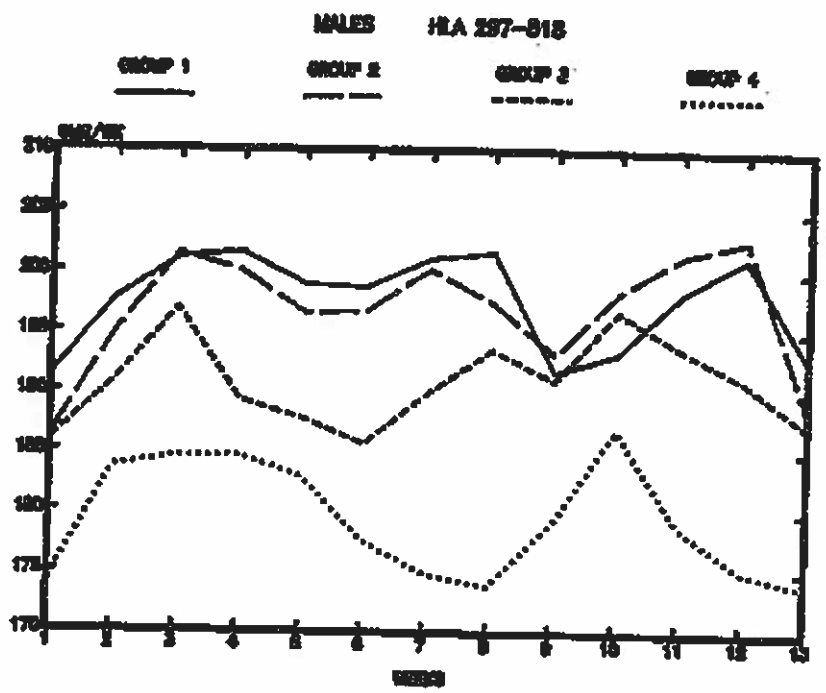
Food Consumption and Compound Consumption

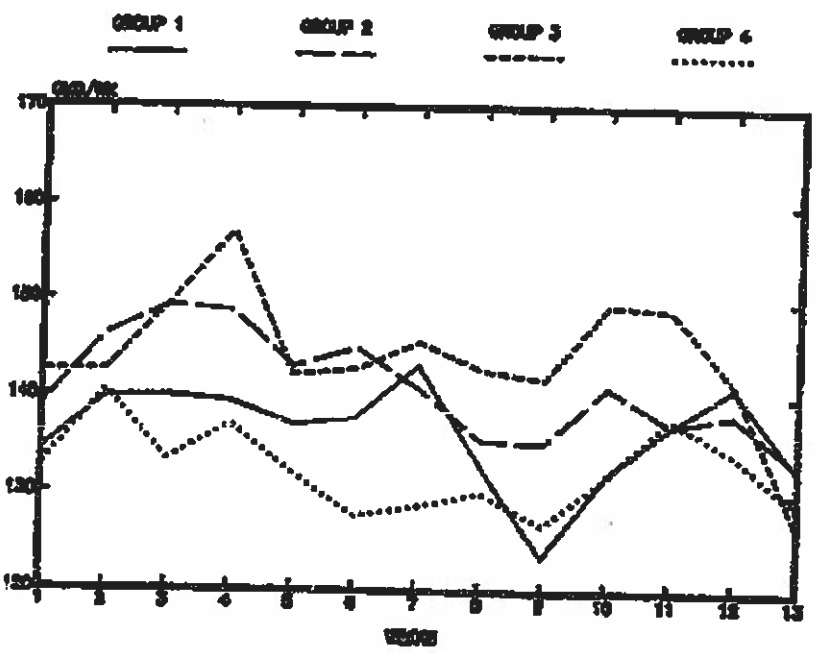
Mean data (food consumption, total food consumption and compound consumption) are presented in Tables 4A, 4B and 5, respectively. Individual data (food consumption and compound consumption) are presented in Appendices 4 and 5, respectively. A graphic presentation of mean food consumption values is presented in Figure 4.

Accompanying the decrease in mean body weights and mean body weight gains for the Group 4 males was a significant decrease in total food consumption. Weekly food consumption was consistently lower than initial control values throughout the 13 weeks for the Group 4 males and in most cases Group 3 males. Total and weekly food consumption was comparable in the remaining male groups and female groups.

The mean compound consumption (mg/kg/day) ranged from 43.82 to 49.97 for Group 2 males, 45.26 to 50.99 for Group 2 females, 129.30 to 149.94 for Group 3 males, 129.94 to 154.43 for Group 3 females, 313.93 to 356.76 for Group 4 males, and 301.14 to 359.47 for Group 4 females.

FIGURE 4 -- MEAN FOOD CONSUMPTION





Clinical Pathology

Mean hematology and clinical chemistry values with standard deviations are presented in Tables 6 and 7, respectively. Individual hematology and clinical chemistry are presented in Appendices 6 and 7, respectively and summarized in the Clinical Pathology Report.

Evaluation of hematology data revealed significantly decreased leukocyte and corrected leukocyte counts in the low- and high-dose males, and a significantly decreased absolute eosinophil count in the high-dose females. Because of the small magnitude of the decreases, high control group mean value, and lack of dose response these changes are felt to be of little biological significance.

Evaluation of clinical chemistry data revealed significantly increased serum alkaline phosphatase, chloride, and inorganic phosphorous in the high-dose males, significantly increased potassium in the high-dose females, and incidentally decreased albumin in the low-dose males and decreased total protein in the high-dose males.

Gross Pathology

A summary of findings is presented in Table 8. Individual gross pathology findings are presented in Appendix 8.

Direct necropsy observations were unremarkable and well distributed among the dose groups. Most commonly noted was dilated renal pelvis (more frequent in males) a common finding in young rats of this strain. All other findings were also of the kinds frequently encountered as spontaneous lesion in this strain. None were suggestive of a compound effect.

Organ Weights

Mean absolute and relative organ weights with standard deviations are presented in Tables 9 and 10, respectively. Individual terminal body weights, organ weights and organ/body weight ratios are presented in Appendix 8.

Evaluation of the data provided no finding showing evidence of direct organ toxicity. Statistical analyses revealed a significant regression for the male terminal body weights, a significant regression for the male absolute

liver weights, a significant regression for the male relative kidney and testes/epididymides weights and a significant regression in the male and female relative brain weights. A significant decrease in the Group 4 male terminal body weights and the absolute liver weights was observed. A significant increase was noted for the Group 4 males relative brain, kidney and testes weights. These statistically significant findings for the Group 4 males are considered to be due to a reflection of general loss of body mass. The Group 3 females were found to have increased mean terminal liver weights. This was due to one animal (#1263) that had a markedly enlarged liver because of a malignant lymphoma.

Histopathology

A summary of histopathological findings is presented in Table 11 and in the Pathology Report. Individual histopathological data are presented in Appendix B.

Histologic alterations attributable to dietary exposure to P2252 were not encountered. All findings were of the kind that are encountered frequently or sporadically in rats of this strain and age.

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CONCLUSION

P2252 was administered in the diet at dose levels of 0, 0.05, 0.15, and 0.35 g/kg/day for at least 91 days to groups of 10 per sex Sprague-Dawley rats. All animals survived to termination and the only significant findings were decrease in mean body weights and body weight gains in the Group 4 males. There were several slight clinical chemistry changes. Since there were no histopathological, hematologic, or clinical chemistry changes of a substantive nature, the decrease in body weights in the Group 4 males is most likely the result of a palatability problem with the formulated diet rather than a toxic effect measurable within the limits of this study.

Study Director:

Gary W. Wolfe 6/13/90
GARY W. WOLFE, Ph.D., D.A.B.T. /Date
Life Sciences Division

Study Coordinator:

Cathy L. Murphy 6/13/90
CATHY L. MURPHY, B.S. /Date
Life Sciences Division

2070/ID:445

HAZLETON
LABORATORIES AMERICA, INC.
9200-B RICCARD DRIVE, ROCKVILLE, MARYLAND 20850

[redacted]

OPHTHALMOLOGY REPORT

Pre-study ophthalmology exams were performed on 11/25/87. Those animals exhibiting ocular lesions were eliminated from the study.

Ophthalmology exams were performed on 2/29/88. All animals appeared normal.

Based on the above findings there appears to be no ophthalmologic or toxic effect of the compound.

Veterinarian:

5/24/90

JOSEPH A. MAKDA, III, D.V.M., M.S., M.B.A. /Date
Life Sciences Division

2070/10:445

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[redacted]

CLINICAL PATHOLOGY SUMMARY

Evaluation of the hematology data revealed significantly decreased leukocyte and corrected leukocyte counts in the low- and high-dose males, and a significantly decreased absolute eosinophil count in the high-dose females. These changes were felt to be of little biological importance, due to the low magnitude of the change, high control group mean value, and/or lack of a dose response.

The elevated mean values in the mid-dose females for leukocyte count, corrected leukocyte count, blasts, and band neutrophils were solely the result of markedly increased results for a single animal, 01263. This animal also exhibited a moderately low erythrocyte count, hemoglobin, hematocrit, and platelet count, and grade "3" anisocytosis, grade "1" microcytosis, and Howell-Jolly bodies (grading "F," i.e.,FEW).

Evaluation of the clinical chemistry data revealed significantly increased serum alkaline phosphatase, chloride, and inorganic phosphorus in the high-dose males; significantly increased potassium in the high-dose females; and incidentally significantly decreased albumin in the low-dose males.

Several of the mean clinical chemistry values in the mid-dose females had changes that were due entirely to marked differences in one animal 01263. This animal exhibited increased blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, and gamma glutamyltransferase. The hematologic and clinical chemistry changes suggested the presence of an undifferentiated leukemia with secondary organ involvement, particularly in the liver. Histopathologically, this animal was diagnosed as having an undifferentiated malignant lymphoma involving multiple tissues.

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In conclusion, potentially treatment-related clinical laboratory findings were limited to slight clinical chemistry changes in the high-dose animals. No important differences were detected in the mid-dose rats.

Clinical Pathologist:

Richard D. Alsaker 4/14/85

RICHARD D. ALSAKER, D.V.M., M.S., D.A.B.T. /Date
Diplomate, American College of Veterinary Pathologists
Life Sciences Division

2070/10:445

HAZLETON
LABORATORIES AMERICA, INC.
1900-B PICCARD DRIVE ROCKVILLE MARYLAND 20850

[redacted]

Pathology Summary

METHOD

Pathological examinations were conducted on 10 male and 10 female Crl:CD®(SD)Br/Charles River rats given P2252 in the food for at least 91 days at each of the following dose levels: Group 1 (Control), 0.00 g/kg; Group 2, 0.05 g/kg; Group 3, 0.15 g/kg; Group 4, 0.35 g/kg.

All animals were examined grossly, and each animal was weighed prior to necropsy. Sacrifice was by exsanguination under sodium pentobarbital anesthesia. The most recent clinical observations were reviewed at necropsy, and all grossly observed abnormalities were entered, as encountered, directly into the computerized data capture system. Brain, liver, gonads, and kidneys were weighed from each animal killed at the scheduled time.

After examination, appropriate samples of the following organs were preserved in 10% buffered formalin:

Adrenals (both)
Aorta (thoracic)
Bone (femur with marrow)
Brain (cerebellum, mid-brain, and cerebrum)
Cervical Lymph Node (if found)
Esophagus, trachea, and thyroid
Eyes (both)
Gluteal plus Biceps Femoris Muscle (caudal to femur)
Gross Lesions that are suspected to potentially represent a test compound effect, including tissue masses/tumors and associated regional lymph nodes and to include a border of apparently normal tissue.
Heart
Kidneys (left midsaggital, right transverse)
Large Intestine (to include caecum and colon)
Larynx
Lymph Node (submandibular)
Ovaries
Pancreas
Pituitary
Prostate/Seminal vesicles
Psoas Muscle (left)
Rectum
Salivary gland (submandibular)
Sciatic Nerve
Skin (inguinal to include mammary gland, if visible)
Small Intestine (to include duodenum, jejunum, and ileum)
Spinal Cord (thoraco-lumbar)
Spleen
Stomach (to include fundic and pyloric regions)
Testes with Epididymus
Thymic Lymph Nodes
Thymus
Tongue
Ureters
Urinary Bladder

Liver
Lung
Lymph Node (ileocecocolic)
Uterus (cross section of each horn)
Vagina

Each lobe of the liver was incised several times to enhance penetration by fixative, and each kidney was bisected - the left longitudinally and the right transversely. The lungs were inflated with formalin via the trachea, and contracted bladders were also inflated with formalin. After fixation, all bony tissues were decalcified prior to processing. Tissues to be examined histologically were embedded in paraffin, sectioned at five to seven microns, and stained with hematoxylin and eosin.

As required by the protocol, histopathological evaluations were conducted on those tissues listed above from all Group 1 and Group 4 male and female rats. In addition, grossly observed abnormalities were examined histologically from all animals on study. Most non-tumor lesions were graded as to severity or relative degree of involvement (1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe, 5 = severe). The grades are subjective evaluations based on morphology alone and are not intended by themselves to imply any degree of functional impairment.

Occasional tissues were lost in processing and not available for microscopic evaluation. Histologic preparations were adequate for evaluation.

GROSS FINDINGS

Gross findings are summarized in detail in the accompanying tables.

All animals survived until the scheduled termination of the study. There was, however, a significant depression in the mean terminal body weight of Group 4 males compared to the other male groups which likely occurred as a result of significantly depressed food consumption that also occurred in the Group 4 males. Whether or not this was purely the result of a palatability problem cannot be surmized with certainty from these data; however, there were no deviations in measured biochemical, hematologic, or histopathologic parameters suggestive of toxicity that might otherwise explain this phenomenon.

Mean terminal liver weights were also reduced in Group 4 males, a reflection of general loss of body mass. Other organs were unaffected in males. In Group 3 females the increased mean terminal liver weight was due to one animal (#1263) that had a markedly enlarged liver because of malignant lymphoma.

Direct necropsy observations were unremarkable and well distributed among the dose groups. Most commonly noted was dilated renal pelvis (more frequent in males) a common finding in young rats of this strain. All other findings were also of the kinds frequently encountered as spontaneous lesions in this strain. None were suggestive of compound effect.

HISTOLOGIC FINDINGS

Incidences of histologic findings are summarized in detail in the accompanying tables. Text references to incidences or grades should be referred to Appendix B for greater detail.

Histologic alterations attributable to dietary exposure to P2252 were not encountered. Indeed all findings were of the kind that are encountered frequently or sporadically in rats of this strain and age.

Chronic progressive nephropathy, a ubiquitous disease in rats of this strain as they age, was seen frequently in its earliest manifestations in this study, mostly in males. It was usually minimal and observable as occasional small scattered groups of regenerative proximal tubular cells.

Dilated renal pelves were also seen frequently as were scattered mineralized tubular cells. Both are commonly seen in young rats of this strain and were unrelated to exposure.

Bronchiolar associated lymphoid tissue (B.A.L.T.) was prominent in most animals examined. This is a usual finding in laboratory rats, and was not unusual in degree in these animals. This, however, together with the presence of perivascular eosinophilic infiltrates in a number of animals is suggestive of mild early Mycoplasmosis.

Hemorrhage was seen in the retrobulbar ocular tissues of most animals examined. It was always unilateral and certainly a result of the pre-necropsy orbital sinus bleeding for hematology.

"Physiologic" hyperplasia was seen in the mammary glands of all males from which they were examined. The hyperplastic cells, presumably alveolar, were eosinophilic and foamy, sometimes containing distinct lipid droplets. They were arranged into alveolar configurations, usually without a distinct lumen and in this characteristic the males were markedly different from the females in which duct development was distinct. This hyperplasia is a normal finding in young male rats and occurs as a response to their endocrine status. It seems likely that it may be related to the very active appearing pituitary glands also seen in most of the male rats of this study. In these there were variable numbers of large, pale staining "secretory cells" that often exhibited a distinct juxtanuclear structure presumed to represent a prominent golgi apparatus. These cells were not present in the females.

Nearly all livers examined exhibited a few small foci of mixed mononuclear inflammatory cells associated with portal radicles or scattered throughout the parenchyma. This lesion is found in most laboratory rats of this age and older. It was unrelated to exposure.

SUMMARY

The absence of histopathological changes attributable to dietary exposure to P2252 was corroborated by the absence of any substantive change in any of the hematologic or clinical chemical parameters measured. In view of this, it is probable that the depression in food consumption and subsequent depression in weight gain in Group 4 males occurred as a result of a palatability problem with the dosed diet rather than any toxic effect measurable within the limits of this study.

Pathologist:

5/24/90

RICHARD H. CARDY, D.V.M., D.A.B.T. /Date
Diplomate, American College of Veterinary Pathologists

2070/10:445

REFERENCES

Hematology

Absolute Reticulocyte Count (A RETIC)

Reticulocyte Count x Erythrocyte Count = Absolute Reticulocyte (Calculated).

Cell Morphology

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DAVIDSOHN, I., AND HENRY, J. (1974). Todd-Sanford Clinical Diagnosis by Laboratory Methods, 15th ed. W. B. Saunders Co., Philadelphia, PA, pp. 135-150.

KAPFF, C., AND JAHOL, J. (1981). BLOOD, Atlas and Sourcebook of Hematology, 1st ed. Little, Brown and Company, Boston, MA.

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WINTROBE, M. (1974). Clinical Hematology, 7th ed. Lea and Febiger, Philadelphia, PA.

ZUBER-FRANKLIN, D., GREAVES, M., GROSSI, C., AND MARMONT, A. (1981). Atlas of Blood Cells, Vols. I and II. Lea and Febiger, Philadelphia, PA.

Corrected Leukocyte Count (COR WBC)

(WBC x 100) ÷ (NRBC + 100) = COR WBC (Calculated).

Hematology (Continued)

Erythrocyte Count (RBC)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

Coulter Counter® Model S+IV Data Terminal with Data Handling PN4235456 (1986, January). Coulter Electronics, Inc., Hialeah, FL.

Hematocrit (HCT)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

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Hemoglobin (HGB)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

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Leukocyte Count (WBC)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

Coulter Counter® Model S+IV Data Terminal with Data Handling PN4235456 (1986, January). Coulter Electronics, Inc., Hialeah, FL.

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ZUBER-FRANKLIN, D., GREAVES, M., GROSSI, C., AND HARMONT, A. (1981). Atlas of Blood Cells, Vols. I and II. Lea and Febiger, Philadelphia, PA.

Mean Corpuscular Hemoglobin (MCH)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

Coulter Counter® Model S+IV Data Terminal with Data Handling PN4235456 (1986, January). Coulter Electronics, Inc., Hialeah, FL.

Mean Corpuscular Hemoglobin Concentration (MCHC)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

Coulter Counter® Model S+IV Data Terminal with Data Handling PN4235456 (1986, January). Coulter Electronics, Inc., Hialeah, FL.

Mean Corpuscular Volume (MCV)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

Coulter Counter® Model S+IV Data Terminal with Data Handling PN4235456 (1986, January). Coulter Electronics, Inc., Hialeah, FL.

Hematology (Continued)

Platelet Count (PLATELET)

Coulter Counter® Model S+IV System, product reference manual 42353238 (1983, November). Coulter Electronics, Inc., Hialeah, FL.

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Reticulocyte Count (RETIC)

DAVIDSOHN, I., AND HENRY, J. (1974). Todd-Sanford Clinical Diagnosis by Laboratory Methods, 15th ed. W. B. Saunders Co., Philadelphia, PA, pp. 177-178.

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NAZI, K. H. (1986, December). The Miller Disk: An improvement in the performance of manual reticulocyte counts. Laboratory Medicine 17(12), pp. 742-744.

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Clinical Chemistry (Continued)

Aspartate Aminotransferase (AST) - BMD/Hitachi® 737

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Blood Urea Nitrogen (BUN) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #052205403-0187 (1987). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Calcium (CALCIUM) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #052215002-0985 (1985). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Chloride (CHLORIDE) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #s 052777803-0787 and 052776603-0787 (1987). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Creatinine (CREAT) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #051187200-0586 (1986). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Glucose (GLUCOSE) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #052213301-0985 (1985). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Gamma Glutamyltransferase (GGT) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #051188600-0686 (1986). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Inorganic Phosphorus (IN PHOS) - BMD/Hitachi® 737

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Potassium (POTAS) - BMD/Hitachi® 737

BMD/Hitachi® 737 Chemistry Analyzer, reagent package insert #s 052777803-0787 and 052776603-0787 (1987). Boehringer Mannheim Diagnostics, Indianapolis, IN.

Clinical Chemistry (Continued)

Sodium (SODIUM) - BMD/Hitachi® 737

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COCHRAN, W. G. (1964). Approximate significance levels of Behrens-Fisher test. Biometrics 20, 191-195.

Fisher's lsd Test

MILLER, R. G., JR. (1966). Simultaneous Statistical Inference Ch. 6.1. McGraw-Hill, NY.

TABLE 1

91-Day Subchronic Oral Toxicity Study in Rats
Results of Test Diet Analyses

MALES

Group:	Target Level (ppm)					Assayed Level (ppm)				Percent Target			
Dose Level: (MG/KG/DAY)	1 0	2 50	3 150	4 350		1 0	2 50	3 150	4 350	1 0	2 50	3 150	4 350
Homogeneity													
Top	0.0	--	--	3360	A	NS	--	--	3252	--	--	--	96.79
					B	--	--	--	3196	--	--	--	95.12
Middle	--	--	--	3360	A	--	--	--	3140	--	--	--	93.45
					B	--	--	--	3252	--	--	--	96.79
Bottom	--	--	--	3360	A	--	--	--	3252	--	--	--	96.79
					B	--	--	--	3200	--	--	--	98.45
Week 1	0.0	488.6	1445	3360	A	NS	451.8	1292	3220[a]	--	92.47	89.41	96.12
					B	--	451.9	1340	-	--	92.47	93.28	--
Week 2	0.0	526.1	1577	3853	A	NS	560.1	1615	3943	--	104.5	102.4	102.9
					B	--	560.1	1638	3941	--	104.5	103.9	102.3
Week 3	0.0	603.9	1829	4296	A	NS	560.1	1729	4020	--	92.74	94.53	93.59
					B	--	560.1	1751	4077	--	92.74	95.73	94.91
Week 6	0.0	808.7	2469	5573	A	NS	793.3	2306	5443	--	98.10	93.38	97.67
					B	--	793.3	2362	5611	--	98.10	95.65	100.7
Week 10	0.0	--	--	6562	A	NS	--	--	6681	--	--	--	101.8
					B	--	--	--	6692	--	--	--	102.0
Week 13	0.0	1017	3173	7128	A	NS	958.9	3130	7137	--	94.27	98.65	100.1
					B	--	1015	3074	7137	--	99.79	96.88	100.1

NS = Not Significant
[a]The reported value is the average of the Group 4 homogeneity values.

2070/1U:453-1

TABLE I (Continued)

91-Day Subchronic Oral Toxicity Study in Rats
Results of Test Diet Analysis

FEMALES

Group:	Target Level (ppm)					Assayed Level (ppm)				Percent Target			
	1	2	3	4		1	2	3	4	1	2	3	4
Dose Level: (MG/KG/DAY)	0	50	150	350		0	50	150	350	0	50	150	350
Homogeneity													
Top	0.0	471.5	--	--	A	NS	429.4	--	--	--	91.08	--	--
					B	--	451.8	--	--	--	95.83	--	--
Middle	--	471.5	--	--	A	--	451.8	--	--	--	95.83	--	--
					B	--	451.8	--	--	--	95.83	--	--
Bottom	--	471.5	--	--	A	--	429.4	--	--	--	91.08	--	--
					B	--	451.8	--	--	--	95.83	--	--
Week 1	0.0	471.5	1459	3257	A	NS	440.6[A]	1270	3084	--	93.45	87.39	94.67
					B	--	--	1292	3196	--	--	88.90	98.11
Week 2	0.0	468.7	1393	3315	A	NS	537.4	1297	3113	--	114.6	93.14	93.92
					B	--	560.1	1297	3169	--	119.5	93.14	95.61
Week 3	0.0	517.8	1592	3553	A	NS	503.4	1581	3340	--	97.22	99.29	94.00
					B	--	480.7	1638	3240	--	92.84	102.9	94.09
Week 6	0.0	652.9	2010	4715	A	NS	625.3	2115	4714	--	95.77	105.2	99.99
					B	--	681.3	2082	4770	--	104.3	103.6	101.2
Week 10	0.0	--	--	5514	A	NS	--	--	5631	--	--	--	102.1
					B	--	--	--	5574	--	--	--	101.1
Week 13	0.0	820.2	2422	5437	A	NS	791.5	2462	5201	--	96.55	101.7	97.50
					B	--	791.9	2517	5412	--	96.55	103.9	99.54

NS = Not Significant.
[A]The reported value is the average of the Group 2 homogeneity values.

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TABLE 2

SUMMARY INCIDENCE OF CLINICAL SIGNS

OBSERVATION	MALES				FEMALES			
	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
HUNCHED	0	0	0	0	0	0	1	0
THIN	1	0	0	0	0	0	1	0
TEETH CUT	2	0	0	3	0	1	0	1
MALOCCLUSION	1	0	0	1	0	1	0	1
WHEEZING	1	0	0	0	0	0	0	0
URINE STAINS	0	0	0	0	0	0	1	0
ALOPECIA[a]	2	1	0	0	1	0	0	0
SORES[b]	1	0	1	0	0	1	0	0
BLOODY CRUST[c]	0	0	1	0	0	0	0	0
LACRIMATION	1	0	0	0	0	0	0	0
CHROMODACRYORRHEA	2	0	0	1	0	1	0	1
ULCERATED[d]	1	0	0	0	0	0	0	0
ENLARGED[e]	0	0	0	0	0	0	1	0
SWOLLEN[f]	1	0	0	0	0	0	0	0
MISSING[g]	1	0	0	0	0	0	0	0

[a] paws-fore-both, legs-fore-both, paw-fore-left

[b] paw-fore-left, shoulder-left, mouth

[c] shoulder-left

[d] eye-right

[e] abdomen

[f] paw-fore-left

[g] digits on paw-fore-left

TABLE 3A
MEAN BODY WEIGHTS AND STANDARD DEVIATIONS (G)
91-DAY SUBCHRONIC ORAL TOXICITY

HLA [redacted]

GROUP AND DOSE LEVEL (MG/KG)	WEEK:	START	1	2	3	4	5	6	7	8	9	10	11	12	13
MALES															
1	MEAN	224.3	289.4	342.9	387.6	425.9	463.1	484.6	512.3	533.6	552.5	569.1	582.9	594.8	608.2
	S.D.	9.36	9.33	8.90	14.88	19.02	21.15	24.66	24.99	27.21	43.22	58.36	48.14	48.01	54.58
.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2	MEAN	224.1	285.4	340.5	384.9	421.8	454.1	482.5	505.5	529.3	548.9	560.8	577.1	589.3	591.9
	S.D.	7.70	7.79	10.17	13.24	18.45	17.90	20.37	19.28	19.84	21.15	29.53	29.99	33.79	24.59
50.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3	MEAN	222.9	279.2	332.4	374.8	408.8	441.5	466.6	490.5	511.3	529.9	546.8	564.2	577.4	588.9
	S.D.	9.96	7.47	10.57	15.09	16.70	26.87	27.87	31.17	33.29	34.96	36.35	39.96	44.94	48.72
150.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4	MEAN	222.2	273.8	322.8	358.8	392.8	415.9	434.6	453.3	463.7	479.7	491.4	502.7	509.6	514.6
	S.D.	7.40	13.09	19.10	24.83	28.97	36.73	42.74	44.95	46.91	48.69	50.78	54.49	57.60	60.12
350.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
FEMALES															
1	MEAN	196.4	185.6	204.8	230.8	246.1	256.4	270.2	281.1	287.8	291.1	301.8	308.5	311.8	310.3
	S.D.	9.02	6.02	8.42	7.83	13.74	15.33	16.52	16.92	15.82	12.93	15.89	22.76	24.14	21.15
.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2	MEAN	198.9	186.4	216.5	240.5	254.9	267.8	279.4	291.9	299.4	305.1	312.1	320.8	324.8	318.4
	S.D.	9.01	7.62	11.97	9.05	10.86	12.81	13.93	11.46	9.60	14.09	15.94	15.11	13.44	19.57
50.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3	MEAN	169.3	189.0	216.4	239.9	260.4	272.3	284.4	295.8	301.1	307.8	314.4	325.9	326.2	319.2
	S.D.	9.09	11.09	16.63	20.36	19.03	24.79	26.11	27.41	28.75	30.60	31.67	32.90	35.71	38.72
150.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4	MEAN	194.9	179.8	203.9	224.9	243.3	254.1	264.8	268.3	277.5	285.3	291.6	296.8	297.8	299.1
	S.D.	7.15	7.73	8.38	13.13	15.35	14.18	14.96	14.82	16.15	18.92	16.99	15.83	16.22	16.46
350.000	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10

TABLE 38
MEAN BODY WEIGHT CHANGE (G)
91-DAY SUBCHRONIC ORAL TOXICITY

GROUP AND DOSE LEVEL (MG/KG)		WEEK 0 - 13 RM
MALES		
1	MEAN	375.8
	S.D.	50.96
.000	N	10
2	MEAN	365.8
	S.D.	21.77
50.000	N	10
3	MEAN	358.0
	S.D.	47.85
150.000	N	10
4	MEAN	292.4 SA-
	S.D.	55.69
350.000	N	10
FEMALES		
1	MEAN	154.8
	S.D.	19.82
.000	N	10
2	MEAN	159.7
	S.D.	20.19
50.000	N	10
3	MEAN	155.9
	S.D.	35.45
150.000	N	10
4	MEAN	142.2
	S.D.	16.17
350.000	N	10

HLA [redacted]

TABLE 4R
MEAN FOOD CONSUMPTION AND STANDARD DEVIATIONS (G/WK)
91-DAY SUBCHRONIC ORAL TOXICITY

GROUP AND DOSE LEVEL (MG/KG)	WEEK:	1	2	3	4	5	6	7	8	9	10	11	12	13
MALES														
1	MEAN	191.4	197.6	201.1	201.6	196.9	198.7	201.1	201.7	191.7	193.3	198.3	201.3	192.8
	S.D.	10.29	6.11	6.48	8.44	8.12	9.99	7.66	11.91	29.82	32.13	6.63	9.44	14.19
.000	N	10	8	7	8	7	8	6	9	10	9	7	7	10
2	MEAN	186.4	194.8	201.5	208.0	196.9	196.7	200.2	197.9	193.2	198.4	201.5	202.8	188.7
	S.D.	10.32	10.19	9.88	8.35	9.85	11.02	9.82	9.65	8.44	9.88	6.39	6.17	4.43
50.000	N	9	9	8	9	8	9	8	9	9	8	8	8	9
3	MEAN	186.9	190.8	196.9	189.3	187.7	189.6	189.9	193.6	191.8	194.8	193.6	191.1	187.2
	S.D.	14.29	11.19	9.54	12.68	13.98	11.91	15.95	15.68	12.76	14.90	14.47	15.86	18.21
150.000	N	10	9	9	9	8	9	9	10	10	9	9	7	10
4	MEAN	174.1	187.7	184.9	184.6	182.8	177.5	174.8	175.8	179.1	187.0	178.8	179.2	173.9
	S.D.	12.49	8.81	15.91	13.53	18.23	16.93	10.71	10.48	16.16	16.04	11.77	12.76	16.17
350.000	N	10	10	10	9	10	10	9	10	10	10	9	9	10
FEMALES														
1	MEAN	134.5	139.8	140.8	139.5	137.1	137.8	143.3	132.8	123.9	132.8	137.8	141.1	132.6
	S.D.	7.72	11.25	11.32	12.35	14.54	12.77	16.23	9.38	2.74	13.11	17.45	19.87	12.01
.000	N	10	10	10	10	9	9	9	9	9	8	9	10	10
2	MEAN	139.2	146.4	149.3	148.9	143.1	145.8	140.9	135.5	135.2	141.1	137.2	139.2	133.0
	S.D.	10.88	10.42	17.61	18.46	14.29	15.86	9.22	7.37	13.88	15.79	16.56	10.78	25.60
50.000	N	10	10	10	10	9	8	10	7	9	10	10	10	10
3	MEAN	142.5	142.7	149.6	157.1	142.3	142.9	145.7	142.8	141.9	149.4	149.8	141.4	127.8
	S.D.	17.42	11.99	16.44	23.87	17.92	14.29	18.15	23.89	17.91	14.79	24.94	26.68	34.31
150.000	N	10	9	9	9	9	10	9	9	10	10	10	10	9
4	MEAN	132.9	140.6	133.4	137.1	132.8	127.2	128.8	130.1	126.8	131.8	127.8	134.2	128.9
	S.D.	7.94	9.90	8.32	12.69	9.28	14.15	12.49	8.85	23.02	12.76	9.40	9.00	17.73
350.000	N	10	10	10	10	9	9	10	10	10	10	8	10	10

TABLE 4B HLA [redacted]
MEAN TOTAL FOOD CONSUMPTION AND STANDARD DEVIATIONS (G)
91-DAY SUBCHRONIC ORAL TOXICITY

GROUP AND DOSE LEVEL (MG/KG)		WEEK 1 - 13 RH
MALES		
1	MEAN	2538.2
	S.D.	50.27
.000	N	4
2	MEAN	2536.1
	S.D.	63.21
50.000	N	7
3	MEAN	2418.6
	S.D.	165.36
150.000	N	6
4	MEAN	2302.5 SA-
	S.D.	131.43
350.000	N	9
FEMALES		
1	MEAN	1736.9
	S.D.	122.69
.000	N	7
2	MEAN	1814.7
	S.D.	123.03
50.000	N	4
3	MEAN	1833.6
	S.D.	218.36
150.000	N	8
4	MEAN	1711.9
	S.D.	115.44
350.000	N	6

TABLE 5
MEAN COMPOUND CONSUMPTION (MG/KG/DAY)
91-DAY SUBCHRONIC ORAL TOXICITY

MLA [redacted]

GROUP AND DOSE LEVEL (MG/KG)	WEEK:	1	2	3	4	5	6	7	8	9	10	11	12
						MALES							
1 .000	MEAN	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
2 90.000	MEAN	57.50	43.82	49.74	45.29	45.69	47.09	48.57	47.46	47.08	49.97	49.35	49.29
3 150.000	MEAN	172.41	129.38	137.27	131.18	137.78	140.31	146.62	146.66	142.84	149.94	142.60	144.98
4 350.000	MEAN	375.25	313.93	319.93	319.95	327.37	325.17	338.47	340.11	348.68	356.76	323.98	341.48
						FEMALES							
1 .000	MEAN	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
2 90.000	MEAN	49.07	45.26	45.94	46.65	46.25	48.42	46.50	46.87	48.97	50.99	47.42	49.77
3 150.000	MEAN	181.31	131.18	141.80	149.13	129.94	144.22	147.44	144.08	148.14	154.43	144.21	142.19
4 350.000	MEAN	344.64	326.49	301.14	332.37	322.84	329.71	347.34	341.96	331.72	356.10	339.47	339.62

TABLE 5
MEAN COMPOUND CONSUMPTION (MG/KG/DAY)
91-DAY SUBCHRONIC ORAL TOXICITY

HLA [redacted]

GROUP AND DOSE LEVEL (MG/KG)	WEEK:	13
MALES		
1 .000	MEAN	.00
2 50.000	MEAN	46.32
3 150.000	MEAN	146.18
4 350.000	MEAN	344.88
FEMALES		
1 .000	MEAN	.00
2 50.000	MEAN	48.90
3 150.000	MEAN	137.64
4 350.000	MEAN	334.82

MLR [redacted]

TABLE 6
MEAN CLINICAL HEMATOLOGY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		RBC MIL/UL WEEK 14	HGB G/DL WEEK 14	HCT % WEEK 14	MCV FL WEEK 14	MCH PG WEEK 14	MCHC G/DL WEEK 14
				MALE			
1 0 G/KG	MEAN	8.64	16.2	44.0	52.0	18.7	36.1
	S.D.	1.132	1.88	4.96	2.07	.97	.60
	N	10	10	10	10	10	10
2 0.05 G/KG	MEAN	8.99	16.8	44.9	51.7	18.6	36.8
	S.D.	.243	.69	1.32	.51	.26	.27
	N	10	10	10	10	10	10
3 0.15 G/KG	MEAN	8.88	16.8	46.5	52.3	18.9	36.2
	S.D.	.208	.70	1.66	1.31	.39	.64
	N	10	10	10	10	10	10
4 0.35 G/KG	MEAN	8.74	16.9	45.9	52.5	19.8	39.9
	S.D.	.361	.94	1.91	1.84	.44	.83
	N	10	10	10	10	10	10
				FEMALE			
1 0 G/KG	MEAN	8.65	16.0	44.8	94.1	19.8	36.8
	S.D.	.297	.68	1.76	.85	.31	.32
	N	10	10	10	10	10	10
2 0.05 G/KG	MEAN	8.47	16.9	46.9	95.4	19.9	36.9
	S.D.	.367	.71	1.98	1.33	.53	.38
	N	10	10	10	10	10	10
3 0.15 G/KG	MEAN	8.21	16.0	44.3	94.6	19.6	36.0
	S.D.	1.398	2.58	9.69	4.43	1.90	.32
	N	10	10	10	10	10	10
4 0.35 G/KG	MEAN	8.92	16.4	45.7	93.8	19.9	39.9
	S.D.	.405	.87	2.03	1.96	.57	.51
	N	10	10	10	10	10	10

TABLE 6 - CONTINUED
MEAN CLINICAL HEMATOLOGY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		PLATELET TH/UL WEEK 14	RETIC % RBC WEEK 14	A RETIC MI/UL WEEK 14
				MALE
1 0 G/KG	MEAN S.D. N	1104 226.2 10	1.4 1.16 10	.12 .092 10
2 0.05 G/KG	MEAN S.D. N	1074 203.0 10	1.3 .74 10	.12 .060 10
3 0.19 G/KG	MEAN S.D. N	1112 179.6 10	1.7 1.07 10	.11 .099 10
4 0.39 G/KG	MEAN S.D. N	1054 174.2 10	1.3 .77 10	.12 .064 10
				FEMALE
1 0 G/KG	MEAN S.D. N	1099 140.4 10	1.0 .98 10	.09 .057 10
2 0.05 G/KG	MEAN S.D. N	1067 241.4 10	1.1 .46 10	.09 .041 10
3 0.19 G/KG	MEAN S.D. N	991 145.7 10	1.4 .84 10	.12 .061 10
4 0.39 G/KG	MEAN S.D. N	1176 233.8 10	1.0 .79 10	.09 .044 10

TABLE 6 - CONTINUED
MEAN CLINICAL HEMATOLOGY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

MLA [redacted]

GROUP AND DOSAGE LEVEL		WBC TH/UL WEEK 14	NRBC /100 WBC WEEK 14	COR WBC TH/UL WEEK 14	BLAST TH/UL WEEK 14	PRO/MYEL TH/UL WEEK 14	META TH/UL WEEK 14
MALE							
1 0 G/KG	MEAN	10.1	0	10.1	.0	.0	.0
	S.D.	3.64	.0	3.64	.00	.00	.00
	N	10	10	10	10	10	10
2 0.05 G/KG	MEAN	7.6 SL-	0	7.6 SL-	.0	.0	.0
	S.D.	1.49	.0	1.65	.00	.00	.00
	N	10	10	10	10	10	10
3 0.15 G/KG	MEAN	8.4	0	8.4	.0	.0	.0
	S.D.	2.15	.0	2.15	.00	.00	.00
	N	10	10	10	10	10	10
4 0.35 G/KG	MEAN	7.4 SL-	.3	7.4 SL-	.0	.0	.0
	S.D.	2.11	1.0	2.09	.00	.00	.00
	N	10	10	10	10	10	10
FEMALE							
1 0 G/KG	MEAN	9.0	0	9.0	.0	.0	.0
	S.D.	1.99	.0	1.99	.00	.00	.00
	N	10	10	10	10	10	10
2 0.05 G/KG	MEAN	6.9	0	6.9	.0	.0	.0
	S.D.	4.49	.0	4.49	.00	.00	.00
	N	10	10	10	10	10	10
3 0.15 G/KG	MEAN	22.0	1	20.7	12.9	.0	.0
	S.D.	49.78	2.0	49.52	40.76	.00	.00
	N	10	10	10	10	10	10
4 0.35 G/KG	MEAN	6.1	0	6.1	.0	.0	.0
	S.D.	2.24	.0	2.20	.00	.00	.00
	N	10	10	10	10	10	10

TABLE 6 - CONTINUED
MEAN CLINICAL HEMATOLOGY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		BAND TH/UL WEEK 14	SEG TH/UL WEEK 14	LYMPH TH/UL WEEK 14	MONO TH/UL WEEK 14	EOSIN TH/UL WEEK 14	BASO TH/UL WEEK 14
				MALE			
1 0 G/KG	MEAN S.D. N	.0 .00 10	2.8 .97 10	7.0 3.15 10	.1 .09 10	.1 .12 10	.0 .00 10
2 0.05 G/KG	MEAN S.D. N	.0 .00 10	1.6 .67 10	5.7 1.27 10	.1 .06 10	.1 .09 10	.0 .00 10
3 0.15 G/KG	MEAN S.D. N	.0 .00 10	1.9 .62 10	6.6 2.09 10	.1 .10 10	.1 .15 10	.0 .00 10
4 0.35 G/KG	MEAN S.D. N	.0 .00 10	1.0 .69 10	5.9 1.59 10	.1 .07 10	.1 .12 10	.0 .00 10
				FEMALE			
1 0 G/KG	MEAN S.D. N	.0 .00 10	1.1 .73 10	3.7 1.06 10	.1 .08 10	.1 .11 10	.0 .00 10
2 0.05 G/KG	MEAN S.D. N	.0 .00 10	.0 .35 10	6.0 4.36 10	.1 .07 10	.1 .05 10	.0 .00 10
3 0.15 G/KG	MEAN S.D. N	.3 .95 10	1.1 1.39 10	6.1 4.55 10	.1 .08 10	.1 .06 10	.0 .00 10
4 0.35 G/KG	MEAN S.D. N	.0 .00 10	1.4 .91 10	4.6 2.29 10	.1 .09 10	.0 .00 10	.0 .00 10

HLA [redacted]

TABLE 7
MEAN CLINICAL CHEMISTRY VALUES
91-Day SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		GLUCOSE MG/DL WEEK 14	BUN MG/DL WEEK 14	CREAT MG/DL WEEK 14	AST U/L WEEK 14	ALT U/L WEEK 14	ALK P U/L WEEK 14 RH
				MALE			
1	MEAN	143	12	.6	157	39	72
0 G/KG	S.D.	18.1	2.9	.07	41.9	8.8	17.9
	N	10	10	10	10	10	10
2	MEAN	148	13	.6	164	37	74
0.05 G/KG	S.D.	13.4	1.8	.04	24.9	9.3	13.1
	N	10	10	10	10	10	10
3	MEAN	133	13	.6	174	44	69
0.15 G/KG	S.D.	10.4	1.9	.09	48.4	26.2	12.9
	N	10	10	10	10	10	10
4	MEAN	138	14	.6	171	38	69 SH+
0.35 G/KG	S.D.	20.9	2.1	.07	46.9	9.4	21.9
	N	10	10	10	10	10	10
				FEMALE			
1	MEAN	133	15	.7	169	41	50
0 G/KG	S.D.	17.4	2.9	.05	63.7	21.6	14.0
	N	10	10	10	10	10	10
2	MEAN	137	14	.7	159	31	48
0.05 G/KG	S.D.	27.1	3.2	.09	30.9	3.9	12.6
	N	10	10	10	10	10	10
3	MEAN	128	17	.7	182	96	132
0.15 G/KG	S.D.	9.6	5.9	.10	164.9	37.2	272.7
	N	10	10	10	10	10	10
4	MEAN	132	16	.7	172	42	54
0.35 G/KG	S.D.	14.4	2.9	.06	39.9	17.2	19.6
	N	10	10	10	10	10	10

TABLE 7 - CONTINUED
MEAN CLINICAL CHEMISTRY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		T PROT G/DL 14 WEEK	ALBUMIN G/DL 14 WEEK
		MALE	
1 0 G/KG	MEAN	6.2	4.1
	S.D.	.37	.40
	N	10	10
2 0.05 G/KG	MEAN	6.0	4.0 S1-
	S.D.	.16	.13
	N	10	10
3 0.19 G/KG	MEAN	6.1	4.2
	S.D.	.35	.10
	N	10	10
4 0.39 G/KG	MEAN	5.8 S1-	4.1
	S.D.	.33	.20
	N	10	10
		FEMALE	
1 0 G/KG	MEAN	6.3	4.5
	S.D.	.20	.24
	N	10	10
2 0.05 G/KG	MEAN	6.3	4.5
	S.D.	.40	.38
	N	10	10
3 0.15 G/KG	MEAN	6.3	4.6
	S.D.	.61	.43
	N	10	10
4 0.36 G/KG	MEAN	6.3	4.4
	S.D.	.24	.33
	N	10	10

HLA [redacted]

TABLE 7 - CONTINUED
MEAN CLINICAL CHEMISTRY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		CALCIUM MG/DL WEEK 14	T BILI MG/DL WEEK 14	IN PHOS MG/DL WEEK 14	GGT U/L WEEK 14	SODIUM MEQ/L WEEK 14	POTAS MEQ/L WEEK 14
					MALE		
1 0 G/KG	MEAN S.D. N	8.8 .38 10	.1 .03 10	6.3 .83 10	1 .5 10	144 1.8 10	4.8 .50 10
2 0.05 G/KG	MEAN S.D. N	8.9 .36 10	.1 .05 10	6.9 .66 10	1 .4 10	143 1.6 10	4.8 .29 10
3 0.15 G/KG	MEAN S.D. N	8.7 .29 10	.1 .00 10	6.8 .82 10	1 .5 10	145 1.8 10	4.7 .68 10
4 0.35 G/KG	MEAN S.D. N	8.9 .38 10	.1 .00 10	7.2 SIG 1.28 10	1 .5 10	144 1.4 10	4.8 .46 10
					FEMALE		
1 0 G/KG	MEAN S.D. N	8.9 .28 10	.1 .00 10	6.7 1.01 10	1 .9 10	143 1.3 10	4.6 .33 10
2 0.05 G/KG	MEAN S.D. N	8.9 .27 10	.1 .00 10	6.1 .84 10	1 .6 10	144 1.1 10	4.7 .42 10
3 0.15 G/KG	MEAN S.D. N	9.1 .84 10	.4 1.81 10	6.2 .84 10	2 1.4 10	144 1.5 10	4.6 .36 10
4 0.35 G/KG	MEAN S.D. N	9.1 .38 10	.1 .00 10	6.5 1.04 10	1 .6 10	144 1.5 10	5.8 SIG .69 10

TABLE 7 - CONTINUED
MEAN CLINICAL CHEMISTRY VALUES
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROUP AND DOSAGE LEVEL		CHLORIDE MEQ/L WEEK 14 RM
MALE		
1 0 G/KG	MEAN S.D. N	104 2.4 10
2 0.05 G/KG	MEAN S.D. N	105 1.4 10
3 0.19 G/KG	MEAN S.D. N	109 1.2 10
4 0.39 G/KG	MEAN S.D. N	107 SA+ 1.9 10
FEMALE		
1 0 G/KG	MEAN S.D. N	105 1.9 10
2 0.09 G/KG	MEAN S.D. N	105 2.5 10
3 0.19 G/KG	MEAN S.D. N	104 2.0 10
4 0.38 G/KG	MEAN S.D. N	105 1.2 10

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TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER OF ANIMALS AFFECTED ---							
	SEX:	-------MALE-------				-------FEMALE-------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	10	10	10	10	10
** TOP OF LIST **									
BRAIN W/STEM (BR)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	9
H-VENTRICLE, DILATED		0	0	0	0	0	0	0	1
CORD, THORACIC (TC)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
PITUITARY (PI)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	9	10	10	10	10	10	9	10
DARK		1	0	0	0	0	0	0	0
PALE		0	0	0	0	0	0	1	0
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
THYROID (TY)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
PARATHYROID (PT)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10

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TABLE B
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER-OF-ANIMALS-AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
ESOPHAGUS (ES)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
TRACHEA (TR)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
LARYNX (LA)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
LUNG (LU)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
HEART (HT)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
SPLEEN (SP)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	9	10	10	10	10	10	9	10
ENLARGED		0	0	0	0	0	0	1	0
MARGIN, ROUNDED		0	0	0	0	0	0	1	0
PALE		0	0	0	0	0	0	1	0
M-IRREGULARLY SHAPED		1	0	0	0	0	0	0	0
LIVER (LI)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	8	10	10	10	10	10	8	10
ENLARGED		0	0	0	0	0	0	1	0

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TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER-OF-ANIMALS-AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
** FROM PREVIOUS PAGE **									
LIVER (LI)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	8	10	10	10	10	10	8	10
LOBE, THICKENED		0	0	0	0	0	0	1	0
MOTTLED		0	0	0	0	0	0	1	0
RAISED AREA		0	0	0	0	0	0	1	0
H-IRREGULARLY SHAPED		2	0	0	0	0	0	0	0
KIDNEY (KD)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	7	7	10	9	9	10	9	10
PELVIS, DILATED		3	2	0	1	1	0	0	0
PELVIS, FLUID		1	0	0	0	0	0	0	0
DARK		0	0	0	0	0	0	1	0
DARK AREA		0	1	0	0	0	0	0	0
URETER (UE)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
STOMACH, NONGL. (SU)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
STOMACH, GL. (ST)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	9	9	10
MUCOSA, SMOOTH		0	0	0	0	0	0	1	0
MUCOSA, FILMY MATERIAL		0	0	0	0	0	1	1	0
DARK AREA		0	0	0	0	0	0	1	0

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TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER OF ANIMALS AFFECTED ---							
SEX:		-------MALE-------				-------FEMALE-------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
DUODENUM (DU)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
M-PEYER'S PATCH, DARK		0	0	0	0	0	0	1	0
M-PEYER'S PATCH, PROMINENT		0	0	0	0	0	0	1	0
JEJUNUM (JE)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
LUMEN, GAS		0	0	0	0	0	0	1	0
M-PEYER'S PATCH, DARK		0	0	0	0	0	0	1	0
M-PEYER'S PATCH, PROMINENT		0	0	0	0	0	0	1	0
ILEUM (IL)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
LUMEN, GAS		0	0	0	0	0	0	1	0
M-PEYER'S PATCH, DARK		0	0	0	0	0	0	1	0
M-PEYER'S PATCH, PROMINENT		0	0	0	0	0	0	1	0
PANCREAS (PA)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
CECUM (CE)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
LUMEN, GAS		0	0	0	0	0	0	1	0
M-LYMPHOID AREA, PROMINENT		0	0	0	0	0	0	1	0

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TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

CROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER-OF-ANIMALS-AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
COLON (CO)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
H-LYMPHOID AREA, PROMINENT		0	0	0	0	0	0	1	0
RECTUM (RE)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
LN, ILEOCECAL (LNC)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
ENLARGED		0	0	0	0	0	0	1	0
TESTIS (TE)	NUMBER EXAMINED:	10	10	10	10	0	0	0	0
	NOT REMARKABLE:	10	10	10	10	0	0	0	0
EPIDIDYMIS (EP)	NUMBER EXAMINED:	10	10	10	10	0	0	0	0
	NOT REMARKABLE:	9	10	10	10	0	0	0	0
H-MASS		1	0	0	0	0	0	0	0
PROSTATE (PR)	NUMBER EXAMINED:	10	10	10	10	0	0	0	0
	NOT REMARKABLE:	10	10	10	10	0	0	0	0
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	10	10	10	10	0	0	0	0
	NOT REMARKABLE:	10	10	10	10	0	0	0	0

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TABLE B
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER OF ANIMALS AFFECTED ---							
SEX:		-------MALE-------				-------FEMALE-------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
URINARY BLADDER (UB)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	10	10	10	10
	NOT REMARKABLE:	0	0	0	0	9	10	8	9
CYST		1	0	0	0	1	0	1	1
PALE		0	0	0	0	0	0	1	0
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	10	10	10	10
	NOT REMARKABLE:	0	0	0	0	10	10	9	8
PALE		0	0	0	0	0	0	1	0
LUMEN, FLUID		0	0	0	0	0	0	0	2
WALL, THICKENED		0	0	0	0	0	0	0	1
VAGINA (VA)	NUMBER EXAMINED:	0	0	0	0	10	10	10	10
	NOT REMARKABLE:	0	0	0	0	10	10	10	10
LN, MANDIBULAR (MN)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	9	10	9	10
ENLARGED		0	0	0	0	1	0	1	0
MAND SALIVARY GL. (SG)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10

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TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER-OF-ANIMALS-AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
LN, MEDIASTINAL (ML)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
ENLARGED		0	0	0	0	0	0	1	0
THYMUS (TH)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
AORTA, THORACIC (AO)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
EYE (EY)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
MUSCLE, SKELETAL (SM)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
MUSCLE, GLUTEAL (SM0)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
MUSCLE, PSOAS (SM1)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10

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TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER OF ANIMALS AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
SKIN (SK)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
MAMMARY GLAND (MG)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
MARROW, FEMUR (FM)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
BONE, FEMUR (FE)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
TONGUE (TO)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	10	10
^COLLECTED/TAKEN (XW)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	0	0	0	0	0	0	0	0
NO SPECIAL REQUIREMENT		10	10	10	10	10	10	10	10
SKIN, OTHER (SS)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	9	9	10	10	10	10	10
ALOPECIA		0	1	0	0	0	0	0	0
SORE		0	0	1	0	0	0	0	0

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 8
*** PATHTOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY — STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER OF ANIMALS AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
LN, OTHER (LN)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
MULTIPLE, ENLARGED		0	0	0	0	0	0	1	0
CAVITY, ABDOM (PC)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
ADIPOSE TISSUE, REDUCED		0	0	0	0	0	0	1	0
MUSCLE, OTHER (OM)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
REDUCED SIZE		0	0	0	0	0	0	1	0
BONE, OTHER (OB)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	10	10	10	10	10	10	9	10
BRITTLE		0	0	0	0	0	0	1	0
NASAL TURBINATE (NT)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	9	10	10	10	10	10	10	10
FRACTURED		1	0	0	0	0	0	0	0
CAVITY, ORAL (OC)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	9	10	10	10	10	10	10	10
DEPRESSED AREA		1	0	0	0	0	0	0	0
H-RAISED AREA		1	0	0	0	0	0	0	0

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 8
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

GROSS PATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

ORGAN AND KEYWORD(S) OR PHRASE		--- NUMBER-OF-ANIMALS-AFFECTED ---							
SEX:		-------MALE-------				-------FEMALE-------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
SUBCUTANEOUS TIS (SQ)	NUMBER EXAMINED:	10	10	10	10	10	10	10	10
	NOT REMARKABLE:	9	10	10	10	10	10	10	10
DARK MATERIAL		1	0	0	0	0	0	0	0
GELATINOUS		1	0	0	0	0	0	0	0

** END OF LIST **

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 9
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

ABSOLUTE ORGAN WEIGHT MEANS (g)

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH-T;SUBSET=ALL

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
>>> TERMINAL BODY WEIGHT (g) <<< RM								
# IN GRP.:	10	10	10	10	10	10	10	10
MEAN:	570.8	563.0	548.6	503.7 SA-	291.8	297.9	299.8	279.4
STAND DEV:	52.9	29.9	49.7	61.9	20.9	16.4	37.5	16.2
>>> OV - OVARY <<<								
# IN GRP.:	0	0	0	0	10	10	10	10
MEAN:					.144	.146	.158	.147
STAND DEV:					.036	.024	.031	.016
>>> BR - BRAIN W/STEM <<<								
# IN GRP.:	10	10	10	10	10	10	10	10
MEAN:	2.21	2.25	2.19	2.21	2.02	2.01	2.08	2.05
STAND DEV:	.08	.11	.09	.11	.03	.10	.11	.05
>>> KD - KIDNEY <<<								
# IN GRP.:	10	10	10	10	10	10	10	10
MEAN:	3.63	3.72	3.60	3.45	1.93	2.08	2.01	1.97
STAND DEV:	.34	.24	.29	.43	.21	.26	.18	.13
>>> TP - TESTIS/EPIDID <<<								
# IN GRP.:	10	10	10	10	0	0	0	0
MEAN:	9.05	9.11	9.16	8.96				
STAND DEV:	.44	.36	.31	.52				
>>> LI - LIVER <<< RM								
# IN GRP.:	10	10	10	10 SI-	10	10	10	10
MEAN:	19.10	18.16	17.92	17.69	7.12	7.23	11.23	7.11
STAND DEV:	1.82	.90	2.30	2.49	.61	.93	11.68	.71

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 10
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

ORGAN TO TERMINAL BODY WEIGHT RATIO MEANS (%)

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=T;SUBSET=ALL

SEX:	MALE				FEMALE			
GROUP:	1	2	3	4	1	2	3	4
NUMBER:	10	10	10	10	10	10	10	10
>>> OV - OVARY <<<								
# IN GRP.:	0	0	0	0	10	10	10	10
MEAN:					.0496	.0492	.0529	.0528
STAND DEV:					.0121	.0089	.0066	.0064
>>> BR - BRAIN W/STEM <<< RM								
# IN GRP.:	10	10	10	10 SA*	10	10	10	10
MEAN:	.398	.399	.402	.443	.695	.677	.703	.734
STAND DEV:	.042	.024	.039	.054	.049	.043	.076	.046
>>> KD - KIDNEY <<< RM								
# IN GRP.:	10	10	10	10 SA*	10	10	10	10
MEAN:	.636	.662	.698	.716	.662	.698	.675	.704
STAND DEV:	.031	.059	.040	.098	.072	.084	.054	.045
>>> TP - TESTIS/EPIDID <<< RM								
# IN GRP.:	10	10	10	10 SA*	0	0	0	0
MEAN:	.891	.900	.947	1.031				
STAND DEV:	.092	.097	.107	.082				
>>> LI - LIVER <<<								
# IN GRP.:	10	10	10	10	10	10	10	10
MEAN:	2.646	2.516	2.928	2.619	2.444	2.429	4.109	2.552
STAND DEV:	.216	.126	.237	.309	.200	.296	5.189	.294

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 11
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

HISTOPATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	10	10	10	10	10
** TOP OF LIST **									
BRAIN W/STEM (BR)	NUMBER EXAMINED:	10	8	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	9
--VENTRICLE, DILATATION		0	0	0	0	0	0	0	1
CORD, THORACIC (TC)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
PITUITARY (PI)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	0	0	0	1	10	0	0	10
--SECRETORY,CELLS		10	0	0	9	0	0	0	0
--CYST		0	0	0	2	0	0	0	0
--HYPERPLASIA, FOCAL		0	0	0	1	0	0	0	0
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
THYROID (TY)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
PARATHYROID (PT)	NUMBER EXAMINED:	0	0	0	10	10	0	0	0
	NOT REMARKABLE:	0	0	0	10	10	0	0	0

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 11
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

HISTOPATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	10	10	10	10	10
ESOPHAGUS (ES)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
TRACHEA (TR)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
LARYNX (LA)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
LUNG (LU)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	0	0	0	0	2	0	0	2
--BRONCHIOLAR ASSOCIATED LYMPHOID TISSUE		10	0	0	10	8	0	0	7
--EOSINOPHILIC INFILTRATE		6	0	0	1	0	0	0	0
--MINERALIZATION, BLOOD VESSEL		4	0	0	5	3	0	0	2
--ALVEOLAR HISTIOCYTOSIS		0	0	0	2	0	0	0	0
HEART (HT)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	2	0	0	3	8	0	0	9
--CARDIOMYOPATHY, DEGENERATIVE		8	0	0	7	2	0	0	1
SPLEEN (SP)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	9	0	0	10	10	0	0	10
--EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		1	0	0	0	0	0	0	0
--K-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEUPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 11
*** PATH-TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

HISTOPATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---							
SEX:		-------MALE-------				------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
LIVER (LI)	NUMBER EXAMINED:	10	0	8	10	10	0	2	10
	NOT REMARKABLE:	1	0	0	1	0	0	0	0
--INFLAMMATION, CHRONIC, FOCAL		9	0	0	9	10	0	1	10
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	6	0	0	1	0
KIDNEY (KD)	NUMBER EXAMINED:	10	7	0	10	10	0	1	10
	NOT REMARKABLE:	0	0	0	2	1	0	0	1
--DILATATION, PELVIS		6	2	0	5	9	0	0	9
--NEPHROPATHY, CHRONIC PROGRESSIVE		5	1	0	2	0	0	0	2
--MINERALIZATION, TUBULAR		2	0	0	5	4	0	0	5
--0-PAPILLOMA, PELVIS		0	1	0	0	0	0	0	0
--HYPERPLASIA, PELVIC EPITHELIUM		0	0	0	1	1	0	0	0
--MICROCALCULUS, PELVIC EPITHELIUM		0	0	0	0	1	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	6	0	0	1	0
URETER (UE)	NUMBER EXAMINED:	9	0	0	10	10	0	0	10
	NOT REMARKABLE:	9	0	0	10	10	0	0	10
STOMACH, NONGL (SU)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
STOMACH, GL (ST)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	1	10
DUODENUM (DU)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
--HYPERPLASIA, LYMPHOID		0	0	0	0	0	0	1	0

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 11
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

HISTOPATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---							
SEX:		-------MALE-------				-------FEMALE-------			
GROUP:		-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10	10	10	10	10
JEJUNUM (JE)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
--HYPERPLASIA, LYMPHOID		0	0	0	0	0	0	1	0
ILEUM (IL)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
--HYPERPLASIA, LYMPHOID		0	0	0	0	0	0	1	0
PANCREAS (PA)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
CECUM (CE)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	1	10
COLON (CO)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
--HYPERPLASIA, LYMPHOID		0	0	0	0	0	0	1	0
RECTUM (RE)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
LN, ILEOCECAL (LNI)	NUMBER EXAMINED:	9	0	0	10	10	0	1	10
	NOT REMARKABLE:	9	0	0	10	10	0	0	10
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 11
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

HISTOPATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	10	10	10	10	10
TESTIS (TE)	NUMBER EXAMINED:	10	0	0	10	0	0	0	0
	NOT REMARKABLE:	10	0	0	10	0	0	0	0
EPIDIDYMIS (EP)	NUMBER EXAMINED:	10	0	1	10	0	0	0	0
	NOT REMARKABLE:	9	0	0	10	0	0	0	0
--INFLAMMATION, GRANULOMATOUS, SPERMATIC		1	0	0	0	0	0	0	0
PROSTATE (PR)	NUMBER EXAMINED:	10	0	0	10	0	0	0	0
	NOT REMARKABLE:	9	0	0	6	0	0	0	0
--INFLAMMATION, CHRONIC		1	0	0	3	0	0	0	0
--INFLAMMATION, ACUTE AND CHRONIC		0	0	0	1	0	0	0	0
URINARY BLADDER (UB)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	10	0	0	10	0	0	0	0
	NOT REMARKABLE:	10	0	0	10	0	0	0	0
OVARY (OV)	NUMBER EXAMINED:	0	3	0	0	10	0	2	10
	NOT REMARKABLE:	0	0	0	0	10	0	0	10
--BURSA, CYST		0	0	0	0	0	0	1	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	10	0	1	10
	NOT REMARKABLE:	0	0	0	0	8	0	1	4
--DILATATION		0	0	0	0	2	0	0	6

HAZLETON LABORATORIES AMERICA, INC.
DEPARTMENT OF PATHOLOGY

TABLE 11
*** PATH/TOX SYSTEM OUTPUT ***
91-DAY SUBCHRONIC ORAL TOXICITY STUDY IN RATS

HISTOPATHOLOGY INCIDENCE SUMMARY

STUDY NUMBER: [redacted]

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---							
	SEX:	-------MALE-------				------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	10	10	10	10	10
VAGINA (VA)	NUMBER EXAMINED:	0	0	0	0	10	0	0	10
	NOT REMARKABLE:	0	0	0	0	10	0	0	10
LN, MANDIBULAR (MN)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	8	0	0	10
--HYPERPLASIA, LYMPHOID		0	0	0	0	2	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0
MAND SALIVARY GL (SG)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
TONGUE (TO)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
THYMUS (TH)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10
LN, MEDIASTINAL (ML)	NUMBER EXAMINED:	10	0	0	10	10	0	1	10
	NOT REMARKABLE:	10	0	0	10	9	0	0	10
--HYPERPLASIA, LYMPHOID		0	0	0	0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	1	0
AORTA, THORACIC (AO)	NUMBER EXAMINED:	10	0	0	10	10	0	0	10
	NOT REMARKABLE:	10	0	0	10	10	0	0	10

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 5, 2013

SUBJECT: Comments on the Draft Report on Betaine and Alkyl Betaine Ingredients Prepared for the September 2013 CIR Expert Panel Meeting

Data profile - It would have been helpful to indicate which boxes could be checked if the information included in the summaries on the ECHA website were included in the report.

p.3 - How much (or concentration) of Cetyl Betaine and Lauryl Betaine were placed on the skin in the dermal penetration study (reference 14)?

p.4 - How much Cetyl Betaine and Lauryl Betaine were applied to the skin in the barrier function study (reference 15)?

p.4 - Was the LD_{50} for Lauryl Betaine really reported in the units of "mg/g"?

p.4 - When after dosing were the gross necropsies completed in the dermal LD_{50} study (reference 14)?

p.5 - Please correct "md/kg"

p.6 - What vehicle was used in the TEWL study (reference 20)?