Alkyl Benzoates

CIR EXPERT PANEL MEETING

DECEMBER 13-14, 2010

ADMINISTRATIVE

Cosmetic Ingredient Review
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November 11, 2010

MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Subject: Draft Tentative Report for C12-15 Alkyl Benzoate and related Alkyl Benzoates

The Cosmetic Ingredient Review (CIR) Expert Panel issued an Insufficient Data Announcement (IDA) for C12-15 alkyl benzoate and related benzoates (17 total ingredients) in August. The data needs are: 1) dermal penetration data, especially on ingredients smaller than C12-C15 alkyl benzoates; if there is possible dermal penetration then 2) reproduction and developmental toxicity; 3) carcinogenicity; and 4) irritation and sensitization data on ingredients smaller than C12-C15 alkyl benzoates.

CIR was informed that a comprehensive dossier on the C12-15 alkyl benzoates is being prepared for the European REACH program and that it would be provided to CIR in late September or early October. The project is behind schedule and is projected to be ready in January, 2011. Data were provided by the Personal Care Products Council and have been inserted into the report. There are now data on dermal penetration and those data do include the smallest MW ingredient, methyl benzoate, and there are irritation and sensitization data. No data on reproduction/developmental toxicity, however, are available, except for sodium benzoate and several alcohol metabolites and no data are available on carcinogenicity, except for benzoic acid and several alcohol metabolites.

A few of the larger submissions that had more detail than necessary (e.g., raw data, informed consent forms) have had some pages removed in the print version of the Panel book. If these data are necessary for evaluation, you may examine the removed pages in the online version.

As requested, the available data on alcohols that have been reviewed by the Panel and RIFM have been summarized and included in the report.

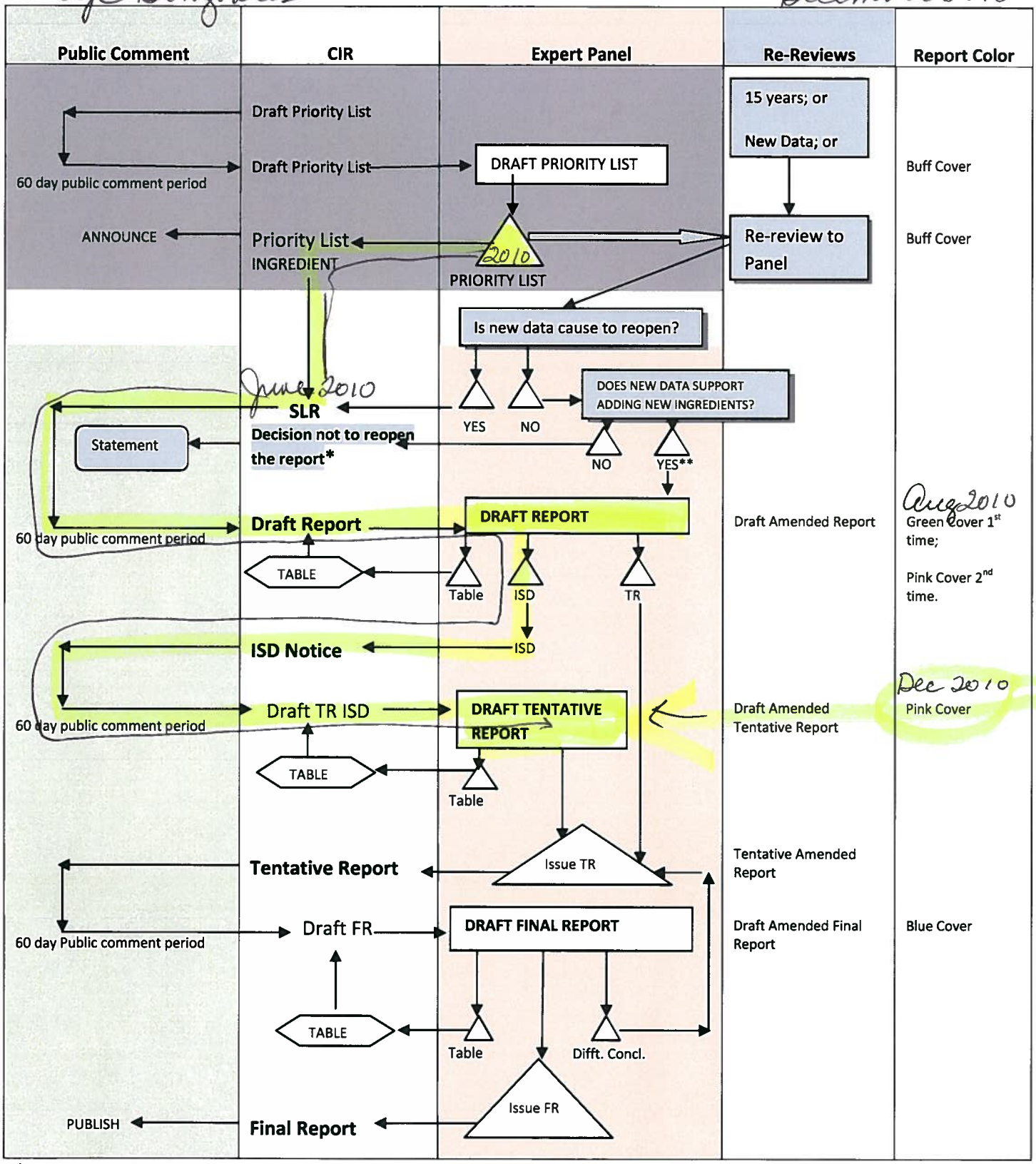
The Panel should review the Draft Tentative Report and decide if the additional data address the data needs of the IDA. If the data are sufficient, then the Panel needs to adjust the safety conclusion. If not, then the Panel may decide to 1) issue a Tentative Report with an insufficient data conclusion or 2) table the report to give industry the additional opportunity to include the data being prepared for REACH and other data on the smaller alkyl benzoates.

1101 17th St., NW, Suite 412 Washington, DC 20036-4702
Phone 202.331.0651 FAX 202.331.0088 http://www.cir-safety.org

SAFETY ASSESSMENT FLOW CHART

Alkyl Benzoates

December 2010



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

Expert Panel Decision

Document for Panel Review

Option for Re-review

History of Alkyl Benzoates

June, 2010 – SLR issued.

August, 2010 – The Panel issued an Insufficient Data Announcement. The data needs are: 1) dermal penetration data, especially on ingredients smaller than C12-C15 alkyl benzoates; if there is possible dermal penetration then 2) reproduction and developmental toxicity; 3) carcinogenicity; and 4) irritation and sensitization data on ingredients smaller than C12-C15 alkyl benzoates.

There is a REACH submission being prepared that is going to be shared with CIR. It was expected before the December meeting. It has been delayed and is not in the December report. Some of the data have been submitted through PCPC and is included in the report.

The Panel decided to issue an Insufficient Data Announcement instead of tabling the report to wait for the REACH data to move the report along the process and to officially identify data needs.

Search Strategy for Benzoates

EXPORATORY SEARCH:

PUBMED: "alkyl benzoate" – 7 hits, 1 useful; CAS No. – 0 hits.
Internet (Dogpile) – "alkyl benzoate" - 1 MSDS

FULL SEARCH:

PUBMED: "lauryl alcohol" – 53 hits, 6 ordered. Learned that Valerie was doing this ingredient.
"tridecyl alcohol" – 0 hits. CAS No. – 0 and 19 hits. 1 useful.
"Amyl benzoate" -0 hits. CAS no – no hits.
"benhyl benzoate" – 0 hits. CAS no – no hits.
"Butyl Benzoate" – 9 hits, 1 useful. CAS no. – no hits.
"Butyloctyl Benzoate" – 0 hits. No CAS no.
"Ethyl Benzoate" – 44 hits, 4 useful.
"Ethylhexyl Benzoate" – no hits.
"Hexyldecyl Benzoate" – no hits.
"Isobutyl Benzoate" – not hits. CAS no. – no hits.
"939-48-0" OR "34364-24-4" OR "Lauryl/Myristyl Benzoate" OR "112-53-8" OR "Octyldodecyl Benzoate" OR "2315-68-6" OR "10578-34-4" – 224 hits, 19 useful

TOXNET: 68411-27-8 – 0 hits; 112-53-8 – 253 hits, 47 useful; 112-70-9 – 33 hits, 2 useful; 26248-42-0 - 10 hits, 4 useful; 629-76-5 – 15 hits, 1 useful (already have); 2049-96-9 – 6 hits, 0 useful; "amyl alcohol" - 798 hits, 86 + 64 hits so far; 103403-38-9 – no hits; 136-60-7 - Butyloctyl Benzoate – no hits; butyloctyl alcohol – no hits; C16-17 Alkyl Benzoate – no hits; palmyl alcohol – no hits; heptadecyl alcohol – no hits; 93-89-0 – 56 hits, 64-17-5 alcohol – 50000 hits, did not explore yet; Hexyldecyl Benzoate – no hits; Hexyldecyl Benzoate – No hits; 120-50-3 – 3 hits, 0 useful; 939-48-0 – 2 hits, 0 useful; 34364-24-4 – no hits, Isopropyl Benzoate – No hits; Isostearyl Benzoate – no hits; Lauryl/Myristyl Benzoate - no hits; 112-53-8 [print toxnet] – 248 hits, 112-53-8 – 248 hits, 61 useful; 93-58-3 – 135 hits, 14 useful; Octyldodecyl Benzoate – no hits; Octyldodecyl Alcohol – no hits; 2315-68-6 – 7 hits, 2 useful; 10578-34-4 – no hits.

EPA – HPV – One relevant report that included methyl benzoate. Data added to report under original citations.

Alkyl Benzoate Data Profile for December, 2010. Writer - Lillian Becker																		
	ADME			Acute toxicity			Repeated dose toxicity			Irritation			Sensitization					
	Dermal Penetration	Log K_{ow}	Use	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
methyl benzoate	X	X	X	X	X					X	X		X	X		X		X
ethyl benzoate	X	X	X	X	X					X	X	X	X	X		X		
propyl benzoate	X	X									X							
butyl benzoate	X	X			X	X				X	X	X						
amyl benzoate		X		X									X					
lauryl/myristyl benzoate		x																
C12-15 alkyl benzoate	X	X	X		X	X				X	X	X	X	X		X		
C16-17 alkyl benzoate			X															
stearyl benzoate		X	X															
behenyl benzoate		X																
isopropyl benzoate		X		X	X	X				X	X							
isobutyl benzoate		X	X		x						X	X	X	X				
isostearyl benzoate		X	X							X				X				
ethylhexyl benzoate		X	X							H		X						X
butyloctyl benzoate		X																
hexyldecyl benzoate		X																
octyldodecyl benzoate		x	x											x				

H – Only human data

TRANSCRIPTS/MINUTES

working on it, when we're done, what do we have? One ingredient. So, we're going to wait until we have the -- back to having toxicology expertise before we go down this route.

DR. MARKS: Okay. Shall we move on?

DR. SHANK: I like the structure given for wheat, ginger root, cucumber.

MR. BAILEY: I would just inject at this time that we will take this table and alert the industry that these are coming up and, you know, to anticipate concentration of use. But we'll need to make sure that we know when those reports are started so that we can get the timing right, because the timing is much shorter than it used to be and we want to make sure we get the information in time for the reviews.

DR. ANDERSEN: (off mic)

DR. MARKS: Thank you, John. Next ingredient is the Green Book on alkyl benzoates. This is a draft report for C12-15, alkyl benzoate and related alkyl benzoates, and this is the first time we've seen this report. So now we need to decide is there enough information to proceed with a tentative report or do we need an insufficient data notice?

DR. BERGFELD: Are there promised data on this particular ingredient?

DR. MARKS: The only promised data, I think, is what I see in front here from Lillian just came, on HRIPT on benzoate esters.

MS. BECKER: There is also a submission being prepared for the REACH program that has been promised later this year, that's been promised to be substantial and increase the amount of data in the report. So, we have the option of tabling and waiting for it, or proceeding with what we have and tabling in December if it doesn't show up.

DR. SLAGA: How detailed are the REACH people? I have no idea.

MS. BECKER: I haven't seen --

DR. ANDERSEN: Detailed with a capital "D."

DR. SLAGA: I say we should wait and table.

MS. BECKER: You can also -- I'm sorry, you can also make a determination now and the stuff will be in the December report and continue to move on, where if you table it now, it will be starting from this point now in December instead of moving on for the (inaudible) meeting. So, whichever you feel is appropriate and beneficial for everybody.

DR. ANDERSEN: I think my concern is just keeping the freight moving. If we table it now, then it's in a holding pattern, we come back in December, and suppose there are one or two pieces of data that are needed, then we're just, at that point, issuing an insufficient data announcement, albeit at that point in time a much better informed announcement because we have what is expected to be a rather massive data submission coming.

If, on the other hand, we flag areas that we know are going to be important now and say these are things that we need data for, likely this data submission is going to address them all and we would be positioned to move forward with a tentative decision in December.

To some extent it's moot because all of -- there's no way this can't get wrapped up in 2011. We're not -- there's no way to get it wrapped up in 2010. So, I'm spitting in the wind to some extent, but I still want to be pushy about the idea of moving things forward expeditiously. But I won't be hugely upset if you think tabling is the better approach.

DR. MARKS: I guess it depends how long the laundry list is we have now versus maybe the (inaudible) will answer it. But I hear you, Alan, in terms of this at least gets us thinking of what data do we need if we issue an insufficient data announcement. I didn't -- for me personally, I didn't see any absorption data. Did I miss that?

DR. SHANK: No, it's not there.

DR. MARKS: Yeah. So I would want to know that from a -- just my own benefit is, if it's absorbed and it's metabolized, a benzoic alcohol or an acid, is contact urticaria from this

an issue? We dealt with that with the benzoic alcohol and acid. We now do have HRIPTs. But when I look at the concentration of the C12-15 it's used up to -- if we look on page 17 of the Panel Book -- it's used up to 50 percent, so this is inadequate to justify use for 50 percent.

And, Lillian, I had a little bit of disconnect in the table, if you look in Table 3, under duration of use on leave-on, the top concentration is 35 percent. When I go to exposure type, dermal is 50 percent. So, I kind of thought dermal would mean that's a leave-on, but am I --

MS. BECKER: I don't have the detail with me, but apparently it's something that gets rinsed off, like a bath product.

DR. MARKS: Is what?

MS. BECKER: Something that gets rinsed off, like a bath product. It's not a -- like, it's not a lotion. It's something that you apply and take off, like nail polish remover or something like that.

DR. MARKS: Oh, okay. That sounds -- yeah, because there are bath products further down here under exposure types.

Now we get into, Ron Shank, your issue of, give me the details because I want to know what that 50 percent dermal is and whether I need to be using that as my limit for an HRIPT or the 35 percent. Either way, I would say there's a data need for the C12-15, that it's got to go up to 35 percent.

DR. SHANK: We just got that, the 35 percent.

DR. MARKS: Did we?

DR. SHANK: We just handed this out.

DR. MARKS: Okay, yes. Thank you.

DR. SHANK: The C12-15 in a hair serum --

DR. MARKS: Okay.

DR. SHANK: -- 35 percent.

DR. MARKS: So, absorption and --

DR. SHANK: But we have no absorption data.

DR. MARKS: No absorption. Do we have photo data? So, we'd need photo. And how about going -- other areas? How about --

REPORTER: Microphone.

DR. EISENMANN: There's a spectrum in here that hasn't been mentioned yet.

DR. MARKS: What page is that? I think I looked at that, but I --

DR. EISENMANN: Twenty-six, Panel Book 26.

DR. MARKS: So, obviously it's absorbed, we need to have photosensitization. Thirty-six, you said?

DR. EISENMANN: Twenty-six.

DR. MARKS: Okay, so that's okay. I missed that. So, we only need absorption. What else do we need?

DR. SHANK: Well, if it's absorbed, then we need reproduction, developmental, mutagenicity, or genotox, on the lipophilic ones, like C12-15.

DR. SLAGA: There is a good bit of genotoxicity, but under carcinogenicity there's -- I wouldn't mind seeing the primary articles that talks about increase in tumor growth which, to me, I don't even know what --

DR. SHANK: But the genotox -- the mutagenicity data is on the water soluble ones -- benzoic acid, sodium benzoate, methyl benzoate -- but not on the lipid soluble ones.

MS. BECKER: On the study with the increased tumor growth, that was, I guess, a small study within a larger paper and they only put in a couple paragraphs and that's pretty much all they gave you.

DR. MARKS: So, Tom, going back, what would you want to see?

DR. SLAGA: Well, there's a good bit of genotoxicity on this. Ron said the water soluble in methyl benzoate, but nothing higher --

DR. MARKS: So, you would want to see the genotox on the lipid soluble if it's absorbed.

DR. SLAGA: -- to get some idea.

DR. SHANK: If it's absorbed.

DR. MARKS: Any other data needs?

DR. HILL: Yeah, we don't have information on some of the alcohols. I just -- Panel Book 9 said, "No uses or concentrations of use were reported for," -- it has a list -- propyl, butyl, amyl, lauryl/myristyl, isopropyl, ethylhexyl, butyloctyl, hexyldecyl, page 9. And I just made the note, "Given this, can the short chain ones be excluded and press on," is what I wrote here. But now I've heard the discussion, so, no.

But there are some of these alcohols that we don't have information on and for sure there will be hydrolysis in skin of these, will be absorbed into skin, and so we need to capture somehow some of these alcohols that aren't in the list that you've given. You have a list on the preceding page, Panel Book 8, but it doesn't include all of those. There are some missing alcohols. But they were ones that didn't have any reported uses, so.

DR. MARKS: So, if I -- let me just summarize this because I think if we -- no, go ahead, Lillian, you wanted to ask --

MS. BECKER: I just want to clarify what he's asking -- what Dr. Hill is asking for.

DR. HILL: Well, I don't know exactly what I'm asking for in terms of missing information because if they weren't searched and we didn't try to put that information into the report or there simply isn't information out there to be had --

MS. BECKER: For?

DR. HILL: For some of those alcohols that are listed on page -- Panel Book page 9. Specifically ethylhexyl, butyloctyl, and hexyldecyl.

MS. BECKER: Okay. The alcohols, not the benzoates?

DR. HILL: The alcohols that are the alcohol portion of the benzoic acid esters.

MS. BECKER: Okay. So, you want the information on the alcohols?

DR. HILL: Yes, whatever exists.

MS. BECKER: Okay.

DR. MARKS: Okay. Any other --

DR. HILL: In the other cases we're relying on some previous reviews, so well and good, but --

DR. MARKS: So, I think actually my suggestion to the team would be follow Alan's lead and issue an insufficient data announcement and really a lot of it hinges whether these ingredients are absorbed or not and if they aren't absorbed, then it would be a pretty straight shot to safe, is that correct?

DR. SLAGA: (off mic)

DR. HILL: Do you want to repeat what you just said?

DR. SLAGA: I mean, because that would --

DR. MARKS: Do you want to --

DR. SLAGA: Oh, yeah, it would eliminate a lot, but still some genotoxicity related to the more lipid soluble ones.

DR. MARKS: So the genotox on lipid solubles -- okay. So, that's actually a pretty short list. Does anybody have a problem with issuing an insufficient data announcement?

And obviously this is going to be filled in by the REACH document and presumably there won't be new issues raised by the REACH document. But at least we've alerted industry where we're heading and actually, as Alan would like, move along and see if we can get the insufficient data.

DR. BERGFELD: I wonder if you could ask John Bailey his opinion?

DR. MARKS: Sure. John, what's your opinion?

MR. BAILEY: I think we should keep this moving. I think, though, that we should keep close tabs on how the REACH package is going and the timing for its availability. And the anticipation is that if we end up with any points that remain insufficient after we go through this next iteration, that those would probably be available in the REACH.

If not, then we're ahead of the game and I think that's the approach that we're talking

about here.

DR. MARKS: I would think our timing would be -- is we would not produce a tentative report until we've reviewed the REACH.

So, I'm not sure what the Belsito Team will move tomorrow, but this is what we will suggest will be our move: Insufficient data announcement.

MS. BECKER: Would you go over your list of needs just to make sure I didn't miss anything, please?

DR. MARKS: Well, I'll also rely on my teammates here, but the most critical is absorption data. If it's absorbed then we need reproduction and development data. And even without that we need genotox data for lipid solubility or for the ingredients which are lipid soluble. Does that summarize things up?

DR. ANDERSEN: And then we have staff assignments to gather data on the other alcohols for which data are not currently in the report.

DR. MARKS: Okay.

DR. HILL: One final comment is, and I made a note of it in here, is that in your PubMed search strategy there is a reasonable chance you will miss some things and I suggested an alternative way to search. I mean, when I do that kind of search I typically use Site Finder because I have it, that does the simultaneous CAS and PubMed search and then it will search all the synonyms, which is nice. But I don't think you have that because I think you're using STN, so I don't know if you can do that or not.

MS. BECKER: Okay.

DR. HILL: Okay, Bart said yes. Yeah.

MS. BECKER: Thanks.

DR. MARKS: Lillian --

DR. HILL: You don't always pick up everything with CAS number and PubMed because they don't always index that way, as you know.

DR. MARKS: Lillian, one other insufficient data need is, if it's absorbed, I'd also like to know whether the ingredients cause contact urticaria.

to the industry about our analysis and we are talking to them and it's trying to put this one study in the context of a number of studies over the years, including some significant human experience.

DR. BELSITO: Any other comments? Okay. So we'll go ahead with it and when the NTP study comes up on retinyl palmitate we'll take a look at that and you figure out when and if you want to do oxy benzo, benzoquinone-3.

Okay. Then we'll move on to the alkyl benzoates, which are Green Book 4. It's the first time that we're looking at this and we're getting some new data as I speak here.

DR. ANDERSEN: These are those tables I told you about that just summarize the bindings out of, what, 10 studies. Rather than inundating you with paper, here's what the table would look like. You'll get all of the raw data the next go-round, so you get a chance to check it.

DR. BELSITO: Okay. And I'm also informed that there will be a dossier on the C-12 to -15 alkyl benzoates coming as a result of the European REACH program, but that's not expected until September or October. And that, as is typical of these dossiers, there will be extensive data in it that may or may not add to what we've already seen.

So this report is pretty thin and I guess the question is do we want to table it because of the expected receipt of this dossier or do we want to just move it ahead, issue sufficient or insufficient -- I think our data is insufficient to support safety, but I'll look at your comments and at least give industry a heads-up as to what kind of data that we're looking at. The advantage of doing that is it'll put it on a quicker time slot or a final than if we table it and then we look at the data from the REACH program that I would expect we would get the next time around.

MS. BECKER: If the data doesn't show up you can always table in December instead of now.

DR. BELSITO: Well, so I guess -- Jay?

DR. ANSELL: We have some concern with concluding in these interim stages that the data is insufficient because we're really working very hard to make the insufficient data conclusion a very negative conclusion, one that presumes the material will move rapidly through the new process that John will talk about into a category of not recommended for use. We want to put a much stronger hammer associated with this insufficiency. And so what we've ended up doing over the last year or two is bifurcating insufficients to one where the report chose not to look at an area which the panel considers to be appropriate or the desire to keep moving forward pursuant with the Alan's discussion this morning that the data books went out before the comment period actually closed.

So we have that type of insufficient, which is kind of a benign insufficient, but we also have insufficient which the industry is working to essentially prohibit the use of these materials. So, you know, I'm troubled when we end up in a situation here where we have lots of data that's potentially going to come with concluding it's insufficient. We would prefer that it be tabled or perhaps the panel can talk about what additional information it would like.

DR. BELSITO: I think the difference there is we're not going final on this. You know, I mean, otherwise what you're saying is that you want us to table everything until we get what we need because you don't even want a tentative insufficient. I mean, this is the first time we've looked at it. And, I mean, I really, I think that it would be very helpful for me when it comes back if I know exactly what we were looking for the last time around to make it sufficient because then it'll shave -- we're going to get a huge data dump. It will shave hours off of my time knowing that I want to see dermal penetration. I want to see this and that in the dossier. And if it's not there then I'm still going ahead with insufficient.

So I sort of like Lillian's suggestion that, you know, we move ahead and then at the next time we look at this meeting, if for whatever reason the REACH data isn't available, at that time, you know, before going to a tentative final insufficient, we can say, okay, we're going to table this. You know, you already know what we want and give it a chance to get that data. I mean, it's just my personal feeling.

I understand what you're saying about coming out with a final insufficient, but, you know, in the very early stages, I mean, I think that's going to happen. And it's not a reflection on, you know, industry or on the specific product. It's simply that industry to date hasn't provided us with all the information that may be out there that would allow us to reach a safe as used conclusion.

DR. SNYDER: We've always used the data deeds.

DR. BELSITO: Right.

DR. SNYDER: And had an indirect indication that we may be going insufficient because we've identified these data gaps. I don't think that's any different --

DR. BELSITO: I don't think it's a problem.

DR. ANSELL: Well, certainly the identification of the data gaps is critical, particular as the CIR staff start using alternative approaches to the SLR. We expect that there may be many cases where the panel comes back and says staff thought this was okay, but we'd like to see data about something else. And I'm just concerned that the tentative conclusion insufficient is ending up being used in two different ways. Insufficient that we'd like to see data and insufficient that the data simply isn't there to substantiate safety. And as we've become more and more aggressive in our tactic insufficients, it's my suggestion that we use a table and the data deficiencies more frequently than tentative conclusions.

DR. BELSITO: Curt, what do you think?

DR. KLAASSEN: Yeah, I can go either

way. I mean, if we know, you know, there is a lot of information coming in in the next couple of weeks, maybe it's okay to wait. On the other hand it's kind of nice to know what we're missing right now also. So I guess I'm right on the fence.

DR. BRESLAWEC: If I could just provide some insight on the basis for one of the recommendations which is to issue an insufficient at this point. First of all, it's not fun. And second, we are under a fair amount of pressure to increase the number of ingredients that we are reviewing. And we're looking at all possible options to speed up the process and having the temporary insufficient data announcement from the panel in fact expedites the process.

DR. ANDERSEN: I think procedurally at this meeting if you identify additional data, those needs would be considered part of an insufficient data announcement. You would not be issuing a tentative report with the insufficient albatross around its neck. This is the first time you've seen this. You can ask for additional data or signal that there are additional data that are needed. And I think that may take some of the sting out of the imprimatur. It's not an insufficient data conclusion; it's your flag that more data are needed.

Given the likely completeness of the REACH dossier, I think it's also not an unreasonable decision to table it. So it's just this is a tough one procedurally. But if we table it and you come back in December and look at everything that you have and you still had data needs, you would be issuing an insufficient data announcement and you would have lost three months. That's my concern. The pressure is on. You know, we're not going to get this done this year no matter what we do, but I want -- my goal is to just keep things moving through the process.

So I don't -- we'll get it done next year regardless, so in that sense maybe it's a lousy example. It doesn't matter whether you table it or not. We're still going to get it done in 2011. But as a rule I'd like to keep them moving forward. And if there are clear data needs, flagging them now I think is a constructive thing to be doing.

DR. LIEBLER: Yeah. I think that's the most important point aside from the issue of whether we hang a bad label on it or not. I mean, if we're going to be effective we need to identify what the data needs are as soon as possible. And they may indeed be coming in the pile that we're awaiting, but they might not be. And if we never said so we simply missed the opportunity.

DR. ANSELL: Yeah. Nothing in my comments. I fully support that. We clearly need to identify what the data needs are. It's just in supporting CIR's desire to move these things forward, to sending out data before the closure of the comment period, with situations where the SLR may have focused on -- ignored large areas because there's a presumption that there was no interest. When we know there's additional data it's just personal feeling that it would be more appropriate to identify data needs and table it for (inaudible). I will leave it at that.

DR. BELSITO: Well, I mean, so I hear Dan saying not table. I'm saying not table. Paul?

DR. SNYDER: Not table.

DR. BELSITO: Curt, you're on the fence. So it's three against the fence sitter. So let's draw up our data needs.

DR. LIEBLER: Isn't the first issue whether we're going to include the other ingredients?

DR. BELSITO: Okay. That's good.

DR. LIEBLER: Which I support.

DR. BELSITO: Okay. Good. So we're going to include all the ingredients. If you look at the log Kow, there's a suggestion that there will be dermal penetration on the lower molecular weight, alkyl benzoates. And I think that raises an issue particularly for the methanol of which we have put restrictions on before. So I think we need some information about dermal penetration during the look at the lower molecular weights,

typically the methanol conjugate.

There was carcinogenicity suggested with methyl benzoate. And I guess I'm throwing this to Paul and Curt, what they thought about that data and do we need a dermal study on that?

DR. SNYDER: What study are you referring to specifically on benzoate.

DR. BELSITO: On page 5, it says, "Methyl Benzoate, A 1970 Review. Poorly administered methyl benzoate and any mgs per kg per day increased tumor growth compared to controls."

DR. KLAASSEN: But it was negative for dermal.

DR. BELSITO: That was benzoic acid. So you're satisfied with that?

DR. BRESLAWEC: I might point out on that one that industry has pointed out that we're referring to the review study --

MS. BECKER: It actually needs to go back. I was mistaken. It's not a review. They actually (unintelligible) the report because I was looking at a secondary before I got the original when I wrote this. It was -- the person who wrote it actually did the study.

DR. BRESLAWEC: Okay. Sorry.

DR. SNYDER: So I had this whole section flagged. I mean, we need a lot more data. They're just too brief. There's no indication of how long and what type studies. And a lot of these studies we need to really know how long these treatments went on for. So a tumor growth, but what tumor? When we talk about the tumor types --

DR. KLAASSEN: Yeah. This entire carcinogenicity.

DR. SNYDER: There's very little data there for us to review, to look at, to evaluate the quality of the study and how it was performed. So I had that tagged.

DR. KLAASSEN: When you have to increase tumor growth it's not clear what that even means. Were they bigger?

MS. BECKER: That's pretty much all he said.

DR. SNYDER: And that's kind of consistent -- going to be consistent throughout all the reports. Brevity has gotten too good and so we need to make sure that we have the relevant data in there.

DR. LIEBLER: That's probably not that particular reference on methyl benzoate number 30. I'm looking at the reference list. It's a broadly titled reference from the 1972 Food and Cosmetology -- Food and Cosmetic Toxicology, excuse me, "Toxicological Evaluation of Some Combinations of Food Preservatives." So it doesn't sound like (unintelligible).

MS. BECKER: Yeah, it was.

DR. LIEBLER: I haven't look at the actual reference, but --

MS. BECKER: It was a small study within a larger.

DR. LIEBLER: Yeah. There must be more stuff out there. That's just my hunch.

DR. BELSITO: Okay. So we need more data from the carcinogenicity studies and any additional carcinogenicity studies that are out there.

Are you happy with the reproductive toxicity studies or do we add if there's significant dermal absorption?

DR. SNYDER: That goes to the dermal absorption question.

DR. BELSITO: Right.

DR. SNYDER: Which I think we definitely need more information on dermal absorption.

DR. BELSITO: And if absorbed, we grow a tox?

DR. SNYDER: Right.

DR. BELSITO: Okay. We also don't have concentration of use for the lower molecular weight products which can be irritating.

DR. SNYDER: They can be very important.

DR. BELSITO: So I think we need more information on concentration and use. And at the time I reviewed this I thought we needed sensitization and irritation on the low and high

molecular weights, but the data we've been provided I think covers the higher molecular weights, but we still need -- that's the summary that was just passed out to you -- but we still need information I believe on the lower molecular weight alkyl benzoates in terms of sensitization.

And that was what was on my list basically.

DR. LIEBLER: Table 3 does have concentrations on ethyl benzoate and isobutyl benzoate for some products. They're relatively low, less than.01 percent.

MS. BECKER: Yeah. For the concentration and use, if they're not listed here they're not being used or there was no uses in the response that Carol got.

Also, I believe it's the isobutyl. We also got a thing about that. That's not being used at all. So the 0.01 percent has disappeared and that's -- there's no concentration and use.

DR. LIEBLER: Now, methyl benzoate, the highest reported is.3 percent in some rinse-offs and eye area exposures.

DR. BELSITO: I guess the problem that I had is that, yeah, it said the highest is.3 in rinse-off and then it's an eye area. So what was that? An eye makeup remover?

MS. BECKER: I don't think I have it. I do not have the original study. Okay. Which one was it again? I'm sorry.

DR. BELSITO: It's in exposure type.

MS. BECKER: In methyl benzoate?

DR. BELSITO: It says eye area for methyl benzoate up to.3. And.3 leave-ons we don't have a concentration range. Rinse-offs it's up to.3.

MS. BECKER: Ah, this has changed. In the newest one there is no "eye."

DR. BELSITO: No "eye?"

MS. BECKER: No. You have face and neck creams, but nothing that's eye makeup.

DR. BELSITO: Okay. I think we need to --

MS. BECKER: Yeah.

DR. BELSITO: -- look at that. Also, looking -- going through the report and the introduction, the benzoic acid and sodium benzoate, that will change. Right?

DR. ANDERSEN: Yes. We've changed it. We're eliminating the aerosol.

DR. BELSITO: The restriction on aerosols. So that should be, I think, changed in this report. Do you see where I'm at?

MS. BECKER: Say that again.

DR. BELSITO: Page 1.

MS. BECKER: Yes.

DR. BELSITO: In the introduction where you summarized the ingredients that we previously looked at. Benzoic acid and sodium benzoate have the old conclusion.

MS. BECKER: Yes.

DR. BELSITO: And we decided to change that so there's no inhalation restriction. That should be in there. It's used -- this group of chemicals is used in some hairspray applications so we need the hairspray inhalation boilerplate in the cosmetic use section.

MS. BECKER: Oh, there it is.

DR. BELSITO: And we don't seem to have any concentration and uses for leave-ons. So is this only used in rinse-off products?

MS. BECKER: According to --

DR. BELSITO: It's used in some leave-on products. And when we come to a safe as used conclusion that would restrict it to rinse-offs.

MS. BECKER: Yes. According to the way we have divided up the last version. That's correct.

DR. BELSITO: Okay. So I think though that industry needs to go out and do a survey, make sure it's not being used in leave-ons because if it is then we need some concentration and uses and product types for those leave-ons. And if it's not --

MS. BECKER: We do have leave-ons for the C12-15.

DR. LIEBLER: Yeah, 858.

DR. BELSITO: Yeah, but then in your

concentration and use there are no use concentrations. Right?

MS. BECKER: No.

DR. BELSITO: I mean, if you --

MS. BECKER: Up to 35 and 50 -- leave-on, up to percent.

DR. LIEBLER: Table 3 near the top left-hand column, C12-C15.

DR. BELSITO: Oh, I'm sorry.

DR. LIEBLER: Up to 35.

DR. BELSITO: Up to 35. Yeah, okay, sorry.

DR. LIEBLER: Actually, up to -- oh, yeah. Up to --

DR. BELSITO: Thirty-five.

DR. LIEBLER: -- for leave-on.

DR. BELSITO: And what does Mike have here? Let me look again.

DR. LIEBLER: That's in a non-coloring hair product.

DR. BELSITO: Okay. Paul, Curt, Dan, any other comments?

DR. SNYDER: Well, one of the issues was on page 6. This benzoic acid for the dermal sensitization and the post-toxicity. That reference is to a CIR report. So it's referencing a former CIR report and referring to studies. So we haven't typically done that, said that things have been covered in other reports. But just presented here is simply saying in four studies test for sensitization for benzoic acid were negative. And then referencing the previous CIR report.

DR. BELSITO: But that's -- I mean, otherwise it would be reduplication of data that's already been published.

MS. BECKER: Right. That's the benzoic acid and sodium benzoate report.

DR. BELSITO: Right. Right.

DR. SNYDER: But I think we need to clearly state in there that rather than giving it as a primary reference and we should probably state that --

DR. BELSITO: That seems to be the way we've been doing it now to when the data has already been reported.

DR. LIEBLER: I think maybe the line could be reasonable drawn referencing original data when you want to reference the data and referencing the reports when you want to reference the conclusion or the reasoning in a report. Because you've got a line that summarizes some data and then a reference. You might as well put the original reference there. I mean, it doesn't change the content in a journal article and it doesn't really violate the idea of republishing old stuff. I mean, many papers are cited multiple times and that doesn't create an editorial problem. So I think citing the primary literature when you're trying to cite the data per se is the most appropriate thing to do. And when you're citing inferences or conclusions or what the panel previously concluded then you cite the report.

DR. BRESLAWEC: Okay.

DR. SNYDER: Well, this goes to the little bit about the organization reports. And so in this entire report the italics versus the text that follows is not italicized. It's just virtually identical. And so, I mean, under dermal sensitization italics versus the summary of all of the data, you're saying in four studies tests for sensitization of benzoic acid were negative. And then under benzoic acid, in four studies tests for sensitization were negative.

MS. BECKER: Yeah, because that's a section of the summary that's going to go into the actual summary. It's repeated in the summary. In the final version of the report that we publish, the italics disappears completely. That's just for you to have the original little bit that's there. That's not actually part of the report that we're going to put out to the public in the final version, but it's part -- it's duplicate of the summary at the end.

Anyway, just to point out in the introduction I do say that we are going to summarize benzoic acid and sodium benzoate information. But if you need more data on that

than what's there -- because at this time we haven't decided that it's actually relevant yet.

DR. BRESLAWEC: Do you need clarification on the italics and how they relate to the summary? Okay. In each section there are -- in each major section there is some wording in italics. That exact wording is repeated in the summary section.

DR. SNYDER: I understand that.

DR. BRESLAWEC: When we go to publish this in IJT or when the final report is issued, the italics at the beginning of each section disappears.

DR. SNYDER: Right.

DR. BRESLAWEC: But for the purposes of panel review, you've told us that it's helpful having a brief little summary before the data. So that's why you see it in two places.

DR. SNYDER: Yeah, my only comment was pursuant to the fact that that's -- it's identical. It's abbreviated there. It tells me the studies were all negative. Now what I want to go do is to go see the text part of the data subset that allowed this to state a conclusion. And if it's identical, just a brief statement, it doesn't allow me to interpret the validity of the study, anything about the data. Or how many animals were involved or anything. It doesn't tell me anything.

DR. BRESLAWEC: When it's something that's in the previous CIR report --

DR. SNYDER: Right.

DR. BRESLAWEC: We generally don't duplicate it. And I think that's where the confusion (inaudible).

DR. SNYDER: But I think for us then we need to then -- well, we can go look up the other report and see what that data was and that's okay in that instance. I was just raising that as a query because we're okay with doing that. I mean, I would prefer to see greater sentences that say this was previously considered in CIR report, blah, blah, blah. It's not obvious to the reader unless they go to the reference and then they see it was in a safety assessment document. So there are two issues. One is that.

And the other issue is I'd like to see more data to support that conclusion and I think not just reiterate that it was negative. There's very little data in here for us to be looking at other than conclusion statements.

DR. BELSITO: In some instances the data that was in the original report can be quite extensive. And then you're just --

DR. SNYDER: No, no. I'm okay. I'm okay with that. I'm just saying that maybe it could be stated differently rather than -- when I read this originally, in four studies for sensitization of benzoic acid were negative. And then it's referencing. So I went to look at the reference and I realized it wasn't until I went to the reference list that I realized it was a former CIR report. I think we should have said up front in a previous safety assessment --

DR. BELSITO: I have no problem with that way of phrasing it.

DR. SNYDER: But I think we need to have (overlapping speakers).

DR. BELSITO: Yeah, right.

DR. SNYDER: Yeah, I'm not (overlapping speakers).

DR. BRESLAWEC: I'm still struggling with making that format because (overlapping speakers).

DR. BELSITO: But I don't want to look at all of the individual data --

DR. SNYDER: No.

DR. BELSITO: -- again because if I do want to I can go to the CIR reports for that.

DR. SNYDER: So that's why I said there are two issues here. That's one issue. I think we should be more upfront exactly where that's referenced instead of just referencing numerically.

DR. BELSITO: That's fine.

DR. SNYDER: And then second of all, these -- I appreciate the italics, but then there has to be substance behind the italics in the data

segment.

DR. BELSITO: The other -- the other issue I'd like to bring up, and we talked about this before as we're looking particularly at these very large groups, it would be nice if upfront, whether -- not necessarily as part of the paper, that there be a tick list of things that we generally look at. And then for each chemical in that group, a tick as to whether there's data there or not. And this really was an issue with me with the vegetable and the nut oils. It would have taken me hours to go through and see what data do we have for what specific oil.

And so, you know, we're going to be looking at, you know, log Kow as a measure of whether it's going to penetrate; whether we have absorption data; you know, whether we have sensitization and irritation data; whether we have concentration and use data; whether there are repro studies, carcinogenicity studies, mutagenicity studies. So you know the broad -- and they may not be relevant if they don't absorb, but you know the broad range of things that we want to look at. And a little tick, you know, would really help me immensely because there may be a specific chemical that one of us has a little bit of concern about in the group and it turns out that there's absolutely data on that. And yet we're missing it because there are so many other ingredients and so much other data in the report that it's hard to fathom that out unless you really spend the time.

So that's just one suggestion for all these large groups, is that the report be proceeded by a tick list and we get an idea is the lead ingredient the group, i.e., the group with the most frequently uses, does it really have -- I mean, that's the ingredient that should have the majority of data on it and doesn't. And if not, then how certain are we that the ingredient that we're looking at is similar to that lead ingredient?

DR. LIEBLER: I think that's a good idea, particularly when we've got really complicated groups with many ingredients to follow. And exactly how to do it I'm not sure. But if we think of something we'll act upon it.

DR. BELSITO: Well, you just do a spreadsheet with, you know, the various columns, you know. And it doesn't tell you the quality of the data.

DR. LIEBLER: Right.

DR. BELSITO: But it simply tells you whether there's data there or not.

DR. ANSELL: Yeah. REACH dossiers often open with just such a table.

DR. BELSITO: Right.

DR. ANSELL: And it's got the compounds on one side and the chem points on the other. And you can look at it and see whether there are any big white spots.

DR. BRESLAWEC: Well, we kind of tried to go in that general direction with the use -- concentration and use tables.

DR. BELSITO: Right. So a similar idea. I wouldn't envision that this would be in the final report; it's just a tool for us to use as we look at this. You know, and then it could be a subject of discussion -- the panel noted that there were absolutely zero safety data for these chemicals, however, we felt that, you know, the data that we had was sufficient.

DR. LIEBLER: Right.

DR. SNYDER: Especially if we are going to make read-across arguments we need to be clear when we're making those as opposed to this is based on data on each and every one of these chemicals. That's rarely going to be the case. We're going to have to be making some argument that the data on a vegetable oil with a specific fatty acid composition is reasonably implied to other vegetable oils with maybe slightly different fatty acid compositions and we're okay with that if that's the case. We can't make that assertion if we don't talk about it.

DR. BELSITO: Okay. So to recap on the alkyl benzoates, the Kow suggests that the lower molecular weights could penetrate so we want

dermal penetration data on the lower molecular weights; and if absorbed, dermal reproductive toxicity. We need more information on the carcinogenicity study that's in this document and any additional carcinogenicity studies that are out there. We need sensitization and irritation of the lower molecular weight compounds. And then within the document, not data needs, but just need to put in the update from the new conclusion of benzoic acid and the respiratory boilerplate and the cosmetic use.

Anything that I missed? Okay. So that should take care of the alkyl benzoates.

Moving along to triclosan. Okay. So I think that this is the first time that we're seeing this report. Huge number of studies that are included in this report. And we got a very nice presentation this morning on additional information as to how to approach the conflicting NOAEL values that have been used for this and the -- I think the CIR staff did a very good job in identifying the six critical issues that would go

DR. BERGFELD: Second? Is there a second?

DR. MARKS: Second.

DR. BERGFELD: Any further discussion? None? Okay. Call for the vote. All those in favor of safe as a conclusion? Thank you, unanimous. Going on to the first -- or second green item is alkyl benzoates. Dr. Belsito.

DR. BELSITO: Oh, I'm getting hit twice in a row here. Okay.

DR. MARKS: I'll present our team if you want that.

DR. BELSITO: Okay -- pardon? No. So, this is the first time that we're seeing this report. The literature was -- literature review was issued in June. We're being told at this time that a dossier on C12-15 alkyl benzoates is being prepared as a result of the European REACH program, and that we could get it as early as late September or early October. So, this caused our team to debate whether we should table this to

await that report or proceed with an evaluation with insufficient data needs.

We thought that at this point, since we had reviewed the document, formulated what we thought our needs were, that it would be best -- in the best interest just to proceed with an insufficient data, list the data needs we have. When that dossier is available, it will make it much easier for the staff as well as for us to go into the dossier and look and see if it contains all of the information we would need to reach a safe as use conclusion.

So at this time we're going insufficient for dermal penetration of the lower molecular weight, alkyl benzoates, especially methyl benzoate. And if absorbed, dermal, reproductive, and developmental toxicity.

More information on the carcinogenicity data. There was just a one line sentence saying that methyl benzoate was a potential carcinogen. We need sensitization and irritation of the lower molecular weight alkyl benzoates. We do need to

update the benzoic acid section, which said that there was insufficient data for respiratory use. We have now changed that conclusion and we need to add the boiler -- respiratory boilerplate to the document since there's some inhalation. But that's what we're recommending, insufficient -- primarily dermal penetration, lower molecular weights, information about this carcinogenicity study, and methyl benzoate, sensitization, irritation, the lower molecular weight compounds -- and that's it.

DR. MARKS: We second the motion for insufficient data announcement. Our team also went through the same thought process and our needs were similar in terms of alerting the writers what we would be looking for in industry in the REACH document.

The only other potential concerns -- Ron Hill also was wanting data on the alcohols, if I recall correctly. Ron, you can elaborate on that. I was concerned if there was absorption, was there metabolism to benzoic alcohol and acid and if so,

then what was the induction of contact or the carrier of these ingredients.

DR. BERGFELD: Ron Shank, did you want to comment on any lipid soluble dermal absorption testing or?

DR. SHANK: Well, you're asking -- yeah. I had not only the low molecular weight ones, but also I recommended C12, C15 benzoate absorption study.

DR. BELSITO: Okay.

DR. BERGFELD: Are there any additions to the want list or the needs list? Alan, do you understand what's been asked or should we have it repeated?

DR. ANDERSEN: Well, I couldn't repeat it back to you right now, but the good news is we're transcribing it all and we'll take it off of the -- directly off of what all of you said.

The expectation remains that with a robust submission to REACH, which we are expecting, that all of these issues will be targeted. It's just I appreciate that the panel went through the effort to list the things that were obviously going to be important. And if you don't see them in subsequent submissions, you need to call.

DR. BERGFELD: Dr. Bailey, do you wish to comment?

DR. BAILEY: No, the only thing I would add is that, you know, the REACH data being available this fall is our expectation. But we can't guarantee that, so we'll certainly, you know, do our best.

DR. BERGFELD: Thank you. With all those comments, then, I will call for the vote. All those in favor of going insufficient with the listed needs, please indicate by raising your hands? Thank you, unanimous.

Moving on to the next document, which is in Pink. The CAPB, Dr. Marks reporting.

DR. MARKS: As you recall, this report was reopened particularly because the concern that impurities within cocamidopropyl betaine were sensitizers and we have tried to come to a

REPORT

Draft Tentative Report

Alkyl Benzoates as Used in Cosmetics

November 18, 2010

The 2010 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Lillian Becker, Scientific Analysts/Writer, CIR.

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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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INTRODUCTION

This is a safety assessment of alkyl benzoate esters that are used in cosmetics mostly as skin-conditioning agents, preservatives, solvents, and plasticizers. The ingredients included in this literature review are: methyl benzoate, ethyl benzoate, propyl benzoate, butyl benzoate, amyl benzoate, lauryl/myristyl benzoate, C12-15 alkyl benzoate, C16-17 alkyl benzoate, stearyl benzoate, behenyl benzoate, isopropyl benzoate, isobutyl benzoate, isostearyl benzoate, ethylhexyl benzoate, butyloctyl benzoate, hexyldecyl benzoate, and octyldodecyl benzoate.

The alkyl benzoate ingredients are esters of benzoic acid and a corresponding alcohol, with the shorter chain alkyl benzoates (methyl, ethyl, propyl, isopropyl, butyl, isobutyl and amyl benzoate) ranging in MW from 136 to 192 and the longer chain alkyl acetates (lauryl/myristyl, C12-15 alkyl, C16-17 alkyl, stearyl, isostearyl, behenyl, ethylhexyl, butyloctyl, hexyldecyl, and octyldodecyl benzoate) ranging in MW from 234 to 431.

There is minimal information as to whether the smaller alkyl benzoates in this report penetrate the skin. If they do, these compounds will be metabolized in the skin to release benzoic acid and the parent alcohol. Since penetration is possible, the safety of these metabolites must be considered when assessing the safety of alkyl benzoates.

Several of the metabolites of the alkyl benzoates in this assessment have been reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel (benzoic acid, sodium benzoate, and the parent alcohols: methyl alcohol, ethyl alcohol, butyl alcohol, myristyl alcohol, behenyl alcohol, isostearyl alcohol). The conclusions are listed below. These conclusions may be relevant if significant penetration and metabolism do occur.

Benzyl alcohol, benzoic acid and sodium benzoate – The conclusion currently states that these ingredients are safe for use in cosmetic formulations at concentrations up to 5%.[1]

Methyl alcohol - safe for use as a denaturant in ethyl alcohol for cosmetic products, with qualifications. The Panel has not stated that methyl alcohol is safe or unsafe as a solvent.[2]

Ethyl alcohol – (as "Alcohol Denat." with methyl alcohol) - safe in the present practices of use and concentration.[3]

Butyl alcohol - safe as a cosmetic ingredient in the present practices of use.[4] In 2005, the panel looked at new data and the safety conclusion in the report was confirmed.

Myristyl alcohol - safe as a cosmetic ingredient in the present practices of use.[5]

Cetyl alcohol - safe as a cosmetic ingredient in the present practices of use.[6] In 2005, the panel looked at new data and the conclusion in the report was confirmed.

Stearyl alcohol - safe as currently used in cosmetics.[7] In 2006, the panel looked at new data and the conclusion in the report was confirmed.

Isostearyl alcohol - safe as cosmetic ingredients in the present practices of use. [6] In 2005, the panel looked at new data and the conclusion in the report was confirmed.

Behenyl alcohol - safe as a cosmetic ingredient in the present practices of use.[5] In 2005, the panel looked at new data and the conclusion in the current report was confirmed.

Propyl alcohol and isopropyl alcohol – safe for use in cosmetic products in the present practices of use and concentration.[8]

The potential metabolites of ethylhexyl benzoate, butyloctyl benzoate, hexyldecyl benzoate, isobutyl benzoate, amyl benzoate, pentadecyl benzoate, heptadecyl benzoate, and octyldecyl benzoate are not current cosmetic ingredients in the dictionary, thus have not been reviewed by CIR.

Some data from the reports on benzoic acid, sodium benzoate, methyl alcohol, ethyl alcohol, butyl alcohol, myristyl alcohol, cetyl alcohol, stearyl alcohol, isostearyl alcohol, behenyl alcohol, propyl alcohol, and isopropyl alcohol are summarized. Data on the other alcohols as well as ethylhexyl alcohol (from Belsito et al. 2010)[9] are included to give a sense of the safety of these possible metabolites.

CHEMISTRY

Definition and Structure

Alkyl benzoates are mostly used as skin-conditioning agents, preservatives, solvents, and plasticizers. The CAS numbers, definitions, functions, as well as technical and trade names of the ingredients under review are presented in Table 1. Structures and potential metabolic pathways of these ingredients are presented in Figures 1 and 2.

Physical and Chemical Properties

The shorter chain alkyl benzoate esters are colorless liquids. Viscosity generally increases as the molecular mass (chain length) increases.[10] The physical and chemical properties of the benzoates are shown in Table 2.

At room temperature and pressure, methyl benzoate, ethyl benzoate, butyl benzoate, and isobutyl benzoate are fragrant, colorless oils, and are insoluble in water.[11]

A UV absorption spectrum or C12-15 alkyl benzoates had peaks at ~200 and 235 nm.[12]

Manufacture and Production

In general, the alkyl benzoates can be produced industrially via esterification of benzoic acid.[10] The manufacture of butyl benzoate, for example, is traditionally accomplished via an acid catalyzed (e.g., sulfuric acid) reactive distillation process between benzoic acid and butyl alcohol (Figure 3).[13]

Methanol and ethanol are normally obtained via fermentation of natural sources. However, some alcohols with chains longer than ethanol are often produced synthetically. An important process for producing C_3- C_{22} industrial alcohols involves a process known as oxo-synthesis (a process for the production of aldehydes which occurs by the reaction of olefins (which can be natural or petroleum sourced) with carbon monoxide, hydrogen and a catalyst [typically cobalt based]), followed by hydrogenation of the aldehyde products, to form the alcohols.[14] Recently, a biocatalytic process developed specifically for the manufacture of esters for use in the formulation of cosmetic and personal care ingredients (i.e. for producing cosmetic grade esters) was developed in 2004.[15]

Impurities

The manufacturing processes of the benzoic esters are typically high yielding (≥90%) and easily purified (e.g., by distillation). Therefore, the starting materials and water, at least, may be expected to be present in preparations of these esters as the major impurities.[10] For example, methyl benzoate is available with a minimum of 99.2% purity, wherein the major contaminants are water (≤0.1%) and benzoic acid (≤0.02%).[16]

Analytical Methods

The benzoic esters can be analyzed using gas chromatography/mass spectroscopy (GC/MS), nuclear magnetic resonance (NMR) spectroscopy, ultraviolet (UV) spectroscopy and infrared (IR) spectroscopy.[10,14,17]

USE

Cosmetic

According to the Voluntary Cosmetic Registration Program (VCRP) administered by the Food and Drug Administration (FDA), the total number of uses of C12-15 alkyl benzoate was 971 (858 leave-on and 113 rinse-off products).[18] A survey conducted by the Personal Care Products Council (Council) found that C12-15 alkyl benzoate was used at 0.0008% - 59% (highest concentration in tonics, dressings, and other hair grooming aids) in leave-on products and 0.0008% - 50% (highest concentration in paste masks [mud packs]) in rinse-off products (Table 3).[19] There were 2 uses reported of C16-17 alkyl benzoates at 0.7% (bath soaps and detergents). Stearyl benzoate at 2% was reported to have 3 uses (including face and neck creams, lotions, and powders). While there were no uses reported by VCRP, the Council reported methyl benzoate use at 0.0005% – 0.3% (highest concentration in perfumes), ethyl benzoate use at 0.0008% - 0.01% (highest concentration in foot powders and sprays), isobutyl benzoate use at 0.01% (perfumes), isostearyl benzoate use at 1% (body and hand creams, lotions, and powders), and octyldodecyl benzoate at 3% - 4% (highest concentration in shaving cream). No uses or concentrations of use were reported for propyl benzoate, butyl benzoate, amyl benzoate, lauryl/myristyl benzoate, behenyl benzoate, isopropyl benzoate, ethylhexyl benzoate, butyloctyl benzoate, and hexyldecyl benzoate.

C12-15 Alkyl benzoate and other benzoates are used in hair sprays and perfumes, and effects on the lungs that may be induced by aerosolized products containing these ingredients are of concern.

The aerosol properties that determine deposition in the respiratory system are particle size and density. The parameter most closely associated with deposition is the aerodynamic diameter, d_a, defined as the diameter of a sphere of unit density possessing the same terminal settling velocity as the particle in question. In humans, particles with an aerodynamic diameter of ≤ 10µm are respirable. Particles with a d_a from 0.1 - 10µm settle in the upper respiratory tract and particles with a d_a < 0.1 µm settle in the lower respiratory tract.[20,21]

Particle diameters of 60-80 µm and ≥80 µm have been reported for anhydrous hair sprays and pump hairsprays, respectively.[22] In practice, aerosols should have at least 99% of their particle diameters in the 10 – 110 µm range and the mean particle diameter in a typical aerosol spray has been reported as ~38 µm.[23] Therefore, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.

In the EU, methyl benzoate, ethyl benzoate, propyl benzoate and butyl benzoate may be used as preservatives in

cosmetics up to 0.5% (acid).[24]

Non-Cosmetic

Alkyl benzoate esters are typically used as solvents in paints, lacquers and coatings, and as intermediates in various chemistry processes.[10] Methyl benzoate is used in flavoring and perfumery, and as a solvent in resins.[25] Ethyl benzoate is used in flavoring and perfumery, and as a solvent in lacquers and resins.[25] Butyl benzoate is used as a solvent for cellulose ether, as a plasticizer, as a perfume ingredient, and for dyeing of textiles.[25] Isobutyl benzoate is used in flavoring and perfumery.[25] Methyl benzoatae, ethyl benzoate, propyl benzoate, isopropyl benzoate, and isobutyl benzoate have been approved by the FDA as flavors.[26]

GENERAL BIOLOGY

Benzoate esters are metabolized into benzoic acid (and the corresponding alcohols) and further metabolized to benzyl glucuronide and benzoyl CoA. Benzoyl CoA metabolizes into hippuric acid, the principal metabolite excreted in the urine. Dermally applied benzoic acid is excreted in the urine within 24 h. Methyl benzoate, ethyl benzoate, propyl benzoate butyl benzoate, and C12-15 alkyl benzoate are not expected to penetrate skin.

Methyl alcohol and ethylhexyl alcohol permeated the skin or nail plates. Aerosolized lauryl alcohol caused mild dyspnea and scattered hemorrhagic areas in the lungs of rats.

Absorption, Distribution, Metabolism, and Excretion

Orally administered benzoate esters are metabolized to benzoic acid and the corresponding alcohols and the acid is further metabolized to benzyl glucuronide and benzoyl CoA.[27] The benzoyl CoA metabolizes to hippuric acid, which is the principal metabolite excreted in the urine.

In general, esters can be hydrolyzed to the parent alcohol and acid under physiologic conditions by carboxylesteases or esterases that are found throughout mammalian tissues, most concentrated in hepatocytes.[10,27] The rate of this reaction can be increased by raising the temperature and decreased by lowering pH. Secondary and tertiary esters are hydrolyzed more slowly than primary esters. Enzymatic hydrolysis occurs via enzymes called esterases. These enzymes are in essentially all tissues, including the respiratory tract, skin, and gastrointestinal tract and in blood.[28,29]

Data on benzyl alcohol show that it is converted to benzoic acid by simple oxidation.[1] Orally consumed benzoic acid is absorbed from the gastrointestinal tract and conjugated with glycine in the liver. The resulting hippuric acid is excreted in the urine (75% - 100% within 6 h). Dermally applied benzoic acid is also excreted in the urine within 24 h.

In general, alcohols can be metabolized by alcohol dehydrogenases to aldehydes or ketones. The aldehydes may be further metabolized by aldehyde dehydrogenases to the corresponding acids.

Benzoic esters are not absorbed through the skin as rapidly as alkyl esters.[30] If alkyl benzoates are absorbed and metabolized, the alcohols resulting from ester hydrolysis can be oxidized via alcohol dehydrogenases to produce the corresponding aldehyde or ketone. As noted above, these aldehydes can be further oxidized via aldehyde dehydrogenases to the corresponding acids.

Alkyl Benzoates

The permeation of methyl benzoate, ethyl benzoate, *n*-propyl benzoate, and *n*-butyl benzoate through excised guinea pig dorsal skin was measured using diffusion cells.[31] The permeability coefficients (K_p) were 20.3 ± 5.8, 34.08 ±1.2, 62.7 ± 13.0, and 79.9 ± 10.1 x 10^{-2} cm/h, respectively. Permeability was increased by removal of the stratum corneum by tape stripping and delipidization using a chloroform-methanol mixture. Permeability was decreased by the addition of *l*-methanol plus 15% ethanol.

Using a penetration cell, the partition coefficient of C12 alkyl benzoate, C13 alkyl benzoate, C14 alkyl benzoate, and C15 alkyl benzoate in 3 product formulations were 8.0, 8.6, 9.1, 9.6 (pH 3), respectively.[32] The amounts of C12-15 alkyl benzoate (7.5 ± 0.5%) from a sun lotion measured in the gently shaved skin of female pigs after 24 h was 93.5%, 6.5%, < 0.05%, and < 0.05% in the horny layer, epidermis, dermis, and receptor fluid, respectively. For a "baby micropigmentcreme" (5.4 ± 0.3% C12-15 alkyl benzoate), the amount of recovered test substance was 91.5%, 8.6%, < 0.07%, and < 0.07%, and for a "protection spray" (6.6 ± 0.3% C12-15 alkyl benzoate), the amount of recovered test substance was 92.5%, 7.5%, < 0.05%, and < 0.05%, respectively.

In a second penetration cell experiment, the partition coefficient of C12 alkyl benzoate, C13 alkyl benzoate, C14 alkyl benzoate, and C15 alkyl benzoate (100%) were found to be the same as above.[33] The amounts of test substance measured in the gently shaved skin of female pigs after 24 h was 84%, 5%, 11%, and none detected in the horny layer, epidermis, dermis, and receptor fluid, respectively.

Alcohols

The permeability constants for methyl alcohol were 0.3, < 0.1, 12.0, 3.0, and 2.5 K_p x 10^3 cm/h in saline, polyethylene glycol 600, isopropyl palmitate, olive oil, and mineral oil, respectively.[34]

The permeability coefficient of methyl alcohol was 5.6 ± 1.2 x 10^3 cm/h through the nail plates of cadavers.[35]

Male Fischer F344 rats (n = 3) were orally administered [13C]-tert-amyl alcohol (250 mg/kg in corn oil) and urine was collected for 48 h.[36] The major metabolites were tert-amyl alcohol glucuronide and 2-methyl-2,3-butanediol and its glucuronide. Free tert-amyl alcohol, 2-hydroxy-2-methylbutric acid, and 2-hydroxy-3-methylbutyric acid were minor metabolites.

Isopropyl alcohol was absorbed in four groups of rabbits (n = 3; strain not specified) exposed to the alcohol by gavage (Group 1: 2 ml/kg; Group 2: 4 ml/kg), whole-body/inhalation combined with dermal application (Group 3: 70% isopropyl alcohol soaked towel applied to the chest), and whole-body/inhalation combined with application over a plastic barrier on the chest.[37] Maximum isopropyl alcohol concentrations in blood after oral exposures were 147 ml/dl (2 ml/kg) and 282 mg/dl (4 ml/kg), which were correlated with inebriation and near coma; the blood concentration was 112 mg/dl 4 h after whole-body/inhalation combined with dermal application. Blood concentrations of acetone (metabolite of isopropyl alcohol) were 74 mg/dl (2 ml/kg by gavage), 73 mg/dl (4 mg/kg by gavage), 19 mg/dl (whole-body/inhalation plus dermal application), and <10 mg/dl (whole-body/inhalation plus application over a plastic barrier). The authors concluded from their results that significant toxicity would require repeated sponging or soaking with isopropyl alcohol for several hours.

In vitro absorption rates for ethylhexyl alcohol through rat and human skin were 0.22 ± 0.09 and 0.38 ± 0.014 mg/cm2/h, respectively.[38] The corresponding permeability constants were 2.59 ± 1.10 x 10^{-4} cm/h for rat skin and 4.54±1.66 x 10^{-5} for human skin.

The absorption rate for ethylhexyl alcohol (1000 mg/kg) applied to the skin of rats for 6 hours was 0.57 mg/cm^2/h; 5.2% of the dose was absorbed during exposure.[39]

Cytotoxicity

Methyl benzoate was cytotoxic to HeLa cells, A. flavus, A. parasiticus, *and lung fibroblasts. Ethyl benzoate was cytotoxic to Hep-2 cells and lung fibroblasts. Propyl benzoate and butyl benzoate were cytotoxic to Hep-2 cells. C12 – 15 alkyl benzoate was cytotoxic to human derived epidermal keratinocytes.*

Methyl alcohol, amyl alcohol, and dodecyl alcohol were cytoxoic.

Methyl Benzoate

In a protein count assay of methyl benzoate, the EC_{50} (50% of the concentration of maximum effect) was 1506.58 (C.I. 1349.27 - 168.22) mM, the NI_{50} (the concentration that reduced the uptake of neutral red by 50%) was 683.30 (466.46 – 1000.91) mM in a neutral red uptake assay, and the ID_{50} (the concentration that inhibited growth by 50%) was 987.19 (605.15-1610.43) mM in a growth inhibition assay using HeLa cells.[40]

Methyl benzoate (2.5 and 5.0 mg/ml) inhibited mycelia growth and aflatoxin release by *Aspergillis flavus* and *A. parasiticus*.[41]

Human diploid embryonic lung fibroblasts (line MRC-5), labeled with [^{3}H]uridine, were incubated in methyl benzoate (25 mM in buffered saline) for 30 min.[42] The amount of cell wall damage was measured by the release of the label. Controls released 3% to 6% of the maximum available label. Incubation in methyl benzoate caused a release of 20% of maximum available label. The authors concluded that methyl benzoate not only caused toxic effects to the cells but also promoted membrane penetration by other substances.

Ethyl Benzoate

Human Hep-2 cells (epithelial cell line derived from human carcinoma of the larynx) were exposed to ethyl benzoate.[43] Total inhibition of cell growth was observed at 500 mg/L. This experiment was repeated and samples were taken for protein determination. There were no effects at 289 mg/L. This experiment was repeated and the cells were stained and examined for morphology. At 289 mg/L, cells lost their typical epithelial shape and became elongated.

The above experiment on human diploid embryonic lung fibroblasts (line MRC-5), labeled with [^{3}H]uridine, was repeated with ethyl benzoate (25 mM).[42] Incubation in ethyl benzoate caused a release of 31% of maximum of available label. The authors concluded that ethyl benzoate not only caused toxic effects to the cells but also promoted membrane penetration by other substances.

Propyl Benzoates

Human Hep-2 cells were exposed to propyl benzoate.[43] Total inhibition of cell growth was observed at 200 mg/L. This experiment was repeated and samples were taken for protein determination. Effects were observed at 122 mg/L and the

cells seemed to recover by day 7. This experiment was repeated and the cells were stained and examined for morphology. At 122 mg/L, the monlayer of the cells was disturbed within 24 h.

Butyl Benzoate

Human Hep-2 cells were exposed to butyl benzoate.[43] Total inhibition of cell growth was observed at 100 mg/L. This experiment was repeated and samples were taken for protein determination. Effects were observed at 61 mg/L and the cells seemed to recover by day 7. This experiment was repeated and the cells were stained and examined for morphology. At 61 mg/L, the monlayer of the cells was disturbed within 24 h.

Human Rhino HeLa cells was incubated in butyl benzoate (in dimthylsulfoxide) for 48 h. The IC_{50} was 0.5 mM.[44]

Alcohols

Methyl alcohol had a 50% production inhibition (PI_{50}) of 1614 mM for Hep G2 cells.[45]

Methyl alcohol was toxic to yeast cells (strain *ade6-60/rad10-198,h*$^-$ of *Schizosaccharomyces pombe* P_1 strain) at 0.05% but not V79 Chinese hamster cells up to 10%.[46] Amyl alcohol was toxic to yeast cells (strain *ade6-60/rad10-198,h*$^-$ of *S. pombe* P_1 strain) and V79 Chinese hamster cells at 0.5%.[46]

Dedecyl alcohol had 50% lysis of human erythrocytes at 15 µM.[47]

TOXICOLOGY

Acute Toxicity

The oral LD_{50} of methyl benzoate was 2170 mg/kg for rabbits, 4100 mg/kg for guinea pigs, 1350-3500 for rats, and 3000-3330 mg/kg for mice. The oral LD_{50} of ethyl benzoate was 2630 mg/kg for rabbits and 2100-6480 mg/kg for rats. The oral LD_{50} for isopropyl benzoate was 3730 mg/kg and 3685 mg/kg for isobutyl benzoate in rats. The dermal LD_{50} of methyl benzoate was ≥ 2000 mg/kg for rabbits. Dermally administered ethyl benzoate at 10% caused no effects to mice and calves; at 100% it was lethal to cats. Dermally administered butyl benzoate caused diarrhea in rabbits. C12-15 alkyl benzoate was not dermally toxic to rabbits. The dermal LD_{50} of isopropyl benzoate was 20 mg/kg for rabbits. Isobutyl benzoate was not dermally toxic to rabbits.

The oral LD_{50} of benzoic acid was reported to be 1996 mg/kg in mice and 2000 – 2500 mg/kg in rats. The oral LD_{100} was reported to be 1520 – 2000 mg/kg for rabbits, and 2000 mg/kg for cats and dogs. The oral LD_{50} for sodium benzoate was 2100 – 4070 mg/kg for rats and 2000 mg/kg for rabbits and dogs.

Methyl alcohol has an oral LD_{50} of 5628 mg.kg for rats and 7300 mg/kg for mice. The oral LD_{50} of amyl alcohol for rats was reported to be 2.69 g/kg. Dodecyl alcohol has an oral LD_{50} of 12,800 mg/kg for rats. Tridecyl alcohol has an oral LD_{50} of 17,200 mg/kg for rats. Tetradecl alcohol has an oral LD_{50} 33,000 mg/kg for rats. Oral LD_{50}s for ethylhexyl alcohol in rats range from 2049 to 7100 mg/kg and 2380 to >5000 mg/kg for rabbits. The oral LD_{50} of hexyldecyl alcohol for rats was reported to be > 8.42 g/kg. The dermal LD_{50} of methyl alcohol was reported to be 15,800 mg/kg in rabbits. The dermal LD_{50} of amyl alcohol for rabbits was reported to be > 3.2 g/kg. The dermal LD_{50} of dodecyl alcohol was reported to be 3560 mg/kg in rabbits. The dermal LD_{50} of tridecyl alcohol was reported to be 5600 mg/kg in rabbits. The dermal LD_{50} of hexadecyl alcohol for rabbits was reported to be > 2.6 g/kg. Aerosolized amyl alcohol caused irritation of the eyes, nose, throat, and respiratory passages of mice and guinea pigs. Aerosolized ethylhexyl alcohol was not toxic to rats.

Methyl Benzoate

The reported oral LD_{50} of methyl benzoate was 2170 mg/kg for rabbits, 4100 mg/kg for guinea pigs, 1350-3500 for rats, and 3000-3330 mg/kg for mice.[48-51]

The dermal LD_{50} of methyl benzoate was ≥ 2000 mg/kg for New Zealand white rabbits (n = 5).[52] There was fecal staining for 3 days after treatment. Irritation was observed at the application site. There was weight loss for 1 – 7 days after treatment. There were no gross findings at necropsy. There were no mortalities.

Ethyl Benzoate

The reported oral LD_{50} of ethyl benzoate was 2630 mg/kg for rabbits, 2100 mg/kg for rats and 6480 mg/kg for female rats.[48,51]

Ethyl benzoate (10% - 100% in acetone) was administered to one-third of the body surface of mice (n = 2 – 4).[53] The mice were observed for 24 h then necropsied. There were no effects at 10%. This experiment was repeated with calves (with no necropsy) except covering the entire body surface and a 15-d observation period. There were no effects at 10%. No further details were provided.

Ethyl benzoate (up to 100% in "various vehicles"; 20 ml) was administered to the clipped backs of cats (n = 2), massaged into the skin with cotton balls.[48] The cats were to be observed for 2 weeks. At 100%, both cats died within 20 h.

Albino rats (n = 6) showed no adverse effects from exposure to aerolized ethyl benzoate ("approaching saturation")

for 8 h.[51]

Intramuscular administration of ethyl benzoate (100%; 0.5 or 1.0 ml) intramuscularly administered to guinea pigs (n not provided) caused musculo-skeletal [sic], moderate deterioration of leg function and muscle toughness at 1.25 ml/kg.[54]

Butyl Benzoate

The reported oral LD_{50} of butyl benzoate was 5.14 g/kg for female rats.[51]

Dermally administered butyl benzoate (5 g/kg) caused no mortalities in rabbits (n =10).[55] Diarrhea was observed during the 14 day observation period.

Intramuscular administration of butyl benzoate (100%; 0.5 or 1.0 ml) intramuscularly administered to guinea pigs (n not provided) caused musculo-skeletal [sic], moderate deterioration of leg function and muscle toughness at 3 ml/kg.[54]

Albino rats (n = 6) showed no adverse effects from exposure to aerosolized butyl benzoate ("approaching saturation") for 8 h.[51]

C12-15 Alkyl Benzoate

Albino rats orally administered C12-15 alkyl benzoate (5.0 g/kg) exhibited no signs of toxicity over a 14-day observation period.[56] At necropsy, enlarged spleens were noted.

In an acute oral study, C12-15 alkyl benzoate (up to 100%; 40 ml/kg) was administered to albino rats.[57] The rats were observed for 14 days and then killed and necropsied. There was no mortality during the observation period. There were no gross internal changes.

At 30.0 g/kg, the females were described as having slight depression up to day 7. At 24 h, loss of ventral body hair and crusty, scabby skin were noted. There were no gross internal changes. At 33.0 g/kg, hair was matted and unkempt, there was a crust-like substance on the skin, and hair loss. One female rat died on day 7. At 37.0 g/kg, 5 rats died. Pyloric and intestinal mucosa were reddened; lung tissue was enlarged and consolidated; and spherical lesions were observed in the lungs.

Female MNRI EOPS mice were orally administered 5000 mg/kg C12-15 alkyl benzoate and observed for 6 days.[58] There were no mortalities and no clinical signs or behavior were observed. Weight gain was comparable to controls.

In a dermal toxicity study, C12-15 alkyl benzoate (100%; 2 g/kg) was applied to the intact skin of albino rabbits (n = 6; 3/sex) under occlusion.[57] The rabbits were observed for 14 days. One male rabbit died which was considered non-treatment related. The authors concluded that C12-15 alkyl benzoate was not a toxic material.

Albino Wistar rats (n = 10) were exposed to aerosolized C12-15 alkyl benzoates (200 mg/L) for 1 h and observed for 2 weeks.[59] There were no toxic effects observed.

Isopropyl Benzoate

The reported oral LD_{50} of isopropyl benzoate was 3730 mg/kg and 3.7 ml/kg for rats.[60,61]

The reported dermal LD_{50} of isopropyl benzoate was 20 ml/kg for rabbits.[61] Dermally administered isopropyl benzoate (5 ml/kg) had no effects to rabbits (n = 2).[60]

There were no observed effects in rats exposed to aerosolized isopropyl benzoate (saturated vapor) for 4 h.[61]

Isobutyl Benzoate

The reported oral LD_{50} of isobutyl benzoate was 3685 mg/kg[27] and 3.7 ml/kg for rats (n = 10).[60]

Isobutyl benzoate (5 ml/kg; 100%) was applied to the intact and abraded clipped skin of albino rabbits (n = 4) for 24 h. There were no mortalities or clinical signs during the 14-day observation period.[60]

Benzoic Acid and Sodium Benzoate

The oral LD_{50} of benzoic acid was reported to be 1996 mg/kg in mice and 2000 – 2500 mg/kg in rats. The oral LD_{100} was reported to be 1520 – 2000 mg/kg for rabbits, and 2000 mg/kg for cats and dogs. The oral LD_{50} for sodium benzoate was 2100 – 4070 mg/kg for rats and 2000 mg/kg for rabbits and dogs.[62]

Alcohols

Methyl alcohol has an oral LD_{50} of 5628 mg/kg for rats and 7300 mg/kg for mice.[63]

The dermal LD_{50} of methyl alcohol was reported to be 15,800 mg/kg in rabbits.[64]

Methyl alcohol has an i.p. LD_{50} of 336 (CI = 299, 373) for mice, 237 (222,252) mmol/kg for rats, 267 (235, 304) mmol/kg for hamsters, and 111 mmol/kg for guinea pigs.[65] The i.v. LD_{50} for mice is 147 (126, 171) mmol/kg, 66.5 (61.5, 71.2) mmol/kg for rats and 278 (185, 371) mmol/kg for rabbits.

The oral LD_{50} of amyl alcohol for Sprague-Dawley rats was reported to be 2.69 g/kg.[66] Deaths occurred within 24 h. Necropsy revealed evidence of gastrointestinal irritation and pooling of blood.

The dermal LD_{50} of amyl alcohol for albino rabbits was reported to be > 3.2 g/kg.[66] There were signs of central nervous system depression. Recovery occurred within 4 to 48 h. Another source reported the dermal LD_{50} for rabbits to be 3600 mg/kg

Swiss mice, Wistar rats, and English short hair guinea pigs (n = 10) were exposed to aerosolized amyl alcohol for 6 h the observed for 14 h.[66] Preconvulsive movements were observed in mice and guinea pigs; the rats tended more toward prostration. Two rats and 7 mice died during exposure. All surviving animals recovered shortly after termination of the exposure. Some animals had irritation of the eyes, nose, throat, and respiratory passages.

Dodecyl alcohol has an oral LD_{50} of 12,800 mg/kg for rats.[63]

The dermal LD_{50} of dodecyl alcohol was reported to be 3560 mg/kg in rabbits.[64]

Tridecyl alcohol has an oral LD_{50} of 17,200 mg/kg for rats.[63]

The dermal LD_{50} of tridecyl alcohol was reported to be 5600 mg/kg in rabbits.[64]

Ssc: CF-1 mice (n = 10) were exposed to *tert*-pentadecyl alcohol (2800 – 14000 ppm), with and without tracheal cannulation, after anaesthetization for 30 min.[67] Sensory irritation of the upper respiratory tract was measured by timing the pauses before exhalation compared with those of untreated controls (n = 37). Stimulation of pulmonary receptors by airborne irritants was measured by the decrease in respiratory rate caused by a pause between the end of expiration and the beginning of the following inspiration, resulting in a net decrease in respiratory rate.

The characteristic sensory irritation pattern was observed in the mice immediately after the onset of the exposure. The pattern was most evident within the first minute and was followed by rapid fading of the responses. The pattern was occasionally seen during the entire exposure period. After a decrease in respiratory rate in the first minute of exposure, the rate partly increased in the next minutes followed by a new slowly progressing decrease. A concentration-dependent recovery of the respiratory rate was seen after cessation of the exposure. In cannulated mice no sensory irritation pattern was observed. The authors stated that this was due to bypass of the trigeminal nerves. The pattern indicated that pulmonary irritation was present.

Myristal alcohol has an oral LD_{50} 33,000 mg/kg for rats.[63]

Oral LD_{50}s for ethylhexyl alcohol in rats range from 2049 to 7100 mg/kg body weight and 2380 to >5000 mg/kg for rabbits.[9]

Rats, mice, and guinea pigs (n = 10 per species) exposed (whole body) to air bubbled through ethylhexyl alcohol for 6 h exhibited signs of irritation of the eyes nose, throat, and respiratory passages, including blinking, lacrimation, nasal discharge, salivation, gasping, and chewing movements, but none died.[66,68]

None of the 6 rats inhaling concentrated ethylhexyl alcohol for up to 8 hours died.[69]

The oral LD_{50} of hexyldecyl alcohol for Sprague-Dawley rats was reported to be > 8.42 g/kg.[66]

The dermal LD_{50} of hexadecyl alcohol for albino rabbits was reported to be > 2.6 g/kg.[66] There were signs of central nervous system depression. Recovery occurred within 4 to 48 h.

Swiss mice, Wistar rats, and English short hair guinea pigs (n = 10) were exposed to aerosolized hexyldecyl alcohol (1060 ppm; 9.6 mg/m^3) for 6 h and observed for 14 h.[66] The alcohol was a slight irritant and no systemic effects were observed.

Repeated Dose Toxicity

Benzoic acid and sodium benzoate were orally toxic to rats and mice in short-term feeding studies at concentrations > 1% in short-term, subchronic, and chronic studies. In Subchronic studies, benzoic acid was toxic to mice at oral doses of 80 mg/kg/d. Sodium benzoate at 880 mg/kg/d incorporated into the feed of rats for 18 – 24 months was not toxic.

Short-term oral exposure to amyl alcohol was not toxic to rats at 100%. Oral NOAELs ranged from 100 to 150 mg/kg in several studies using mice or rats exposed to ethylhexyl alcohol. Dermal exposure to ethylhexyl alcohol caused physiological changes in rats at 500 mg/kg. Inhalation of isobutyl alcohol caused reversible inhibition of responsiveness in rats. Adverse effects of isopropyl alcohol at the LOAEL included clinical signs in rat and mice, hematological changes in rats, and increased liver weights in mice; higher doses caused kidney and testicular effects. Aerosolized n-pentadecyl alcohol was not toxic to rats.

Benzoic Acid and Sodium Benzoate

In multiple-dose oral/feed toxicity studies on rats and mice, decreased feed consumption, depressed growth, and toxic effects were observed at concentrations > 1% benzoic acid or sodium benzoate (Table 4).[1]

Cross bred white mice (n = 100) were orally administered benzoic acid at 80 mg/kg/d for 3 months.[70] The treated group had decreased weight gain. Mortality was increased (68% vs. 60 % in the control group).

Sodium benzoate (0, 1%, or 2%; 735 or 880 mg/kg/d) was incorporated into the feed of Fischer 344 rats (n = 102; 50 males, 52 females) for 18 – 24 months.[71] There were no differences in mortality between groups. Necropsies were unremarkable.

Alcohols

Amyl alcohol (0, 50, 150, 100 mg/kg/d) was administered orally to ASH/CSE rats (n = 30; 15/sex) for 13 weeks.[72] The rats were killed and necropsied 24 h after the last treatment. No adverse effects were observed at any dose.

The NOAEL and LOAEL for the inhalation of isopropyl alcohol was 1230 mg/m^3 and 3690 mg/m^3, respectively, in Fischer 344 rats and CD-1 mice (n = 10/sex) exposed (0, 1230, 3690, or 12,300 mg/m^3) 6 h/d, 5 d/week for 13 weeks.[73] Adverse effect observed at the LOAEL included clinical signs in rats and mice, hematological changes in rats, and increased liver weights in mice.

A NOAEL of 1230 mg/m^3 for both rats and mice was reported (based on kidney and testicular effects) in a study in which Fischer 344 rats (n = 65/sex) and CD-1 mice (n = 55/sex) were exposed by inhalation to isopropyl alcohol (0, 1230, 6150, or 12,300 mg/m3) for 6 h/d, 5 d/week for 104 weeks in rats and 78 weeks in mice.[74]

All of the rats (n = 10) exposed to isobutyl alcohol (0, 770, 3100, or 7700 mg/m^3) by inhalation for 6 h/d, 5 d/week for 14 weeks exhibited a slight reduction in responsiveness to external stimuli, which was reversed by terminating the exposures.[75]

Male Wistar rats (n = 10) were exposed to aerosolized n-pentadecyl alcohol (0, 100, 300 or 600 ppm) 6 h/day, 5 d/week for 7 or 14 weeks.[76] The rats were then killed and necropsied. There were no mortalities and there was no effect on weights. No valeraldehyde as found in the blood, while the *n*-pentadecyl alcohol concentration in blood was linearly correlated to the dose. The brain *n*-pentadecyl alcohol was related to the blood alcohol at 7 weeks. At 14 weeks, this relationship changed because the brain *n*-pentadecyl alcohol concentration decreased. Valeraldehyde was measured in the brain only at the high dose level.

The liver *n*-pentadecyl alcohol dehydrogenase activity did not change at all. The microsomal cytochrome P-450 contents and 7-ethoxycoumarin-*O*-deethylase activities in the liver remained unaffected while the kidney deethylase activity was enhanced in a dose-dependent manner at 7 weeks. This effect lessened after 14 weeks. The kidney *n*-pentadecyl alcohol dehydrogenase activity was slightly deceased at the mid and high doses after 7 weeks. The brain acetylcholinesterase activity was greater than the control range at all doses at 7 weeks. Similar effects were noted in the muscles at the mid and high doses. The authors suggested that moderate pentadecyl alcohol vapour exposure may cause metabolic and functional adaptation in its target organs.

Rats (n=10) dermally treated daily for 17 days with ethylhexyl alcohol (100%; ~1600 mg/kg/d) administered to shaved backs exhibited decreased thymus weights and spermatogenesis, liver granulomas, bronchiectasis, renal tubular epithelial necrosis, edema in heart and testes, and increased lipid levels in the adrenal glands.[77]

Fischer 344 rats (n = 20; 10/sex) were topically administered ethylhexyl alcohol (500 or 1000 mg /kg/d) for 5 days under occlusion, followed by 2 days untreated, and then 4 days treatment with S9.[78,79] Both doses produced exfoliation (minimal severity), and the high dose caused transient erythema of the treated skin. The female rats exhibited elevated serum triglycerides at both doses and reduced peripheral blood lymphocytes and spleen weights at the high dose.

NOAELs ranged from 100 to 150 mg/kg body weight/day in several studies in which mice or rats were exposed orally for 9 to 11 days to ethylhexyl alcohol by gavage, in drinking water, or in feed.[78-82] Doses ≥ 330 mg/kg body weight/day produced CNS depression, lacrimation, and decreased food consumption and body weights.

The NOAEL was 125 mg/kg/d for male and female F344 rats and B6C3Fl mice treated daily with ethylhexyl alcohol (0, 25, 125, 250, or 500 mg/kg/d) by gavage for 13 weeks.[83]

Multiple in vitro and in vivo short-term repeated dose studies showed that ethylhexyl alcohol is a peroxisome proliferator and liver-enzyme inducer in mice and rats, and that doses ≥ 60 mg/kg body weight/day can cause these effects and alpha-2u-nephropathy in male rats.[84]

No local or systemic effects were found in male and female Wistar rats (n = 20; 10/sex) exposed 6 h/day, 5 days/week, for 90 days to 2-ethyl-l-hexanol (15, 40, or 120 ml/m^3; purity 99.9%).[85]

The oral NOAEL for non-cancer systemic toxicity endpoints was 200 and 50 mg/kg/d in mice and rats, respectively, exposed chronically to ethylhexyl alcohol.[86]

Ocular/Mucosal Irritation

Methyl benzoate, ethyl benzoate, isopropyl benzoate, and butyl benzoate were grade 1 ocular irritants at 100%. C12-15 alkyl benzoate, isopropyl benzoate, and isostearyl benzoate were rated as non- to mild ocular irritants.

Methyl alcohol, amyl alcohol, dodecyl alcohol, isopropyl alcohol, and ethylhexyl alcohol were rated as severe ocular irritants. Hexyldecyl alchol was an ocular irritant.

Methyl Benzoate

Methyl benzoate (100%) was administered to the center of the cornea while the lids were retracted in rabbits (n = 5).[51] The eye lids were released after ~1 min. The eyes were scored after 18 – 24 h in daylight and with staining. Methyl benzoate was given a grade 1 (iritis, slight internal congestion).

Ethyl Benzoate

The above experiment was repeated with ethyl benzoate (100%).[51] Ethyl benzoate was given a grade 1.

Butyl Benzoate

The above experiment was repeated butyl benzoate (100%).[51] Butyl benzoate was given a grade 1 (iritis, slight internal congestion).

C12-15 Alkyl Benzoate

In an ocular irritation test of C12-15 alkyl benzoate (1.8% - 2.4%; 0.1 ml) using rabbits, the test material was applied to the eye and washed after 24 h.[56] The eyes were observed for 7 days. There was no ocular irritation to rabbits under these test conditions.

C12-15 alkyl benzoate (100%) administered to the eyes of albino New Zealand rabbits caused diffuse crimson coloration, slight swelling, and some discharge.[87] The reactions were resolved in < 6 days.

In an EpiOcular tissue model toxicity testing system, human-derived epidermal keratinocytes were incubated in C12-15 alkyl benzoate (20% in corn oil), corn oil (negative control), and Triton X-100 (0.3%).[88] Using the instructions of the test kit, it was extrapolated that C12-15 alkyl benzoate was non-irritating at 10% and 100%.

Isopropyl Benzoate

Isopropyl benzoate (100%) was administered to the center of the cornea while the lids were retracted in rabbits (n = 5).[61] The eye lids were released after ~1 min. The eyes were scored after 18 – 24 h in daylight and with staining. Isopropyl benzoate was given a grade 1.

Isostearyl Benzoate

In an ocular irritation assessment of a body lotion containing isostearyl benzoate (0.95%) using neutral red release (NRR) assay, the hen's egg test on the chorio-allantoic membrane (HET-CAM) assay, and the reconstituted human epithelial culture (REC) assay, the authors rated the lotion as slightly irritating.[89]

Alcohols

In a neutral red assay using human keratinocytes and fibroblasts from natal foreskins to assess ocular irritancy, methyl alcohol was rated a mild irritant.[90]

Amyl alcohol (100%, 0.1 ml) was rated as a severe ocular irritant in the Draize test and ocular cell count assay.[91] Severe swelling of conjunctival tissue interfered with accurate assessment of Draize scores and cell washes at l h after instillation.

Amyl alcohol (100%) was rated a severe ocular irritant when applied to rabbits.[66]

Dodecyl alcohol was reported to have a maximum average score (MAS) or 24.2 in an ocular Draize test.[92]

Isopropyl alcohol has been described to be a severe ocular irritant, based on tests in rabbits.[75]

Several in vitro tests to investigate the eye irritation potential showed an irritating effect of ethylhexyl alcohol.[93-99]

Undiluted ethylhexyl alcohol was moderately to severely irritating to the eyes in rabbits.[100-106] Effects ranged from conjunctival redness and swelling, lacrimation, and discharge, which did not clear within 96 hours after treatment, to persistent corneal dullness and vascularization.

Hexyldecyl alcohol (100%) was rated a slight ocular irritant when applied to rabbits.[66]

Dermal Irritation

Methyl benzoate, ethyl benzoate, propyl benzoate, and butyl benzoate at 100% were dermally irritating to rabbits.

Methyl alcohol, amyl alcohol, lauryl alcohol, ethylhexyl alcohol, and hexydecyl alcohol were dermal irritants.

Methyl Benzoate

Methyl benzoate (100%) was applied to both the clipped dorsum (0.5 ml) and external surface of the outer ear (0.2 ml) of male New Zealand albino rabbits (n = 14) daily for 6 days.[107] On the dorsum, there were marked cellular reactions and dermal edema beginning on day 2 followed by dermal hemorrhages, desquamated crust, and thickening of the malpighian stratum beginning on day 5. On the inner ear, there was slight hyperkeratosis at day 6.

Methyl benzoate (100%; 0.01 ml) was applied to the clipped skin of albino rabbits (n = 5) and observed within 24

h.[51] Irritation was rated as grade 3 in a 1 to 10 system (grade 1 = least visible capillary injection from the undiluted material; 6 = necrosis with the undiluted material; and 10 = necrosis from a 0.01% solution).

Methyl benzoate (100%) was administered to the clipped and depilated skin of guinea pigs (n = 3 – 4) using filter paper soaked in the test substance for up to 2 min.[108] Before and after treatment, the guinea pigs were administered Evans Blue dye i.v. The permeability was measured by exuded dye at the treated sites. There was a minimal response observed.

Ethyl Benzoate

Ethyl benzoate was administered to the clipped dorsum (0.5 ml) and external surface of the outer ear (0.2 ml) to male New Zealand albino rabbits (n = 14) daily for 6 days.[107] On the dorsum, there were marked cellular changes, edema, desquamated crusts, and thickening of the malpighian stratum beginning on day 1. On the inner ear, there were slight cellular reaction, no edema or hemorrhages, no necrosis, slight to marked desquamated crusts, marked thickening of malpighian stratum, hyperkeratosis, and slight hyperplasia of sebaceous glands beginning day 1.

Ethyl benzoate (100%; 0.01 ml in water, propylene glycol, or kerosene) was administered to the clipped skin of rabbits (n = 5) and scored after 24 h.[51] Grade 4 irritation was observed.

Propyl Benzoate

Propyl benzoate was applied to the clipped dorsum (0.5 ml) and external surface of the outer ear (0.2 ml) to male New Zealand albino rabbits (n = 14) daily for 6 days.[107] On the dorsum, there were marked cellular reactions, necrosis, thickening of the malpighian stratum beginning on day 1 followed by dermal hemorrhages, desquamated crusts beginning on days 3 or 4.. On the inner ear, there were slight cellular reactions, necrosis, and moderate thickening of the malpighian stratum beginning on day 1 or 3.

Butyl Benzoate

Butyl benzoate was applied to the clipped dorsum (0.5 ml) and external surface of the outer ear (0.2 ml) to male New Zealand albino rabbits (n = 14) daily for 6 days.[107] On the dorsum, there were marked cellular reaction, necrosis, and slight detachment of the dermo-epidermis beginning on day 1 followed by desquamated crusts and thickening of the malpighian stratum beginning on day 3. On the inner ear, there were slight cellular reactions, necrosis beginning on day 1, and moderate desquamed crusts and hyperplasia of the sebaceous glands on day 2 or 3.

Butyl benzoate (100%; 0.5 ml) was applied to the clipped skin of female New Zealand white rabbits (n = 4) under occlusion for 4 h.[55] Observations were made at 1, 24, 48, and 72 h. There were no effects at 1 h, well defined erythema and slight edema at 24 h, and very slight erythema and edema at 72 h.

In a Draize test, butyl benzoate (5 g/kg) had slight to no irritation effects after 24 h.[55] The treated skin was scaly at necropsy.

C12-15 Alkyl Benzoate

In a primary dermal irritation test of C12-15 alkyl benzoate (100%; 0.5 ml), the test material was applied to the intact and abraded clipped skin of albino New Zealand rabbits (n = 6).[56] The primary irritation index was 0.08. C12-15 alkyl benzoate was not a primary irritant to rabbits.

In a repeat 14-day irritation study, C12-15 alkyl benzoate (62% and 100% in corn oil; 0.5ml) was administered to the clipped dorsal skin of New Zealand white rabbits (n = 10; 5/sex).[109] Mineral oil and isopropyl myristate were used as controls. The average combined erythema and edema score/animal/day was 4.64 and 4.11 for the high and low dose of C12-15 alkyl benzoate, respectively. The scores were 2.68 and 5.40 for mineral oil and isopropyl myristate, respectively.

C12-15 alkyl benzoate (100%) administered to the skin of male albino New Zealand rabbits (n = 3) caused slight erythema and edema at 1 h which was resolved at 24 h.[58]

Human derived epidermal keratinocytes (NHEK) were incubated with C12-15 alkyl benzoates (10% and 100% in corn oil) and Triton X-100 (1%; positive control) for 3 h in an EpiDerm in vitro toxicity testing system.[110] The test material was found to be non-irritating at both concentrations.

Isopropyl Benzoate

Isopropyl benzoate (100%) administered to the skin of rabbits (n = 5) in a Draize test had an irritation score of 3 (strong capillary injection).[51]

Isobutyl Benzoate

In an acute toxicity test (see above), isobutyl isobutyl benzoate (100%) administered under occlusion for 24 h to the intact and abraded clipped backs of rabbits (n = 4) produced no effects at 5 ml/kg.[60]

Isobutyl benzoate (100%) administered to the skin of rabbits (n = 5) in a Draize test had an irritation score of 5.[51]

Alcohols

Methyl alcohol (10 and 35 mg in water; 35 mg in paraffin, and 10 mg in oil) was injected intracutaneously into the dorsal skin of shaved rabbits (n = 4). The sizes of the wheals at 24 h were 9, 0, 3, and 1 mm^2, respectively.[111]

Amyl alcohol was rated a severe irritant at 3.2 g/kg when applied to the abraded abdominal skin of albino rabbits.[66]

Amyl alcohol (10 and 35 mg in water; 35 mg in paraffin, and 10 mg in oil) was injected intracutaneously into the dorsal skin of shaved rabbits (n = 4). The sizes of the wheals at 24 h were 74, 19, 53, and 40 mm^2, respectively.[111]

When lauryl alcohol was applied to the skin of CD(SD) hrBI hairless rats, moderate erythema was observed.[112]

Lauryl alcohol was reported to have a primary irritative index (PII) of 0.96 in a dermal Draize test.[92]

A single dermal administration of ethylhexyl alcohol (5000 mg/kg) caused slight or moderate skin irritation in studies using clipped rabbits (n=10).[113]

Slight redness and scabbing was reported in rabbits (n=10) after 10 daily dermal administrations of ethylhexyl alcohol (100%; 2 ml/kg/d).[77]

Occlusive exposures to ethylhexyl alcohol (100%; 3.16 mg/kg/d) administered to the clipped intact skin of rabbits (n = 4) for 7 days caused moderate dermal irritation, erythema, edema, desquamation, necrosis, and eschar formation.[66]

Hexydecyl alcohol was rated a slight irritant at 2.6 g/kg when applied to the abraded abdominal skin of albino rabbits.[66] One rabbit showed transient central nervous system depression and labored respiration.

Dermal Sensitization

Methyl benzoate was not sensitizing up to 10%. Ethyl benzoate was not sensitizing at 8%. Amyl benzoate was not sensitizing at 6%. C12-15 Alkyl benzoate was not sensitizing at 10%. Isobutyl benzoate was not sensitizing up to 2%.

Methyl Benzoate

In a modified Freund's complete adjuvant test using guinea pigs (n not provided), methyl benzoate (10%; 30 mg) was not sensitizing.[114]

A guinea pig open epicutaneous test (OET; n = 6-8) of methyl benzoate (up to 4%; vehicle not provided) was performed.[115] The test material was applied daily for 3 weeks onto shaved skin. Challenge was conducted on days 21 and 35 on the opposite flank. Observations were made at 24, 48, and 72 h. Methyl benzoate at 4% was not sensitizing.

A guinea pig OET (n = 6-8) of methyl benzoate (up to 4%; 0.1 ml; vehicle not provided) was performed.[116] Controls (n = 10) were untreated or treated with the vehicle. The test material was applied daily for 3 weeks to clipped skin (8 cm^2). Challenge was conducted on days 21 and 35 on the opposite flank of the test and control animals. Observations were made at 24, 48, and 72 h. Methyl benzoate at 4% was not sensitizing.

Ethyl Benzoate

A guinea pig OET (n = 6-8) of ethyl benzoate (up to 8%; 0.1 ml; vehicle not provided) was performed.[116] Controls (n = 10) were untreated or treated with the vehicle. The test material was applied daily for 3 weeks onto clipped skin (8 cm^2). Challenge was conducted on days 21 and 35 on the opposite flank to the test and control animals. Observations were made at 24, 48, and 72 h. Ethyl benzoate at 8% was not sensitizing.

A guinea pig OET (n = 6-8) of ethyl benzoate (up to 8%; vehicle not provided) was performed.[115] The test material was applied daily for 3 weeks onto shaved skin. Challenge was conducted on days 21 and 35 on the opposite flank. Observations were made at 24, 48, and 72 h. Ethyl benzoate at 8% was not sensitizing.

Amyl Benzoate

A guinea pig open OET (n = 6-8) of amyl benzoate (up to 6%; vehicle not provided; 0.1 ml) was performed.[116] The test material was applied daily for 3 weeks onto clipped skin. Challenge was conducted on days 21 and 35 on the opposite flank. Amyl benzoate at 6% was not sensitizing.

C12-15 Alkyl Benzoate

In a guinea pig sensitization test, C12-15 alkyl benzoate (10%; 0.5 ml) was administered to the clipped backs and flanks of white male guinea pigs (n = 12) for 6 h/day under occlusion, 3 times/week for 3 weeks.[59] Two challenges were performed 14 days after the last application. There were no topical or systemic reactions observed.

Isobutyl Benzoate

A guinea pig OET (n = 6-8) of isobutyl benzoate (up to 2%; vehicle not provided; 0.1 ml) was conducted.[116] Water or the vehicle were administered to the controls (n = 10). The test material was applied daily for 3 weeks onto shaved skin. Challenge was conducted on days 21 and 35 and read at 24, 48, and 72 h. Isobutyl benzoate at 2% was not sensitizing.

In a guinea pig OET (n = 6 – 8 males; controls = 10), isobutyl benzoate (2%; 0.1 ml; vehicle not provided) was applied to clipped skin (8 cm^2) daily for 21 days.[116] On days 21 and 35, the opposite flank was treated with the minimal

irritation concentration and lower concentrations (not provided). The authors reported that 2% isobutyl benzoate was not irritating or sensitizing.

Comedogencity

Cetyl alcohol, stearyl alcohol, benzyl alcohol, and propyl alcohol had no comedogenic activity. Lauryl alcohol and myristyl alcohol had slight comedogenity. Octyl alcohol had strong comedogenicity.

Alcohols

Alcohols were tested for comedogenciity of alcohols by repeated application to the inner ear of rabbits (5 days/week for 2 weeks).[117] Cetyl alcohol (100%), stearyl alcohol (100%), benzyl alcohol (100%), and propyl alcohol (100%) had no comedogenic activity. Lauryl alcohol (50% in mineral oil) and myristyl alcohol (50% in mineral oil) had slight comedogenity. Octyl alcohol had strong comedogenicity.

Reproductive and Developmental Toxicity

Studies on sodium benzoate and benzoic acid did not show reproductive or developmental toxicity. Where effects of the fetus were noted, they occurred at maternally toxic concentrations (> 4% sodium benzoate in rats).

Aerosolized methyl alcohol caused no maternal effects but caused reduced weights and increased malformations in offspring. Male mating success was reversibly reduced. The oral NOAELs for the maternal and developmental toxicity of isopropyl alcohol were 400 mg/kg in rats (maternal and developmental) and 240 mg/kg (maternal) and 480 mg/kg (developmental) in rabbits.

No data were discovered for reproductive and developmental toxicity of alkyl benzoates. It is necessary to rely on the data on benzoic acid, sodium benzoate and the alcohol metabolites.

Benzoic Acid and Sodium Benzoate

Sodium benzoate (0, 1%, 2%, 4%, or 8%; 0, 667, 1333, 1600, or 710 [sic] mg/kg/d) was administered in the feed of female Wistar rats (n = 27-30) during gestation (number of days not provided).[118] On day 20, 20-25 of each group were killed and necropsied. The rest were allowed to live through pregnancy and nurse for 3 or 8 weeks and then killed. Half of the pups were then killed at each of these times and necropsied. The 2 highest dose groups had an increase in the number of dead fetuses and resorbed embryos. The body weights of the viable pups were decreased and was mild systemic edema was observed. The number of fetal abnormalities was increased in a dose-dependent manner. The number of pups born was decreased, the number of perinatal deaths increased to 100%, lactation rate decreased, and survival rate decreased to 0 in the 2 highest dose groups. The effects on the fetus occurred only at maternally toxic concentrations of ≥ 4% sodium benzoate.

Sodium benzoate (up to 5mg/egg) was injected twice into the air sac of fertilized chicken eggs at 0 and 96 h and incubated to hatching.[119] Surviving chicks were killed and necropsied. There were no teratogenic effects reported. The LD_{50} was 4.74 mg/egg.

Female Wistar rats (n = 20) were orally administered sodium benzoate (0, 1.75, 8.0, or 175 mg/kg/d) during days 6 – 15 of gestation.[120] On day 20 of gestation, the pups were delivered by Caesarean section. There were no differences in the types or incidences of abnormalities observed in any of the treatment groups compared to the control. The fetal and maternal NOAEL was 175 mg/kg.

The study above was repeated with mice (n = 20), hamsters (n = 21 - 22; gestation days 6 - 10), and rabbits (n = 10). Similar results were reported.

In oral teratogencity studies, benzoic acid administered on gestation days 6 - 10 increased the number of resorptions at ≥ 30 mg/kg/d and increased the number of fetal malformations at > 600 mg/kg/d results in hamsters. Results were negative in 2 rat studies up to 500 mg/kg/d.[121]

Cross bred white mice (n = 50; 25/sex) were orally administered benzoic acid (40 mg/kg/d) for 8 months before breeding.[70] This was continued for 5 generations. The parental and F1 cohorts had increased mortality compared to controls after a 5-day 100% food restriction test. Otherwise, there were no effects on reproduction.

A neurobiological study on the effects of sodium benzoate (0.1%, 0.5%, and 1.0%) on the offspring of rats and mice was negative.[122] The dams (n = 8) were administered feed incorporated with sodium benzoate (0, 0.1%, 0.5%, or 1.0%) from gestation day 5 until weaning. Locomotor activity and brain chemistry of the pups were not affected.

Alcohols

Sprague-Dawley rats were exposed to aerosolized methyl alcohol (5000, 10,000 or 20,000 ppm) for 6 h/d on days 1 – 19 of gestation.[123] There were no maternal effects observed at any concentration. The offspring had reduced weights in the

mid and high-dose groups. The high-dose group also had increased incidences of external malformations, skeletal malformations, and visceral malformations compared to controls.

Mating success was not affected in male (n = 18) and female (n = 15) Sprague-Dawley rats exposed to propyl alcohol (8.61 mg/L) via inhalation 7 h/d, 7d/week for 62 days.[124] The decreased mating success of the male rats exposed to a higher dose of propyl alcohol (17.2 mg/L) in this study was reversed 15 weeks after exposure. Changes in activity measures were observed in the offspring of the 8.61 mg/L propyl alcohol maternally-exposed group, and crooked tails were found in 2-3 offspring.

The NOAELs for the maternal and developmental toxicity of isopropyl alcohol were 400 mg/kg in rats (maternal and developmental) and 240 mg/kg (maternal) and 480 mg/kg (developmental) in rabbits exposed by gavage during gestation (rats: 0, 400, 800, or 1200 mg/kg/day on gestation days 6 through 15; rabbits: 0, 120, 240, or 480 mg/kg/day on gestation day 6 through 18).[125]

No effects on rat reproductive cells or organs were observed in several in vitro studies of ethylhexyl alcohol (200 µM) for up to 48 h or in oral in vivo studies using rats (up to 500 mg/kg/d) for up to 90 days.[83,85,126-131]

In Wistar rats administered a single dose of 1,666 mg/kg ethylhexyl alcohol by gavage on day 12 of pregnancy, 22.2% of the surviving fetuses had malformations (compared to 2% and 0% for 833 mg/kg and controls, respectively), and average fetal weight was reduced.[126,132] No maternal toxicity or effects on implantation index or numbers of dead and resorbed fetuses were found in this study.

The embryos of pregnant Sprague-Dawley rats (n = 6) administered a single dose of ethylhexyl alcohol (1,625 mg/kg) by gavage exhibited reduced ^{65}Zn content, although the percentage of resorptions was not affected.[133,134]

No treatment-related increases in the incidences of malformations or variations were found in F344 rats (n = 25) dermally exposed (clipped dorsal skin) to 2-ethyl-1-1hexanol (0, 252, 420, 840, 1680 or 2520 mg/kg/d, 6 h/d, occlusive) on gestation days 6 to 15.[39,125,135] Maternal effects at ≥ 840 mg/kg/d included persistent exfoliation, crusting and erythema at the site of application, and doses ≥1680 mg/kg/d were associated with decreased maternal weight gain.

Maternal and developmental NOAEL was found to be 130 mg/kg/d in Wistar rats (n = 10) exposed to ethylhexyl alcohol (0, 130, 650, and 1,300 mg/kg/d) on days 6 to 19 of gestation.[136]

The pups of Charles River CD-l mice (n = 50) exposed to 2-ethyl-1-1hexanol (1,525 mg/kg/d in corn oil) on days 7 to 14 of pregnancy exhibited reduced viability and body weights on day 3 of lactation.[137] Maternal effects included reduced fertility and pregnancy indexes, body weights, and other signs of toxicity.

No maternal, reproductive, or developmental toxicities were found in CD-1 Swiss mice (n = 28) ingesting microencapsulated ethylhexyl alcohol (0.13, 43, and 129 mg/kg/d; >99% pure) in the diet on days 0 to 17 of pregnancy.[138,139]

The NOAEL for maternal and developmental toxicity was 850 mg/m^3 (160 ml/m^3) ethylhexyl alcohol in Sprague-Dawley rats (n = 15) exposed (whole body) 7 h/d on gestation days 0-19.[124]

GENOTOXICITY

In Ames tests, methyl benzoate, ethyl benzoate, C12-15 alkyl benzoate were not genotoxic. Benzoic acid and sodium benzoate had mixed results in several genotoxic assays.

Methyl alcohol and ethylhexyl alcohol were not genotoxic is various assays.

Methyl Benzoate

In an Ames test using *S. typhimurium* (TA97, TA98, TA100, TA1535, and TA1537), methyl benzoate (6666 µg/plate) was not mutagenic with or without metabolic activation.[140] Methyl benzoate (dose not reported) was not found to be mutagenic using *E. coli* (Sd-4-73).[141]

Ethyl Benzoate

In an Ames test using *S. typhimurium* (TA98, TA100, TA102, TA1535, and TA1537), ethyl benzoate (15 to 5000 µg/plate without metabolic activation and 5 to 5000 µg/plate with metabolic activation) was not mutagenic with or without metabolic activation.[53]

C12-15 Alkyl Benzoate

In an Ames test using *S. typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538), C12-15 alkyl benzoate (100%; 0.1 ml) was not mutagenic to any strain tested, with or without metabolic activation.[142] Saline was the negative control and Dexon and 2-aminofluorene were the positive control.

Benzoic Acid and Sodium Benzoate

Benzoic acid and sodium benzoate had mixed results in genotoxicity assays (Table 5). Sample studies are presented

below.

Benzoic acid was negative in several Ames tests using *Salmonella typhimurium* (including TA98, TA100, TA1535, TA1537 and TA 1538) with and without metabolic activation.[1,143-146]

In one Ames test using *S. typhimurium* (TA98 and TA100), benzoic acid (0.1 mg/plate) and sodium benzoate (0.1 mg/plate) were genotoxic with activation.[145] In a reverse mutation test, benzoic acid (5 mg/disc) was positive for genotoxicity.

In a sister chromatid exchange assay using human lymphocytes, benzoic acid (0 – 2.0 mM) was not genotoxic with metabolic activation.[146]

Sodium benzoate was positive without metabolic activation and negative with metabolic activation in a reverse mutation assay using *Bacillus subtilis*.[143] Benzoic acid (1.5 mg/ml) was positive in a chromosomal aberration test without metabolic activation.

In a sister chromatid exchange assay using hamster lung fibroblasts, sodium benzoate was not clastogenic without metabolic activation.[147]

Alcohols

In an Ames test, methyl alcohol (5 – 5000 µg/plate) was not mutagenic to *Salmonella typhimuium* (strains TA98, TA100, TA1535, TA1537, and TA1538) and *Escherichia coli* (WP2uvrA).[148]

Genotoxicity tests of ethylhexyl alcohol were generally negative, including in vitro tests for chromosome aberrations[124,149-151], unscheduled DNA synthesis,[152] mutagenicity (Ames, $TK^{+/-}$ mouse lymphoma, and HPRT assays),[153-155] and cell transformation.[38,156,157] The exceptions include one positive result in one of two rec-assays[158,159] and another in a test of mutagenicity in *S. typhimurium* TA-l00 (mutation resistance to 8-azaguanine),[160] both of which were questionable.[68]

Urine samples from Sprague-Dawley rats exposed to ethylhexyl alcohol (1000 mg /kg/d) by gavage for 15 days tested negative for mutagenicity in *S. typhimurium* with and without rat liver microsomes or beta-glucuronidase/arylsulfatase.[161,162]

In vivo tests of ethylhexyl alcohol genotoxicity were also negative, including assays for covalent binding to liver DNA,[163,164] dominant lethal mutations,[165] and bone marrow micronuclei.[166,167]

CARCINOGENICITY

No data was discovered on the carcinogenicity of alkyl benzoates.

Benzoic acid (40 mg/kg/d) orally administered to mice increased tumor growth compared to controls. Benzoic acid was negative for carcinogenicity when dermally applied to mice at 0.016% in a non-oxidative hair dye.

Orally administered methyl alcohol, amyl alcohol, lauryl alcohol, and dodecyl alcohol caused increases in polyploidy cells, cells with gaps, and cells with aberrations in the bone marrow of rats. Ethylhexyl alcohol was a weak liver tumor promoter in female mice.

Benzoic Acid

Cross bred white mice (n = 100) were orally administered benzoic acid (40 mg/kg/d in a paste).[70] After 8 months the mice were bred and also administered the benzoic acid paste. This was repeated for 5 generations. Eight of 100 mice in the first generation and 1 of 100 in the third generation were found to have malignant tumors. No tumors were found in the control group. In a follow up tumor transplantation test, benzoic acid fed to mice for 3 months did not increased tumor growth.

A non-oxidative hair dye containing benzoic acid (0.016%) and benzyl alcohol (2.0%) was negative for carcinogenicity when dermally applied to mice (n = 60) 3 times per week for 20 months.[71] In a feeding study of rats and mice (n = 102), feed containing sodium benzoate (1% or 2%; 102-151 or 202-280 mg/d) was not carcinogenic after 6 weeks.[168]

Alcohols

Orally administered methyl alcohol, amyl alcohol, lauryl alcohol at one-fifth of the lethal dose, caused increases in polyploidy cells, cells with gaps, and cells with aberrations in the bone marrow of rats.[169]

Male rats exhibited a concentration-dependent increase in the incidence of interstitial (Leydig) cell adenomas of the testes at all doses in a study in which Fischer 344 rats (n = 65/sex) and CD-1 mice (55/sex) were exposed by inhalation to isopropyl alcohol (0, 1230, 6150, or 12,300 mg/m^3) for 6 h/d, 5 d/week for 104 weeks in rats and 78 weeks in mice.[170] No other tumors or neoplastic lesions were found in the rats or mice. International Agency for Research on Cancer (IARC)[171] has determined that isopropyl alcohol is not classifiable as to its carcinogenicity to humans (Group 3).

Male and female rats and mice were exposed chronically to ethylhexyl alcohol by gavage 5 times a week (rats: 0, 50, 150, 500 mg/kg/d for 24 months; mice: 0, 50, 200, 750 mg/kg/d for 18 months).[86] The results of this study suggested that ethylhexyl alcohol was a weak liver tumor promoter in female mice. Mechanistic studies suggest that tumor promotion in mice is attributable to the induction of peroxisome proliferation by ethylhexyl alcohol, which has questionable relevance for human exposures. Belsito et al.[68] concluded that "while this mechanism cannot be completely discounted, it is reasonable to assume that humans are less sensitive than rodents."

CLINICAL ASSESSMENT OF SAFETY

Toxicity

Benzoic Acid

In clinical studies, toxic symptoms (including: discomfort, malaise, nausea, headache, weakness, esophageal burning, irritation, hunger, indigestion, vomiting, itching, perspiration) were observed following oral doses far exceeding the acceptable daily intake (ADI) established by the World Health Organization.[1] The Registry of Toxic Effects of Chemical Substances (RTECS) cited the human low lethal oral dose of benzoic acid to be 500 mg/kg.[172]

Ocular/Mucosal Irritation

Ethylhexyl benzoate was not an ocular irritant in a sunscreen at 2.5%.

Ethylhexyl alcohol increased nasal and eye irritation and perceived odor intensity.

Ethylhexyl Benzoate

A sunscreen liquid containing ethylhexyl benzoate (3.5%) was randomly administered to the eyes of subjects (n = 30; 10 male, 20 female) with an eye swab.[173] The reactions were scored at 5 min then the eyes were washed. Scoring was repeated at 15 and 60 min. The control was a baby shampoo (10%) with no ethylhexyl benzoate. The test material and the control exhibited no differences at all scoring times and all reactions were cleared at 1 h. The test material caused no tearing.

Alcohols

Self-reported nasal and eye irritation and perceived odor intensity were increased in a concentration-related manner in male volunteers exposed to ethylhexyl alcohol ($\geq$10 ml/m^3) for 4 h in an exposure chamber.[174]

Dermal Irritation

Ethyl benzoate and butyl benzoate were not dermally irritating at 8%. C12-15 Alkyl benzoate was not irritating at 100%. Isobutyl benzoate was non irritating at 2% as was ethylhexyl benzoate at3.5%. In multiple clinical studies, the benzoates were recognized to produce non-immunologic contact uriticaria or non-immunologic immediate contact reactions, but it was not clear whether the reactions were histamine or prostaglandin mediated.

Benzoic acid at 0.2% was not irritating to subjects. Benzoic acid at 0.2% caused mild, transient irritation when applied daily in a liquid foundation product at least twice/day for 45 days.

Little or no dermal irritation was observed in tests of propyl alcohol, lauryl alcohol, cetyl alcohol, isostearyl alcohol, and ethylhexyl alcohol on humans.

Ethyl Benzoate

In a 48-h closed patch test (n = 5 males), ethyl benzoate (8% in petrolatum) produced no effects.[175]

Butyl Benzoate

In a 48-h closed patch test (n = 5 males), butyl benzoate (8% in petrolatum) produced no effects.[175]

C12-15 Alkyl Benzoate

In an irritation study, C12-15 alkyl benzoate (0, 3%, 10%, 30, and 100% in vegetable oil) was applied to the backs of subjects (n = 21) under occlusion for 48 h.[176] No signs of irritation were observed at 48 and 72 h.

Isobutyl Benzoate

Isobutyl benzoate (2%) administered in a 24 h patch test (n = 5 males) produced no effects.[60]

Ethylhexyl Benzoate

In a 14-day cumulative irritation test of a sunscreen liquid containing ethylhexyl benzoate (3.5%), the test material was applied to the skin of subjects (n = 28).[177] The positive control was SLS (0.25%) and the negative control was saline. There was a total dermal irritations score of 5.0 out of 1120. The authors concluded that there was no potential for eliciting cumulative dermal irritation.

A sunscreen lotion spray containing ethylhexyl benzoate (3.5%) was administered to the skin on the arms and legs of subjects (n = 35; male and female; 7 months to 8 years old) daily for 4 weeks.[178] There were no increases in erythema,

edema or dryness of the arms and no increase in erythema and edema of the legs. One subject exhibited mild dryness of the legs following the four-week use period.

Benzoic Acid

Benzoic acid (0.2%) was not irritating to subjects (n = 12) after 3 occlusive patches were applied over 1 week.[179] Benzoic acid (0.2%) caused mild, transient irritation when applied daily in a liquid foundation product at least twice/day for 45 days.[180]

Alcohols

Propyl alcohol produced no dermal irritation or skin sensitization in several clinical studies in which it was used as a vehicle and control.[181-186] These studies include a cumulative irritation study (n = 20 males) in which Al-test® patches containing propyl alcohol were applied daily for 10 days to the interscapular area of each subject, each application remaining in place for 24 hours.[184]

In a patch test lauryl alcohol (C12), subjects had scores of ~ 0.02, 0, and 0.05 for irritation for 2, 1, and 0.5 mg in petrolatum, respectively. In a nitrocellulose-replica test, the scores were ~ 0.35, 0.2, and 0.1, respectively.[187]

One subject of 80 males (21 to 52 years old) exposed to cetyl alcohol (11.5%) in a cream base five times daily (every 3 h) for 10 days developed erythema, folliculitis, and pustules (forearm site).[5,184] Mild cumulative irritation (total score 418 for 21 applications) was reported in 12 female subjects (18 to 60 years old) exposed to cetyl alcohol (6.0%) using the same protocol.

No irritation was found in female subjects (n = 110) exposed to cetyl alcohol (8.4%), 10 patch application sites per subject, followed 14 days later by a challenge patch.[5,184]

Isostearyl alcohol (25.0% in petrolatum and 25.0%, 27.0%, and 28.0% in lipstick) did not induce skin irritation in subjects (n = 19; 18 - 65 years old).[5,184]

No skin irritation was found in 29 healthy male volunteers in an occlusive patch test with 4% ethylhexyl alcohol in petrolatum.[188]

Dermal Sensitization

In HRIPTs, methyl benzoate at 4%, ethyl benzoate at 8%, C12-15 alkyl benzoate at 100%, stearyl benzoate at 2%, isobutyl benzoate at 2%, isostearyl benzoate at 0.95%, ethylhexyl benzoate at 3.5% and octyldodecyl benzoate at 0.4% were not sensitizing. In 4 studies, tests for the sensitization of benzoic acid were negative up to 2%.

Dodecyl alcohol, cetyl alcohol, isopropyl alcohol, isopropyl alcohol, isostearyl alcohol, and ethylhexyl alcohol were not sensitizing. A product containing isostearyl alcohol at 5.0% was sensitizing.

The results of several human insult patch tests (HRIPT) of products containing various benzoate esters are summarized in Table 6. None were irritating or sensitizing.

Methyl Benzoate

Human repeated insult patch tests (HRIPT) were conducted on methyl benzoate (0.05% – 0.5% in a perfumed base cream, a non-perfumed base cream, or 99% ethanol) in multiple studies (total n = 4737; 2341 Japanese men, 2396 Japanese women).[189] There were no visible reactions to the test substance observed.

In a HRIPT (n = 25), methyl benzoate (4% in petrolatum) was not sensitizing.[189]

Ethyl Benzoate

HRIPTs were conducted on ethyl benzoate (0.05% – 0.5% in a perfumed base cream, a non-perfumed base cream, or 99% ethanol) in multiple studies (total n = 4737; 2341 Japanese men, 2396 Japanese women).[189] There were no visible reactions to the test substance observed.

In a HRIPT (n = 25), methyl benzoate (8% in petrolatum) was not sensitizing.[189]

C12-15 Alkyl Benzoate

An HRIPT (n = 101) was conducted on C12-15 alkyl benzoate (100%).[190] There were no visible reactions to the test substance observed.

An HRIPT (n = 48) was conducted on C12-15 alkyl benzoate (20% in corn oil).[191] Induction consisted of 10 applications under occlusion over 3.5 weeks. The challenge was applied ~14 days after last application on a naïve site. There were no signs of irritation or sensitization.

Isobutyl Benzoate

In a human maximization test, isobutyl benzoate (in petrolatum) was applied to the volar surface of male subjects (n = 25) on 5 alternate days. [60] The test surfaces were pretreated with 5% aqueous sodium lauryl sulfate (SLS) under occlusion

for 24 h. After 10 days, fresh sites were treated with 10% SLS for 1 h then isobutyl alcohol was applied. Test sites were read at removal and 24 h. There was no sensitization at 2% isobutyl alcohol.

Isostearyl Benzoate

A HRIPT (n = 107) was conducted on a body lotion product containing isostearyl benzoate (0.95%) under semi-occlusion.[192] Except for one subject, who also reacted to several other test substances on the shared panel, there were no visible reactions to the product containing isostearyl benzoate at 0.95%.

Octyldodecyl Benzoate

An HRIPT (n = 105) was conducted on a shaving cream product containing octyldodecyl benzoate (4%) under semi-occlusion.[193] The product was diluted to a 10% aqueous solution. There were no visible reactions to the product containing octyldodecyl benzoate at 0.4%.

Benzoic Acid

In 4 studies, tests for the sensitization of benzoic acid were negative.[1] A liquid/powder foundation containing benzoic acid (0.2%) produced no reactions at induction or challenge (n = 75).[194] Benzoic acid (2.0%) in petrolatum produced no reactions at induction or challenge (n = 25).[195,196] Benzoic acid (5% in petrolatum) produced no reaction at induction or challenge (n = 10).[197] In a cosmetic intolerance assay, a reaction to benzoic acid (concentration not provided) was observed in 34 of 5202 subjects; a reaction was observed in 1 of 155 subjects described as having a cosmetic allergy.[198]

Alcohols

No primary sensitization was found in female subjects (n = 110) exposed to cetyl alcohol (8.4%), 10 patch application sites per subject, followed 14 days later by a challenge patch.[5]

An isopropyl alcohol (80.74%) spray concentrate did not exhibit any potential for dermal sensitization in human subjects (n = 9).[199]

An HRIPT study on test subjects (n = 9) showed that a hair dye base formulation of isopropyl alcohol (2.85%) and a isopropyl acetate (1.95%) caused no dermal sensitization in humans.[200]

No reactions were observed in healthy individuals (n = 12; 8 males, 4 females; 18 to 64 years old) exposed to isopropyl alcohol (Finn chambers, occlusive patches) on the flexor side of the right and left forearm for 24 h.[201]

Three of 12 male subjects (21-60 years old) exposed to isostearyl alcohol (25% v/v in 95.0% isopropyl alcohol) exhibited erythema during induction, but none of the subjects exhibited evidence of sensitization when challenged 2 weeks later.[5]

Three of 12 male subjects (21 - 60 years old) exposed to isostearyl alcohol (25% v/v in 95.0% isopropyl alcohol) exhibited erythema during induction.[5,184] However, 12 of 148 male and female subjects exhibited signs of sensitization after exposure to a pump spray antiperspirant containing isostearyl alcohol (5.0%) using a occlusive patch applied to the upper arm for 24 h, 3 times/week for 3 weeks. Six of 10 of these subjects had reactions during the re-challenge 2 months later, and all 4 of the 6 subjects re-challenged with isostearyl alcohol (5.0%) in ethanol tested positive 6 weeks after the first re-challenge. In a second study, 5 of 60 male and female subjects had positive responses after the first challenge with the same product and test protocol; one of which was later re-challenged with isostearyl alcohol (5.0%), and again tested positive.

No skin sensitization was found in healthy male subjects (n = 29) in an occlusive patch test with ethylhexyl alcohol (4% in petrolatum).[202,203] Like other saturated alcohols, 2-ethyl-1-hexanol has little skin sensitizing potential.[68]

Phototoxicity

Methyl benzoate at 0.1% and ethylhexyl benzoate at 3.5% were not phototoxic.

A product containing cetyl alcohol was not photoxic.

Methyl Benzoate

Human erythrocytes in suspension (0.4 ml) in methyl benzoate (0.1 ml) were exposed to UVA and UVB for 1 h.[204] Photohemolysis was not induced.

Ethylhexyl Benzoate

A sunscreen liquid containing ethylhexyl benzoate (3.5%; 0.2 ml) was administered to a 2 4-cm^2 area of the backs of subjects (n = 21) that had fair skin.[205] Patches were removed and the test area cleanted 24 h later. After scoring, UVA was applied to 1 of the test sites and to a naïve site. Sites were graded at 24, 48, and 72 h. There were no signs of photoxicity observed.

Benzoic Acid

Phototoxicity and photosensitivity tests of benzoic acid were negative for a matte eye shadow (0.1%; n = 77) and a liquid/powder foundation (0.2%; n = 10 and 30).[206-208]

Alcohols

No photosensitization reactions were found in subjects (n = 52) exposed to cetyl alcohol (40%) in a lipstick product or to cetyl alcohol (1.0%) in subjects (n = 407) tested (product and experimental procedure not stated).[5]

Case Reports

Alcohols

Over 19 months, 33 cases of acute allergic contact dermatitis from epilating waxes and/or accompanying tissue were presented.[209] Patch tests of 26 of the patients resulted in 9 positive tests for lauryl alcohol (10% in petrolatum) varying from minor to severe.

Patients (n = 34) with allergic reactions to fatty alcohols had no positive reactions to lauryl alcohol (5% in petrolatum).[210]

A 37-year-old man presented with severe genital swelling and inflammation that was not responding to treatment.[211] Prolonged oral prednisolone and antihistamines relieved the symptoms. Patch testing revealed a persistent 3+ reaction to octydodecyl alcohol (13.5% in liquid paraffin) at 48 and 96 h.

A 62-year-old man had a 5-year history of eczamatous eruption that he treated with a topical corticosteroids, emollients and an itch reliever.[212] Patch test revealed a + reaction to octyldodecyl alcohol (3% in petrolatum).

SUMMARY

This is a safety assessment of alkyl benzoates that are used in cosmetics. Alkyl benzoates are mostly used as skin-conditioning agents, preservatives, solvents, and plasticizers. In general, the alkyl benzoates can be produced industrially via esterification of benzoic acid. The manufacturing processes of the benzoic esters are typically high yielding (≥90%) and easily purified (e.g., by distillation). The esters, acids and alcohols can be analyzed using gas chromatography/mass spectroscopy (GCMS), nuclear magnetic resonance (NMR) spectroscopy, ultraviolet (UV) spectroscopy and infrared (IR) spectroscopy.

The toxicity of the metabolites (benzoic acid and the parent alcohols) is taken into consideration in this safety assessment.

Alkyl Benzoates, Benzoic Acid, and Sodium Benzoate

The total number of uses of C12-15 alkyl benzoate was 971 (858 leave-on and 113 rinse-off products) at concentrations up to 35% and 50% in leave-on and rinse-off products, respectively. The highest concentrations of use for C16-17 alkyl benzoates, stearyl benzoate, behenyl benzoate, ethyl benzoate, isobutyl benzoate, isostearyl benzoate, methyl benzoate, and octyldodecyl benzoate were reported to be from 0.01% to 4%. No uses or concentrations of use were reported for propyl benzoate, butyl benzoate, amyl benzoate, lauryl/myristyl benzoate, isopropyl benzoate, ethylhexyl benzoate, butyloctyl benzoate, and hexyldecyl benzoate.

Benzoate esters are metabolized into benzoic acid (and the corresponding alcohols) and further metabolized to benzoyl glucuronide and benzoyl CoA. The benzoyl CoA metabolizes into hippuric acid, the principal metabolite excreted in the urine. Methyl benzoate, ethyl benzoate, propyl benzoate butyl benzoate, and C12-15 alkyl benzoate are not expected to penetrate skin. Dermally applied benzoic acid is also excreted in the urine within 24 h. There were no human absorption, distribution, metabolism, and excretion data discovered for alkyl benzoates.

Ethyl benzoate did not increase penetration of malachite green, Rhodamine B, or orcein. Dermal penetration of several chemicals was increased by various alcohols.

Methyl benzoate was cytotoxic toHeLa cells, *A. flavus*, *A. parasiticus*, and lung fibroblasts. Ethyl benzoate was cytotoxic to Hep-2 cells and lung fibroblasts. Propyl benzoate and butyl benzoate were cytotoxic to Hep-2 cells. C12 – 15 alkyl benzoate was cytotoxic to Human derived epidermal keratinocytes.

The oral LD_{50} of methyl benzoate was 2170 mg/kg for rabbits, 4100 mg/kg for guinea pigs, 1350-3500 for rats, and 3000-3330 mg/kg for mice. The oral LD_{50} of ethyl benzoate was 2630 mg/kg for rabbits and 2100-6480 mg/kg for rats. The oral LD_{50} for isopropyl benzoate was 3730 mg/kg and 3685 mg/kg for isobutyl benzoate in rats. The dermal LD_{50} of methyl benzoate was ≥ 2000 mg/kg for rabbits. Dermally administered ethyl benzoate at 10% caused no effects to mice and calves; at 100% it was lethal to cats. Dermally administered butyl benzoate caused diarrhea in rabbits. C12-15 alkyl benzoate was not dermally toxic to rabbits. The dermal LD_{50} of isopropyl benzoate was 20 mg/kg for rabbits. Isobutyl benzoate was not dermally toxic to rabbits.

Benzoic acid and sodium benzoate were orally toxic to rats and mice in feed studies at concentrations > 1% in short-term, subchronic, and chronic studies.

Methyl benzoate, ethyl benzoate, and butyl benzoate were grade 1 ocular irritants. C12-15 alkyl benzoate, isopropyl benzoate, and isostearyl benzoate were rated as non- to mild ocular irritants.

Methyl benzoate, ethyl benzoate, propyl benzoate, and butyl benzoate at 100% were dermally irritating to rabbits.

Methyl benzoate was not sensitizing up to 10%. Ethyl benzoate was not sensitizing at 8%. Amyl benzoate was not sensitizing at 6%. C12-15 Alkyl benzoate was not sensitizing at 10%. Isobutyl benzoate was not sensitizing up to 2%.

In Ames tests, methyl benzoate, ethyl benzoate, C12-15 alkyl benzoate were not genotoxic.

Ethylhexyl benzoate was not an ocular irritant in a sunscreen at 2.5%.

Ethyl benzoate and butyl benzoate were not dermally irritating at 8%. C12-15 Alkyl benzoate was not irritating at 100%. Isobutyl benzoate was non irritating at 2% as was ethylhexyl benzoate at3.5%. In multiple clinical studies, the benzoates were recognized to produce non-immunologic contact uriticaria or non-immunologic immediate contact reactions, but it was not clear whether the reactions are histamine or prostaglandin mediated.

In HIRPTs, methyl benzoate at 4%, ethyl benzoate at 8%,C12-15 alkyl benzoate at 100%, stearyl benzoate at 2%, isobutyl benzoate at 2%, isostearyl benzoate at 0.95%, ethylhexyl benzoate at 3.5% and octyldodecyl benzoate at 0.4% were not sensitizing.

Methyl benzoate at 0.1% and ethylhexyl benzoate at 3.5% were not phototoxic.

Benzoic Acid and Sodium Benzoate

The oral LD_{50} of benzoic acid was reported to be 1996 mg/kg in mice and 2000 – 2500 mg/kg in rats. The oral LD_{100} was reported to be 1520 – 2000 mg/kg for rabbits, and 2000 mg/kg for cats and dogs. The oral LD_{50} for sodium benzoate was 2100 – 4070 mg/kg for rats and 2000 mg/kg for rabbits and dogs.

Benzoic acid and sodium benzoate were orally toxic to rats and mice in short-term feeding studies at concentrations > 1% in short-term, subchronic, and chronic studies. In Subchronic studies, benzoic acid was toxic to mice at oral doses of 80 mg/kg/d. Sodium benzoate at 880 mg/kg/d incorporated into the feed of rats for 18 – 24 months was not toxic.
Studies on sodium benzoate and benzoic acid did not show reproductive or developmental toxicity. Where effects of the fetus were noted, they occurred at maternally toxic concentrations (> 4% sodium benzoate in rats).

Benzoic acid and sodium benzoate had mixed results in several genotoxic assays.
Benzoic acid was negative for carcinogenicity when dermally applied to mice at 0.016% in a non-oxidative hair dye.

Benzoic acid at 0.2% was not irritating to subjects. Benzoic acid at 0.2% caused mild, transient irritation when applied daily in a liquid foundation product at least twice/day for 45 days.

In 4 studies, tests for the sensitization of benzoic acid were negative up to 2%.

Alcohols

Methyl alcohol and ethylhexyl alcohol permeated the skin or nail plates. Aerosolized lauryl alcohol caused mild dyspnea and scattered hemorrhagic areas in the lungs of rats.

Methyl alcohol, amyl alcohol, and dodecyl alcohol were cytoxoic.

Methyl alcohol has an oral LD_{50} of 5628 mg.kg for rats and 7300 mg/kg for mice. The oral LD_{50} of amyl alcohol for rats was reported to be 2.69 g/kg. Dodecyl alcohol has an oral LD_{50} of 12,800 mg/kg for rats. Tridecyl alcohol has an oral LD_{50} of 17,200 mg/kg for rats. Tetradecl alcohol has an oral LD_{50} 33,000 mg/kg for rats. Oral LD_{50}s for ethylhexyl alcohol in rats range from 2049 to 7100 mg/kg and 2380 to >5000 mg/kg for rabbits. The oral LD_{50} of hexyldecyl alcohol for rats was reported to be > 8.42 g/kg. The dermal LD_{50} of methyl alcohol was reported to be 15,800 mg/kg in rabbits.

The dermal LD_{50} of amyl alcohol for rabbits was reported to be > 3.2 g/kg. The dermal LD_{50} of dodecyl alcohol was reported to be 3560 mg/kg in rabbits. The dermal LD_{50} of tridecyl alcohol was reported to be 5600 mg/kg in rabbits. The dermal LD_{50} of hexadecyl alcohol for rabbits was reported to be > 2.6 g/kg. Aerosolized amyl alcohol caused irritation of the eyes, nose, throat, and respiratory passages of mice and guinea pigs. Aerosolized ethylhexyl alcohol was not toxic to rats.

Short-term oral exposure to amyl alcohol was not toxic to rats at 100%. Oral NOAELs ranged from 100 to 150 mg/kg in several studies using mice or rats exposed to ethylhexyl alcohol. Dermal exposure to ethylhexyl alcohol caused physiological changes in rats at 500 mg/kg. Inhalation of isobutyl alcohol caused reversible inhibition of responsiveness in rats. Adverse effects of isopropyl alcohol at the LOAEL included clinical signs in rat and mice, hematological changes in rats, and increased liver weights in mice; higher doses caused kidney and testicular effects. Aerosolized n-pentadecyl alcohol was not toxic to rats.

Methyl alcohol, amyl alcohol, dodecyl alcohol, isopropyl alcohol, and ethylhexyl alcohol were rated as severe ocular irritants. Hexyldecyl alchol was an ocular irritant.

Methyl alcohol, amyl alcohol, lauryl alcohol, ethylhexyl alcohol, and hexydecyl alcohol were dermal irritants.

Cetyl alcohol, stearyl alcohol, benzyl alcohol, and propyl alcohol had no comedogenic activity. Lauryl alcohol and myristyl alcohol had slight comedogenity. Octyl alcohol had strong comedogenicity.

Aerosolized methyl alcohol caused no maternal effects but caused reduced weights and increased malformations in offspring. Male mating success was reversibly reduced. The oral NOAELs for the maternal and developmental toxicity of isopropyl alcohol were 400 mg/kg in rats (maternal and developmental) and 240 mg/kg (maternal) and 480 mg/kg (developmental) in rabbits.

Methyl alcohol and ethylhexyl alcohol were not genotoxic is various assays.

Orally administered methyl alcohol, amyl alcohol, lauryl alcohol, and dodecyl alcohol caused increases in polyploidy cells, cells with gaps, and cells with aberrations in the bone marrow of rats. ethylhexyl alcohol was a weak liver tumor promoter in female mice.

Ethylhexyl alcohol increased nasal and eye irritation and perceived odor intensity.

Little or no dermal irritation was observed in tests of propyl alcohol, lauryl alcohol, cetyl alcohol, cetyl alcohol, isostearyl alcohol, and ethylhexyl alcohol on humans.

Dodecyl alcohol, cetyl alcohol, isopropyl alcohol, isopropyl alcohol, isostearyl alcohol, and ethylhexyl alcohol were not sensitizing. A product containing isostearyl alcohol at 5.0% was sensitizing.

A product containing cetyl alcohol was not photoxic.

DISCUSSION

The Expert Panel had issued an insufficient data announcement for dermal penetration, in particular of the smaller alkyl benzoates. If there is the possibility of dermal penetration, the Expert Panel also requested data on reproduction and developmental toxicity, carcinogenicity, and irritation and sensitization data on those ingredients smaller than C12-C15 alkyl benzoates.

There has been new data submitted and the Expert Panel will develop a new discussion after examining the information.

CONCLUSION

The CIR Expert Panel will provide a conclusion after examination of new data.

TABLES AND FIGURES

Table 1. Definitions, functions and structures of alkyl benzoate ingredients in this safety assessment.

Ingredient	CAS No.	Definition	Function(s)	Technical names	Trade names
Alkyl Benzoates					
Methyl Benzoate	93-58-3	Methyl benzoate is the ester of methyl alcohol and benzoic acid that conforms to the formula in Figure 1.	Fragrance ingredient, skin-conditioning agent-emollient, solvent	Benzoic Acid, Methyl Ester; Methyl Benzenecarboxylate Methyl benzoate (RIFM)	Morflex Methyl Benzoate
Ethyl Benzoate	93-89-0	Ethyl benzoate is the ester of ethyl alcohol and benzoic acid.	Fragrance	Benzoic Acid, Ethyl Ester; Ethyl benzoate (RIFM)	-
Propyl Benzoate	2315-68-6	Propyl benzoate is the ester of n-propyl alcohol and benzoic acid.	Fragrance ingredient, preservative	Benzoic Acid, n-Propyl Ester; Propyl benzoate (RIFM)	-
Butyl Benzoate	136-60-7	Butyl benzoate is the ester of butyl alcohol and benzoic acid.	Fragrance ingredient, preservative	Benzoic Acid, n-Butyl Ester; Butyl benzoate (RIFM)	-
Amyl Benzoate	2049-96-9	Amyl benzoate is the ester of amyl alcohol and benzoic acid that conforms to the formula in Figure 1.	Fragrance ingredient	Benzoic Acid, Pentyl Ester; Pentyl Benzoate Pentyl benzoate (RIFM)	-
Lauryl/ Myristyl Benzoate	No CAS No.	Lauryl/Myristyl benzoate is the organic compound that conforms to the formula in Figure 1.	Skin-conditioning agent-miscellaneous	-	Corum 5014
C12-15 Alkyl Benzoate	68411-27-8	C12-15 alkyl benzoate is the mixture of esters of benzoic acid and C12-15 alcohols.	Skin-conditioning agents - emollient	Alkyl (C12-C15) Benzoate; Benzoic Acid, C12-15 Alkyl Esters; C12-15 Alcohols Benzoate	AEC C12-15 Alkyl Benzoate; Botanester AB; Cetiol AB; Corum 5012; Crodamol AB; Crodamol AB; Dub B1215; Finsolv TN; Hest 25B; Liponate NEB; OriStar AKB; Saboderm AB; Sterol B 125; Tegosoft TN; Tegosoft TN 2
C16-17 Alkyl Benzoate	669700-05-2	C16-17 alkyl benzoate is a mixture of esters of C16-17 alcohols and benzoic acid that conforms generally to the formula in Figure 1.	Skin-conditioning agents-emollient, solvent	-	Finsolv G-2
Stearyl Benzoate	10578-34-4	Stearyl benzoate is the ester of stearyl alcohol and benzoic acid that conforms to the formula in Figure 1.	Skin-conditioning agent-emollient, solvent	Benzoic Acid, Octadecyl Ester; Benzoic Acid, Stearyl Ester; Octadecyl Benzoate	Dub PG; Finsolv 116
Behenyl Benzoate	103403-38-9	Behenyl benzoate is the ester of behenyl alcohol and benzoic acid that conforms to the formula in Figure 1.	Skin-conditioning agent – emollient	Benzoic Acid, Docosyl Ester	Finsolv 137
Branched Alkyl Benzoates					
Isopropyl Benzoate	939-48-0	Isopropyl benzoate is the ester of isopropyl alcohol and benzoic acid.	Fragrance ingredient	Benzoic Acid, Isopropyl Ester; Benzoic Acid, 1-Methylethyl Ester; Isopropyl benzoate (RIFM); 1-Methylethyl Benzoate	-

Table 1. Definitions, functions and structures of alkyl benzoate ingredients in this safety assessment.

Ingredient	CAS No.	Definition	Function(s)	Technical names	Trade names
Isobutyl Benzoate	120-50-3	Isobutyl benzoate is the ester of isobutyl alcohol and benzoic acid.	Fragrance ingredient, solvent	Benzoic Acid, Isobutyl Ester; Benzoic Acid, 2-Methylpropyl Ester; Isobutyl benzoate (RIFM); 2-Methylpropyl Benzoate	-
Isostearyl Benzoate	34364-24-4	Isostearyl benzoate is the ester of isostearyl alcohol and benzoic acid.	Skin-conditioning agent-emollient	Benzoic Acid, Isooctadecyl Ester; Benzoic Acid, Isostearyl Ester	Finsolv SB
Ethylhexyl Benzoate	5444-75-7	Ethylhexyl benzoate is the ester of 2-ethylhexanol and benzoic acid.	Skin-conditioning agent-emollient, solvent	Benzoic Acid, 2-Ethylhexyl Ester; 2-Ethylhexyl Benzoate Octyl Benzoate	Bernel Ester OB; Finsolv EB
Butyloctyl Benzoate	1888038-97-3	Butyloctyl benzoate is the organic compound that conforms to the formula in Figure 2.	Plasticizer; skin-conditioning agent-emollient, solvent	Benzoic Acid, 2-Butyloctyl Ester	-
Hexyldecyl Benzoate	163883-40-7	Hexyldecyl benzoate is the organic compound that conforms to the formula in Figure 2.	Plasticizer, skin-conditioning agent-emollient, solvent	Benzoic Acid, 2-Hexyldecyl Ester	-
Octyldodecyl Benzoate	108347-89-3	Octyldodecyl benzoate is the ester of octyldodecanol and benzoic acid.	Skin-conditioning agent-emollient	Benzoic Acid, 2-Octyldodecyl Ester	Finsolv BOD

Table 2. Physical and Chemical properties of the alkyl benzoate ingredients.[14,25,25,213,213]

	Methyl Benzoate	Ethyl Benzoate	Propyl Benzoate	Butyl Benzoate	Amyl Benzoate	Lauryl/Myristal Benzoate
Molecular Weight (g/mol)	136.15	150.17	164.20	178.23	192.25	290.44/318.49
Boiling Point (°C)	198.6	212.9	230.0	247.3	248	225 (Lauryl at 20 mmHg)
Density (g/cm^3)	1.09	1.04	1.04	1.00	0.95	0.93(Lauryl)
Vapor pressure (mm Hg @ 20°C)	0.38	0.267	0.136	0.01	0.009	-
Solubility (g/1000g water @ 20°C)	2.1	0.72	0.351	0.059	0.028	-
Log K_{ow}	2.12	2.64	3.01	3.84	4.16 (est.)	7.23 (est. Lauryl)

	C12-15 Alkyl Benzoate	C16-17 Alkyl Benzoate	Stearyl Benzoate	Behenyl Benzoate	Isopropyl Benzoate	Isobutyl Benzoate
Molecular Weight (g/mol)	290.44-332.52	346.55-360.57	374.60	430.71	164.20	178.23
Boiling Point (°C)	363 (est.)	-	433 (est.)	518.3	266	237
Density (g/cm^3)	-	-	-	0.908	-	1.02
Vapor pressure (mm Hg @ 20°C)	1×10^{-5} (est.)	-	6×10^{-8} (est.)	7×10^{-11}	0.161 (est.)	0.0417 (est.)
Solubility (g/1000g water @ 20°C)	9×10^{-6} (est.)	-	9×10^{-6} (est.)	7×10^{-7}	0.126 (est.)	0.098 (est.)
Log K_{ow}	7.23 (est.)	-	10.18 (est.)	13.35	3.18	3.23 (est.)

	Isostearyl Benzoate	Ethylhexyl Benzoate	Butyloctyl Benzoate	Hexyldecyl Benzoate	Octyldodecyl Benzoate
Molecular Weight (g/mol)	374.60	234.33	290.44	346.55	402.65
Boiling Point (°C)	426 (est.)	169-170 (at 20 mmHg)	376.9	434.8	449 (est.)
Density (g/cm^3)	-	0.91	0.939	0.923	-
Vapor pressure (mm Hg @ 20°C)	1×10^{-7}	5×10^{-4} (est.)	7×10^{-6}	9×10^{-8}	2×10^{-8} (est.)
Solubility (g/1000g water @ 25°C)	1×10^{-5}	1.1×10^{-3}	5.8×10^{-4}	1.5×10^{-5}	1×10^{-6} (est.)
Log K_{ow}	10.10 (est.)	5.7 (est.)	7.857	9.982	11.09 (est.)

est.= Values were estimated using the EPI Suite, Version 4.0 program or Advanced Chemistry Development (ACD/Labs) Software V11.02.
- Not found

Table 3. Frequency of use according to duration and exposure.[18,19]

Use type	Uses	Concentration (%)	Uses	Concentration (%)	Uses	Concentration (%)	Uses	Concentration (%)
	Methyl benzoate		**Ethyl benzoate**		**C12-15 Alkyl benzoate**		**C16-17 Alkyl benzoate**	
Total/range	**NR**	**0.0005-0.3**	**NR**	**0.0008-0.01**	**971**	**0.0008-59**	**2**	**NR**
Duration of use								
Leave-on	NR	0.005-0.3	NR	0.0008-0.01	858	0.0008-59	NR	NR
Rinse-off	NR	0.007-0.3	NR	NR	113	0.1-50	2	NR
Exposure type								
Eye area	NR	NR	NR	NR	69	0.0008-11	NR	NR
Possible ingestion	NR	NR	NR	NR	66	3-16	NR	NR
Inhalation	NR	NR	NR	0.003-0.01	25	0.3-12	NR	NR
Dermal	NR	0.0005-0.3	NR	0.0008-0.01	870	0.0008-59	2	NR
Deodorant (underarm)	NR	0.004	NR	NR	6	2-12	NR	NR
Hair-noncoloring	NR	NR	NR	NR	98	0.3-35	NR	NR
Hair-coloring	NR	NR	NR	NR	-	0.5-2	NR	NR
Nail	NR	NR	NR	NR	2	0.008-10	NR	NR
Mucous Membrane	NR	0.03	NR	NR	12	0.01-0.04	2	NR
Bath products	NR	0.07	NR	NR	-	0.008-10	NR	NR
Infant	NR	NR	NR	NR	9	10	NR	NR

	Stearyl benzoate		**Isobutyl benzoate**		**Isostearyl benzoate**		**Ethylhexyl benzoate**	
Total/range	**3**	**2**	**NR**	**0.01**	**NR**	**1**	**NR**	**3-4**
Duration of use								
Leave-on	3	2	NR	0.01	NR	1	NR	3-4
Rinse-off	NR	NR	NR	NR	NR	NR	NR	NR
Exposure type				NR		NR		
Eye area	NR	NR	NR	NR	NR	NR	NR	NR
Possible ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Inhalation	NR	NR	NR	0.01	NR	NR	NR	NR
Dermal	3	2	NR	0.01	NR	1	NR	3-4
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair – coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Bath products	NR	NR	NR	NR	NR	NR	NR	NR
Infant	2	NR	NR	NR	NR	NR	NR	NR

	Octyldodecyl benzoate	
Total/range	**NR**	**3-4**
Duration of use		
Leave-on	NR	NR
Rinse-off	NR	3-4
Exposure type		
Eye area	NR	NR
Possible ingestion	NR	NR
Inhalation	NR	NR
Dermal	NR	3-4
Deodorant (underarm)	NR	NR
Hair-noncoloring	NR	NR
Hair-coloring	NR	NR
Nail	NR	NR
Mucous Membrane	NR	NR
Bath products	NR	3
Infant	NR	NR

Table 4. Repeated Dose Oral/Feed Toxicity Studies on Benzoic Acid and Sodium Benzoate

Protocol	Results/Comments	Reference
Benzoic Acid		
Royal Wistar rats dosed with 3% for 1, 2, 3, or 5 days (~1500 mg/kg/day); basal diet followed for 19-30 days	14/35 Rats dosed for five days died; necrosis of parenchymal cells noted in brain in all 5-day treated rats and occasionally in 3-day treated rats	Kreis et al. 1967[214]
Royal Wistar rats (no. not stated) dosed with 1.1% for 7, 14, or 35 days (~550 mg/kg/day)	Significantly poor weight gain; no signs of neurotoxicity or pathological changes in the brain	Kreis et al. 1967[214]
100 Mice (50 each sex) dosed for 3 mo with 80 mg/kg/day (oral intubation)	Weight gain in treated animals was 66% (females) and 71% (females) of gain in controls, values significant; however, feed intake comparable	Shtenberg & Ignatév 1970[70]
40 Sprague-Dawley rats (20 each sex) received feed containing either 0.5% or 2% for 1 yr. Some other groups also received sorbic acid	No effect noted at 0.5%; slight reduction of growth rate noted at 2%. No additive toxicity noted of Benzoic Acid plus sorbic acid	Ohno et al. 1978[215]
50 Mice (25 each sex) dosed with 40 mg/kg/day; fed as a paste for 17 mo, followed by 5 d of oral intubation	Major finding was a reduced response to physiological stress in treated animals compared to controls	Shtenberg & Ignatév 1970[70]
Mice (no. not stated) dosed with 40 or 80 mg/kg/day for 3, 8, or 18 mo	Negative effects on body weight and viability; treatment-related carcinogenic effects noted (not specified); increased liver weights, enlarged spleens, ovaries and lungs	Ignatév 1965[216]
20 Rats (10 each sex) dosed with 40 mg/kg/day; fed as a paste for 18 mo, followed by 13 d of oral intubation	Developed increased tolerance to lethal doses of Sodium Benzoate; daily feed and water intake significantly less for treated males; limited data reported	Shtenberg & Ignatév 1970[70]
Rats (no. not stated) dosed with 40 or 80 mg/kg/day for 3, 8, or 18 mo	No apparent affect on body weight or viability; no changes noted in parenchymatous organs; developed increased tolerance to lethal doses of Benzoic Acid	Ignatév 1965[216]
50 Wistar rats (20 female, 30 male), 20 male Wistar rats and 20 male Osborne-Mendel rats, dosed with 1.5% in feed for 18 mo	Decreased feed intake and reduced growth	Marquardt 1960[217]
4 Generations of Bayer-Elberfeid rats dosed with 0.5 or 1.0% in feed	No adverse effect noted; increased life-span noted in treated rats	Kieckebusch & Lang 1960[218]
Sodium Benzoate		
28 Rats dosed with 5% in feed	19/28 Died within two wks of dosing; remaining 9 died by end of wk 3	Kieckebusch & Lang 1960[218]
12 Sherman rats (6 each sex) dosed with 2% or 5% in feed for 28 days	Slight weight depression (significant in males) noted at 2%; 5% toxic to all rats.	Fanelli & Halliday 1963[219]
Groups of 10 Sherman rats (5 each sex) dosed with 16-1090 mg/kg/day (four doses) for 30 days	No toxic effects; increased body weight, reduced appetite (compared to control), noted. Lesions of adrenal glands, upper intestine, kidneys, liver and spleen	Smyth & Carpenter 1948[220]
Rats (no. not stated) dosed with 1947-2195 mg/kg/day for 3-6 wk	Severe reduction of growth rate	White 1941[221]
Wistar rats dosed with 1.5% in feed for 6 or 8 wk (after wk 4, carotene was added to diet)	No significant effect noted. Vitamin A content in liver and kidneys comparable to control	Kramer & Tarjan 1962[222]
Groups of 10 Sherman rats (5 each sex) dosed with 1, 2, 4, 8% in feed for 90 days	No adverse effects at ≤ 4%. At 8% reduced growth rate (feed consumption comparable to control), significantly increased liver and kidneys weight with lesions noted	Deuel et al. 1954[223]
White rats (no. not stated) dosed with 1.5, 2.0, 2.5, 3.0% in feed for unknown duration	No effects noted in rats of ≤2.5% groups; distinct growth reduction noted in rats of 3.0% group though feed intake was comparable to control. One third of rats of this group died	Griffith 1929[224]

**Reviewing the studies, the GRAS report (Informatics Inc., 1972) concluded, " . . . at a level of approximately 1%, the benzoates are at maximum non-toxic level; higher than this, they result in decreased food intake, depressed growth, and toxic effects on test animals."

Table 5. Genotoxicity tests of sodium benzoate and benzoic acid.

Assay	Concentration/method	Results/comments	Reference
Bacterial cells			
Host-mediated	Mice orally dosed (either single dose or 5 doses 24 h apart) with 50, 500, 5000 mg/kg sodium benzoate, then inoculated with *Salmonella* TA 1530, G46, and *Saccharomyces* D3; after 3 h, mice were killed and the bacteria removed (by peritoneal wash) and plated	Negative (slight increase in mutation frequencies ntoed; non-dose dependent)	Litton Bionetics 1974[225]
Ames; *S. typhimurium* (TA97A, TA102)	33 – 10,000 µg Benzoic acid/plate ± S9	Negative	Fujita and Sasaki 1986[226]
Ames; *S. typhimurium* (TA97, TA98, TA100, TA1535, TA1537)	Benzoic acid at 100 – 6666 µg/plate or 100 – 10,000 µg/plate ± S9 (either rat or hamster liver)	Negative	Zeiger et al 1988[227]
Ames; *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) *E. coli* (WP2)	0.033 – mg Sodium benzoate/plate ± S9	Negative	Prival, et al. 1991[228]
Reverse mutation assay; *Bacillus subtilis*	Not reported; no metabolic activation	Positive	Kawachi et al 1980[147]
Ames; *S. typhimurium* (TA98, TA100)	0.1 mg/Disc; ± metabolic activation	Positive	Kuboyama and Fufii 1992[145]
Ames; *S. typhimurium* (TA98, TA100, TA1535, TA1537)	Not reported; with metabolic activation	Positive	McCann et al 1975[144]
Mammal cells			
SCE in CHO	1, 3, 10 mM Benzoic acid	Negative	Oikawa et al. 1980[229]
SCE in CHO	1, 2, 5, 10 mM Sodium benzoate	Positive at ≥ 2 mM (considered a high dose)	Abe and Sasaki 1977[230]
ABS in CHO	Maximum effective dose: 2.00 mg/ml (138.8 x $1.^{-4}$ M) sodium benzoate	Positive: aberrations noted in 38%	Ishidate and Odashima 1977[231]
Cytogenetics (human embryonic lung cells)	2, 20, 200 mg/kg Sodium benzoate	Negative (checked for aberrations in anaphase chromosomes)	Litton Bionetics 1974[225]
Human lymphocytes	0 – 2.0 mM Benzoic acid with metabolic activation; sister chromatid assay	Negative	Jansson et al 1986[146]
In vivo: Mammalian			
Dominant lethal (rats)	Following dosing by oral intubation (50, 500, 5000 mg/kg sodium benzoate either single dose or 5 doses 24 h apart), male rats were mated with 2 females/week for 8 weeks. Corpora lutea, early and late fetal deaths, and total implantations monitored	Negative	Litton Bionetics 1974[225]
Cytogenetic (rats)	Rats dosed by gastric intubation (50, 500, 5000 mg/kg sodium benzoate either single dose or 5 doses 24 h apart, killed at various times after dosing (were given colcemid to arrest cells in metaphase)	Negative (checked for aberrations in bone marrow metaphase chromosomes)	Litton Bionetics 1974[225]

Table 6. HRIPTs of products containing benzoate esters.

Benzoate	Product type	Concentration	N	Results	Reference
Methyl benzoate	Perfume	0.028%	110	No irritation or sensitization	Product Investigations, Inc. 2006 [232]
C12-15 Alkyl Benzoate	Hand cream	0.07398%	49	No irritation or sensitization	Consumer Product Testing Co. 2010[233]
C12-15 Alkyl Benzoate	Concealer	4.2%	108	No irritation or sensitization	Clinical Research Laboratories, Inc. 2008 [234]
C12-15 Alkyl benzoate	Blush	14.15%	116	No irritation or sensitization	Product Investigations, Inc. 2009[225,235]
C12-15 Alkyl benzoate	Lipstick	16%	104	No irritation or sensitization	TKL Research[236]
C12-15 Alkyl benzoate	Lipstick	16%	107[a]	No irritation or sensitization	TKL Research 2005[237]
C12-15 Alkyl benzoate	Lipstick	16%	107[a]	No irritation or sensitization	TKL Research 2005 [238]
C12-15 Alkyl benzoate	Body oil	19.5%	100	No irritation or sensitization	Product Investigations, Inc. 2009[225,239]
C12-15 Alkyl benzoate	Hair serum	35%	208	No irritation or sensitization	Consumer Product Testing Co. 2003[240]
Stearyl benzoate	Face lotion	2%	206	No irritation or sensitization	TKL Reasearch 2010[241]
Isobutyl benzoate	Perfume	0.01%	103	No irritation or sensitization	Product Investigations, Inc. 2006[232]
Isostearyl bemzoate	Body lotion	0.95	107	No irritation or sensitization	Consumer Product Testing Co. 2005[242]
Ethylhexyl benzoate	Sunscreen liquid	3.5%	212	No irritation or sensitization	Clinical Research Lab. Inc. 2010 16 page 19
Octyldodecyl benzoate	Perfumed bar soap	2.0473%	101	No irritation or sensitization	Clinical Research Services 1998[243]
Octyldodecyl benzoate	Shaving cream	0.4% (diluted from 4% in water)	105	No irritation or sensitization	Personal Care Products Council 2010[244]

[a] These two studies were completed on the same panel of subjects.

Figure 1. Straight chain alkyl benzoates: structures, esterase metabolism, and metabolites.

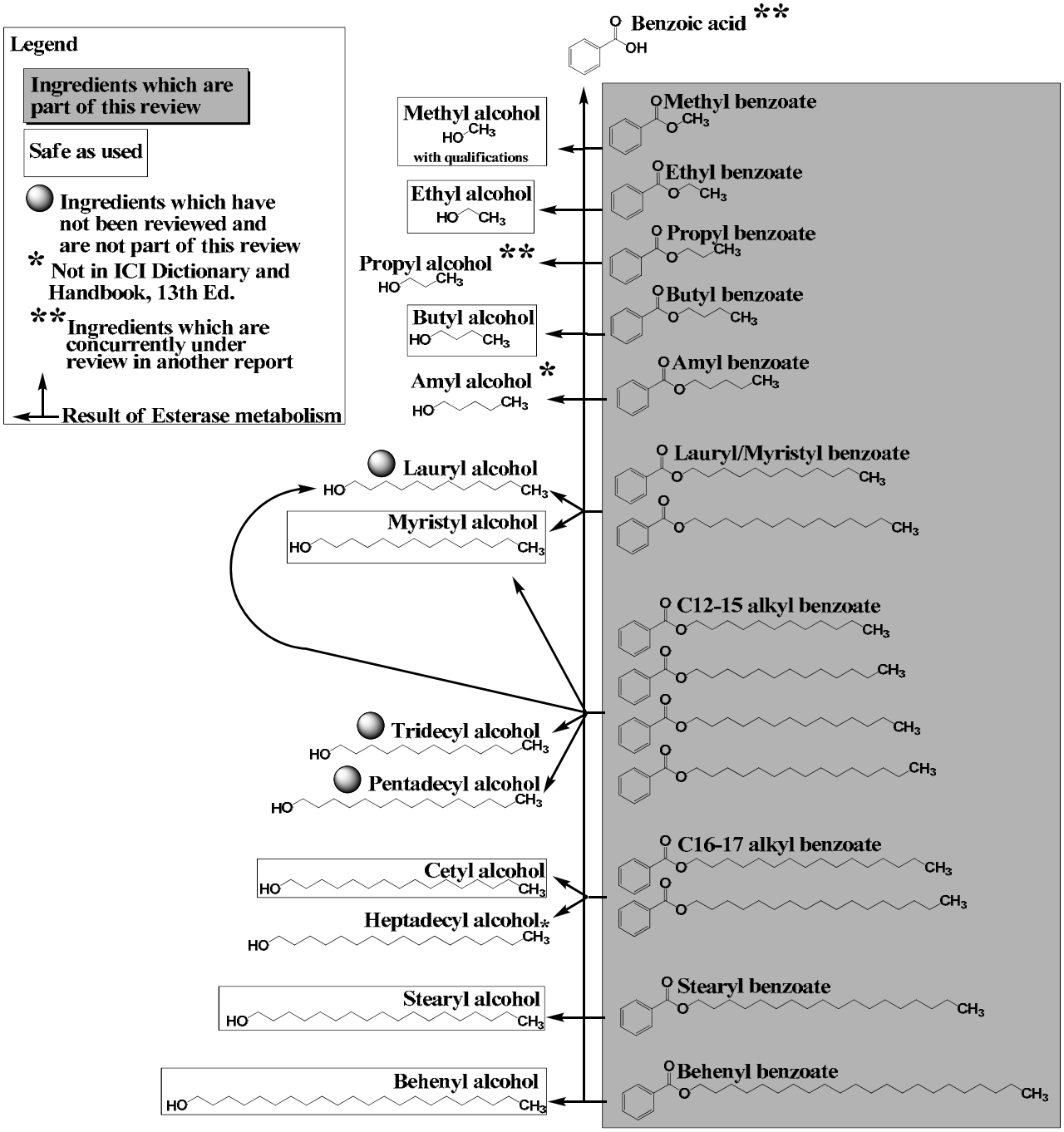


Figure 2. Branched-chain alkyl benzoates: structures, esterase metabolism, and metabolites.

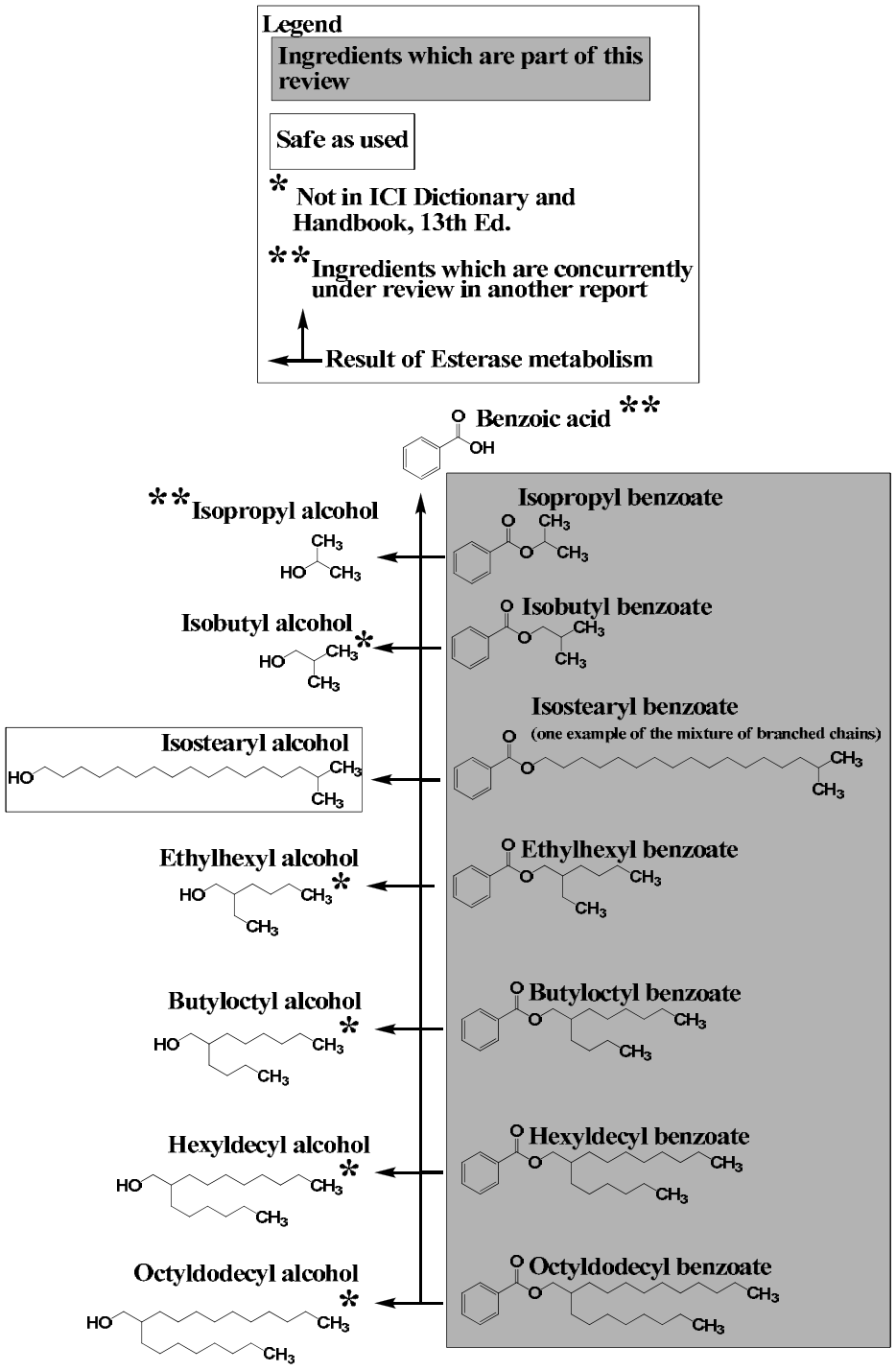
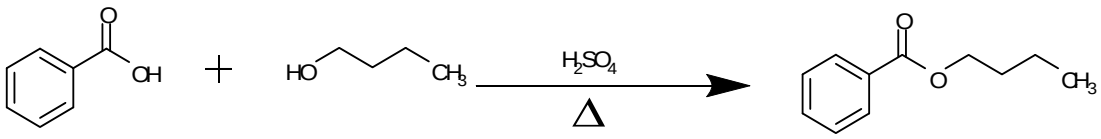


Figure 3. The synthesis of butyl benzoate.



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DATA

Unpublished Data

Unpublished data have been submitted by the Council and incorporated into the benzoate report. The following is a list of the types of data in the order that they are presented in this section.

A few of the larger submissions that had more detail than necessary (e.g., raw data, informed consent forms) have had some pages removed in the print version (marked with an *) below. The complete versions are available in the on line version.

1 – Concentration of Use survey by PCPC

2 – Skin Penetration of C12-15 Alkyl Benzoates

3 – RIFM Summaries for: methyl benzoate, ethyl benzoate, propyl benzoate, isopropyl benzoate, butyl benzoate, isobutyl benzoate, pentyl benzoate, and ethylhexyl benzoate.

4 – Various tests of a product containing ethylhexyl benzoate – ocular irritation, phototoxicity, HRIPT*, cumulative irritation, and pediatric safety.

5 – Various tests of a product containing C12-C15 alkyl benzoate – dermal irritation, acute dermal toxicity, acute inhalation, dermal irritation (14-day), EpiDerm*, EpiOcular*, HRIPT*, and Ames.

6 – Various tests of C12-15 alkyl benzoate – acute oral and skin toxicity, acute eye irritation/corrosion*.

7 – HRIPTs of products containing mostly C12-15 alkyl benzoate*. Other studies contain isobutyl benzoate*, octyldodecyl benzoate*, methyl benzoate*, and stearyl benzoate*.

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. 10/1/10
Industry Liaison to the CIR Expert Panel

DATE: October 1, 2010

SUBJECT: Updated Concentration of Use Information Alkyl Benzoates

Uses of Ethylhexyl Benzoate have now been reported

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use

C12-15 Alkyl Benzoate, Amyl Benzoate, Behenyl Benzoate, Butyl Benzoate, Butyloctyl Benzoate, C16-17 Benzoate, Ethyl Benzoate, Ethylhexyl Benzoate, Hexyldecyl Benzoate, Isobutyl Benzoate, Isopropyl Benzoate, Isostearyl Benzoate, Lauryl/Myristyl Benzoate, Methyl Benzoate, Octyldodecyl Benzoate, Propyl Benzoate and Stearyl Benzoate*

Ingredient	Product Category	Concentration of Use
C12-15 Alkyl Benzoate	Baby lotions, oils, powders and creams	10%
C12-15 Alkyl Benzoate	Other baby products	10%
C12-15 Alkyl Benzoate	Eyebrow pencil	2%
C12-15 Alkyl Benzoate	Eyeliner	0.008-0.4%
C12-15 Alkyl Benzoate	Eye shadow	2-9%
C12-15 Alkyl Benzoate	Eye lotion	2-11%
C12-15 Alkyl Benzoate	Eye makeup remover	4%
C12-15 Alkyl Benzoate	Mascara	0.0008-0.08%
C12-15 Alkyl Benzoate	Colognes and toilet waters	0.4%
C12-15 Alkyl Benzoate	Perfumes	0.5-10%
C12-15 Alkyl Benzoate	Powders (dusting and talcum)	3%
C12-15 Alkyl Benzoate	Other fragrance preparations	0.6-11%
C12-15 Alkyl Benzoate	Hair conditioners	0.3-2%
C12-15 Alkyl Benzoate	Hair sprays (aerosol fixatives)	0.3-12%
C12-15 Alkyl Benzoate	Shampoos (noncoloring)	3%
C12-15 Alkyl Benzoate	Tonics, dressings and other hair grooming aids	0.5-35%
C12-15 Alkyl Benzoate	Hair rinses (coloring)	0.5%
C12-15 Alkyl Benzoate	Other hair coloring preparations	2%
C12-15 Alkyl Benzoate	Blushers (all types)	0.5-16%
C12-15 Alkyl Benzoate	Face powders	0.008-7%
C12-15 Alkyl Benzoate	Foundations	6-16%
C12-15 Alkyl Benzoate	Leg and body paints	3%
C12-15 Alkyl Benzoate	Lipstick	3-16%
C12-15 Alkyl Benzoate	Makeup bases	2-6%
C12-15 Alkyl Benzoate	Other makeup preparations	0.0008-11%
C12-15 Alkyl Benzoate	Cuticle softeners	0.008-10%
C12-15 Alkyl Benzoate	Bath soaps and detergents	0.01-0.04%

C12-15 Alkyl Benzoate	Deodorants (underarm)	2-12%
C12-15 Alkyl Benzoate	Aftershave lotions	2-3%
C12-15 Alkyl Benzoate	Preshave lotions (all types)	9%
C12-15 Alkyl Benzoate	Shaving creams (aerosol, brushless and lather)	6%
C12-15 Alkyl Benzoate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.1-9%
C12-15 Alkyl Benzoate	Face and neck creams, lotions and powders	0.05-11%
C12-15 Alkyl Benzoate	Body and hand creams, lotions and powders	0.005-20%
C12-15 Alkyl Benzoate	Body and hand sprays	2%
C12-15 Alkyl Benzoate	Foot powders and sprays	1%
C12-15 Alkyl Benzoate	Moisturizing creams, lotions and powders	0.03-10%
C12-15 Alkyl Benzoate	Night creams, lotions and powders	0.05-5%
C12-15 Alkyl Benzoate	Paste masks (mud packs)	50%
C12-15 Alkyl Benzoate	Other skin care preparations	0.07-11%
C12-15 Alkyl Benzoate	Suntan gels, creams and liquids	2-59%
C12-15 Alkyl Benzoate	Indoor tanning preparations	3-5%
C12-15 Alkyl Benzoate	Other suntan preparations	10-24%
Ethyl Benzoate	Perfumes	0.003%
Ethyl Benzoate	Face and neck creams, lotions and powders	0.0008%
Ethyl Benzoate	Body and hand creams, lotions and powders	0.002%
Ethyl Benzoate	Foot powders and sprays	0.01%
Ethylhexyl Benzoate	Suntan gels, creams and liquids	3%
Ethylhexyl Benzoate	Other suntan preparations	4%
Isobutyl Benzoate	Perfumes	0.01%
Isostearyl Benzoate	Body and hand creams, lotions and powders	1%
Methyl Benzoate	Bath oils, tablets and salts	0.007%
Methyl Benzoate	Perfumes	0.3%
Methyl Benzoate	Bath soaps and detergents	0.03%
Methyl Benzoate	Deodorants (underarm)	0.004%
Methyl Benzoate	Face and neck creams, lotions and powders	0.0005%
Methyl Benzoate	Body and hand creams, lotions and powders	0.04%
Methyl Benzoate	Other skin care preparations	0.002%

Octyldodecyl Benzoate	Bath soaps and detergents	3%
Octyldodecyl Benzoate	Shaving cream (aerosol, brushless and lather)	4%
Stearyl Benzoate	Face and neck creams, lotions and powders	2%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2010
Table prepared May 12, 2010
Updated July 8, 2010
Updated August 19, 2010 C16-17 Alkyl Benzoate deleted
Updated October 1, 2010, Ethylhexyl Benzoate added

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. 11/3/10
Industry Liaison to the CIR Expert Panel

DATE: November 3, 2010

SUBJECT: Dermal Penetration Studies of C12-15 Alkyl Benzoate

Beiersdorf AG. 2000. Dermal absorption and penetration of C12-15 Alkyl Benzoate [Finsolv TN]. (PEN.248) (material applied neat)

Beiersdorf AG. 2000. Dermal absorption and penetration of C12-15 Alkyl Benzoate [Finsolve TN] (PEN.247) (material applied in formulation)

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Beiersdorf AG

Research & development

Report

Report-Nr.: 2193-2010

Date: 01.11.2010

Date of Study-procedure: 20.07.2000

Dermal absorption and penetration of C12-15 Alkyl Benzoate [Finsolv TN]

(PEN.248)

Order No. : 8857

Authors : 4218 Schepky, Dr. Andreas
4218 Akhiani, Mehdi

Attn. : 4280 Teichert, Dr. Thomas
4280 Schuler, Dr. Jürgen

1. INTRODUCTION

In this study dermal absorption and percutaneous penetration of C12-15 Alkyl Benzoate [Finsolv TN] (lipid) after topical application to excised pig skin and 24 hours exposure was examined.

2. MATERIALS AND METHODS

2.1 Responsibility

Study Directors:	Dr. W. Diembeck / H.-J. Düsing
Technical Staff:	M. Akhiani
Lab-protocols:	16.03 - 28.06. 2000

2.2 Test Sample [SAM] and Test Substance [SUB]

	Notation	Tradename	P.nr.	Charge
SUB=SUB	C12-15 Alkyl Benzoate	Finsolv TN	11431.9	31.132

2.3 Reagents and apparatus

Analytical determination of the test substance was carried out with a HPLC (VWR-Hitachi).

Substances, special materials and tools

- Methanol, HPLC Grade, Merck
- Isopropanol, HPLC Grade, Merck
- Purified Water, Millipore Milli-Q grade
- Ortho phosphoric acid (85%, Merck, Germany)
- C12-15 Alkyl Benzoate, LOT: 31.132
- NaCl (Merck, Germany)
- Bovine serum albumin (96%, Sigma-Aldrich Chemie, Germany)
- gentamicine sulfate (from micromonospora purpurea, Fluka Germany)
- Unboiled back skin of pigs
- Electric knife
- Electric clipper for animals (Aesculap®, Germany), shaver head GH 703 1/10 mm)
- Butcher knife
- Hot plate (Ceram) with temperature control
- Metallic cylinder (weight: 400 g, pressure: 80 g/cm^2)
- Precision scale (0.001 mg)
- Scalpel, forceps, spatula, micropipette, magnetic stirrer, magnetic bars, timer

- Franz type static diffusion cell (-receptor volume: 7-8 ml, exposure area: 4.9 cm²)
- Water bath (34° C)

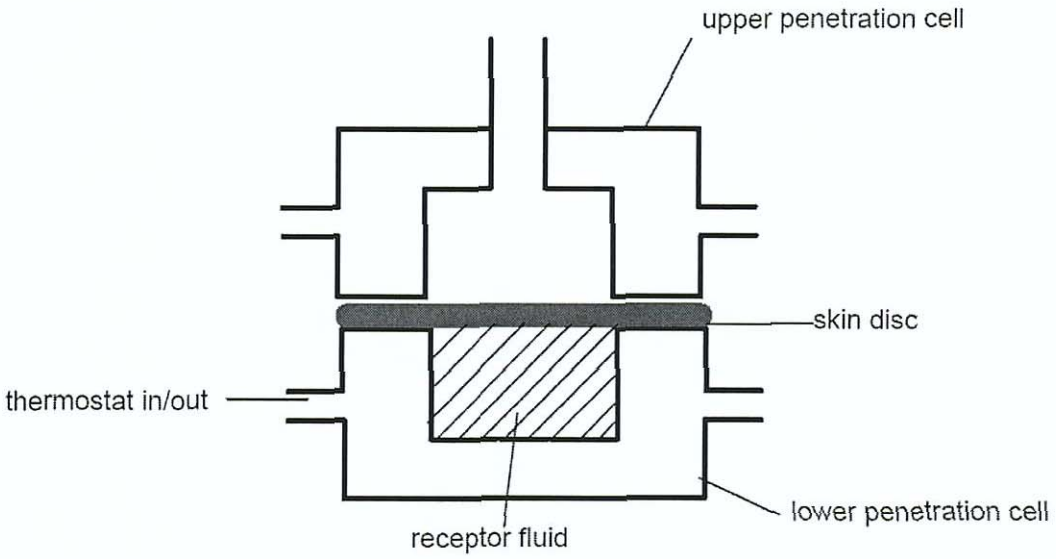
2.4 Experimental

2.4.1 Standard-Protocol (method)

PEN "In vitro penetration of cosmetic ingredients without radiolabelled tracers"09.12.1999 (BDF internal SOP according to OECD guideline No. 428 and Diembeck W, Beck H, Benech-Kieffer F et al., Test guidelines for in vitro assessment of dermal absorption and percutaneous penetration of cosmetic ingredients. European Cosmetic, Toiletry and Perfumery Association. *Food Chem Toxicol* 1999; 37: 191-205.)

This *ex vivo / in vitro*-model was used for the estimation of the dermal absorption and penetration of ingredients of cosmetics and pharmaceuticals which are being developed for topical administration. It complements our existing *in vitro* dermatoxicological test battery for the compatibility and risk assessment, supports the selection of suitable raw materials and the optimization of preparations to achieve desirable liberation, absorption and penetration characteristics. The experiments are carried out with especially prepared, unboiled (!) back skin of selected female pigs (about 130 days old, weighing about 100 kg, delivered by a local, commercial butcher according to our specifications).
Pig skin is recommended in the literature for the *in vitro* estimation of the dermal absorption and penetration behavior of human skin.

Schematic diagram of the penetration cell



The experiments are carried out in penetration cells of glass (Franz type static diffusion cell) kept at 32°C (skin temperature) with a thermostat. Skin discs (gently dry-shaved surface, thickness about 3-4 mm, diameter 5 cm) are used.

The receptor fluid, which is suitable to solute hydrophilic and lipophilic test samples / substances, is composed as follows: 0.9% sodium chloride, 1% bovine serum albumin, and 0.02% gentamycine sulphate in water.

The analytical samples are prepared as follows:

- extraction: solvent: isopropanol, 20 hours at rest, 5 min in the ultrasonic bath
- skin surface : gentle scraping with a spatula
- horny layer : tesa™ tape (#4129), 16 strips, pressure: 80 g/cm^2 during 5 seconds
- epidermis : heating of the skin disc (epidermal side for 45 seconds on a 80°C hotplate (Ceram), separation epidermis <=> dermis with forceps
- dermis : total dermis cut to small pieces
- receptor fluid: 1 aliquot

Test samples / test substances are examined in triplicate (3 skin discs, Application area: ca. 5 cm², Application amount: ca. 4mg test samples/ cm²)

POW "Determination of the partition coefficient (HPLC-method of OECD Guideline 117)" (available as methods short description)

2.4.2 Analysis conditions

Method (HPLC)

-solvent:	100% isopropanol
-column:	Merck, Select B
-mobile phase:	MeOH/H2O
-injection volume:	10 µl
-flow:	1 ml/min

Calibration, linearity, detection limit and quantitative determination limit

-calibration function:	$A = a * x^b$	
	$(x = 1.65E\text{-}04 * A^{1.000})$	A: area (counts) X: concentration [µg/ml] b: 0.998
-linearity interval:	3.46 – 280.20 µg/ml	variation coefficient: 1.4 %
-detection limit:	1.0 µg/ml	correlation factor = 0.9998
-quantitative determination limit:	3.0 µg/ml	

3. RESULTS AND DISCUSSION

3.1 Recovery

The total recovery of the test substance amounted to approx. 82 % of the applied Finsolv TN in this study.

3.2 Partition coefficient

The partition coefficient of Finsolv TN was carried out with a HPLC (VWR-Hitachi).

For C12-Benzoic acid-alkyl ester to: log P_{OW} = 8.0
For C13-Benzoic acid-alkyl ester to: log P_{OW} = 8.6
For C14-Benzoic acid-alkyl ester to: log P_{OW} = 9.1
For C15-Benzoic acid-alkyl ester to: log P_{OW} = 9.6 (pH 3).

Therefore, a percutaneous penetration of Finsolv TN cannot be expected.

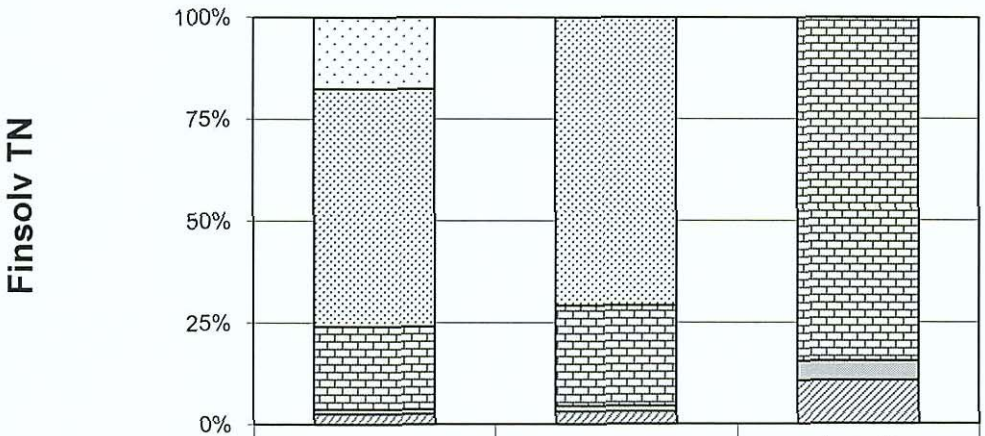
3.3 dermal absorption and penetration*

24 hours exposure	Finsolv TN 100 %	
	%	µg/cm²
applied dose	100	3860
total recovery of the unchanged, parent test substance	82	3181
dermal absorption	29**	933
distribution of the dermally absorbed test substance		
horny layer	84	787
epidermis	5	45
dermis	11	100
receptor fluid	n. d.***	n. d.

* see attached 1

** referred to total recovery of the unchanged, parent test substance

*** no detectable (< detection limit)



	applied	recovered	dermally absorbed
loss	17,6%		
surface	58,3%	70,7%	
horny layer	20,4%	24,7%	84,4%
epidermis	1,2%	1,4%	4,8%
dermis	2,6%	3,1%	10,7%
receptor fluid	0,0%	0,0%	0,0%

'applied' = percentages of applied test substance (recovered plus loss)
'recovered' = percentages of recovered amounts (extrapolated to 100% recovery)
'dermally absorbed' = percentages of dermally absorbed test substance (extrapolated to 100% recovery of dermally absorbed test substance)

The largest amount of Finsolv TN after 24 hours incubation was found in the horny layer (stratum corneum).

Approx. 29 % of the applied test substance was dermally absorbed within 24 hours post application. Approx. 84 % of the dermally absorbed Finsolv TN were found in the horny layer (stratum corneum), approx. 5 % in the epidermis and approx. 11 % in the dermis. No Finsolv TN was detected in the receptor fluid. A percutaneous penetration is not found here for Finsolv TN. The application of the "raw" Finsolv TN (not as part of a cosmetic formulation) shows a distinctly different penetration behavior in comparison to the emulsion. (Report 8822)

Because of the non-representativeness and furthermore of the very small size of the sample (N=3 taken from the same pig) an inductive statistical analysis could not be performed. Therefore the descriptive results achieved in this study have to be considered as a trend and interpreted as such.

4218 - Bioavailability

Dr. A. Schepky

Mehdi Akhiani

Attached 1 (raw data penetration)

ORDER : 8857 From 19.01.2000		TEST	START	BATCH	OPERATOR
SAMPLE:	Finsolv TN	PEN	16.03.2000	Feb 2000	Ak
SUBSTANCE:	Finsolv TN	24h / 32°C	2-Propanol	Analytic:	DAD

due:	100 %
is:	100 %

	#1	#2	#2	mean	VC [%]
weight (mg)	18.56	17.13	21.15	18.95	11
substance [µg]	18560	17130	21150	18946	11
top (upper penetration cell) [µg]	160	156	0	105.6	87
Skin surface [µg]	11105	8753	12943	10933	19
horny layer [µg]	2901	3744	4948	3864	27
epidermis [µg]	253	261	149	221	28
dermis [µg]	536	351	584	490	25
receptor fluid [µg]	0	0	0	0	0
SUM [µg]	14955	13266	18625	15615	18
total recovery	80.5%	77.4%	88.0%	82.4 %	
strips 1 - 2 [µg]	1164	1602	2343		35
strips 3 - 4 [µg]	656	743	740		7
strips 5 - 6 [µg]	288	406	707		46
strips 7 - 8 [µg]	228	298	430		32
strips 9 - 10 [µg]	218	218	247		7
strips 11 - 12 [µg]	148	185	181		12
strips 13 - 14 [µg]	125	166	178		18
strips 15 - 16 [µg]	75	127	124		27

application area	4,9	cm^2
doping test substance	3860	µg/ cm^2

distribution	µg	µg/cm^2	% applied substance dermally absorbed	% normalized applied substance dermally absorbed	% abs. norm.
skin surface	11039	2249	58,3%	70,69%	**********
horny layer	3864	787	20,4%	24,75%	84,45%
epidermis	221	45	1,2%	1,42%	4,84%
dermis	490	100	2,6%	3,14%	10,71%
receptor fluid	0	0	0,0%	0,00%	0,00%
SUM	15615	3181	82,4%	100,0%	100,0%

Beiersdorf AG

Research & development

Report

Report-Nr. 2194-2010

Date: 28.10.2010

Date of Study-procedure: 15.03.2000

Dermal absorption and penetration of C12-15 Alkyl Benzoate [Finsolv TN]

(PEN.247)

Order No. : 8822

Authors : 4218 Schepky, Dr. Andreas
4218 Akhiani, Mehdi

Attn. : 4280 Teichert, Dr. Thomas
4280 Schuler, Dr. Jürgen

1. INTRODUCTION

In this study dermal absorption and percutaneous penetration of C12-15 Alkyl Benzoate [Finsolv TN] (lipid) from Formulations STEO98 427, PHYW/O 137 and SUNPIT 22 after topical application to excised pig skin and 24 hours exposure was examined.

2. MATERIALS AND METHODS

2.1 Responsibility

Study Directors:	Dr. W. Diembeck / H.-J. Düsing
Technical Staff:	M. Akhiani
Lab-protocols:	Feb. 16. – Mar. 03. 2000

2.2 Test Sample [SAM] and Test Substance [SUB]

	Notation	Code
SAM	Nivea Sun-Lotion LF 12	STEO98 427
	Nivea Baby Micropigmentcreme LF30	PHYW/O 137
SUB	Eucerin protection spray LF 20 Finsolv TN (Solvin, PNR 11431.9) Ch# 31132	SUNPIT 22

2.3 Reagents and apparatus

Analytical determination of the test substance was carried out with a HPLC (VWR-Hitachi).

Substances, special materials and tools

- Methanol, HPLC Grade, Merck
- Isopropanol, HPLC Grade, Merck
- Purified Water, Millipore Milli-Q grade
- Ortho phosphoric acid (85%, Merck, Germany)
- C12-15 Alkyl Benzoate, LOT: 31.132
- NaCl (Merck, Germany)
- Bovine serum albumin (96%, Sigma-Aldrich Chemie, Germany)
- gentamicine sulfate (from micromonospora purpurea, Fluka Germany)
- Unboiled back skin of pigs
- Electric knife
- Electric clipper for animals (Aesculap®, Germany), shaver head GH 703 1/10 mm)
- Butcher knife

- Hot plate (Ceram) with temperature control
- Metallic cylinder (weight: 400 g, pressure: 80 g/cm^2)
- Precision scale (0.001 mg)
- Scalpel, forceps, spatula, micropipette, magnetic stirrer, magnetic bars, timer
- Franz type static diffusion cell (-receptor volume: 7-8 ml, exposure area: 4.9 cm²)
- Water bath (34° C)

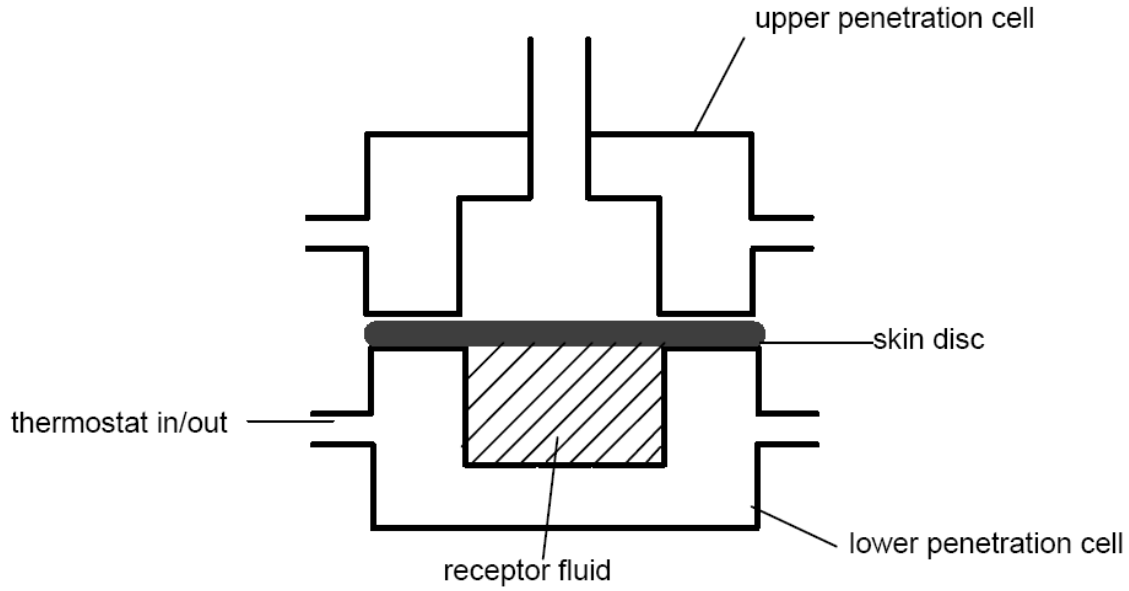
2.4 Experimental

2.4.1 Standard-Protocol (method)

PEN "In vitro penetration of cosmetic ingredients without radiolabelled tracers"09.12.1999 (BDF internal SOP according to OECD guideline No. 428 and Diembeck W, Beck H, Benech-Kieffer F et al., Test guidelines for in vitro assessment of dermal absorption and percutaneous penetration of cosmetic ingredients. European Cosmetic, Toiletry and Perfumery Association. *Food Chem Toxicol* 1999; 37: 191-205.)

This *ex vivo / in vitro*-model was used for the estimation of the dermal absorption and penetration of ingredients of cosmetics and pharmaceuticals which are being developed for topical administration. It complements our existing *in vitro* dermatoxicological test battery for the compatibility and risk assessment, supports the selection of suitable raw materials and the optimization of preparations to achieve desirable liberation, absorption and penetration characteristics. The experiments are carried out with especially prepared, unboiled (!) back skin of selected female pigs (about 130 days old, weighing about 100 kg, delivered by a local, commercial butcher according to our specifications).
Pig skin is recommended in the literature for the *in vitro* estimation of the dermal absorption and penetration behavior of human skin.

Schematic diagram of the penetration cell



The experiments are carried out in penetration cells of glass (Franz type static diffusion cell) kept at 32°C (skin temperature) with a thermostat. Skin discs (gently dry-shaved surface, thickness about 3-4 mm, diameter 5 cm) are used.
The receptor fluid, which is suitable to solute hydrophilic and lipophilic test samples / substances, is composed as follows: 0.9% sodium chloride, 1% bovine serum albumin, and 0.02% gentamycine sulphate in water.

The analytical samples are prepared as follows:

- extraction: solvent: isopropanol, 20 hours at rest, 5 min in the ultrasonic bath
- skin surface : gentle scraping with a spatula
- horny layer : tesa™ tape (#4129), 16 strips, pressure: 80 g/cm^2 during 5 seconds
- epidermis : heating of the skin disc (epidermal side for 45 seconds on a 80°C hotplate (Ceram), separation epidermis <=> dermis with forceps
- dermis : total dermis cut to small pieces
- receptor fluid: 1 aliquot

Test samples / test substances are examined in triplicate (3 skin discs, Application area: ca. 5 cm², Application amount: ca. 4mg test samples/ cm²)

POW "Determination of the partition coefficient (HPLC-method of OECD Guideline 117)" (available as methods short description)

2.4.2 Analysis conditions

Method (HPLC)

-solvent:	100% isopropanol
-column:	Merck, Select B
-mobile phase:	MeOH/H2O
-injection volume:	10 µl
-flow:	1 ml/min

Calibration, linearity, detection limit and quantitative determination limit

-calibration function:	$A = a * x^b$ $(x = 1.73E\text{-}04 * A^{1.000})$	A: area (counts) X: concentration [µg/ml] b: 0.999
-linearity interval:	2.16 – 174.80 µg/ml	variation coefficient: 0.6 %
-detection limit:	0.7 µg/ml	correlation factor = 0.9998
-quantitative determination limit:	2.1 µg/ml	

3. RESULTS AND DISCUSSION

3.1 Recovery

The total recovery of the unchanged Finsolv TN after topical application to excised pig skin and 24 hours exposure:

Sample	recovery [%]
STEO98 427	**90**
PHYW/O 137	**88**
SUNPIT 22	**95**

3.2 Determination of Finsolv TN amount in the test sample

Recovery of Finsolv TN in the test sample was determined in triplicates

SAMPLE	due [%]	is [%]
STEO98 427	9.0	7.5 ± 0.5
PHYW/O 137	6.0	5.4 ± 0.3
SUNPIT 22	7.0	6.6 ± 0.3

3.3 Partition coefficient

The partition coefficient of Finsolv TN was carried out with a HPLC (VWR-Hitachi).

For C12-Benzoic acid-alkyl ester to: log P_{OW} = 8.0
For C13-Benzoic acid-alkyl ester to: log P_{OW} = 8.6
For C14-Benzoic acid-alkyl ester to: log P_{OW} = 9.1
For C15-Benzoic acid-alkyl ester to: log P_{OW} = 9.6 (pH 3).

Therefore, a percutaneous penetration of Finsolv TN cannot be expected.

3.4 dermal absorption and penetration after 24 hours exposure*

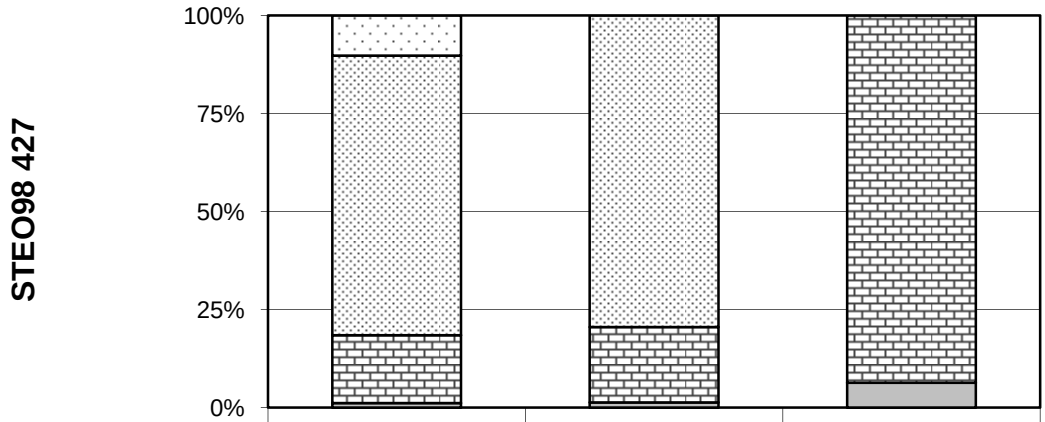
Samples	applied dose		total recovery of the unchanged, parent test substance		dermal absorption**	
	[µg/cm²]	[%]	[µg/cm²]	[%]	[µg/cm²]	[%]
STEO98 427	326	100	293	90	61	21
PHYW/O 137	200	100	177	88	61	34
SUNPIT 22	268	100	255	95	67	26

	distribution of the dermally absorbed test substance					
	STEO98 427		**PHYW/O 137**		**SUNPIT 22**	
	[µg/cm²]	[%]	[µg/cm²]	[%]	[µg/cm²]	[%]
horny layer	56	93.5	55	91.5	62	92.5
epidermis	4	6.5	5	8.6	5	7.5
dermis	< 0.15***	<0.05***	< 0.15	< 0.07	< 0.15	< 0.05
receptor fluid	< 0.15	<0.05	< 0.15	< 0.07	< 0.15	< 0.05

***** see attached 2.

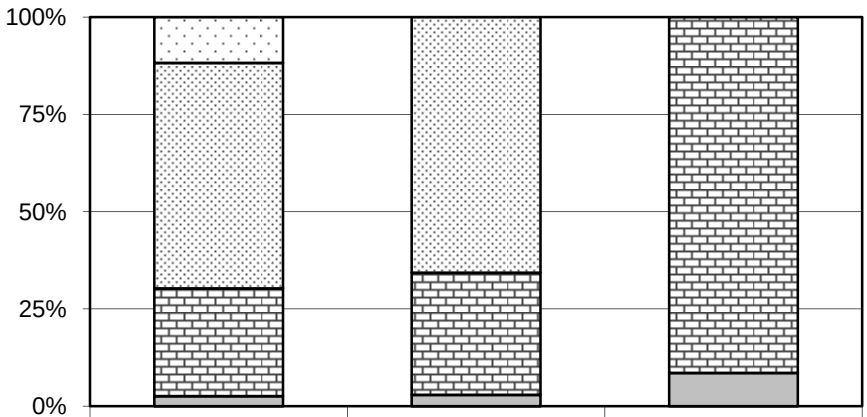
****** referred to total recovery of the unchanged, parent test substance

******* Detection limit



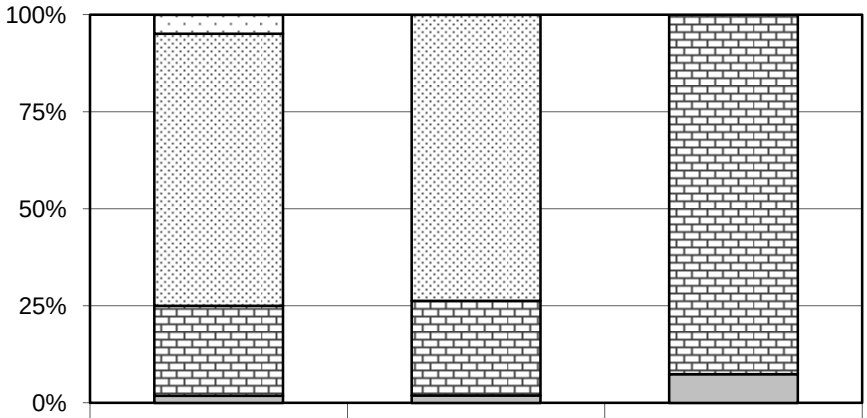
	applied	recovered	dermally absorbed
loss	10,2%		
surface	71,3%	79,4%	
horny layer	17,3%	19,3%	93,6%
epidermis	1,2%	1,3%	6,4%
dermis	0,0%	0,0%	0,0%
receptor fluid	0,0%	0,0%	0,0%

PHYW/O 137



	applied	recovered	dermally absorbed
loss	11,7%		
surface	58,0%	65,7%	
horny layer	27,7%	31,4%	91,4%
epidermis	2,6%	2,9%	8,6%
dermis	0,0%	0,0%	0,0%
receptor fluid	0,0%	0,0%	0,0%

SUNPIT 22



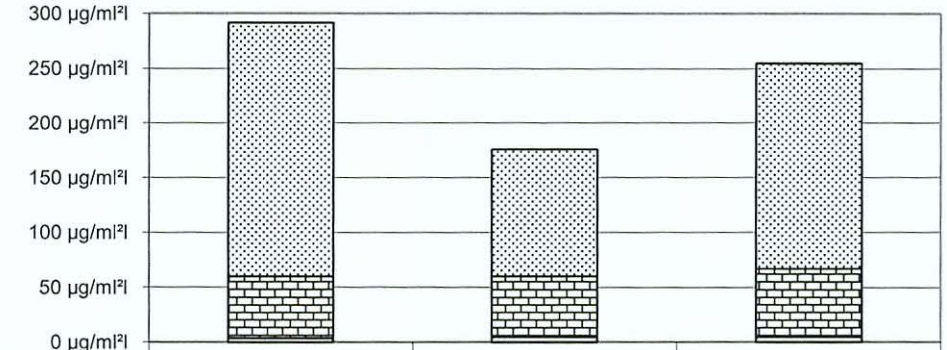
	applied	recovered	dermally absorbed
loss	4,8%		
surface	70,1%	73,7%	
horny layer	23,2%	24,3%	92,6%
epidermis	1,9%	2,0%	7,4%
dermis	0,0%	0,0%	0,0%
receptor fluid	0,0%	0,0%	0,0%

‘applied‘ = percentages of applied test substance (recovered plus loss)

‘recovered‘ = percentages of recovered amounts (extrapolated to 100% recovery)

‘dermally absorbed‘ = percentages of dermally absorbed test substance (extrapolated to 100% recovery of dermally absorbed test substance)

Comparison of samples



	STEO98 427	PHYW/O 137	SUNPIT 22
surface	232 µg/ml²l	116 µg/ml²l	188 µg/ml²l
horny layer	56 µg/ml²l	55 µg/ml²l	62 µg/ml²l
epidermis	4 µg/ml²l	5 µg/ml²l	5 µg/ml²l
dermis	0 µg/ml²l	0 µg/ml²l	0 µg/ml²l
receptor fluid	0 µg/ml²l	0 µg/ml²l	0 µg/ml²l

From the examined samples approx. 21% (STEO98 427), approx. 34% (PHYWE/O 137) and approx. 26% (SUNPIT 22) of applied Finsolv TN was dermally absorbed within 24 hours post application.

The relative distribution of the dermal absorbed Finsolv TN in the skin is for all samples comparable. Approx. 91 – 94 % of the dermally absorbed Finsolv TN was found in the horny layer (stratum corneum) and approx. 6 - 9 % in the epidermis (equivalent to approximately 1 – 3 % of the applied amount). No Finsolv TN was detected in the dermis and receptor fluid. A percutaneous penetration could not be found here for Finsolv TN.

Benzoic acid as a possible degradation product (hydrolysis, enzymatic degradation) in the test samples STEO98 427, PHYWE/O 137 and SUNPIT 22 is detectable (traces, not quantifiable). After penetration no amounts of benzoic acid was detected in the pig skin.

Because of the non-representativeness and furthermore of the very small size of the sample (N=3 taken from the same pig) an inductive statistical analysis could not be performed. Therefore the descriptive results achieved in this study have to be considered as a trend and interpreted as such.

4218 - Bioavailability

Dr. A. Schepky

Mehdi Akhiani

Attached 1 (ANCOL Formulation) **STEO98 427**

class	nart	inci	amount [%]
uv filter	02250-90000-00	4-Methylbenzylidene Camphor	4.0000
solvent	00398-90000-00	Alcohol Denat.	3.5000
uv filter	01573-90000-00	Butyl Methoxydibenzoylmethane	2.0000
moisturiser	11350-90000-00	Butylene Glycol	2.5000
emollient	11431-90000-00	C12-15 Alkyl Benzoate	9.0000
emollient	00823-90000-00	Caprylic/Capric Triglyceride	2.5000
emollient	00137-90000-00	Cetearyl Alcohol	0.7500
emollient	12775-90000-00	Cetyl Dimethicone	0.5000
emollient	12508-90000-00	Dicaprylyl Ether	5.0000
uv filter	11910-90000-00	Ethylhexyl Triazone	1.5000
fragrance	12934-90000-00	Fragrance	0.4000
moisturiser	00091-90000-00	Glycerin	7.5000
emulsifier	00848-90000-00	Glyceryl Stearate SE	4.1600
emulsifier	11270-70000-00	Lanolin Alcohol (Eucerit®)	0.1000
emollient	00079-90000-00	Octyldodecanol	3.0000
preservative	00965-90000-00	Phenoxyethanol + Methyl/Ethyl/n-Butyl/Isopropyl/Propylparaben	0.5000
preservative	12696-90000-00	Sodium Bicarbonate + Iodopropynyl Butylcarbamate	0.1000
emulsifier	00693-90000-00	Stearic Acid	2.2400
uv filter	12117-90000-00	Titanium Dioxide + Trimethoxycaprylylsilane	1.0000
active	10597-90000-00	Tocopheryl Acetate	0.5000
solvent	04034-90000-00	Water	47.7060
neutralising agent	02285-90000-00	Water + Sodium Hydroxide	0.0440
stabiliser	00285-70000-00	Water + Trisodium EDTA	1.0000
rheology modifier	02162-90000-00	Xanthan Gum	0.5000

(ANCOL Formulation) **PHYW/O 137**

class	nart	inci	amount [%]
emulsifier	11872-90000-00	Cetyl PEG/PPG-10/1 Dimethicone	4.0000
emulsifier	12609-90000-00	Polyglyceryl-2 Dipolyhydroxystearate	2.0000
emulsifier	11270-70000-00	Lanolin Alcohol (Eucerit®)	0.1000
emollient	00823-90000-00	Caprylic/Capric Triglyceride	6.0000
emollient	12508-90000-00	Dicaprylyl Ether	6.0000
emollient	02008-90000-00	Isohexadecane	3.0000
emollient	10481-90000-00	Cyclomethicone	5.0000
emollient	11431-90000-00	C12-15 Alkyl Benzoate	6.0000
filler	12676-90000-00	Sodium Starch Octenylsuccinate	0.5000
active	10597-90000-00	Tocopheryl Acetate	0.5000
uv filter	12117-90000-00	Titanium Dioxide + Trimethoxycaprylylsilane	8.0000
moisturiser	00091-90000-00	Glycerin	5.0000
stabiliser	00037-90000-00	Magnesium Sulfate	0.3000
moisturiser	11350-90000-00	Butylene Glycol	5.0000
active	11855-90000-00	Glycine	0.5000
preservative	01376-90000-00	Phenoxyethanol	0.6500
preservative	12672-90000-00	Hexamidine Diisethionate	0.1000
color pigment	12918-90000-00	Zinc Oxide + Dimethicone	8.0000
active	11570-90000-00	Panthenol	1.4000
solvent	04034-90000-00	Water	37.9500

(ANCOL Formulation) **SUNPIT 22**

class	nart	inci	amount (%)
active	10597-90000-00	Tocopheryl Acetate	0.5000
emollient	12776-90000-00	Butylene Glycol Dicaprylate/Dicaprate	2.0000
emollient	11431-90000-00	C12-15 Alkyl Benzoate	7.0000
emollient	12508-90000-00	Dicaprylyl Ether	1.5000
emulsifier	13099-90000-00	Ceteareth-20	2.6000
emulsifier	13110-90000-00	Glyceryl Stearate + Ceteareth-20 + Cetearyl Alcohol + Cetyl Palmitate + Ceteareth-12	5.4000
film former	11637-90000-00	VP/Hexadecene Copolymer	0.5000
moisturiser	00091-90000-00	Glycerin	5.0000
neutralising agent	02285-90000-00	Water + Sodium Hydroxide	0.5500
preservative	01376-90000-00	Phenoxyethanol	0.4000
preservative	12779-90000-00	DMDM Hydantoin	0.4000
solvent	04034-90000-00	Water	62.1500
stabiliser	00285-70000-00	Water + Trisodium EDTA	1.0000
uv filter	11910-90000-00	Ethylhexyl Triazone	2.0000
uv filter	01573-90000-00	Butyl Methoxydibenzoylmethane	2.0000
uv filter	02250-90000-00	4-Methylbenzylidene Camphor	4.0000
uv filter	12945-90000-00	Diethylhexyl Butamido Triazone	1.0000
uv filter	01164-90000-00	Phenylbenzimidazole Sulfonic Acid	2.0000

Attached 2 (raw data penetration)

ORDER # 8822	From 31.01.2000	TEST	START	BATCH	OPERATOR
SAMPLE:	STEO98 427	PEN	07.02.2000	Feb.00	Ak
SUBSTANCE:	Finsolv TN	24h / 32°C	2-Propanol	Analytic:	DAD

sample	weight (mg)	area	volume(ml)	content (%)	due:	9.00 %
	16.93	345900	20	7.08 %	is:	7.52 %
	38.80	848500	20	7.57 %		
	25.37	479700	20	7.91 %	variation coefficient (VC)	5.6%

	#1	#2	#3		mean	VC [%]
weight (mg)	20.16	21.37	22.28		21.27	5
substance [µg]	1516	1607	1676		1600	5
top (upper penetration cell) [µg]	0	0	0		0.0	#DIV/0!
Skin surface [µg]	1012	1096	1316		1141	14
horny layer [µg]	232	384	215		277	34
epidermis [µg]	17	20	18		18.8	8
dermis [µg]	0	0	0		0.0	#DIV/0!
receptor fluid [µg]	0	0	0		0,0	#DIV/0!
SUM [µg]	1261	1500	1549		1436	11
total recovery	83.3%	93.4%	92.4%		89.8%	
strips 1 - 2 [µg]	156.4	247.3	149.9		184.5	30
strips 3 - 4 [µg]	39.4	76.9	37.6		51.3	43
strips 5 - 6 [µg]	19.4	32.7	20.1		24.1	31
strips 7 - 8 [µg]	11.0	13.9	7.6		10.8	29
strips 9 - 10 [µg]	5.4	12.8	0.0		6.1	106
strips 11 - 12 [µg]	0.0	0.0	0.0		0.0	#DIV/0!
strips 13 - 14 [µg]	0.0	0.0	0.0		0.0	#DIV/0!
strips 15 - 16 [µg]	0.0	0.0	0.0		0.0	#DIV/0!

application surface	4.9 cm²	cm²
doping test substance	326	µg/ cm²

distribution	µg	µg/cm²	% applied substance dermally absorbed	% norm. applied substance dermally absorbed	% abs. norm.
skin surface	1141	232	71.3%	79.43%	**********
horny layer	277	56	17.3%	19.26%	93.64%
epidermis	19	4	1.2%	1.31%	6.36%
dermis	0	0	0.0%	0.00%	0.00%
receptor fluid	0	0	0.0%	0.00%	0.00%
SUM	1437	293	89.8%	100.0%	100.0%

ORDER # 8822	From 31.01.2000	TEST	START	BATCH	OPERATOR
SAMPLE:	PHWO-137	PEN	07.02.2000	Feb.00	Ak
SUBSTANCE:	Finsolv TN	24h / 32°C	2-Propanol	Analytic:	DAD

sample	weight (mg)	area	volume(ml)	content (%)	due:	6.00%
	15.66	257500	20	5.70%	is:	5.42%
	18.26	275400	20	5.22%		
	44.88	691000	20	5.33%	variation coefficient (VC)	4.6%

	#1	#2	#3		mean	VC [%]
weight (mg)	19.91	16.54	18.01		18.15	9
substance [µg]	1078.6	896.0	975.7		983.4	9
top (upper penetration cell) [µg]	0	0	0		0	#DIV/0!
Skin surface [µg]	757	483	469		570	28
horny layer [µg]	237	214	365		272	30
epidermis [µg]	31	18	27		25	27
dermis [µg]	0	0	0		0	#DIV/0!
receptor fluid µg]	0	0	0		0	#DIV/0!
SUM [µg]	1025	715	862		868	18
total recovery	95 %	79.8 %	88.3 %		88.3 %	
strips 1 - 2 [µg]	142.6	125.0	215.4		161.0	30
strips 3 - 4 [µg]	35.5	30.8	65.0		43.8	42
strips 5 - 6 [µg]	23.3	25.7	30.9		26.6	15
strips 7 - 8 [µg]	14.0	15.3	17.5		15.6	11
strips 9 - 10 [µg]	10.4	10.1	15.9		12.1	27
strips 11 - 12 [µg]	7.7	7.1	14.0		9.6	39
strips 13 - 14 [µg]	3.7	0.0	7.2		3.6	99
strips 15 - 16 [µg]	0.0	0.0	0.0		0.0	#DIV/0!

application surface	4.9 cm²	cm²
doping test substance	200	µg/ cm²

distribution	µg	µg/cm²	% applied substance dermally absorbed	% norm. applied substance dermally absorbed	% abs. norm.
skin surface	570	116	58.0%	65.67%	**********
horny layer	272	55	27.7%	31.38%	91.42%
epidermis	26	5	2.6%	2.95%	8.58%
dermis	0	0	0.0%	0.00%	0.00%
receptor fluid	0	0	0.0%	0.00%	0.00%
SUM	868	177	88.3%	100.0%	100.0%

ORDER # 8822	From 31.01.2000	TEST	START	BATCH	OPERATOR
SAMPLE:	SUNPIT 22	PEN	07.02.2000	Feb.00	Ak
SUBSTANCE:	Finsolv TN	24h / 32°C	2-Propanol	Analytic:	DAD

sample	weight (mg)	area	volume(ml)	content (%)	due:	7.00%
	23.85	434000	20	6.30%	is:	6.56%
	48.82	978500	20	6.94%		
	22.79	423500	20	6.44%	variation coefficient (VC)	5.1%

	#1	#2	#3		mean	VC [%]
weight (mg)	20,36	20,69	19,12		20,06	4
substance [µg]	1335	1357	1254		1316	4
top (upper penetration cell) [µg]	0	0	0		0,0	#DIV/0!
Skin surface [µg]	859	981	927		922	7
horny layer [µg]	399	330	184		304	36
epidermis [µg]	27	25	21		25	12
dermis [µg]		0	0		0	#DIV/0!
receptor fluid [µg]	0	0	0		0	#DIV/0!
SUM [µg]	1286	1337	1133		1252	8
total recovery	96.3 %	98.5 %	90.3 %		95.1 %	
strips 1 - 2 [µg]	238.7	200.8	99.2		179.5	40
strips 3 - 4 [µg]	50.3	60.9	32.9	50.3	60.9	23
strips 5 - 6 [µg]	42.2	30.7	27.2	42.2	30.7	32
strips 7 - 8 [µg]	26.8	18.8	14.3	26.8	18.8	63
strips 9 - 10 [µg]	21.9	10.1	6.4	21.9	10.1	59
strips 11 - 12 [µg]	11.1	4.4	4.3	11.1	4.4	96
strips 13 - 14 [µg]	8.5	4.9	0.0	8.5	4.9	#DIV/0!
strips 15 - 16 [µg]	0.0	0.0	0.0	0.0	0.0	4

application surface	4.9 cm²	cm²
doping test substance	268	µg/ cm²

distribution	µg	µg/cm²	% applied substance dermally absorbed	% norm. applied substance dermally absorbed	% abs. norm.
skin surface	923	188	70.1%	73.70%	**********
horny layer	305	62	23.2%	24.34%	92.55%
epidermis	25	5	1.9%	1.96%	7.45%
dermis	0	0	0.0%	0.00%	0.00%
receptor fluid	0	0	0.0%	0.00%	0.00%
SUM	1252	255	95.2%	100.0%	100.0%

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 28, 2010

SUBJECT: Research Institute for Fragrance Materials, Inc. (RIFM) Reviews of Alkyl Benzoate Ingredients

RIFM. 2010. Synopsis Methyl Benzoate.

RIFM. 2010. Synopsis Ethyl Benzoate.

RIFM. 2010. Synopsis Propyl Benzoate.

RIFM. 2010. Synopsis Isopropyl Benzoate.

RIFM. 2010. Synopsis Butyl Benzoate.

RIFM. 2010. Synopsis Isobutyl Benzoate.

RIFM. 2010. Synopsis Pentyl Benzoate.

RIFM. 2010. Synopsis 2-Ethylhexyl Benzoate.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Methyl benzoate

Synonyms	
Benzoic acid, methyl ester	CAS
Methyl benzenecarboxylate	
Methyl benzoate	Principal EINECS RIFM
Oil of niobe	

93-58-3

CAS Number	RIFM ID	FEMA	EINECS	Registration
93-58-3	174	2683	202-259-7	EINECS DSL TSCA

RIFM Monograph: 174 (Published 1974: FCT,v12,p937, Special Issue I (Binder, p537))

Fragrance Structure-Activity Group: Esters/Simple C1-C4 Alcohol Aryl Alkyl Acid Ester/Benzoic Acid Derivatives (Benzoates)

Formula $C_8H_8O_2$ **Structure** C_6H_5-OCO-CH_3 **Molecular Weight** 136.15

SMILES Notation O=C(OC)c(cccc1)c1

Generic Class Aromatic Esters

Description A clear, colorless to very pale yellow liquid having a fruity odor; reminiscent of cananga.

Physical Data

Acid Value (XV.B)	1.0 Max.	FMA	5.0 g.
Boiling Point	200□C	FMA	
Boiling Point (calculated)	195.93 □C	EPI Suite	
Flash Point	180□F;CC	FMA	
Halogenated Compounds (XXVIII.B.)	Negative	FMA	
Henry's Law (calculated)	3.471e-005 Pa□m^3/mol	EPI Suite	
Log K_{ow} (calculated)	1.83	EPI Suite	
Log K_{ow} (measured)	LogK pdms/w = 1.882 (n=12)	Xia,2007	
Melting Point (calculated)	-11.87 □C	EPI Suite	
Purity	100.0%	Quest,1995b	
Purity (X.B.2.b.)	98.0 Min.	FMA	1.0 g.|Calc.as C8H8
Refractive Index @ 20□C (I.B.)	1.514 - 1.518	FMA	
Specific Gravity	1.09	Quest,1995b	
Specific Gravity 20□C (I.A.)	1.084 - 1.090	FMA	

Specific Gravity 25□C (I.A.)	1.082 - 1.088	FMA
Vapor Pressure (calculated)	0.379 mm Hg @ 25□C	EPI Suite
Vapor Pressure (calculated)	0.3 mm Hg 20C	FMA
Water Solubility (calculated)	1344 mg/L	EPI Suite

Preparation	By esterification of methyl alcohol and benzoic acid
Natural Occurrence	**Methyl benzoate** is reported to occur in nature.
Use Levels	In public use since the 1900s.

Flavor Consumption (in kg)

1995	EUROPE	329
1995	USA	1724
1987	USA	350
1982	USA	4850
1975	USA	558
1970	USA	300

Uses (in ppm)

Product	Average Usual	Average Maximum	Mean Daily Consumption (gms)	Updated
Alcoholic Beverage	1	3	32.5	21-Jul-88
Baked Goods	10.72	17.1	137.2	21-Jul-88
Chewing Gum	45.63	45.63	0.2	21-Jul-88
Frozen Dairy	3.51	6.97	25.6	21-Jul-88
Gelatin Pudding	2.58	4.32	20.4	21-Jul-88
Hard Candy	12.59	19.49	0.6	21-Jul-88
Non-alcoholic Beverage	2.06	3.81	104.0	21-Jul-88
Soft Candy	5.88	9.67	5.8	21-Jul-88

PADI 1.911.911.911.911.911.911.911.91

Food Products Containing Methyl benzoate (in ppm)

Product	Code	Lower Limit	Upper Limit
Bilberry (Vaccinium myrtillus L.)	>6-I	0.007	
American cranberry (V. macrocarpon Ait.)	>6-II	0.01	0.1
Guava (Psidium guajava L.)	>8-I	0.02	1.25

Status

Methyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 260) , was approved by the FDA as a flavor (21 CFR 172.515) .

Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2683) Hall,1965

Indicative Non-Exhaustive List states: Listed

The Industrial Safety and Health Law (Japan) states: ISHL Number ((3)-1356)

Joint Expert Committee on Food Additives states: The JECFA Evaluation Summary is available here.

Joint Expert Committee on Food Additives states: The Benzyl Derivatives Safety Evaluation is available here.

Joint Expert Committee on Food Additives states: The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent. (851)

United Nations Transport Classification Codes states: Not regulated

FFIDS Volume VI Updated 1-Nov-85

European Hazard Classification Labeling	
R22	Harmful if swallowed.
	Harmful

Global Harmonized System Hazard Statements			
Hazard Category	**Signal Word**	**Code**	**Hazard Statement**
FL 4	Warning	H227	Combustible liquid
SCI 3	Warning	H316	Causes mild skin irritation
ATO 4	Warning	H302	Harmful if swallowed

Precautionary Code	Precautionary Statement
P210	Keep away from heat/sparks/open flames/hot surfaces. □ No smoking
P264	Wash .. (hands/face) thoroughly after handling
P270	Do not eat, drink or smoke when using this product
P280	Wear protective gloves/protective clothing/eye protection/face protection
	IF SWALLOWED: call a POISON CENTER or doctor/physician if you feel

P301/312	unwell
P330	Rinse mouth
P332/313	If skin irritation occurs: Get medical advice/attention
P403/235	Store in a well-ventilated place. Keep cool
P501	Dispose of contents/container to ... (in accordance with local/regional/national/international regulation).

Human Health Data

Acute toxicity

Route: oral. **Species:** rat. Groups of 5 non-fasted, Carworth-Wistar male rats weighing 90 - 120 grams, were administered a single dose of the test material orally. When necessary, the test material was tested as a solution in water, corn oil, or as a 1% solution of sodium 3,9-diethyl-6-tridecanol sulfate (Tergitol Penetrant 7), to bring the volume given to each rat to 1 □ 10 ml. The animals were observed for mortality during a 14-day period and the LD50 was estimated by the Thompson and Weil method. No further details were provided. **LD50** 3.42 G/KG 3.42 g/kg calculated LD50, C.I. 2.83-4.13. (Smyth,1954)

Route: gavage. **Species:** rat. Test materials were administered to 5 male and 5 female Osborne-Mendel rats per dose. Animals were subjected to an 18 hour predose fast. All doses were given by intubation. The animals were observed over a 2 week period for mortality and/or systemic effects. LD50 results were calculated per Litchfield-Wilcoxon (1949). No further details were provided. **LD50** 1350 MG/KG 1350 mg/kg calculated LD50, 95% confidence limit=1290-1410mg/kg. Slope=1.1(0.7-1.5). Death from 2-18 hrs. Toxic signs were depression, porphyrin-like deposit around nose, rough fur,and wet posterior. Survivors excitable. (Jenner,1964)

Route: gavage. **Species:** mouse. Oral doses of test materials were given to mice. Mice were treated on full stomachs. All doses were given by intubation. Mice were observed for mortality and/or systemic effects over a 2 week period. LD 50 results were calculated per Litchfield-Wilcoxon (1949). Number, type or sex of mice were not provided. No necropsy mentioned. 50% in corn oil.**LD50** 3330 MG/KG 3330 mg/kg calculated LD50, 95% confidence limits=2920-3800 mg/kg. Slope=1.2 (1.1-1.4). Death from a few minutes to 18 hours. Toxic signs were excitation and tremors. (Jenner,1964)

Route: oral. **Species:** rat. The LD50, orally by stomach tube was determined. Animals observed for 2 wk or until death. Strain not specified. Method for determining LD50 not specified. **LD50** 2.17 G/KG 2.17 g/kg calculated LD50, using 25 rats. (Graham,1945)

Route: oral. **Species:** rabbit. The LD50, orally by stomach tube was determined. Animals observed for 2 wk or until death. Strain not specified. Method for determining LD50 not specified. **LD50** 2.17 G/KG 2.17 g/kg calculated LD50, using 12 rabbits. (Graham,1945)

Route: oral. **Species:** mouse. The vehicle was a 2% starch solution Data from CA abstract only. **LD50** 3 G/KG 3 g/kg calculated LD50. (Kravets-Bekker,1971)

Route: oral. **Species:** rat. The vehicle was a 2% starch solution Data from CA abstract only. **LD50** 3.5 G/KG 3.5 g/kg calculated LD50. (Kravets-Bekker,1971)

Route: oral. **Species:** guinea pig. The vehicle was a 2% starch solution Data from CA abstract only. **LD50** 4.1 G/KG 4.1 g/kg calculated LD50. (Kravets-Bekker,1971)

Route: intragastric. **Species:** mouse. White mice were used. The vehicle was a 2% starch solution. The observation period was 14 days. The LD50 was calculated per Berens. **LD50** 3 G/KG 3 g/kg calculated LD50. (Kravets-Bekker,1970)

Route: intragastric. **Species:** rat. White rats were used. The vehicle was a 2% starch solution. The

observation period was 14 days. The LD50 was calculated per Berens. **LD50** 3.5 G/KG 3.5 g/kg calculated LD50. (Kravets-Bekker,1970)

Route: intragastric. **Species:** guinea pig. The vehicle was a 2% starch solution. The observation period was 14 days. The LD50 was calculated per Berens. **LD50** 4.1 G/KG 4.1 g/kg calculated LD50. (Kravets-Bekker,1970)

Route: oral. **Species:** rat. In an acute oral toxicity study, the LD50 of the test material in rats was analyzed by using various parameters of the test material, and was reported. No further details were provided. Article in Japanese. **LD50** 1177 MG/KG 1177 mg/kg calculated LD50. (Nishimura,1994a)

Route: inhalation. **Species:** rat. Rats were raised in cylindrical lucite cages, 20 cm in diameter and 20 cm high, with closed tops. The cages were placed on a coarse grid, and the droppings were removed twice a day. Fresh air was blown through filters of charcoal and molecular sieves. The flow of air was monitored with a venturimeter for each of two sets of 6 cages and kept at about 0.6 litre x sec(-1) through each cage. Each substance was introduced into the air stream from a glass bottle, the content of which was weighed before and after the experiments to measure the concentration of substance in the stream. The rats (Wistar) weighed between 28 and 39 grams and were about 2 weeks old when placed in the cages. For every 5 substances there was a group of control animals exposed to filtered fresh air only. The animals were sacrificed at about 1, 2, and 3 months of age. Some 250 animals were used in the experiments. A variety of different chemicals were used for odour exposure; they were selected to represent different qualities of odours as judged by man, and they also represented substance with different physico-chemical properties. A series of cycloketones was included, as they are currently being studied by physiological means. Since the previous study indicated that few additional changes occur after 1 month either in the morphology of the mitral cells or in the pattern of degeneration, the exposure times were limited up to 3 months. For each odour at least two different exposure times were used. The animals were then perfused in buffered saline and fixed with a formaldehyde-glutaraldehyde mixture. In this study the emphasis was placed upon the distribution of changes in the mitral cells of the olfactory bulbs following exposure to different odours, rather than on the nature and progression of the morphological alterations. The morphological changes noted were principally a darkening and shrinkage of the cell bodies (both cytoplasm and nucleus) as observed by light microscope. Positive effects, Moderate and severe changes noted. Lateral, medial and dorsal areas affected. [Exposure concentration was 3.1 x 10(-8) M]. (Pinching,1974)

Route: skin. **Species:** cat. Benzyl compounds neat or in various vehicles was applied one or two times to the clipped backs (4 x 6 in) by cotton balls held firmly with hemostatic forceps. The area was massaged during application to facilitate absorption. Animals observed for 2 wk or until death. 100%. 20 ml lethal, 2 cats used; died within 21 hr. (Graham,1945)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. On the next Day, serial dilutions of test cpd were added to the cells, beginning in ea. case w/ a conc. close to saturation level. 1100 mg/l positive effects, 100% inhibition of growth noted (Data taken from graph). (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. After 24 hr The IC50 conc. of test cpd was added to ea. flask. Flasks were kept @ 37 C w/ the screw plugs well closed for 7 days. Two flasks from ea. test grp were removed daily for protein determination. 495 mg/l positive effects, greater inhibition of growth than the estimated 50% was noted on day 7. (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells were plated in 25 cm2 flasks @ a density of 200 cells/flask in the presence of test cpd @ the IC50 conc. Cultures were incubated @ 37 C for 8 days &

then the cells were stained w/ May-Grunwald & Giemsa and colonies of @ least 10 cells were counted. 495 mg/l positive effects, cell morphology was affected. Cells lost their typical epithelial shape & became elongated. (DeAngelis,1986)

Route: inhalation. **Species:** rat. Male or female albino rats were exposed to a flowing stream of air approaching saturation with vapors, which was prepared by passing dried air through a fritted disc gas washing bottle. Groups of 6 rats were exposed for periods of time in a logarithmic series with a ratio of 2, for up to 8 hours, until the period of time that killed 50% of the animals within 14 days of inhalation was determined. No further details were provided. The results reflect the longest inhalation period that allowed all the rats to survive the 14-day observation period. 8 hours no effects. (Smyth,1954)

Irritation

Route: skin. **Species:** rabbit. Primary skin irritation was determined using groups of 5 albino rabbits. A 0.01-ml aliquot of the test material was applied to the clipped skin of the animals undiluted or as a solution in water, propylene glycol or kerosene. Scoring of the reactions was conducted within 24 hours of the application, using irritation grades that ranged from 1 to 10 (grade 1 = least visible capillary injection from the undiluted material; 6 = necrosis with the undiluted material; and 10 = necrosis from a 0.01% solution). No further details were provided. The specific vehicle used with each test material was not mentioned. 100 % irritant effects, grade = 3. (Smyth,1954)

Route: surface of eye. **Species:** rabbit. Eye injury was evaluated using groups of 5 rabbits per dose. The test material was applied to the center of the cornea while the lids were retracted. Approximately 1 minute later, the lids were released, and 18 - 24 hours later, the eye was examined in strong diffuse daylight, then stained with fluorescein, and the injury scored. The test material was tested undiluted or as a solution in propylene glycol and water. Eye injury was graded according to a 10-grade series: 1 = 0.5 ml undiluted gives injury of 0 to 1.0 points; 2 = 0.5 ml undiluted gives injury of over 1.0 up to 5.0 points; 3 = 0.1 ml undiluted gives injury of up to 5.0 points (0.5 ml gives over 5.0); 4 = 0.02 ml undiluted gives injury of up to 5.0 points (0.1 ml gives over 5.0); 5 = 0.005 ml undiluted gives injury of up to 5.0 points (0.02 ml gives over 5.0); 6 = Excess of 40% solution gives injury of up to 5.0 points (0.005 ml gives over 5.0); 7 = Excess of 15% solution gives injury of up to 5.0 points (40% gives over 5.0); 8 = Excess of 5% solution gives injury of up to 5.0 points (15% gives over 5.0); 9 = Excess of 1% solution gives injury of up to 5.0 points (5% gives over 5.0); 10 = Excess of 1% solution gives injury of over 5.0 points. The points were equivalent to the following symptoms visible before fluorescein staining: 1 point = iritis, slight internal congestion; 2 points = cornea dull; 4 points = cornea opaque, less than half of area; and 6 points = cornea opaque, more than half of area. The points were equivalent to the following symptoms visible after fluorescein staining: 1 point = necrosis on less than 5% of the cornea; 2 points = necrosis on 5 □ 12%; 3 points = necrosis on 13 □ 37%; 4 points = necrosis on 38 - 62%; 5 points = necrosis on 63 - 87%; and 6 points = 88 - 100%. No further details were provided. (Carpenter,1946) 100 % eye effects, grade = 1. (Smyth,1954)

Route: skin. **Species:** guinea pig. Batches of 3-4 albino animals weighing 500-700 grams were used. Skin of the dorsal trunk from the lumbo-sacral function below to the cervical hump above, and extending laterally to the mid-axillary line was closely clipped and depilated, washed and dried. Discs of filter paper (Whatman No. 5), 5 mm diameter were soaked in the test substance, drained and applied to the skin. After appropriate intervals, disc was removed and treated site wiped dry. Chemicals were applied both before and after animals had received Evans Blue dye iv. Lesions were inspected hourly in good daylight and arbitrarily graded by comparison with normal skin using a scale of 1 to 6. 1 corresponded to the faintest pink erythema that can be observed and 6 to bright red lesions. Permeability changes were measured by the intensity of colour of the exuded dye in the treated sites as estimated by comparison with a chart prepared by dropping known concentrations of dye on to good quality filter paper. The colour intensity of all lesions was graded by comparison with the chart as +/-, +, + +/-, ++, up to ++++ and then recorded as the equivalent concentration of Evans blue. Positive effects, only the feeblest permeability response was noted when applied for periods up to 2 minutes.

(Steele,1966)

Route: skin. **Species:** rabbit. Non-occluded irritation tests using 10-14 New Zealand males/material according to the Draize method (1944). About 0.5ml undiluted material applied daily to one side of the clipped dorsal area for up to 6 days & 0.2ml applied to the external surface of the rt. outer ear. Test areas examined at 4 & 24 hr after each treatment. 2/grp killed daily & skin of back & ear examined microscopically. Micro exam of organs from n-PB & n-BB rabbits. >99% pure. Irritant effects, skin effects, dorsum: erythema increased with no. of treatments; mod-severe edema on last day. Ear not affected. Micro of 2 areas showed different responses. (Branca,1988)

Sensitization

Route: skin. **Species:** guinea pig. A modified Freund's complete adjuvant test was conducted. For induction with crude material or with fractions, 30 mg was used. In the case of pure isolated, synthesized or purchased compounds, 15 mg was applied. For challenge, vehicle was acetone. The challenge dose appears in the results. The mean response was computed as the quotient of the sum of all reactions obtained divided by the total number of treated animals. A mean response of 0 to 1 was considered as weak, 1 to 2 as moderate, and greater than 2 as strong No further details were provided. (Hausen,1992b) 10 % no effects. (Hausen,1995)

Route: skin. **Species:** guinea pig. An open epicutaneous test was conducted in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6-8 guinea pigs per group. One to six experimental and one control group was used. Open challenge applications were made on days 21 and 35. Reactions were read at 24, 48 and 72 hours. No further details were provided. Vehicles were not specified by material. (Klecak,1977) Greater than or equal to 98% purity. 4 % no effects. (Klecak,1979)

Route: skin. **Species:** guinea pig. A guinea pig open epicutaneous test (OET) was conducted on groups of 6 - 8 male and female guinea pigs weighting 300 - 450 grams. Daily applications were made for 3 weeks to a clipped 8-cm2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 hours after each application. A total of 21 applications of 0.1 ml test material in an unspecified vehicle were made for 21 days. The 10 controls were either left untreated or treated with 0.1 ml of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with the test material at the minimal irritating concentration and some lower primary non-irritating concentrations. The results were not reported for the irritation pre-screen. It was not specified if the concentration reported in the results was the minimal irritating concentration or 1 of the lower primary nonirritating concentration. 4 % no effects, No sensitization (-) was produced. (Klecak,1985)

Route: skin. **Species:** human 18+ yrs. A 24 to 48 hour occluded patch test was conducted on 30-200 patients. The test material concentration was 0.05 - 0.5% in a vehicle which was either a base cream or 99% ethanol (type of vehicle per test material was not specifically identified). Patch tests were always carried out with both perfumed and non-perfumed cream base at the same time. Patches consisted of a piece of 1 cm2 lint with a 2 cm2 cellophane disc placed on the lint and then covered with 4 cm2 plaster. The patches were applied to the back, the forearm and the inside of the upper arm for 24 to 48 hours. Reactions were evaluated 30 minutes after removal of patch. The skin reaction was graded as follows: - No visible reaction; +- Slight erythema; + Erythema; ++ Erythema and swelling or marked erythema. The period of study extended over 4 yrs and 3 months and patch testing was performed 1-3 times per month; September to May (summer months were excluded). The total number of subjects was 4737 (2341 Japanese men and 2396 Japanese women aged 16-45, mostly 17-30, living in Tokyo and Osaka). Volume dose not provided. 99% purity. No effects, 0/96. (Takenaka,1986)

Route: skin. **Species:** man. Vehicle was petrolatum. 25 subjects completed the study. 4 % no effects. (RIFM,1970) [Kligman,1970]

Phototoxicity

Route: *in vitro*. **Species:** human 18+ yrs. A study was conducted to evaluate the phototoxic potential of a series of test materials invitro with a photohemolysis test using suspensions of human erythrocytes

exposed to radiation sources rich in UV-A (320-460 nm) and UV-B (275-365 nm) in the presence of the test materials. Red blood cells obtained from healthy human volunteers were washed and suspended at a dilution of 1:200 in TCM buffer containing 0.03% human albumin. A 0.4 ml volume of this suspension and a correspondingly prepared erythrocyte-free sample (blank) were incubated with 0.1 ml of test material preparations for 1 hour at 37 C. Both the test material sample nd the blank were exposed to 0, 5, 25, or 50 J/cm2 UVA or to 0, 500, 1000 or 2000 mJ/cm2 UVB from the TL 20 W/12 light bulbs. During irradiation, samples were kept in a shaking bath at 37 C. 100% heamolysis was obtained by exposure of the erythrocytes to distilled water. After an incubation period of 30 minutes in the dark supernatants were recovered by centrifugation. The released heamologlobin in the supernatants was determined after incubating the samples for 15 minutes in Drabkin's solution. Hemolysis was measured by reading the absorbance values at 550 nm with a MR 700 microplate reader, and photoheamolysis was calculated as a percentage of total heamolysis. Values of >5% were regarded as significant photohemolysis. **Summary** Under the conditions of this study, test material did not induce photohemolysis. No effects, Test material did not induce photohemolysis. (Placzek,2007)

Pharmacokinetics

Route: intraduodenal,cannula insert. **Species:** rat. The intestinal absorption rate of the test material was evaluated. Vehicle was 0.9% saline adjusted to pH 6.0. Anesthetized male rats, weighing 270 +/- 30 grams were used after a 24 hour fast. Water was allowed ad libitum. The perfusion sample size was 50, 100 or 200 ml maintained at 37 C and was perfused by recirculation at a rate of 20 ml per minute. After recirculation, the sample solution was recovered and the intestinal lumen was washed. The remaining drug was completely collected in a measuring flask and measured. The experiments for each sample solution were repeated three times or more with alteration in the perfusion period. 2 millimolar , the absorption rate coefficient was 1.43. (Nogami,1968)

Pharmacokinetics and metabolism studies

Route: *in vitro*. **Species:** rabbit. Metabolites were identified after a 1 hour incubation with liver microsomes. 25-100 umoles of test material were added in ethanol. Benzoic acid (35 umoles) was identified as a metabolite. (Daly,1968)

Route: *in vitro*. **Species:** human 18+ yrs. The rate of hydrolysis of the test material by 80% human plasma was studied. Vehicle was acetonitrile. The t1/2 for the in vitro hydrolysis of methyl benzoate to benzoic acid by human plasma was 108 minutes. (Nielsen,1987)

Pharmacology

Route: oral. **Species:** mouse. Female DBA/2 Cr mice (6-week-old, 17-19 g) were used. Animals were housed 5 per cage in a temperature-controlled room, with food and water ad libitum and under a 12 hour light/12 hour dark diurnal cycle (light at 0700 hrs). The temperature in the room was kept at 24 C. The mice were acclimated for at least 4 days before starting any experimental procedure. Mouse-adapted influenza virus (A/PR/8/34 (H1N1)) was prepared from the lungs of infected mice. Mice were intranasally infected or mock-infected with 2000-3000 plaque forming units of influenza virus under ether anesthesia. Hot water-extract of each herbal component (5 mg 0.25 ml(-1)/mouse) or water (0.25 ml) was orally applied by gavage to the mice 3 times daily (approximately 8-hour interval) for 4 days starting a day before infection. Since Kakkon-to of 750 mg/kg/day for mice corresponds to dose for human use and was effective in reducing fever in infected mice, the dose of each herbal component was used as its maximum dose for mice in this experiment. Five to ten mice were used in each group. The rectal temperature was monitored by a thermometer at 42-46 hours after infection, at which times fever has been shown to be produced in the murine model. After the measurement of rectal temperature, sera were prepared from four to five mice in each group under ether anesthesia and interleukin-1- alpha concentrations in sera were determined by the enzyme-linked immunosorbent assay (ELISA) using ELISA kits for mouse interleukin-1-alpha. The fractions of C. cassia (0.25 ml/mouse) and cinnamyl derivatives and related compounds (4.7-14.1 mg 0.25/ml/kg) were orally administered to evaluate their possible antipyretic activity. Test material was dissolved or suspended in dimethylsulfoxide (DMSO)

and then diluted with distilled water for administration. Doses of compounds for mice were used as dose corresponding to the dose of aspirin which was calculated from doses for human use and exhibited antipyretic activity in mice. The rectal temperature of each mouse was monitored and interleukin-1-alpha concentrations in sera were determined. No effects. (Kurokawa,1998)

Interaction

Route: *in vitro*. **Species:** guinea pig. In vitro skin penetration of drugs was examined. The skin preparations were mounted in a two-chamber diffusion cell with water jackets (37 C). The available diffusion area was about 0.65 cm2, and each half-cell volume was about 5.4 ml. The donor cells were filled with saline either in the presence or absence of the enhancer unless otherwise mentioned, and the receiver cells with PBS (pH 7.4). Cells were pretreated for 12 h with stirring at 450 rpm by a magnetic stirrer. After washing of both compartments, the suspension of excess amount of benzoic acid or its 4-alkyl substituents in saline either in the presence or absence of the penetration enhancer was added to the donor compartments unless otherwise mentioned, and the penetration experiment was begun. One hundred fifty ul of sample was taken from the receiver cells periodically over a maximum period of 29 h, diluted with PBS twenty times or more and analyzed by UV absorbance at 224 nm for benzoic acid, at 235 nm for 4-methyl and 4-ethyl substituents and at 236 nm for 4-n-propyl and 4-n-butyl substituents. Solubilized components from skin and penetrated enhancers did not interfere with the UV absorbance. Dependency on pH in the donor compartment of the permeability coefficients of the derivatives was examined with suspension of their excess amount (pH 3.0, 4.0) or a 50 mM solution of the derivative (pH 5.0-7.0), using either phosphate buffer (pH 3.0, 60, 7.0) or citrate buffer (pH 4.0, 5.0). Permeability coefficients of benzoic acid and the derivatives decreased with the increase of pH. (Kitagawa,1999)

Route: *in vitro*. **Species:** guinea pig. The permeability coefficient was calculated and the solubility of benzoic acid and its 4-alkyl substituents was measured after their incubation in an excess amount in saline either in the presence or absence of the enhancer at 37 C for 24 h. After quick centrifugation at 1000xg for 2 min at 37 C, concentration of the supernatant was obtained by measuring UV absorbance. The solubility (Cd) and permeability (Kp) coefficients were 3.80 mM and 20.3 x 10(-2)cm/h, resp. (Kitagawa,1999)

Subchronic toxicity

Route: unreported. A six month study was conducted Data from CA abstract only. Species and route not given. Neurological effects, central nervous system damage was seen at "high doses". Doses of 500 mg/kg reduced cholinesterase and ascorbic acid. (Kravets-Bekker,1971)

Route: intragastric. **Species:** rat. A 1.5 month study was conducted using white rats. The observations were survival, body weight, condition, behavior, blood analyses, necropsy and histology. 111 mg/kg blood effects. 500 mg/kg blood effects, decreased ascorbic acid level in the adrenals. (Kravets-Bekker,1970)

Route: intragastric. **Species:** rat. A 6 month study was conducted using white rats for evaluation of biochemical effects and grey rats for evaluation of conditional reflexes. Other observations were survival, body weight, necropsy and histology. 0.05 mg/kg blood effects, liver, neurological effects, altered conditional reflexes were seen. Increased sulfhydryl groups in the cerebral tissue. 0.005 mg/kg no effect level. (Kravets-Bekker,1970)

Carcinogenesis and mutagenesis studies

Route: *in vitro*. **Species:** *Salmonella typhimurium*. The mutagenicity of the test material was determined by the preincubation method. Cultures of Salmonella typhimurium strains TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538 were grown at 37oC overnight with shaking in Oxoid #2 broth, or in supplemented defined minimal medium. A 0.05 ml aliquot of the test material at doses of 0.1 - 10,000 ug/plate, the overnight culture of S. typhimurium, and 0.5 ml of buffer or S9 mix (derived from the livers of Aroclor 1254-induced male Sprague-Dawley and Syrian hamsters) were mixed in tubes and incubated at 37oC without shaking for 20 minutes. The top agar was added, and the

contents of the tubes were mixed and poured onto the surface of petri dishes containing Vogel-Bonner medium. Incubation was conducted for 2 days at 37oC, and the histidine-independent colonies arising on the plates were counted. The vehicles used included distilled water, dimethyl sulfoxide (DMSO), 95% ethanol, and acetone. Vehicle was DMSO; doses were 10, 33, 100, 333, 666, 1000, 1666, 3333, and 6666 ug/plate; strains TA100, TA1535, TA1537, TA97, and TA98 were used. No effects. (Zeiger,1992)

Route: *in vitro*. **Species:** *Escherichia coli*. Assays were conducted by a paper-disk method or its simplified modification. The increase in the frequency of reversion from streptomycin dependence to independence in E. coli strain Sd-4-73 was used as a measure of mutagenicity. Mutagenicity was manifested as a zone of streptomycin-independent mutant, colonies around a filter paper disk saturated with mutagenic agent (0.01 to 0.025 ml or containing a small crystal) and resting on the surface of streptomycin-free nutrient agar heavily seeded with a streptomycin-dependent parental population. No further details were provided. No effects. (Szybalski,1958)

Cytotoxicity

Route: *in vitro*. **Species:** human 18+ yrs. The ability of the test material to increase the permeability of the membranes of human lung fibroblasts was studied by measuring the release of an intracellular nucleotide marker. Human diploid embryonic lung fibroblasts (line MRC-5) were cultivated to a cell density of 10 to the fifth cells/cm2 (approximately 7 x 10(5) cells/well). The cells were then labeled with [3H]uridine. The labelled cultures were incubated with 25 mM of the test material for 30 minutes at 37 C in Tris-buffered saline. The solution containing the leaked radioactive marker was removed and centrifuged and the radioactivity was measured. A maximal release of the radioactivity was obtained by treating control cells for 30 minutes with 0.06 M sodium borate buffer and scraping with a rubber policeman. This treatment ruptured the cell membranes leaving the nuclei intact. The results notes section indicates the percentage of nucleotide released. Positive effects, 20%. (Thelestam,1980)

Miscellaneous

Route: *in vitro*. Antimicrobial activity was evaluated using a standardized petri plate procedure. Organisms used were Staphylococcus aureus (SA), Escherichia coli (EC), Candida albicans and, if active in the others, a Diphtheroid (D). All materials were tested at 10% (w/v) in 95% (v/v) ethanol unless solubility problems occurred. Not all materials were tested in all 4 organisms. Controls were 3,4,4'-trichlorocarbanilide and 2,4,4'-trichloro-2'-hydroxydiphenyl ether and hexachlorophene. No effects. (Morris,1979)

Route: *in vitro*. **Species:** bacteria. The effect of test chemicals on growing cultures of four bacterial strains [Bacillus subtilis, ATCC 9524, Escherichia coli ATCC 11229, Staphylococcus aureus Ox-H (penicillin sensitive) and Staphylococcus aureus ATCC 10390 (penicillin resistant)] was studied. Dilutions of each chemical were prepared in sterile nutrient broth at 1:500. Tubes were inoculated with 0.5 ml of a 24 hour broth culture of test organism. Tubes were incubated at 37 C for 24 hours and the presence or absence of visible growth observed. Failure of growth to occur in the subculture was taken as evidence that the organisms had been killed in the original chemical-broth tubes. When this occurred, additional concentrations were prepared (1:1000, 1:2000, 1:10,000) and tested in a similar manner. No effects, bacterial growth was noted at 1:500 dilution in all four strains tested. (Maruzzella,1961)

Route: *in vitro*. **Species:** bacteria. Antibacterial activity against 9 bacteria was evaluated - Escherichia coli, Eberthella typhosa, Neisseria, Streptococcus faecalis, Streptococcus pyogenes, Staphylococcus aureus, Bacillus megatherium, Corynebacterium diphtheriae, and Oidium albicans Document in German. Data from abstract and table only. No effects. (Kellner,1955)

Route: inhalation. **Species:** rat. Rats were exposed to 22 different odorants combining five different functional groups with five different hydrocarbon structural features to test whether all functional group-associated glomerular modules respond independently of hydrocarbon structure. All liquid odorants were tested neat. Solid odorants were diluted in light mineral oil using a ratio of 1 g/10 ml for

2,3-dimethylpyrazine and 1 g/20 ml for cinnamyl alcohol, methyl cinnamate, thymol and methyl nicotinate. Odorants were volatilized using high-purity nitrogen. Most of the odorants were volatilized at a nitrogen flow rate of 250 ml/min and then were diluted 1/8 in ultra zero air before reaching the exposure chamber at 2 L/min. One exception was methyl isocaproate which had a tendency to foam excessively at higher flow rates. For this odorant, a nitrogen flow rate of 181 ml/min and dilution of 1/11 was used. Separate FEP tubing was dedicated to each odorant. Rats (postnatal day 17-19) were transferred together with their dams into clean cages at least 1 hour before any experiment to reduce carryover of odors from soiled bedding into the odorant exposure chamber. Both male and female rats were used, with their numbers balanced across the different odorant conditions. Approximately 3-4 animals were tested with each odorant. Immediately before exposure, each rat was given a subcutaneous injection of [14C]2-deoxyglucose (2-DG; 16 ul/g, 0.1 mCi/ml, 52 mCi/mmol) at the back of the neck, after which the animal was placed in a 2 L glass mason jar. Odorant or control vapor then began to enter the jar through the lid, which also was fitted with a vent. Odorant exposures continued for 45 minutes, after which the rat was decapitated and its brain was removed rapidly and frozen at -45 C in 2-methylbutane. The olfactory bulbs were sectioned and mapped uptake of 2-DG was measured (Johnson et al, 1999, 2004). The bulbs were sectioned in a cornonal plane using a cryostat and regularly spaced, 20 um sections were taken for autoradiography. Images of alternate, cresyl violet-stained sections were used to locate anatomical landmarks and to direct measurements from the glomerular layer of the autoradiograph section. Measurements were taken at the intersection of the glomerular layer and each gridline of a polar grid chosen for each section on the basis of its relative rostral-caudal location in the bulb. Individual section files were merged into matrices that were standardized with respect to anatomical landmarks to correct for differences in bulb size. Left and right bulbs of each rat were mapped and their matrices averaged. A vehicle blank was subtracted, followed by converting each matrix into units of z scores with respect to the mean and standard deviation of values in that matrix. These z score-standardized matrices then were either averaged across a given odorant condition to generate plots of activity patterns or used for other statistical analyses. The spatial distributions of activity represented by the averaged matrices were illustrated by means of contour charts plotted by using Microsoft Excel. **Study Length**: 45 minutes **Summary** Methyl benzoate evoked 2-DG uptake in paired modules "b" (lateral), "B" (medial), "c" (lateral), "C" (medial), "f" (lateral), "F" (medial), "a" (lateral), "M" (medial). 100 % , Methyl benzoate evoked 2-DG uptake in paired modules "b" (lateral), "B" (medial), "c" (lateral), "C" (medial), "f" (lateral), "F" (medial), "a" (lateral), "M" (medial). (Johnson,2005)

A model for studying specific effects.

Xu,2002 . The LSER model was employed in analyses of soil organic partition coefficients, octanol-water coefficients, capacity factors in soil leaching column chromatography (SLCC), the influence of methanol-water mixtures on capacity factors, and to make clear the intrinsic relationship between soil organic partition coefficients and capacity factors in SLCC.

Serra,2003 . A classification model using genotoxicity data from an invitro chromosomal aberration assay with Chinese hamster lung cells is presented.

Analytical methodology. Can also refer to physical properties such as boiling temperature, flash point, etc.

Stull,1947 . Vapor pressures were collected for over 1200 organic compounds.

Chakraborty,1967 . The retention time in various stationary phases was determined.

Appell,1970 .

Morris,1973 .

Kaiser,1988 .

Vaughan,1988 .

Reverchon,1997 .

Orav,2002 . The composition of volatile components from blackcurrent berries were determined.

Mazza,2002 . The constituents of coriander oil were determined.

Fernandez,2003 . The chemical composition of the volatile extracts obtained from Siam and Sumatra benzoin gums by direct GC injection.

Jantan,2005 . The percentage composition of the leaf oil of Cinnamomum pubescens was determined.

Sacchetti,2005 . Compound found in C. odorata.

Ranadive,2006 . The chemical constituents of vanilla flavor were determined.

Xia,2007 . The partition coefficients were determined for several materials. LogK pdms/w = 1.882 (n=12)

Article in foreign language; no English translation available.

Mashimo,1953 . No English abstract or translation available. Article cited in published monograph.

Barthelmess,1962 .

Fuhrer,1972 .

Grubner,1972 .

Biological tests other than classical toxicology testing and pharmacological tests.

Valette,1954 . Measured & compared skin penetration of various hydrocarbons, alcohols, etc., in rats. In french.

Ottoson,1964 . Methyl benzoate has been used in studies of olfactory mechanisms in frogs

Ishikawa,1965 . No English translation available.

Mozell,1968 . Methyl benzoate has been used in studies of olfactory mechanisms in frogs

Boch,1971 . Methyl benzoate was a repellent to honey bees, Apis mellifera

Atkins,1975 . The suitability of the test material as a honeybee repellent was studied

Zhou,1993 . The test material was used as a model odorant in the study of the mechanism of vertebrate olfactory reception

Genotoxicity. See also mutagenicity (MUT), Ames (AMES), DNA (DNA) binding (BIND), sister chromatid exchange (SCE), etc.

Zeiger,1997 .

How materials are used. A use test/study conducted in humans as a means of measuring the elicitation potential of a material in an actual consumer product.

Somogyi,1996 .

Metabolism or non-ecological (not bacteria or fungi) biotransformation studies . See also pharmacokinetics (PKIN).

Harkiss,1973 .

Mixture.

Baker,2004 . The potential chemical changes and biological activity of smoke from cigarettes with added ingredients was investigated. The ingredients of the experimental cigarettes were listed.

Baker,2004a . The effects of flavoring and additives on smoke chemistry was investigated. The individual ingredients used in the experimental ingredient mixtures were reported.

Papers that are a review on a general subject, not a specific material. TREV papers may contain one or more RIFM materials. TREV papers do not contain original data.

Hjorth,1962 . This paper is a capsule preview of two detailed papers to be published in Arch Pharm Chemi

Stofberg,1987 .

Fort,1992 .

DeGroot,1997 . A clinical review of adverse reactions to fragrances

Photochemistry. See also phototoxicity (PTOX) and photosensitization (PSEN).

Shibamoto,1985 .

Reports on patients such as patch testing.

Sugawara,1984 . Results not specified by material in English abstract

Review articles. Papers that are reviews on a material or a class of materials. A review paper may be on one or more RIFM materials. Review papers do not contain original data.

AFAMRL,1982 .

Mathew,2001 . An analytical review of Piper nigrum L. oil is conducted, and the oxygenated components listed.

WHO,2002 . Summary of results of safety evaluation of benzyl derivatives used as flavouring agents, annual volumes, acute toxicity, short term studies of toxicity and genotoxicity was shown.

Adams,2005 . The key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances are presented.

Structure activity relationships such as studies of a group of aldehydes or other similar structures.

Tosato,1991 .

Nishimura,1994 . Mouse LD50 values were taken from RTECS. Paper in Japanese

Muller,1997 .

Cronin,1997 .

Karelson,2000 . The solvent and conformational dependence of different molecular descriptors was analyzed. The compounds used in the analysis of solvent effects were mentioned.

Serra,2001 . Three quantitative structure toxicity relationships were developed, to predict 50% population growth impairment concentrations for industrially important aromatic solvents.

Chen,2004 . The quantitative structure-activity relationship of selected esters was studied using the HQSAR method.

Studies of odor.

Hosokawa,1978 .

Bergstrom,2000 . The most common volatile compounds of floral odor belonging to various chemical classes are presented.

Brown,2002 . Benzoic acid carboxyl methyltransferase was isolated and characterized as the enzyme helping to form methyl benzoate.

Test methodology (new or altered classical), testing procedures, guidelines, etc. If applicable, see also biological tests (BIOTS).

Scholz,1998 . Several carboxylic acid methyl esters were utilized as reactants in a synthesis procedure which was part of a reporter gene assay.

Environmental Data

Acute toxicity

Route: Freshwater. **Species/Media :** fish. The acute toxicity of the test materials to fish at extremely low concentrations was evaluated. Trout (Salmo trutta), bluegill sunfish (Lepomis macrochirus), yellow perch (Perca flavescens) and goldfish (Carassius auratus) of fingerling size under 4 inches used. The

test was conducted in an 8-L aquarium containing 5 liters of water, and the temperature was maintained at 55 oF by submerging the aquaria in troughs of water at that temperature. Aeration of the aquarium by compressed air was conducted, and the water characteristics were: pH = 7; dissolved oxygen = 7.5 ppm; total hardness = 300 ppm; methyl orange alkalinity = 310 ppm; phenolphthalein alkalinity = 0; and free carbon dioxide = 5 ppm. The test materials were dispersed in the aquarium before the fish were added, and any test material that were insoluble in water, were first dissolved in a small amount of acetone or alcohol. The fish were exposed to the test material for 24 hours, and the time to produce death or obvious distress was recorded for each aquarium. For each test, 2 fish of each species were used. Not all test materials were tested in duplicate. The test materials were classified to be "sufficiently toxic" if they were toxic enough to kill at least 1 species in 24 hours, but not toxic enough to kill goldfish in 24 hours or less, and kill all other fish in 8 hours or less. All materials reported were classified "sufficiently toxic" at 5 ppm. If no death occurred in a species, the time to sickness was reported. An inadequate supply of yellow perch did not allow for all the materials to be tested on this species. 5 ppm lethal, no effects, The time to produce death in the trout and bluegill sunfish was 5 and 23 hours, respectively. No effects were observed in the goldfish. (EPA,1987)

Route: unclassified. **Species/Media :** *Daphnia magna*. On the basis of test data generated for a minimum number of specific compounds (subreference 01), the aquatic toxicities of 84 nontested monosubstituted benzenes were predicted using a statistically validated model. Ranked #56 among nontested monosubstituted benzenes. Ypredicted=-1.99. (Tosato,1991)

Skin absorption

Route: multiple routes. **Species/Media :** fish. The absorption of the test material through the gills or skin of goldfish was studied. The goldfish weighed 13+/-2 g. For gill exposure, the fish bodies were enclosed in a rubber bag with only the gills exposed. For body suface exposure, the fish were suspended in the test solution only below the gills. Disappearance of test material from the exposure solution was measured. Samples were collected 0, 2, 4, 6, 8, 10, 20 and 30 minutes after the start of exposure. The vehicle was water adjusted to pH 6.0 using diluted HCl or NaOH. 6 or 7 experiments were conducted in each case. 1.0 millimolar skin absorption, The absorption rate constant in (min-1g-1) x10(4) was 9.394+/-0.373 for the body and 9.965+/-0.548 for gills. (Sakiya,1988)

Pharmacology

Route: *in vitro*. **Species/Media :** fish. Giant axons were dissected from the mantles of freshly killed Loligo foibesi. The axons were cleaned of surrounding fibres and were usually between 600 and 1000 um in diameter. Axons were mounted in a voltage-clamp chamber described by Hayden. Sodium currents were measured following treatment w/ test compounds. 2 millimolar positive effects, sodium current was reduced in both intact axons and axons internally perfused w/ cesium fluoride. (Elliott,1987)

Cytotoxicity

Route: *in vitro*. **Species/Media :** fish. A 96-well tissue culture microtiter plate was inoculated with 0.2 ml of medium containing 4 x 10(4) bluegill sunfish fibroblast BF-2 cells. The plates were incubated at 32oC for 24 hours, the old medium was removed, and the cells were re-fed with fresh medium only or with fresh medium containing the test material. An additional 24 hours of incubation was conducted, and the old medium was removed with the addition of 0.33% of neutral red solution. Following incubation for a 3-hour period and removal of the medium, the cells were rinsed with neutral red assay fixative and washed with phosphate buffered saline (PBS). The wash solution was removed and the incorporated dye was solubilized in neutral red assay solubilization solution for 10 minutes. Absorption was measured and the cytotoxicity of the test material was evaluated by calculating the concentration required to induce a 50% inhibition in neutral red uptake (NI50). 10.727 millimolar positive effects, The NI50. (Shen,2000)

Route: *in vitro*. **Species/Media :** fish. Bluegill sunfish fibroblast BF-2 cells were seeded at 5 x 10(-4) per 35-mm petri dish and were incubated for 24 hours, followed by removal of the medium and re-

feeding of the cells with fresh medium only or with fresh medium containing the test material. After 3 day of incubation, the cells were trypanized and counted by the trypan blue exclusion test using a hemocytometer. The cytotoxicity of the test material was evaluated by calculating the concentration required to induce a 50% inhibition in cell growth (ID50). 4.919 millimolar positive effects, The ID50. (Shen,2000)

Route: *in vitro*. **Species/Media :** fish. A cell protein assay was conducted to evaluate the total mass of bluegill sunfish fibroblast BF-2 cells treated with the test material, and the cytotoxicity of the test material was evaluated by calculating the concentration required to induce a 50% inhibition in protein synthesis (ED50). No further details were provided. 8.754 millimolar positive effects, The EC50. (Shen,2000)

Route: *in vitro*. **Species/Media :** fish. Bluegill sunfish fibroblast BF-2 cells (300) were inoculated into 35-mm tissue culture dishes and incubated for 24 hours. The medium was removed and the cells were refed with medium only or medium containing the test material. Following 48 horus of incubation, the medium was removed, the cells were washed with phosphate buffered saline (PBS), and fresh medium only was added. After incubation for 12 days, the medium was removed and the cells were washed with PBS, fised in methanol and stained with Giemsa. The colonies were counted, and the cytotoxicity of the test material was evaluated by calculating the concentration required to induce a 50% decrease in colony formation (CI50). 15.140 millimolar positive effects, The CI50. (Shen,2000)

Miscellaneous

Route: *in vitro*. **Species/Media :** insect. The effect of test compound(s) on mites was determined. Mites were kept in contact with the volatiles for 24 hours at 25 C, in 75% relative humidity in an airtight petri dish to determine the amount (ul) of the test compound required to give 100% mortality of the mites. Mites species used were Dermatophagoides pteronyssinus, Dermatophagoides farinae and Tyrophagus putrescentiae. In addition, test compounds were further tested for their inhibiting effect on cholinesterase (ChE) activities, to study the mechanism of the killing activity Article in Japanese. Information obtained from English abstract and tables. There was no correlation between the mite-killing effect and the inhibiting effect on ChE activities. Positive effects, LD100 values (ul) for Dermatophagoides pteronyssinus, Dermatophagoides farinae and Tyrophagus putrescentiae were 1, 2, and 1 respectively. No inhibition of ChE activity. (Watanabe,1989)

Environmental

Species/Media : sludge. A study was conducted to determine the ready and ultimate biodegradability of the test material using the sealed vessel test. The test was conducted in 160 ml vessels (hypovials) containing 100 ml mineral salts medium inoculated with secondary effluent and the respective test or reference substance. The inoculum used was 10% by volume of activated sludge plant secondary effluent, filtered through a Whatman filter paper (541) to remove coarse particulate matter. The level of dissolved inorganic carbon (DIC) was reduced by sparging the filtered effluent with nitrogen after prior adjustment of the pH to 6.5. Test concentration was nominal 10 mg/l organic carbon. Test temperature range was 17-20 C. Multiple vessels were prepared per test material sealed with a butyl rubber septum and an aluminium crimp seal. The headspace in each vessel had a volume of 60 ml and when filled with air, contained approximately 6 times the mass of oxygen required for the complete oxidation of the test material. The sealed vessels were incubated at 20 C on a rotary shaker. At intervals during the 28 day test period a vessel was removed and concentration of carbon dioxide in the headspace gas determined. The seal is then broken and the concentration of inorganic carbon in the test medium was determined. Analysis of both the headspace gas and the liquid medium for CO2/DIC was performed on day numbers: 3, 8, 10, 14, 17, 21, 24 and 28 using the Ionics 555 Inorganic Carbon Analyser. The total inorganic carbon in the vessel was calculated and corrected by subtracting the inorganic carbon produced in the control. The control vessels were identical to the test vessels except for the omission of the test material. From a knowlege of the initial organic carbon concentration added as test substance, the extent of mineralisation was determined. A test substance was considered readily and ultimately

biodegradable if the material exceeded 60% biodegradation within a 10 day window over 28 days Reference material not provided. Nominal carbon concentrations of the test materials were used based on the calculated percentage carbon (from molecular formula) assuming 100% purity of the test material. **Study Length**: 28 days **Regulatory Compliance** OECD Guideline 301(B) **GLP** No (Birch, 1991) R541/545103.**Summary** The test material was classified as readily and ultimately biodegradable. 10 mg/l readily biodegradable, % biodegradation (nominal) of test material after 28 days (95% confidence limits) = 95.3 (93.7-96.9). (Quest,1995)

Species/Media : water. This is a report of the analysis of test materials as organic micro-contaminants in the water of the Rhine (Rijn) River and Lake IJsselmeer in the Netherlands. Water samples were analyzed monthly for the years 1990-1991. This project has been conducted by RIWA, an association of waterworks companies that extract surface water as a resource for drinking water, and who have joined forces to fight pollution in the water. Methodology for analysis involved concentration techniques by XAD-resin and solid phase, followed by identification by GC/MS. The lower limit of detection was 0.1 microgram per liter. Test material was detected in the Rhine River with an annual average and maximum concentration of <0.1 and <0.1 ug/liter for 1991. (RIWA,1993)

Ecotoxicity studies, tests or effects. Definition: Studies such as algal growth & inhibition, aquatic toxicity on various species (D. magna, shrimp, fish, mollusks, etc.). Includes studies on other species (earthworm, birds, plants, etc.)

Kool,1982 .

Environmental studies, tests or effects. Definition: fate and effect studies that include biodegradation, bioaccumulation, biotransformation, bioconcentration, soil adsorption, soil migration, sediment and water studies, etc.

Keith,1976 .

Kopfler,1977 .

Akiyama,1980 .

Botta,1984 .

Walker,1988 .

Meylan,1992 .

Paxeus,1996 .

Paxeus,1996a .

Kordel,1997 .

Flint,1998 .

Szabo,1999 . An HPLC screening method for determination of soil sorption constants was tested.

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No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Ethyl benzoate

Synonyms	
Benzoic acid, ethyl ester	CAS
Ethyl benzene carboxylate	
Ethyl benzoate	Principal EINECS RIFM

93-89-0

CAS Number	RIFM ID	FEMA	EINECS	Registration
93-89-0	332	2422	202-284-3	EINECS DSL TSCA

RIFM Monograph: 332 (Published 1974: FCT,v12,p717 (Binder, p352))

Fragrance Structure-Activity Group: Esters/Simple C1-C4 Alcohol Aryl Alkyl Acid Ester/Benzoic Acid Derivatives (Benzoates)

Formula $C_9H_{10}O_2$ **Structure** CH_3-CH_2-OCO-C_6H_5 **Molecular Weight** 150.18

SMILES Notation O=C(OCC)c(cccc1)c1

Generic Class Aromatic Esters

Description A clear, colorless to very pale yellow liquid having a somewhat fruity odor, reminiscent of Ylang. Its odor is somewhat milder than Methyl Benzoate.

Physical Data

Acid Value (XV.B.)	1.0 Max.	FMA	5.0 g.
Boiling Point	212□C	FMA	
Boiling Point (calculated)	215.57 □C	EPI Suite	
Flash Point	190□F;CC	FMA	
Halogenated Compounds (XXVIII.)	Negative	FMA	
Henry's Law (calculated)	4.608e-005 Pa□m^3/mol	EPI Suite	
Log K_{ow} (calculated)	2.32	EPI Suite	
Log K_{ow} (measured)	LogK pdms/w = 2.400 (n=12)	Xia,2007	
Melting Point (calculated)	-0.5 □C	EPI Suite	
Purity (X.B.2.b.)	98.0 Min.	FMA	1.1 g.|Calc.as C9H10<
Refractive Index @ 20□C (I.B.)	1.502 - 1.506	FMA	
Specific Gravity 20□C (I.A.)	1.045 - 1.048	FMA	
Specific Gravity 25□C (I.A.)	1.043 - 1.046	FMA	
Vapor Pressure (calculated)	0.1 mm Hg 20C	FMA	
Vapor Pressure (calculated)	0.197 mm Hg @ 25□C	EPI Suite	

Water Solubility (calculated)	421.5 mg/L	EPI Suite

Preparation	By esterification of ethyl alcohol and benzoic acid
Natural Occurrence	**Ethyl benzoate** is reported to occur in nature.
Use Levels	In public use since the 1940s.

Flavor Consumption (in kg)

1995	EUROPE	787
1995	USA	798
1987	USA	490
1982	USA	581
1975	USA	671
1970	USA	420

Uses (in ppm)

Product	Average Usual	Average Maximum	Mean Daily Consumption (gms)	Updated
Alcoholic Beverage	0.93	3.27	32.5	21-Jul-88
Baked Goods	14.94	18.41	137.2	21-Jul-88
Chewing Gum	2.09	6.82	0.2	21-Jul-88
Fats Oils	1.07	1.47	17.5	21-Jul-88
Frozen Dairy	10.35	14.12	25.6	21-Jul-88
Gelatin Pudding	11.56	14.24	20.4	21-Jul-88
Hard Candy	0.83	10.01	0.6	21-Jul-88
Non-alcoholic Beverage	9.78	14.72	104.0	21-Jul-88
Soft Candy	15.38	20.1	5.8	21-Jul-88

PADI 3.73.73.73.73.73.73.73.7

Food Products Containing Ethyl benzoate (in ppm)

Product	Code	Lower Limit	Upper Limit
Raspberry (Rubus idaeus L.)	>15-I	0.04	0.2
Bilberry (Vaccinium myrtillus L.)	>6-I	0.002	
American cranberry (V. macrocarpon Ait.)	>6-II	0.03	0.1
Rum category II (total volatiles 1100-3600 ppm)	>65-II	0.5	
Port wine	>70-IV	0.2	2
Guava (Psidium guajava L.)	>8-I	0.01	0.5
Mushroom (raw)	>93-I	0.15	

Status

Ethyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 261) , was approved by the FDA as a flavor (21 CFR 172.515) .

Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2422) Hall,1965

Indicative Non-Exhaustive List states: Listed

The Industrial Safety and Health Law (Japan) states: ISHL Number ((3)-1356)

Joint Expert Committee on Food Additives states: The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent. (852)

Joint Expert Committee on Food Additives states: The JECFA Evaluation Summary is available here.

Joint Expert Committee on Food Additives states: The Benzyl Derivatives Safety Evaluation is available here.

United Nations Transport Classification Codes states: Not regulated

FFIDS Volume VI Updated 1-Nov-85

European Hazard Classification Labeling	
NC	Based on available data, classification and labeling was not considered necessary as per the EFFA Code of Practice.

US OSHA Health Hazard Statements			
NONE	NONE	None Assigned	1-Jan-00

Global Harmonized System Hazard Statements			
Hazard Category	**Signal Word**	**Code**	**Hazard Statement**
FL 4	Warning	H227	Combustible liquid
SCI 3	Warning	H316	Causes mild skin irritation
ATO 5	Warning	H303	May be harmful if swallowed

Precautionary Code	Precautionary Statement
P210	Keep away from heat/sparks/open flames/hot surfaces. □ No smoking

P280	Wear protective gloves/protective clothing/eye protection/face protection
P312	Call a POISON CENTER or doctor/physician if you feel unwell
P332/313	If skin irritation occurs: Get medical advice/attention
P403/235	Store in a well-ventilated place. Keep cool
P501	Dispose of contents/container to ... (in accordance with local/regional/national/international regulation).

Human Health Data

Acute toxicity

Route: oral. **Species:** rat. The LD50, orally by stomach tube was determined. Animals observed for 2 wk or until death. Strain not specified. Method for determining LD50 not specified. **LD50** 2.1 G/KG 2.1 g/kg calculated LD50, using 45 rats. (Graham,1945)

Route: oral. **Species:** rabbit. The LD50, orally by stomach tube was determined. Animals observed for 2 wk or until death. Strain not specified. Method for determining LD50 not specified. **LD50** 2.63 G/KG 2.63 g/kg calculated LD50, using 12 rabbits. (Graham,1945)

Route: oral. **Species:** rat. In an acute oral toxicity study, the LD50 of the test material in rats was analyzed by using various parameters of the test material, and was reported. No further details were provided. Article in Japanese. **LD50** 2100 MG/KG 2100 mg/kg calculated LD50. (Nishimura,1994)

Route: oral. **Species:** rat. Groups of 5 non-fasted, Carworth-Wistar male rats weighing 90 - 120 grams, were administered a single dose of the test material orally. When necessary, the test material was tested as a solution in water, corn oil, or as a 1% solution of sodium 3,9-diethyl-6-tridecanol sulfate (Tergitol Penetrant 7), to bring the volume given to each rat to 1 □ 10 ml. The animals were observed for mortality during a 14-day period and the LD50 was estimated by the Thompson and Weil method. No further details were provided. Female rats were used.**LD50** 6.48 G/KG 6.48 g/kg calculated LD50, C.I. 5.66-7.42. (Smyth,1954)

Route: gavage. **Species:** rat. An unspecified number of rats were given the test material perorally. No further details were given. **LD50** 6480 MG/KG 6480 mg/kg calculated LD50. (Bar,1967)

Route: inhalation. **Species:** rat. Male or female albino rats were exposed to a flowing stream of air approaching saturation with vapors, which was prepared by passing dried air through a fritted disc gas washing bottle. Groups of 6 rats were exposed for periods of time in a logarithmic series with a ratio of 2, for up to 8 hours, until the period of time that killed 50% of the animals within 14 days of inhalation was determined. No further details were provided. The results reflect the longest inhalation period that allowed all the rats to survive the 14-day observation period. 8 hours no effects. (Smyth,1954)

Route: skin. **Species:** mouse. Doses of 10% to 100% test material were applied to 1/3 of the skin surface of 2-4 mice per dose. The vehicle was acetone. The observation period was 7 days. The observations were survival, body weight, skin temperature, behavior, food intake and autopsy The only result given by dose was the highest tolerable dose. 10 % no effects. (Lebedev,1969)

Route: skin. **Species:** domestic animals. A single application of test material in the form of an emulsion or solution was made to the entire body surface of calves. The observation period was 15 days. Survival and signs were monitored. 10 % no effects. (Lebedev,1969)

Route: skin. **Species:** cat. Benzyl compounds neat or in various vehicles was applied one or two times to the clipped backs (4 x 6 in) by cotton balls held firmly with hemostatic forceps. The area was massaged during application to facilitate absorption. Animals observed for 2 wk or until death. 100%. 20 ml lethal, 2 cats used; died within 20 hr. (Graham,1945)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human

carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. On the next Day, serial dilutions of test cpd were added to the cells, beginning in ea. case w/ a conc. close to saturation level. 500 mg/l positive effects, 100% inhibition of growth noted (Data taken from graph). (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. After 24 hr The IC50 conc. of test cpd was added to ea. flask. Flasks were kept @ 37 C w/ the screw plugs well closed for 7 days. Two flasks from ea. test grp were removed daily for protein determination. 289 mg/l no effects, no variation from the estimated 50% growth inhibition noted. (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells were plated in 25 cm2 flasks @ a density of 200 cells/flask in the presence of test cpd @ the IC50 conc. Cultures were incubated @ 37 C for 8 days & then the cells were stained w/ May-Grunwald & Giemsa and colonies of @ least 10 cells were counted. 289 mg/l positive effects, cell morphology was affected. Cells lost their typical epithelial shape & became elongated. (DeAngelis,1986)

Route: intramuscular. **Species:** guinea pig. Liquid esters of organic acids were examined for use as solvents in parenteral administrations. Sterilized esters (0.5 or 1.0 ml) were injected into the gluteal muscles & the animals observed continuously for several hours & then daily for 1 wk for systemic toxicity, local irritation & action upon, neuromuscular functions of the injected leg. Strain & no. tested/ester not specified. 1.25 ml/kg musculo-skeletal, moderate deterioration of leg function & caused muscle toughness. (Lipschitz,1942)

Irritation

Route: skin. **Species:** man. A 48 hour closed patch test on the backs of 5 male volunteers. 72-8-137 Vehicle was petrolatum. 8 % no effects. (RIFM,1972) [Kligman,1972]

Route: skin. **Species:** rabbit. Non-occluded irritation tests using 10-14 New Zealand males/material according to the Draize method (1944). About 0.5ml undiluted material applied daily to one side of the clipped dorsal area for up to 6 days & 0.2ml applied to the external surface of the rt. outer ear. Test areas examined at 4 & 24 hr after each treatment. 2/grp killed daily & skin of back & ear examined microscopically. Micro exam of organs from n-PB & n-BB rabbits. >99% pure. Irritant effects, skin effects, dorsum: erythema increased with no. of treatments; severe edema on last day. Ear not affected. Micro of 2 areas showed different responses. (Branca,1988)

Route: skin. **Species:** rabbit. Primary skin irritation was determined using groups of 5 albino rabbits. A 0.01-ml aliquot of the test material was applied to the clipped skin of the animals undiluted or as a solution in water, propylene glycol or kerosene. Scoring of the reactions was conducted within 24 hours of the application, using irritation grades that ranged from 1 to 10 (grade 1 = least visible capillary injection from the undiluted material; 6 = necrosis with the undiluted material; and 10 = necrosis from a 0.01% solution). No further details were provided. The specific vehicle used with each test material was not mentioned. 100 % irritant effects, grade = 4. (Smyth,1954)

Route: surface of eye. **Species:** rabbit. Eye injury was evaluated using groups of 5 rabbits per dose. The test material was applied to the center of the cornea while the lids were retracted. Approximately 1 minute later, the lids were released, and 18 - 24 hours later, the eye was examined in strong diffuse daylight, then stained with fluorescein, and the injury scored. The test material was tested undiluted or as a solution in propylene glycol and water. Eye injury was graded according to a 10-grade series: 1 = 0.5 ml undiluted gives injury of 0 to 1.0 points; 2 = 0.5 ml undiluted gives injury of over 1.0 up to 5.0 points; 3 = 0.1 ml undiluted gives injury of up to 5.0 points (0.5 ml gives over 5.0); 4 = 0.02 ml undiluted gives injury of up to 5.0 points (0.1 ml gives over 5.0); 5 = 0.005 ml undiluted gives injury of up to 5.0 points (0.02 ml gives over 5.0); 6 = Excess of 40% solution gives injury of up to 5.0 points

(0.005 ml gives over 5.0); 7 = Excess of 15% solution gives injury of up to 5.0 points (40% gives over 5.0); 8 = Excess of 5% solution gives injury of up to 5.0 points (15% gives over 5.0); 9 = Excess of 1% solution gives injury of up to 5.0 points (5% gives over 5.0); 10 = Excess of 1% solution gives injury of over 5.0 points. The points were equivalent to the following symptoms visible before fluorescein staining: 1 point = iritis, slight internal congestion; 2 points = cornea dull; 4 points = cornea opaque, less than half of area; and 6 points = cornea opaque, more than half of area. The points were equivalent to the following symptoms visible after fluorescein staining: 1 point = necrosis on less than 5% of the cornea; 2 points = necrosis on 5 □ 12%; 3 points = necrosis on 13 □ 37%; 4 points = necrosis on 38 - 62%; 5 points = necrosis on 63 - 87%; and 6 points = 88 - 100%. No further details were provided. (Carpenter,1946) 100 % eye effects, grade = 1. (Smyth,1954)

Sensitization

Route: skin. **Species:** guinea pig. A guinea pig open epicutaneous test (OET) was conducted on groups of 6 - 8 male and female guinea pigs weighting 300 - 450 grams. Daily applications were made for 3 weeks to a clipped 8-cm2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 hours after each application. A total of 21 applications of 0.1 ml test material in an unspecified vehicle were made for 21 days. The 10 controls were either left untreated or treated with 0.1 ml of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with the test material at the minimal irritating concentration and some lower primary non-irritating concentrations. The results were not reported for the irritation pre-screen. It was not specified if the concentration reported in the results was the minimal irritating concentration or 1 of the lower primary nonirritating concentration. 8 % no effects, No sensitization (-) was produced. (Klecak,1985)

Route: skin. **Species:** guinea pig. An open epicutaneous test was conducted in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6-8 guinea pigs per group. One to six experimental and one control group was used. Open challenge applications were made on days 21 and 35. Reactions were read at 24, 48 and 72 hours. No further details were provided. Vehicles were not specified by material. (Klecak,1977) 8 % no effects. (Klecak,1979)

Route: skin. **Species:** human 18+ yrs. A 24 to 48 hour occluded patch test was conducted on 30-200 patients. The test material concentration was 0.05 - 0.5% in a vehicle which was either a base cream or 99% ethanol (type of vehicle per test material was not specifically identified). Patch tests were always carried out with both perfumed and non-perfumed cream base at the same time. Patches consisted of a piece of 1 cm2 lint with a 2 cm2 cellophane disc placed on the lint and then covered with 4 cm2 plaster. The patches were applied to the back, the forearm and the inside of the upper arm for 24 to 48 hours. Reactions were evaluated 30 minutes after removal of patch. The skin reaction was graded as follows: - No visible reaction; +- Slight erythema; + Erythema; ++ Erythema and swelling or marked erythema. The period of study extended over 4 yrs and 3 months and patch testing was performed 1-3 times per month; September to May (summer months were excluded). The total number of subjects was 4737 (2341 Japanese men and 2396 Japanese women aged 16-45, mostly 17-30, living in Tokyo and Osaka). Volume dose not provided. 99% purity. No effects, 0/46. (Takenaka,1986)

Route: skin. **Species:** man. 25 subjects completed the study. 72-8-137 Vehicle was petrolatum. 8 % no effects. (RIFM,1972) [Kligman,1972]

Skin absorption

Route: *in vitro*. **Species:** guinea pig. Test compounds were tested as to their penetrating agent properties by assessing depth to which various "active principles" such as malachite greeen, Rhodamine B., orcein penetrated skin of animals in the presence of a test compound. Histological findings in epithelium (epi), hair follicles (hf), corium (c), and subcutis (sc) were evaluated by following criterion: + (strong coloring/fluorescence), (+) (noticeable coloring/fluorescence), = (slight coloring/fluorescence), and - (no coloring/fluorescence). Genuine penetration defined as active principle detected in corium or subcutis. t = duration of action. Vehicle usually 50% ethyl glycol. 50 %

no effects, rhodamine, t=2 hr, epi,hf,c,sc: =,=,-,-, resp. (Meyer,1965)

Pharmacokinetics and metabolism studies

Route: *in vitro*. **Species:** human 18+ yrs. The rate of hydrolysis of the test material by 80% human plasma was studied. Vehicle was acetonitrile. The t1/2 for the in vitro hydrolysis of ethyl benzoate to benzoic acid by human plasma was 210 minutes. (Nielsen,1987)

Route: *in vitro*. **Species:** human 18+ yrs. The ability of human plasma derived arylesterase to hydrolyse the test material was evaluated. Most test materials were solubilized using Tween 80. The Warburg manometric technique was used for esterase determination at pH 7.4, during which the initial substrate concentration was 8 mM. Not hydrolysed. (Augustinsson,1962)

Interaction

Route: *in vitro*. **Species:** guinea pig. In vitro skin penetration of drugs was examined. The skin preparations were mounted in a two-chamber diffusion cell with water jackets (37 C). The available diffusion area was about 0.65 cm2, and each half-cell volume was about 5.4 ml. The donor cells were filled with saline either in the presence or absence of the enhancer unless otherwise mentioned, and the receiver cells with PBS (pH 7.4). Cells were pretreated for 12 h with stirring at 450 rpm by a magnetic stirrer. After washing of both compartments, the suspension of excess amount of benzoic acid or its 4-alkyl substituents in saline either in the presence or absence of the penetration enhancer was added to the donor compartments unless otherwise mentioned, and the penetration experiment was begun. One hundred fifty ul of sample was taken from the receiver cells periodically over a maximum period of 29 h, diluted with PBS twenty times or more and analyzed by UV absorbance at 224 nm for benzoic acid, at 235 nm for 4-methyl and 4-ethyl substituents and at 236 nm for 4-n-propyl and 4-n-butyl substituents. Solubilized components from skin and penetrated enhancers did not interfere with the UV absorbance. Dependency on pH in the donor compartment of the permeability coefficients of the derivatives was examined with suspension of their excess amount (pH 3.0, 4.0) or a 50 mM solution of the derivative (pH 5.0-7.0), using either phosphate buffer (pH 3.0, 60, 7.0) or citrate buffer (pH 4.0, 5.0). Permeability coefficients of benzoic acid and the derivatives decreased with the increase of pH. (Kitagawa,1999)

Route: *in vitro*. **Species:** guinea pig. The permeability coefficient was calculated and the solubility of benzoic acid and its 4-alkyl substituents was measured after their incubation in an excess amount in saline either in the presence or absence of the enhancer at 37 C for 24 h. After quick centrifugation at 1000xg for 2 min at 37 C, concentration of the supernatant was obtained by measuring UV absorbance. The solubility (Cd) and permeability (Kp) coefficients were 3.34 mM and 34.8 x 10(-2)cm/h, resp. (Kitagawa,1999)

Subchronic toxicity

Route: food. **Species:** rat. A 12 wk study was conducted using 12 rats per sex per dose. The test material was a mixture of flavor materials. Sacrifice was at the end of feeding. The observations were survival, body weight, behavior, appearance, food intake, EFU, urinalysis, blood hemoglobin, liver & kidney weights and autopsy The test mixture contained ethyl benzoate, isobutyl benzoate, benzyl acetate, benzyl butyrate, ethyl methyl phenyl glycidate and glycidate M-116. 0.16 mg/kg no effects. (Trubek,1957)

Carcinogenesis and mutagenesis studies

Route: *in vitro*. **Species:** *Salmonella typhimurium*. The mutagenicity potential of the test substance was studied in five mutant strains of Salmonella typhimurium using a standard plate incorporation assay with and without liver homogenate (S9). The strains used in the study were TA1535, TA1537, TA98, TA100 and TA102. Vehicle was dimethyl sulfoxide (DMSO). Dose levels were based on an intial toxicity test and were 15 to 5000 ug/plate in the presence and 5 to 5000 ug/plate in the absence of S9 mix. The tester strain cultures in this study were grown in Oxoid nutrient broth No. 2 (2.5%) at 37 □C on a shaker for 11 □ 12 hours to a density of 1-3 x 10(9) cells/ml. The cultures were then kept at 4 □C

and only fresh cultures were used for the assays. To determine the cell titer, 0.1 ml aliquots of the 1 x 10-6 dilution were spread over the surface of complete medium plates and incubated overnight at 37 □C. The rat liver homogenate fractions were prepared from Sprague-Dawley male rats (8 □ 10 weeks old) induced with Aroclor 1254. S9 mix, which contains 10% S9, was freshly prepared for each mutagenicity assay. To each culture tube containing 2 ml of top agar, bacteria (0.1 ml) was added, then test substance and then 0.5 ml of S9 mix or phosphate buffer for the assays without metabolic activation. The components were vortexed and immediately poured onto minimal agar plates and spread to achieve uniform distribution. The minimal agar plates consisted of 20 □ 25 ml of 1.5% Vogel-bonner medium E with 2% glucose. Three parallel plates were prepared for each experimental point and the plates kept for 48 □ 72 hours at 37□C in the dark. The plates were then examined for the number of revertant colonies (his+ revertants), the existence of normal background lawn and/or precipitates and microscopically for microcolony growth. Any reduction in the number of revertant colonies and/or a diminished background lawn was taken as a sign of bacteriotoxicity. If there was a questionable colony and when positive mutagenic results were obtained, the genotype are spot checked by picking and streaking on histidine free plates. The experiment was repeated in full after an interval of at least 3 days. To confirm the reversion properties and specificity of the bacterial strains as well as the activity of the liver homogenates used, positive mutagenesis controls were run simultaneously. Positive controls were sodium azide, 2-nitrofluorene, 9-aminoacridine, mitomycin C and 2-aminoanthracene. **Regulatory Compliance** OECD Guidelines for Testing of Chemicals 471, adopted July 21, 1997 (Bacterial Reverse Mutation Test). **GLP** Yes (Ames et al, 1975; Maron and Ames, 1983) HR 613001; Batch No. 50494310 vehicle was dimethyl sulfoxide (DMSO).**Summary** Under the conditions of the study, the test material was not mutagenic to Salmonella typhimurium strains TA1535, TA537, TA98, TA100 and TA102 in the presence and absence of a metabolizing system. Test material concentrations were 15 to 5000 ug/plate in the presence of S9 and 5 to 5000 ug/plate in the absence of S9. In the absence of S9 mix the test material was bacteriotoxic towards the strain TA102 at 500 ug/plate, towards the strain TA98 at 1500 ug/plate and towards the strains TA100, TA1535, and TA1537 at 5000 ug/plate. In the presence of S9 mix the test material was bacteriotoxic towards the strain TA102 at 1500 ug/plate and towards the strains TA98, TA100 and TA1535 at 5000 ug/plate. Precipitation of the test material on the plates was not observed. The test material did not induce a significant increase in the mutation frequency of the test strains in the absence or presence of S9 mix. The positive control substance confirmed the known reversion properties and specificity of the tester strains as well as the full activity of the metabolizing system (S9). Under the conditions of the study, the test material was not mutagenic to Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 in the presence and absence of a metabolizing system. (Symrise,1991)

Route: *in vitro*. **Species:** *Escherichia coli*. The antimutagenic activity of flavorings was evaluated in Escherichia coli strain WP2s (uvrA, trpE). Cultures were pre-treated with 4-nitroquinoline-1-oxide, furylfuramide (AF-2), or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), washed and then spread on agar plates containing the test material. Plates were then incubated at 37 C for 3 days. Article in Japanese. Information obtained from English abstract and translated tables. No effects. (Ohta,1995)

Route: *in vitro*. **Species:** *Escherichia coli*. A log phase culture of E. coli PQ37 (sulA::lacZ, rfa, uvrA, Phoc) was irradiated with 1-1.5 J/m2 UV and then immediately subjected to culture for 2 hours in medium containing test materials at concentrations up to 500 ug/ml, unless toxicity was observed. Induction of SOS was assayed by measuring B-galactosidase in the sulA::lacZ fusion cells. The levels of constitutive alkaline phosphatase were assayed in parallel to examine inhibitory effects on protein synthesis. 233 compounds tested. Unspecified effects, Results not specific except to say "many antibiotics and inorganics inhibited protein synthesis, because they caused a decrease in both enzyme activities.". (Ohta,1986a)

Route: *in vitro*. **Species:** *Escherichia coli*. Antimutagenic activity was evaluated in strain E. coli WP2s (uvrA, trpE). Mutagens were Captan, Methylglyoxal, 4-Nitroquinoline 1-oxide and furylfuramide. All materials tested up to 200 ug/ml unless toxic. Cultures were treated with mutagen, washed and then

spread on agar plates containing test material. Plates were incubated at 37 C for 3 days. No effects. (Ohta,1986)

Route: *in vitro*. **Species:** *Salmonella typhimurium*. Antimutagenic activity was evaluated in strain TA98 (uvrB. hisD). Mutagens were 3-Amino-1-methyl-5H-pyrido(4,3-b)indole and 2-Amino-3-methyl-imidazo[4,5-f]quinoline. Dose up to 200 ug/ml unless toxic. Cultures were treated with mutagen, washed and then spread on agar plates containing test material. Plates were incubated at 37 C for 3 days. No effects. (Ohta,1986)

Cytotoxicity

Route: *in vitro*. **Species:** human 18+ yrs. The ability of the test material to increase the permeability of the membranes of human lung fibroblasts was studied by measuring the release of an intracellular nucleotide marker. Human diploid embryonic lung fibroblasts (line MRC-5) were cultivated to a cell density of 10 to the fifth cells/cm2 (approximately 7 x 10(5) cells/well). The cells were then labeled with [3H]uridine. The labelled cultures were incubated with 25 mM of the test material for 30 minutes at 37 C in Tris-buffered saline. The solution containing the leaked radioactive marker was removed and centrifuged and the radioactivity was measured. A maximal release of the radioactivity was obtained by treating control cells for 30 minutes with 0.06 M sodium borate buffer and scraping with a rubber policeman. This treatment ruptured the cell membranes leaving the nuclei intact. The results notes section indicates the percentage of nucleotide released. Positive effects, 31%. (Thelestam,1980)

Enzyme processes

Route: *in vitro*. **Species:** pig. Liver esterase was prepared according to Adler & Kistinkowsky. Fresh liver obtained from the slaughterhouse was converted to an acetone powder. Powder was extracted & subjected to DEAE-cellulose column chromatography followed by gel electrophoresis for purification. Substrate specificity was ass essed through analysis of ester hydrolysis activity. Esteratic hydrolysis rate = 1000 - 5000 umoles/min. (Levy,1969)

Miscellaneous

Route: *in vitro*. **Species:** fungi. The antifungal effect of test material on wheat seeds was evaluated. The moisture content of the seeds was raised to a level of 20% by mixing the seeds with requisite quantity of sterile distilled water in a tightly stoppered bottle, which was stored at 4 C for 3 days. During this period the bottle was shaken several times per day to facilitate uniform distribution of the moisture. Samples of wheat from these bottles were transferred to 10 ml glass vials, about 1 gram of grains to each vial. The test material was added by volume with a microsyringe or a micropipette over the surface of seeds at levels of 1, 2, 4 and 8 ul/vial. The vials were then closed tightly and shaken vigorously. They were kept in darkness at 30 C for 5 days. Each series included three replicates for each test material tested and one control series with three replicates to which no test material was added. The treated seeds were then transferred to fresh vials with plastic lids where three small holes were punched in each lid. The vials were incubated at 30 C for 5 days at an ambient relative humidity of 70%. Fungal growth and germinability of the seeds were evaluated. Results were evaluated in the following way: first a number of seeds were examined under a dissecting microscope for the determining of any fungal growth; a second set of treated seeds were cut longitudinally and placed on malt agar (2% malt extract) and on salt-malt-agar (10% NaCl) and incubated for 7 days at 30 C; a third set of treated seeds were soaked in sterilized distilled water for 2 hours, then placed on moist filter paper in petri dishes and incubated at 30 C for 7 days. Seeds producing a small coleoptile and a root were counted as having germinated Except in a few cases, all treatments which effectively controlled fungal growth destroyed the germinability of seeds. Positive effects. (Nandi,1976)

Route: *in vitro*. **Species:** fungi. Vapors of the test material were tested for antifungal properties on Candida albicans ATCC 10231, Phoma betae ATCC 6504, Geotrichum candidum Coll. No. 4762 and Oospora lactis ATCC 4798. 0.5 ml of the test organism was streaked onto the agar surface. 0.5 ml of the test material was placed in a cup in the center of a petri dish top. Dishes with culture were inverted over the top and incubated. Vapors of the test material were allowed to emanate throughout the five day

incubation period at 22 degrees. After incubation, the presence of a definite clear zone of inhibition on the surface of the agar indicated that the vapor possessed antifungal activity and the larger the zone the greater the activity in this test system. All test materials were tested in triplicate with one cup per dish. Positive effects, in all four fungi. (Maruzzella,1961)

Route: *in vitro*. **Species:** fungi. The fungicidal properties of the test material upon the conidial stage of the hop powdery mildew (Sphaerotheca humuli (DC) Burr.) were evaluated. Test material was sprayed on young hop leaves on which the mildew was growing. Fungicidal and phytocidal properties were evaluated No further details provided. Vehicle was 0.25% agral I. 1 % positive effects, fungicidal to super-fungicidal with some leaf injury. 0.5 % no effects. (Martin,1934)

Route: *in vitro*. **Species:** bacteria. The effect of test chemicals on growing cultures of four bacterial strains [Bacillus subtilis, ATCC 9524, Escherichia coli ATCC 11229, Staphylococcus aureus Ox-H (penicillin sensitive) and Staphylococcus aureus ATCC 10390 (penicillin resistant)] was studied. Dilutions of each chemical were prepared in sterile nutrient broth at 1:500. Tubes were inoculated with 0.5 ml of a 24 hour broth culture of test organism. Tubes were incubated at 37 C for 24 hours and the presence or absence of visible growth observed. Failure of growth to occur in the subculture was taken as evidence that the organisms had been killed in the original chemical-broth tubes. When this occurred, additional concentrations were prepared (1:1000, 1:2000, 1:10,000) and tested in a similar manner. No effects, bacterial growth was observed at 1:500 dilution in all four strains tested. (Maruzzella,1961a)

Route: *in vitro*. **Species:** fungi. Antifungal activity was evaluated using the filter paper disk method. The organisms used were Lenzites trabea ATCC 8715, Polyporus versicolor ATCC 11235 and Lentinus lepideus ATCC 11453. Sterile disks (6.35 mm diameter) were thoroughly saturated with test material. The saturated disks were then placed on the surface of Sabauraud's maltose agar plates which had been previously seeded with 1 ml of fungal broth culture. All dishes were conducted in triplicate with one disk per dish and incubated for 12 to 14 days at room temperature. The presence of zones of inhibition surround the saturated paper disks indicated anti-fungal activity and the zones were recorded in millimeters with a metric ruler with the aid of an illuminated Quebec colony counter. (Vincent and Vincent, 1944) Positive effects. (Maruzzella,1960)

Route: *in vitro*. **Species:** bacteria. The vapors of 192 aromatic chemicals were tested in vitro against growing cultures of Bacillus subtilis (var. aterrimus ATCC 6461), Serratia marcescens (ATCC 9986), Staphylococcus aureus (OX-H), Escherichia coli (ATCC 11229) and Mycobacterium avium (ATCC 4676). All of the test organisms were cultivated on nutrient agar and broth at 37 degrees C, except M. avium which was grown in nutrient agar and broth containing 5% glycerol. Using the method of Maruzzella, J.C. (see methology reference in comments section) 15 mls of nutrient agar were poured into Petri dishes and allowed to harden; the surface of the agar was streaked with 0.5 ml of a 48 hour broth culture of the test organisms (M. avium broth culture was 72 hrs old); aluminum caps with 0.5 ml of aromatic chemical were placed on the inner surface of the petri dish top; dishes were inverted and incubated 48 hours(M. avium - 72 hours). After incubation, a definate zone of inhibition on the surface of the agar indicated that the vapor possessed antibacterial activity. The larger the zone, the greater the activity. The diameters of the zones of inhibition were measured by a metric ruler with the aid of an illuminated Quebec Colony Counter. All dishes were conducted in triplicate. In some tests the vapor permitted no growth to occur on the surface of the entire dish. In this case, the zones of inhibition were recorded as 90 mm. which is the inside diameter of the Petri dish. The method used to test the effects of the vapors on bacteria was by Maruzzella, J.C., in Soap, Perfumery and Cosmetics, vol. 33, pg. 835, 1960. Positive effects, Vapors exhibited activity against B. subtilis, S. marcescens, S. aureus and M. avium. (Maruzzella,1961b)

Route: *in vitro*. Antimicrobial activity was evaluated using a standardized petri plate procedure. Organisms used were Staphylococcus aureus (SA), Escherichia coli (EC), Candida albicans and, if active in the others, a Diphtheroid (D). All materials were tested at 10% (w/v) in 95% (v/v) ethanol

unless solubility problems occurred. Not all materials were tested in all 4 organisms. Controls were 3,4,4'-trichlorocarbanilide and 2,4,4'-trichloro-2'-hydroxydiphenyl ether and hexachlorophene. No effects. (Morris,1979)

Route: *in vitro*. **Species:** guinea pig. The effect of immersion in test material on the penetration of light through stratum corneum was evaluated. The spectral transmission curve for isolated stratum corneum was measured under ambient conditions and after complete immersion of the stratum corneum in neat test material The wavelengths measured were 320 and 400 nm. 100 % skin effects, a substantial increase in transmision was noted. (Solan,1977)

A model for studying specific effects.

Xu,2002 . The LSER model was employed in analyses of soil organic partition coefficients, octanol-water coefficients, capacity factors in soil leaching column chromatography (SLCC), the influence of methanol-water mixtures on capacity factors, and to make clear the intrinsic relationship between soil organic partition coefficients and capacity factors in SLCC.

Analytical methodology. Can also refer to physical properties such as boiling temperature, flash point, etc.

Stull,1947 . Vapor pressures were collected for over 1200 organic compounds.

Morris,1973 .

Tressl,1978 . The components characterized in beer was determined.

Ramsey,1980 .

Williams,1983 . Compounds identified in commercial port wines upon examination of aroma extracts.

Vaughan,1988 .

Reverchon,1997 .

El-Ghorab,2003 . The components of clove bud and cinnamon bark oil were determined.

Fernandez,2003 . The chemical composition of the volatile extracts obtained from Siam and Sumatra benzoin gums by direct GC injection.

Figueredo,2005 . Composition of various Oregano populations determined.

Cai,2006 . The volatile components of clary sage oil was determined.

Ranadive,2006 . The chemical constituents of vanilla flavor were determined.

Xia,2007 . The partition coefficients were determined for several materials. LogK pdms/w = 2.400 (n=12)

Article in foreign language; no English translation available.

Meyer,1960 . No English abstract or translation available.

Grubner,1972 .

Biological tests other than classical toxicology testing and pharmacological tests.

Valette,1954 . Measured & compared skin penetration of various hydrocarbons, alcohols, etc., in rats. In french.

Ishikawa,1965 . No English translation available.

Carcinogenicity studies and/or effects.

Gaworski,1999 . The flavor ingredients were tested in combination; not individually.

Mixture.

Gaworski,1998 . Mixtures were tested not individual ingredients

Larsen,1998a .

Roemer,2000 . The flavor components of cigarettes are listed.

Baker,2004 . The potential chemical changes and biological activity of smoke from cigarettes with

added ingredients was investigated. The ingredients of the experimental cigarettes were listed.

Baker,2004a . The effects of flavoring and additives on smoke chemistry was investigated. The individual ingredients used in the experimental ingredient mixtures were reported.

Papers that are a review on a general subject, not a specific material. TREV papers may contain one or more RIFM materials. TREV papers do not contain original data.

Mitchell,1975 .

Ruth,1986 .

Stofberg,1987 .

Schreiber,1997 .

Larsen,1998 .

Photochemistry. See also phototoxicity (PTOX) and photosensitization (PSEN).

Shibamoto,1985 .

Predictability of a test. See also model (MODEL), relevance (RELEV).

Eldred,1999 .

Pavan,2006 . The predictive capability of the proposed model for the SIDS data set was compared with five well known literature QSAR models for acute fish toxicity to Pimephales promelas. 408 heterogeneous chemicals were selected for this study.

Reports on patients such as patch testing.

DeGroot,1994 . Synonyms, patch test concentrations and vehicles with references, in addition to comments for each chemical are listed.

Review articles. Papers that are reviews on a material or a class of materials. A review paper may be on one or more RIFM materials. Review papers do not contain original data.

WHO,2002 . Summary of results of safety evaluation of benzyl derivatives used as flavouring agents, annual volumes, acute toxicity, short term studies of toxicity and genotoxicity was shown.

Adams,2005 . The key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances are presented.

Statistical methods and calculations.

Ran,2002 . A proposed general solubility equation (GSE) was used to estimate the aqueous solubility of 1026 non-electrolytes.

Structure activity relationships such as studies of a group of aldehydes or other similar structures.

Bearden,1998 .

Karelson,2000 . The solvent and conformational dependence of different molecular descriptors was analyzed. The compounds used in the analysis of solvent effects were mentioned.

Serra,2001 . Three quantitative structure toxicity relationships were developed, to predict 50% population growth impairment concentrations for industrially important aromatic solvents.

Studies of odor.

Bergstrom,2000 . The most common volatile compounds of floral odor belonging to various chemical classes are presented.

Studies on flavor and flavor components other than processed flavors (PF).

Swaine,1994 .

Test methodology (new or altered classical), testing procedures, guidelines, etc. If applicable, see also biological tests (BIOTS).

Martin,2001 . A general group combination method was used to correlate the toxicity (LC50) of

various compounds.

Environmental Data

Acute toxicity

Route: *in vitro*. **Species/Media :** invertebrate. The impairment of the population growth of ciliate Tetrahymena pyriformis strain GL-C by industrial organic compounds was assessed. A 40-hour static assay was conducted under test conditions that allowed for 8 - 9 cell cycles in the controls. Erlenmeyer flasks containing sterile, semidefined proteose-peptone-based medium were inoculated to an initial density of approximately 2500 cells/mL with log-growth- phase ciliates, in the presence of the test materials in DMSO. Each test material was evaluated for 2 additional replicates, and each replicate consisted of 6 - 8 different concentrations of individual test materials with duplicate flasks of each concentration. The controls were inoculated with T. pyriformis in the absence of the test materials. Blanks were also used, which consisted of no ciliate or test materials. Following incubation at 27 +/- 1 oC, the population density of T. pyriformis was quantitated spectrophotometrically with absorbance at 540 nM. The 50% growth inhibitory concentration (IGC50) was determined for each test material, with the dependent variable being the absorbance normalized as a percentage of the control, and the independent variable being the test concentration in mg/l. The IGC50 was reported as log 1/IGC50. Positive effects, The log 1/IGC50 = -0.01 (or approximately 0.98 mM). (Schultz,1997)

Route: Freshwater. **Species/Media :** *Daphnia magna*. Acute toxicity test to Daphnia based on the OECD Guideline. The toxicity was expressed as 24 hr-EC(50), i.e. the concentration of toxicant causing immobilization of 50% of the population after 24 hr exposure, which was estimated by the method of Litchfield & Wilcoxon (1949). 16 mg/l , EC50 with 95% confidence limits of 13.0-19.7 mg/l. Yobserved=-2.03. Ycalculated=-2.09. (Tosato,1991)

Pharmacology

Route: *in vitro*. **Species/Media :** fish. Giant axons were dissected from the mantles of freshly killed Loligo foibesi. The axons were cleaned of surrounding fibres and were usually between 600 and 1000 um in diameter. Axons were mounted in a voltage-clamp chamber described by Hayden. Sodium currents were measured following treatment w/ test compounds. 1 millimolar positive effects, sodium current was reduced in both intact axons and axons internally perfused w/ cesium fluoride. (Elliott,1987)

Miscellaneous

Route: *in vitro*. **Species/Media :** insect. The effect of test compound(s) on mites was determined. Mites were kept in contact with the volatiles for 24 hours at 25 C, in 75% relative humidity in an airtight petri dish to determine the amount (ul) of the test compound required to give 100% mortality of the mites. Mites species used were Dermatophagoides pteronyssinus, Dermatophagoides farinae and Tyrophagus putrescentiae. In addition, test compounds were further tested for their inhibiting effect on cholinesterase (ChE) activities, to study the mechanism of the killing activity Article in Japanese. Information obtained from English abstract and tables. There was no correlation between the mite-killing effect and the inhibiting effect on ChE activities. Positive effects, LD100 values (ul) for Dermatophagoides pteronyssinus, Dermatophagoides farinae and Tyrophagus putrescentiae were 1, 2, and 1 respectively. No inhibition of ChE activity. (Watanabe,1989)

Environmental

Species/Media : sludge. Testing of ready biodegradability over a period of 28 days with activated sludge bacteria was conducted in order to check the rate of biodegradation in percent. The inoculum was activated sludge from secondary effluent at a domestic sewage treatment plant. Filtration was used to separate coarse particles and mineral medium plus inoculum was aerated for 5 days. Sodium benzoate was used as the reference item to confirm that the effluent was sufficiently active. Test

solutions were prepared and cultivated as follows: 10 test suspension bottles which contain mineral medium with a known concentration of the test material(3.1 mg) and inoculum (2.9 mg); 10 procedure control bottles containing the reference chemical sodium benzoate (2 flasks in parallel to the normal test runs); 10 inoculum blank bottles containing a measured volume of mineral medium containing only inoculum; and 10 toxicity control bottles containing test material, reference substance and inoculum to determine toxic effects of test material. The incubation was conducted at 20 +/-1 degree C for 28 days, under aerobic conditions in completely full, closed bottles. The reduction of dissolved oxygen was measured in the test solution over the 28 day period. The amount of oxygen taken up by the test material (corrected for that in the blank inoculum control) is expressed as a percentage of theoretical oxygen demand (ThOD) or chemical oxygen demand (COD). **Study Length**: 28 days **Regulatory Compliance** Council Directive 92/69/EEC Method C.4-E: Closed Bottle Test. OECD Guideline 301 D. **GLP** Yes 103933; Batch 50494310.**Summary** Under the conditions of the study, the test material should be classified as "Readily Biodegradable". 3.1 mg readily biodegradable, the test material showed 42% degradation after 7 days; 58% degradation after 14 days; 68% degradation after 21 days; 69% degradation after 28 days. The reference compound showed 75% degradation after 14 days. Under the conditions of the study, the test material should be classified as "Readily Biodegradable". (Symrise,2000)

Environmental studies, tests or effects. Definition: fate and effect studies that include biodegradation, bioaccumulation, biotransformation, bioconcentration, soil adsorption, soil migration, sediment and water studies, etc.

Akiyama,1980 .

McFall,1985 .

Meylan,1992 .

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No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Propyl benzoate

Synonyms	
Benzoic acid, propyl ester	CAS
Benzoic acid, *n*-propyl ester	
n-Propyl benzenecarboxylate	
Propyl benzoate	Principal EINECS

2315-68-6

CAS Number	RIFM ID	FEMA	EINECS	Registration
2315-68-6	6192	2931	219-020-8	EINECS DSL TSCA

Fragrance Structure-Activity Group: Esters/Simple C1-C4 Alcohol Aryl Alkyl Acid Ester/Benzoic Acid Derivatives (Benzoates)

Formula $C_{10}H_{12}O_2$ **Molecular Weight** 164.2

SMILES Notation O=C(OCCC)c(cccc1)c1

Generic Class Aromatic Esters

Physical Data

Boiling Point	231□C	FMA
Boiling Point (calculated)	234.31 □C	EPI Suite
Flash Point	186□F;CC	FMA
Henry's Law (calculated)	6.117e-005 Pa□m^3/mol	EPI Suite
Log K_{ow} (calculated)	2.81	EPI Suite
Melting Point (calculated)	10.6 □C	EPI Suite
Specific Gravity	1.020	FMA
Vapor Pressure (calculated)	0.09 mm Hg 20C	FMA
Vapor Pressure (calculated)	0.207 mm Hg @ 25□C	EPI Suite
Water Solubility (calculated)	176 mg/L	EPI Suite

Natural Occurrence	**Propyl benzoate** is reported to occur in nature.

Flavor Consumption (in kg)

1995	EUROPE	0.1
1995	USA	0
1987	USA	0

1982	USA	0
1970	USA	2

Uses (in ppm)

Product	Average Usual	Average Maximum	Mean Daily Consumption (gms)	Updated
Baked Goods	15	20	137.2	21-Jul-88

PADI 2.05

Status

Propyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 677) , was approved by the FDA as a flavor (21 CFR 172.515) .

Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2931) Hall,1965

Indicative Non-Exhaustive List states: Listed

Joint Expert Committee on Food Additives states: The JECFA Evaluation Summary is available here.

Joint Expert Committee on Food Additives states: The Benzyl Derivatives Safety Evaluation is available here.

Joint Expert Committee on Food Additives states: The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent. (853)

FFIDS Volume VII Updated 1-Nov-85

Human Health Data

Acute toxicity

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. On the next Day, serial dilutions of test cpd were added to the cells, beginning in ea. case w/ a conc. close to saturation level. 200 mg/l positive effects, 100% inhibition of growth noted (Data taken from graph). (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to

complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. After 24 hr The IC50 conc. of test cpd was added to ea. flask. Flasks were kept @ 37 C w/ the screw plugs well closed for 7 days. Two flasks from ea. test grp were removed daily for protein determination. 122 mg/l positive effects, on day 7, cells seemed to recover from exposure to test cpd. (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells were plated in 25 cm2 flasks @ a density of 200 cells/flask in the presence of test cpd @ the IC50 conc. Cultures were incubated @ 37 C for 8 days & then the cells were stained w/ May-Grunwald & Giemsa and colonies of @ least 10 cells were counted. 122 mg/l positive effects, monolayers of treated cells were disturbed w/in 24 hrs. (DeAngelis,1986)

Irritation

Route: skin. **Species:** rabbit. Non-occluded irritation tests using 10-14 New Zealand males/material according to the Draize method (1944). About 0.5ml undiluted material applied daily to one side of the clipped dorsal area for up to 6 days & 0.2ml applied to the external surface of the rt. outer ear. Test areas examined at 4 & 24 hr after each treatment. 2/grp killed daily & skin of back & ear examined microscopically. Micro exam of organs from n-PB & n-BB rabbits. 98% pure. Irritant effects, skin effects, dorsum & ear: immediate erythematous reaction that increased in severity. Different patterns of micro lesions. No organ lesions. (Branca,1988)

Pharmacokinetics and metabolism studies

Route: *in vitro*. **Species:** human 18+ yrs. The rate of hydrolysis of the test material by 80% human plasma was studied. Vehicle was acetonitrile. The t1/2 for the in vitro hydrolysis of propyl benzoate to benzoic acid by human plasma was 46 minutes. (Nielsen,1987)

Interaction

Route: *in vitro*. **Species:** guinea pig. In vitro skin penetration of drugs was examined. The skin preparations were mounted in a two-chamber diffusion cell with water jackets (37 C). The available diffusion area was about 0.65 cm2, and each half-cell volume was about 5.4 ml. The donor cells were filled with saline either in the presence or absence of the enhancer unless otherwise mentioned, and the receiver cells with PBS (pH 7.4). Cells were pretreated for 12 h with stirring at 450 rpm by a magnetic stirrer. After washing of both compartments, the suspension of excess amount of benzoic acid or its 4-alkyl substituents in saline either in the presence or absence of the penetration enhancer was added to the donor compartments unless otherwise mentioned, and the penetration experiment was begun. One hundred fifty ul of sample was taken from the receiver cells periodically over a maximum period of 29 h, diluted with PBS twenty times or more and analyzed by UV absorbance at 224 nm for benzoic acid, at 235 nm for 4-methyl and 4-ethyl substituents and at 236 nm for 4-n-propyl and 4-n-butyl substituents. Solubilized components from skin and penetrated enhancers did not interfere with the UV absorbance. Dependency on pH in the donor compartment of the permeability coefficients of the derivatives was examined with suspension of their excess amount (pH 3.0, 4.0) or a 50 mM solution of the derivative (pH 5.0-7.0), using either phosphate buffer (pH 3.0, 60, 7.0) or citrate buffer (pH 4.0, 5.0). Permeability coefficients of benzoic acid and the derivatives decreased with the increase of pH. (Kitagawa,1999)

Route: *in vitro*. **Species:** guinea pig. The permeability coefficient was calculated and the solubility of benzoic acid and its 4-alkyl substituents was measured after their incubation in an excess amount in saline either in the presence or absence of the enhancer at 37 C for 24 h. After quick centrifugation at 1000xg for 2 min at 37 C, concentration of the supernatant was obtained by measuring UV absorbance. The solubility (Cd) and permeability (Kp) coefficients were 0.70 mM and 62.7 x 10(-2)cm/h, resp. (Kitagawa,1999)

Analytical methodology. Can also refer to physical properties such as boiling temperature, flash point, etc.

Stull,1947 . Vapor pressures were collected for over 1200 organic compounds.

Fernandez,2003 . The chemical composition of the volatile extracts obtained from Siam and Sumatra benzoin gums by direct GC injection.

Biological tests other than classical toxicology testing and pharmacological tests.

Ishikawa,1965 . No English translation available.

Review articles. Papers that are reviews on a material or a class of materials. A review paper may be on one or more RIFM materials. Review papers do not contain original data.

WHO,2002 . Summary of results of safety evaluation of benzyl derivatives used as flavouring agents, annual volumes, acute toxicity, short term studies of toxicity and genotoxicity was shown.

Adams,2005 . The key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances are presented.

Statistical methods and calculations.

Ran,2002 . A proposed general solubility equation (GSE) was used to estimate the aqueous solubility of 1026 non-electrolytes.

Structure activity relationships such as studies of a group of aldehydes or other similar structures.

Tosato,1991 .

Environmental Data

Acute toxicity

Route: unclassified. **Species/Media :** *Daphnia magna*. On the basis of test data generated for a minimum number of specific compounds (subreference 01), the aquatic toxicities of 84 nontested monosubstituted benzenes were predicted using a statistically validated model. Ranked #62 among nontested monosubstituted benzenes. Ypredicted=-2.05. (Tosato,1991)

References

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Fernandez X. , Lizzani-Cuvelier L. , Loiseau A.-.M. , Perichet C. and Delbecque C. (2003) Volatile constiuents of benzoin gums: Siam and Sumatra. Part 1. Flavour and Fragrance Journal, 18(4), 328-333.

Ishikawa S. and Hirao T. (1965) Studies on olfactory sensation in the larvae of the silkworm, Bombyx mori. III. Attractants and repellents of hatched larvae. Bull. Sericul. Exp. Sta., 20(1), 21-36. [Sanshi Shikenjo]

Kitagawa S. and Li H. (1999) Effects of removal of stratum corneum, delipidization and addition of enhancers, ethanol and l-menthol, on skin permeation of benzoic acid and its 4-n-alkyl substituents in excised guinea pig dorsal skin. Chemical and Pharmaceutical Bulletin, 47(1), 44-47.

Nielsen N.M. and Bundgaard H. (1987) Prodrugs as drug delivery systems. 68. Chemical & plasma-

catalyzed hydrolysis of various esters of benzoic acid: A reference system for designing prodrug esters of carboxylic acid agents. International Journal of Pharmacology, 39, 75-85.

Ran Y. , He Y. , Yang G. , Johnson J.L.H. and Yalkowsky S.H. (2002) Estimation of aqueous solubility of organic compounds by using the general solubility equation. Chemosphere, 48(5), 487-509.

Stull D.R. (1947) Vapor pressure of pure substances organic compounds. Industrial and Engineering Chemistry, 39(4), 517-540.

Tosato M.L. , Vigano L. , Skagerberg B. and Clementi S. (1991) A new strategy for ranking chemical hazards. Framework and application. Environmental Science & Technology, 25(4), 695-702.

World Health Organization (2002) Benzyl derivatives. WHO Food Additives Series, 48, 227-271.

No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Isopropyl benzoate

Synonyms	
Benzoic acid, isopropyl ester	
Benzoic acid, 1-methylethyl ester	CAS
Isopropyl benzoate	Principal EINECS
1-Methylethyl benzoate	

939-48-0

CAS Number	RIFM ID	FEMA	EINECS	Registration
939-48-0	6176	2932	213-361-6	EINECS DSL TSCA

Fragrance Structure-Activity Group: Esters/Simple C1-C4 Alcohol Aryl Alkyl Acid Ester/Benzoic Acid Derivatives (Benzoates)

Formula $C_{10}H_{12}O_2$ **Molecular Weight** 164.2

SMILES Notation O=C(OC(C)C)c(cccc1)c1

Generic Class Aromatic Esters

Physical Data

Boiling Point	219□C	FMA
Boiling Point (calculated)	223.12 □C	EPI Suite
Henry's Law (calculated)	6.117e-005 Pa□m^3/mol	EPI Suite
Log K_{ow} (calculated)	2.74	EPI Suite
Melting Point (calculated)	-0.17 □C	EPI Suite
Specific Gravity	1.01	FMA
Vapor Pressure (calculated)	0.1 mm Hg 20C	FMA
Vapor Pressure (calculated)	0.161 mm Hg @ 25□C	EPI Suite
Water Solubility (calculated)	126 mg/L	EPI Suite

Natural Occurrence	**Isopropyl benzoate** is reported to occur in nature.

Flavor Consumption (in kg)

1995	EUROPE	0.03
1995	USA	0
1987	USA	0
1970	USA	2

Uses (in ppm)

Product	Average Usual	Average Maximum	Mean Daily Consumption (gms)	Updated
Baked Goods	0.1	0.9	137.2	21-Jul-88

PADI 0.01

Status

Isopropyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 652) , was approved by the FDA as a flavor (21 CFR 172.515) .

Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2932) Hall,1965

Indicative Non-Exhaustive List states: Listed

The Industrial Safety and Health Law (Japan) states: ISHL Number ((3)-1356)

Joint Expert Committee on Food Additives states: The Benzyl Derivatives Safety Evaluation is available here.

Joint Expert Committee on Food Additives states: The JECFA Evaluation Summary is available here

Joint Expert Committee on Food Additives states: The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent. (855)

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Human Health Data

Acute toxicity

Route: gavage. **Species:** rat. An unspecified number of rats were given the test material perorally. No further details were given. **LD50** 3750 MG/KG 3750 mg/kg calculated LD50. (Bar,1967)

Route: oral. **Species:** rat. A singe dose of the test material was administered to 5 rats per group, and the LD50 was determined after an observation period of 14 days. The animals were not fasted overnight. The LD50 was calculated per the Thompson method, but in some cases it was obtained graphically. No further details were provided. (Smyth,1949)**LD50** 3.73 G/KG 3.73 g/kg calculated LD50, +/- 1.96 standard deviations=2.43-5.73 gm/kg. (Smyth,1951)

Route: skin. **Species:** rabbit. The test material was administered to 5 rabbits per group, and the LD50 was determined after an observation period of 14 days. The LD50 was calculated per the Thompson method, but in some cases it was obtained graphically. No further details were provided. (Smyth,1949)

LD50 obtained graphically.**LD50** 20 ML/KG 20 ml/kg calculated LD50. (Smyth,1951)

Route: inhalation. **Species:** rat. Groups of an unspecified number of rats were exposed to a saturated vapor of the test material, and the maximum time at which no death occurred was determined. No further details were provided. (Smyth,1949) 4 hours no effects. (Smyth,1951)

Irritation

Route: skin. **Species:** rabbit. Primary skin irritation was determined using groups of 5 rabbits each. The scoring used was similar to the Draize scoring method, and the irritation grades ranged from 1 to 10. No further details were provided. Grade 1 = no irritation from the undiluted material, 2 = reaction equivalent to a trace capillary injection, 3 = strong capillary injection, 4 = slight erythema, 5 = strong erythema, edema or slight necrosis, 6 = higher necrosis with the diluted test material or no reaction more severe than edema with 10% test material in acetone, 7 = no reaction more severe that edema with 1% in acetone, 8 = 0.1% in acetone, 9 = 0.01% in acetone and 10 = any weaker solution in acetone. (Smyth,1949) 100 % irritant effects, score=3. (Smyth,1951)

Route: surface of eye. **Species:** rabbit. Groups consisting of 5 albino rabbits were tested per dose. The test material was applied to the center of the cornea while the lids were retracted. Approximately 1 minute later, the lids were released, and 18 - 24 hours later, the eye was examined in strong diffuse daylight, then stained with fluorescein, and the injury scored. The vehicle were propylene glycol, water or Deobase. Eye injury was graded on the following 10 point scale: 1 = 0.5 ml undiluted gives injury of 0 - 1 points; 2 = 0.5 ml undiluted gives injury over 1 point and up to 5 points; 3 = 0.5 ml undiluted gives injury over 5 points or 0.1 ml undiluted gives injury up to 5 points; 4 = 0.1 ml undiluted gives injury over 5 points or 0.02 ml undiluted gives injury up to 5 points; 5 = 0.02 ml undiluted gives injury over 5 points or 0.005 ml undiluted gives injury up to 5 points; 6 = 0.005 ml undiluted gives injury over 5 points or excess of 40% solution gives injury up to 5 points; 7 = excess of 40% gives injury over 5 points or excess of 15% solution gives injury up to 5 points; 8 = excess of 15% gives injury over 5 points or excess of 5% solution gives injury up to 5 points; 9 = excess of 5% gives injury over 5 points or excess of 1% solution gives injury up to 5 points; 10 = excess of 1% gives injury of over 5 points. The points were equivalent to the following symptoms visible before fluorescein staining: 1 point = iritis, slight internal congestion; 2 points = cornea dull; 4 points = cornea opaque, less than half of area; and 6 points = cornea opaque, more than half of area. The points were equivalent to the following symptoms visible after fluorescein staining: 1 point = necrosis on less than 5% of the cornea; 2 points = necrosis on 5 □ 12%; 3 points = necrosis on 13 □ 37%; 4 points = necrosis on 38 - 62%; 5 points = necrosis on 63 - 87%; and 6 points = 88 - 100%. The specific vehicle used with each test material was not mentioned. (Carpenter,1946) 100 % eye effects, irritant effects, score=1. (Smyth,1951)

Interaction

Route: *in vitro*. **Species:** rat. Adipocytes were prepared from the epididymal fat of two Sprague-Dawley animals. The permeation of [14C]test cpd or [3H]test cpd was measured as described by Abumrad. After incubation w/ test cpd, cells were recovered on glass fibre filters & transferred to scintillation fluid for counting of Radioactivity. 75 micromole interaction, [14C]linoleate uptake was 50 pmol/15 sec-10 ul cells in the presence of isopropyl benzoate (38% inhibition). (Abumrad,1984)

Papers that are a review on a general subject, not a specific material. TREV papers may contain one or more RIFM materials. TREV papers do not contain original data.

Bentley,1993 .

Review articles. Papers that are reviews on a material or a class of materials. A review paper may be on one or more RIFM materials. Review papers do not contain original data.

WHO,2002 . Summary of results of safety evaluation of benzyl derivatives used as flavouring agents, annual volumes, acute toxicity, short term studies of toxicity and genotoxicity was shown.

Adams,2005 . The key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances are presented.

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Bar V.F. and Griepentrog F. (1967) Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel fur Lebensmittel. (Where we stand concerning the evaluation of flavoring substances from the viewpoint of health). Medizin Ernahr., 8, 244-251.

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World Health Organization (2002) Benzyl derivatives. WHO Food Additives Series, 48, 227-271.

No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Butyl benzoate

Synonyms	
Benzoic acid, butyl ester	CAS
Benzoic acid, *n*-butyl ester	
Butyl benzoate	Principal EINECS RIFM

136-60-7

CAS Number	RIFM ID	EINECS	Registration
136-60-7	1046	205-252-7	EINECS DSL TSCA

RIFM Monograph: 1046 (Published 1983: FCT,v21,p651)

Fragrance Structure-Activity Group: Esters/Simple C1-C4 Alcohol Aryl Alkyl Acid Ester/Benzoic Acid Derivatives (Benzoates)

Formula $C_{11}H_{14}O_2$ **Structure** C_6H_5-COO-$(CH_2)_3CH_3$ **Molecular Weight** 178.23

SMILES Notation O=C(OCCCC)c(cccc1)c1

Generic Class Aromatic Esters

Description Merck Index (1976); colorless, viscous liquid with characteristic odor

Physical Data

Boiling Point	250□C	FMA
Boiling Point (calculated)	252.15 □C	EPI Suite
Flash Point	> 200□F;CC	FMA
Henry's Law (calculated)	8.121e-005 Pa□m^3/mol	EPI Suite
Log K_{ow} (calculated)	3.3	EPI Suite
Melting Point (calculated)	21.44 □C	EPI Suite
Specific Gravity	1.00	FMA
Vapor Pressure (calculated)	0.0268 mm Hg @ 25□C	EPI Suite
Vapor Pressure (calculated)	0.02 mm Hg 20C	FMA
Water Solubility (calculated)	29.52 mg/L	EPI Suite

Preparation	By direct esterification of n-butyl alcohol with benzoic acid under azeotropic conditions (Arctander,1969)
Natural Occurrence	**Butyl benzoate** is reported to occur in nature.
Use Levels	In public use since the 1960s.

Status

Butyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 740) .

Indicative Non-Exhaustive List states: Listed

The Industrial Safety and Health Law (Japan) states: ISHL Number ((3)-1356)

United Nations Transport Classification Codes states: Not regulated

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European Hazard Classification Labeling	
R38	Irritating to skin.
	Irritant

US OSHA Health Hazard Statements			
Irritation Data	IR1	Liquid may be irritating to skin and eyes.	1-Apr-89

Global Harmonized System Hazard Statements			
Hazard Category	**Signal Word**	**Code**	**Hazard Statement**
SCI 2	Warning	H315	Causes skin irritation
ATD 5	Warning	H313	May be harmful in contact with skin
ATO 5	Warning	H303	May be harmful if swallowed
EDI 2A	Warning	H319	Causes serious eye irritation

Precautionary Code	Precautionary Statement
P264	Wash .. (hands/face) thoroughly after handling
P280	Wear protective gloves/protective clothing/eye protection/face protection
P302/352	IF ON SKIN: Wash with plenty of soap and water
P305/351/358	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
P312	Call a POISON CENTER or doctor/physician if you feel unwell
P332/313	If skin irritation occurs: Get medical advice/attention
P337/313	If eye irritation persists: Get medical advice/attention
P362	Take off contaminated clothing and wash before reuse

Human Health Data

Acute toxicity

Route: skin. **Species:** rabbit. Ten (8 male and 2 female) healthy, New Zealand White rabbits with initial body weights of 2.0-2.7 kilograms received one dermal application of test material. Food and water were available freely. The test material was applied to clipped, intact or abraded abdominal skin under occluded patches for 24 hours of contact. The exposure site was wiped, but not washed, to remove excess material. Observations for mortality and/or systemic effects were made daily for 14 days. Dermal reactions were scored at 24 hours by the Draize scoring system. Body weights were recorded pretrest and in the survivors at 14 days. Gross necropsy was conducted on all animals. 5 g/kg nonspecific effects, 0 deaths. Acute dermal LD50>5 gm/kg. Diarrhea. All normal at necropsy. (RIFM,1980) [Moreno,1980]

Route: oral. **Species:** rat. Groups of 5 non-fasted, Carworth-Wistar male rats weighing 90 - 120 grams, were administered a single dose of the test material orally. When necessary, the test material was tested as a solution in water, corn oil, or as a 1% solution of sodium 3,9-diethyl-6-tridecanol sulfate (Tergitol Penetrant 7), to bring the volume given to each rat to 1 □ 10 ml. The animals were observed for mortality during a 14-day period and the LD50 was estimated by the Thompson and Weil method. No further details were provided. Female rats were used.**LD50** 5.14 G/KG 5.14 g/kg calculated LD50, C.I. 4.70-5.62. (Smyth,1954)

Route: gavage. **Species:** rat. An unspecified number of rats were given the test material perorally. No further details were given. **LD50** 5140 MG/KG 5140 mg/kg calculated LD50. (Bar,1967)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. On the next Day, serial dilutions of test cpd were added to the cells, beginning in ea. case w/ a conc. close to saturation level. 100 mg/l positive effects, 100% inhibition of growth noted. (Data taken from graph). (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells, an epithelial cell line derived from human carcinoma of the larynx, were routinely cultured in monolayers @ 37 C. Test cpds were added to complete medium, which after vigorous shaking was left overnite @ 24 C. Hep-2 cells were plated in 25 cm2 flasks @ 10(5) cells/flask. After 24 hr The IC50 conc. of test cpd was added to ea. flask. Flasks were kept @ 37 C w/ the screw plugs well closed for 7 days. Two flasks from ea. test grp were removed daily for protein determination. 61 mg/l positive effects, on day 7, cells seemed to recover from exposure to test cpd. (DeAngelis,1986)

Route: *in vitro*. **Species:** human 18+ yrs. Hep-2 cells were plated in 25 cm2 flasks @ a density of 200 cells/flask in the presence of test cpd @ the IC50 conc. Cultures were incubated @ 37 C for 8 days & then the cells were stained w/ May-Grunwald & Giemsa and colonies of @ least 10 cells were counted. 61 mg/l positive effects, monolayers of treated cells were disturbed w/in 24 hrs. (DeAngelis,1986)

Route: intramuscular. **Species:** guinea pig. Liquid esters of organic acids were examined for use as solvents in parenteral administrations. Sterilized esters (0.5 or 1.0 ml) were injected into the gluteal muscles & the animals observed continuously for several hours & then daily for 1 wk for systemic toxicity, local irritation & action upon, neuromuscular functions of the injected leg. Strain & no. tested/ester not specified. 3 ml/kg musculo-skeletal, slight deterioration of leg function & caused muscle toughness. (Lipschitz,1942)

Route: inhalation. **Species:** rat. Male or female albino rats were exposed to a flowing stream of air approaching saturation with vapors, which was prepared by passing dried air through a fritted disc gas washing bottle. Groups of 6 rats were exposed for periods of time in a logarithmic series with a ratio of 2, for up to 8 hours, until the period of time that killed 50% of the animals within 14 days of inhalation was determined. No further details were provided. The results reflect the longest inhalation period that allowed all the rats to survive the 14-day observation period. 8 hours no effects. (Smyth,1954)

Irritation

Route: skin. **Species:** rabbit. Primary skin irritation was determined using groups of 5 albino rabbits. A 0.01-ml aliquot of the test material was applied to the clipped skin of the animals undiluted or as a solution in water, propylene glycol or kerosene. Scoring of the reactions was conducted within 24 hours of the application, using irritation grades that ranged from 1 to 10 (grade 1 = least visible capillary injection from the undiluted material; 6 = necrosis with the undiluted material; and 10 = necrosis from a 0.01% solution). No further details were provided. The specific vehicle used with each test material was not mentioned. 100 % irritant effects, grade = 5. (Smyth,1954)

Route: surface of eye. **Species:** rabbit. Eye injury was evaluated using groups of 5 rabbits per dose. The test material was applied to the center of the cornea while the lids were retracted. Approximately 1 minute later, the lids were released, and 18 - 24 hours later, the eye was examined in strong diffuse daylight, then stained with fluorescein, and the injury scored. The test material was tested undiluted or as a solution in propylene glycol and water. Eye injury was graded according to a 10-grade series: 1 = 0.5 ml undiluted gives injury of 0 to 1.0 points; 2 = 0.5 ml undiluted gives injury of over 1.0 up to 5.0 points; 3 = 0.1 ml undiluted gives injury of up to 5.0 points (0.5 ml gives over 5.0); 4 = 0.02 ml undiluted gives injury of up to 5.0 points (0.1 ml gives over 5.0); 5 = 0.005 ml undiluted gives injury of up to 5.0 points (0.02 ml gives over 5.0); 6 = Excess of 40% solution gives injury of up to 5.0 points (0.005 ml gives over 5.0); 7 = Excess of 15% solution gives injury of up to 5.0 points (40% gives over 5.0); 8 = Excess of 5% solution gives injury of up to 5.0 points (15% gives over 5.0); 9 = Excess of 1% solution gives injury of up to 5.0 points (5% gives over 5.0); 10 = Excess of 1% solution gives injury of over 5.0 points. The points were equivalent to the following symptoms visible before fluorescein staining: 1 point = iritis, slight internal congestion; 2 points = cornea dull; 4 points = cornea opaque, less than half of area; and 6 points = cornea opaque, more than half of area. The points were equivalent to the following symptoms visible after fluorescein staining: 1 point = necrosis on less than 5% of the cornea; 2 points = necrosis on 5 □ 12%; 3 points = necrosis on 13 □ 37%; 4 points = necrosis on 38 - 62%; 5 points = necrosis on 63 - 87%; and 6 points = 88 - 100%. No further details were provided. (Carpenter,1946) 100 % eye effects, grade = 1. (Smyth,1954)

Route: skin. **Species:** rabbit. As part of an associated acute dermal LD50 study, ten (8 male and 2 female) healthy, New Zealand White rabbits with initial body weights of 2.0-2.7 kilograms received one dermal application of test material. Food and water was available freely. The test material was applied to clipped, intact or abraded abdominal skin under occluded patches for 24 hours of contact. The exposure site was wiped, but not washed to remove excess material. Irritation was evaluated on day 1 (24 hours) according to the Draize scoring system. 5 g/kg irritant effects, absent to slight. Scaly treated skin at necropsy. (RIFM,1980) [Moreno,1980]

Route: skin. **Species:** human 18+ yrs. In a pretest for a human maximization study, a 48 hour closed patch test on the forearms of 25 healthy, male and female volunteers was conducted. Vehicle was petrolatum. 80-6-3. 6 % no effects. (RIFM,1980) [Kligman,1980]

Route: skin. **Species:** rabbit. Non-occluded irritation tests using 10-14 New Zealand males/material according to the Draize method (1944). About 0.5ml undiluted material applied daily to one side of the clipped dorsal area for up to 6 days & 0.2ml applied to the external surface of the rt. outer ear. Test areas examined at 4 & 24 hr after each treatment. 2/grp killed daily & skin of back & ear examined microscopically. Micro exam of organs from n-PB & n-BB rabbits. 99% pure. Irritant effects, skin effects, dorsum & ear: immediate erythematous reaction that increased in severity. Different patterns of micro lesions. No organ lesions. (Branca,1988)

Route: skin. **Species:** rabbit. Healthy female New Zealand white rabbits, 10 □ 12 weeks old and weighing approximately 2 kilograms, were obtained and allowed to acclimatize for at least 5 days. The animals were individually housed in metal cages, and allowed food and water ad libitum. The animals were maintained at 5 □ 24oC, relative humidity of 37 □ 68% and a 12-hour light/dark cycle. The day before the treatment period, the animals were restrained and the hair on the dorsal surface of the trunk was clipped. Groups of 4 animals with healthy, intact skin were selected for the treatment period, and

the next day, the animals were restrained and treated with the test material. A 0.5-ml aliquot of the test material was placed over a 2.5-cm square surgical lint, which was then placed on the left flank skin. The patches were secured by wrapping the trunk with Elastoplast elastic adhesive bandage. Four different test materials were simultaneously tested at different sites on the backs of the animals. After the 4-hour treatment period, the adhesive tape was removed and the treated sites were cleaned with warm water. Skin reactions such as erythema and edema were assessed at 1, 24, 48 and 72 hours and 7 days after the patch removal, and scored according to a numerical scale. The average scores were calculated from the numerical values assigned to the irritation observed at the 24-, 48- and 72-hour observations, and when the mean value obtained for either erythema or edema was equal or greater than 2, the material was considered to be an irritant. **Regulatory Compliance** pages 106-108 of EEC document L251. **GLP** Yes 1046-88. 100 % no effects, Classified as a non-irritant based on the average scores (erythema = 1.9, edema = 0.8). At 24 hours, erythema (well-defined in 4/4) and edema (absent to very slight in □ and very slight to slight in 1/4) were observed. By 72 hours, erythema (very slight in □, very slight to well-defined in □ and well-defined in 2/4) and edema (absent to very slight in □ and very slight in 2/4) were observed. (RIFM,1988) [Haynes,1988]

Route: skin. **Species:** human 18+ yrs. This study presents data from human 4-hour patch tests for skin irritation that can be used to determine appropriate classification for labeling according to EC rules. The results are also intended to provide gold standard data for the development and acceptance of in vitro alternatives to the rabbit skin test. A panel of human volunteers were used to apply 0.2 ml (0.2 g for solid test materials) on a 25 mm plain Hill Top Chamber containing a Webril pad, moistened for solid test materials, to the skin of the upper outer arm. To avoid unacceptable strong reactions, test materials were applied progressively from 15 and 30 minutes through 1, 2, 3 and 4 hours. Each progressive application was placed at a new skin site. The presence of irritation was assessed at 24, 48 and 72 hours after patch removal as follows: 0 = no reaction; + = weakly positive reaction (usually characterized by mild erythema or dryness across most of the treatment site); ++ = moderately positive reaction (usually distinct erythema possibly spreading beyond the treatment site); +++ = strongly positive reaction (strong, often spreading erythema with edema). A response of + or greater at times less than 4 hours was sufficient and further progressive applications were not required. Twenty percent sodium dodecyl sulfate (SDS), a known irritant, was used as a positive control in the same panelists. The results with test materials were interpreted for EC purposes as either NC (not classified) or R38 (irritant). When the incidence of positive reactions to the test material was significantly greater than or not significantly different (based on Fisher□s exact test) than the level of reaction in the same panel of volunteers to 20% SDS, the test material was classified as an irritant (R38). When the level of reaction was substantially and significantly lower than the response to SDS, the test material was not considered to be an irritant (NC). 100 % no effects, Test material was labelled as NC based on 0 positive reactions with 30 panelists treated with the human 4-hour patch test. (Basketter,2004)

Sensitization

Route: skin. **Species:** human 18+ yrs. Vehicle was petrolatum. 25 subjects completed the study. (KLIGMAN,1975A) 80-6-3. 6 % no effects. (RIFM,1980) [Kligman,1980]

Pharmacokinetics and metabolism studies

Route: *in vitro*. **Species:** human 18+ yrs. The rate of hydrolysis of the test material by 80% human plasma was studied. Vehicle was acetonitrile. The t1/2 for the in vitro hydrolysis of butyl benzoate to benzoic acid by human plasma was 40 minutes. (Nielsen,1987)

Interaction

Route: *in vitro*. **Species:** guinea pig. In vitro skin penetration of drugs was examined. The skin preparations were mounted in a two-chamber diffusion cell with water jackets (37 C). The available diffusion area was about 0.65 cm2, and each half-cell volume was about 5.4 ml. The donor cells were filled with saline either in the presence or absence of the enhancer unless otherwise mentioned, and the receiver cells with PBS (pH 7.4). Cells were pretreated for 12 h with stirring at 450 rpm by a magnetic

stirrer. After washing of both compartments, the suspension of excess amount of benzoic acid or its 4-alkyl substituents in saline either in the presence or absence of the penetration enhancer was added to the donor compartments unless otherwise mentioned, and the penetration experiment was begun. One hundred fifty ul of sample was taken from the receiver cells periodically over a maximum period of 29 h, diluted with PBS twenty times or more and analyzed by UV absorbance at 224 nm for benzoic acid, at 235 nm for 4-methyl and 4-ethyl substituents and at 236 nm for 4-n-propyl and 4-n-butyl substituents. Solubilized components from skin and penetrated enhancers did not interfere with the UV absorbance. Dependency on pH in the donor compartment of the permeability coefficients of the derivatives was examined with suspension of their excess amount (pH 3.0, 4.0) or a 50 mM solution of the derivative (pH 5.0-7.0), using either phosphate buffer (pH 3.0, 60, 7.0) or citrate buffer (pH 4.0, 5.0). Permeability coefficients of benzoic acid and the derivatives decreased with the increase of pH. (Kitagawa,1999)

Route: *in vitro*. **Species:** guinea pig. The permeability coefficient was calculated and the solubility of benzoic acid and its 4-alkyl substituents was measured after their incubation in an excess amount in saline either in the presence or absence of the enhancer at 37 C for 24 h. After quick centrifugation at 1000xg for 2 min at 37 C, concentration of the supernatant was obtained by measuring UV absorbance. The solubility (Cd) and permeability (Kp) coefficients were 0.37 mM and 79.9 x 10(-2)cm/h, resp. (Kitagawa,1999)

Cytotoxicity

Route: *in vitro*. **Species:** human 18+ yrs. Growth inhibition of mammalian cells by test materials was investigated. Strain Rhino HeLa cells seeded at 2.5x10(5) cells and grown in either 60 mm petri dishes or in 75 cm2 T-flasks, were incubated for 20 hours in Eagle minimal essential medium containing 10% fetal calf serum and antibiotics. The medium was replaced with fresh medium containing test material and incubation was continued for 48 hours. The number of viable cells was counted when test material was added to the culture and again 48 hours later. Growth, ATP synthesis, amino acid uptake and oxygen consumption were measured. The positive concentration in the results is that which gives 50% inhibition. Vehicle was dimethylsulfoxide. 0.5 millimolar positive effects. (Sheu,1975)

Miscellaneous

Route: *in vitro*. Antimicrobial activity was evaluated using a standardized petri plate procedure. Organisms used were Staphylococcus aureus (SA), Escherichia coli (EC), Candida albicans and, if active in the others, a Diphtheroid (D). All materials were tested at 10% (w/v) in 95% (v/v) ethanol unless solubility problems occurred. Not all materials were tested in all 4 organisms. Controls were 3,4,4'-trichlorocarbanilide and 2,4,4'-trichloro-2'-hydroxydiphenyl ether and hexachlorophene. No effects. (Morris,1979)

Route: *in vitro*. **Species:** *Bacillus subtilis*. A prototroph-sporulating strain derived from strain 168. Growth, ATP synthesis, amino acid uptake and oxygen consumption were measured Positive concentration in results is that which gives 50% inhibition. Vehicle was dimethylsulfoxide. 0.6 millimolar positive effects. (Sheu,1975)

Route: *in vitro*. **Species:** *Escherichia coli*. Strain dec-16 oldD88 was used. Growth, ATP synthesis, amino acid uptake and oxygen consumption were measured Positive concentration in results is that which produces 50% inhibition. Vehicle was dimethylsulfoxide. >10 MM needed for 50% growth inhibition. (Sheu,1975)

Analytical methodology. Can also refer to physical properties such as boiling temperature, flash point, etc.

Indrayan,2007 . The constituents of Alpinia officinarum were determined.

Biological tests other than classical toxicology testing and pharmacological tests.

Ishikawa,1965 . No English translation available.

Statistical methods and calculations.

Ran,2002 . A proposed general solubility equation (GSE) was used to estimate the aqueous solubility of 1026 non-electrolytes.

Structure activity relationships such as studies of a group of aldehydes or other similar structures.

Serra,2001 . Three quantitative structure toxicity relationships were developed, to predict 50% population growth impairment concentrations for industrially important aromatic solvents.

Environmental studies, tests or effects. Definition: fate and effect studies that include biodegradation, bioaccumulation, biotransformation, bioconcentration, soil adsorption, soil migration, sediment and water studies, etc.

Meylan,1992 .

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No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Isobutyl benzoate

Synonyms		
Benzoic acid, isobutyl ester		
Benzoic acid, 2-methylpropyl ester	CAS	120-50-3
Isobutyl benzoate	Principal EINECS RIFM	
2-Methylpropyl benzoate		

CAS Number	RIFM ID	FEMA	EINECS	Registration
120-50-3	405	2185	204-401-3	EINECS DSL TSCA

RIFM Monograph: 405 (Published 1975: FCT,v13,p553 (Binder, p452))

Fragrance Structure-Activity Group: Esters/Simple C1-C4 Alcohol Aryl Alkyl Acid Ester/Benzoic Acid Derivatives (Benzoates)

Formula $C_{11}H_{14}O_2$ **Structure** C_6H_5-COO-CH_2-CH(CH_3)-CH_3 **Molecular Weight** 178.23

SMILES Notation O=C(OCC(C)C)c(cccc1)c1

Generic Class Aromatic Esters

Description Givaudan Index (1961)

Physical Data

Boiling Point	237□C	FMA
Boiling Point (calculated)	241.5 □C	EPI Suite
Flash Point	>200□F;CC	FMA
Henry's Law (calculated)	8.121e-005 Pa□m^3/mol	EPI Suite
Log K_{ow} (calculated)	3.23	EPI Suite
Melting Point (calculated)	10.84 □C	EPI Suite
Specific Gravity	0.996	FMA
Vapor Pressure (calculated)	0.03 mm Hg 20C	FMA
Vapor Pressure (calculated)	0.0417 mm Hg @ 25□C	EPI Suite
Water Solubility (calculated)	98.34 mg/L	EPI Suite

Preparation	By direct esterification of isobutyl alcohol with benzoic acid, under azeotropic conditions (Arctander,1969)
Natural Occurrence	**Isobutyl benzoate** is reported to occur in nature.

Use Levels	In public use since the 1920s.

Flavor Consumption (in kg)

1995	EUROPE	2.6
1995	USA	5.9
1987	USA	2
1982	USA	12
1975	USA	23
1970	USA	46

Uses (in ppm)

Product	Average Usual	Average Maximum	Mean Daily Consumption (gms)	Updated
Baked Goods	9.18	23.48	137.2	21-Jul-88
Frozen Dairy	6.17	8.12	25.6	21-Jul-88
Gelatin Pudding	12	21	20.4	21-Jul-88
Non-alcoholic Beverage	3.07	8.54	104.0	21-Jul-88
Soft Candy	11.23	15.8	5.8	21-Jul-88

PADI 2.042.042.042.042.04

Status

Isobutyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 567) , was approved by the FDA as a flavor (21 CFR 172.515) .

Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 3. (2185) Hall,1965

Indicative Non-Exhaustive List states: Listed

The Industrial Safety and Health Law (Japan) states: ISHL Number ((3)-1356)

Joint Expert Committee on Food Additives states: The Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the substance does not present a safety concern at current levels of intake when used as a flavouring agent. (856)

Joint Expert Committee on Food Additives states: The JECFA Evaluation Summary is available here.

Joint Expert Committee on Food Additives states: The Benzyl Derivatives Safety Evaluation is available here.

United Nations Transport Classification Codes states: Not regulated

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European Hazard Classification Labeling	
NC	Based on available data, classification and labeling was not considered necessary as per the EFFA Code of Practice.

US OSHA Health Hazard Statements			
NONE	NONE	None Assigned	1-Jan-00

Global Harmonized System Hazard Statements			
Hazard Category	**Signal Word**	**Code**	**Hazard Statement**
ATO 5	Warning	H303	May be harmful if swallowed

Precautionary Code	Precautionary Statement
P312	Call a POISON CENTER or doctor/physician if you feel unwell

Human Health Data

Acute toxicity

Route: gavage. **Species:** rat. A total of 110 CFE Carworth strain animals (5 male and 5 female per dose), weighing 200-300 grams, were used. Animals were fasted 18 hours prior to dosing. Animals were housed in raised wire mesh cages in an air conditioned room. Animals received food and water ad libitum. The test material was fed as received and administered via a stomach tube. Animals were observed for 14 days for signs of toxicity. Animals were examined daily, mortality recorded and if autolysis had not occurred an autopsy was performed. LD50 calculated per Litchfield-Wilcoxon, 1949. **Subjects:** 55 Male 55 Female EOA# 72-167.**LD50** 3.7 ML/KG 3.7 ml/kg calculated LD50, acute oral LD50 = 3.7 ml/kg with a 19/20 confidence limit=3.19-4.29 ml/kg. 3 ml/kg lethal, 2/10 deaths. 3.5 ml/kg lethal, 4/10 deaths. 5 ml/kg lethal, 7/10 deaths. 4 ml/kg lethal, 7/10 deaths. (RIFM,1973) [Levenstein,1973]

Route: skin. **Species:** rabbit. A dermal toxicity study was conducted on albino rabbits weighing 2 □ 3.5 kg. The animals were acclimated to the laboratory conditions for at least 2 weeks prior to the test. The undiluted test material at a dose of 5 ml/kg was applied for 24 hours to the clipped, intact and abraded skin on the backs of 4 rabbits per dose. The test sites were covered with a sleeve, and in some cases, more than 1 dose was tested. Following the exposure period, the skin reactions were recorded and the animals were observed for mortality and/or systemic effects for a 14-day period. 5 ml/kg no effects. (RIFM,1973) [Levenstein,1973a]

Irritation

Route: skin. **Species:** rabbit. Irritation was assessed during an acute dermal toxicity study. Groups of 4 albino rabbits per dose were administered a single dermal application of the undiluted test material under occlusion for 24 hours. Applications were made to the clipped intact and abraded backs. 5 ml/kg no effects. (RIFM,1973) [Levenstein,1973a]

Route: skin. **Species:** man. In a pre-test for a human maximization study, a 48-hour closed patch test

with the test material in petrolatum was conducted on the backs of 5 healthy male volunteers. 72-2-167. 2 % no effects. (RIFM,1973) [Kligman,1973]

Sensitization

Route: skin. **Species:** guinea pig. An open epicutaneous test was conducted in guinea pigs. Induction consisted of 21 daily open applications to the shaved flank of 6-8 guinea pigs per group. One to six experimental and one control group was used. Open challenge applications were made on days 21 and 35. Reactions were read at 24, 48 and 72 hours. No further details were provided. Vehicles were not specified by material. (Klecak,1977) 2 % no effects. (Klecak,1979)

Route: skin. **Species:** guinea pig. A guinea pig open epicutaneous test (OET) was conducted on groups of 6 - 8 male and female guinea pigs weighting 300 - 450 grams. Daily applications were made for 3 weeks to a clipped 8-cm2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 hours after each application. A total of 21 applications of 0.1 ml test material in an unspecified vehicle were made for 21 days. The 10 controls were either left untreated or treated with 0.1 ml of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with the test material at the minimal irritating concentration and some lower primary non-irritating concentrations. The results were not reported for the irritation pre-screen. It was not specified if the concentration reported in the results was the minimal irritating concentration or 1 of the lower primary nonirritating concentration. 2 % no effects, No sensitization (-) was produced. (Klecak,1985)

Route: skin. **Species:** man. A human maximization test was conducted on 25 healthy male volunteers with the test material in petrolatum. Applications were made under occlusion to the same site on the volar forearms for five alternate-day 48-hour periods. The test sites were pretreated for 24 hours with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. Following a 10-day rest period, the challenge patches were applied to fresh sites for 48 hours under occlusion. Before the challenge patches were applied, the sites were pretreated with 10% SLS for 1 hour under occlusion. The challenge sites were read at patch removal and 24 hours after patch removal. 72-2-167. 2 % no effects. (RIFM,1973) [Kligman,1973]

Subchronic toxicity

Route: food. **Species:** rat. A 12 wk study was conducted using 12 rats per sex per dose. The test material was a mixture of flavor materials. Sacrifice was at the end of feeding. The observations were survival, body weight, behavior, appearance, food intake, EFU, urinalysis, blood hemoglobin, liver & kidney weights and autopsy The test mixture contained ethyl benzoate, isobutyl benzoate, benzyl acetate, benzyl butyrate, ethyl methyl phenyl glycidate and glycidate M-116. 26.2 mg/kg no effects. (Trubek,1957)

Miscellaneous

Route: *in vitro*. **Species:** fungi. Vapors of the test material were tested for antifungal properties on Candida albicans ATCC 10231, Phoma betae ATCC 6504, Geotrichum candidum Coll. No. 4762 and Oospora lactis ATCC 4798. 0.5 ml of the test organism was streaked onto the agar surface. 0.5 ml of the test material was placed in a cup in the center of a petri dish top. Dishes with culture were inverted over the top and incubated. Vapors of the test material were allowed to emanate throughout the five day incubation period at 22 degrees. After incubation, the presence of a definite clear zone of inhibition on the surface of the agar indicated that the vapor possessed antifungal activity and the larger the zone the greater the activity in this test system. All test materials were tested in triplicate with one cup per dish. Positive effects, in all four fungi. (Maruzzella,1961)

Route: *in vitro*. **Species:** bacteria. The effect of test chemicals on growing cultures of four bacterial strains [Bacillus subtilis, ATCC 9524, Escherichia coli ATCC 11229, Staphylococcus aureus Ox-H (penicillin sensitive) and Staphylococcus aureus ATCC 10390 (penicillin resistant)] was studied. Dilutions of each chemical were prepared in sterile nutrient broth at 1:500. Tubes were inoculated with 0.5 ml of a 24 hour broth culture of test organism. Tubes were incubated at 37 C for 24 hours and the

presence or absence of visible growth observed. Failure of growth to occur in the subculture was taken as evidence that the organisms had been killed in the original chemical-broth tubes. When this occurred, additional concentrations were prepared (1:1000, 1:2000, 1:10,000) and tested in a similar manner. Positive effects, a 2+ reaction (bacterial growth at 1:1000 dilution, but not at 1:500 dilution) in B. subtilis. (Maruzzella,1961a)

Route: *in vitro*. **Species:** fungi. Antifungal activity was evaluated using the filter paper disk method. The organisms used were Lenzites trabea ATCC 8715, Polyporus versicolor ATCC 11235 and Lentinus lepideus ATCC 11453. Sterile disks (6.35 mm diameter) were thoroughly saturated with test material. The saturated disks were then placed on the surface of Sabauraud's maltose agar plates which had been previously seeded with 1 ml of fungal broth culture. All dishes were conducted in triplicate with one disk per dish and incubated for 12 to 14 days at room temperature. The presence of zones of inhibition surround the saturated paper disks indicated anti-fungal activity and the zones were recorded in millimeters with a metric ruler with the aid of an illuminated Quebec colony counter. (Vincent and Vincent, 1944) Positive effects. (Maruzzella,1960)

Route: *in vitro*. **Species:** bacteria. The vapors of 192 aromatic chemicals were tested in vitro against growing cultures of Bacillus subtilis (var. aterrimus ATCC 6461), Serratia marcescens (ATCC 9986), Staphylococcus aureus (OX-H), Escherichia coli (ATCC 11229) and Mycobacterium avium (ATCC 4676). All of the test organisms were cultivated on nutrient agar and broth at 37 degrees C, except M. avium which was grown in nutrient agar and broth containing 5% glycerol. Using the method of Maruzzella, J.C. (see methology reference in comments section) 15 mls of nutrient agar were poured into Petri dishes and allowed to harden; the surface of the agar was streaked with 0.5 ml of a 48 hour broth culture of the test organisms (M. avium broth culture was 72 hrs old); aluminum caps with 0.5 ml of aromatic chemical were placed on the inner surface of the petri dish top; dishes were inverted and incubated 48 hours(M. avium - 72 hours). After incubation, a definate zone of inhibition on the surface of the agar indicated that the vapor possessed antibacterial activity. The larger the zone, the greater the activity. The diameters of the zones of inhibition were measured by a metric ruler with the aid of an illuminated Quebec Colony Counter. All dishes were conducted in triplicate. In some tests the vapor permitted no growth to occur on the surface of the entire dish. In this case, the zones of inhibition were recorded as 90 mm. which is the inside diameter of the Petri dish. The method used to test the effects of the vapors on bacteria was by Maruzzella, J.C., in Soap, Perfumery and Cosmetics, vol. 33, pg. 835, 1960. Positive effects, Vapors exhibited activity against B. subtilis and M. avium. (Maruzzella,1961b)

Analytical methodology. Can also refer to physical properties such as boiling temperature, flash point, etc.

Stull,1947 . Vapor pressures were collected for over 1200 organic compounds.

Thomas,1981 .

Fernandez,2003 . The chemical composition of the volatile extracts obtained from Siam and Sumatra benzoin gums by direct GC injection.

Biological tests other than classical toxicology testing and pharmacological tests.

Ishikawa,1965 . No English translation available.

Review articles. Papers that are reviews on a material or a class of materials. A review paper may be on one or more RIFM materials. Review papers do not contain original data.

WHO,2002 . Summary of results of safety evaluation of benzyl derivatives used as flavouring agents, annual volumes, acute toxicity, short term studies of toxicity and genotoxicity was shown.

Adams,2005 . The key data relevant to the safety evaluation of benzyl alcohol, benzaldehyde, or benzoic acid and 34 structurally related substances are presented.

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No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

Pentyl benzoate

Synonyms	
Amyl benzoate	
Benzoic acid, pentyl ester	CAS
Pentyl benzoate	Principal EINECS

2049-96-9

CAS Number	RIFM ID	EINECS	Registration
2049-96-9	6184	218-077-6	EINECS DSL TSCA

Fragrance Structure-Activity Group: Esters/Straight Chain Alcohol Aryl Alkyl Acid Ester/Saturated/Primary Alcohol Benzoic Acid Derivatives (Benzoates)

Formula $C_{12}H_{16}O_2$ **Structure** C_6H_5-$COO(CH_2)_4CH_3$ **Molecular Weight** 192.26

SMILES Notation O=C(OCCCCC)c(cccc1)c1

Generic Class Aromatic Esters

Physical Data

Boiling Point (calculated)	269.08 □C	EPI Suite
Henry's Law (calculated)	0.0001078 Pa□m^3/mol	EPI Suite
Log K_{ow} (calculated)	3.79	EPI Suite
Melting Point (calculated)	32.02 □C	EPI Suite
Vapor Pressure (calculated)	0.00859 mm Hg @ 25□C	EPI Suite
Vapor Pressure (calculated)	0.02 mm Hg 20C	FMA
Water Solubility (calculated)	27.63 mg/L	EPI Suite

Natural Occurrence	**Pentyl benzoate** is reported to occur in nature.

Status

Pentyl benzoate was included by the Council of Europe in the list of substances granted B - information required - hydrolysis study (COE No. 2307) .

Indicative Non-Exhaustive List states: Listed

The Industrial Safety and Health Law (Japan) states: ISHL Number ((3)-1356)

United Nations Transport Classification Codes states: Not regulated

European Hazard Classification Labeling	
NC	Based on available data, classification and labeling was not considered necessary as per the EFFA Code of Practice.

US OSHA Health Hazard Statements			
NONE	NONE	None Assigned	1-Jan-00

Global Harmonized System Hazard Statements			
Hazard Category	**Signal Word**	**Code**	**Hazard Statement**
NC		H000	Not Classified

Human Health Data

Sensitization

Route: skin. **Species:** guinea pig. A guinea pig open epicutaneous test (OET) was conducted on groups of 6 - 8 male and female guinea pigs weighting 300 - 450 grams. Daily applications were made for 3 weeks to a clipped 8-cm2 area on the flank of each guinea pig. The test sites were not covered and the reactions were read 24 hours after each application. A total of 21 applications of 0.1 ml test material in an unspecified vehicle were made for 21 days. The 10 controls were either left untreated or treated with 0.1 ml of the vehicle for 21 days. At the challenge phase, both the test and control animals were treated on days 21 and 35 on the contralateral flank with the test material at the minimal irritating concentration and some lower primary non-irritating concentrations. The results were not reported for the irritation pre-screen. It was not specified if the concentration reported in the results was the minimal irritating concentration or 1 of the lower primary nonirritating concentration. 6 % no effects, No sensitization (-) was produced. (Klecak,1985)

Pharmacokinetics and metabolism studies

Route: *in vitro*. **Species:** human 18+ yrs. The rate of hydrolysis of the test material by 80% human plasma was studied. Vehicle was acetonitrile. The t1/2 for the in vitro hydrolysis of pentyl benzoate to benzoic acid by human plasma was 24 minutes. (Nielsen,1987)

Miscellaneous

Route: *in vitro*. **Species:** bacteria. The effect of test chemicals on growing cultures of four bacterial strains [Bacillus subtilis, ATCC 9524, Escherichia coli ATCC 11229, Staphylococcus aureus Ox-H (penicillin sensitive) and Staphylococcus aureus ATCC 10390 (penicillin resistant)] was studied. Dilutions of each chemical were prepared in sterile nutrient broth at 1:500. Tubes were inoculated with 0.5 ml of a 24 hour broth culture of test organism. Tubes were incubated at 37 C for 24 hours and the presence or absence of visible growth observed. Failure of growth to occur in the subculture was taken as evidence that the organisms had been killed in the original chemical-broth tubes. When this occurred, additional concentrations were prepared (1:1000, 1:2000, 1:10,000) and tested in a similar manner. No effects, bacterial growth was noted at 1:500 dilution in all strains tested. (Maruzzella,1961)

Analytical methodology. Can also refer to physical properties such as boiling temperature, flash point, etc.

Appell,1970 .

Article in foreign language; no English translation available.

Mashimo,1953 . No English abstract or translation available. Article cited in published monograph.

Environmental studies, tests or effects. Definition: fate and effect studies that include biodegradation, bioaccumulation, biotransformation, bioconcentration, soil adsorption, soil migration, sediment and water studies, etc.

Thruston,1978 .

References

Appell L. (1970) Physical foundations in perfumery. X. Equivalent weights of aromatic products. American Perfumer Cosm., 85, 49-54.

Klecak G. (1985) The Freund's Complete Adjuvant Test and the Open Epicutaneous Test. In: Current Problems in Dermatology, Vol. 14, 152-171.

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Nielsen N.M. and Bundgaard H. (1987) Prodrugs as drug delivery systems. 68. Chemical & plasma-catalyzed hydrolysis of various esters of benzoic acid: A reference system for designing prodrug esters of carboxylic acid agents. International Journal of Pharmacology, 39, 75-85.

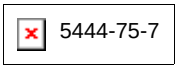
Thruston A.D. (1978) High pressure liquid chromatography techniques for the isolation and identification of organics in drinking water extracts. Journal of Chromatographic Science, 16, 254-259.

No data added since 22-Sep-10.

DATA IN THIS SYNOPSIS HAVE NOT BEEN PEER REVIEWED BY A SCIENTIFIC PANEL

2-Ethylhexyl benzoate

Synonyms	
Benzoic acid, 2-ethylhexyl ester	
2-Ethyl-1-hexanol benzoate	
Ethylhexyl benzoate	
2-Ethylhexyl benzoate	Principal
1-Hexanol, 2-ethyl-, benzoate	



CAS Number	FEMA	Registration
5444-75-7	4630	No Registrations

Formula $C_{15}H_{22}O_2$ **Molecular Weight** 234.33

SMILES Notation O=C(OCC(CCCC)CC)c(cccc1)c1

Generic Class Aromatic Esters

Physical Data

Boiling Point (calculated)	305.98 □C	EPI Suite
Henry's Law (calculated)	0.0002522 Pa□m^3/mol	EPI Suite
Log K_{ow} (calculated)	5.19	EPI Suite
Melting Point (calculated)	52.2 □C	EPI Suite
Vapor Pressure (calculated)	0.000797 mm Hg @ 25□C	EPI Suite
Water Solubility (calculated)	1.061 mg/L	EPI Suite

Uses (in ppm)

Product	Average Usual	Average Maximum	Mean Daily Consumption (gms)	Updated
Frozen Dairy	0.01	0.2	25.6	6-May-09
Gelatin Pudding	0.02	0.5	20.4	6-May-09
Non-alcoholic Beverage	0.01	0.1	104.0	6-May-09

PADI 000

Status

Flavor and Extract Manufacturers' Association states: Generally Recognized as Safe as a flavor ingredient - GRAS 24 (4630) Smith,2009

Human Health Data

None on file.

Studies on flavor and flavor components other than processed flavors (PF).

Smith,2009 . This article includes the results of the FEMA Expert Panel's review of 236 new GRAS 24 flavoring substances.

References

Smith R.L. , Waddell W.J. , Cohen S.M. , Feron V.J. , Marnett L.J. , Portoghese P.S. , Rietjens I.M.C.M. , Adams T.B. , Lucas Gavin C. , McGowen M.M. , Taylor S.V. and Williams M.C. (2009) GRAS 24: The 24th publication by the FEMA Expert Panel presents safety and usage data on 236 new generally recognized as safe flavoring ingredients. Food Technology, 63(6), 46-48, 51,52,55,56,58,60,62,64-66,68,70,72,74,76,78,80,82,84,86,88,90,92,94,96,98-105.

No data added since 22-Sep-10.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 6, 2010

SUBJECT: Studies on a Sunscreen Containing 3.5% Ethylhexyl Benzoate

Harrison Research Laboratories, Inc. 2010. Ocular sting and/or lacrimation test of a sunscreen containing 3.5% Ethylhexyl Benzoate. Study #0110291.

Clinical Research Laboratories, Inc. 2010. Evaluation of a topically applied test material for phototoxic potential (sunscreen containing 3.5% Ethylhexyl Benzoate). CRL Study No: CRL19110.

Clinical Research Laboratories, Inc. 2010. Repeated insult patch test (sunscreen containing 3.5% Ethylhexyl Benzoate). CRL Study No: CRL17610.

Clinical Research Laboratories, Inc. 2010. 14-day cumulative irritation test (sunscreen containing 3.5% Ethylhexyl Benzoate). CRL Study No: CRL20310.

Clinical Research Laboratories, Inc. 2010. Pediatric safety in-use evaluation (sunscreen containing 3.5% Ethylhexyl Benzoate). CRL Study No: CRL21710.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

HARRISON RESEARCH LABORATORIES, INC.

2497 Vauxhall Road • Union, NJ 07083
E-Mail: info@hrlabs.us.com Phone (908) 688-7600
Website: www.hrlabs.us.com Fax (908) 688-7601

FINAL REPORT
OCULAR STING AND/OR LACRIMATION TEST (OSLT)

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**
Contains 3.5% Ethylhexyl Benzoate

PURPOSE: To assess the potential of the Test Materials to induce ocular sting and/or lacrimation in human subjects.

IRB APPROVAL: Both the HRL Standard Protocol #475 and the Informed Consent were approved by the Clarus Institutional Review Board (CIRB) on 3/24/09. A Sponsor-signed Protocol is retained in HRL files.

SPONSOR: [redacted]

AUTHORIZATION AND SAFETY ASSURANCE: Sponsor authorization and Safety Assurance dated February 12, 2010.

PRINCIPAL INVESTIGATOR: Lynne B Harrison, PhD
CO-INVESTIGATORS: Peter Nussbaum, MD
Joanna D Pruzon, DO

TEST FACILITY: Harrison Research Laboratories, Inc. (HRL)
2497 Vauxhall Road
Union, New Jersey 07083

TEST MATERIALS:

Product B: Sunscreen Liquid 50, NB#3043-16, MF#3078-16, Study #0110291
Product W: Control Baby Shampoo

Test Material Sunscreen Liquid 50, NB#3043-87, a white liquid, was received from [redacted] at Harrison Research Laboratories, Inc. (HRL) on February 12, 2010, with the following instructions: Test as received as per Sponsor's instructions and HRL Protocol #475. A second jar of the Test Material was received on February 19, 2010. The Control, Baby Shampoo, was supplied by HRL and diluted to 10% with sterile water according to HRL Protocol 475.

-CONTINUED-

HARRISON RESEARCH LABORATORIES, INC.

2497 Vauxhall Road • Union, NJ 07083
E-Mail: info@hrlabs.us.com Phone (908) 688-7600
Website: www.hrlabs.us.com Fax (908) 688-7601

FINAL REPORT
OCULAR STING AND/OR LACRIMATION TEST (OSLT)

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

PRODUCT APPLICATION: Sterile disposable eye swab.

SUBJECTS: A total of 30 subjects, 10 male and 20 female, were empanelled. Subjects range in age from 19 to 70.

METHOD: This test was conducted according to HRL Standard Protocol #475 and HRL Standard Operating Procedures (including any Sponsor alterations).

TEST DATES: February 16, 2010 and February 23, 2010

SCORING SYSTEM: See p 4.

RESULTS: See Tables II-III.

CONCLUSIONS: The Test Material and the Control exhibited no statistically significant differences at all scoring intervals for Sting. All reactions cleared within 1 hour.

No Lacrimation was elicited by the Test Material Sunscreen Liquid 50, NB#3043-16 and the Control. The claim "No Tearing" was substantiated in this test.

QUALITY ASSURANCE (QA): The QA Unit performed an in-phase audit of this study.

Peter Nussbaum, MD	Debra Harrison, MA	Lynne B Harrison, PhD
Co-Investigator (Ophthalmologist)	Project Manager	Principal Investigator

Date: 2/24/10

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

SUBJECTS: Each potential subject completed an HRL Subject History Form (HRL Form:SHF), including relevant medical history. (An updated Subject History Form is secured approximately every two years.) Each accepted subject was assigned a permanent HRL Identification Number. No subject was used if he or she exhibited any dermatological or other medical or physical condition that would preclude topical application of the Test Material. No subject reported using any medication that would interfere with the results. No known pregnant nor nursing women were used on this test. No minor subjects were used on this test.

At least one week had elapsed since a subject participated in an Ocular Study.

Legally valid written IRB-approved Informed Consent, in conformity with: 21 CFR 50.25, Subtitle A, Protection of Human Subjects, was secured from each subject.

All subjects had ocular sting, lacrimation, bulbar conjunctiva irritation and palpebral conjunctiva irritation scores of zero (0), within normal limits, prior to the application of the Test Materials. (See Scoring System, below.)

Subjects did not use false eyelashes, eye makeup and/or any other eye product that might have interfered with the results during the study. Contact lens wearers were acceptable if they did not wear them on the day of the study. No swimming was permitted for 12 hours prior to the start of the test.

METHOD: Each subject was given a Snellen Eye Chart Visual Acuity Test prior to instillation of the Test Materials.

The subject's eyes were examined by the Ophthalmological Investigator immediately before treatment. Scoring was as per the Scoring System (see below).

This was a controlled, double-blind, randomized study. At all stages of the procedure, the subject and the Ophthalmological Investigator were unaware of which eye was treated with which Test Material and of the previous assessment scores.

A sterile disposable eye swab was used for the right eye of the subject for instillation of the appropriate Test Material or Control as per the randomization schedule. A new sterile disposable eye swab was used for the left eye. (See Randomization Schedule—Table I.)

Each instillation was performed by a trained HRL technician. Two white tissues (one for each eye) were provided to blot any tears which fell from the eye. Within 30 sec, or as closely as possible following instillation, the eyes were examined by the Ophthalmological Investigator and graded according to the Scoring System.

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

METHOD: (continued) The eyes were examined and scored again at 5 minutes, followed by the washing of the eyes to flush them out. The eyes were again examined and scored at 15 minutes and 1 hour post-instillation. If residual reactions had remained, the Ophthalmological Investigator would have flushed the eyes with an irrigation solution. Any subject who exhibited a 1-level or higher score at 1 hour would not have been dismissed until he or she returned to a zero (0) level score. (See Results; this procedure was not necessary for any subject in this test.)

During the study, the subjects were not permitted to apply any product directly to the eyes, eyelids or eyelashes, and were seated in a secure area.

SCORING SYSTEM:

Stinging (pain, etc.)

0 = Within normal limits
1 = Mild, very slight
2 = Moderate
3 = Severe

Lacrimation

0 = Within normal limits
1 = Excessive wetness (no distinct tears)
2 = A few formed tears (contained in orbit)
3 = Intense tearing (leave orbit)

RESULTS: See Tables II-III. No serious adverse events occurred during this test. This test was conducted under the supervision of a Board-Certified Ophthalmologist, a Co-Investigator. A complete set of Individual Data Forms (OSLT) are included with this Report; these Data Forms are an integral part of this Final Report. A total of 30 subjects, 10 male and 20 female, completed the test. Subjects range in age from 19 to 70.

RETENTION: All original Data Forms will be retained at HRL for a period of three years, or such other time as may be required by law. A laboratory retainer bottle of the Test Material shall be retained, in ambient conditions, for at least two years, or as required by law.

Return or disposal of unused Test Material shall be as per the Sponsor's instructions—to be communicated within 30 days of receipt of this Final Report. HRL shall appropriately dispose of any Test Material after six months if no Sponsor instructions have been communicated

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

TABLE I

RANDOMIZATION SCHEDULE

Test Materials: B = Sunscreen Liquid 50, NB#3043-87
W = Control Baby Shampoo

Subject #	HRL #	Subject's Initials	Right Eye	Left Eye
01	29103	XC	B	W
02	22311	CL	B	W
03	18742	LM	W	B
04	35057	DD	B	W
05	26559	BR	B	W
06	35775	AS	W	B
07	22032	SP	W	B
08	21322	TF	B	W
09	05342	AM	W	B
10	08261	CC	W	B
11	19923	PB	B	W
12	30560	MB	W	B
13	19413	MR	B	W
14	15129	SR	W	B
15	17121	JM	B	W
16	31458	JM	W	B
17	09971	CB	W	B
18	27197	DS	B	W
19	20917	JS	W	B
20	10454	CS	B	W
21	09126	HD	W	B
22	08854	LP	B	W
23	09011	DP	W	B
24	10457	PP	B	W
25	26363	JB	W	B
26	21407	CV	B	W
27	26217	IF	B	W
28	26198	EG	W	B
29	17331	AM	W	B
30	04954	BM	B	W

FINAL REPORT
OCULAR STING AND/OR LACRIMATION TEST (OSLT)

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

TABLE II - STINGING

SUB #	HRL #	INIT	AGE	Test Material 30 sec	Control 30 sec	Test Material 5 min	Control 5 min	Test Material 15 min	Control 15 min	Test Material 1 hr	Control 1 hr
01	29103	XC	43	1	0	0	0	0	0	0	0
02	22311	CL	27	0	0	1	0	0	0	0	0
03	18742	LM	49	0	0	0	0	0	0	0	0
04	35057	DD	30	1	0	0	0	0	0	0	0
05	26559	BR	41	0	0	0	0	0	0	0	0
06	35775	AS	19	0	1	0	0	0	0	0	0
07	22032	SP	46	0	1	0	1	0	1	0	0
08	21322	TF	45	0	0	0	0	0	0	0	0
09	05342	AM	48	0	1	0	0	0	0	0	0
10	08261	CC	49	0	0	0	0	0	0	0	0
11	19923	PB	55	0	0	0	0	0	0	0	0
12	30560	MB	20	0	0	0	0	0	0	0	0
13	19413	MR	56	0	0	0	0	0	0	0	0
14	15129	SR	53	0	0	0	0	0	0	0	0
15	17121	JM	43	0	0	0	0	0	0	0	0
16	31458	JM	36	1	0	0	0	0	0	0	0
17	09971	CB	60	0	0	0	0	0	0	0	0
18	27197	DS	45	0	0	0	0	0	0	0	0
19	20917	JS	28	0	0	0	0	0	0	0	0
20	10454	CS	29	0	0	0	0	0	0	0	0
21	09126	HD	61	0	0	0	0	0	0	0	0
22	08854	LP	61	0	0	0	0	0	0	0	0
23	09011	DP	26	0	0	0	0	0	0	0	0
24	10457	PP	33	0	0	0	0	0	0	0	0
25	26363	JB	27	0	0	0	0	0	0	0	0
26	21407	CV	42	0	0	0	0	0	0	0	0
27	26217	IF	47	0	0	0	0	0	0	0	0
28	26198	EG	34	0	0	0	0	0	0	0	0
29	17331	AM	54	0	0	0	0	0	0	0	0
30	04954	BM	70	0	0	0	0	0	0	0	0
Mean				0.1	0.1	0.33333	0.33333	0	0.33333	0	0
t-Test											
t				0		0		-1		NA	
df				29		29		29		29	
Two-tailed p<				1		1		0.325582		NA	
r				-0.11111		-0.03448		NA		NA	
Conclusion				n.s.		n.s.		n.s		n.s	

Summary The Test Material and the Control exhibited no significant differences at all scoring intervals.

Scoring System:

Sig	=	significant difference	p	=	probability of chance
NS	=	no significant difference	r	=	pearson correlation
df	=	degrees of freedom	NA	=	not applicable

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

TABLE III - LACRIMATION

SUB #	HRL #	INIT	AGE	Test Material 30 sec	Control 30 sec	Test Material 5 min	Control 5 min	Test Material 15 min	Control 15 min	Test Material 1 hr	Control 1 hr
01	29103	XC	43	0	0	0	0	0	0	0	0
02	22311	CL	27	0	0	0	0	0	0	0	0
03	18742	LM	49	0	0	0	0	0	0	0	0
04	35057	DD	30	0	0	0	0	0	0	0	0
05	26559	BR	41	0	0	0	0	0	0	0	0
06	35775	AS	19	0	0	0	0	0	0	0	0
07	22032	SP	46	0	0	0	0	0	0	0	0
08	21322	TF	45	0	0	0	0	0	0	0	0
09	05342	AM	48	0	0	0	0	0	0	0	0
10	08261	CC	49	0	0	0	0	0	0	0	0
11	19923	PB	55	0	0	0	0	0	0	0	0
12	30560	MB	20	0	0	0	0	0	0	0	0
13	19413	MR	56	0	0	0	0	0	0	0	0
14	15129	SR	53	0	0	0	0	0	0	0	0
15	17121	JM	43	0	0	0	0	0	0	0	0
16	31458	JM	36	0	0	0	0	0	0	0	0
17	09971	CB	60	0	0	0	0	0	0	0	0
18	27197	DS	45	0	0	0	0	0	0	0	0
19	20917	JS	28	0	0	0	0	0	0	0	0
20	10454	CS	29	0	0	0	0	0	0	0	0
21	09126	HD	61	0	0	0	0	0	0	0	0
22	08854	LP	61	0	0	0	0	0	0	0	0
23	09011	DP	26	0	0	0	0	0	0	0	0
24	10457	PP	33	0	0	0	0	0	0	0	0
25	26363	JB	27	0	0	0	0	0	0	0	0
26	21407	CV	42	0	0	0	0	0	0	0	0
27	26217	IF	47	0	0	0	0	0	0	0	0
28	26198	EG	34	0	0	0	0	0	0	0	0
29	17331	AM	54	0	0	0	0	0	0	0	0
30	04954	BM	70	0	0	0	0	0	0	0	0
	Mean			0	0	0	0	0	0	0	0
	t-Test										
	t				NA		NA		NA		NA
	df				29		29		29		29
	Two-tailed p<				NA		NA		NA		NA
	r				NA		NA		NA		NA
	Conclusion				n.s		n.s		n.s		n.s

Summary: The Test Material and the Control exhibited no significant differences at all scoring intervals.

Scoring System:

Sig	=	significant difference	p	=	probability of chance
NS	=	no significant difference	r	=	pearson correlation
df	=	degrees of freedom	NA	=	not applicable

HARRISON RESEARCH LABORATORIES, INC.

2497 Vauxhall Road • Union, NJ 07083
E-Mail: info@hrlabs.us.com Phone (908) 688-7600
Website: www.hrlabs.us.com Fax (908) 688-7601

FINAL REPORT
OCULAR STING AND/OR LACRIMATION TEST (OSLT)

[redacted]

Test Material: **Sunscreen Liquid 50, NB#3043-87, MF#3078-16, Study #0110291**

QUALITY ASSURANCE MEMORANDUM

This Final Report was reviewed for accuracy and conformity with both HRL Standard Protocol #475 (including any Sponsor alterations) and HRL Standard Operating Procedures (including any Sponsor alterations) and any written communication from the Sponsor.

Inspections were accomplished by a random sampling approach and reported to the Project Manager and Principal Investigator immediately following their completion.

The raw data for this study are retained at Harrison Research Laboratories, Inc.

HARRISON RESEARCH LABORATORIES, INC.

Susan D Lauck

SUSAN LAUCK
Quality Assurance Manager

QUALITY ASSURANCE UNIT

Dated: February 24, 2010

This report is only submitted for the use of the party to whom it is addressed, and neither it nor the name of our company or any member of our staff may be used in connection with any advertising, promotional material, or sale without our written authorization

Clinical
Research
Laboratories, Inc.

Final Report

Evaluation of a Topically Applied Test Material for Phototoxic Potential

CLIENT: [redacted]

ATTENTION: [redacted]

TEST MATERIAL: Sunscreen Liquid SPF 50, NB#3043-92, MF#3078-16 Containing 3.5% Ethyl hexyl Benzoate

CRL STUDY NUMBER: CRL19110

[redacted] **STUDY #:** 0110308

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

REPORT DATE: March 31, 2010

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical
Research
Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: CRL19110

Start Date: March 15, 2010

Completion Date: March 19, 2010

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

Karen Hayley
Signature of QA Auditor

March 31, 2010
Date

FINAL REPORT

EVALUATION OF A TOPICALLY APPLIED TEST MATERIAL FOR PHOTOTOXIC POTENTIAL

PURPOSE

The purpose of this study was to determine the phototoxic potential of a topically applied test material.

RATIONALE

Photodermatitis is a form of contact dermatitis characterized by an eczematous reaction confined to light-exposed skin sites. When such a skin response develops after a single exposure, it is considered a phototoxic effect analogous to a primary irritant effect. Phototoxicity implies a non-immunologic state in which a photosensitizing molecule absorbs quantities of light in the long wave (UVA) region (320-400 nm). The absorbed energy then produces toxic biochemical changes. A phototoxic effect on skin resembles a severe sunburn clinically.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
732-981-1616

TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on March 5, 2010:

Sunscreen Liquid SPF 50, NB#3043-92, MF#3078-16

The test material was numbered with the following CRL identification number:

CRL19110

STUDY DATES

This study was initiated on March 15, 2010 and was completed on March 19, 2010.

PANEL SELECTION

A total of 22 female and male subjects ranging in age from 19 to 61 years who met all of the inclusion criteria and none of the exclusion criteria listed below were selected for the study. Each subject was assigned a permanent CRL Identification Number (Appendix I – Subject Demographics).

Subject Inclusion Criteria:

a. Twenty or more male and/or female subjects between the ages of 18 and 65.

b. Subjects with fair skin, Types I – III*, according to the following criteria:

I Always burns easily; never tans (sensitive)
II Always burns easily; tans minimally (sensitive)
III Burns moderately; tans gradually (normal)

c. Subjects in generally good health who have a current Subject Profile/Medical History on file.

d. Subjects who do not exhibit any skin disease which may influence test results.

e. Subjects willing to sign an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects".

f. Subjects who have completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164.

g. Subjects who are dependable and able to follow directions.

Subject Exclusion Criteria:

a. Subjects with a history of an abnormal response to the sun or heat or those taking medication (e.g. a dosage of medication causing a photosensitizing or anti-inflammatory response) which may produce an abnormal response to sunlight will be excluded from the study.

b. Subjects exhibiting sunburn, suntan, uneven skin tone, blemishes, moles or excess hair in the test site area.

c. Subjects who are pregnant or nursing.

* Fitzpatrick T.B., et al (editors). (1974). *In Sunlight and Man, Normal and Abnormal Photobiologic Responses*. Tokyo: University of Tokyo Press, 751.

TEST METHOD

Light Source:
A Xenon Arc Solar Simulator (150w, Model 15S or 16S) (Solar Light Company, Philadelphia, PA)** was used as a source of ultraviolet light irradiation and filtered to provide a basic solar-like spectrum in compliance with the International Sun Protection Factor (SPF) Test Method (May 2006). The lamp output was measured with an UV Intensity Meter (Model DCS-1 or Model PMA2100, Solar Light Company, Philadelphia, PA). A Schott WG-345 filter was employed to block UVB wave length 290-300 nanometers.

The spectral distributions of the optical output of the solar simulators are validated annually by Rapid Precision Testing Laboratories.

Test Sites:
Test sites measuring approximately 4 cm^2 were located on the back between the belt line and shoulder blade, lateral to the midline.

Phototoxicity Evaluation:
Approximately 0.2 ml of material was applied to semi-occlusive patches***. Patches were then placed on the designated test site. Twenty-four hours later, the patches were removed, the skin was gently wiped and the test sites were evaluated according to the following scale:

Scoring System	
0	Negative, no visible reaction
±	Minimal erythema
1+	Defined slight erythema
2+	Moderate erythema
3+	Severe erythema

After examination of test sites, one test site was irradiated with a filtered light source (320 - 400 nanometers) equivalent to 10.0 J/cm^2 of UVA irradiation. The second site was the treated non-irradiated site and a third site was irradiated as described above as a non-treated, irradiated control. Test sites were delineated with a skin marker to assure continuity of the patch site.

Test and control sites were examined and graded at 24 hours, 48 hours and 72 hours following irradiation of the test sites.

** Berger, D.S.. (1969). Specification and Design of Solar Ultraviolet Simulators. *J. Invest Dermatol*, 53, 192.

*** Semi-occlusive Strip (manufactured by Brady Medical, Mesquite, TX) consisting of breathable tape, with center portion of ¾" x ¾" fabric.

TEST METHOD (Continued)

The results obtained determined if the test material was a phototoxic material based on a comparison of the reactions on treated and non-treated test sites as follows:

Phototoxic Potential			
Test Condition			Conclusion
Treated (Irradiated)	Treated (Non-Irradiated)	Non Treated (Irradiated)	
Positive Response	No Reaction*	No Reaction*	Phototoxic Response
No Reaction*	No Reaction*	No Reaction*	Non Phototoxic

*No reaction refers to phototoxic effect

TEST RESULTS

A total of 21 subjects completed the study. One subject (#12) discontinued for reasons unrelated to the test material.

A Summary of Dermal Scores appears in Table I for the test material identified as Sunscreen Liquid SPF 50, NB#3043-92, MF#3078-16 (CRL19110). No visible skin reactions were observed for the product on the irradiated or the non-irradiated test sites.

CONCLUSION

The clinical evaluation of the test material, Sunscreen Liquid SPF 50, NB#3043-92, MF#3078-16 (CRL19110), did not exhibit any evidence of Phototoxic potential.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

REFERENCES

1. Kaidbey, K. H. and Kligman, A. M. (1978). "Identification of Topical Photosensitizing Agents in Humans." *J. Invest. Derm.* 70, 149.
2. Kligman, A.M. and Breit, R. (1968). "The Identification of Phototoxic Drugs by Human Assay." *J. Invest. Derm.* 51, 90.
3. Stott, C. W., Stasse, J., Bonomo, R. & Campbell, A. H. (1970). "Evaluation of the Phototoxic Potential of Topically Applied Agents Using Long Wave (Ultraviolet Light)." *J. Invest. Derm.* 55, 335.

Table I

Summary of Phototoxicity Scores

Test Material: Sunscreen Liquid SPF 50, NB#3043-92, MF#3078-16 (CRL19110)

Dermal Scores - Post Irradiation										
Subject Number	**CRL ID #**	**24 Hour**			**48 Hour**			**72 Hour**		
		I	**NI**	**C**	**I**	**NI**	**C**	**I**	**NI**	**C**
1	21024	0	0	0	0	0	0	0	0	0
2	26524	0	0	0	0	0	0	0	0	0
3	15917	0	0	0	0	0	0	0	0	0
4	18742	0	0	0	0	0	0	0	0	0
5	13519	0	0	0	0	0	0	0	0	0
6	00086	0	0	0	0	0	0	0	0	0
7	24195	0	0	0	0	0	0	0	0	0
8	26232	0	0	0	0	0	0	0	0	0
9	26239	0	0	0	0	0	0	0	0	0
10	24401	0	0	0	0	0	0	0	0	0
11	26520	0	0	0	0	0	0	0	0	0
12	06279	DISCONTINUED								
13	07891	0	0	0	0	0	0	0	0	0
14	19221	0	0	0	0	0	0	0	0	0
15	13918	0	0	0	0	0	0	0	0	0
16	07257	0	0	0	0	0	0	0	0	0
17	15618	0	0	0	0	0	0	0	0	0
18	22008	0	0	0	0	0	0	0	0	0
19	21927	0	0	0	0	0	0	0	0	0
20	20747	0	0	0	0	0	0	0	0	0
21	17689	0	0	0	0	0	0	0	0	0
22	12494	0	0	0	0	0	0	0	0	0

Dermal Scores

0	Negative, no reaction	NI	Non-Irradiated Site (Treated)
±	Minimal erythema	I	Irradiated Site (Treated)
1+	Defined slight erythema	C	Control Irradiated Site (Non-Treated)
2+	Moderate rythema		
3+	Severe erythema		

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex	Skin Type
1	NB	21024	47	F	III
2	JH	26524	50	M	II
3	LC	15917	47	F	II
4	DM	18742	55	F	II
5	SJ	13519	59	F	I
6	AH	00086	59	F	II
7	PD	24195	47	F	II
8	KS	26232	32	F	II
9	LE	26239	48	F	I
10	RC	24401	30	M	I
11	DS	26520	26	M	II
12	KD	06279	50	F	II
13	JD	07891	19	M	II
14	KY	19221	40	F	II
15	CR	13918	31	F	I
16	EC	07257	53	F	II
17	KH	15618	61	F	III
18	RB	22008	21	F	II
19	AF	21927	48	F	III
20	AS	20747	39	F	III
21	JL	17689	42	F	II
22	GD	12494	46	F	I

Clinical Research Laboratories, Inc.

Final Report

Repeated Insult Patch Test

CLIENT: [redacted]

ATTENTION: [redacted]

TEST MATERIAL: **Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92**
Containing 3.5% Ethyl hexyl Benzoate

CRL STUDY NUMBER: **CRL17610**

[redacted] **STUDY #:** **0110306**

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

Anita Lee Cham

Anita Lee Cham, M.D.
Dermatologist

REPORT DATE: **May 17, 2010**
REVISED REPORT DATE: **May 25, 2010**

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical
Research
Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: CRL17610

Start Date: March 15, 2010

Completion Date: May 7, 2010

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

Karen Hayley
Signature of QA Auditor

May 25, 2010
Date

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
732-981-1616

TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on March 4, 2010:

Test Material	Test Condition	Patch Type
Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92	Test as Received / Shake well prior to application	Occlusive*

The test material was coded with the following CRL identification number:

CRL17610

STUDY DATES

This study was initiated on March 15, 2010 and was completed on May 7, 2010.

* Occlusive Strip with Flexcon® (Brady Medical, Mesquite,TX)

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I). Subjects who met the following Inclusion Criteria and none of the Exclusion Criteria were impaneled:

Inclusion Criteria

a. Male and female subjects between the ages of 18 and 70 years;

b. Subjects who do not exhibit any skin diseases which might be confused with a skin reaction from the test material;

c. Subjects who agree to avoid exposure of the test sites to the sun and to refrain from visits to tanning salons during the course of this study;

d. Subjects willing to sign an Informed Consent in conformance with 21CFR Part 50: "Protection of Human Subjects;"

e. Subjects who have completed a HIPAA Authorization Form in conformance with 45CFR Parts 160 and 164;

f. Subjects in generally good health who have a current Subject Profile/Medical History on file;

g. Subjects who are dependable and able to follow directions as outlined in the protocol.

Exclusion Criteria

a. Female subjects who are pregnant or nursing;

b. Subjects who are currently using any systemic or topical corticosteroids, anti-inflammatory drugs, or antihistamines on a regular basis;

c. Subjects exhibiting any skin disorder, sunburn, scars, excessive tattoos, etc. in the test area.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of 24 hours.

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on 2 consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation 24 hours after removal of the patches by the subjects on Tuesday and Thursday and 48 hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

The sites were graded according to the following scoring system:

Dermal Scoring Scale

0 No visible skin reaction
± Barely perceptible erythema
1+ Mild erythema
2+ Well defined erythema
3+ Erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a 2-week rest period, the challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a 96-hour reading.

RESULTS

This study was initiated with 224 subjects. Twelve subjects discontinued study participation for reasons unrelated to the test material. A total of 212 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

CONCLUSION

Based on the test population of 212 subjects and under the conditions of this study, the test material identified as Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92 did not demonstrate a clinically significant potential for eliciting dermal irritation or sensitization.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

TABLE I

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
1A	0	0	0	0	0	0	0	0	0	0	0	0
2A	0	0	0	0	0	0	0	0	0	0	0	0
3A	0	0	0	0	0	0	0	0	0	0	0	0
4A	0	0	0	0	0	0	0	0	0	0	0	0
5A	0	0	0	0	0	0	0	0	0	0	0	0
6A	0	0	0	0	0	0	0	0	0	0	0	0
7A	0	0	0	0	0	0	0	0	0	0	0	0
8A	0	0	0	0	0	0	0	0	0	0	0	0
9A	0	0	0	0	0	0	0	0	0	0	0	0
10A	0	0	0	0	0	0	0	0	0	0	0	0
11A	0	0	0	0	0	0	0	0	0	0	0	0
12A	0	±	0	0	0	±	0	0	0	0	0	0
13A	0	0	0	0	0	0	0	0	0	0	0	0
14A	0	0	0	0	0	0	0	0	0	0	0	0
15A	0	0	0	0	0	0	0	0	0	0	0	0
16A	0	0	0	0	0	0	0	0	0	0	0	0
17A	0	0	0	0	0	0	0	0	0	0	0	0
18A	0	0	0	0	0	0	0	0	0	0	0	0
19A	0	0	0	0	0	0	0	0	0	0	0	0
20A	0	0	0	0	0	0	0	0	0	0	0	0
21A	0	0	0	0	0	0	0	0	0	0	0	0
22A	0	0	0	0	0	0	0	0	0	0	0	0
23A	0	0	0	0	Discontinued							
24A	0	0	0	0	0	0	0	0	0	0	0	0
25A	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
26A	0	0	0	0	0	0	0	0	0	0	0	0
27A	0	0	0	0	0	0	0	0	0	0	0	0
28A	0	0	0	0	0	0	0	0	0	0	0	0
29A	0	0	0	0	0	0	0	0	0	0	0	0
30A	0	0	0	0	0	0	0	0	0	0	0	0
31A	0	0	0	0	0	0	0	0	0	0	0	0
32A	0	0	0	0	0	0	0	0	0	0	0	0
33A	0	0	0	0	0	0	0	0	0	0	0	0
34A	0	0	0	0	0	0	0	0	0	0	0	0
35A	0	Discontinued										
36A	±	±	±	0	0	0	0	0	0	0	0	0
37A	0	0	0	0	0	0	0	0	0	0	0	0
38A	0	0	0	0	0	0	0	0	0	0	0	0
39A	0	0	0	0	0	0	0	0	0	0	0	0
40A	0	0	0	0	0	0	0	0	0	0	0	0
41A	0	0	0	0	0	0	0	0	0	0	0	0
42A	0	0	0	0	0	0	0	0	0	0	0	0
43A	0	0	0	0	0	0	0	0	0	0	0	0
44A	0	0	0	0	0	0	0	0	0	0	0	Disc.
45A	0	0	0	0	0	0	0	0	0	0	0	0
46A	0	0	0	0	0	0	0	0	0	0	0	0
47A	0	0	0	0	0	0	0	0	0	0	0	0
48A	0	0	0	0	0	0	0	0	0	0	0	0
49A	0	0	0	0	0	0	0	0	0	0	0	0
50A	0	0	0	0	0	0	0	0	0	0	0	0

Disc. = Discontinued

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
51A	0	0	0	0	0	0	0	0	0	0	0	0
52A	0	0	0	0	0	0	0	0	0	0	0	0
53A	0	0	0	0	0	0	0	0	0	0	0	0
54A	0	0	0	0	0	0	0	0	0	0	0	0
55A	0	0	0	0	0	0	0	0	0	0	0	0
56A	0	0	0	0	0	0	0	0	0	0	0	0
57A	0	0	0	0	0	0	0	0	0	0	0	0
58A	0	0	0	0	0	0	0	0	0	0	0	0
59A	0	0	0	0	0	0	0	0	0	0	0	0
60A	0	0	0	0	0	0	0	0	0	0	0	0
61A	0	0	0	0	0	0	0	0	X	0	0	0
62A	0	0	0	0	0	0	0	0	0	0	0	0
63A	0	0	0	0	0	0	0	0	0	0	0	0
64A	0	0	0	0	0	0	0	0	0	0	0	0
65A	0	0	0	0	0	0	0	0	0	0	0	0
66A	0	0	0	0	0	0	0	0	0	0	0	0
67A	0	0	0	0	0	0	0	0	0	0	0	0
68A	0	0	0	0	0	0	0	0	0	0	0	0
69A	0	0	0	0	0	0	0	0	0	0	0	0
70A	0	0	0	0	0	0	0	0	0	0	0	0
71A	0	0	0	0	0	0	0	0	0	0	0	0
72A	0	0	0	0	0	0	0	0	0	0	0	0
73A	0	0	0	0	0	0	0	0	0	0	0	0
74A	0	0	0	0	0	0	0	0	0	0	0	0
75A	0	0	0	0	0	0	0	0	0	0	0	0

X = Subject Absent

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
76A	Discontinued											
77A	0	0	0	0	0	0	0	Discontinued				
78A	0	0	0	0	0	0	0	0	0	0	0	0
79A	0	0	0	0	0	0	0	0	0	0	0	0
80A	0	0	0	0	0	0	0	0	0	0	0	0
81A	0	0	0	0	0	0	0	0	0	0	0	0
82A	0	0	0	0	0	0	0	0	0	0	0	0
83A	0	0	0	0	0	0	0	0	0	0	0	0
84A	0	0	0	0	0	0	0	0	0	0	0	0
85A	0	0	±d	0	0	0	0	0	0	0	0	0
86A	0	0	0	0	0	0	0	0	0	0	0	0
87A	0	0	0	0	0	0	0	0	0	0	0	0
88A	0	0	0	0	0	0	0	0	0	0	0	0
89A	0	0	0	0	0	0	0	0	0	0	0	0
90A	0	0	0	0	0	0	0	0	0	0	0	0
91A	0	0	0	0	0	0	0	0	0	0	0	0
92A	0	0	0	0	0	0	0	0	0	0	0	0
93A	0	0	0	0	0	0	0	0	0	0	0	0
94A	0	0	0	0	0	0	0	0	0	0	0	0
95A	0	0	0	0	0	0	0	0	0	0	0	0
96A	0	0	0	0	0	0	0	0	0	0	0	0
97A	0	0	0	0	0	0	0	0	0	0	0	0
98A	0	0	0	0	0	0	0	0	0	0	0	0
99A	0	0	0	0	0	0	0	0	0	0	0	0
100A	0	0	0	0	0	0	0	0	0	0	0	0

d = Dryness

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
101A	0	0	0	0	0	0	0	0	0	0	±	±
102A	0	0	0	0	0	0	0	0	0	0	0	0
103A	0	0	0	0	0	0	0	0	0	0	1+	1+*
104A	0	0	0	0	0	0	0	0	0	0	0	0
105A	0	0	0	0	0	0	0	0	0	0	0	0
106A	0	0	0	0	0	0	0	0	0	0	0	0
107A	0	0	0	0	0	0	0	0	0	0	0	0
108A	0	0	0	0	0	0	0	0	0	0	0	0
109A	0	0	0	0	0	0	0	1+P	±d	0	0	0
110A	0	0	0	0	0	0	0	0	0	0	0	0
111A	0	0	0	0	0	0	0	0	0	0	0	0
112A	0	0	0	0	0	0	0	0	0	0	0	0

P = Peeling
d = Dryness
* A reaction of 1+ was observed at the 96 hour reading.

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
1B	0	0	0	0	0	0	0	0	0	0	0	0
2B	0	0	0	0	0	0	0	0	0	0	0	0
3B	0	0	0	0	0	0	0	0	0	0	0	0
4B	0	0	0	0	0	0	0	0	0	0	0	0
5B	0	0	0	0	0	0	0	0	0	0	0	0
6B	0	0	0	0	0	0	0	0	0	0	0	0
7B	0	0	0	0	0	0	0	0	0	0	0	0
8B	0	0	0	0	0	0	0	0	0	0	0	0
9B	0	0	0	0	0	0	0	0	0	0	0	0
10B	0	0	0	0	0	0	0	0	0	0	0	0
11B	0	0	0	0	0	0	0	0	0	0	0	0
12B	0	0	0	0	0	0	0	0	0	0	0	0
13B	0	0	0	0	0	0	0	0	0	0	0	0
14B	0	0	0	0	0	0	0	0	0	0	0	0
15B	0	0	0	0	0	0	0	0	0	0	0	0
16B	Discontinued											
17B	Discontinued											
18B	0	0	0	0	0	0	0	0	0	0	0	0
19B	0	0	0	0	0	0	0	0	0	0	0	0
20B	0	0	0	0	0	0	0	0	0	0	0	0
21B	0	0	0	0	0	0	0	0	0	0	0	0
22B	0	0	0	0	0	0	0	0	0	0	0	0
23B	0	0	0	0	0	0	0	0	0	0	0	0
24B	0	0	0	0	0	0	0	0	0	0	0	0
25B	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
26B	0	0	0	0	0	0	0	0	0	0	0	0
27B	0	0	0	0	0	0	0	0	0	0	0	0
28B	0	0	0	0	0	0	0	0	0	0	0	0
29B	0	0	0	0	0	0	0	0	0	0	0	0
30B	0	0	0	0	0	0	0	0	0	0	0	0
31B	0	0	0	0	0	0	0	0	0	0	0	0
32B	0	0	0	0	0	0	0	0	0	0	0	0
33B	0	0	0	0	0	0	0	0	0	0	0	0
34B	0	0	0	0	0	0	0	0	0	1+	1+	±
35B	Discontinued											
36B	0	0	0	0	0	0	0	0	0	0	0	0
37B	0	0	0	0	0	0	0	0	0	0	0	0
38B	0	0	0	0	0	0	0	0	0	0	0	0
39B	0	0	0	0	0	0	0	0	0	0	0	0
40B	0	0	0	0	0	0	0	0	0	0	0	0
41B	0	0	0	0	0	0	0	0	0	0	0	0
42B	0	0	0	0	0	0	0	0	0	0	0	0
43B	0	0	0	0	0	0	0	0	0	0	0	0
44B	0	0	0	0	0	0	0	0	0	0	0	0
45B	0	0	0	0	0	0	0	0	0	0	0	0
46B	0	0	0	0	0	0	0	0	0	0	0	0
47B	0	0	0	0	0	0	0	0	0	0	0	0
48B	0	0	0	0	0	0	0	0	0	0	0	0
49B	0	0	0	0	0	0	0	0	0	0	0	0
50B	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
51B	0	0	0	0	0	0	0	0	0	0	0	0
52B	0	0	0	0	0	0	0	0	0	0	0	0
53B	0	0	0	0	0	0	0	0	0	0	0	0
54B	0	0	0	0	0	0	0	0	0	0	0	0
55B	0	0	0	0	0	0	0	0	0	0	0	0
56B	0	0	0	Discontinued								
57B	0	0	0	0	0	0	0	0	0	0	0	0
58B	0	0	0	0	0	0	0	0	0	0	0	0
59B	0	0	0	0	0	0	0	0	0	0	0	0
60B	Discontinued											
61B	0	0	0	0	0	0	0	0	0	0	0	0
62B	0	0	0	0	0	0	0	0	0	0	0	0
63B	0	0	0	0	0	0	0	0	0	0	±	±*
64B	0	0	0	0	0	0	0	0	0	0	0	0
65B	0	0	0	0	0	0	0	0	0	0	0	0
66B	0	0	0	0	0	0	0	0	0	0	0	0
67B	0	0	0	0	0	0	0	0	0	0	0	0
68B	0	0	0	0	0	0	0	0	0	0	0	0
69B	0	0	0	0	0	0	0	0	0	0	0	0
70B	0	0	0	0	0	0	0	0	0	0	0	0
71B	0	0	0	0	0	0	0	0	0	0	0	0
72B	0	0	0	0	0	0	0	0	0	0	0	0
73B	0	0	0	0	0	0	0	0	0	0	0	0
74B	0	0	0	0	0	0	0	0	0	0	0	0
75B	0	0	0	0	0	0	0	0	0	0	0	0

* No reaction was observed at the 96 hour reading.

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
76B	0	0	0	0	0	0	0	0	0	0	0	0
77B	0	0	0	0	0	0	0	0	0	0	0	0
78B	0	0	0	0	0	0	0	0	0	0	0	0
79B	0	0	0	0	0	0	0	0	0	0	0	0
80B	0	0	0	0	0	0	0	0	0	0	0	0
81B	0	0	0	0	0	0	0	0	0	0	0	±*
82B	0	0	0	0	0	0	0	0	0	0	0	0
83B	0	0	0	0	0	0	0	0	0	0	0	0
84B	0	0	0	0	Discontinued							
85B	0	0	0	0	0	0	0	0	0	0	0	0
86B	0	0	0	0	0	0	0	0	0	0	0	0
87B	0	0	0	0	0	0	0	0	0	0	0	0
88B	0	0	0	0	0	0	0	0	0	0	0	0
89B	0	0	0	0	0	0	0	0	0	0	0	1+**
90B	0	0	0	0	0	0	0	0	0	0	0	0
91B	0	0	0	0	0	0	0	0	0	0	0	0
92B	0	0	0	0	0	0	0	0	0	0	0	0
93B	0	0	0	0	0	0	0	0	0	0	0	0
94B	0	0	0	0	0	0	0	0	0	0	0	0
95B	0	0	0	0	0	0	0	0	0	0	0	0
96B	0	0	0	0	0	0	0	0	0	0	0	0
97B	0	0	0	0	0	0	0	0	0	0	0	0
98B	0	0	0	0	0	0	0	0	0	0	0	0
99B	0	0	0	0	0	0	0	0	0	0	0	0
100B	0	0	0	0	0	0	0	0	0	0	0	0

* No reaction was observed at the 96 hour reading.
** A reaction of ± was observed at the 96 hour reading.

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Sunscreen Liquid SPF50 TA#10-0037, MF#3078-16, NB#3043-92

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
101B	0	0	0	0	0	0	0	0	0	0	0	0
102B	0	0	0	0	0	0	0	0	0	0	0	0
103B	0	0	0	0	0	0	0	0	0	0	0	0
104B	0	0	0	0	0	0	0	0	0	0	0	0
105B	0	0	0	0	0	0	0	0	0	0	0	0
106B	0	0	0	0	0	0	0	0	0	0	0	0
107B	0	0	0	0	0	0	0	0	0	0	0	0
108B	0	0	0	0	0	0	0	0	0	0	0	0
109B	0	0	0	0	0	0	0	0	0	0	0	0
110B	0	0	0	0	0	0	0	0	0	0	0	0
111B	0	0	0	0	0	0	0	0	0	0	0	0
112B	Discontinued											

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1A	DG	16950	52	F
2A	JB	14759	58	F
3A	CS	25934	57	F
4A	KC	26179	41	M
5A	JS	00467	48	F
6A	SR	02417	62	F
7A	PR	25905	29	F
8A	LR	25309	51	F
9A	AT	14294	68	F
10A	CV	26046	49	F
11A	JM	10420	40	F
12A	CY	14780	54	F
13A	HL	26115	49	M
14A	KW	08856	51	M
15A	AG	23159	45	F
16A	SM	25694	56	F
17A	JS	24167	55	M
18A	FR	24101	39	M
19A	AP	15450	29	F
20A	MV	25235	63	F
21A	CL	09171	37	F
22A	LH	23724	49	F
23A	CG	17817	29	F
24A	KW	19599	35	F
25A	DL	21338	39	F
26A	PG	13984	48	F
27A	BW	26154	57	F
28A	SJ	26156	66	M

Subject Number	Subject Initials	CRL ID #	Age	Sex
29A	AK	21288	63	F
30A	DP	23728	39	M
31A	SP	26003	31	F
32A	HP	26009	60	M
33A	UP	26008	53	F
34A	MA	20331	52	F
35A	JL	11685	54	M
36A	AK	06659	53	F
37A	MR	23590	61	F
38A	MR	21928	34	F
39A	MM	18694	40	F
40A	CR	24445	34	M
41A	LL	21886	65	F
42A	PK	10971	68	F
43A	EK	26293	68	M
44A	JP	06213	44	F
45A	AJ	23193	47	F
46A	CV	15532	39	F
47A	PP	15763	58	F
48A	JP	17665	37	F
49A	CK	02313	49	F
50A	JM	13991	69	F
51A	WG	18828	63	F
52A	MW	23452	68	F
53A	LJ	23672	53	F
54A	SB	22825	48	F
55A	MB	22824	45	M
56A	WM	23824	51	F

Appendix I
(Continued)

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
57A	EH	24951	62	M
58A	AA	25921	19	F
59A	KT	12777	44	F
60A	LS	25019	55	F
61A	RA	23557	38	F
62A	RB	05376	61	M
63A	KS	18451	50	F
64A	KM	00302	56	F
65A	EP	17560	52	F
66A	LD	25080	51	F
67A	MR	10668	67	F
68A	CA	17565	54	F
69A	EJ	24955	48	M
70A	PR	00278	49	F
71A	KW	16112	55	M
72A	SF	10327	44	F
73A	SK	10172	40	M
74A	RS	21197	28	F
75A	SR	22294	50	F
76A	EC	22293	45	M
77A	KM	26029	50	F
78A	WV	17705	45	M
79A	MS	17112	31	F
80A	LR	26119	38	F
81A	LD	25937	44	F
82A	CR	05923	56	F
83A	RR	10112	51	F
84A	RS	05543	44	M

Subject Number	Subject Initials	CRL ID #	Age	Sex
85A	DJ	17980	27	M
86A	LC	20318	49	F
87A	KM	09210	57	F
88A	MG	25048	56	F
89A	DT	24320	25	F
90A	DW	25982	52	M
91A	MB	03505	52	F
92A	CR	20675	53	F
93A	KB	25526	46	F
94A	LT	12033	62	F
95A	MR	26200	36	F
96A	BB	04876	60	F
97A	JP	22148	34	F
98A	AG	21336	37	F
99A	KC	09142	55	F
100A	RS	23449	21	F
101A	HS	24646	50	F
102A	FK	04033	61	M
103A	SC	17212	52	F
104A	LJ	20048	44	F
105A	JS	23688	46	F
106A	LN	24351	43	F
107A	JG	24987	50	M
108A	MH	11093	52	F
109A	BP	23069	49	F
110A	GF	24982	48	F
111A	DS	25493	53	F
112A	LH	13288	66	F

Appendix I
(Continued)

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1B	PP	22202	57	M
2B	RP	16060	27	F
3B	JS	25062	52	F
4B	RJ	26063	48	F
5B	DS	26709	20	F
6B	MM	24933	39	F
7B	NG	25711	36	M
8B	GE	26227	54	M
9B	NS	24150	19	F
10B	GS	24198	46	F
11B	JK	14141	70	F
12B	DD	19959	49	F
13B	EN	14911	56	F
14B	BC	20385	45	F
15B	RA	20421	52	F
16B	CR	26396	56	F
17B	ET	26685	39	F
18B	AP	26576	66	M
19B	LD	26508	43	F
20B	FA	21228	46	M
21B	DL	00674	50	F
22B	TH	17294	47	F
23B	RS	18672	28	F
24B	WM	19523	52	F
25B	AW	26678	39	M
26B	LB	14480	46	F
27B	ML	20303	65	F
28B	MC	20533	56	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
29B	MP	18796	27	F
30B	PL	25562	54	F
31B	JH	23464	63	F
32B	MV	15003	61	F
33B	MP	15018	32	F
34B	AP	24717	52	F
35B	DT	25302	46	M
36B	BL	20206	61	F
37B	AP	00708	50	M
38B	CS	26616	55	F
39B	AI	21944	57	F
40B	KS	25108	45	F
41B	CD	25061	41	F
42B	YP	11690	53	F
43B	SP	25963	67	F
44B	RP	25964	34	F
45B	TS	01448	40	F
46B	DB	21823	61	F
47B	LP	15115	45	F
48B	TP	22284	19	M
49B	CM	22448	18	M
50B	JW	24346	54	F
51B	LT	26357	48	F
52B	DC	26674	19	F
53B	EC	26673	21	F
54B	AP	23745	26	F
55B	LL	09586	51	M
56B	CS	26676	32	M

Appendix I
(Continued)

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
57B	GS	25005	51	M
58B	ES	18090	33	F
59B	SP	26205	45	F
60B	MM	22344	52	F
61B	DL	03280	39	F
62B	VP	24129	26	M
63B	BP	22742	55	M
64B	SB	14190	48	F
65B	AB	23860	64	M
66B	BW	06687	62	F
67B	LW	06688	64	M
68B	CP	21493	53	F
69B	NP	00707	43	F
70B	CC	26694	57	M
71B	CL	14668	66	F
72B	MI	14669	66	F
73B	DA	24796	56	F
74B	AA	23089	44	F
75B	SA	25315	25	F
76B	MA	25404	60	F
77B	MD	06584	42	F
78B	RC	25488	62	M
79B	GC	06412	50	F
80B	CC	25487	61	F
81B	PP	26656	34	F
82B	TR	13698	43	F
83B	JR	26625	45	M
84B	VB	26607	42	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
85B	OA	26608	18	F
86B	DC	19990	52	F
87B	DP	16953	52	F
88B	GM	10424	53	F
89B	DP	24464	39	F
90B	JK	03896	39	M
91B	MN	24619	19	F
92B	SL	24903	53	F
93B	PP	24006	54	M
94B	SM	21575	44	F
95B	VZ	23723	47	F
96B	MP	20531	43	F
97B	KM	20892	35	F
98B	KT	06277	54	F
99B	LN	14920	54	F
100B	RO	23734	47	F
101B	SJ	24550	46	F
102B	AS	02343	60	F
103B	CC	23591	63	F
104B	DK	21563	48	F
105B	MF	26627	69	F
106B	AR	25676	34	F
107B	MC	15840	28	F
108B	LL	08362	56	F
109B	LD	18531	49	F
110B	JD	18532	46	F
111B	KS	23456	51	F
112B	NS	26687	19	F

Clinical Research Laboratories, Inc.

Final Report

14-Day Cumulative Irritation Test

CLIENT: [redacted]

ATTENTION: [redacted]

TEST MATERIALS: **Study#0110307, Sunscreen liquid SPF 50**
NB#3043-92, MF#3078-16
Containing 3.5 % Ethylhexyl Benzoate

CRL STUDY NUMBER: **CRL20310**

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

Anita Lee Cham, M.D.

Anita Lee Cham, M.D.
Dermatologist

REPORT DATE: **April 12, 2010**

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical Research Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: CRL20310

Start Date: March 15, 2010

End Date: March 29, 2010

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted, as indicated below.

Signature of QA Auditor

4/12/10
Date

FINAL REPORT

14-Day Cumulative Irritation Test

PURPOSE

The purpose of this study was to determine the cumulative irritation potential of a test material topically applied to the skin of human subjects.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc. (CRL)
371 Hoes Lane
Piscataway, New Jersey 08854
732-981-1616

INVESTIGATOR:

Anita Lee Cham, M.D.
Dermatologist

SPONSOR

[redacted]

TEST MATERIALS

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on March 5, 2010:

Test Material Identification		Dilution	Patch Type
CRL20310	Study#0110307, Sunscreen liquid SPF 50 NB#3043-92, MF#3078-16	Neat	Occlusive

The test material was labeled with the CRL identification number listed above.

CONTROL MATERIALS

The following control materials were provided by Clinical Research Laboratories, Inc. and were labeled with the CRL identification listed below.

Test Material Identification	Dilution	Patch Type	CRL Identification
SLS (Positive Control)	0.25%	Occlusive	PC
Saline (Negative Control)	Neat	Occlusive	NC

STUDY DATES

This study was initiated on March 15, 2010 and completed on March 29, 2010.

PANEL SELECTION

A total of 28 male and female subjects, ranging in age from 19 to 60 years, were selected for the study (Subject Demographics - Appendix I). Subjects who met all of the inclusion criteria and none of the exclusion criteria listed in the study protocol were enrolled.

TEST METHOD

This study was conducted according to the attached study protocol, CRL20310 CL 9.0 (Attachment I).

TEST RESULTS

Completed and Discontinued Subjects

A total of 28 subjects completed the study.

Dermal Evaluations

Individual dermal scores for each test material are listed in Table I. The total score for the test material is listed below.

Test/Control Material	Total Score	Potential Maximum Score
Study#0110307, Sunscreen liquid SPF 50 NB#3043-92, MF#3078-16	5.0	1120

Adverse Events

There were no adverse events reported during the study period.

CONCLUSION

Under the conditions of the study, test material Study#0110307, Sunscreen liquid SPF 50 NB#3043-92, MF#3078-16 did not demonstrate a potential for eliciting cumulative dermal irritation.

Table I

Dermal Scores

Study#0110307, Sunscreen liquid SPF 50 NB#3043-92, MF#3078-16											
Subject Number	**Dermal Reading**										**Total**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0.5	0	0.5
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0.5	0.5	0	1
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0.5	0.5	1
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0.5	0.5	1
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0.5	0	0.5	1
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0.5	0	0.5
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
Potential Maximum Score: 1120							**Maximum Score: 5.0**				

Table I
(Continued)

Dermal Scores

Positive Control (Sodium Lauryl Sulfate)											
Subject Number	Dermal Reading										Total
	1	2	3	4	5	6	7	8	9	10	
1	0	0	0	0	1	0	0	1	0	0	2
2	0	0	0	0.5	0.5	0.5	1	1	1	1	5.5
3	0	0	0	0.5	0	1	1	0	1	1	4.5
4	0	0	0	0.5	0.5	1	1	1	1	1	6.0
5	0	0	0	0.5	0.5	0.5	0.5	1	1	1	5.0
6	0	0	0	0	0	0	0	0.5	0	0.5	1.0
7	0	0	0	0	0	0	0.5	0	0.5	0	1.0
8	0	0.5	0	0.5	0.5	0.5	0.5	1	1	1	5.5
9	0	0	0	0	0	1	1	1	1	1	5.0
10	0	0	0	0	0	0.5	0.5	0.5	1	1	3.5
11	0	0	0	0	0.5	1	1	1	1	1	5.5
12	0	0	0	0.5	0.5	0	0	0	0	1	2.0
13	0	0	0	0	0	0	1	1	1	1	4.0
14	0	0	0	0	0	0	0.5	1	1	1	3.5
15	0	0	0	0	0	1	1	1	0	0	3.0
16	0	0	0	0	0	0.5	0.5	1	1	1	4.0
17	0	0	0	0	0.5	0.5	0.5	0.5	0	1	3.0
18	0	0	0.5	1	1	0	1	1	1	1	6.5
19	0	0	0	0	0	0	0	0.5	0	1	1.5
20	0	0	0	0.5	0.5	0	0.5	1	1	1	4.5
21	0	0	0	0	0	0	0	1	1	1	3.0
22	0	0	0	0	0	0	0	0.5	0.5	0	1.0
23	0	0	0	0	0	0.5	1	1	1	1	4.5
24	0	0	0.5	0	0.5	0.5	1	1	1	1	5.5
25	0	0	0	0	1	1	1	1	1	1	6.0
26	0	0	0	0	0.5	0.5	1	1	1	1	5.0
27	0	0	0	0	0.5	0.5	1	1	1	1	5.0
28	0	0	0	0	0.5	1	1	1	1	1	5.5
Potential Maximum Score: 1120							Maximum Score: 112				

Table I
(Continued)

Dermal Scores

Negative Control (Saline)											
Subject Number	**Dermal Reading**										**Total**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
Potential Maximum Score:					**1120**		**Maximum Score:**				**0.0**

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	CN	18898	47	M
2	PC	13910	50	F
3	AV	21550	45	F
4	MM	23373	46	M
5	MR	22766	35	F
6	CG	17817	27	F
7	FC	23784	50	M
8	MS	24690	40	M
9	LL	07031	53	F
10	AM	21393	43	F
11	DW	20173	34	F
12	TC	20722	40	F
13	MK	12040	60	F
14	KS	17559	43	F
15	LZ	08758	44	F
16	CT	24011	22	F
17	LP	16510	59	F
18	KP	23293	24	F
19	BD	22250	59	F
20	MW	15770	35	F
21	SE	20804	50	F
22	BP	24412	46	F
23	BD	14500	34	F
24	AM	22773	42	F
25	TH	08056	50	F
26	LF	00390	56	M
27	LL	11650	35	M
28	MF	24257	19	M

Clinical
Research
Laboratories, Inc.

Final Report

Pediatric Safety In-Use Evaluation

CLIENT: [redacted]

ATTENTION: [redacted]

TEST MATERIAL: Sunscreen Lotion Spray SPF 50+, MF#3078-16, Batch#AL025-010, TA#10-0037

CRL STUDY NUMBER: CRL21710

[redacted] **STUDY NUMBER:** 0110318

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

Louis M. Diemer, M.D.
Diplomate American Board of Pediatrics

REPORT DATE: May 11, 2010

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical
Research
Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: CRL21710

Start Date: April 1, 2010

Completion Date: April 30, 2010

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted.

Karen Hayberg
QA Auditor

May 11, 2010
Date

FINAL REPORT

Pediatric Safety In-Use Evaluation

PURPOSE

The objective of this study was to determine the dermal irritation potential of a test material, when applied to the arms and legs once daily for four weeks, in a population consisting of children ages six months to eight years.

INVESTIGATOR

Louis M. Diemer, M.D.
Diplomate American Board of Pediatrics

Clinical Research Laboratories, Inc.
371 Hoes Lane, Suite 100
Piscataway, New Jersey 08854
732-981-1616

SPONSOR

[redacted]

INSTITUTIONAL REVIEW BOARD

Allendale Investigational Review Board
Robert J. Staab, Ph.D.
President
30 Neck Road
Old Lyme, CT 06371

TEST MATERIAL

The following test material was provided by [redacted] and was received by Clinical Research Laboratories, Inc. on March 18, 2010:

Client Identification	CRL Identification
Sunscreen Lotion Spray SPF 50+, MF#3078-16, Batch#AL025-010, TA#10-0037	CRL21710

Test materials were labeled with CRL identification and subject numbers.

STUDY DATES

This study was initiated on April 1, 2010 and was completed on April 30, 2010.

STUDY POPULATION

A total of 35 male and female subjects, ranging in age from 7 months to 8 years and in generally good health, were selected for the study (Subject Demographics - Appendix I). Subjects who met all of the inclusion criteria and none of the exclusion criteria listed in the study protocol were enrolled for study participation.

STUDY EXECUTION

This study was conducted according to the attached study protocol, CRL21710 CL 3.0.

TEST RESULTS

Thirty-four subjects completed the study. One subject (#20) discontinued study participation for reasons unrelated to test material use.

Dermal Examinations

Dermal examination results appear in Table I. There were no increases in erythema, edema or dryness of the arms and no increase in erythema and edema of the legs. One subject exhibited mild dryness of the legs following the four-week use period.

Daily Diaries

At the final visit, one parent reported that the child developed a papular rash at the 3rd week of use on the arms and legs. The parent discontinued test material application for two days. The rash cleared up and test material application was resumed with no further reaction. No evidence of irritation was observed at the final visit.

CONCLUSION

The dermal evaluation of Sunscreen Lotion Spray SPF 50+, MF#3078-16, Batch#AL025-010, TA#10-0037 following a four-week use period revealed no evidence of increases in erythema or edema and only a mild increase in dryness in one subject. In this test population, the test material did not demonstrate a potential for eliciting dermal irritation or sensitization when applied to the arms and legs once daily for four weeks.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

Table I

Dermal Examination Results

Subject Number	Baseline						Final					
	Right Arm			Left Arm			Right Arm			Left Arm		
	Erythema	Edema	Dryness	Erythema	Edema	Dryness	Erythema	Edema	Dryness	Erythema	Edema	Dryness
1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	Discontinued					
21	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1	1	1	1	1	1

Dermal Scoring Scale
1 = None
2 = Mild
3 = Moderate
4 = Severe

Table I
(Continued)

Dermal Examination Results

Subject Number	Baseline						Final					
	Right Leg			Left Leg			Right Leg			Left Leg		
	Erythema	Edema	Dryness	Erythema	Edema	Dryness	Erythema	Edema	Dryness	Erythema	Edema	Dryness
1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	2	1	1	2	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	Discontinued					
21	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1
25	1	1	2	1	1	2	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1	1	2	1	1	2
30	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1	1	1	1	1	1

Dermal Scoring Scale
1 = None
2 = Mild
3 = Moderate
4 = Severe

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	NM	23559	6	M
2	AS	26568	4	F
3	SS	26569	2	M
4	AF	23488	4	F
5	AF	23487	5	F
6	MF	26606	7 months	M
7	LS	26581	3	F
8	QS	26580	4	F
9	BE	24701	4	M
10	HM	26563	4	F
11	DR	26589	3	M
12	SC	26260	4	M
13	MS	25213	1	F
14	RD	26601	1	M
15	TC	25120	3	F
16	KL	23623	5	F
17	DL	23624	3	F
18	JC	26543	6	F
19	MM	24533	6	F
20	PB	23602	4	M
21	ME	26668	2	F
22	TF	26639	5	F
23	AR	23620	3	M
24	IR	24617	6	F
25	NM	21809	8	M
26	BM	26487	8	F
27	NT	26211	6	M
28	BB	26247	6	M
29	EB	26246	5	F
30	RF	26566	4	M
31	KF	26565	2	F
32	HD	25091	6	F
33	AR	23670	5	F
34	JM	26752	7	F
35	LM	26753	3	F

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 27, 2010

SUBJECT: Studies on C12-15 Alkyl Benozate (Finsolv TN)

Consumer Product Testing Company Inc. 1978. Primary dermal irritation (rabbit), ocular irritation (rabbit), acute oral toxicity (rabbit) Finsolv TN (C12-15 Alkyl Benzoate). Experiment Reference No.: 78426-1.

Consumer Product Testing Company Inc. 1979. Acute dermal toxicity (rabbit), acute oral LD_{50} (rat) Finslov TN (C12-15 Alkyl Benzoate). Experiment Reference No.: 78516.

Consumer Product Testing Company Inc. 1979. Acute inhalation toxicity (rat), guinea pig sensitization Finsolv TN (C12-15 Alkyl Benzoate). Experiment Reference No.: 7950.

Consumer Product Testing Company Inc. 1988. Repeat 14-day dermal irritation study in rabbits Finsolv TN (C12-15 Alkyl Benzoate). Experiment Reference No.: 88420.

Consumer Product Testing Company. 1998. The MatTek Corporation EpiDerm™ skin model *in vitro* toxicity testing system Finsolv TN (C12-15 Alkyl Benzoate). Experiment Reference No.: V98-0014-6.

Consumer Product Testing Company. 1998. The MatTek Corporation EpiOcular™ tissue model *in vitro* toxicity testing system Finsolv TN (C12-15 Alkyl Benzoate). Experiment Reference No.: V98-0014-6.

Consumer Product Testing Company. 1994. Repeated insult patch test Finsolv TN (C12-15 Alkyl Benzoate). Experiment Reference Number: C94-0297.

NamSA. 1994. Ames Salmonella/mammalian microsome mutagenicity assay for mutagens Finsolv TN (C12-15 Alkyl Benzoate). MG019-223/S.

Consumer Product Testing

Company Incorporated

Bldg. No. 2-15B
1275 Bloomfield Avenue
Fairfield, New Jersey 07006

(201) 575-7688
(201) 575-7689

FINAL REPORT

CLIENT:	Finetex Inc. 418 Falmouth Avenue Elmwood Park, New Jersey 07407
ATTENTION:	Herman Brown Vice President, Chemical Specialties
TESTS:	Primary Dermal Irritation (Rabbit) Ocular Irritation (Rabbit) Acute Oral Toxicity (Rat)
TEST MATERIAL:	1. Finsolv TN, Lot#1950 C12-15 Alkyl Benzoate
EXPERIMENT REFERENCE NO.:	78426-1

Christine A. Lewis, B.S.
Laboratory Supervisor
Project Director

Allen L. Palanker
President

Date November 2, 1978
CAL/we

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed and neither the repo

This report details :

a primary dermal irritation study and
an ocular irritation study in albino rabbits, and
an acute oral toxicity study in albino rats

performed at the behest of :

Finetex Inc.
418 Falmouth Avenue
Elmwood Park, New Jersey 07407

The test material, supplied by :

Finetex Inc.

was received on :

October 9, 1978

and identified as :

1. Finsolv TN, Lot #1950

and was used as indicated in the Final Report Summaries.

Study Interval : October 13, 1978 to October 31, 1978

All animals are fed and watered ad libitum; with Wayne animal feeds used exclusively. Animals are received from Summit View Farm, Belvidere, New Jersey and are conditioned prior to use. Samples, raw data and final report copies are retained at these laboratories unless otherwise indicated.

Project Director and study supervisor are signatory to this report. Date of submission is recorded on title page. Names of persons performing these tests are recorded on raw data sheets and laboratory log books and are available upon request.

Final Report Summary

DATE: November 2, 1978
CLIENT: Finetex Inc.
STUDY NO.: 78426-1
REFERENCE NO.: H. Brown
MATERIAL: 1. Finsolv TN, Lot#1950

Primary Dermal Irritation

Method: Six rabbits, mixed sex, each abraded and non-abraded, 0.5 ml single application under occluded patch, 24 and 72 hour observation. Material used as received.

Result: Primary Irritation Index:*
0.08

Not a primary dermal irritant to rabbits under the conditions of this test.

* See Table 1A for evaluation.

Final Report Summary

DATE: November 2, 1978
CLIENT: Finetex Inc.
STUDY NO.: 78426-1
REFERENCE NO.: H. Brown
MATERIAL: 1. Finsolv TN, Lot #1950

Ocular Irritation

Method: Six rabbits, mixed sex, 1.8 - 2.4 kg, 0.1 ml single administration, all with no wash for 24 hours and up to seven day observation. Material used as received.

Result:

	Draize Score				
	Day				
Group	1	2	3	4-------7	
No Wash	0.0	0.0	0.0	---	---

Not an ocular irritant to rabbits under conditions of this test.

Final Report Summary

DATE: November 2, 1978
CLIENT: Finetex Inc.
STUDY NO.: 78426-1
REFERENCE NO.: H. Brown
MATERIAL: 1. Finsolv TN, Lot#1950

Acute Oral Toxicity

Method: Albino rats in groups of ten (5M:5F), 188-256g, single dosed orally, observed fourteen days. Material used as received (Specific Gravity:0.95).

Result: LD_{50}: > 5g/kg

Dose Level g/kg	Sex	#dead/#dosed	%
5.0	5M:5F	0/5 : 0/5	0

Not a toxic material orally to rats under conditions of this test.

Gross Pathology: Indication of enlarged spleen noted.

METHOD:

Primary Dermal Irritation in Rabbits

A group of albino New Zealand rabbits as indicated in the summary were used in this study. The test method was essentially that of Draize et.al.[1]

Briefly paraphrased, it consisted of application of 0.5 ml of the test material to clipped areas of intact and abraded skin. The abrasions were longitudinal epidermal incisions sufficiently deep to penetrate the stratum corneum, but not so deep as to destroy the integrity of the derma. Applications were made under occlusive patches (2" x 2" gauze, covered by adhesive tape). Following application of the test material the entire trunk of each animal was covered with an impermeable occlusive wrapping. The wrapping and test material were removed 24 hours following application. The sites were individually examined and scored separately for erythema and edema at 24 and 72 hours. The mean scores for 24 and 72 hour gradings were averaged to determine final irritation indices.

[1]Draize, John H., Woodward, Geoffrey, and Calvery, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", J. Pharm. & Ex. Ther. 82, 377, (1944).

Primary Eye Irritation in Rabbits (FHSA)

New Zealand rabbits as indicated in the summary without ocular defects were used. The procedure followed was a modification of that used by Dr. J.H. Draize in Appraisal of The Safety of Chemicals in Foods, Drugs and Cosmetics, compiled by the staff of the Division of Pharmacology, Food and Drug Administration - Department of Health, Education and Welfare.

In the technique of determining toxicity of substances to eye mucosa, observations of injuries were made on the cornea, iris and the bulbar and palpebral conjunctivae. Numerical scores were assigned to lesions observed according to the Draize[1] standard scoring system. In this system of scoring, the injuries to the cornea and iris account for approximately eighty (80%) percent of the total score; these structures are purposely weighted because of their vital role in vision. Healthy New Zealand rabbits were used for this test. One-tenth of a milliliter of each test substance was instilled in the right eye; the left eye, remaining untreated, served as a control. The treated eyes of all rabbits remained unwashed for 24 hours.

Readings facilitated by hand-held lenses were made 1, 2, and 3 days after treatment, and up to seven (7) days when necessary.

[1]Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", J. Pharm. & Ex. Ther. 82, 377, (1944).

Acute Oral Toxicity in Rats

Acute oral toxicity in rats was determined according to the procedure suggested by Hagan.[1] Wistar-derived albino rats, as indicated in the summary were maintained under standard laboratory conditions for a minimum of seven days, and fasted overnight prior to adminstration of the test material.

The rats were dosed individually by gavage, according to bodyweight, after which they were returned to quarters where food and water were available ad libitum.

Animals were observed for signs of pharmacologic activity and drug toxicity at 1, 3, 6, and 24 hours post-dosage. Observations were made daily thereafter to a total of fourteen days.

Animals sacrificed at the end of the 14-day observation period were subjected to complete gross necropsy.

[1]Hagan, E.C. (1959) Acute Toxicity; Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, pp. 17 - 25.

RESULTS:

Primary Dermal Irritation in Rabbits

The scoring scale used is presented in Table 1. The individual results are presented in Table 2.

Primary Eye Irritation in Rabbits

Table 3 details scoring criteria. The individual results are presented in Table 4.

Acute Oral Toxicity in Rats

Individual results are presented in Table 5.

Summaries of all results are found preceding the text.

Table 1

Scoring Criteria for Skin Reactions

Erythema and Edema Formation	
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Total possible erythema score = 4

Edema Formation	
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Total possible edema score = 4

Total possible primary irritation score = 8

Table 1A

Scale of Interpreting
Primary Dermal Irritation Scores
(Draize - Rabbit)

Score	Interpretation
C	Corrosive - highly dangerous, warning labels must be used
5.0 and above	Primary Dermal Irritant - highly dangerous, warning label must be used
3.0 - 4.9	Potential for severe irritation - warning label would be advised
2.0 - 2.9	Potential for moderate irritation - may be irritating to humans under conditions similar to test (may consider warning label)
1.0 - 1.9	Potential for mild irritation - possibly irritating to some people under occlusive wrap conditions - usually no warning required
0.1 - 0.9	Potential for slight irritation - rarely irritating to people - no warning required
0.0	No irritation potential - no warning required

Table 2

Primary Skin Irritation - Rabbits
SUMMARY OF SCORES FOR SKIN IRRITATION

Finsolv TN, Lot#1950

Rabbit Number	Skin	Hours: 24 E[1]	/	Ed	72 E	/	Ed
1	NA[2]	0		0	0		0
	A[3]	0		0	0		0
2	NA	0		0	0		0
	A	1		0	0		0
3	NA	0		0	0		0
	A	0		0	0		0
4	NA	0		0	0		0
	A	1		0	0		0
5	NA	0		0	0		0
	A	0		0	0		0
6	NA	0		0	0		0
	A	0		0	0		0
Average	NA	0.0		0.0	0.0		0.0
	A	0.3		0.0	0.0		0.0

Combined Averages: 0.3
Primary Irritation Index: 0.08

[1] E/Ed= Erythema and Edema

[2] NA = Non-abraded skin

[3] A = Abraded Skin

Table 3

Eye Irritation Test
Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Grading
Cornea	Opacity (A)	
	Opacity - degree of density (area which is dense is taken for reading)	
	Scattered or diffuse area, details of iris clearly visible.	1
	Easily discernible translucent areas, details of iris slightly obscured.	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible.	3
	Opaque, iris invisible.	4
	Area of Cornea Involved (B)	
	One-quarter (or less), but not zero.	1
	Greater than one-quarter, but less than one-half.	2
	Greater than one-half, but less than three-quarters.	3
	Greater than three-quarters, up to whole area.	4
	Score equals A x B x 5	Total maximum = 80
Iris	Values (A)	
	Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combinations of any thereof), iris still reacting to light.	
	Sluggish reaction is positive.	1
	No reaction to light hemorrhage, gross destruction, (any or all of these).	2
	Score equals A x 5	Total maximum = 10

Table 3 (cont'd.)

Eye Irritation Test
Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Grading
Conjunctivae	Redness (A)	
	Redness (refers to palpebral conjunctivae only). Vessels definitely injected above normal.	1
	More diffuse, crimson red, individual vessels not easily discernible.	2
	Diffuse beefy red.	3
	Chemosis (B)	
	Any swelling above normal (includes nictitating membrane).	1
	Obvious swelling with partial eversion of the lids.	2
	Swelling with lids about half-closed.	3
	Swelling with lids about half-closed to completely closed.	4
	Discharge (C)	
	Any amount different from normal (does not include small amount observed in inner canthus of normal animals).	1
	Discharge with moistening of the lids and hairs just adjacent to the lids.	2
	Discharge with moistening of the lids and hairs and considerable area around eye.	3
	Score equals (A + B + C) x 2	Total maximum = 20

Note: The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctivae.

Table 4

Primary Eye Irritation - Rabbits
SUMMARY OF EYE IRRITATION

Finsolv TN, Lot#1950

Rabbit Number	Day	Cornea: A x B x 5 +		Iris: A x 5 +	Conjunctivae: (A + B + C) x 2 =			Total Scores*
				----No Wash Group----				
1	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-
2	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-
3	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-
4	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-
5	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-
6	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-
Average	1							0.0
	2							0.0
	3							0.0
	4							---
	7							---

*Total score possible/animal/observation interval = 110

Table 5

Acute Oral Toxicity

Finsolv TN, Lot#1950

Dose g/kg	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2	3	4	5	6	7--14	Bodyweight (grams)
5.0	1 M	204	N	N	N	N	N	N	N	N	N	N	356
	2 M	226	N										300
	3 M	194	N										280
	4 M	256	N										358
	5 M	208	N	NO CHANGES OBSERVED									268
	6 F	190	N										234
	7 F	188	N										240
	8 F	204	N										250
	9 F	206	N										258
	10 F	208	N										246

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

Comments: Animal#1: No gross changes observed.
Animal#2-10: No gross changes observed.
Spleens appear enlarged.

Consumer Product Testing

Company Incorporated

Bldg. No. 2-15B
1275 Bloomfield Avenue
Fairfield, New Jersey 07006

(201) 575-7688
(201) 575-7689

F I N A L R E P O R T

CLIENT:	Finetex Inc. 418 Falmouth Avenue Elmwood Park, New Jersey 07407
ATTENTION:	Herman Brown Vice President, Chemical Specialties
TESTS:	Acute Dermal Toxicity (Rabbit) Oral LD_{50} (Rat)
TEST MATERIAL:	Finsolv TN, Lot #1950 C 12-15 Alkyl Benzoate
EXPERIMENT REFERENCE NO.:	78516

Christine A. Lewis

Christine A. Lewis, B.S.
Laboratory Supervisor
Project Director

Allen L. Palanker

Allen L. Palanker
President

Date February 7, 1979
CAL/pap

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any member of its staff, may be used in connection with

This report details :

a dermal toxicity study in albino rabbits, and
an oral LD_{50} study in albino rats

performed at the behest of :

Finetex Inc.
418 Falmouth Avenue
Elmwood Park, New Jersey 07407

The test material, supplied by :

Finetex Inc.

was received on :

November 29, 1978

and identified as :

Finsolv TN, Lot#1950

and was used as indicated in the Final Report Summaries.

Study Interval : December 14, 1978 to February 6,1979

All animals are fed and watered ad libitum; with Wayne animal feeds used exclusively. Animals are received from Summit View Farm, Belvidere, New Jersey and are conditioned prior to use. Samples, raw data and final report copies are retained at these laboratories unless otherwise indicated.

Project Director and study supervisor are signatory to this report. Date of submission is recorded on title page. Names of persons performing these tests are recorded on raw data sheets and laboratory log books and are available upon request.

Final Report Summary

DATE: February 7, 1979
CLIENT: Finetex Inc.
STUDY NO.: 78516
REFERENCE NO.: H. Brown
MATERIAL: Finsolv TN, Lot #1950

Dermal Toxicity

Method: Six rabbits, 1/2 male, 1/2 female, 1/2 abraded and 1/2 non-abraded, 2 g/kg single application under occluded patch, 14 day observation. Material used received. (Specific Gravity: 0.94).

Result: LD_{50}: > 2 g/kg

Dose Level (g/kg)	Sex	# dead/ # dosed	%
2.0	3M:3F	1/3:0/3	17

Not a toxic material dermally to rabbits under conditions of this test.

Consumer Product Testing Company, Inc.

Final Report Summary

DATE: February 7, 1979
CLIENT: Finetex Inc.
STUDY NO.: 78516
REFERENCE NO.: H. Brown
MATERIAL: Finsolv TN, Lot #1950

Oral LD_{50}

Method: Albino rats in groups of six (3M:3F), 174-252g, single dosed orally, (40 ml/kg top dose mechanically feasible) observed fourteen days. Material used as received. (Specific Gravity:0.94).

Result: LD_{50}: 34.5 (32.54-36.57) g/kg

	Dose Level (g/kg)	Sex	#dead/#dosed	%
Range Finding:	0.5	1M	0/1	0
	1.0	1M	1/1	100*
	3.0	1F	0/1	0
	5.0	1F	0/1	0
	10.0	1F	0/1	0
	25.0	1F	0/1	0
	40.0	1F	0/1	0
Test Dosage:	30.0	3M:3F	0/3:0/3	0
	33.0	3M:3F	2/3:2/3	67
	37.0	3M:3F	3/3:2/3	83
	40.0	3M:3F	1/3:3/3	67

Not a toxic material to rats under the conditions of this test.

* Probable non-dose related death.

Consumer Product Testing Company, Inc.

METHOD:

Acute Dermal Toxicity in Rabbits

A modification of the techniques described in Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, compiled by the staff of the Division of Pharmacology, Food and Drug Administration, was followed. Healthy albino rabbits as indicated in the summary were used. The animals were prepared by clipping the skin of the trunk free of hair. One-half of the animals were further prepared by introducing epidermal abrasions over the clipped skin surface, thus enhancing penetrability of the test material through the stratum corneum.

A single dermal application as indicated, according to bodyweight was used. The trunk of each animal was encased in a sleeve of plasticized material to insure contact of the test material and the skin for a 24 hour period.

Following the 24 hour exposure period the sleeve was removed and the skin sites gently cleansed. All animals were then observed for mortality, skin response and general behavior for 14 days subsequent to exposure to the test material.

Acute Oral Toxicity in Rats (LD_{50})

Acute oral toxicity in rats was determined according to the procedure suggested by Hagan.[1] Wistar-derived albino rats, as indicated in the summary were assigned to groups, and maintained under standard laboratory conditions for a minimum of seven days, and fasted overnight prior to administration of the test material.

The rats were dosed individually by gavage, at graded dose levels (following range finding) after which they were returned to quarters where food and water were available ad libitum.

Animals were observed for signs of pharmacologic activity and drug toxicity at 1, 3, 6 and 24 hours post-dosage. Observations were made daily thereafter to a total of fourteen days.

Animals sacrificed at the end of the 14-day observation period were subjected to complete gross necropsy.

LD_{50} was calculated, (including the 95% confidence limits) where possible, using the method of Litchfield and Wilcoxin[2] except when the highest maximum dose mechanically possible (40 ml/kg) is used.

[1]Hagan, E.C. (1959) Acute Toxicity; Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, pp. 17 - 25.

[2]Litchfield, J.T. and Wilcoxin, F. (1949) J. Pharmacol. Exptl. Therap. pp. 96, 99.

RESULTS:

Acute Dermal Toxicity in Rabbits

Individual results are presented in Table 1.

Oral LD_{50} in Rats

Individual results are presented in Tables 2-6.

Summaries of all results are found preceding the text.

Table 1

Acute Dermal Toxicity in Rabbits

Finsolv TN, Lot#1950

Dose g/kg	Animal Number and Sex	Bodyweight (kg)	Hours: 1	3	6	24 *	Days: 2	3	4	5	6	7--14	Bodyweight (kg)
2.0	1Ma	1.50	N	N	N	N	N	N	N	N	N	+	0.92
	2M	1.60	N	N	N	N	N	N	N	N	N	N	1.88
	3Ma	1.66	N	N	N	N	N	N	N	N	N	N	1.75
	4F	1.87	N	N	N	N	N	N	N	N	N	N	1.84
	5Fa	1.51	N	N	N	N	N	N	N	N	N	N	1.80
	6F	1.74	N	N	N	N	N	N	N	N	N	N	1.32

a = abraded skin

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
\+ = Animal Death

Comments: Animal #1: Skin pliable and non-irritated. No gross changes observed. (Died on day 13).
#2-#6: Skin pliable and non-irritated. No gross changes observed.

* Draize scores at 24 hours: Animal #1 - 1/1
2 - 1/1
3 - 2/2
4 - 2/2
5 - 2/1
6 - 2/1

Table 2

Acute Oral Toxicity

Finsolv TN, Lot # 1950

Range Finding:

Dose g/kg	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2	3	4	5	6	7--14	Bodyweight (grams)
0.5	1 M	196	N	N	N	N	N	N	N	N	N	N	344
1.0	2 M	202	N	N	N	N	N	N	N	N	N	+	226
3.0	3 F	212	N	N	N	N	N	N	N	N	N	N	286
5.0	4 F	196	N	N	N	N	N	N	N	N	N	N	260
10.0	5 F	196	N	SD	SD	SD*	SD*	N*	N*	N	SD	N	246
25.0	6 F	200	N	SD	SD	SD*	SD*	N*	N*	SD	SD	N	242
40.0	7 F	182	SD	SD	D	SD*	SD*	N*	N*	SD	SD	N	238

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

* Hair matted and moist

Comments: Animal #1, #3, #4-#7: No gross changes observed.
#2: Fibrous tissue encasing heart and lungs.
(Died on day 13).

Table 3

Acute Oral Toxicity

Finsolv TN, Lot #1950

Dose g/kg	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2*	3*	4*	5*	6*	7--14	Bodyweight (grams)
30.0	1 M	250	SD	SD	N	D*	D	D	D	D	D	D SD	200
	2 M	196	SD	SD	N	D*	D	D	D	D	D	D SD	244
	3 M	188	SD	SD	N	D*	D	D	D	D	D	D SD	246
	4 F	192	SD	SD	SD*	SD*	SD	SD	D	D	SD	SD N	210
	5 F	194	SD	SD	SD*	SD*	SD	SD	D	D	SD	SD N	246
	6 F	198	SD	SD	SD*	SD*	SD	SD	D	D	SD	SD N	240

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

Comments: Animal #1: Marked loss of hair on ventral portion of body, all lung tissue consolidated.
#2: Marked loss of body hair. No gross internal changes observed.
#3: Marked loss of ventral body hair. No gross internal changes observed.
#4-#6: No gross internal changes observed.

*Hair matted and moist (All animals for days 2-7).

Table 4

Acute Oral Toxicity

Finsolv TN, Lot #1950

Dose g/kg	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6*	24*	Days: 2	* 3	* 4*	5*	6*	7-14		Bodyweight (grams)
33.0	1 M	252	SD	SD	SD	D	SD	SD	SD	SD	D	SD+	-	164
	2 M	222	SD	SD	SD	D	SD	SD	SD	SD	D	SD+	-	188
	3 M	244	SD	SD	SD	D	SD	SD	SD	SD	D	SD	N	300
	4 F	204	SD	SD	SD	D	SD	SD	SD	SD	D	+	-	148
	5 F	208	SD	SD	SD	D	SD	SD	+	-	-	-	-	180
	6 F	224	SD	SD	SD	D	SD	SD	SD	SD	D	SD	N	228

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

Comments: Animal #1: Hair matted and unkempt. Crust-like substance covering entire skin surface. Pyloric and intestinal mucosa distended and gas filled. (Died on day 8).
#2: Hair matted and unkempt. Crust-like substance covering entire skin surface. No gross internal changes.(Died on day 8).
#3: Hair loss on all areas of body. No gross internal changes observed.
#4: Pyloric and intestinal mucosa moderately reddened. (Died on day
#5: Hair matted and unkempt. No gross changes observed.
#6: Near complete loss of hair on all body surfaces. Small scabs covering dorsum. No gross internal changes observed.

* Hair matted and moist for all animals at 6 and 24 hours, and 2-7 days.

Table 5

Acute Oral Toxicity

Finsolv TN, Lot #1950

Dose g/kg	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2	3	4	5	6	7--14	Bodyweight (grams)
37.0	1 M	220	SD	SD	SD*	SD*	+	-	-	-	-	- -	186
	2 M	250	SD	SD	SD*	SD*	SD*	SD*	SD*	+	-	- -	160
	3 M	244	SD	SD	SD*	SD*	SD*	SD*	SD*	D*	D*	SD* +	162
	4 F	240	SD	SD	SD*	SD*	SD*	SD*	SD*	D*	D*	+ -	190
	5 F	238	SD	SD	SD*	SD*	SD*	SD*	SD*	D*	D*	SD* N	244
	6 Fa	218	SD	SD	SD	SD*	+	-	-	-	-	- -	190

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

Comments: Animal #1: Hair matted and unkempt. No gross internal changes.
#2: Hair matted and unkempt. Pyloric and intestinal mucosa moderately reddened.
#3: Hair matted and unkempt. Pyloric and intestinal mucosa moderately reddened. (Died on day 12).
#4: Hair matted and unkempt. All lung tissue enlarged and consolidated. (Died on day 7).
#5: Near complete hair loss on all body surfaces. Small scabs noted on ventrum. All lobes of lungs contain roughly spherical lesions from 0.5 to 2 cm. in diameter, firm, some containing yellow white pus.
#6a: Hair matted and unkempt. Pyloric and intestinal mucosa severely reddened.

* Hair matted and moist

Original animal number 6 replaced: misdirected dose

Table 6

Acute Oral Toxicity

Finsolv TN, Lot # 1950

Dose g/kg	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2	3	4	5	6	7--14	Bodyweight (grams)
40.0	1 M	174	SD	SD	SD	SD*	N*	N*	N*	SD*	SD*	SD*SD	258
	2 Ma	192	SD*	SD*	SD*	N*	D*	+	-	-	-	- -	168
	3 M	194	SD	SD	SD	SD*	N*	N*	N*	SD*	SD*	SD*SD	280
	4 F	190	SD	SD	SD*	D*	D*	XD*	+	-	-	- -	168
	5 F	178	SD	SD	SD*	D*	D	XD*	+	-	-	- -	180
	6 F	200	SD	SD	SD*	D*	+	-	-	-	-	- -	170

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

* Hair moist

Comments: Animal #1, #3: No gross changes observed.
#2a: Hair moist and matted. Intestinal mucosa slightly reddened. No other gross changes observed.
#4: Intestinal mucosa moderately reddened.
#5: No gross changes observed.
#6: Hair moist and matted. No gross internal changes apparent.

Original animal number 2 replaced: misdirected dose

RECEIVED MAY 2 4 1979

Consumer Product Testing

Company Incorporated

Bldg. No. 2-15B
1275 Bloomfield Avenue
Fairfield, New Jersey 07006

(201) 575-7688
(201) 575-7689

FINAL REPORT

CLIENT: Finetex Inc.
418 Falmouth Avenue
Elmwood Park, New Jersey 07407

ATTENTION: Herman Brown
Vice President, Chemical Specialties

TESTS: Acute Inhalation Toxicity (Rat)
Guinea Pig Sensitization

TEST MATERIAL: Finsolv TN, Lot No. 24, 755
C 12-15 Alkyl Benzoate

EXPERIMENT REFERENCE NO.: 7950

Christine A. Lewis, B.S.
Laboratory Supervisor
Project Director

Allen L. Palanker
President

Date May 7, 1979
CAL/pap

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed and neither the report nor the name of these Laboratories nor of any member of its staff may be used in connection with the advertising or sale of any product

This report details :

an acute inhalation study in albino rats, and
a guinea pig sensitization study

performed at the behest of :

Finetex Inc.
418 Falmouth Avenue
Elmwood Park, New Jersey 07407

The test material, supplied by :

Finetex Inc.

was received on :

February 7, 1979

and identified as :

Finsolv TN, Lot No. 24, 755

and was used as indicated in the Final Report Summaries.

Study Interval : February 16, 1979 to April 27, 1979

All animals are fed and watered ad libitum; with Wayne animal feeds used exclusively. Animals are received from Summit View Farm, Belvidere, New Jersey and are conditioned prior to use. Samples, raw data and final report copies are retained at these laboratories unless otherwise indicated.

Project Director and study supervisor are signatory to this report. Date of submission is recorded on title page. Names of persons performing these tests are recorded on raw data sheets and laboratory log books and are available upon request.

Final Report Summary

DATE: May 7, 1979
CLIENT: Finetex Inc.
STUDY NO.: 7950
REFERENCE NO.: H. Brown
MATERIAL: Finsolv TN, Lot No. 24,755

Acute Inhalation Toxicity

Method: Albino rats in groups of ten (5M:5F), 200-246g, exposed to concentrations of 200 mg/liter for one hour, observed two weeks. Material used as received.

Result: LC_{50} > 200 mg/l

Dose Level (mg/l)	Sex	# dead/ # dosed	%
200	5M:5F	1/5:0/5	10

Not a toxic material by inhalation to rats under the conditions of this test.

Consumer Product Testing Company, Inc.

Final Report Summary

DATE: May 7, 1979
CLIENT: Finetex Inc.
STUDY NO.: 7950
REFERENCE NO.: H. Brown
MATERIAL: Finsolv TN, Lot No. 24,755

Guinea Pig Sensitization (Buehler)

Method: Twelve guinea pigs (male) 260-352 g, 9 sensitizing topical, occluded applications (6 hours/day) over 21 day period, two challenge applications 14 days later, to determine local and systemic effects. Since nonirritating concentrations were used, any reaction indicated positive response; the severity of which was proportional to the score obtained. Material used in 10% gravimetric aqueous suspension.

Result:	Topical Challenge	Systemic Challenge
	No positive reactions	No positive reactions

Not a potential sensitizer to guinea pigs under conditions of this test.

Consumer Product Testing Company, Inc.

METHOD:

Acute Inhalation Toxicity in Rats

A group of adult Wistar-derived albino rats as indicated in the summary, was housed in a three-cubic-foot plastic chamber for the single one (1) hour dynamic exposure period. The chamber was initially charged with test material at a concentration of 200 mg/l. Preconditioned supportive air was supplied at five (5) liters per minute, and the test material was presented from a compressor activated generator or timer operated solenoid to continue a nominal chamber concentration of 200 mg/liter air, over the one hour period.

At the end of the one-hour period, the animals were returned to individual quarters and observed daily for two weeks after which time they were sacrificed and autopsied.

Guinea Pig Sensitization (Buehler)

White male guinea pigs subsisting on a commercial guinea pig diet were identified, and hair removed from back and flanks by close clipping. Prior to testing for sensitization, the primary irritation threshhold was determined and a concentration in an appropriate vehicle below that which caused the least irritation response was then used to sensitize. A 3" x 3" gauze pad wetted with the test solution was applied to the shaved back, occluded with 2" wide Dermicel hypoallergenic adhesive tape and patches were left on the animal for 6 hours. The applications were made three times weekly, until a total of nine had been made. The nine sensitizing applications were made along the dorsal midline of the back. This area measures three or four centimeters square. The retest application was made in the same area as well as ventrally to determine systemic as well as local effects. All applications consisted of 0.5 ml of test material. Two weeks after the ninth application, the retest applications were made. Challenge sites were graded on a scale of zero to four, with four representing a severe erythematous or edematous response. The presence of edema was also noted. Since a non-irritating concentration of the sensitizer was used, any reaction at challenge was considered positive; the severity of which was proportional to the score obtained.

RESULTS:

Acute Inhalation Toxicity

Individual results are presented in Table 1.

Guinea Pig Sensitivity

Individual results are presented in Tables 2 and 3.

Summaries of all results are found preceding the text

Table 1

Acute Inhalation Toxicity

Finsolv TN, Lot No. 24, 755

Dose mg/l	Animal Number and Sex	Bodyweight (grams)	Hours: 1	3	6	24	Days: 2	3	4	5	6	7--14		Bodyweight (grams)
200	1 M	202	SD	N	N	N	N	N	N	N	N	N+	-	156
	2 M	230	SD	N	N	N	N	N	N	N	N	N	N	296
	3 M	232	SD	N	N	N	N	N	N	N	N	N	N	324
	4 M	230	SD	N	N	N	N	N	N	N	N	N	N	290
	5 M	246	SD	N	N	N	N	N	N	N	N	N	N	312
	6 F	204	SD	N	N	N	N	N	N	N	N	N	N	222
	7 F	200	SD	N	N	N	N	N	N	N	N	N	N	226
	8 F	200	SD	N	N	N	N	N	N	N	N	N	N	262
	9 F	200	SD	N	N	N	N	N	N	N	N	N	N	210
	10 F	200	SD	N	N	N	N	N	N	N	N	N	N	220

N = Normal
D = Depression
SD = Slight Depression
XD = Severe Depression
+ = Animal Death

Comments: Animal #1: All lobes of right lung enlarges and consolidated. (Died on day 9).
#2-#10: No gross changes observed.

Table 2

Guinea Pig Screen
(Buehler)

Finsolv TN, Lot No. 24, 755

Animal Number	Concentration	Scores: 16 Hours	40 Hours
1	50% v/v Aqueous	0/0	0/0
2	25% v/v Aqueous	1/0	1/0
3	10% v/v Aqueous	0/0	0/0
4	5% v/v Aqueous	0/0	0/0

Individual Results
Guinea Pig Sensitization
(Buehler)

Finsolv TN, Lot No. 24, 755

Dosed at: (concentration): 10%
Vehicle: Water

Topical Application No.	Volume (ml)	Animal Number 1a Er/Ed[1]	2a Er/Ed	3 Er/Ed	4 Er/Ed	5 Er/Ed	6 Er/Ed
1	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
2	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
3	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
4	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
5	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
6	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
7	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
8	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
9	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
[2]Dorsal Challenge	6 hrs.	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	0/0	0/0	0/0	0/0	0/0	0/0
Ventral Challenge	6 hrs.	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	0/0	0/0	0/0	0/0	0/0	0/0

[1]Draize Scale 0-4

[2]Dorsal Challenge for topical effects
Ventral Challenge for systemic effects

Finsolv TN, Lot No. 24, 755

Dosed at: (concentration): 10%
Vehicle: Water

Topical Application No.	Volume (ml)	Animal Number 7 Er/Ed[1]	8 Er/Ed	9a Er/Ed	10a Er/Ed	11 Er/Ed	12 Er/Ed
1	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
2	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
3	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
4	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
5	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
6	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
7	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
8	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
9	.5	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	---	---	---	---	---	---
[2]Dorsal Challenge	6 hrs.	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	0/0	0/0	0/0	0/0	0/0	0/0
Ventral Challenge	6 hrs.	0/0	0/0	0/0	0/0	0/0	0/0
	24 hrs.	0/0	0/0	0/0	0/0	0/0	0/0

[1]Draize Scale 0-4

[2]Dorsal Challenge for topical effects
Ventral Challenge for systemic effects

Table 5
(continued)
Individual Results

Autopsy Comments

Comments: Animal #1a,#2a,#3-#8,#9a,#10a,#11,#12: No gross changes observed.

Original animal number 1 replaced:	died one day after ninth application; inferior portion of left lung consolidated.
Original animal number 2 replaced:	died on eighth application day; fibrous-filled sac encasing heart and right lung; whitish yellow in color.
Original animal number 9 replaced:	died after second application day; fibrous tissue throughout abdominal cavity.
Original animal number 10 replaced:	died on eighth application day; fibrous tissue encasing heart and lungs.

Consumer Product Testing

Company Incorporated

Bldg. No. 2-158
1275 Bloomfield Avenue
Fairfield, New Jersey 07006

(201) 575-7688
(201) 575-7689

FINAL REPORT

CLIENT: Finetex, Inc.
PO Box 216
Elmwood Park, New Jersey 07407

ATTENTION: Herman Brown

TEST: Repeat 14-Day Dermal Irritation Study in Rabbits

TEST ARTICLE: FINSOLV TN, (Patented), LOT # 31492

C12-15 Alkyl Benzoate

EXPERIMENT REFERENCE NO.: 88420

Steven Nitka
Laboratory Director
Vice President

Date November 16, 1988
SN/gt

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor of any member of its staff, may be used in connection with the advertising or sale of any product or process without written authorization

Consumer Product Testing
Company Incorporated
132 West Greenbrook Road
North Caldwell, New Jersey 07006-4729
(201) 403-0303

QUALITY ASSURANCE UNIT SUMMARY

Study No.: 88420

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance to standard operating procedures and standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies lasting six months or more are inspected every three months; and studies lasting less than six months are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and Study Director. All materials and data pertinent to this study will be stored in the Archives Facility, unless specified otherwise, in writing, by the sponsor.

Date(s) of inspections: September 21, 1988
October 12, 1988
October 19, 1988
October 26, 1988
November 21, 1988

Professional personnel involved:

Steven Nitka, B.S.	- Vice President Laboratory Director (Study Director)
Jill Powers, B.S.	- Laboratory Supervisor
Douglas Beach, B.S.	- Technician
Lillian Deniza, B.S.	- Technician
Garry Innocent, B.S.	- Technician
Joseph F. Kleha, B.S.	- Technician
Michele P. Weitz, A.A.	- Technician
Kathleen R. Paladino	- Animal Care Supervisor
Deborah A. Worman	- General Manager Member, Quality Assurance Unit
Gwendolyn Hill	- Secretary - Archivist Member, Quality Assurance Unit

The following has been assured by signing below that this study has been performed in accordance with standard operating procedures, study procotols and the Good Laboratory Practice principles.

Melvin F. Weiss
Acting Director,

Consumer Product Testing
Company Incorporated
132 West Greenbrook Road
North Caldwell, New Jersey 07006-4729
(201) 403-0303

Final Report Summary

DATE: November 16, 1988
CLIENT: Finetex Inc.
STUDY NO.: 88420
REFERENCE: H. Brown
TEST ARTICLE: FINSOLV TN, (Patented), LOT # 31492
TEST ARTICLE RECEIPT DATE: September 15, 1988
STUDY INTERVAL: October 6, 1988 to October 20, 1988

14-Day Repeat Dermal in Rabbits

Method: Ten (10) New Zealand white rabbits each received daily applications of 0.5 milliliter of the test article, at two (2) concentrations, isopropyl myristate and mineral oil, on four (4) test sites, all intact. The applications were carried out for 14 consecutive days. The test sites were scored individually for erythema, edema and other effects prior to each application and approximately 24 hours after the last application. Mean combined erythema and edema scores were determined per test and control article, per animal, per day. The test article was used as received and as a 62% gravimetric corn oil suspension. The mineral oil and isopropyl myristate were used as purchased.

Average Combined Erythema and Edema Score/Animal/Day

Test Article (neat):	4.64
Test Article (62% w/v c.o.):	4.11
Mineral Oil:	2.68
Isopropyl Myristate:	5.40

14-Day Repeat Dermal in Rabbits

This test was designed to determine the cumulative dermal effects of substances on rabbit skin.

Ten (10) New Zealand white rabbits, ½ male, weighing approximately 2 kilograms and about 3 months of age were obtained from a suitably licensed dealer. Animals were checked carefully upon receipt for diarrhea and dehydration, respiratory difficulties, postural deficiencies, and general condition.

Animals were acclimated at least 7 days prior to test initiation. They were individually housed in galvanized or stainless steel cages, in a room with a 12 hour light/dark cycle. The room temperature was controlled to comply with Animal Welfare Regulations with an approximate range of 65° to 75° F. The humidity was also monitored. Diet consisted of Agway Pro-Pet Big Red Rabbit Feed, as well as water, ad libitum. Animals were identified through individual markings on the outer ear of each animal, as well as a cage label. Purina Liquid Sulfa-Nox, (Water Medication), was administered on the day of delivery and on the following four days. This medication aids in the prevention of coccidiosis, accentuated by the process of delivery. The medication may have been administered after the initial five days, if symptoms occurred.

Twenty-four (24) hours prior to test initiation, the animals were reexamined. Any animals in poor condition, and particularly animals with skin eruptions or dermal lesions, were not used. Animals were prepared for testing by close-clipping the skin of the mid-dorsal area of the trunk, between the scapulae and the pelvis, using an Oster small animal clipper equipped with a #40 (surgical) head.

Immediately prior to test initiation, the animals were weighed and placed in wooden restrainers. The shaved dorsal surface of each animal was divided into four quadrants. All test sites remained intact. Each site on each animal was assigned a test or control article as follows:

Test Site #1: Test article (neat)

Test Site #2: Test article (62% w/v corn oil)

Test Site #3: Mineral Oil (Medi Mart Drug Stores)

Test Site #4: Isopropyl Myristate (Eastman Kodak Company)

All sites were initially examined and scored according to the attached Draize scale. Each animal was observed for pharmacotoxic signs. A single application of one-half (0.5) of a milliliter of the respective article was then made to each test site. No wrappings were used. This procedure was carried out daily, for 14 consecutive days. Approximately 24 hours following the fourteenth dosage, the animals were observed and all sites were scored for the final time. Terminal bodyweight were recorded. All animals were then scacrificed with an injection of T-61® Euthanasia Solution, American Hoechst Corportation. Gross necropsies were performed with all finding noted.

Following the final reading, the combined erythema and edema scores for each site were averaged to determine the average combined erythema and edema score per site, per animal, per day.

Repeat 14-Day Dermal Irritation in Rabbits

The scoring scale used is presented in Table 1. The individual test results are presented in Table 2.

Summaries of all results are found preceding the text.

Table 1

Scoring Criteria for Skin Reactions

Erythema Formation	
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Total possible erythema score = 4

Edema Formation	
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema raised more than 1 mm and extending beyond area of exposure)	4

Total possible edema score = 4

Total possible primary irritation score = 8

Table 1
(continued)

Scoring Criteria for Skin Reactions - Addendum

Notation	Lesion/Condition	Description
B	Blanching	Loss of color; skin is left pale, grey-white.
	Blister	See vesicle.
Bu	Bulla	A vesicle greater than 1 cm in diameter.
C	Crust	Scab. Dried exudate on the surface of a lesion.
D	Dry	Skin feels dry to the touch (dehydrated).
Dy	Dye	Dye from the test article remains after excess removed. (Noted because it may cause difficulty in scoring.)
E	Eschar	A slough.
F	Fissure	A linear cleavage into the epidermis, or through epidermis into dermis. May be single or multiple tiny cracks, or large clefts.
P	Pustule	Small circumscribed elevation of skin filled with pus, usually yellow.

Table 1
(continued)

Scoring Criteria for Skin Reactions - Addendum

Notation	Lesion/Condition	Description
R	Red ring	Red ring formed around test site where blanching and possible necrosis / irreversible damage observed at 24 and/or 72 hours. Ring usually forms between 5 to 7 days, indiciative of irreversible damage.
	Scab	See crust.
S	Scale	Accumulation of loose fragments of horny layer of skin (stratum corneum). Peeling. Only uppermost layer involved.
Sc	Scar	An area of fibrous tissue that has replaced damaged dermis or subcutaneous tissues. Found after a crust has sloughed off. Usually does not develop within 72 hours.
U	Ulcer	A break in the continuity of epidermis with exposure of the underlying dermis. An 'open sore'. If test induced, indiciates a 'corrosive' compound. Score test site as appears, note U.
V	Vesicle	Sharply circumscribed elevation of skin filled with clear, free fluid, up to 1 cm in diameter.

Table 2

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 0	1	2	3	4	5	6	7
1	M	1	0/0	2/0	2/0	2/0	2/2	2/2	2/2	2/2
	M	2	0/0	2/0	2/0	2/0	2/1	2/2	2/2	2/2
	M	3	0/0	1/0	1/0	1/0	1/0	1/1	1/0	1/0
	M	4	0/0	2/0	2/1	2/1	2/2	2/3	3/3F,S	4/4F,S
2	M	1	0/0	2/1	2/1	2/1	2/2	2/2F,S	2/2F,S	2/2
	M	2	0/0	2/1	2/1	2/1	2/1	2/1	2/2	2/2
	M	3	0/0	2/1	2/1	2/1	2/1	2/1	2/1	2/1
	M	4	0/0	2/1	2/1	2/1	2/1	2/2F,S	3/2F,S	3/3F,S
DAILY OBSERVATIONS										
1			N	N	N	N	N	N	N	N
2			N	N	N[4]	N[4]	N	N	N	N

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[1]
[2]Collar removed; animal has developed diarrhea.
[3]Collar removed; animal has lost considerable amount of weight.
[4]Collar removed; caught in animal's mouth.
[4]Animal shaved.

Comments: Animal #1, #2: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 8	9	10	11	12	13	14	Bodyweight (kg) Day 0	Day 14 (Term
1	M	1	2/3	2/3	2/3	2/3	2/3	2/3F	2/3F	2.19	2.58
	M	2	2/2	2/2	2/2	2/2	2/2	2/2S	2/2S		
	M	3	1/0	1/0	1/0	2/1	2/1	2/1	2/1		
	M	4	4/4F,S	3/4F,S	4/4F,S	3/4F,S	3/4F,S	3/4F,S	3/4F,S		
2	M	1	2/2	3/3	3/2F	3/2F	3/2F,S	3/3F,S	3/3F,S	2.51	2.77
	M	2	2/2	2/2	2/2	2/2	2/2	3/2S	3/2S		
	M	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	M	4	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S		
DAILY OBSERVATIONS											
1			N	N	N	N	N	N	N		
2			N	N	N	N	N	N	N		

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[1][2]Collar removed; animal has developed diarrhea.
[3]Collar removed; animal has lost considerable amount of weight.
[4]Collar removed; caught in animal's mouth.
[4]Animal shaved.

Comments: Animal #1, #2: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 0	1	2	3	4	5	6	7
3	M	1	0/0	2/0	2/1	2/1	2/1	2/2F,S	2/3F,S	2/3F,S
	M	2	0/0	2/0	2/1	2/1	2/1	2/2	2/2	2/2
	M	3	0/0	2/1	2/0	2/0	2/0	2/1	2/1	2/1
	M	4	0/0	2/1	2/1	2/1	2/1	2/3F,S	3/3F,S	3/3F,S
4	M	1	0/0	2/0	2/1	2/1	2/1	2/2	2/2F,S	2/2F,S
	M	2	0/0	2/1	2/1	2/1	2/1	2/2	2/2	2/2
	M	3	0/0	2/1	1/1	2/1	2/0	2/1	2/1	2/1
	M	4	0/0	2/1	2/1	2/0	2/1	2/2	3/3F,S	3/3F,S
3 DAILY			N	N	N[4]	N	N	N	N[4]	N
4 OBSERVATIONS			N	N	N[4]	N	N	N	N[4]	N

Raw Data Page 577

[1]Collar removed; animal has developed diarrhea.
[2]Collar removed; animal has lost considerable amount of weight.
[3]Collar removed; caught in animal's mouth.
[4]Animal shaved.

Comments: Animal #3, #4: No gross changes observed.

Table 2
(continued)

21-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 8	9	10	11	12	13	14	Bodyweight (kg. Day 0	Day 14 (Te
3	M	1	2/3F,S	2/3F,S	2/3F,S	2/3F,S	2/3F,S	3/3F,S	3/3F,S	2.56	2.44
	M	2	2/2	2/3	2/3	2/3	2/3	3/3F,S	3/2F,S		
	M	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	M	4	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/4F,S	3/4F,S	3/4F,S		
4	M	1	2/2F,S	2/2F,S	2/2F,S	2/2F,S	2/2F,S	3/3F,S	3/3F,S	2.49	2.32
	M	2	2/2	2/2	2/2	2/2	2/2	3/2F,S	3/2F,S		
	M	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	M	4	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/4F,S	3/4F,S		
3 DAILY			N[1]	N[1]	N	N[1]	N	N	N		
4 OBSERVATIONS			N[1]	N[1]	N	N[1]	N	N	N		

Raw Data Page 577

[1]Collar removed; animal has developed diarrhea.
[2]Collar removed; animal has lost considerable amount of weight.
[3]Collar removed; caught in animal's mouth.
[4]Animal shaved.

Comments: Animal #3, #4: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 0	1	2	3	4	5	6	7
5	M	1	0/0	2/1	2/1	2/1	2/1	2/3	3/3F,S	3/3F,S
	M	2	0/0	2/1	2/1	2/1	2/1	2/3	2/2	2/2
	M	3	0/0	2/0	1/0	1/0	2/0	2/1	2/1	2/1
	M	4	0/0	2/0	2/1	2/1	2/1	2/2F,S	3/3F,S	3/3F,S
6	F	1	0/0	2/0	2/1	2/1	2/1	2/2	2/2	2/2
	F	2	0/0	2/0	2/1	2/1	2/1	2/2	2/2	2/2
	F	3	0/0	2/0	1/0	2/0	2/0	2/1	2/1	2/1
	F	4	0/0	1/0	2/1	2/1	2/2F	2/2F,S	2/2F,S	2/2F,S
5 DAILY			N	N	N[4]	N	N[1]	N	N[4]	N[1,4]
6 OBSERVATIONS			N	N	N[4]	N	N[1]	N1	N[1,4]	N[1,4]

Raw Data Page 578

[1]Collar removed; animal has developed diarrhea.
[2]Collar removed; animal has lost considerable amount of weight.
[3]Collar removed; caught in animal's mouth.
[4]Animal shaved.

Comments: Animal #5, #6: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 8	9	10	11	12	13	14	Bodyweight (kg) Day 0	Day 14 (T
5	M	1	3/3F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	2.88	2.71
	M	2	2/2	4/3F,S	4/3F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S		
	M	3	2/1	2/2	2/2	2/2	2/2	2/2	2/2		
	M	4	3/3F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S		
6	F	1	2/2	2/3	2/3	2/3S	3/3S	3/3F,S	3/3F,S	2.50	2.52
	F	2	2/2	2/2	2/2	2/2S	2/2S	3/3S	3/3S		
	F	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	F	4	2/2F,S	3/2F,S	3/2F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S		
5 DAILY			N[1]	N	N	N	N	N	N		
6 OBSERVATIONS			N[1]	N	N	N	N	N	N		

Raw Data Page 578

[1]Collar removed; animal has developed diarrhea.
[2]Collar removed; animal has lost considerable amount of weight.
[3]Collar removed; caught in animal's mouth.
[4]Animal shaved.

Comments: Animal #5, #6: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN. (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 0	1	2	3	4	5	6	7
7	F	1	0/0	2/0	2/1	2/1	2/1	2/3	2/2F,S	2/2
	F	2	0/0	2/0	2/1	2/1	2/1	2/2	2/2	2/2
	F	3	0/0	1/0	2/0	2/0	2/1	2/1	2/1	2/1
	F	4	0/0	1/0	2/1	2/1	2/1	2/2	3/3F,S	3/3F,S
8	F	1	0/0	2/0	2/2	2/1	2/1	2/2	2/2	2/2
	F	2	0/0	2/0	2/2	2/1	2/1	2/2	2/2	2/2
	F	3	0/0	1/0	2/0	2/0	2/0	2/1	2/1	2/1
	F	4	0/0	1/0	2/1	2/1	2/2	3/3	3/3	3/3F,S
DAILY OBSERVATIONS										
7			N	N	N[4]	N	N	N	N[4]	N
8			N	N	N[4]	N	N	N	N[4]	N

Raw Data Page 579

[1]
[2]Collar removed; animal has developed diarrhea.
[3]Collar removed; animal has lost considerable amount of weight.
[4]Collar removed; caught in animal's mouth.
Animal shaved.

Comments: Animal #7, #8: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 8	9	10	11	12	13	14	Bodyweight (kg) Day 0	Day 14 (Te
7	F	1	2/2	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S	2.41	2.82
	F	2	2/2	2/2	2/2	3/2	3/2S	3/2F,S	3/2F,S		
	F	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	F	4	3/3F,S	3/3F,S	3/3F,S	3/3F,S	4/3F,S	4/3F,S	4/3F,S		
8	F	1	2/2	2/2	2/2	3/2S	3/2S	3/2F,S	3/2F,S	2.18	2.45
	F	2	2/2	2/2	2/2	2/2S	2/2S	2/2	2/2		
	F	3	2/1	2/2	2/2	2/1	2/1	2/2	2/2		
	F	4	3/3F,S	3/3F,S	3/4F,S	3/3F,S	3/3F,S	3/3F,S	3/3F,S		
7 DAILY			N	N	N	N	N	N	N		
8 OBSERVATIONS			N	N	N	N	N	N	N		

Raw Data Page 579

[1]
[2]Collar removed; animal has developed diarrhea.
[3]Collar removed; animal has lost considerable amount of weight.
[4]Collar removed; caught in animal's mouth.
Animal shaved.

Comments: Animal #7, #8: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented), LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 0	1	2	3	4	5	6	7
9	F	1	0/0	2/0	2/1	2/1	2/1	3/2F,S	2/2F,S	2/2
	F	2	0/0	2/0	2/1	2/0	2/1	3/2F	2/2F,S	2/2
	F	3	0/0	1/0	2/0	2/0	2/0	2/1	2/1	2/1
	F	4	0/0	1/0	2/1	2/1	2/1	4/3F,S	4/3F,S	4/3F,S
10	F	1	0/0	2/0	2/1	2/1	2/1S	2/2F,S	3/2F,S	4/4F,S
	F	2	0/0	2/0	2/1	2/1	2/1	2/2S	2/2F,S	2/3F
	F	3	0/0	2/0	2/0	2/0	2/0	2/0	2/1	2/1
	F	4	0/0	1/0	2/1	2/1	2/2F	4/2F,S	4/3D,F,S	4/3F,S
DAILY OBSERVATIONS										
9			N	N	N[4]	N	N	N	N[4]	N
10			N	N	N[4]	N	N	N	N	N

Raw Data Page 580

[1]
[2]Collar removed; animal has developed diarrhea.
[3]Collar removed; animal has lost considerable amount of weight.
[4]Collar removed; caught in animal's mouth.

Comments: Animal #9, #10: No gross changes observed.

Table 2
(continued)

14-Day Subacute Dermal Application

Daily Skin Scores

FINSOLV TN, (Patented); LOT # 31492

Group I

Animal Number	Sex	Site NA	Day 8	9	10	11	12	13	14	Bodyweight (kg Day 0	Day 14 (T
9	F	1	2/2	3/2S	3/2S	4/3F,S	4/3F,S	4/3F,S	4/3F,S	2.60	2.55
	F	2	2/2	2/2	2/2	2/3	3/3S	3/3F,S	3/3F,S		
	F	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	F	4	4/3F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S		
10	F	1	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	2.34	2.39
	F	2	3/3F	3/3S	3/3S	3/3S	3/3S	3/3F,S	3/3F,S		
	F	3	2/1	2/1	2/1	2/1	2/1	2/1	2/1		
	F	4	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S	4/4F,S		
9 DAILY			N	N	N	N	N	N	N		
10 OBSERVATIONS			N	N	N	N	N	N	N		

Raw Data Page 580

[1]Collar removed; animal has developed diarrhea.
[2]Collar removed; animal has lost considerable amount of weight.
[3]Collar removed; caught in animal's mouth.

Comments: Animal #9, #10: No gross changes observed.

Table 2

Total Combined Scores by Site

Animal Number	Skin Site 1	2	3	4
1	57	49	23	82
2	62	53	42	69
3	62	58	39	74
4	55	54	40	71
5	83	75	42	81
6	60	55	37	61
7	64	55	38	71
8	56	52	41	72
9	66	58	37	86
10	84	66	37	87
	649	575	376	754
(÷ 10)	64.9	57.5	37.6	75.4
(÷ 14)	4.64	4.11	2.68	5.40

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT:	Finetex, Inc. 418 Falmouth Avenue Elmwood Park, NJ 07407
ATTENTION:	Ismail Walele Director of Research & Development
TEST:	The MatTek Corporation EpiDerm™ Skin Model *In Vitro* Toxicity Testing System
TEST ARTICLE:	FINSOLV® TN (#35962/ref.#104-218-B) C12-15 Alkyl Benzoate
EXPERIMENT REFERENCE NO.:	V98-0014-6

Nattina Rachkiree, B.S.
Quality Assurance Associate

5/1/98

Steven Nitka
Vice President
Laboratory Director

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QUALITY ASSURANCE UNIT STATEMENT

Study No.: V98-0014-6

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance to standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections may have been reported to management and the Study Director.

Date of biophase/data inspection: April 29, 1998
April 30, 1998

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	-	Laboratory Supervisor
Melissa Pandorf, B.S.	-	Technician
Kathleen Alworth, B.A.	-	Director of Quality Assurance

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures, study protocols, and the Good Laboratory Practice principles.

4) MTT Transfer -

After the appropriate exposure period, each insert will be individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual test material. Each insert will then be rinsed a second time. Excess liquid will be shaken off and each EpiDerm sample will be placed into 300 microliters of MTT solution. Each insert will be checked to insure that no air bubbles are trapped beneath the Millicells. The 24 well trays will then be returned to the incubator for three hours, under the same atmospheric conditions as detailed above.

5) Extraction -

After the three hour MTT exposure, each insert will be removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS will be shaken from the inserts. The bottoms of the inserts will then be blotted. Each insert will then be placed into a well of a 24 well extraction plate. Two milliliters of extraction solution will then be added to each well, completely covering the EpiDerm sample. The extractions will be run overnight, at room temperature, ambient atmospheric conditions and in the dark. Each extraction plate will be placed in a sealed plastic bag to minimize extractant evaporation. After the extraction period is complete, the liquid within each insert will be decanted back into the well from which the insert was taken. The inserts will be discarded.

Construction of a Dose Response Curve:

The extractant solution within each well will be pipetted up and down several times to insure that each is well mixed. Two hundred microliters of each of the mixed extraction solutions will then be pipetted into separate wells of a 96 well microtiter plate.

A Dynatech MR 4000 Automatic Microplate Reader will be used to determine the optical density of each extract at 570nm. With the absorbance of a negative control defined as 100%, the percent absorbencies of the articles will be determined (% viability = 100 x (OD [article]/OD [negative control]). The calculated percentages directly correlate with the cell metabolism in the EpiDerm samples.

Using a semi-log scale, the percent viability (liner "y" axis) will be plotted versus the dosing time (log "x" axis). By interpolation, the time at which the viability has dropped to 50 percent will be determined.

Objective:

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiDerm *in vitro* toxicity testing system.

Introduction:

"MatTek's patented EpiDerm Skin Model consists of normal, human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis. Keratinocytes are cultured on specially prepared, permeable cell culture inserts . . . " This system " . . . closely parallels human skin. EpiDerm consists of highly organized basal, spinous, granular and cornified layers analogous to those found *in vivo*. Epiderm cultured keratinocytes are mitotically and metabolically active."[1]

EpiDerm, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

Test Article: FINSOLV® TN (#35962/ref.#104-218-B)

Experimental Start Date: February 24, 1998

Experimental Termination Date: February 26, 1998

Method:

After the appropriate tissue preparation, 100 microliters of the test article, at 100% and 10%, and the negative and positive control (Triton X-100 @ 1%) articles, were added to the Millicells containing the EpiDerm samples. The six (6) well plates containing the dosed EpiDerm samples were then incubated at 37°C, five (5)% carbon dioxide and $\geq$ 90% humidity. After the appropriate exposure periods, each insert was individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual test material. Each were then rinsed a second time. Excess liquid was shaken off and each EpiDerm sample was placed into 300 microliters of MTT solution. The EpiDerm samples were then returned to the incubator.

[1]MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721

Method (continued):

After the three (3) hour MTT exposure, each insert was removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS was shaken from each of the inserts which were then blotted on the bottom on paper towels. The inserts were then each placed into one (1) well of a 24 well extraction plate. Each insert was then immersed in two (2) milliliters of extraction solution overnight. After the exposure, the liquid within each insert was decanted back into the well from which it was taken. The remaining extractant solution was then agitated and a 200 microliter aliquot of each extract was removed for evaluation. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of a negative control (corn oil) defined as 100%, the percent absorbencies of the test and reference articles were determined. The data listed in Table 1 directly correlate with the cell metabolism in the EpiDerm samples.

Discussion:

For each article, a semi-log scale was used to plot the percent viabilities, on the linear y axis, versus the dosing times, on the log x axis. By interpolation and where possible, the time at which the percent viability would be 50% (ET-50) was estimated for each article.

The test article, FINSOLV® TN, at both 100% and at 10%, elicited ET-50's greater than 24 hours. The positive control article, 1% Triton X 100, elicited an ET-50 of approximately 6 hours.

According to MatTek Corporation, as a general guideline, the following groupings can be used in assigning expected *in vivo* irritancy responses based on the ET-50 results obtained using MatTek's EpiDerm:

ET-50 (hrs)	Expected *In vivo* Irritancy	Example
<0.5	Severe, probably corrosive	Conc. Nitric Acid
0.5-4	Moderate	1% Sodium Dodecyl Sulfate
4-12	Moderate to Mild	1% Triton X-100
12-24	Very Mild	Baby Shampoo
24	Non-irritating	10% Tween 20

Conclusion:

Under the conditions of this test, the results indicate that the test article, FINSOLV® TN, both at both 100% and at 10%, has expected *in vivo* dermal irritancy potentials in the non-irritating range; the positive control article, 1% Triton X 100, has an expected *in vivo* dermal irritancy potential in the moderate to mild range.

Table 1
Results

Article	Concentration	Exposure	Raw OD @ 570nm	Mean OD @ 570nm	Stnd. Deviation	Avg. % Viability	ET50	Classification
FINSOLV® TN	100%	18 hrs	1.149					
	100%	18 hrs	1.327					
	100%	18 hrs	1.398	1.291	0.128	97		
FINSOLV® TN	100%	4 hrs	1.446					
	100%	4 hrs	1.374					
	100%	4 hrs	1.557	1.459	0.092	110		
FINSOLV® TN	100%	2 hrs	1.498					
	100%	2 hrs	1.541					
	100%	2 hrs	1.719	1.586	0.117	119	>24 hrs	Non-irritating
FINSOLV® TN	10%	18 hrs	1.209					
	10%	18 hrs	1.190	1.200	0.013	90		
FINSOLV® TN	10%	4 hrs	1.333					
	10%	4 hrs	1.404	1.369	0.050	103		
FINSOLV® TN	10%	2 hrs	1.500					
	10%	2 hrs	1.602	1.551	0.072	117	>24 hrs	Non-irritating

Table 1
Results (continued)

Article	Concentration	Exposure	Raw OD @ 570nm	Mean OD @ 570nm	Stnd. Deviation	Avg. % Viability	ET50	Classification
Corn Oil	100%	4 hrs	1.126					
	100%	4 hrs	1.308					
	100%	4 hrs	1.361					
	100%	4 hrs	1.308					
	100%	4 hrs	1.532					
	100%	4 hrs	1.349	1.331	0.130	100	N/A	N/A
Triton X-100	1%	18 hrs	0.137					
	1%	18 hrs	0.167	0.152	0.021	11		
Triton X-100	1%	4 hrs	0.844					
	1%	4 hrs	1.188	1.016	0.243	76		
Triton X-100	1%	2 hrs	1.099					
	1%	2 hrs	0.873	0.986	0.160	74	6 hrs	Moderate to mild

Figure 1: Cell Viability

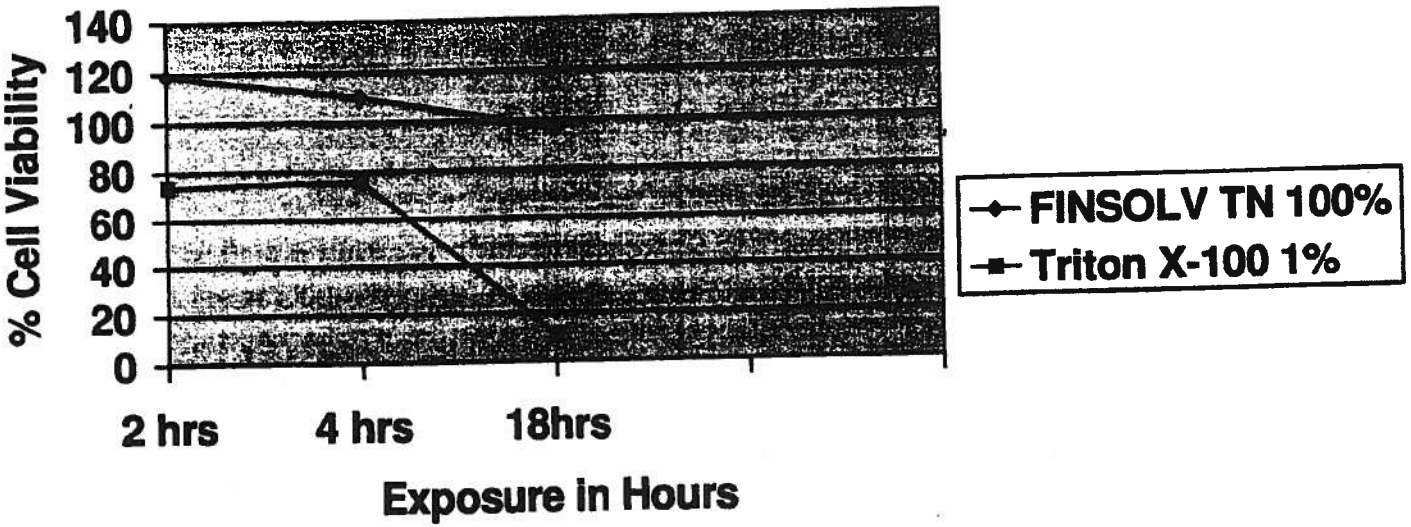
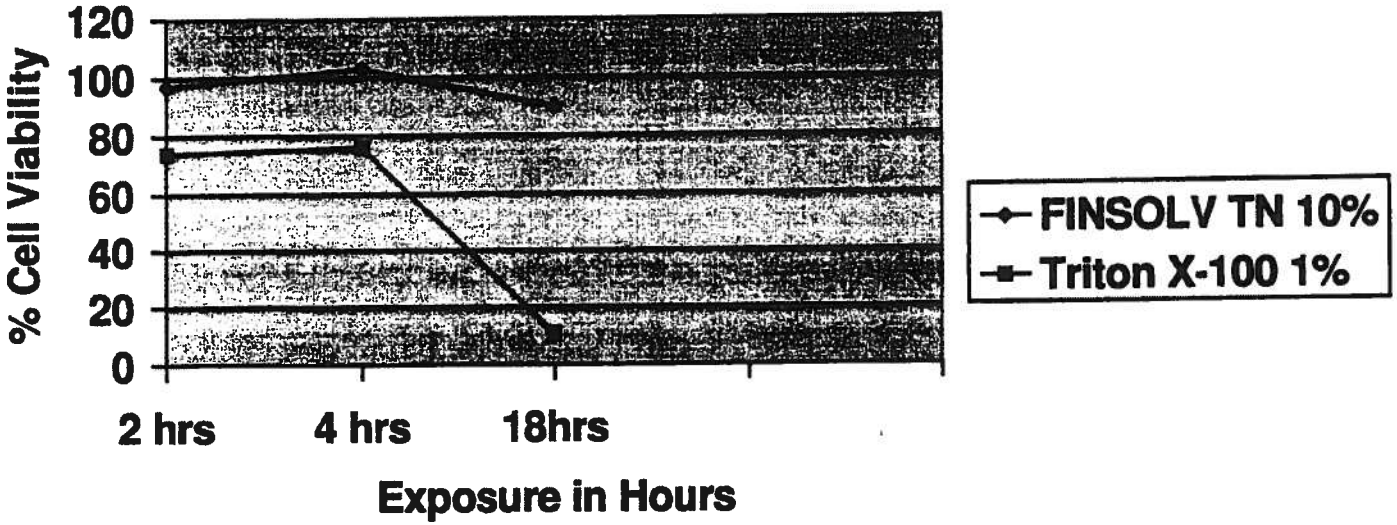


Figure 2: Cell Viability



APPENDIX

Irritation Testing Utilizing The MatTek Corporation EpiDerm™ *In Vitro* Toxicity Testing System

Objective:

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiDerm™ *in vitro* toxicity testing system.

Introduction:

"MatTek's patented EpiDerm Skin Model consists of normal, human-derived epidermal keratinocytes (NHEK) which have been cultured to form a multilayered, highly differentiated model of the human epidermis. Keratinocytes are cultured on specially prepared, permeable cell culture inserts . . . " This system "_ . . . closely parallels human skin. EpiDerm consists of highly organized basal, spinous, granular and cornified layers analogous to those found *in vivo*. Epiderm cultured keratinocytes are mitotically and metabolically active."[1]

EpiDerm, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

Test Article:

As supplied by the sponsor and labeled according to the sponsor's requirements and specifications. If dilutions are to be tested, the sponsor may consider sending those dilutions or informing the study director on how those dilutions are to be prepared.

Reference Article(s):

As suggested by the sponsor and/or the study director.

Proposed Initiation Date:

Within two weeks of receipt of sample and written authorization.

[1] MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721.

Method:

1) Preparation –

The assay medium will be warmed to 37° C. Using sterile technique, 0.9 ml of the warmed assay medium will be transferred into each well of the sterile, 6-well plates. The plates will be labeled indicating the test or control article and the dosing time to be used.

One hour before the dosing is to begin, using sterile technique, the EpiDerm samples will be removed from the transport medium and transferred to the 6-well plates containing the pre-warmed assay medium. Care will be taken to remove all adherent agarose from the outside of the Millicells (insert) containing the EpiDerm samples.

The 6-well plates containing the EpiDerm samples will be placed into a humidified, 37° C, 5% CO_2 incubator for 1 hour prior to dosing. After the hour, the plates will be removed from the incubator. The assay medium will be removed from the plates. Fresh, 37° C, assay medium will be placed into each well, 0.9 ml per well.

2) Dosing –

After the appropriate tissue preparation, 100 microliters of the test articles, reference articles and/or negative control articles will be added to the Millicells containing the EpiDerm samples. The six (6) well plates containing the dosed EpiDerm samples will then again be incubated at 37°C, five (5)% carbon dioxide and $\geq$ 90% humidity.

Several exposure periods of between 1 and 24 hours will be chosen for each article to determine each article's ET-50 (estimated time for 50% cellular dysfunction). The more irritating the articles are expected to be, the shorter the exposure periods should be. The study director and sponsor will choose the durations prior to initiation of the testing. The dosing times may be adjusted so that all exposures end nearly simultaneously. The exposure period for the negative controls will be approximately 4 hours.

3) MTT Preparation -

Approximately one hour before the end of the exposure periods, the MTT solution will be prepared. The MTT concentrate will be thawed and mixed with the MTT diluent. Approximately 15 minutes before the end of the exposure periods, 24 well plates will be prepared with the MTT solution. The MTT solution will be added to each well of the 24 well tray, at 0.3 ml per well. The top of the 24 well tray will be labeled to indicate to which wells the inserts will be transferred.

Consumer Product Testing Co.

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FINAL REPORT

CLIENT: Finetex, Inc.
418 Falmouth Avenue
Elmwood Park, NJ 07407

ATTENTION: Ismail Walele
Director of Research & Development

TEST: The MatTek Corporation EpiOcular™ Tissue Model *In Vitro* Toxicity Testing System

TEST ARTICLE: FINSOLV® TN (#35962/ref.#104-218-B)

C 12-15 Alkyl Benzoate

EXPERIMENT REFERENCE NO.: V98-0014-2

05/07/98
Nattina Rachkiree, B.S.
Quality Assurance Associate

5/11/98
Steven Nitka
Vice President
Laboratory Director

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70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

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QUALITY ASSURANCE UNIT STATEMENT

Study No.: V98-0014-2

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of nonclinical laboratory studies. These studies have been performed under Good Laboratory Practice principles (including government regulations to the extent applicable) and in accordance to standard operating procedures and applicable standard protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections may have been reported to management and the Study Director.

Date of biophase/data inspection: April 29, 1998
April 30, 1998

Professional personnel involved:

Steven Nitka, B.S.	-	Vice President Laboratory Director (Study Director)
Lillian Deniza, B.S.	-	Laboratory Supervisor
Melissa Pandorf, B.S.	-	Technician
Kathleen Alworth, B.A.	-	Director of Quality Assurance

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures, study protocols, and the Good Laboratory Practice principles.

Objective:

To evaluate the test article for irritancy potential utilizing the MatTek Corporation EpiOcular *in vitro* toxicity testing system.

Introduction:

"MatTek's patented EpiOcular corneal Model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, squamous epithelium similar to that found in the cornea. The epidermal cells, which are cultured on specially prepared cell culture inserts using serum free medium, differentiate to form a multilayered structure which closely parallels the corneal epithelium . . . " This system " . . . provides a predictive, morphologically relevant *in vitro* means to assess ocular irritancy."[1]

EpiOcular, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

In order to use results from EpiOcular to predict an approximate Draize rabbit score, it is necessary to dilute to 20% all materials with a density of >0.95 g/ml. Therefore the results for the test and reference articles at 20% correspond to Draize results at 100% and results for the test and reference articles at 2% correspond to Draize results at 10%.

Test Article: FINSOLV® TN (#35962/ref.#104-218-B)

Experimental Start Date: February 18, 1998

Experimental Termination Date: February 19, 1998

Method:

As per MatTek's protocol, the specific gravities (density) of the test articles was determined. Because the article's specific gravity was greater than 0.95 grams/milliliter, the article was diluted to 20% in corn oil (1 part TA to 4 parts corn oil). After the appropriate tissue preparation, 100 microliters of the test article, at 20% and 2%, and the negative and positive control (Triton X-100 @ 0.3%) articles, were added to the Millicells containing the EpiOcular samples. The six (6) well plates containing the dosed EpiOcular samples were then incubated at 37°C, five (5)% carbon dioxide and ≥ 90% humidity.

[1]MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721

Method (continued):

After the appropriate exposure period, each insert was individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual test or reference material. Each were then rinsed a second and third time. Following the 3 rinses, each Millicell was submerged in 5 milliliters of assay media for 10 minutes, at room temperature. This final soak removed any residual, absorbed test or reference article. After the 10 minutes, excess liquid was shaken off and each EpiOcular tissue was placed into 300 microliters of MTT solution. The EpiOcular samples were then returned to the incubator.

After the three (3) hour MTT exposure, each insert was removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS was shaken from each of the inserts which were then blotted on the bottom with paper towels. The inserts were then each placed into one (1) well of a 24 well extraction plate.- Each insert was then immersed in two (2) milliliters of extraction solution, at room temperature, overnight. After the extraction procedure, the liquid within each insert was decanted back into the well from which it was taken. The remaining extractant solution was then agitated and a 200 microliter aliquot of each extract was removed for evaluation. A Dynatech MR 4000 Automatic Microplate Reader was used to determine the absorbance of each extract at 570nm. With the absorbance of a negative control (corn oil) defined as 100%, the percent absorbencies of the test and reference articles were determined. The data listed in Table 1 directly correlate with the cell metabolism in the EpiOcular samples.

Using a semi-log scale, the percent viabilities for the test article were plotted on the linear y axis versus the dosing time on the log x axis. By interpolation, the time at which the percent viability for each would be 50% was determined (ET-50), where possible. As a general guideline (provided by MatTek) the following equation can be used to estimate the rabbit Draize eye score:

$$\textbf{Log Draize} = 2.067 - (0.679 \times \text{Log ET-50})$$

Based on the literature (Kay, J.H. and Calandra, J.C., "Interpretation of eye irritation tests," *J. Soc. Cosmetic Chem.*, 13, 281-289 (1962)), the ocular irritancy estimated potential has been categorized by MatTek into the following groups, based on the Draize score:

Draize Score	Irritancy Classification	Example	EpiOcular ET-50 (min)
0-15	Non-irritating, Minimal	PEG-75 Lanolin, Tween	>240-20.5
15.1 – 25	Mild	3% Sodium Dodecyl Sulfate	<20.5 – 9.67
25.1 – 50	Moderate	5% Triton X-100	<9.67 – 3.48
50.1 – 110	Severe, Extreme	5% Benzalkonium Chloride	<3.48

Discussion:

The Triton X positive control article, at 0.3%, elicited *in vitro* results which place its ET-50 at approximately 21 minutes. Therefore, using the equation supplied by MatTek, the Triton X positive control article's estimated Draize ocular irritation score is approximately 15 with a "minimal to mild" irritancy classification. The test article (FINSOLV® TN, at both 20 and 2%, elicited *in vitro* results which indicate that its ET-50's are greater than 240 minutes. Therefore the article, at both 100% and 10%, has estimated Draize ocular irritation scores of approximately 0 with a "non-irritating" classification.

Conclusion:

Under the conditions of this test, the results indicate that the sponsor-submitted test article, at both percentages tested, has a "non-irritating" classification, similar to that elicited by PEG-75 Lanolin.

A tabulation of the results are presented in Table 1. Graphic representations of those results are presented in Figures 1 and 2. The protocol is attached as an appendix.

Table 1
Results

Article	Concentration	Exposure	Raw OD @ 570nm	Mean OD @ 570nm	Stnd. Deviation	Avg. % Viability	ET50	Classification
FINSOLV® TN	20%	3 hrs	1.725					
	20%	3 hrs	1.860					
	20%	3 hrs	1.963	1.849	0.119	108		
FINSOLV® TN	20%	1 hr	1.660					
	20%	1 hr	1.789					
	20%	1 hr	2.099	1.849	0.226	108		
FINSOLV® TN	20%	20 min	1.814					
	20%	20 min	1.814					
	20%	20 min	1.969	1.866	0.089	109	>240 m	Non-irritating
FINSOLV® TN	2%	3 hrs	1.644					
	2%	3 hrs	1.767	1.706	0.087	100		
FINSOLV® TN	2%	1 hr	1.687					
	2%	1 hr	1.885	1.786	0.140	105		
FINSOLV® TN	2%	20 min	1.549					
	2%	20 min	1.713	1.631	0.116	95	>240 m	Non-irritating
Corn Oil	100%	1 hr	1.737					
	100%	1 hr	1.750					
	100%	1 hr	1.593					
	100%	1 hr	1.647					
	100%	1 hr	1.673					
	100%	1 hr	1.856	1.709	0.092	100	N/A	N/A

Table 1
Results (continued)

Article	Concentration	Exposure	Raw OD @ 570nm	Mean OD @ 570nm	Stnd. Deviation	Avg. % Viability	ET50	Classification
Triton X-100	0.3%	1 hr	0.272					
	0.3%	1 hr	0.254	0.263	0.013	15		
Triton X-100	0.3%	15 min	1.147					
	0.3%	15 min	1.099	1.123	0.034	66		
Triton X-100	0.3%	5 min	1.389					
	0.3%	5 min	1.559	1.474	0.120	86	21 min	Minimal to mild

Figure 1: Cell Viability

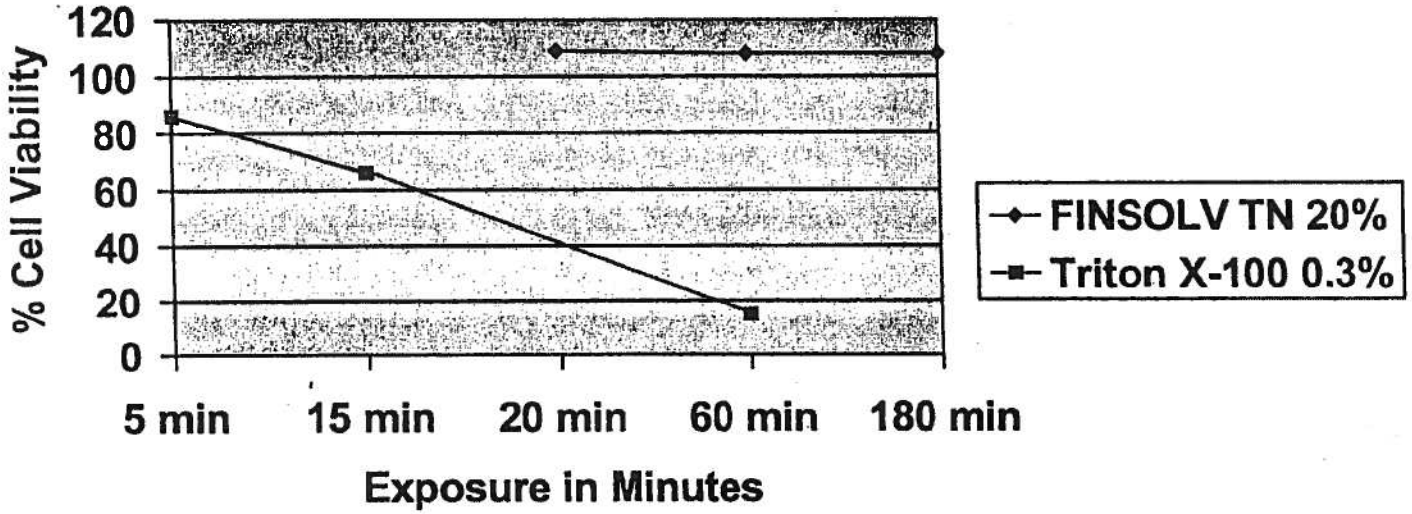
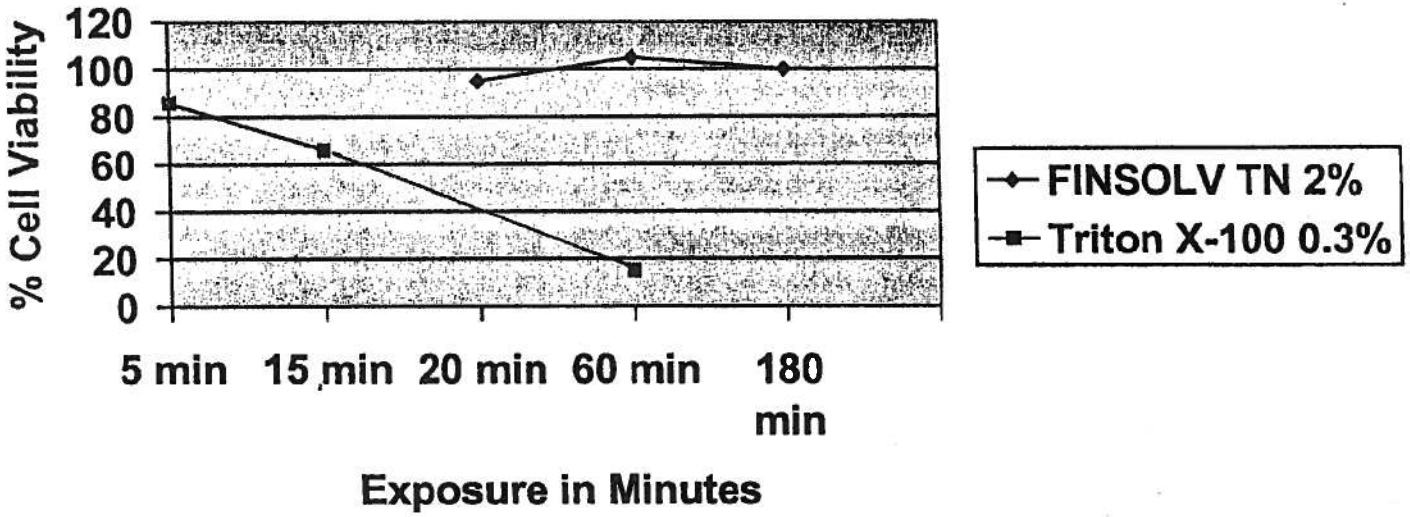


Figure 2: Cell Viability



APPENDIX

Irritation Testing Utilizing The MatTek Corporation EpiOcular™ *In Vitro* Toxicity Testing system

Objective:

To evaluate the test article for irritancy potential utilizing the MatTek Corporation's EpiOcular™ *in vitro* toxicity testing system.

Introduction:

"MatTek's patented EpiOcular corneal model consists of normal, human-derived epidermal keratinocytes which have been cultured to form a stratified, squamous epithelium similar to that found in the cornea. The epidermal cells, which are cultured on specially prepared cell culture inserts using serum free medium, differentiate to form a multi-layered structure which closely parallels the corneal epithelium."[1]

EpiOcular, when used with the recommended cell metabolism assay, can quickly provide toxicological profiles. The procedure utilizes a water-soluble, yellow, tetrazolium salt (MTT {3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide}), which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Substances which damage this mitochondrial enzyme inhibit the reduction of the tetrazolium salt. The amount of MTT reduced by a culture is therefore proportional to the number of viable cells.

Test Article:

As supplied by the sponsor and labeled according to the sponsor's requirements and specifications. If dilutions are to be tested, the sponsor may consider sending those dilutions or informing the study director on how those dilutions are to be prepared.

Reference Article(s):

As suggested by the sponsor and/or the study director.

Proposed Initiation Date:

Within two (2) weeks of receipt of sample and written authorization.

[1] MatTek Corporation, 200 Homer Avenue, Ashland, Massachusetts 01721.

Method:

1) Preparation –

The assay medium will be warmed to 37° C. Using sterile technique, 0.9 ml of the warmed assay medium will be transferred into each well of the sterile, 6-well plates. The plates will be labeled indicating the test or control article and the dosing time to be used.

One hour before the dosing is to begin, using sterile technique, the EpiOcular samples will be removed from the transport medium and transferred to the 6-well plates containing the pre-warmed assay medium. Care will be taken to remove all adherent agarose from the outside of the Millicells (insert) containing the EpiOcular samples.

The 6-well plates containing the EpiOcular samples will be placed into a humidified, 37° C, 5% CO_2 incubator for 1 hour prior to dosing. After the hour, the plates will be removed from the incubator. The assay medium will be removed from the plates. Fresh, 37° C, assay medium will be placed into each well, 0.9 ml per well.

2) Dosing –

In order to use results from EpiOcular to predict an approximate Draize rabbit eye score, it is necessary to dilute to 20% all articles having a density of greater than 0.95 g/ml. If the article's density is greater than 0.95 g/ml, it will be diluted to 20% in distilled water. If the article's density is less than 0.95 g/ml it will be applied neat.

After the appropriate tissue and article preparation, 100 microliters of the test articles, reference articles and/or negative control articles will be added to the Millicells containing the EpiOcular samples. The six (6) well plates containing the dosed EpiOcular samples will then again be incubated at 37°C, five (5)% carbon dioxide and $\geq$ 90% humidity.

Several exposure periods of between 5 and 240 minutes will be chosen for each article to determine each article's ET-50 (estimated time for 50% cellular dysfunction). The more irritating the articles are expected to be, the shorter the exposure periods should be. The study director will choose the durations prior to initiation of the testing. The dosing times may be adjusted so that all exposures end nearly simultaneously. The exposure period for the negative controls will be 60 minutes.

3) MTT Preparation -

Approximately one hour before the end of the exposure periods, the MTT solution will be prepared. The MTT concentrate will be thawed and mixed with the MTT diluent. Approximately 15 minutes before the end of the exposure periods, 24 well plates will be prepared with the MTT solution. The MTT solution will be added to each well of the 24 well tray, at 0.3 ml per well. The top of the 24 well tray will be labeled to indicate to which wells the inserts will be transferred.

4) MTT Transfer -

After the appropriate exposure period, each insert will be individually removed from its plate and rinsed with phosphate buffered saline (PBS) to remove any residual test material. Each insert will then be rinsed a second and third time. Following the third rinse, each insert will be submerged in 5 milliliters of assay media for 10 minutes to remove any residual article.

After the 10 minute soak, the media will be decanted and excess liquid will be shaken off. Each EpiOcular sample will then be placed into 300 microliters of MTT solution. Each insert will be checked to insure that no air bubbles are trapped beneath the Millicells. The 24 well trays will then be returned to the incubator for three hours, under the same atmospheric conditions as detailed above.

5) Extraction -

After the three hour MTT exposure, each insert will be removed and gently rinsed with PBS to remove any residual MTT solution. Excess PBS will be shaken from the inserts. The bottoms of the inserts will then be blotted. Each insert will then be placed into a well of a 24 well extraction plate. Two milliliters of extraction solution will then be added to each well, completely covering the EpiOcular sample. The extractions will be run overnight, at room temperature, ambient atmospheric conditions and in the dark. Each extraction plate will be placed in a sealed plastic bag to minimize extractant evaporation. After the extraction period is complete, the liquid within each insert will be decanted back into the well from which the insert was taken. The inserts will be discarded.

Construction of a Dose Response Curve:

The extractant solution within each well will be pipetted up and down several times to insure that each is well mixed. Two hundred microliters of each of the mixed extraction solutions will then be pipetted into separate wells of a 96 well microtiter plate.

A Dynatech MR 4000 Automatic Microplate Reader will be used to determine the optical density of each extract at 570nm. With the absorbance of a negative control defined as 100%, the percent absorbencies of the articles will be determined (% viability = 100 x (OD [article]/OD [negative control]). The calculated percentages directly correlate with the cell metabolism in the EpiOcular samples.

Using a semi-log scale, the percent viability (liner "y" axis) will be plotted versus the dosing time (log "x" axis). By interpolation, the time at which the viability has dropped to 50 percent will be determined.

Correlation of *In vitro* and *In vivo* Results:

As a general guideline, the following equation can be used to estimate the rabbit Draize eye score:

$$\text{Log Draize} = 2.067 - (0.679 \times \text{Lot ET-50 (min)})$$

Based on the literature (Kay, J.H. and Calandra, J.C., "Interpretation of eye irritation tests," *J. Soc. Cosmetic Chem.*, 13, 281 – 289 (1962), the ocular irritancy can be categorized into the following groups based on the Draize score:

Draize Score	Irritancy Classification	Example	EpiOcular et-50 (min)
0	Non-irritating	PEG-75 Lanolin	>240
0.1 – 15	Minimal	Tween 20	240 – 20.5
15.1 – 25	Mild	3% Sodium Dodecyl Sulfate	20.5 – 9.7
25.1 – 50	Moderate	5% Triton X-100	9.7 – 3.5
50.1 – 80	Severe	5% Benzalkonium Chloride	3.5 – 1.7
80 – 110	Extreme	10% NaOH	1.7 < 1.0

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT: Finetex Inc.
418 Falmouth Avenue
Elmwood Park, New Jersey 07407

ATTENTION: Ismail Walele
Research Director

TEST: Repeated Insult Patch Test

TEST MATERIALS:
1. Finsolv TN Lot No. 34308 Ref. No. 93-198-A
2. Finsolv TN Lot No. 34449 Ref. No. 93-198-B

C12-15 Alkyl Benzoate

EXPERIMENT REFERENCE NUMBER: C94-0297

Richard R. Eisenberg, M.D.

Oliver Shapiro, M.S.
Director of Quality Assurance
and Regulatory Compliance

Joy Frank, R.N.
Executive Vice President
Clinical Evaluations

Date September 8, 1994
JF/rt

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the [illegible] of any members of its staff, may be used in connection with the advertising or sale of any product or process

Consumer Product Testing Co.

EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: C94-0297

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. Studies lasting six months or more are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to management and the Clinical Director. All materials and data pertinent to this study will be stored in the Archives Facility unless otherwise requested by the sponsor.

Date of inspection: September 6, 1994

Senior personnel involved:

Joy Frank, R.N.	-	Executive Vice President Clinical Evaluations
Beverly Pinsky	-	Clinical Laboratory Supervisor
Michele Bull, B.A.	-	Quality Assurance Associate

The representative signature of the Quality Assurance Unit on the front page signifies that this study has been performed in accordance with standard operating procedures and study protocol as well as government regulations regarding such procedures and protocols as outlined in the Federal Register (Vol. 46, No. 17 of Tuesday, January 27, 1981).

12 Spielman Road • Fairfield, New Jersey 07004-3404 • (201) 808-7111 • Fax (201) 808-7234
Corporate Headquarters • Sales • Clinical • Toxicology

10 Industrial Road • Fairfield, New Jersey 07004-3018 • (201) 882-8755 • Fax: (201) 882-8956
Analytical Chemistry • Microbiology

Objective: To determine by epidermal contact the primary or cumulative irritation and/or sensitization potential of test materials.

Participants: A panel comprised of fifty-three (53) subjects, ranging in age from 18 to 70 years, who qualified were selected for this study. Forty-eight (48) subjects completed this evaluation. The remaining subjects discontinued their participation for various reasons, none of which were related to the use of the test materials.

The criteria for selection was:

1. Willingness to cooperate,
2. Absence of any visible skin disease which might be confused with skin reactions from the test materials,
3. Avoidance of use of topical or systemic steroids and/or antihistamines for several days prior to study initiation,
4. Dependability and intelligence in following directions, and
5. Reading, understanding, and signing an informed consent contract.

Test Materials:

1. Finsolv TN Lot No. 34308 Ref. No. 93-198-A
2. Finsolv TN Lot No. 34449 Ref. No. 93-198-B

Study Schedule:

Initiation Date	Completion Date
July 22, 1994	September 1, 1994

Methodology: The upper back between the scapulae served as the treatment site. Prior to the initiation of this study, the test materials were prepared as a 20% dilution using corn oil. Approximately 0.2 ml of each test material was applied to the 1" x 1" gauze portion of a Readi-Bandage® adhesive dressing*. These were then applied to the appropriate treatment site to form a semi-occluded patch.

This procedure was followed three times per week: Monday, Wednesday, and Friday for a total of ten applications. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction Period. The sites were marked to ensure the continuity of patch application. The participants were instructed to remove these patches after twenty-four hours. The evaluation of each site was made just prior to re-application.

*Manufactured by Professional Medical Products, Inc., Greenwood, SC

Methodology (continued):

If the test site exhibited a moderate (2+) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2+) reaction was observed on this new test site. Applications would also be discontinued if a marked (3+) reaction was noted.

Rest periods consisted of twenty-four hours following the Tuesday and Thursday removal, and forty-eight hours following the Saturday removal.

At the conclusion of a rest period of approximately fourteen days following the tenth application, a challenge patch was applied to the original site and to a virgin site. Each site was evaluated at twenty-four and forty-eight hours after application. The volar forearm served as the virgin test site. Should an individual have exhibited a response during this test phase, a Rechallenge Patch Test would have been scheduled to confirm the previously recorded reaction.

Evaluation Key:

0 - No visible reaction
1+ - Mild erythema
2+ - Well-defined erythema, possible presence of barely perceptible edema
3+ - Erythema and edema
4+ - Erythema and edema with vesiculation and ulceration

Results:

The results of each participant are appended. Observations of both treated areas remained negative throughout the test interval.

Summary:

Under the conditions of this study, the following test materials do not indicate a potential for dermal irritation and/or sensitization:

1. Finsolv TN Lot No. 34308 Ref. No. 93-198-A
2. Finsolv TN Lot No. 34449 Ref. No. 93-198-B

Table 1

Individual Results

Finsolv TN Lot No. 34308 Ref. No. 93-198-A

Subject Number	Induction Exposures 1	2	3	4	5	6	7	8	9	10	Original Site 24	48	Virgin Site 24	48
1	0	0	0m	0	0	0	0m*	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0m	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0m	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0m	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0m	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0m	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0m	0	--	0	--

m = Makeup day
* = Additional makeup day granted due to a medical emergency.
-- = Did Not Complete Study

Table 1
(continued)

Individual Results

Finsolv TN Lot No. 34308 Ref. No. 93-198-A

Subject Number	Induction Exposures 1	2	3	4	5	6	7	8	9	10	Original Site 24	48	Virgin Site 24	48
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0[m]	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	----------DID NOT COMPLETE STUDY----------												
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0[m]	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0[m]	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0[m]	0	0	0	0	0	0	0	0
43	0	0	0[m]	0[m*]	0	0	0	0	0	0	0	0	0	0
44	----------DID NOT COMPLETE STUDY----------													
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0[m]	0	0	0	0	0
48	0	0	0	0	----------DID NOT COMPLETE STUDY----------									
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	----------DID NOT COMPLETE STUDY----------												
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0[m]	0	0	0	0	0	0

m = Makeup day
* = Additional makeup day granted due to a medical emergency.

Table 2

Individual Results

Finsolv TN Lot No. 34449 Ref. No. 93-198-B

Subject Number	Induction Exposures 1	2	3	4	5	6	7	8	9	10	Original Site 24	48	Virgin Site 24	48
1	0	0	0m	0	0	0	0m*	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0m	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0m	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0m	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0m	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0m	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0m	0	--	0	--

m = Makeup day
* = Additional makeup day granted due to a medical emergency.
-- = Did Not Complete Study

Table 2
(continued)

Individual Results

Finsolv TN Lot No. 34449 Ref. No. 93-198-B

Subject	Induction Exposures										Original Site		Virgin Site	
Number	1	2	3	4	5	6	7	8	9	10	24	48	24	48
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0[m]	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	----------DID NOT COMPLETE STUDY----------												
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0[m]	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0[m]	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0[m]	0	0	0	0	0	0	0	0
43	0	0	0[m]	0[m*]	0	0	0	0	0	0	0	0	0	0
44	----------DID NOT COMPLETE STUDY----------													
45	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0[m]	0	0	0	0	0
48	0	0	0	0	----------DID NOT COMPLETE STUDY----------									
49	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
51	0	----------DID NOT COMPLETE STUDY----------												
52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0[m]	0	0	0	0	0	0

m = Makeup day
* = Additional makeup day granted due to a medical emergency.

Table 3

Subject Data

Subject Number	Initials	Age	Sex
1	AB	43	F
2	EP	64	F
3	KG	40	F
4	PR	64	M
5	RS	43	F
6	AS	67	F
7	JW	42	M
8	ML	63	F
9	VT	64	F
10	JC	58	F
11	MS	69	F
12	WB	31	F
13	MS	60	F
14	FS	42	M
15	BS	39	F
16	PP	69	F
17	DB	31	F
18	MR	60	F
19	HT	61	F
20	ML	31	F
21	ST	65	M
22	MU	36	F
23	ED	35	F
24	KR	41	F
25	FC	68	F
26	KT	38	F
27	MW	52	F

Table 3
(continued)

Subject Data

Subject Number	Initials	Age	Sex
28	JP	38	F
29	MD	25	F
30	MW	40	F
31	AB	61	F
32	CG	18	F
33	NS	47	F
34	JG	55	F
35	GS	54	F
36	BR	23	F
37	ST	44	F
38	MR	34	F
39	AC	23	F
40	CW	47	F
41	EG	45	F
42	CA	36	F
43	TB	20	F
44	LF	18	F
45	WM	29	M
46	EG	70	F
47	MS	27	F
48	PM	19	M
49	HP	56	F
50	PJ	43	F
51	AB	18	F
52	RF	52	F
53	JP	28	F

Lab No. 94T 09184 00

MG019-223/S

P.O. No. 55384

STUDY TITLE:

AMES SALMONELLA/MAMMALIAN

MICROSOME MUTAGENICITY ASSAY FOR MUTAGENS

TEST ARTICLE:

FINSOLV TN - C_{12-15} ALKYL BENZOATE

IDENTIFICATION NO.:

LOT #34449

SPONSOR:

JOHN BRODZINSKI
FINETEX INC
PO BOX 216
ELMWOOD PARK, NJ 07407

NAmSA® *World Leader in Testing Services for the Medical Device Industry*

2261 Tracy Road
Northwood, OH 43619
Phone 419-666-9455
FAX 419-666-2954

SUMMARY

An Ames Mutagenicity assay was conducted to determine whether a test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, would cause mutagenic changes in histidine-dependent Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of S-9 metabolic activation. The methodology of Ames et al (1975) was followed but modified to use a test article solution.

The test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, (prepared by the sponsor) was found to be non-inhibitory to growth of tester strains TA98, TA100, TA1535, TA1537, and TA1538 (see Table II). Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution were inoculated with 0.1 ml of culture for each of five tester strains, and 0.1 ml of the test article solution. A 0.5 ml aliquot of S-9 homogenate simulating metabolic activation was added when necessary. The mixture was poured across triplicate Minimal E. plates. Parallel testing was also conducted with a negative control, and four (4) positive controls. The mean number of revertants of the triplicate test plates were compared to the mean number of revertants of the triplicate negative control plates for each of the five tester strains employed. The values (means) obtained for the positive controls were used as points of reference.

Under the conditions of this assay, a test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, was not considered mutagenic to Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538. Data obtained from this study does meet NAmSA criteria for a valid assay.

Study and Supervisory Personnel: Jamie M. Tonich, BS
Barry Phelps, BS

Approved by: Barry Phelps, BS — 08-10-94
In Vitro Supervisor — Date Completed

/pjf
MMQ

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FAX 419-666-2954

INTRODUCTION

An Ames Mutagenicity Standard plate incorporation assay was conducted to determine whether a test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, would cause mutagenic changes in Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537 and TA1538. The test was performed utilizing the presence and absence of S-9 metabolic activation. The methodologies of Ames et al, as reported in Mutation Research (1975), was employed with test methods modified to use a test article solution. The Ames mutagenicity test system has been widely used as a rapid screening procedure for the determination of mutagenic and potential carcinogenic hazards of pure compounds, complex compounds and commercial products. The preliminary toxicity screen began on July 29, 1994, and the testing ended on August 8, 1994.

MATERIALS AND METHODS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	Finsolv TN - C_{12-15} Alkyl Benzoate
Identification No.:	Lot #34449
Storage Conditions:	Room temperature
Preparation:	The test article solution was prepared by the sponsor. A negative (0.85% SC) control (vehicle without test material) was similarly prepared.
Condition of Solutions:	Test: clear Control: clear

Test System[1] - The Ames test is based upon the use of five specially constructed strains of Salmonella typhimurium containing a specific mutation in the histidine operon and other mutations that increase their ability to detect mutagens. These genetically altered strains, TA98, TA100, TA1535, TA1537, and TA1538 cannot grow in the absence of histidine. When they are placed in a histidine-free medium, only those cells which mutate spontaneously back to their wild type state (nonhistidine-dependent by manufacturing their own histidine) are able to form colonies. The spontaneous mutation rate (or reversion rate) for any one strain is relatively constant, but if a mutagen is added to the test system, the mutation rate is significantly increased.

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[1] Originally developed by Dr. Bruce Ames, University of California at Berkeley

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Phone 419-666-9455
FAX 419-666-2954

Tester Strain	Mutations
TA98	hisD3052, rfa, ΔuvrB, frameshift, R-factor
TA100	hisG46, rfa, ΔuvrB, missense R-factor
TA1538	hisD3052, rfa, ΔuvrB, frameshift
TA1537	hisC3076, rfa, ΔuvrB, frameshift
TA1535	hisG46, rfa, ΔuvrB, missense

rfa = cause partial loss of the lipopolysaccharide wall which increases permeability of the cell to large molecules (i.e crystal violet inhibition)
ΔuvrB = deficient DNA excision - repair system (i.e. ultraviolet sensitivity)
frameshift = Base-pair addition/deletion
missense = Base-pair substitution
R-factor = contain plasmid PKM101 (i.e. ampicillin resistance)

TABLE I

	Tester Strains				
Characteristics (expected)	TA98	TA100	TA1535	TA1537	TA1538
Ampicillin-TA98 & TA100 = (Resistant) TA1535, TA1537 & TA1538= (Sensitive)	R	R	S	S	S
Rfa Mutation; CV (Sensitive)	S	S	S	S	S
UVrb (No Growth)	NG	NG	NG	NG	NG
Histidine Requirement; (Growth)	G	G	G	G	G
Biotin (No Growth)	NG	NG	NG	NG	NG
Purity (Pure)	PURE	PURE	PURE	PURE	PURE
Total Plate Count Plate CFU's (10^{-7})	74 78 76	136 214 175	65 41 53	39 42 41	26 29 28
Titer (Organisms/ml)	7.6×10^8	1.8×10^9	5.3×10^8	4.1×10^8	2.8×10^8

R = Resistant S = Sensitive NG = No Growth G = Growth N/A = Not Applicable

Metabolic Activation[2] - Aroclor 1254 - induced rat liver (S-9 homogenate) is used as metabolic activation. The S-9 homogenate is prepared from male Sprague-Dawley rats. The rats are induced with one intraperitoneal injection of Aroclor 1254 (500 mg/ml), five days prior to sacrifice. Prior to use the S-9 homogenate will be mixed with a buffer containing 0.4 M $MgCl_2$/1.65 M KCl, 1.0 M Glucose-6-phosphate, 0.1 M NADP, 0.2 M sodium phosphate buffer and sterile deionized water.

[2] S-9, Lot 38265, obtained from Organon Teknika Corp., Box 15969, Durham, NC - 27704-0969

Negative Control[3] - Vehicle without test material, 0.85% saline, was tested with each tester strain to determine the spontaneous reversion rate. These data represent a base rate to which the number of revertant colonies that develop in each test plate are compared to determine whether the test article has significant mutagenic properties.

Positive Control[4,5,6,7] - A known mutagen, Dexon (paradimethylaminobenzene diazosulfonic acid sodium salt), was used as a positive control to demonstrate that tester strains TA98, TA100, and TA1537, were sensitive to mutation to the wild type state. For tester strain TA1535, sodium azide was used as a positive control. For tester strain TA1538, 2-nitrofluorene was used as a positive control. For tester strains TA100 and TA1538, 2-aminofluorene was also used as a positive control. Although metabolic activation is only required with 2-aminofluorene to induce mutagenic results; all positive controls are tested with and without S-9 homogenate.

Preliminary Toxicity Screen - Separate tubes containing 2 ml of molten top agar supplemented with histidine - biotin solution were inoculated with 0.1 ml of culture for each of the five tester strains. After mixing, the agar was poured across the surface of separate Minimal E. plates labelled with assigned NAmSA lab number, and appropriate tester strain. Once the agar solidified, sterile filter discs were placed in the center of the plates. A 0.1 ml aliquot of the test article solution of Finsolv TN - $C_{12\text{-}15}$ Alkyl Benzoate, Lot #34449, was added to the filter discs on each of the labelled plates. Parallel testing was conducted with a negative control, and to demonstrate a positive zone of inhibition, 10x Dexon was utilized.

---

3 0.85% Saline, Lot 6-29-94, obtained from NAmSA®.

4 Dexon, Lot FCX01, obtained from TCI America, Inc., 9211 N. Harborgate St., Portland, OR - 97203

5 Sodium azide, Lot 43H0291, obtained from Sigma, Chemical Company, P.O. Box 14508, St. Louis, MO - 63178-9916

6 2-nitrofluorene, Lot 01215AZ, obtained from Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI - 53201

7 2-aminofluorene, Lot 01606EY, obtained from Sigma, Chemical Company, P.O. Box 14508, St. Louis, MO - 63178-9916

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Northwood, OH 43619
Phone 419-666-9455
FAX 419-666-2954

The plates were incubated at 37°C ± 2°C for 48-72 hours. Following the incubation period, the zone of growth inhibition was observed and recorded (see Table II).

TABLE II

	Zone of Inhibition (mm)				
	TA98	TA100	TA1535	TA1537	TA1538
0.85% Saline (- control)	0	0	0	0	0
Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449 test article solution	0	0	0	0	0
Dexon (+ control)	37	35	30	29	32

Standard Plate Incorporation - Separate tubes containing 2 ml of molten top agar supplemented with histidine-biotin solution were inoculated with 0.1 ml of culture for each of the five tester strains and 0.1 ml of the test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449. A 0.5 ml aliquot of S-9 homogenate simulating metabolic activation was added when necessary. The mixture was poured across triplicate Minimal E. plates labelled with assigned NAmSA lab number, appropriate tester strain, and S-9 metabolic activation (when it applied). Parallel testing was also conducted with a negative control, and four (4) positive controls.

Histidine-free Minimal E. plates were prepared in triplicate as follows:

1. Test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, with and without S-9 activation
2. Negative control with and without S-9 activation
3. 1x Dexon (known mutagen) with and without S-9 activation with strains TA98, TA100, and TA1537
4. 1x 2-nitrofluorene (known mutagen) with and without S-9 activation with strain TA1538
5. 1x 2-aminofluorene (known mutagen) with and without S-9 activation with strains TA100 and TA1538
6. 1x Sodium azide (known mutagen) with and without S-9 activation with strain TA1535

Plates were incubated at 37°C ± 2°C for 48-72 hours. Following the incubation period, spontaneous revertants from each plate were recorded. The mean number of revertants from triplicate testing was determined for the negative control, test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, and the positive controls. Data are summarized in TABLE III.

TABLE III

	Salmonella typhimurium Tester Strains									
	TA98		TA100		TA1535		TA1537		TA1538	
	CFTP	$\bar{x}$	CFTP	$\bar{x}$	CFTP	$\bar{x}$	CFTP	$\bar{x}$	CFTP	$\bar{x}$
0.85% saline (- control)	40 32 36	36	146 155 162	154	10 9 14	11	3 5 4	4	11 15 17	14
Finsolv TN - C_{12-15} Alkyl Benzoate, Lot 34449 test article solution	41 40 30	37	149 143 158	150	11 12 15	13	6 8 7	7	16 12 9	12
0.85% saline w/ S-9 (- control)	42 40 36	39	161 164 139	155	17 10 12	13	2 5 9	5	10 8 7	8
Finsolv TN - C_{12-15} Alkyl Benzoate, Lot 34449 test article solution w/S-9	41 40 40	40	155 159 171	162	11 11 16	13	5 2 2	3	11 13 8	11
Dexon (+ control)	1248 1280 1328	1285	1280 1296 1456	1344	NA	NA	1616 1728 1664	1669	NA	NA
Dexon w/S-9 (+ control)	1376 1360 1408	1381	1479 1424 1440	1448	NA	NA	1712 1744 1648	1701	NA	NA
Sodium azide (+ control)	NA	NA	NA	NA	3552 3584 3648	3595	NA	NA	NA	NA
Sodium azide w/ S-9 (+ control)	NA	NA	NA	NA	3600 3632 3664	3632	NA	NA	NA	NA
2-nitrofluorene (+ control)	NA	NA	NA	NA	NA	NA	NA	NA	1536 1616 1488	1547
2-nitrofluorene w/S-9 (+ control)	NA	NA	NA	NA	NA	NA	NA	NA	1568 1456 1648	1557
2-aminofluorene (+ control) (- control for S-9)	NA	NA	161 153 149	154	NA	NA	NA	NA	13 21 27	20
2-aminofluorene (+ control) (+ control for S-9)	NA	NA	1504 1568 1376	1483	NA	NA	NA	NA	1552 1632 1440	1541

CFTP = Counts from triplicate plates
$\bar{x}$ = Mean of triplicate plates
NA = Not Applicable

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CONCLUSIONS

Test System - Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 exhibited appropriate genetic characteristics pertaining to this assay.

Preliminary Toxicity Screen - The test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, was evaluated by a spot plate technique, modeled after the antimicrobial zone of inhibition test. This screen was used to evaluate the extracts toxicity to determine whether dilution of the extract was required to provide an extract non-inhibitory to the Salmonella typhimurium. No significant inhibition was observed (see Table II).

Standard Plate Incorporation - Data are summarized in Table III. The mean number of revertants of the test plates were compared to the mean number of revertants of the negative control for each of the five tester strains employed. For the test article solution to be evaluated as a test failure or "potential mutagen" there must be a two-fold or greater increase in the number of mean revertants over the values (means) obtained from the negative control. In no case was there a two-fold or greater increase in the mean number of revertants of tester strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence of a test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449.

Test Validity - Data obtained from this study meets NAmSA criteria for a valid assay.

RESULTS

Under the conditions of this assay, a test article solution of Finsolv TN - C_{12-15} Alkyl Benzoate, Lot #34449, was not considered mutagenic to Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, and TA1538.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive files at North American Science Associates, Inc. (NAmSA®), 2261 Tracy Road, Northwood, Ohio 43619-1397.

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Northwood, OH 43619
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FAX 419-666-2954

Personal Care Products Council
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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. 8/9/10
Industry Liaison to the CIR Expert Panel

DATE: August 9, 2010

SUBJECT: Unpublished Studies on C12-15 Alkyl Benzoate

EviC-CEBA. 1994. Attestation of biological test: Acute oral toxicity and acute skin irritation and/or corrosion of C12-15 Alkyl Benzoate. Study References: K 685/4048 - K 686/4048.

EviC-CEBA. 1994. Attestation of biological test: Acute eye irritation and/or corrosion in the rabbit of C12-15 Alkyl Benzoate. Study Reference: T 208/4086.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

LABORATOIRE DE RECHERCHE ET D'EXPERIMENTATION

Blanquefort, June 7 1994

ATTESTATION OF BIOLOGICAL TEST

Study References : K 685/4048 - K 686/4048

Study Sponsor : **LABORATOIRES STEARINERIE DUBOIS**
3 rue des Longs Près

92100 BOULOGNE

TEST MATERIAL : BENZOATE C12-C15
Batch 1803 EL

1. ACUTE ORAL TOXICITY

Test system : 5 female mice NMRI EOPS, weighing 19 to 20 g.

Protocol : . Assessment of the acute oral toxicity of the product at a single dose of 5000 mg/kg.

. Observation of the animals for 6 days following the treatment.

Start date : April 4, 1991

2. ACUTE SKIN IRRITATION AND/OR CORROSION

Test system : 3 New-Zealand albino males rabbits

Protocol : OECD 404 guide line

Interpretation of the results : 67/548/EEC guide line

Start date : March 25, 1991

RESULTS AND CONCLUSIONS

1. ACUTE ORAL TOXICITY

a. Results

- absence of mortality,

- clinical and behaviour examination : no anomaly observed,

- weight growth : satisfactory.

b. Conclusion

Absence of toxicity of the product under the experimental conditions adopted.

2. ACUTE SKIN IRRITATION AND/OR CORROSION

a. Results

Erythema

N° AIs	1 hour	24 hours	48 hours	72 hours	Mean
9403	1	0	0	0	0
9404	1	0	0	0	0
9405	2	1	0	0	0,3

Oedema

N° AIs	1 hour	24 hours	48 hours	72 hours	Mean
9403	1	0	0	0	0
9404	0	0	0	0	0
9405	1	0	0	0	0

- Reactions totally reversible 48 hours after application of the product.

b. Conclusion

Test material **no irritant** for the skin.

Ph. DUFOUR
Doctor of Pharmacy
Toxicologist-Pharmacologist Expert

EVIC-CEBA
LABORATOIRE DE RECHERCHE ET D'EXPERIMENTATION

Blanquefort, May 20 1994

ATTESTATION OF BIOLOGICAL TEST

Study reference : T 208/4086

Study Sponsor : **LABORATOIRES STEARINERIE DUBOIS**
3 rue des Longs Près

92100 BOULOGNE

TEST MATERIAL : BENZOATE DE $C_{12}C_{15}$
Code 14101 – BATCH 1410 G

1. ACUTE EYE IRRITATION AND/OR CORROSION IN THE RABBIT

Test System : 3 New–Zealand albino rabbits

Protocol : CECD 405 guide line

Interpretation of the results : 67/548/EEC guide line

Start date : April 11, 1994

Z.I. - 33295 BLANQUEFORT - FRANCE
TÉLEX 550 717 - FAX 56 95 05 22
TÉL. 56 35 02 25

1, rue Massue - 94300 VINCENNES - FRANCE
FAX (1) 48 08 62 31
TÉL. (1) 43 65 03 03

S.A. AU CAPITAL DE 2 400 000 FRS - RC 70B70 BORDEAUX - SIRET 470 200 700 00016 - FR 79470200700

2. RESULTS

One hour after instillation of the test material, as such into the eye, slight irritation reactions are noticed at the level of the palpebral and bulbar conjonctivae of the 3 rabbits : diffuse crimson coloration, slight swelling and some discharge. These reactions are quickly decreased and disappeared totally in less than 6 days.
No lesion of the iris and cornea is visible

3. CONCLUSION

Test material non irritant for the eye.

Ph. DUFOUR
Doctor of Pharmacy
Toxicologist-Pharmacologist Expert

OECD 405

ACUTE EYE IRRITATION and/or CORROSION IN THE RABBIT

Product : BENZOATE DE C12/C15 – batch n° 1410 G

Study N° : T 208 / 4086

Mode d'application : 0.1 ml of undiluted product in the right eye
Start Date : 11.04.94

Als N°	D1 (1h)	24 hours	48 hours	72 hours	Mean	D5	D6
CONJUNCTIVA							
Chemosis (A)							
3240	1	1	1	0	**0,7**	0	0
2341	0	0	0	0	**0,0**	0	0
3242	1	1	1	1	**1,0**	0	0
Discharge (B)							
3240	1	0	0	0	**0,0**	0	0
2341	1	0	0	0	**0,0**	0	0
3242	1	0	0	0	**0,0**	0	0
Redness (C)							
3240	2	2	2	1	**1,7**	0	0
2341	2	1	1	0	**0,7**	0	0
3242	2	2	1	1	**1,3**	1	0
IRIS (D)							
3240	0	0	0	0	**0,0**	0	0
2341	0	0	0	0	**0,0**	0	0
3242	0	0	0	0	**0,0**	0	0
CORNEA							
Opacity (E)							
3240	0	0	0	0	**0,0**	0	0
2341	0	0	0	0	**0,0**	0	0
3242	0	0	0	0	**0,0**	0	0
Opacity Area (F)							
3240	0	0	0	0	**0,0**	0	0
2341	0	0	0	0	**0,0**	0	0
3242	0	0	0	0	**0,0**	0	0

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	No visible skin reaction	E	=	Edema
0.5	=	Barely perceptible	D	=	Dryness
1	=	Mild	S	=	Staining
2	=	Moderate	P	=	Papules
3	=	Marked	V	=	Vesicles
4	=	Severe	B	=	Bullae
			U	=	Ulceration
			Sp	=	Spreading

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Results:

The results of each participant are appended (Table 1).

Observations remained within normal limits throughout the test interval.

Subject demographics are presented in Table 2.

Summary:

Under the conditions of this study, test material, Hand Crème – , did not indicate a potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20100091

Individual Results

Hand Crème – I

Subject Number	Induction Phase									Virgin Challenge Site	
	24*hr	1	2	3	4	5	6	7	8	9	24*hr	72 hr
1	0	0[W]	DID NOT COMPLETE STUDY									
2	0	0[W]	0	0	0	0	0	0	0	0	0	0
3	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
4	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
5	-	0[W]	0	0	0	0	0	0	0	0	0	0
6	-	0[W]	0	0	0	0	0	0	0	0	0	0
7	-	0[W]	0	0	0	DID NOT COMPLETE STUDY						
8	0	0[W]	0	0	0	0	0	0	0	0	0	0
9	0	0[W]	0	0	0	0	0	0	0	0	0	0
10	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
11	-	0[W]	0	0	0	0	0	0	0	0	0	0
12	0	0[W]	0	0	0	0	0	0	0	0	0	0
13	0	0[W]	0	0	0	0	0	0	0	0	0	0
14	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
15	-	0[W]	0	0	0	0	0	0	DID NOT COMPLETE STUDY			
16	0	0[W]	0	0	0	0	0	0	0	0	0	0
17	0	0[W]	0	0	0	0	0	0	0	0	0	0
18	-	0[W]	0	0	0	0	0	0	0	0	0	0
19	-	0[W]	0	0	DID NOT COMPLETE STUDY							
20	0	0[W]	0	0	0	0	0	0	0	0	0	0
21	0	0[W]	0	0	0	0	0	0	0	0	0	0
22	0	0[W]	0	0	0	0	0	0	0	0	0	0
23	0	0[W]	0	0	0	0	0	0	0	0	0	0
24	DID NOT COMPLETE STUDY											
25	0	0[W]	0	0	0	0	0	0	0	0	0	0
26	0	0[W]	0	0	0	0	0	0	0	0	0	0
27	-	0[W]	0	0	0	0	0	0	0	0	0	0
28	-	0[W]	0	0	0	0	0	0	0	0	0	0
29	DID NOT COMPLETE STUDY											

24* = Supervised removal of 1st Induction and Challenge Patch
W = Inclement weather. Subject unable to report as scheduled
- = Subject not present for supervised removal

Table 1
(continued)
Panel #20100091

Individual Results

Hand Crème – 2-4

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
30	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
31	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
32	-	0[W]	0	0	0	0	0	0[W]	0[W]	0	0	0
33	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
34	0	0[W]	0	0	0	0	0	0	0	DNC		
35	-	0[W]	0	0	0	0	0	0	0	0	0	0
36	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
37	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
38	-	0[W]	0	0	0	0	0	0	0	0	0	0
39	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
40	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
41	-	0.5[W]	0.5	0	0	0	0[W]	0[W]	0	0	0	0
42	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
43	DID NOT COMPLETE STUDY											
44	-	0[W]	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0[W]	0	0	0	0	0
46	0	0	0	0	0	0	0[W]	0	0	0	0	0
47	0	0	0	0	0	0	0[W]	0	0	0	0	0
48	0	0	0	0	0	0	0[W]	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	DID NOT COMPLETE STUDY										
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0[W]	0[W]	0	0	0	0
57	0	0	0	0	0	0	0[W]	0[W]	0	0	0	0
58	0	0	0	0	0	0	0[W]	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch
W = Inclement weather. Subject unable to report as scheduled
- = Subject not present for supervised removal
DNC = Did not complete study

Table 2
Panel #20100091

Subject Demographics

Subject Number	Initials	Age	Sex
1	CMS	30	F
2	JMM	33	F
3	GMG	59	F
4	RM	46	M
5	AV	66	M
6	AJS	22	F
7	AMW	19	F
8	JLP	25	F
9	DMD	22	M
10	JMG	35	F
11	CAM	21	F
12	GSB	19	M
13	RAS	61	F
14	LV	31	F
15	JAS	40	M
16	MRZ	41	F
17	MNM	42	F
18	GMV	27	M
19	DEC	19	M
20	MDN	34	F
21	EAF	55	F
22	BAM	63	F
23	MRB	19	M
24	MB	62	F
25	JLM	34	F
26	TJG	22	F
27	DJB	54	F
28	SS	62	M
29	MCS	27	M

Table 2
(continued)
Panel #20100091

Subject Demographics

Subject Number	Initials	Age	Sex
30	CJC	28	M
31	JAH	59	F
32	CS	25	F
33	TOK	25	M
34	MB	58	F
35	VVL	16	F
36	SLK	30	F
37	TDK	32	F
38	MMH	49	F
39	KAD	56	F
40	TJD	57	M
41	DPB	32	M
42	MAC	60	F
43	GIA	19	F
44	CMG	27	F
45	BMM	45	F
46	NPM	47	M
47	HC	32	M
48	RJD	54	M
49	LSG	60	F
50	LAB	44	F
51	JL	68	F
52	MML	42	F
53	GDS	41	F
54	SPM	41	F
55	YV	21	F
56	JPM	76	M
57	DJM	66	M
58	ELL	63	F

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: August 19, 2010

SUBJECT: Studies on Products Containing Alkyl Benzoates

Product Investigations, Inc. 2009. Determination of the irritating and sensitizing propensities of a perfume containing 0.01% Isobutyl Benzoate on human skin.

Product Investigations, Inc. 2009. Determination of the irritating and sensitizing propensities of a blush containing 14.15% C12-15 Alkyl Benzoate on human skin.

Product Investigations, Inc. 2009. Determination of the irritating and sensitizing propensities of a body oil containing 19.5% C12-15 Alkyl Benzoate on human skin.

Clinical Research Services. 1998. Human repeat insult patch test on a perfumed soap bar containing 2.0473% Octyldodecyl Benzoate. Study C98-0012.

Product Investigations, Inc. 2006. Determination of the irritating and sensitizing propensities of a perfume containing 0.028% Methyl Benzoate on human skin.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

PRODUCT INVESTIGATIONS, INC.

151 East Tenth Avenue
Conshohocken, PA 19428
610-825-5855 • fax 610-825-7288

[redacted]

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

PREPARED FOR

Isobutyl Benzoate - 0.01%
RIPT (0/103)
Product Type - Perfume

[redacted]

[redacted]

[redacted]

[redacted]

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DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

1.00 OBJECTIVES:

.01 To identify and characterize the skin-damaging propensities that [redacted] can be induced to exercise under the conditions of this modified patch test procedure.

.02 To adjudge whether the exercise of such propensities under the test conditions contraindicates the kind of skin contact that would be occasioned during the appropriate use of the product.

2.00 DESIGN:

.01 A modified version of the Repeated Insult Patch Test [redacted] was conducted under double blind conditions on a panel composed of more than one hundred subjects at the outset.

.02 The regimen comprised nine sequential 24-hour induction applications and two concurrently conducted 24-hour challenge applications, one on the initial induction site and one on a naive site.

.03 During the initial phase, the skin of the contact sites was graded and the grades recorded on Wednesdays, Fridays (i.e. twenty-four hours after patches had been removed), and Mondays (i.e. forty-eight hours after patches had been removed).

.04 During the challenge phase, the skin of the contact sites was graded within moments after the patches had been removed (24 hours post application) and again twenty-four hours later. Follow-up examinations were conducted thereafter only if adverse effects were present.

.05 This study was conducted in compliance with the standards of good clinical practices generally applicable for the protection of the privileges and well-being of individuals who participate in patch test procedures.

3.00 SPONSOR: [redacted]

Project Director: [redacted]

Authorization:

Purchase Order:

4.00 STUDY PRODUCT:

Type of Product:	Fragrance
Sponsor Identification:	[redacted]
Date received:	3/12/09
Quantity rec'd:	>701 g. gross wt.
Form used in study:	Volatilized [redacted]

5.00 SITE OF STUDY:

Product Investigations, Inc.
142 North Ninth Street
Suite 16
Modesto, CA 95350

Study Personnel:

Medical Director:	Morris V. Shelanski, MDCM
Dir. Derm. Services:	Joseph E. Nicholson III
Dermatologist:	Clinton E. Prescott Jr., MD
Technicians:	Lisa Cortez, Henry Cortez
Quality Assurance:	Samuel J. Charles III

6.00 DATES OF STUDY: [redacted]

7.00 SELECTION OF SUBJECTS:

.01 RECRUITING:

Prospective subjects were recruited from surrounding localities via phone, posters and personal contact.

.02 INFORMED CONSENT:

All individuals who expressed interest in participating were given an informed consent document to read. This document, which each candidate had to read and sign before being entered into the study, presented the following information:

a. How many subjects were to be enrolled in the study;
b. The intended use of the product;
c. Why the product was being tested;
d. How the test was to be performed;
e. That the regimen was not intended to benefit a subject's health, well being, or quality of life.
f. The different ways that participation may be detrimental to a subject's health, well being, or quality of life.
g. That not all detrimental effects could be foreseen and made known at the time the informed consent was presented for the prospective subject's signature.
h. What commitments a subject had to make to be in compliance; and
i. What considerations a subject was entitled to receive and the conditions for receiving them.

.03 DETERMINATION OF ELIGIBILITY:

Information concerning a prospective subject's qualifications was obtained from the answers the subject gave in filling out a medical history form and in responding to specific questions. Those who did not meet the following criteria were rejected.

a. **Inclusion Criteria: Satisfaction of all the following items was obligatory:**
   i. The candidate was at least eighteen years old, and
   ii. agreed to comply fully with the scheduled study regimen, and
   iii. expressed awareness that a participant would incur risks that would affect her/his well-being, and
   iv. denied that the amount of the stipend had induced her/him to participate against her/his better judgment, and
   v. had read the informed consent agreement, and
   vi. had assured the interviewer that she/he had no questions about the informed consent's contents that had not been answered to her/his satisfaction, and
   vii. had signed the consent form willingly and without reservation.

b. **Exclusion Criteria:** Any one of the following items was cause for rejection:
   i. The candidate had an illness that contraindicated participation; or
   ii. a condition that rendered the skin unsuitable for use in this study; or
   iii. was using dosages of medications that could alter the skin's tolerance; or
   iv. had a documented history of intolerance to the category of products submitted for study; or
   v. was a female who was pregnant or was breast feeding an infant.

.04 PANEL INFORMATION:

a. [redacted]

b. **Demographics:**

SEX	Number	Age Range
Female	65	18 - 65
Male	49	18 - 68

c. **Dedication:** This was a shared panel, i.e. the subjects were engaged in the evaluation of materials submitted by sponsors other than [redacted]

8.00 SITE INFORMATION:

.01 LOCATION:

[redacted] was assigned Band #3 on the left side of the back of each subject.

.02 IDENTIFICATION OF A CONTACT SITE:

At each visit the skin around the contact site was marked to facilitate examinations after the device was removed and positioning of subsequently-applied devices as precisely as was feasible on the same site.

9.00 PATCHING DEVICES:

.01 TYPE OF DEVICE:

Partially-occlusive patching devices consisting of a 2cm x 2cm absorbent pad centered on the adhesive-coated surface of a 2cm x 4cm plastic film were used to convey and maintain the product on the skin.

.02 PREPARATION OF A PATCHING DEVICE:

The webril pad of a patching device was infused with 200µl of the test material. Prepared devices were exposed to ambient air for at least 30 minutes prior to application.

.03 POSITIONING AND REMOVING A PATCHING DEVICE:

a. A prepared device was positioned on its designated site on each subject with the product-treated surface of the pad in contact with the skin.
b. Firm pressure was applied to the backing of the device to effect intimate contact of the pad with the skin and to bond the flanges of the device securely to the skin.
c. When the time came for removing the device, the device was peeled off the skin as gently as was feasible under the circumstances.

10.00 DATA ACQUISITION:

.01 GRADING PROCEDURE:

a. Examinations of the contact sites to grade the effects elicited by the product were conducted on Mondays, Wednesday and Fridays. When a subject came in on a scheduled examination day, the technician examined the skin of the contact site.
   i. If no adverse effect was detected, a "0" was recorded in the subject's Case Report Form.
   ii. If an adverse effect was detected, the technician entered a grade indicating her assessment of the response's intensity.
b. The subject was then sent into the patching room where the site was examined again by a second technician to ascertain independently whether or not the site should be used again. If she disagreed with the first technician's assessment, the application was held in abeyance until the issue could be resolved with the help of the supervisor and/or the investigator.
c. The supervisor or the investigator was called in not only when a disagreement had to be resolved, but also to validate substantial sudden changes, e.g. when a response is deemed to merit a grade ≥3 or when a response has been judged to have decreased by two or more points from the previous day's status.

.02 CRITERIA FOR GRADING RESPONSE INTENSITY:

The following scale was used in this procedure to designate the intensities of those gross skin changes that may be occasioned by exposing the surface of the skin to a product.

Morphology	Visible Change	Grade
Subclinical Stage	None	0
Inflammation		
Vascular Dilation:	Faint redness with poorly defined margins	1
	Redness with well-defined margins	2
Infiltration:	Redness plus well-defined edema	3
	Redness plus papules, or vesicles or ulceration	4

.04 SITE CHANGES:

a. **Switch to a Naive Site:**
   i. If the product had elicited a Grade 2 response on a subject, application of the product would have been switched immediately to a naive site on the subject.
b. **Discontinuation of Applications:**
   i. If the product had elicited a second Grade 2 on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.
   ii. If the product had elicited a Grade 3 response on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.

11.00 OVERVIEW OF STUDY REGIMEN:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week #1	Apply-	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #2	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #3	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #4	Grade	–	–	–	–	–	–
Week #5	Apply	Remove/Grade	Grade	Grade*	Grade*	–	–

*If necessary

12.00 STUDY REGIMEN:

.01 INITIAL/INDUCTION PHASE-

Week #1:

Monday:

i. As each subject presented herself/himself at the clinic, the skin of the contact site assigned to the product submitted for study was examined and ascertained to be suitable before applications were begun.
ii. A freshly-prepared patching device was applied on its assigned site.
iii. The skin around the device was marked and the subject was instructed to return on Tuesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Wednesday

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Monday for resumption of the regimen.

Week #2:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday, Friday:

The procedures followed were the same as those followed on corresponding days during Week 1.

Week #3:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday, Friday:

The procedures followed were the same as those followed on corresponding days during Week 1.

Week #4:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. a) If the subject had undergone all nine induction applications, she/he was dismissed after being instructed as follows:
i) to report back to the clinic on the following Monday to receive the challenge applications, and
ii) to notify the investigator without delay should any significant changes occur in the skin of the contact site before Monday of the challenge week.

b) If the subject had not received the required number of induction applications and was deficient without valid reason, applications were continued. As many as two missed applications could be made up during this week. When the subject had undergone the required number of make up applications, she/he was dismissed after being instructed as in section a) ii, above.

.02 HIATUS/MAKE UP PHASE-
Week # 4:

After the examination on Monday of Week 4, no procedures other than make-up cycles were scheduled during this period.

.03 CHALLENGE PHASE-
Week #5:

Monday:

i. As each subject returned, the skin of the initial induction site was examined and ascertained to be free of any conditions that would have rendered it unfit for undergoing the challenge applications.
ii. A prepared device was applied on the initial induction site.
iii. A second prepared device was applied on a naive site.
iv. The skin around both devices was marked and the subject was instructed to return on Tuesday.

Tuesday: (Note: If a subject was absent on Monday, she/he was patched on Tuesday.)

i. As each subject returned, the site-identifying marks around both contact sites were reinforced.
ii. Both patching devices were removed by a technician.
iii. The skin of both contact sites was graded; the grades were recorded.
iv. The subject was instructed to return on Wednesday.

Wednesday:

i. As each subject returned, the skin of both contact sites was graded; the grades were recorded.
ii. If follow-up was indicated, the subject was instructed to return on Thursday, otherwise the subject was dismissed from the study of this material.

.04 FOLLOW-UP PHASE:

Week No. 6 and Week No. 7:

During the two weeks following the exit examiuation, the subjects were given the opportunity to relay any information concerning effects that were relevant to the characterization of the product as well as to communicate the need for treatment of persistent or newly-occurring responses.

13.00 PROCEDURE DEVIATIONS:

No deviations in procedure were necessary.

14.00 COMPLIANCE

PHASE	No. Of AEC's Required	EXCUSED	COMPLIANT YES	NO
Induction	8	0	106	8
Challenge	1/1	0	103	11

106 of the 114 Subjects were in compliance with the number of required application/examination cycles during induction.
103 of the 114 Subjects were in compliance with the number of required application/examination cycles during challenge.

15.00 INCIDENCE OF RESPONSES:

GRADE	TYPE OF RESPONSE	INDUCTION PHASE	CHALLENGE PHASE	
			ORIGINAL CONTACT SITE	NAIVE CONTACT SITE
0	NO VISIBLE CHANGE	113 SUBJECTS	103 SUBJECTS	103 SUBJECTS
1	FAINT REDNESS, UNDEFINED BORDER	0 "	0 "	0 "
2	INTENSE REDNESS, DEFINED BORDER	0 "	0 "	0 "
3	REDNESS + DEFINITE EDEMA	0 "	0 "	0 "
4	REDNESS + PAPULES, OR VESICLES,	0 "	0 "	0 "
[redacted]	NO. OF RESPONDERS	0 SUBJECTS	0 SUBJECTS	0 SUBJECTS
[redacted]	NO DATA ACQUIRED	1 SUBJECT	11 SUBJECTS	11 SUBJECTS

16.00 SIGNIFICANCE OF THE RESPONSES:

.01 INITIAL/INDUCTION PHASE:

No responses were noted on any of the 113 subjects who underwent at least one post-application examination. The absence of responses characterizes the product as one which is devoid of clinically significant skin-irritating propensities.

.02 CHALLENGE PHASE:

a. **Original Contact Sites:**
No responses were noted on any of the 103 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

b. **Naive Contact Sites:**
No responses were noted on any of the 103 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

17.00 CONCLUSIONS:

.01 [redacted] was found to be neither a clinically significant skin irritant nor a skin sensitizer under the conditions of this study.

.02 [redacted] is not contraindicated for usages entailing repeated applications on human skin under conditions appropriate for such products.

PRODUCT INVESTIGATIONS, INC.

5/13/09
Date

Joseph E. Nicholson III
Director, Dermatological Studies

18.00 COMPLIANCE WITH GOOD QUALITY ASSURANCE STANDARDS:

I have audited the results presented in this report and believe that, to the best of my knowledge, they accurately reflect the raw data acquired during the course of this study.

Samuel Charles
Director, Quality Assurance

[redacted]

Site: L3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
1	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
2	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
3	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
4	B	[redacted]	0	[redacted]	Dropped		[redacted]		[redacted]			[redacted]		[redacted]												
5	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
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(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Site: L3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE				
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5				
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37	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	A	[redacted]	A	[redacted]	Dropped										
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(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Site: L3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
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(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Site: L3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
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(Grade) / (Grade) = Original Site / Naive Site

PRODUCT INVESTIGATIONS, INC.
151 East Tenth Avenue
Conshohocken, PA 19428
610-825-5855 • fax 610-825-7288

REPORT: [redacted]

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted]ON HUMAN SKIN

PREPARED FOR

C12-C15 Alkyl Benzoates - 14.15%
RIPT (0/116)
Product Type - Blush

[redacted]

[redacted]

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17.00	Conclusions	"
18.00	Compliance with Good QA Standards	"

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

1.00 OBJECTIVES:

.01 To identify and characterize the skin-damaging propensities that [redacted] can be induced to exercise under the conditions of this modified patch test procedure.

.02 To adjudge whether the exercise of such propensities under the test conditions contraindicates the kind of skin contact that would be occasioned during the appropriate use of the product.

2.00 DESIGN:

.01 A modified version of the Repeated Insult Patch Test [redacted] was conducted under double blind conditions on a panel composed of more than one hundred subjects at the outset.

.02 The regimen comprised nine sequential 24-hour induction applications and two concurrently conducted 24-hour challenge applications, one on the initial induction site and one on a naive site.

.03 During the initial phase, the skin of the contact sites was graded and the grades recorded on Wednesdays, Fridays (i.e. twenty-four hours after patches had been removed), and Mondays (i.e. forty-eight hours after patches had been removed).

.04 During the challenge phase, the skin of the contact sites was graded within moments after the patches had been removed (24 hours post application) and again twenty-four hours later. Follow-up examinations were conducted thereafter only if adverse effects were present.

.05 This study was conducted in compliance with the standards of good clinical practices generally applicable for the protection of the privileges and well-being of individuals who participate in patch test procedures.

3.00 SPONSOR: [redacted]

Project Director: [redacted]

Authorization: [redacted]

Purchase Order: [redacted]

4.00 STUDY PRODUCT:

Type of Product:	Cream Blush
Sponsor Identification:	[redacted]
Date received:	4/10/09
Quantity rec'd:	>408 g. gross wt.
Form used in study:	As supplied

[redacted]

5.00 SITE OF STUDY:

Product Investigations, Inc.
142 North Ninth Street
Suite 16
Modesto, CA 95350

Study Personnel:

Medical Director:	Morris V. Shelanski, MDCM
Dir. Derm. Services:	Joseph E. Nicholson III
Dermatologist:	Clinton E. Prescott Jr., MD
Technicians:	Lisa Cortez, Henry Cortez
Quality Assurance:	Samuel J. Charles III

6.00 DATES OF STUDY: [redacted]

7.00 SELECTION OF SUBJECTS:

.01 RECRUITING:

Prospective subjects were recruited from surrounding localities via phone, posters and personal contact.

.02 INFORMED CONSENT:

All individuals who expressed interest in participating were given an informed consent document to read. This document, which each candidate had to read and sign before being entered into the study, presented the following information:

a. How many subjects were to be enrolled in the study;
b. The intended use of the product;
c. Why the product was being tested;
d. How the test was to be performed;
e. That the regimen was not intended to benefit a subject's health, well being, or quality of life.
f. The different ways that participation may be detrimental to a subject's health, well being, or quality of life.
g. That not all detrimental effects could be foreseen and made known at the time the informed consent was presented for the prospective subject's signature.
h. What commitments a subject had to make to be in compliance; and
i. What considerations a subject was entitled to receive and the conditions for receiving them.

.03 DETERMINATION OF ELIGIBILITY:

Information concerning a prospective subject's qualifications was obtained from the answers the subject gave in filling out a medical history form and in responding to specific questions. Those who did not meet the following criteria were rejected.

a. **Inclusion Criteria: Satisfaction of all the following items was obligatory:**
   i. The candidate was at least eighteen years old, and
   ii. agreed to comply fully with the scheduled study regimen, and
   iii. expressed awareness that a participant would incur risks that would affect her/his well-being, and
   iv. denied that the amount of the stipend had induced her/him to participate against her/his better judgment, and
   v. had read the informed consent agreement, and
   vi. had assured the interviewer that she/he had no questions about the informed consent's contents that had not been answered to her/his satisfaction, and
   vii. had signed the consent form willingly and without reservation.

b. **Exclusion Criteria:** Any one of the following items was cause for rejection:
   i. The candidate had an illness that contraindicated participation; or
   ii. a condition that rendered the skin unsuitable for use in this study; or
   iii. was using dosages of medications that could alter the skin's tolerance; or
   iv. had a documented history of intolerance to the category of products submitted for study; or
   v. was a female who was pregnant or was breast feeding an infant.

.04 PANEL INFORMATION:

a. [redacted]

b. **Demographics:**

SEX	Number	Age Range
Female	82	18 - 83
Male	39	18 - 62

c. **Dedication:** This was a shared panel, i.e. the subjects were engaged in the evaluation of materials submitted by sponsors other than [redacted]

8.00 SITE INFORMATION:

.01 LOCATION:

[redacted] was assigned Band #3 on the left side of the back of each subject.

.02 IDENTIFICATION OF A CONTACT SITE:

At each visit the skin around the contact site was marked to facilitate examinations after the device was removed and positioning of subsequently-applied devices as precisely as was feasible on the same site.

9.00 PATCHING DEVICES:

.01 TYPE OF DEVICE:

Partially-occlusive patching devices consisting of a 2 cm x 2 cm absorbent pad centered on the adhesive-coated surface of a 2 cm x 4 cm plastic film were used to convey and maintain the product on the skin.

.02 PREPARATION OF A PATCHING DEVICE:

The webril pad of a patching device was infused with 200μl of the test material.

.03 POSITIONING AND REMOVING A PATCHING DEVICE:

a. A prepared device was positioned on its designated site on each subject with the product-treated surface of the pad in contact with the skin.
b. Firm pressure was applied to the backing of the device to effect intimate contact of the pad with the skin and to bond the flanges of the device securely to the skin.
c. When the time came for removing the device, the device was peeled off the skin as gently as was feasible under the circumstances.

10.00 DATA ACQUISITION:

.01 GRADING PROCEDURE:

a. Examinations of the contact sites to grade the effects elicited by the product were conducted on Mondays, Wednesday and Fridays. When a subject came in on a scheduled examination day, the technician examined the skin of the contact site.
   i. If no adverse effect was detected, a "0" was recorded in the subject's Case Report Form.
   ii. If an adverse effect was detected, the technician entered a grade indicating her assessment of the response's intensity.
b. The subject was then sent into the patching room where the site was examined again by a second technician to ascertain independently whether or not the site should be used again. If she disagreed with the first technician's assessment, the application was held in abeyance until the issue could be resolved with the help of the supervisor and/or the investigator.
c. The supervisor or the investigator was called in not only when a disagreement had to be resolved, but also to validate substantial sudden changes, e.g. when a response is deemed to merit a grade ≥ 3 or when a response has been judged to have decreased by two or more points from the previous day's status.

.02 CRITERIA FOR GRADING RESPONSE INTENSITY:

The following scale was used in this procedure to designate the intensities of those gross skin changes that may be occasioned by exposing the surface of the skin to a product.

Morphology	Visible Change	Grade
Subclinical Stage	None	0
Inflammation		
Vascular Dilation:	Faint redness with poorly defined margins	1
	Redness with well-defined margins	2
Infiltration:	Redness plus well-defined edema	3
	Redness plus papules, or vesicles or ulceration	4

.04 SITE CHANGES:

a. Switch to a Naive Site:
i. If the product had elicited a Grade 2 response on a subject, application of the product would have been switched immediately to a naive site on the subject.

b. Discontinuation of Applications:
i. If the product had elicited a second Grade 2 on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.
i If the product had elicited a Grade 3 response on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.

11.00 OVERVIEW OF STUDY REGIMEN:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week #1	Apply-	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #2	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #3	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #4	Grade	–	–	–	–	–	–
Week #5	Apply	Remove/Grade	Grade	Grade*	Grade*	–	–

*If necessary

12.00 STUDY REGIMEN:

.01 INITIAL/INDUCTION PHASE-

Week #1:

Monday:

i. As each subject presented herself/himself at the clinic, the skin of the contact site assigned to the product submitted for study was examined and ascertained to be suitable before applications were begun.
ii. A freshly-prepared patching device was applied on its assigned site.
iii. The skin around the device was marked and the subject was instructed to return on Tuesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Wednesday

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Monday for resumption of the regimen.

Week #2:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday, Friday:

The procedures followed were the same as those followed on corresponding days during Week 1.

Week #3:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday, Friday:

The procedures followed were the same as those followed on corresponding days during Week 1.

Week #4:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. a) If the subject had undergone all nine induction applications, she/he was dismissed after being instructed as follows:
i) to report back to the clinic on the following Monday to receive the challenge applications, and
ii) to notify the investigator without delay should any significant changes occur in the skin of the contact site before Monday of the challenge week.

b) If the subject had not received the required number of induction applications and was deficient without valid reason, applications were continued. As many as two missed applications could be made up during this week. When the subject had undergone the required number of make up applications, she/he was dismissed after being instructed as in section a) ii, above.

.02 HIATUS/MAKE UP PHASE-
Week # 4:

After the examination on Monday of Week 4, no procedures other than make-up cycles were scheduled during this period.

.03 CHALLENGE PHASE-
Week #5:

Monday:

i. As each subject returned, the skin of the initial induction site was examined and ascertained to be free of any conditions that would have rendered it unfit for undergoing the challenge applications.
ii. A prepared device was applied on the initial induction site.
iii. A second prepared device was applied on a naive site.
iv. The skin around both devices was marked and the subject was instructed to return on Tuesday.

Tuesday: (**Note:** If a subject was absent on Monday, she/he was patched on Tuesday.)

i. As each subject returned, the site-identifying marks around both contact sites were reinforced.
ii. Both patching devices were removed by a technician.
iii. The skin of both contact sites was graded; the grades were recorded.
iv. The subject was instructed to return on Wednesday.

Wednesday:

i. As each subject returned, the skin of both contact sites was graded; the grades were recorded.
ii. If follow-up was indicated, the subject was instructed to return on Thursday, otherwise the subject was dismissed from the study of this material.

.04 FOLLOW-UP PHASE:

Week No. 6 and Week No. 7:

During the two weeks following the exit examination, the subjects were given the opportunity to relay any information concerning effects that were relevant to the characterization of the product as well as to communicate the need for treatment of persistent or newly-occurring responses.

13.00 PROCEDURE DEVIATIONS:

No deviations in procedure were necessary.

14.00 COMPLIANCE

PHASE	No. Of AEC's Required	EXCUSED	COMPLIANT YES	COMPLIANT NO
Induction	8	0	117	4
Challenge	1/1	0	116	5

117 of the 121 Subjects were in compliance with the number of required application/examination cycles during induction
116 of the 121 Subjects were in compliance with the number of required application/examination cycles during challenge.

15.00 INCIDENCE OF RESPONSES:

Grade	Type of Response	Induction Phase	Challenge Phase	
			Original Contact Site	Naive Contact Site
0	No visible change	121 subjects	116 subjects	116 subjects
1	Faint redness, undefined border	0 "	0 "	0 "
2	Intense redness, defined border	0 "	0 "	0 "
3	Redness + definite edema	0 "	0 "	0 "
4	Redness + papules, or vesicles,	0 "	0 "	0 "
[redacted]	No. of Responders	0 subjects	0 subjects	0 subjects
[redacted]	No Data Acquired	0 subjects	5 subjects	5 subjects

16.00 SIGNIFICANCE OF THE RESPONSES:

.01 Initial/Induction Phase:

No responses were noted on any of the 121 subjects who underwent at least one post-application examination. The absence of responses characterizes the product as one which is devoid of clinically significant skin-irritating propensities.

.02 Challenge Phase:

a. **Original Contact Sites:**
No responses were noted on any of the 116 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

b. **Naive Contact Sites:**
No responses were noted on any of the 116 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

17.00 CONCLUSIONS:

.01 [redacted] was found to be neither a clinically significant skin irritant nor a skin sensitizer under the conditions of this study.

.02 [redacted] is not contraindicated for usages entailing repeated applications on human skin under conditions appropriate for such products.

PRODUCT INVESTIGATIONS, INC.

6/2/09
Date

Joseph E. Nicholson III
Director, Dermatological Studies

18.00 COMPLIANCE WITH GOOD QUALITY ASSURANCE STANDARDS:

I have audited the results presented in this report and believe that, to the best of my knowledge, they accurately reflect the raw data acquired during the course of this study.

Samuel Charles
Director, Quality Assurance

[redacted]

Subj #	Panel: [redacted]					Sample No. [redacted]					PI No: [redacted]					Site: L3									
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE				
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5				
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F
1	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
2	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
3	B	[redacted]	0	[redacted]	0	A	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	A		0			B	0/0	0/0		
4	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
5	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
6	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
7	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
8	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
9	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
10	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
11	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
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13	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
14	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
15	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
16	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
17	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
18	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
19	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
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21	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
22	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
23	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
24	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
25	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
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27	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
28	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
29	B	[redacted]	0	[redacted]	0	0	[redacted]	A	[redacted]	0	0	[redacted]	0	[redacted]	0	0		0			B	0/0	0/0		
30	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Subj #	Panel: [redacted]					Sample No [redacted]					PI No: [redacted]					Site: L3									
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE				
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5				
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F
31	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0		
32	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
33	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
34	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
35	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
36	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
37	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
38	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
39	B		0		A	0		0		0	0		0		0	0		0			B	0/0	0/0		
40	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
41	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
42	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
43	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
44	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
45	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
46	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
47	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
48	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
49	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
50	B		0		0	0		0		A	0		0		0	0		0			B	0/0	0/0		
51	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
52	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
53	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
54	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
55	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
56	B		0		0	A		0		0	0		0		0	0		0			B	0/0	0/0		
57	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
58	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
59	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		
60	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0		

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

	Pane[redacted]					Sample No[redacted]								PI No:[redacted]						Site: L3					
Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
61	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
62	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
63	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
64	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
65	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
66	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
67	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
68	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
69	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
70	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
71	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
72	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
73	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
74	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
75	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
76	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
77	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
78	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
79	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
80	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
81	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
82	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
83	B	[redacted]	0	[redacted]	0	A	[redacted]	A	[redacted]	Dropped																
84	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
85	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
86	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
87	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
88	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
89	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
90	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

	Panel: [redacted]					Sample No. [redacted]						PI No: [redacted]						Site: L3					
Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
91	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
92	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
93	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
94	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
95	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
96	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
97	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
98	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
99	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
100	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
101	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
102	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
103	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
104	B	[redacted]	0	[redacted]	Dropped		[redacted]		[redacted]			[redacted]		[redacted]												
105	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
106	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
107	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
108	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
109	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	A	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
110	B	[redacted]	0	[redacted]	Dropped		[redacted]		[redacted]			[redacted]		[redacted]												
111	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
112	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
113	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
114	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
115	B	[redacted]	0	[redacted]	0	A	[redacted]	0	[redacted]	0	A	[redacted]	A	[redacted]	Dropped											
116	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
117	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
118	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
119	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
120	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			
121	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

PRODUCT INVESTIGATIONS, INC.

151 East Tenth Avenue
Conshohocken, PA 19428
610-825-5855 • fax 610-825-7288

REPORT: [redacted]

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

PREPARED FOR

[redacted]

C12-C15 Alkyl Benzoates - 19.5%
RIPT (0/100)
Product Type - Body Oil

[redacted]

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DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

1.00 **OBJECTIVES:**

.01 To identify and characterize the skin-damaging propensities that [redacted] can be induced to exercise under the conditions of this modified patch test procedure.

.02 To adjudge whether the exercise of such propensities under the test conditions contraindicates the kind of skin contact that would be occasioned during the appropriate use of the product.

2.00 **DESIGN:**

.01 A modified version of the Repeated Insult Patch Test [redacted] was conducted under double blind conditions on a panel composed of more than one hundred subjects at the outset.

.02 The regimen comprised nine sequential 24-hour induction applications and two concurrently conducted 24-hour challenge applications, one on the initial induction site and one on a naive site.

.03 During the initial phase, the skin of the contact sites was graded and the grades recorded on Wednesdays, Fridays (i.e. twenty-four hours after patches had been removed), and Mondays (i.e. forty-eight hours after patches had been removed).

.04 During the challenge phase, the skin of the contact sites was graded within moments after the patches had been removed (24 hours post application) and again twenty-four hours later. Follow-up examinations were conducted thereafter only if adverse effects were present.

.05 This study was conducted in compliance with the standards of good clinical practices generally applicable for the protection of the privileges and well-being of individuals who participate in patch test procedures.

3.00 **SPONSOR:** [redacted]

Project Director: [redacted]
Authorization:
Purchase Order:

4.00 **STUDY PRODUCT:**

Type of Product:	Blue liquid (fragrance)
Sponsor Identification:	[redacted]
Date received:	8/10/09
Quantity rec'd:	>600 g. gross wt.
Form used in study:	As supplied

[redacted]

5.00 **SITE OF STUDY:**

Product Investigations, Inc.
151 East Tenth Avenue
Conshohocken, PA 19428

Study Personnel:

Medical Director:	Morris V. Shelanski, MDCM
Dir. Derm. Services:	Joseph E. Nicholson III
Dermatologist:	Julia H. Cohen, MD
Technicians:	Kay Walk, Margaret Reilly
Quality Assurance:	Samuel J. Charles III

6.00 **DATES OF STUDY:** [redacted]

7.00 SELECTION OF SUBJECTS:

.01 RECRUITING:

Prospective subjects were recruited from surrounding localities via phone, posters and personal contact.

.02 INFORMED CONSENT:

All individuals who expressed interest in participating were given an informed consent document to read. This document, which each candidate had to read and sign before being entered into the study, presented the following information:

a. How many subjects were to be enrolled in the study;
b. The intended use of the product;
c. Why the product was being tested;
d. How the test was to be performed;
e. That the regimen was not intended to benefit a subject's health, well being, or quality of life.
f. The different ways that participation may be detrimental to a subject's health, well being, or quality of life.
g. That not all detrimental effects could be foreseen and made known at the time the informed consent was presented for the prospective subject's signature.
h. What commitments a subject had to make to be in compliance; and
i. What considerations a subject was entitled to receive and the conditions for receiving them.

.03 DETERMINATION OF ELIGIBILITY:

Information concerning a prospective subject's qualifications was obtained from the answers the subject gave in filling out a medical history form and in responding to specific questions. Those who did not meet the following criteria were rejected.

a. **Inclusion Criteria: Satisfaction of all the following items was obligatory:**
   i. The candidate was at least eighteen years old, and
   ii. agreed to comply fully with the scheduled study regimen, and
   iii. expressed awareness that a participant would incur risks that would affect her/his well-being, and
   iv. denied that the amount of the stipend had induced her/him to participate against her/his better judgment, and
   v. had read the informed consent agreement, and
   vi. had assured the interviewer that she/he had no questions about the informed consent's contents that had not been answered to her/his satisfaction, and
   vii. had signed the consent form willingly and without reservation.

b. **Exclusion Criteria:** Any one of the following items was cause for rejection:
   i. The candidate had an illness that contraindicated participation; or
   ii. a condition that rendered the skin unsuitable for use in this study; or
   iii. was using dosages of medications that could alter the skin's tolerance; or
   iv. had a documented history of intolerance to the category of products submitted for study; or
   v. was a female who was pregnant or was breastfeeding an infant.

.04 PANEL INFORMATION:

a. [redacted] [redacted]

b. **Demographics:**

SEX	Number	Age Range
Female	84	18 - 86
Male	20	19 - 78

c. **Dedication:** This was a shared panel, i.e. the subjects were engaged in the evaluation of materials submitted by sponsors other than [redacted]

8.00 SITE INFORMATION:

.01 LOCATION:

[redacted] was assigned Band #3 on the right side of the back of each subject.

.02 IDENTIFICATION OF A CONTACT SITE:

At each visit the skin around the contact site was marked to facilitate examinations after the device was removed and positioning of subsequently-applied devices as precisely as was feasible on the same site.

9.00 PATCHING DEVICES:

.01 TYPE OF DEVICE:

Partially-occlusive patching devices consisting of a 2 cm x 2 cm absorbent pad centered on the adhesive-coated surface of a 2 cm x 4 cm plastic film were used to convey and maintain the product on the skin.

.02 PREPARATION OF A PATCHING DEVICE:

The webril pad of a patching device was infused with 200µl of the test material.

.03 POSITIONING AND REMOVING A PATCHING DEVICE:

a. A prepared device was positioned on its designated site on each subject with the product-treated surface of the pad in contact with the skin.
b. Firm pressure was applied to the backing of the device to effect intimate contact of the pad with the skin and to bond the flanges of the device securely to the skin.
c. When the time came for removing the device, the device was peeled off the skin as gently as was feasible under the circumstances.

10.00 DATA ACQUISITION:

.01 GRADING PROCEDURE:

a. Examinations of the contact sites to grade the effects elicited by the product were conducted on Mondays, Wednesday and Fridays. When a subject came in on a scheduled examination day, the technician examined the skin of the contact site.
   i. If no adverse effect was detected, a "0" was recorded in the subject's Case Report Form.
   ii. If an adverse effect was detected, the technician entered a grade indicating her assessment of the response's intensity.
b. The subject was then sent into the patching room where the site was examined again by a second technician to ascertain independently whether or not the site should be used again. If she disagreed with the first technician's assessment, the application was held in abeyance until the issue could be resolved with the help of the supervisor and/or the investigator.
c. The supervisor or the investigator was called in not only when a disagreement had to be resolved, but also to validate substantial sudden changes, e.g. when a response is deemed to merit a grade ≥3 or when a response has been judged to have decreased by two or more points from the previous day's status.

.02 CRITERIA FOR GRADING RESPONSE INTENSITY:

The following scale was used in this procedure to designate the intensities of those gross skin changes that may be occasioned by exposing the surface of the skin to a product.

Morphology	Visible Change	Grade
Subclinical Stage	None	0
Inflammation		
Vascular Dilation:	Faint redness with poorly defined margins	1
	Redness with well-defined margins	2
Infiltration:	Redness plus well-defined edema	3
	Redness plus papules, or vesicles or ulceration	4

.04 SITE CHANGES:

a. **Switch to a Naive Site:**
   i. If the product had elicited a Grade 2 response on a subject, application of the product would have been switched immediately to a naive site on the subject.
b. **Discontinuation of Applications:**
   i. If the product had elicited a second Grade 2 on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.
   ii. If the product had elicited a Grade 3 response on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.

11.00 OVERVIEW OF STUDY REGIMEN:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week #1	Apply-	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #2	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #3	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #4	–	Grade	–	–	–	–	–
Week #5	Apply	Remove/Grade	Grade	Grade*	Grade*	–	–

*If necessary

12.00 STUDY REGIMEN:

.01 INITIAL/INDUCTION PHASE-

Week #1:

Monday:

i. As each subject presented herself/himself at the clinic, the skin of the contact site assigned to the product submitted for study was examined and ascertained to he suitable before applications were begun.
ii. A freshly-prepared patching device was applied on its assigned site.
iii. The skin around the device was marked and the subject was instructed to return on Tuesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Wednesday

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Monday for resumption of the regimen.

Week #2:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday, Friday:

The procedures followed were the same as those followed on corresponding days during Week 1.

Week #3:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday:

The procedures followed were the same as those followed on corresponding days during Week 1.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Tuesday for resumption of the regimen.

Week #4:

Tuesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.

ii. The time at which the patch was removed on Saturday was recorded.

iii. a) If the subject had undergone all nine induction applications, she/he was dismissed after being instructed as follows:

i) to report back to the clinic on the following Monday to receive the challenge applications, and

ii) to notify the investigator without delay should any significant changes occur in the skin of the contact site before Monday of the challenge week.

b) If the subject had not received the required number of induction applications and was deficient without valid reason, applications were continued. As many as two missed applications could be made up during this week. When the subject had undergone the required number of make up applications, she/he was dismissed after being instructed as in section a) ii, above.

.02 HIATUS/MAKE UP PHASE-

Week # 4:

After the examination on Tuesday of Week 4, no procedures other than make-up cycles were scheduled during this period.

.03 CHALLENGE PHASE-

Week #5:

Monday:

i. As each subject returned, the skin of the initial induction site was examined and ascertained to be free of any conditions that would have rendered it unfit for undergoing the challenge applications.

ii. A prepared device was applied on the initial induction site.

iii. A second prepared device was applied on a naive site.

iv. The skin around both devices was marked and the subject was instructed to return on Tuesday.

Tuesday: (Note: If a subject was absent on Monday, she/he was patched on Tuesday.)

i. As each subject returned, the site-identifying marks around both contact sites were reinforced.

ii. Both patching devices were removed by a technician.

iii. The skin of both contact sites was graded; the grades were recorded.

iv. The subject was instructed to return on Wednesday.

Wednesday:

i. As each subject returned, the skin of both contact sites was graded; the grades were recorded.

ii. If follow-up was indicated, the subject was instructed to return on Thursday, otherwise the subject was dismissed from the study of this material.

.04 FOLLOW-UP PHASE:

Week #6 and Week #7:

During the two weeks following the exit examination, the subjects were given the opportunity to relay any information concerning effects that were relevant to the characterization of the product as well as to communicate the need for treatment of persistent or newly-occurring responses.

13.00 PROCEDURE DEVIATIONS:

The lab was closed on Monday of Week #4 due to the Labor Day holiday. Subjects returned on Tuesday as noted above.

14.00 COMPLIANCE :

PHASE	No. Of AEC's Required	EXCUSED	COMPLIANT YES	NO
Induction	8	0	100	4
Challenge	1/1	0	100	4

100 of the 104 Subjects were in compliance with the number of required application/examination cycles during induction.
100 of the 104 Subjects were in compliance with the number of required application/examination cycles during challenge

15.00 INCIDENCE OF RESPONSES:

Grade	Type of Response	Induction Phase	Challenge Phase	
			Original Contact Site	Naive Contact Site
0	No visible change	104 subjects	100 subjects	100 subjects
1	Faint redness, undefined border	0 "	0 "	0 "
2	Intense redness, defined border	0 "	0 "	0 "
3	Redness + definite edema	0 "	0 "	0 "
4	Redness + papules, or vesicles, etc.	0 "	0 "	0 "
[redacted]	No. of Responders	0 subjects	0 subjects	0 subjects
[redacted]	No Data Acquired	0 subjects	4 subjects	4 subjects

16.00 SIGNIFICANCE OF THE RESPONSES:

.01 Initial/Induction Phase:

No responses were noted on any of the 104 subjects who underwent at least one post-application examination. The absence of responses characterizes the product as one which is devoid of clinically significant skin-irritating propensities.

.02 Challenge Phase:

a. **Original Contact Sites:**
No responses were noted on any of the 100 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

b. **Naive Contact Sites:**
No responses were noted on any of the 100 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

17.00 CONCLUSIONS:

.01 [redacted] was found to be neither a clinically significant skin irritant nor a skin sensitizer under the conditions of this study.

.02 [redacted] is not contraindicated for usages entailing repeated applications on human skin under conditions appropriate for such products.

PRODUCT INVESTIGATIONS, INC.

7 Oct. 2009
Date

Joseph E. Nicholson III
Director, Dermatological Studies

18.00 COMPLIANCE WITH GOOD QUALITY ASSURANCE STANDARDS:

I have audited the results presented in this report and believe that, to the best of my knowledge, they accurately reflect the raw data acquired during the course of this study.

Samuel J. Charles III
Director, Quality Assurance

[redacted]

Site: R3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
1	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
2	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
3	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
4	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
5	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
6	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
7	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
8	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
9	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
10	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
11	B	[redacted]	0	[redacted]	0	A	[redacted]	A	[redacted]	Dropped																
12	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
13	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
14	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
15	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
16	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
17	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
18	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
19	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
20	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
21	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
22	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
23	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
24	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
25	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	A	0	[redacted]	0	[redacted]	0	[redacted]	0		0		B	0/0	0/0			
26	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
27	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
28	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
29	B	[redacted]	0	[redacted]	0	A	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0		0		B	0/0	0/0			
30	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Site: R3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
31	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
32	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
33	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
34	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
35	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
36	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
37	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
38	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
39	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
40	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
41	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
42	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
43	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
44	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
45	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
46	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
47	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
48	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
49	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
50	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
51	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
52	B		0		0	0		0		0	0		A		0		0		0		B	0/0	0/0			
53	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
54	B		A		0	0		0		0	0		0		0		0		0		B	0/0	0/0			
55	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
56	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
57	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
58	B		0		0	A		0		0	0		0		0		0		0		B	0/0	0/0			
59	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
60	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Site: R3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
61	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
62	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
63	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
64	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
65	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
66	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
67	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
68	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
69	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
70	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
71	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
72	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
73	B		A		0	0		0		0	0		0		0		0		0		B	0/0	0/0			
74	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
75	B		0		0	0		0		A	0		0		0		0		0		B	0/0	0/0			
76	B		0		0	0		0		A	0		0		0		0		0		B	0/0	0/0			
77	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
78	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
79	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
80	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
81	B		0		0	A		0		0	0		0		0		0		0		B	0/0	0/0			
82	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
83	B		0		0	A		A		Dropped																
84	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
85	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
86	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
87	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
88	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
89	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			
90	B		0		0	0		0		0	0		0		0		0				B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

[redacted]

Site: R3

Subj #	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
91	B	[redacted]	A	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0		0		B	0/0	0/0			
92	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
93	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
94	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	A	[redacted]										
95	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	A	[redacted]										
96	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
97	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
98	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
99	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
100	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
101	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
102	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
103	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			
104	B	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	[redacted]	0				B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

Clinical Research Services
CLINICAL & BIOMEDICAL TESTING

Human Repeat Insult Patch Test
Study C98-0012

Octyldodecyl Benzoate - 2.0473%
RIPT (0/101)
Product Type - Perfumed Soap Bar

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Human Repeat Insult Patch Test

[redacted]

Abstract

One hundred one (101) male and female subjects between the ages of 18 and 70 completed a six-week human repeat insult patch test.

During the 'Induction Phase' samples [redacted] D, F and G showed no irritation. The remaining samples in the series showed mild to moderate irritation except L and N. These samples exhibited papular irritation. Sample N required a reduction in concentration to 50% to complete the induction phase.

Results of the 'Challenge Phase' were as follows:

	48 Hours		96 Hours		
Sample [redacted]	Original (frequency)	Alternate (frequency)	Original (Frequency)	Alternate (Frequency)	Contact Allergen
A	0	0	0	0	No
B	0	0 (100) 1 (1)	0	0 (100) 1 (1)	No
C	0	0	0	0	No
D	0	0	0 (100) 1 (1)	0	No
H	0 (98) 1 (3)	0 (99) 1 (2)	0	0	No
I	0	0	0	0	No
J	0	0	0	0	No
K	0	0	0 (100) 1 (1)	0	No
L	0	0	0	0	No
M	0	0	0	0	No
N	0	0	0	0	No
O	0	0	0	0	No
E	0	0	0	0	No
F	0	0	0	0	No
G	0	0	0	0	No

Purpose:

This study was conducted to evaluate the potential of the Sponsor's test materials to induce irritant and allergic contact dermatitis.

Materials:

The following samples were supplied to Clinical Research and prepared according to the Sponsor's instructions:

TABLE 1

Sample [redacted]	Concentration	Patch
A	Neat	Occlusive
B	Neat	Occlusive
C	Neat	Occlusive
D	Neat	Occlusive
E	5% in Distilled Water	Semi-Occlusive
F	20% in Distilled Water	Semi-Occlusive
G	Neat	Semi-Occlusive
H	Neat	Occlusive
I	1.3% in Distilled water	Occlusive
J	0.3% in Distilled Water	Occlusive
K	1% in Castor Oil	Semi-Occlusive
L	4% in Distilled Water	Occlusive
M	0.2% in Glycerin	Occlusive
N	Neat	Occlusive
O	Neat	Occlusive

Test Material Identification Forms describe the sample received by Clinical Research. (Appendix I) The stability, purity and microbial testing of the samples was left to the Sponsor.

Subject Selection:

One-hundred one (101) male and female subjects between the ages of 18 and 70 completed the study. An Informed Consent Agreement was obtained from all subjects before testing. (Appendix II) Subjects were admitted to the study at the discretion of the Principal Investigator

based on the medical history, study criteria and pre-study interview and examination. (Appendices III & IV) Anyone with an uncontrolled metabolic disorder was excluded from the study. Also excluded were individuals with active psoriasis, eczema, sunburn, tattoos or acne (on test areas), skin infection, skin cancer or history of atopic dermatitis. Subjects who were taking medications that would interfere with the study (e.g., steroids, anticancer, immunosuppressive or anti-inflammatory drugs, etc.) were also excluded. Female subjects who were pregnant, nursing or planning a pregnancy within six months were excluded. No subject had participated in other patch tests within the past 30 days.

Subject Numbering, Subject Withdrawals

Each subject admitted to the study was assigned a unique three digit number. This number remained with the subject throughout the study. Subjects 006, 010, 014, 017, 019, 022, 026, 076 and 106 withdrew from the study for reasons unrelated to testing. (Appendix V) The data for these subjects is not used. Subject 012 was withdrawn from the panel due to adverse reactions to samples K and N. These reactions are documented on the Adverse Clinical Reaction Form. (Appendix VI)

Confidentiality

To maintain confidentiality, only the study number and subject number are provided for identification. Signed copies of the consent forms, health histories and eligibility forms are maintained on file at Clinical Research.

Methods:[1,2]

Induction Phase

The three-week induction phase began on August 3, 1998 and continued through August 24, 1998. Occlusive and semi-occlusive patches were used for the present study according to Table 1. The patches consisted of a 2-cm diameter Webril® cotton pad held on all sides with 3M Micropore® tape. Blenderm® covertape was used for occlusion where required. The test materials were prepared according to the Sponsor's instructions. (Table 1) They were applied at a dose of approximately 200 μl per patch for liquids and 200 mg for creams and semi-solids.

Patches were applied to the back at 48 hour intervals for a total of nine (9) exposures. Panelists were instructed to remove the patches 2 hours before their next visit. At each visit a clinical grader scored the test site before the application of the next series of patches. A modified scoring scale, adapted from the American Academy of Dermatology, **Diagnostic Patch Testing Technique**, was used.[3] (Appendix VII) Subsequent patches in the induction series were returned to the original location. However, a patch was moved to an adjacent, untreated area when scores reached 2+ (with or without a letter grade). Patches could be moved twice. A patch was stopped if residual reactions persisted on the original and alternate areas. (Noted as 'X' in the data) Residual reactions to the test product were monitored and treated as necessary. An 'N9G'

shows that the subject received all nine induction patches but was unable to attend the final reading day.

Subjects were provided written instructions for care of the patches. (Appendix VIII) A single missed visit was allowed during this phase with a required make-up visit on August 26.

Rest Phase

A rest phase of 10 to 11 days began on [redacted] and continued through September 7, 1998. The actual duration of the rest phase was dependent on any required makeup visits. No patches were applied during this phase.

Challenge Phase

The Challenge Phase began on [redacted] and continued through September 12, 1998. Challenge patches, identical to those used in the induction phase, were returned to the original sites on the back. In addition, alternate patches were placed on the upper arms. The alternate patch system is used to cross-check reactions on the original site. This helps to reduce the incidence of false positive scores. Both patches were worn for approximately 48 hours and were removed by the test subjects approximately 2 hours before their scheduled visit. Test areas were then scored at 48 and 96 hours. No absences were allowed during this phase.

Records:

All original forms are retained by Clinical Research Services, Inc. for two years. These records are considered confidential, and will be available only to the Sponsor. At the end of two years the Sponsor may retrieve the archived files at their expense or have the records destroyed.

Results:

Descriptive statistics and raw data for the study are shown in the tabbed sections labeled 'Induction Phase' and 'Challenge Phase'.

Conclusion:

Based on the results of this test, there is no indication of allergic contact sensitization to the test agents. However, there is a likelihood of contact irritation to samples [redacted] and N with repeated exposure. In addition, sample H exhibited a high frequency of mild irritation in the 'Induction Phase'. This sample might produce irritation on sensitive skin with repeated use.

John C. Seitz, PA-C
Clinical Research Services
9/22/98
Date

Ken Pechman, M.D., Ph.D.
Medical Advisor
9/22/98
Date

PRODUCT INVESTIGATIONS, INC.

151 East Tenth Avenue
Conshohocken, PA 19428
610-825-5855 • fax 610-825-7288

[redacted]

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

PREPARED FOR

[redacted]

Methyl Benzoate - 0.028%
RIPT (0/110)
Product Type - Perfume

[redacted]

[redacted]

[redacted]

[redacted]

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[redacted]

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF [redacted] ON HUMAN SKIN

1.00 **OBJECTIVES:**

.01 To identify and characterize the skin-damaging propensities that [redacted] can be induced to exercise under the conditions of this modified patch test procedure.

.02 To adjudge whether the exercise of such propensities under the patch test conditions contraindicates the kind of skin contact that would be occasioned during the appropriate use of the product.

2.00 **DESIGN:**

.01 A modified version of the Repeated Insult Patch Test [redacted] was conducted under double blind conditions on a panel composed of more than one hundred subjects at the outset.

.02 The regimen comprised nine sequential 24-hour induction applications and two concurrently conducted 24-hour challenge applications, one on the initial induction site and one on a naive site.

.03 During the initial phase, the skin of the contact sites was graded and the grades recorded on Wednesdays, Fridays (i.e. twenty-four hours after patches had been removed), and Mondays (i.e. forty-eight hours after patches had been removed).

.04 During the challenge phase, the skin of the contact sites was graded within moments after the patches had been removed and again twenty-four and forty-eight hours later. Follow-up examinations were conducted thereafter only if adverse effects were present.

.05 This study was conducted in compliance with the standards of good clinical practices generally applicable for the protection of the privileges and well-being of individuals who participate in patch test procedures.

3.00 **SPONSOR:** [redacted]

Project Director: [redacted]

Authorization: [redacted]

4.00 **STUDY PRODUCT:**

Type of Product:	Fragrance
Sponsor Identification:	[redacted]
Date received:	2/7/06
Quantity rec'd:	> 600 g. gross wt.
Form used in study:	As supplied, volatilized

[redacted]

5.00 **SITE OF STUDY:**

Product Investigations, Inc.
151 East Tenth Avenue
Conshohocken, PA 19428

Study Personnel:

Medical Director:	Morris V. Shelanski, MDCM
Dir. Derm. Services:	Joseph E. Nicholson III
Sr. Technician:	June Zummo
Quality Assurance:	Samuel J. Charles III

6.00 **DATES OF STUDY:** [redacted]

[redacted]

7.00 SELECTION OF SUBJECTS:

.01 RECRUITING:

Prospective subjects were recruited from surrounding localities via phone, posters and personal contact.

.02 INFORMED CONSENT:

All individuals who expressed interest in participating were given an informed consent document to read. This document, which each candidate had to read and sign before being entered into the study, presented the following information:

a. How many subjects were to be enrolled in the study;
b. The intended use of the product;
c. Why the product was being tested;
d. How the test was to be performed;
e. That the regimen was not intended to benefit a subject's health, well being, or quality of life.
f. The different ways that participation may be detrimental to a subject's health, well being, or quality of life.
g. That not all detrimental effects could be foreseen and made known at the time the informed consent was presented for the prospective subject's signature.
h. What commitments a subject had to make to be in compliance; and
i. What considerations a subject was entitled to receive and the conditions for receiving them.

.03 DETERMINATION OF ELIGIBILITY:

Information concerning a prospective subject's qualifications was obtained from the answers the subject gave in filling out a medical history form and in responding to specific questions. Those who did not meet the following criteria were rejected.

a. **Inclusion Criteria: Satisfaction of all the following items was obligatory:**
   i. The candidate was at least eighteen years old, and
   ii. agreed to comply fully with the scheduled study regimen, and
   iii. expressed awareness that a participant would incur risks that would affect her/his well-being, and
   iv. denied that the amount of the stipend had induced her/him to participate against her/his better judgement, and
   v. had read the informed consent agreement, and
   vi. had assured the interviewer that she/he had no questions about the informed consent's contents that had not been answered to her/his satisfaction, and
   vii. had signed the consent form willingly and without reservation.

b. **Exclusion Criteria:** Any one of the following items was cause for rejection:
   i. The candidate had an illness that contraindicated participation; or
   ii. a condition that rendered the skin unsuitable for use in this study; or
   iii. was using dosages of medications that could alter the skin's tolerance; or
   iv. had a documented history of intolerance to the category of products submitted for study; or
   v. was a female who was pregnant or was breast feeding an infant.

.04 PANEL INFORMATION:

a. [redacted]

b. **Demographics:**

SEX	Number	Age Range
Female	86	21 - 72
Male	25	19 - 60

c. **Dedication:** The subjects in this panel were engaged concomitantly in the studies of products submitted by more than one sponsor.

8.00 SITE INFORMATION:

.01 LOCATION:

[redacted] was assigned Band #1 on the left side of the back of each subject.

.02 IDENTIFICATION OF A CONTACT SITE:

At each visit the skin around the contact site was marked to facilitate examinations after the device was removed and positioning of subsequently-applied devices as precisely as was feasible on the same site.

9.00 PATCHING DEVICES:

.01 TYPE OF DEVICE:

Partially-occlusive patching devices consisting of a 2 cm x 2 cm absorbent pad centered on the adhesive-coated surface of a 2 cm x 4 cm plastic film were used to convey and maintain the product on the skin.

.02 PREPARATION OF A PATCHING DEVICE:

a. The webril pad of a patching device was infused with 150µl of the test material and allowed to volatilize for at least 30 minutes prior to application..

.03 POSITIONING AND REMOVING A PATCHING DEVICE:

a. A prepared device was positioned on its designated site on each subject with the product-treated surface of the pad in contact with the skin.
b. Firm pressure was applied to the backing of the device to effect intimate contact of the pad with the skin and to bond the flanges of the device securely to the skin.
c. When the time came for removing the device, the device was peeled off the skin as gently as was feasible under the circumstances.

10.00 DATA ACQUISITION:

.01 GRADING PROCEDURE:

a. Examinations of the contact sites to grade the effects elicited by the product were conducted on Mondays, Wednesday and Fridays. When a subject came in on a scheduled examination day, the technician examined the skin of the contact site.
   i. If no adverse effect was detected, a "0" was recorded in the subject's Case Report Form.
   ii. If an adverse effect was detected, the technician entered a grade indicating her assessment of the response's intensity.
b. The subject was then sent into the patching room where the site was examined again by a second technician to ascertain independently whether or not the site should be used again. If she disagreed with the first technician's assessment, the application was held in abeyance until the issue could be resolved with the help of the supervisor and/or the investigator.
c. The supervisor or the investigator was called in not only when a disagreement had to be resolved, but also to validate substantial sudden changes, e.g. when a response is deemed to merit a grade ≥3 or when a response has been judged to have decreased by two or more points from the previous day's status.

.02 CRITERIA FOR GRADING RESPONSE INTENSITY:

The following scale was used in this procedure to designate the intensities of those gross skin changes that may be occasioned by exposing the surface of the skin to a product.

Morphology	Visible Change	Grade
Subclinical Stage	None	0
Inflammation		
Vascular Dilation:	Faint redness with poorly defined margins	1
	Redness with well-defined margins	2
Infiltration:	Redness plus well-defined edema	3
	Redness plus papules, or vesicles or ulceration	4

.04 SITE CHANGES:

a. **Switch to a Naive Site:**
   i. If the product had elicited a Grade 2 response on a subject, application of the product would have been switched immediately to a naive site on the subject.
b. **Discontinuation of Applications:**
   i. If the product had elicited a second Grade 2 on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.
   ii. If the product had elicited a Grade 3 response on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.

11.00 OVERVIEW OF STUDY REGIMEN:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week #1	Apply	Remove	Rem/Gr/Appl	Remove	Rem/Gr/Appl	(Removed)	--
Week #2	Grade/Apply	Remove	Rem/Gr/Appl	Remove	Grade/Apply	(Removed)	--
Week #3	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	--
Week #4	Grade	---	—	--	--	--	--
Week #5	Apply	Remove/Grade	Grade	Grade*	Grade*	--	-

*If necessary

12.00 STUDY REGIMEN:

.01 INITIAL/INDUCTION PHASE-

Week #1:

Monday:

i. As each subject presented herself/himself at the clinic, the skin of the contact site assigned to the product submitted for study was examined and ascertained to be suitable before applications were begun.
ii. A freshly-prepared patching device was applied on its assigned site.
iii The skin around the device was marked and the subject was instructed to return on Tuesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii.. The patching device was removed by a technician and the subject was instructed to return on Wednesday.

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii.. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii.. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Monday for resumption of the regimen.

Week #2 / Week #3:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii.. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday, Wednesday, Thursday, Friday:

The same procedures followed during Week 1 were followed during Weeks 2 and 3.

Week #4:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii a) If the subject had undergone all nine induction applications, she/he was dismissed after being instructed as follows:
i) to report back to the clinic on the following Monday to receive the challenge applications, and
ii) to notify the investigator without delay should any significant changes occur in the skin of the contact site before Monday of the challenge week.
b) If the subject had not received the required number of induction applications and was deficient without valid reason, applications were continued. As many as two missed applications could be made up during this week. When the subject had undergone the required number of make up applications, she/he was dismissed after being instructed as in sections ii(a), above.

.02 HIATUS/MAKE UP PHASE-

Week# 4:

After the examination on Monday of Week 4, no procedures were scheduled during this period except make-up applications.

.03 CHALLENGE PHASE-

Week #5:

Monday:
i. As each subject returned, the skin of the initial induction site was examined and ascertained to be free of any conditions that would have rendered it unfit for undergoing the challenge applications.
ii. A prepared device was applied on the initial induction site.
iii A second prepared device was applied on a naive site.
iv. The skin around both devices was marked and the subject was instructed to return on Tuesday.

Tuesday: (Note: If a subject was absent on Monday, she/he was patched on Tuesday.)
i. As each subject returned, the site-identifying marks around both contact sites were reinforced.
ii. Both patching devices were removed by a technician.
iii The skin of both contact sites was graded; the grades were recorded.
iv. The subject was instructed to return on Wednesday.

Wednesday:
i. As each subject returned, the skin of both contact sites was graded; the grades were recorded.
ii. If follow-up was indicated, the subject was instructed to return on Thursday, otherwise the subject was dismissed from the study of this material..

.04 FOLLOW-UP PHASE:

Week No. 6 and Week No. 7:

During the two weeks following the exit examination, the subjects were given the opportunity to relay any information concerning effects that were relevant to the characterization of the product as well as to communicate the need for treatment of persistent or newly-occurring responses.

13.00 PROCEDURE DEVIATIONS:

None were necessary.

[redacted]

14.00 COMPLIANCE:

PHASE	No. Of AEC's Required	EXCUSED	COMPLIANT YES	COMPLIANT NO
Induction	8	0	111	0
Challenge	1/1	0	110	1

All of the 111 Subjects were in compliance with the number of required application/examination cycles during induction.
110 of the 111 Subjects were in compliance with the number of required application/examination cycles during challenge.

15.00 INCIDENCE OF RESPONSES:

GRADE	TYPE OF RESPONSE	INDUCTION PHASE	CHALLENGE PHASE	
			Original Contact Site	Naive Contact Site
0	No visible change	111 subjects	110 subjects	110 subjects
1	Faint redness, undefined border	0 "	0 "	0 "
2	Intense redness, defined border	0 "	0 "	0 "
3	Redness + definite edema	0 "	0 "	0 "
4	Redness + papules, or vesicles, etc.	0 "	0 "	0 "
	No. of Responders	0 subjects	0 subjects	0 subjects
	No Data Acquired	0 subjects	1 subject	1 subject

16.00 SIGNIFICANCE OF THE RESPONSES:

.01 INITIAL/INDUCTION PHASE:

No responses were noted on any of the 111 subjects who participated in all or part of this study. The absence of responses characterizes the product as one which does not possess clinically significant skin-irritating propensities.

.02 CHALLENGE PHASE:

a. **Original Contact Sites:**
The absence of responses on all of the 110 subjects who participated in this phase of the study characterizes the product as one which does not possess clinically significant skin sensitizing propensities.

b. **Naive Contact Sites:**
The absence of responses on all of the 110 subjects who participated in this phase of the study characterizes the product as one which does not possess clinically significant skin sensitizing propensities.

[redacted]

17.00 CONCLUSIONS:

.01 [redacted] was found to be neither a clinically significant skin irritant nor a skin sensitizer under the conditions of this study.

.02 [redacted] is not contraindicated for usages entailing repeated applications on human skin under conditions appropriate for such products.

PRODUCT INVESTIGATIONS, INC.

3/22/06
Date

Joseph E. Nicholson III
Director, Clinical Studies

18.00 COMPLIANCE WITH GOOD QUALITY ASSURANCE STANDARDS:

I have audited the results presented in this report and believe that, to the best of my knowledge, they accurately reflect the raw data acquired during the course of this study.

Samuel Charles
Director, Quality Assurance

[redacted]

Subj #	Sponsor Code:																Site: L1									
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE WEEK					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M
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[redacted]

Subj #	Sponsor Code:															Site: L1										
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE WEEK					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M
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Subj #	Sponsor Code:									Site: L1														
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE WEEK					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M
061	B/0	[redacted]	0	[redacted]	0	A	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0				0	0/0	0/0	0/0			
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070	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
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085	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
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Subj #	Sponsor Code: [redacted]																				Site: L1					
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE WEEK					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 5					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M
091	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
092	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	A	0	[redacted]	0	[redacted]	0	0		0			0/0	0/0	0/0			
093	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
094	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
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098	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
099	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
100	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
101	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
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108	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
109	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
110	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					0/0	0/0	0/0			
111	B/0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0	[redacted]	0	[redacted]	0	0					B/0	0/0	0/0			

LEGEND

A = Absent
D = Dropped

Monday week 6: Only if any reactions on the last Friday week 5 (challenge week)

Personal Care Products Council
Committed to Safety,
Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: August 19, 2010

SUBJECT: Information on Lipstick Containing C12-15 Alkyl Benzoate

The three products tested differed only by color.

TKL Research. 2005. Repeated insult patch test of a lipstick containing 16% C12-15 Alkyl Benzoate. TKL Study No. DS106805-3.

The following two studies were completed in the same panel of volunteers.

TKL Research. 2005. Repeated insult patch test of a lipstick containing 16% C12-15 Alkyl Benzoate. TKL Study No. DS107305-5.

TKL Research. 2005. Repeated insult patch test of a lipstick containing 16% C12-15 Alkyl Benzoate. TKL Study No. DS107305-6.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

C12-15 Alkyl Benzoate at 16% in Lipstick

TKL RESEARCH

SUMMARY REPORT

TKL Study No.: DS106805-3

Date of Report: December 8, 2005

Study Title:	Repeated Insult Patch Test
Study Sponsor:	
TKL Protocol No.:	TKL-1000
Sponsor Reference No.:	
Study Objective:	To assess the sensitization potential of topically applied study material.
Study Design:	Standard RIPT methodology with nine 24-hour induction applications and a single 24-hour challenge application following a 10-15 day rest period.
Principal Investigator:	Jonathan S. Dosik, MD - Dermatologist
Manager, Dermatologic Safety Testing:	Kathleen Georgeian
Study Center:	TKL Research, Inc. 4 Forest Avenue Paramus, NJ 07652
Study Site:	Paramus, NJ
Study Dates:	Date Study Initiated: October 3, 2005 Date Study Completed: November 10, 2005

Study Material:

SPL Code and Category	Amount Applied	Patch Condition
Lip Stick	0.2mL	Occlusive

Number of Subjects:	Enrolled: 116	Completed: 104
	Discontinued: 12	
	Lost to follow-up: 4	Voluntary withdrawal: 8

Listing of Attached Tables:

Appendix I	Summary Tables
Appendix II	Data Listings

SUMMARY AND CONCLUSION:

There were no adverse events reported.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

Under the conditions employed in this study, there was no evidence of sensitization to

SIGNATURES:

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Jonathan S. Dosik, MD
Dermatologist/Principal Investigator

Kathleen Georgeian
Clinical Research Coordinator and Manager, Dermatologic Safety Testing

This report has been reviewed by the TKL Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Henry Briscoe
Quality Assurance

12/8/05
Date

APPENDIX I

SUMMARY TABLES

TKL STUDY NO. DS106805

TABLE 1: SUMMARY OF SUBJECT ENROLLMENT AND DISPOSITION

	n	(%)
Subjects enrolled	116	
Subjects completed all phases	104	(89.7)
Total subjects discontinued	12	(10.3)
Lost to follow-up	4	(3.4)
Voluntary withdrawal	8	(6.9)

Note: All percentages are relative to total subjects enrolled

See Data Listing 1 for further detail

Program: DISPSMY.SAS/USES: FINAL/30NOV05:13:01:58

TKL STUDY NO. DS106805
TABLE 2: SUMMARY OF SUBJECT DEMOGRAPHICS
ALL ENROLLED SUBJECTS

Age	
n (%) 18 to 44	45 (38.8)
n (%) 45 to 64	61 (52.6)
n (%) 65 and up	10 (8.6)
Mean (SD)	48.1 (12.1)
Median	47.9
Range	20.6 to 70.4
Gender	
n (%) Male	34 (29.3)
n (%) Female	82 (70.7)
Race	
n (%) Asian	1 (0.9)
n (%) Black	6 (5.2)
n (%) Caucasian	89 (76.7)
n (%) Hispanic	20 (17.2)

See Data Listing 2 for further detail

Program: DEMOSMY.SAS/USES: DEMOGS/30NOV05:13:02:00

TKL STUDY NO. DS106805
TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES
NUMBER OF SUBJECTS BY PRODUCT

PRODUCT=

	Induction Reading									Make-	Challenge Phase		
Response	1	2	3	4	5	6	7	8	9	Up	48hr	72hr	96hr(*)
-	110	108	107	107	105	105	101	101	104	7	103	103	
?	0	0	0	0	0	0	0	0	0	0	0	1	
+	0	0	0	0	0	0	0	0	0	0	1	0	
Total evaluable	110	108	107	107	105	105	101	101	104	7	104	104	
Number absent	0	0	0	0	1	0	3	3	0		0	0	
Number discontinued	6	8	9	9	10	11	12	12	12		12	12	

MAXIMUM ELICITED RESPONSE DURING INDUCTION
ALL SUBJECTS COMPLETING INDUCTION (N=104)

Response	n(%) Subjects
-	104 (100.0%)

(*) when required

Key to Symbols:
- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50%

Program: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/30NOV05:13:02:12

APPENDIX II

DATA LISTINGS

TKL STUDY NO. DS106805
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
1	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
2	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
3	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
4	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
5	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
6	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
7	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
8	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
9	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
10	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
11	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
12	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
13	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
14	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
15	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
16	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
17	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
18	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
19	10/03/05	10/03/05		10/12/05	I2	L	10
20	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
21	10/03/05	10/03/05		10/05/05	I0	S	3
22	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
23	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
24	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
25	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
26	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
27	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
28	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
29	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
30	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
31	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
32	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
33	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
34	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
35	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
36	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
37	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
38	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
39	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/30NOV05:13:01:36

Subject	Study Dates				Last	Completion	Days on
No.	Screened	1st Applic	Chall Applic	Ended	Reading #	Status	Study
40	10/03/05	10/03/05		10/19/05	I5	S	17
41	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
42	10/03/05	10/03/05		10/05/05	I0	S	3
43	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
44	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
45	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
46	10/03/05	10/03/05		10/05/05	I0	S	3
47	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
48	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
49	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
50	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
51	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
52	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
53	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
54	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
55	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
56	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
57	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
58	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
59	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
60	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
61	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
62	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
63	10/03/05	10/03/05		10/05/05	I0	S	3
64	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
65	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
66	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
67	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
68	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
69	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
70	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
71	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
72	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
73	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
74	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
75	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
76	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
77	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
78	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/30NOV05:13:01:36

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
79	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
80	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
81	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
82	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
83	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
84	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
85	10/03/05	10/03/05		10/05/05	I0	S	3
86	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
87	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
88	10/03/05	10/03/05		10/07/05	I0	L	5
89	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
90	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
91	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
92	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
93	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
94	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
95	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
96	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
97	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
98	10/03/05	10/03/05		10/10/05	I1	L	8
99	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
100	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
101	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
102	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
103	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
104	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
105	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
106	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
107	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
108	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
109	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
110	10/03/05	10/03/05		10/10/05	I1	S	8
111	10/03/05	10/03/05		10/21/05	I6	L	19
112	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
113	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
114	10/03/05	10/03/05		10/17/05	I4	S	15
115	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39
116	10/03/05	10/03/05	11/07/05	11/10/05	C2	C	39

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/30NOV05:13:01:36

TKL STUDY NO. DS106805
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
1	59.7	Male	Caucasian
2	66.3	Male	Caucasian
3	50.6	Female	Caucasian
4	47.7	Female	Hispanic
5	60.6	Female	Caucasian
6	24.9	Female	Caucasian
7	42.3	Female	Caucasian
8	45.2	Female	Caucasian
9	33.9	Female	Hispanic
10	67.1	Female	Caucasian
11	59.5	Male	Caucasian
12	35.2	Female	Caucasian
13	43.5	Male	Caucasian
14	65.8	Female	Caucasian
15	44.3	Female	Black
16	60.3	Female	Caucasian
17	40.0	Female	Caucasian
18	31.9	Female	Hispanic
19	67.2	Female	Caucasian
20	59.8	Male	Hispanic
21	35.5	Female	Caucasian
22	39.1	Female	Caucasian
23	55.4	Female	Caucasian
24	45.2	Female	Caucasian
25	50.2	Female	Caucasian
26	51.8	Male	Caucasian
27	63.8	Male	Caucasian
28	63.2	Male	Caucasian
29	67.7	Female	Black
30	46.6	Male	Caucasian
31	62.6	Female	Caucasian
32	44.8	Female	Caucasian
33	63.4	Female	Hispanic
34	70.0	Female	Caucasian
35	69.7	Female	Caucasian
36	31.7	Female	Caucasian
37	41.5	Female	Caucasian
38	23.1	Male	Caucasian
39	45.4	Female	Caucasian
40	46.4	Female	Caucasian

Subject No.	Age	Gender	Race
41	46.3	Female	Caucasian
42	21.4	Male	Caucasian
43	56.8	Female	Caucasian
44	45.4	Female	Asian
45	32.4	Female	Caucasian
46	45.9	Male	Caucasian
47	35.2	Female	Caucasian
48	39.4	Female	Caucasian
49	46.5	Female	Caucasian
50	48.1	Female	Caucasian
51	45.1	Male	Black
52	70.4	Male	Caucasian
53	36.8	Female	Caucasian
54	41.9	Male	Caucasian
55	58.0	Female	Caucasian
56	65.8	Male	Caucasian
57	68.3	Female	Caucasian
58	48.3	Female	Caucasian
59	54.5	Female	Caucasian
60	51.0	Female	Caucasian
61	51.7	Male	Caucasian
62	60.8	Female	Caucasian
63	49.2	Female	Caucasian
64	41.5	Female	Caucasian
65	49.3	Female	Hispanic
66	40.1	Male	Hispanic
67	56.8	Female	Hispanic
68	36.0	Female	Caucasian
69	38.5	Female	Caucasian
70	32.9	Male	Black
71	43.4	Female	Hispanic
72	44.9	Female	Hispanic
73	43.1	Male	Hispanic
74	59.5	Male	Hispanic
75	30.2	Female	Hispanic
76	49.9	Female	Caucasian
77	61.7	Male	Caucasian
78	56.1	Female	Caucasian
79	20.6	Male	Caucasian
80	52.9	Female	Hispanic

Subject No.	Age	Gender	Race
81	47.3	Female	Caucasian
82	57.2	Female	Caucasian
83	63.3	Male	Caucasian
84	31.1	Female	Black
85	49.0	Female	Caucasian
86	49.9	Female	Black
87	36.2	Female	Caucasian
88	54.0	Female	Caucasian
89	26.0	Female	Caucasian
90	61.8	Female	Caucasian
91	58.0	Female	Caucasian
92	50.7	Male	Caucasian
93	33.4	Male	Hispanic
94	41.6	Female	Caucasian
95	42.7	Female	Caucasian
96	45.2	Male	Hispanic
97	29.5	Female	Caucasian
98	34.9	Male	Caucasian
99	23.3	Female	Hispanic
100	59.8	Female	Hispanic
101	41.8	Male	Caucasian
102	63.1	Female	Caucasian
103	56.7	Female	Caucasian
104	54.5	Female	Caucasian
105	24.7	Female	Caucasian
106	43.4	Male	Caucasian
107	56.1	Female	Caucasian
108	49.9	Male	Caucasian
109	53.0	Female	Caucasian
110	54.8	Male	Caucasian
111	49.4	Female	Caucasian
112	61.6	Male	Hispanic
113	43.5	Female	Caucasian
114	36.0	Male	Caucasian
115	35.9	Female	Caucasian
116	56.3	Female	Hispanic

TKL STUDY NO. DS106805
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
1	-	-	-	-	-	-	-	-	-		-	-	
2	-	-	-	-	-	-	-	-	-		-	-	
3	-	-	-	-	-	-	X	-	-	-	-	-	
4	-	-	-	-	-	-	-	-	-		-	-	
5	-	-	-	-	-	-	-	-	-		-	-	
6	-	-	-	-	-	-	-	-	-		-	-	
7	-	-	-	-	-	-	-	-	-		-	-	
8	-	-	-	-	-	-	-	-	-		-	-	
9	-	-	-	-	-	-	X	-	-	-	-	-	
10	-	-	-	-	-	-	-	-	-		-	-	
11	-	-	-	-	-	-	-	-	-		-	-	
12	-	-	-	-	-	-	-	-	-		-	-	
13	-	-	-	-	-	-	-	-	-		-	-	
14	-	-	-	-	-	-	-	-	-		-	-	
15	-	-	-	-	-	-	-	-	-		-	-	
16	-	-	-	-	-	-	-	-	-		-	-	
17	-	-	-	-	-	-	-	-	-		-	-	
18	-	-	-	-	-	-	-	-	-		-	-	
19	-	-	X	X	X	X	X	X	X		X	X	
20	-	-	-	-	-	-	-	-	-		-	-	

Key to Symbols:

- = No reaction

? = Minimal or doubtful response, slightly different from surrounding normal skin

+ = Definite erythema, no edema

++ = Definite erythema, definite edema

+++ = Definite erythema, definite edema and vesiculation

N9G = No ninth grading NA=Not applied NP=Not patched due to reaction achieved
X = Reading not performed due to missed visit or subject discontinuation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50% NR=Data not recorded
MU = Make-up reading for missed induction visit

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/30NOV05:13:01:40

TKL STUDY NO. DS106805
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
21	X	X	X	X	X	X	X	X	X		X	X	
22	-	-	-	-	-	-	-	-	-		-	-	
23	-	-	-	-	-	-	-	-	-		-	-	
24	-	-	-	-	-	-	-	-	-		-	-	
25	-	-	-	-	-	-	-	-	-		-	-	
26	-	-	-	-	-	-	-	-	-		-	-	
27	-	-	-	-	-	-	-	-	-		-	-	
28	-	-	-	-	-	-	-	-	-		-	-	
29	-	-	-	-	-	-	-	-	-		-	-	
30	-	-	-	-	-	-	-	-	-		-	-	
31	-	-	-	-	-	-	-	-	-		-	-	
32	-	-	-	-	-	-	-	-	-		+	?	
33	-	-	-	-	-	-	-	-	-		-	-	
34	-	-	-	-	-	-	-	-	-		-	-	
35	-	-	-	-	-	-	-	-	-		-	-	
36	-	-	-	-	-	-	-	-	-		-	-	
37	-	-	-	-	-	-	-	-	-		-	-	
38	-	-	-	-	-	-	-	-	-		-	-	
39	-	-	-	-	-	-	-	-	-		-	-	
40	-	-	-	-	-	X	X	X	X		X	X	
41	-	-	-	-	-	-	-	-	-		-	-	
42	X	X	X	X	X	X	X	X	X		X	X	
43	-	-	-	-	-	-	-	-	-		-	-	
44	-	-	-	-	-	-	-	-	-		-	-	
45	-	-	-	-	-	-	-	-	-		-	-	
46	X	X	X	X	X	X	X	X	X		X	X	
47	-	-	-	-	-	-	-	-	-		-	-	
48	-	-	-	-	-	-	-	-	-		-	-	
49	-	-	-	-	-	-	-	-	-		-	-	
50	-	-	-	-	-	-	-	-	-		-	-	
51	-	-	-	-	-	-	-	-	-		-	-	
52	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/30NOV05:13:01:40

IRL STUDY NO. DS106805
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
53	-	-	-	-	-	-	-	-	-		-	-	
54	-	-	-	-	-	-	-	-	-		-	-	
55	-	-	-	-	-	-	-	-	-		-	-	
56	-	-	-	-	-	-	-	-	-		-	-	
57	-	-	-	-	-	-	-	-	-		-	-	
58	-	-	-	-	-	-	-	-	-		-	-	
59	-	-	-	-	-	-	-	X	-	-	-	-	
60	-	-	-	-	-	-	-	-	-		-	-	
61	-	-	-	-	-	-	-	-	-		-	-	
62	-	-	-	-	-	-	-	-	-		-	-	
63	X	X	X	X	X	X	X	X	X		X	X	
64	-	-	-	-	-	-	X	-	-	-	-	-	
65	-	-	-	-	-	-	-	-	-		-	-	
66	-	-	-	-	-	-	-	-	-		-	-	
67	-	-	-	-	-	-	-	-	-		-	-	
68	-	-	-	-	-	-	-	-	-		-	-	
69	-	-	-	-	-	-	-	-	-		-	-	
70	-	-	-	-	-	-	-	-	-		-	-	
71	-	-	-	-	-	-	-	-	-		-	-	
72	-	-	-	-	-	-	-	-	-		-	-	
73	-	-	-	-	-	-	-	-	-		-	-	
74	-	-	-	-	-	-	-	-	-		-	-	
75	-	-	-	-	-	-	-	-	-		-	-	
76	-	-	-	-	-	-	-	-	-		-	-	
77	-	-	-	-	-	-	-	-	-		-	-	
78	-	-	-	-	-	-	-	X	-	-	-	-	
79	-	-	-	-	X	-	-	-	-	-	-	-	
80	-	-	-	-	-	-	-	X	-	-	-	-	
81	-	-	-	-	-	-	-	-	-		-	-	
82	-	-	-	-	-	-	-	-	-		-	-	
83	-	-	-	-	-	-	-	-	-		-	-	
84	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/30NOV05:13:01:40

TKL STUDY NO. DS106805
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
85	X	X	X	X	X	X	X	X	X		X	X	
86	-	-	-	-	-	-	-	-	-		-	-	
87	-	-	-	-	-	-	-	-	-		-	-	
88	X	X	X	X	X	X	X	X	X		X	X	
89	-	-	-	-	-	-	-	-	-		-	-	
90	-	-	-	-	-	-	-	-	-		-	-	
91	-	-	-	-	-	-	-	-	-		-	-	
92	-	-	-	-	-	-	-	-	-		-	-	
93	-	-	-	-	-	-	-	-	-		-	-	
94	-	-	-	-	-	-	-	-	-		-	-	
95	-	-	-	-	-	-	-	-	-		-	-	
96	-	-	-	-	-	-	-	-	-		-	-	
97	-	-	-	-	-	-	-	-	-		-	-	
98	-	X	X	X	X	X	X	X	X		X	X	
99	-	-	-	-	-	-	-	-	-		-	-	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	-	-		-	-	
103	-	-	-	-	-	-	-	-	-		-	-	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	-	-	-	-	-	-	-	-		-	-	
109	-	-	-	-	-	-	-	-	-		-	-	
110	-	X	X	X	X	X	X	X	X		X	X	
111	-	-	-	-	-	-	X	X	X		X	X	
112	-	-	-	-	-	-	-	-	-		-	-	
113	-	-	-	-	-	-	-	-	-		-	-	
114	-	-	-	-	X	X	X	X	X		X	X	
115	-	-	-	-	-	-	-	-	-		-	-	
116	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/30NOV05:13:01:40

C12-15 Alkyl Benzoate at 16% in Lipstick

TKL RESEARCH

SUMMARY REPORT

TKL Study No.: DS107305-5

Date of Report: December 14, 2005

Study Title:	Repeated Insult Patch Test
Study Sponsor:	
TKL Protocol No.:	TKL-1000
Sponsor Reference No.:	
Study Objective:	To assess the sensitization potential of topically applied study material.
Study Design:	Standard RIPT methodology with nine 24-hour induction applications and a single 24-hour challenge application following a 10-15 day rest period.
Principal Investigator:	Jonathan S. Dosik, MD - Dermatologist
Manager, Dermatologic Safety Testing:	Kathleen Georgeian
Study Center:	TKL Research, Inc. 4 Forest Avenue Paramus, NJ 07652 **Study Site:** Paramus, NJ
Study Dates:	Date Study Initiated: October 10, 2005 Date Study Completed: November 18, 2005

Study Material:

SPL Code and Category	Amount Applied	Patch Condition
Lip Stick	0.2g	Occlusive

Number of Subjects:	Enrolled: 122	Completed: 107
	Discontinued: 15	
	Lost to follow-up: 14	Voluntary withdrawal: 1

Listing of Attached Tables:

Appendix I	Summary Tables
Appendix II	Data Listings

SUMMARY AND CONCLUSION:

One non-product-related adverse event was reported. See Data Listing 4, Appendix II

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

Under the conditions employed in this study, there was no evidence of sensitization to 2005.319A.001.

SIGNATURES:

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Jonathan S. Dosik, MD
Dermatologist/Principal Investigator

Kathleen Georgeian
Clinical Research Coordinator and Manager, Dermatologic Safety Testing

This report has been reviewed by the TKL Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Henry Brisson
Quality Assurance

12/14/05
Date

APPENDIX I

SUMMARY TABLES

TKL STUDY NO. DS107305

TABLE 1: SUMMARY OF SUBJECT ENROLLMENT AND DISPOSITION

	n (%)
Subjects enrolled	122
Subjects completed induction phase	110 (90.2)
Subjects completed all phases	107 (87.7)
Total subjects discontinued	15 (12.3)
Lost to follow-up	14 (11.5)
Voluntary withdrawal	1 (0.8)

Note: All percentages are relative to total subjects enrolled

See Data Listing 1 for further detail

Program: DISPSMY.SAS/USES: FINAL/07DEC05:09:17:29

TKL STUDY NO. DS107305
TABLE 2: SUMMARY OF SUBJECT DEMOGRAPHICS
ALL ENROLLED SUBJECTS

Age	
n (%) 18 to 44	50 (41.0)
n (%) 45 to 64	69 (56.6)
n (%) 65 and up	3 (2.5)
Mean (SD)	45.9 (11.3)
Median	47.4
Range	18.3 to 66.6
Gender	
n (%) Male	25 (20.5)
n (%) Female	97 (79.5)
Race	
n (%) Asian	2 (1.6)
n (%) Black	14 (11.5)
n (%) Caucasian	99 (81.1)
n (%) Hispanic	7 (5.7)

See Data Listing 2 for further detail

Program: DEMOSMY.SAS/USES: DEMOGS/07DEC05:09:17:31

TKL STUDY NO. DS107305

TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES
NUMBER OF SUBJECTS BY PRODUCT

PRODUCT=

	Induction Reading									Make-Up	Challenge Phase		
Response	1	2	3	4	5	6	7	8	9		48hr	72hr	96hr(*)
-	115	111	110	108	110	108	105	110	108	7	107	107	
?	0	0	0	0	0	1	0	0	0	0	0	0	
Total evaluable	115	111	110	108	110	109	105	110	108	7	107	107	
Number absent	4	4	5	6	4	4	7	0	2		0	0	
Number discontinued	3	7	7	8	8	9	10	12	12		15	15	

MAXIMUM ELICITED RESPONSE DURING INDUCTION
ALL SUBJECTS COMPLETING INDUCTION (N=110)

Response	n(%) Subjects
-	109 (99.1%)
?	1 (0.9%)

(*) when required

Key to Symbols:
- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50%

Program: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/07DEC05:09:17:41

APPENDIX II

DATA LISTINGS

TKL STUDY NO. DS107305

DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
1	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
2	10/10/05	10/10/05		10/26/05	I6	L	17
3	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
4	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
5	10/10/05	10/10/05		10/14/05	I0	L	5
6	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
7	10/10/05	10/10/05		11/15/05	I9	L	37
8	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
9	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
10	10/10/05	10/10/05		11/15/05	I9	L	37
11	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
12	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
13	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
14	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
15	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
16	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
17	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
18	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
19	10/10/05	10/10/05		10/24/05	I5	L	15
20	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
21	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
22	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
23	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
24	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
25	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
26	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
27	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
28	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
29	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
30	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
31	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
32	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
33	10/14/05	10/14/05		11/04/05	I7	L	22
34	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
35	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
36	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
37	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
38	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
39	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
40	10/14/05	10/14/05		10/19/05	I0	L	6
41	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
42	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
43	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
44	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
45	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
46	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
47	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
48	10/14/05	10/14/05		10/19/05	I1	S	6
49	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
50	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
51	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
52	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
53	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
54	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
55	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
56	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
57	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
58	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
59	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
60	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
61	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
62	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
63	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
64	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
65	10/14/05	10/14/05		10/21/05	I1	L	8
66	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
67	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
68	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
69	10/14/05	10/14/05		10/26/05	I3	L	13
70	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
71	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
72	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
73	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
74	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
75	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
76	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
77	10/14/05	10/14/05		10/21/05	I1	L	8
78	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
79	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
80	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
81	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
82	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
83	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
84	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
85	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
86	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
87	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
88	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
89	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
90	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
91	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
92	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
93	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
94	10/14/05	10/14/05		10/19/05	I0	L	6
95	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
96	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
97	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
98	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
99	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
100	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
101	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
102	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
103	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
104	10/14/05	10/14/05		11/04/05	I7	L	22
105	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
106	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
107	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
108	10/14/05	10/14/05		11/15/05	I9	L	33
109	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
110	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
111	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
112	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
113	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
114	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
115	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
116	10/14/05	10/14/05		10/21/05	I1	L	8
117	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
118	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
119	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
120	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
121	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
122	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
1	48.2	Female	Caucasian
2	44.5	Female	Caucasian
3	37.5	Female	Hispanic
4	56.9	Female	Caucasian
5	36.8	Male	Black
6	56.2	Female	Caucasian
7	58.5	Female	Caucasian
8	54.5	Female	Caucasian
9	47.5	Female	Caucasian
10	40.9	Female	Caucasian
11	48.6	Female	Caucasian
12	51.5	Female	Caucasian
13	57.7	Female	Caucasian
14	22.8	Female	Caucasian
15	38.2	Female	Black
16	42.7	Female	Caucasian
17	48.2	Female	Caucasian
18	51.1	Female	Caucasian
19	37.5	Female	Hispanic
20	45.2	Female	Caucasian
21	23.8	Female	Black
22	56.0	Male	Caucasian
23	42.8	Female	Caucasian
24	49.0	Female	Caucasian
25	66.5	Male	Caucasian
26	51.1	Female	Caucasian
27	33.0	Female	Black
28	58.0	Male	Caucasian
29	55.3	Female	Caucasian
30	43.5	Male	Caucasian
31	48.7	Female	Caucasian
32	45.9	Male	Caucasian
33	58.4	Female	Caucasian
34	23.9	Male	Caucasian
35	49.1	Male	Caucasian
36	48.5	Female	Caucasian
37	48.2	Female	Caucasian
38	38.2	Male	Black
39	46.5	Male	Caucasian
40	38.3	Female	Caucasian

Subject No.	Age	Gender	Race
41	44.5	Female	Caucasian
42	61.8	Female	Caucasian
43	35.3	Female	Caucasian
44	61.7	Female	Caucasian
45	45.8	Female	Caucasian
46	37.4	Male	Caucasian
47	20.4	Female	Caucasian
48	44.9	Female	Caucasian
49	50.0	Female	Caucasian
50	54.7	Female	Caucasian
51	47.3	Female	Caucasian
52	54.1	Female	Caucasian
53	66.6	Male	Caucasian
54	34.6	Female	Caucasian
55	46.0	Female	Caucasian
56	53.3	Male	Caucasian
57	46.2	Female	Caucasian
58	56.0	Female	Caucasian
59	59.5	Female	Caucasian
60	52.5	Female	Caucasian
61	59.9	Female	Black
62	60.3	Female	Caucasian
63	46.1	Female	Hispanic
64	41.4	Female	Caucasian
65	27.1	Female	Hispanic
66	54.6	Female	Caucasian
67	41.7	Female	Caucasian
68	56.0	Female	Caucasian
69	44.4	Female	Caucasian
70	42.5	Female	Caucasian
71	48.1	Male	Caucasian
72	28.7	Female	Caucasian
73	52.8	Female	Caucasian
74	54.5	Female	Caucasian
75	39.8	Female	Hispanic
76	18.3	Female	Caucasian
77	58.9	Female	Caucasian
78	49.3	Female	Caucasian
79	42.9	Female	Caucasian
80	59.7	Female	Caucasian

Subject No.	Age	Gender	Race
81	39.7	Female	Caucasian
82	58.1	Female	Caucasian
83	61.8	Male	Caucasian
84	29.3	Female	Caucasian
85	52.9	Female	Caucasian
86	56.5	Male	Caucasian
87	47.0	Female	Caucasian
88	43.0	Female	Caucasian
89	65.7	Female	Caucasian
90	50.8	Female	Caucasian
91	21.8	Male	Caucasian
92	21.4	Female	Caucasian
93	56.9	Female	Caucasian
94	60.7	Female	Caucasian
95	40.6	Female	Black
96	55.1	Female	Caucasian
97	18.6	Male	Hispanic
98	60.5	Female	Asian
99	47.4	Female	Caucasian
100	60.4	Male	Caucasian
101	35.8	Female	Black
102	50.4	Female	Black
103	50.0	Female	Caucasian
104	37.0	Female	Caucasian
105	60.3	Male	Asian
106	50.6	Male	Hispanic
107	48.6	Male	Black
108	46.4	Female	Caucasian
109	28.7	Male	Black
110	45.7	Female	Caucasian
111	23.9	Female	Caucasian
112	40.2	Female	Caucasian
113	48.5	Female	Caucasian
114	49.0	Female	Caucasian
115	40.0	Female	Caucasian
116	33.7	Female	Black
117	32.9	Female	Caucasian
118	44.8	Male	Black
119	41.8	Female	Black
120	19.1	Female	Caucasian

Subject No.	Age	Gender	Race
121	44.0	Female	Caucasian
122	35.7	Male	Caucasian

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
1	-	-	-	-	-	-	-	-	-		-	-	
2	-	-	X	-	-	-	X	X	X		X	X	
3	-	-	-	-	-	-	-	-	-		-	-	
4	-	X	-	-	-	-	-	-	-	-	-	-	
5	X	X	X	X	X	X	X	X	X		X	X	
6	-	-	-	-	-	-	-	-	-		-	-	
7	-	-	-	-	-	-	-	-	-		X	X	
8	-	X	-	-	-	-	-	-	-	-	-	-	
9	-	-	-	-	-	-	-	-	-		-	-	
10	-	X	-	-	-	-	-	-	-	-	X	X	
11	-	-	-	-	-	-	-	-	-		-	-	
12	-	-	-	-	-	-	-	-	-		-	-	
13	-	-	-	-	X	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-		-	-	
15	-	-	-	-	-	-	-	-	-		-	-	
16	-	-	-	-	-	-	-	-	-		-	-	
17	-	-	-	-	-	-	-	-	-		-	-	
18	-	-	-	-	X	-	-	-	-	-	-	-	
19	-	-	-	X	-	X	X	X	X		X	X	
20	-	-	-	-	-	-	-	-	-		-	-	

Key to Symbols:

- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation

N9G = No ninth grading NA=Not applied NP=Not patched due to reaction achieved
X = Reading not performed due to missed visit or subject discontinuation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50% NR=Data not recorded
MU = Make-up reading for missed induction visit

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
21	-	-	-	-	-	-	-	-	-		-	-	
22	-	-	-	-	-	-	-	-	-		-	-	
23	-	-	-	-	-	X	-	-	-	-	-	-	
24	-	-	-	-	-	-	X	-	-	-	-	-	
25	-	-	-	-	-	-	-	-	-		-	-	
26	-	-	-	-	-	-	-	-	-		-	-	
27	-	-	-	X	-	-	-	-	-	N9G	-	-	
28	-	-	-	-	-	-	-	-	-		-	-	
29	-	-	-	-	-	-	-	-	-		-	-	
30	-	-	-	-	-	-	-	-	-		-	-	
31	-	-	-	-	-	-	-	-	-		-	-	
32	-	-	X	-	-	-	-	-	-	N9G	-	-	
33	-	-	-	-	-	-	-	X	X		X	X	
34	-	-	-	-	-	-	-	-	-		-	-	
35	-	-	-	-	-	-	-	-	-		-	-	
36	-	-	-	-	-	-	-	-	-		-	-	
37	-	-	-	-	-	-	-	-	-		-	-	
38	-	-	-	-	-	-	-	-	-		-	-	
39	-	-	-	-	-	-	-	-	-		-	-	
40	X	X	X	X	X	X	X	X	X		X	X	
41	-	-	-	-	-	-	X	-	-	N9G	-	-	
42	-	-	-	-	-	-	-	-	-		-	-	
43	-	-	-	-	-	-	-	-	-		-	-	
44	-	-	-	-	-	-	-	-	-		-	-	
45	-	-	-	-	-	-	-	-	-		-	-	
46	-	-	-	-	-	-	-	-	-		-	-	
47	-	-	-	-	-	X	-	-	-	N9G	-	-	
48	-	X	X	X	X	X	X	X	X		X	X	
49	-	-	-	-	-	-	-	-	-		-	-	
50	-	-	-	-	-	-	-	-	-		-	-	
51	-	-	-	-	-	-	-	-	-		-	-	
52	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase	
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr 96hr(*)
53	-	-	-	-	-	-	-	-	-		-	-
54	-	-	-	X	-	-	-	-	-	N9G	-	-
55	-	-	-	-	-	-	-	-	-		-	-
56	-	-	-	-	-	-	-	-	-		-	-
57	-	-	-	-	-	-	X	-	-	N9G	-	-
58	-	-	-	-	-	X	-	-	-	N9G	-	-
59	-	-	-	-	-	-	-	-	-		-	-
60	-	-	-	-	-	-	-	-	-		-	-
61	-	-	-	-	X	-	-	-	-	N9G	-	-
62	-	-	-	-	-	-	-	-	-		-	-
63	-	-	-	-	-	-	-	-	N9G		-	-
64	-	-	-	-	-	-	-	-	-		-	-
65	-	X	X	X	X	X	X	X	X		X	X
66	-	-	-	-	-	-	-	-	-		-	-
67	-	-	-	-	X	-	-	-	-	N9G	-	-
68	-	-	-	-	-	-	-	-	-		-	-
69	-	-	-	X	X	X	X	X	X		X	X
70	-	-	X	-	-	-	-	-	-	N9G	-	-
71	-	-	-	-	-	-	-	-	-		-	-
72	-	-	-	-	-	-	X	-	-	N9G	-	-
73	-	-	-	-	-	-	-	-	-		-	-
74	-	-	-	-	-	-	-	-	-		-	-
75	-	-	-	-	-	-	-	-	-		-	-
76	X	-	-	-	-	-	-	-	-	N9G	-	-
77	-	X	X	X	X	X	X	X	X		X	X
78	-	-	-	-	-	-	-	-	-		-	-
79	-	-	-	-	-	-	-	-	-		-	-
80	-	-	-	-	-	-	-	-	-		-	-
81	-	-	-	-	-	-	-	-	-		-	-
82	-	-	-	-	-	-	-	-	-		-	-
83	-	-	-	-	-	-	-	-	-		-	-
84	X	-	-	-	-	-	-	-	-	N9G	-	-

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
85	-	-	-	-	-	-	-	-	-		-	-	
86	-	-	-	-	-	-	-	-	-		-	-	
87	-	-	-	-	-	-	X	-	-	N9G	-	-	
88	-	-	-	-	-	-	-	-	-		-	-	
89	-	-	-	-	-	-	-	-	-		-	-	
90	-	-	-	-	-	-	-	-	-		-	-	
91	-	-	X	-	-	-	-	-	-	N9G	-	-	
92	-	-	-	-	-	-	X	-	-	N9G	-	-	
93	-	-	-	-	-	-	-	-	-		-	-	
94	X	X	X	X	X	X	X	X	X		X	X	
95	-	-	-	-	-	-	-	-	-		-	-	
96	-	-	-	-	-	-	-	-	-		-	-	
97	-	-	-	X	-	-	-	-	-	N9G	-	-	
98	-	-	-	-	-	-	-	-	-		-	-	
99	-	-	-	-	-	-	-	-	-		-	-	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	X	-	-	-	-	-	N9G	-	-	
102	-	-	-	X	-	-	-	-	-	N9G	-	-	
103	-	-	-	-	-	?	X	-	-	N9G	-	-	
104	-	-	-	-	-	X	-	X	X		X	X	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	X	-	-	-	-	-	-	-	N9G	X	X	
109	-	-	X	-	-	-	-	-	-	N9G	-	-	
110	-	-	-	-	-	-	-	-	-		-	-	
111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	-	-	-	-	-	-	-		-	-	
113	-	-	-	-	-	-	-	-	-		-	-	
114	-	-	-	-	-	-	-	-	-		-	-	
115	-	-	-	-	-	-	-	-	-		-	-	
116	-	X	X	X	X	X	X	X	X		X	X	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
117	-	-	-	-	-	-	-	-	-		-	-	
118	X	-	-	-	-	-	-	-	-	N9G	-	-	
119	X	-	-	-	-	-	-	-	-	N9G	-	-	
120	-	-	-	-	-	-	-	-	N9G		-	-	
121	-	-	-	-	-	-	-	-	-		-	-	
122	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 4: ADVERSE EVENTS

Subject No. 79

Adverse Event: TOPICAL INFECTION

Date of Onset: 10/28
Frequency: Single episode
Duration:
Rel. to Study Product: Unrelated
Action Taken/Study Product: None
Action Taken/Treatment?: YES
Serious? NO

Date of Resolution: 11/07
Severity: Mild
Outcome: Resolved

Comment: SUBJECT HAD TOPICAL INFECTION ON LOWER LEFT SIDE OF HER STOMACH AND WAS SEEN BY EMERGENCY ROOM DOCTOR WHO PRESCRIBED AUGMENTIN XR1000-62.5 TAB 1 TAB BID FOR 7 DAYS. SUBJECT HAD TO RETURN TO DOCTOR FOR FOLLOW-UP VISIT ON 11/02/05. SUBJECT RETURNED TO TKL RESEARCH ON 11/2/05 AFTER VISIT WITH DOCTOR WHO PRESCRIBED CLINDAMYCIN HCL 300 MG CAPS. 1 CAPSULE QID AND ALSO WAS GIVEN AN INJECTION OF CLINDAMYCIN. SUBJECT RETURNED TO TKL RESEARCH FOR CHALLENGE VISIT ON 11/15 AND AT THE TIME HER TOPICAL INFECTION HAD BEEN RESOLVED. THE DATE OF RESOLUTION WAS 11/7/05.

C12-15 Alkyl Benzoate at 1690 in Lipstick

TKL RESEARCH

SUMMARY REPORT

TKL Study No.: DS107305-6

Date of Report: December 14, 2005

Study Title:	Repeated Insult Patch Test
Study Sponsor:	
TKL Protocol No.:	TKL-1000
Sponsor Reference No.:	
Study Objective:	To assess the sensitization potential of topically applied study material.
Study Design:	Standard RIPT methodology with nine 24-hour induction applications and a single 24-hour challenge application following a 10-15 day rest period.
Principal Investigator:	Jonathan S. Dosik, MD - Dermatologist
Manager, Dermatologic Safety Testing:	Kathleen Georgeian
Study Center:	TKL Research, Inc. 4 Forest Avenue Paramus, NJ 07652
Study Site:	Paramus, NJ
Study Dates:	Date Study Initiated: October 10, 2005 Date Study Completed: November 18, 2005

Study Material:

SPL Code and Category	Amount Applied	Patch Condition
Lip Stick	0.2g	Occlusive

Number of Subjects:	Enrolled: 122	Completed: 107
	Discontinued: 15	
	Lost to follow-up: 14	Voluntary withdrawal: 1

Listing of Attached Tables:

Appendix I	Summary Tables
Appendix II	Data Listings

SUMMARY AND CONCLUSION:

One non-product-related adverse event was reported. See Data Listing 4, Appendix II

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

Under the conditions employed in this study, there was no evidence of sensitization to

SIGNATURES:

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Jonathan S. Dosik, MD
Dermatologist/Principal Investigator

Kathleen Georgeian
Clinical Research Coordinator and Manager, Dermatologic Safety Testing

This report has been reviewed by the TKL Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Henry Briscoe
Quality Assurance

12/14/05
Date

APPENDIX I

SUMMARY TABLES

TKL STUDY NO. DS107305

TABLE 1: SUMMARY OF SUBJECT ENROLLMENT AND DISPOSITION

	n (%)
Subjects enrolled	122
Subjects completed induction phase	110 (90.2)
Subjects completed all phases	107 (87.7)
Total subjects discontinued	15 (12.3)
Lost to follow-up	14 (11.5)
Voluntary withdrawal	1 (0.8)

Note: All percentages are relative to total subjects enrolled

See Data Listing 1 for further detail

Program: DISPSMY.SAS/USES: FINAL/07DEC05:09:17:29

TKL STUDY NO. DS107305
TABLE 2: SUMMARY OF SUBJECT DEMOGRAPHICS
ALL ENROLLED SUBJECTS

Age	
n (%) 18 to 44	50 (41.0)
n (%) 45 to 64	69 (56.6)
n (%) 65 and up	3 (2.5)
Mean (SD)	45.9 (11.3)
Median	47.4
Range	18.3 to 66.6
Gender	
n (%) Male	25 (20.5)
n (%) Female	97 (79.5)
Race	
n (%) Asian	2 (1.6)
n (%) Black	14 (11.5)
n (%) Caucasian	99 (81.1)
n (%) Hispanic	7 (5.7)

See Data Listing 2 for further detail

Program: DEMOSMY.SAS/USES: DEMOGS/07DEC05:09:17:31

TKL STUDY NO. DS107305

TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES
NUMBER OF SUBJECTS BY PRODUCT

PRODUCT=

	Induction Reading									Make-	Challenge Phase		
Response	1	2	3	4	5	6	7	8	9	Up	48hr	72hr	96hr(*)
-	115	111	110	108	110	109	105	110	108	7	107	107	
Total evaluable	115	111	110	108	110	109	105	110	108	7	107	107	
Number absent	4	4	5	6	4	4	7	0	2		0	0	
Number discontinued	3	7	7	8	8	9	10	12	12		15	15	

MAXIMUM ELICITED RESPONSE DURING INDUCTION
ALL SUBJECTS COMPLETING INDUCTION (N=110)

Response	n(%) Subjects
-	110 (100.0%)

(*) when required

Key to Symbols:
- = No reaction
? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema
++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50%

Program: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/07DEC05:09:17:41

APPENDIX II

DATA LISTINGS

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
1	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
2	10/10/05	10/10/05		10/26/05	I6	L	17
3	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
4	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
5	10/10/05	10/10/05		10/14/05	I0	L	5
6	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
7	10/10/05	10/10/05		11/15/05	I9	L	37
8	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
9	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
10	10/10/05	10/10/05		11/15/05	I9	L	37
11	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
12	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
13	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
14	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
15	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
16	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
17	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
18	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
19	10/10/05	10/10/05		10/24/05	I5	L	15
20	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
21	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
22	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
23	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
24	10/10/05	10/10/05	11/15/05	11/18/05	C2	C	40
25	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
26	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
27	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
28	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
29	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
30	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
31	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
32	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
33	10/14/05	10/14/05		11/04/05	I7	L	22
34	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
35	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
36	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
37	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
38	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
39	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
40	10/14/05	10/14/05		10/19/05	I0	L	6
41	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
42	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
43	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
44	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
45	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
46	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
47	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
48	10/14/05	10/14/05		10/19/05	I1	S	6
49	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
50	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
51	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
52	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
53	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
54	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
55	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
56	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
57	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
58	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
59	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
60	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
61	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
62	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
63	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
64	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
65	10/14/05	10/14/05		10/21/05	I1	L	8
66	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
67	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
68	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
69	10/14/05	10/14/05		10/26/05	I3	L	13
70	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
71	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
72	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
73	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
74	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
75	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
76	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
77	10/14/05	10/14/05		10/21/05	I1	L	8
78	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
79	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
80	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
81	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
82	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
83	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
84	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
85	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
86	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
87	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
88	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
89	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
90	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
91	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
92	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
93	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
94	10/14/05	10/14/05		10/19/05	I0	L	6
95	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
96	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
97	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
98	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
99	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
100	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
101	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
102	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
103	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
104	10/14/05	10/14/05		11/04/05	I7	L	22
105	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
106	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
107	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
108	10/14/05	10/14/05		11/15/05	I9	L	33
109	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
110	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
111	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
112	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
113	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
114	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
115	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
116	10/14/05	10/14/05		10/21/05	I1	L	8
117	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 1: SUBJECT ENROLLMENT AND DISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
118	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
119	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
120	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
121	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36
122	10/14/05	10/14/05	11/15/05	11/18/05	C2	C	36

Key: Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal
V=Protocol violation, AE=Adverse event, O=Other)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/07DEC05:09:17:09

TKL STUDY NO. DS107305
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
1	48.2	Female	Caucasian
2	44.5	Female	Caucasian
3	37.5	Female	Hispanic
4	56.9	Female	Caucasian
5	36.8	Male	Black
6	56.2	Female	Caucasian
7	58.5	Female	Caucasian
8	54.5	Female	Caucasian
9	47.5	Female	Caucasian
10	40.9	Female	Caucasian
11	48.6	Female	Caucasian
12	51.5	Female	Caucasian
13	57.7	Female	Caucasian
14	22.8	Female	Caucasian
15	38.2	Female	Black
16	42.7	Female	Caucasian
17	48.2	Female	Caucasian
18	51.1	Female	Caucasian
19	37.5	Female	Hispanic
20	45.2	Female	Caucasian
21	23.8	Female	Black
22	56.0	Male	Caucasian
23	42.8	Female	Caucasian
24	49.0	Female	Caucasian
25	66.5	Male	Caucasian
26	51.1	Female	Caucasian
27	33.0	Female	Black
28	58.0	Male	Caucasian
29	55.3	Female	Caucasian
30	43.5	Male	Caucasian
31	48.7	Female	Caucasian
32	45.9	Male	Caucasian
33	58.4	Female	Caucasian
34	23.9	Male	Caucasian
35	49.1	Male	Caucasian
36	48.5	Female	Caucasian
37	48.2	Female	Caucasian
38	38.2	Male	Black
39	46.5	Male	Caucasian
40	38.3	Female	Caucasian

Subject No.	Age	Gender	Race
41	44.5	Female	Caucasian
42	61.8	Female	Caucasian
43	35.3	Female	Caucasian
44	61.7	Female	Caucasian
45	45.8	Female	Caucasian
46	37.4	Male	Caucasian
47	20.4	Female	Caucasian
48	44.9	Female	Caucasian
49	50.0	Female	Caucasian
50	54.7	Female	Caucasian
51	47.3	Female	Caucasian
52	54.1	Female	Caucasian
53	66.6	Male	Caucasian
54	34.6	Female	Caucasian
55	46.0	Female	Caucasian
56	53.3	Male	Caucasian
57	46.2	Female	Caucasian
58	56.0	Female	Caucasian
59	59.5	Female	Caucasian
60	52.5	Female	Caucasian
61	59.9	Female	Black
62	60.3	Female	Caucasian
63	46.1	Female	Hispanic
64	41.4	Female	Caucasian
65	27.1	Female	Hispanic
66	54.6	Female	Caucasian
67	41.7	Female	Caucasian
68	56.0	Female	Caucasian
69	44.4	Female	Caucasian
70	42.5	Female	Caucasian
71	48.1	Male	Caucasian
72	28.7	Female	Caucasian
73	52.8	Female	Caucasian
74	54.5	Female	Caucasian
75	39.8	Female	Hispanic
76	18.3	Female	Caucasian
77	58.9	Female	Caucasian
78	49.3	Female	Caucasian
79	42.9	Female	Caucasian
80	59.7	Female	Caucasian

Subject No.	Age	Gender	Race
81	39.7	Female	Caucasian
82	58.1	Female	Caucasian
83	61.8	Male	Caucasian
84	29.3	Female	Caucasian
85	52.9	Female	Caucasian
86	56.5	Male	Caucasian
87	47.0	Female	Caucasian
88	43.0	Female	Caucasian
89	65.7	Female	Caucasian
90	50.8	Female	Caucasian
91	21.8	Male	Caucasian
92	21.4	Female	Caucasian
93	56.9	Female	Caucasian
94	60.7	Female	Caucasian
95	40.6	Female	Black
96	55.1	Female	Caucasian
97	18.6	Male	Hispanic
98	60.5	Female	Asian
99	47.4	Female	Caucasian
100	60.4	Male	Caucasian
101	35.8	Female	Black
102	50.4	Female	Black
103	50.0	Female	Caucasian
104	37.0	Female	Caucasian
105	60.3	Male	Asian
106	50.6	Male	Hispanic
107	48.6	Male	Black
108	46.4	Female	Caucasian
109	28.7	Male	Black
110	45.7	Female	Caucasian
111	23.9	Female	Caucasian
112	40.2	Female	Caucasian
113	48.5	Female	Caucasian
114	49.0	Female	Caucasian
115	40.0	Female	Caucasian
116	33.7	Female	Black
117	32.9	Female	Caucasian
118	44.8	Male	Black
119	41.8	Female	Black
120	19.1	Female	Caucasian

TKL STUDY NO. DS107305
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
121	44.0	Female	Caucasian
122	35.7	Male	Caucasian

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
1	-	-	-	-	-	-	-	-	-		-	-	
2	-	-	X	-	-	-	X	X	X		X	X	
3	-	-	-	-	-	-	-	-	-		-	-	
4	-	X	-	-	-	-	-	-	-	-	-	-	
5	X	X	X	X	X	X	X	X	X		X	X	
6	-	-	-	-	-	-	-	-	-		-	-	
7	-	-	-	-	-	-	-	-	-		X	X	
8	-	X	-	-	-	-	-	-	-	-	-	-	
9	-	-	-	-	-	-	-	-	-		-	-	
10	-	X	-	-	-	-	-	-	-	-	X	X	
11	-	-	-	-	-	-	-	-	-		-	-	
12	-	-	-	-	-	-	-	-	-		-	-	
13	-	-	-	-	X	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-		-	-	
15	-	-	-	-	-	-	-	-	-		-	-	
16	-	-	-	-	-	-	-	-	-		-	-	
17	-	-	-	-	-	-	-	-	-		-	-	
18	-	-	-	-	X	-	-	-	-	-	-	-	
19	-	-	-	X	-	X	X	X	X		X	X	
20	-	-	-	-	-	-	-	-	-		-	-	

Key to Symbols:

- = No reaction

? = Minimal or doubtful response, slightly different from surrounding normal skin

+ = Definite erythema, no edema

++ = Definite erythema, definite edema

+++ = Definite erythema, definite edema and vesiculation

N9G = No ninth grading NA=Not applied NP=Not patched due to reaction achieved
X = Reading not performed due to missed visit or subject discontinuation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50% NR=Data not recorded
MU = Make-up reading for missed induction visit

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
21	-	-	-	-	-	-	-	-	-		-	-	
22	-	-	-	-	-	-	-	-	-		-	-	
23	-	-	-	-	-	X	-	-	-	-	-	-	
24	-	-	-	-	-	-	X	-	-	-	-	-	
25	-	-	-	-	-	-	-	-	-		-	-	
26	-	-	-	-	-	-	-	-	-		-	-	
27	-	-	-	X	-	-	-	-	-	N9G	-	-	
28	-	-	-	-	-	-	-	-	-		-	-	
29	-	-	-	-	-	-	-	-	-		-	-	
30	-	-	-	-	-	-	-	-	-		-	-	
31	-	-	-	-	-	-	-	-	-		-	-	
32	-	-	X	-	-	-	-	-	-	N9G	-	-	
33	-	-	-	-	-	-	-	X	X		X	X	
34	-	-	-	-	-	-	-	-	-		-	-	
35	-	-	-	-	-	-	-	-	-		-	-	
36	-	-	-	-	-	-	-	-	-		-	-	
37	-	-	-	-	-	-	-	-	-		-	-	
38	-	-	-	-	-	-	-	-	-		-	-	
39	-	-	-	-	-	-	-	-	-		-	-	
40	X	X	X	X	X	X	X	X	X		X	X	
41	-	-	-	-	-	-	X	-	-	N9G	-	-	
42	-	-	-	-	-	-	-	-	-		-	-	
43	-	-	-	-	-	-	-	-	-		-	-	
44	-	-	-	-	-	-	-	-	-		-	-	
45	-	-	-	-	-	-	-	-	-		-	-	
46	-	-	-	-	-	-	-	-	-		-	-	
47	-	-	-	-	-	X	-	-	-	N9G	-	-	
48	-	X	X	X	X	X	X	X	X		X	X	
49	-	-	-	-	-	-	-	-	-		-	-	
50	-	-	-	-	-	-	-	-	-		-	-	
51	-	-	-	-	-	-	-	-	-		-	-	
52	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
53	-	-	-	-	-	-	-	-	-		-	-	
54	-	-	-	X	-	-	-	-	-	N9G	-	-	
55	-	-	-	-	-	-	-	-	-		-	-	
56	-	-	-	-	-	-	-	-	-		-	-	
57	-	-	-	-	-	-	X	-	-	N9G	-	-	
58	-	-	-	-	-	X	-	-	-	N9G	-	-	
59	-	-	-	-	-	-	-	-	-		-	-	
60	-	-	-	-	-	-	-	-	-		-	-	
61	-	-	-	-	X	-	-	-	-	N9G	-	-	
62	-	-	-	-	-	-	-	-	-		-	-	
63	-	-	-	-	-	-	-	-	N9G		-	-	
64	-	-	-	-	-	-	-	-	-		-	-	
65	-	X	X	X	X	X	X	X	X		X	X	
66	-	-	-	-	-	-	-	-	-		-	-	
67	-	-	-	-	X	-	-	-	-	N9G	-	-	
68	-	-	-	-	-	-	-	-	-		-	-	
69	-	-	-	X	X	X	X	X	X		X	X	
70	-	-	X	-	-	-	-	-	-	N9G	-	-	
71	-	-	-	-	-	-	-	-	-		-	-	
72	-	-	-	-	-	-	X	-	-	N9G	-	-	
73	-	-	-	-	-	-	-	-	-		-	-	
74	-	-	-	-	-	-	-	-	-		-	-	
75	-	-	-	-	-	-	-	-	-		-	-	
76	X	-	-	-	-	-	-	-	-	N9G	-	-	
77	-	X	X	X	X	X	X	X	X		X	X	
78	-	-	-	-	-	-	-	-	-		-	-	
79	-	-	-	-	-	-	-	-	-		-	-	
80	-	-	-	-	-	-	-	-	-		-	-	
81	-	-	-	-	-	-	-	-	-		-	-	
82	-	-	-	-	-	-	-	-	-		-	-	
83	-	-	-	-	-	-	-	-	-		-	-	
84	X	-	-	-	-	-	-	-	-	N9G	-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
85	-	-	-	-	-	-	-	-	-		-	-	
86	-	-	-	-	-	-	-	-	-		-	-	
87	-	-	-	-	-	-	X	-	-	N9G	-	-	
88	-	-	-	-	-	-	-	-	-		-	-	
89	-	-	-	-	-	-	-	-	-		-	-	
90	-	-	-	-	-	-	-	-	-		-	-	
91	-	-	X	-	-	-	-	-	-	N9G	-	-	
92	-	-	-	-	-	-	X	-	-	N9G	-	-	
93	-	-	-	-	-	-	-	-	-		-	-	
94	X	X	X	X	X	X	X	X	X		X	X	
95	-	-	-	-	-	-	-	-	-		-	-	
96	-	-	-	-	-	-	-	-	-		-	-	
97	-	-	-	X	-	-	-	-	-	N9G	-	-	
98	-	-	-	-	-	-	-	-	-		-	-	
99	-	-	-	-	-	-	-	-	-		-	-	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	X	-	-	-	-	-	N9G	-	-	
102	-	-	-	X	-	-	-	-	-	N9G	-	-	
103	-	-	-	-	-	-	X	-	-	N9G	-	-	
104	-	-	-	-	-	X	-	X	X		X	X	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	X	-	-	-	-	-	-	-	N9G	X	X	
109	-	-	X	-	-	-	-	-	-	N9G	-	-	
110	-	-	-	-	-	-	-	-	-		-	-	
111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	-	-	-	-	-	-	-		-	-	
113	-	-	-	-	-	-	-	-	-		-	-	
114	-	-	-	-	-	-	-	-	-		-	-	
115	-	-	-	-	-	-	-	-	-		-	-	
116	-	X	X	X	X	X	X	X	X		X	X	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT=

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
117	-	-	-	-	-	-	-	-	-		-	-	
118	X	-	-	-	-	-	-	-	-	N9G	-	-	
119	X	-	-	-	-	-	-	-	-	N9G	-	-	
120	-	-	-	-	-	-	-	-	N9G		-	-	
121	-	-	-	-	-	-	-	-	-		-	-	
122	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/07DEC05:09:17:13

TKL STUDY NO. DS107305
DATA LISTING 4: ADVERSE EVENTS

Subject No. 79

Adverse Event: TOPICAL INFECTION

Date of Onset: 10/28
Frequency: Single episode
Duration:
Rel. to Study Product: Unrelated
Action Taken/Study Product: None
Action Taken/Treatment?: YES
Serious? NO

Date of Resolution: 11/07
Severity: Mild
Outcome: Resolved

Comment: SUBJECT HAD TOPICAL INFECTION ON LOWER LEFT SIDE OF HER STOMACH AND WAS SEEN BY EMERGENCY ROOM DOCTOR WHO PRESCRIBED AUGMENTIN XR1000-62.5 TAB 1 TAB BID FOR 7 DAYS. SUBJECT HAD TO RETURN TO DOCTOR FOR FOLLOW-UP VISIT ON 11/02/05. SUBJECT RETURNED TO TKL RESEARCH ON 11/2/05 AFTER VISIT WITH DOCTOR WHO PRESCRIBED CLINDAMYCIN HCL 300 MG CAPS. 1 CAPSULE QID AND ALSO WAS GIVEN AN INJECTION OF CLINDAMYCIN. SUBJECT RETURNED TO TKL RESEARCH FOR CHALLENGE VISIT ON 11/15 AND AT THE TIME HER TOPICAL INFECTION HAD BEEN RESOLVED. THE DATE OF RESOLUTION WAS 11/7/05.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: August 19, 2010

SUBJECT: Information on Products Containing Alkyl Benzoates

Consumer Product Testing Co. 2003. Human repeat insult patch test of formula no. 1001449G (hair serum containing 35% C12-15 Alkyl Benzoate). Experiment reference number C03-0542-07.

TKL Research. 2010. Human repeated insult patch test formula no. 740017 15 (face lotion containing 2% Stearyl Benzoate). TKL study no. DS1111309/100310-1.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Consumer Product Testing Co.

EST. 1975

HUMAN REPEAT INSULT PATCH TEST
SUMMARY
Formula N°. 1001449G

Hair serum contains 35% C12-15 Alkyl Benzoate

Study N° (C03-0542.07)
Study N° (U03192.07)

Study Objective:

The main objective of this study is to confirm that the application of the product under the test conditions does not induce delayed contact allergic responses.
Secondarily the potential irritant effect of the product may be evaluated during the induction phase of the protocol.

Method

Study under dermatological supervision.
Induction: Patches applied on the back, 3 times each week for 3 weeks (total of nine patches). Patches applied for 24 hours.
Rest phase: 2 weeks.
Challenge: one 24-hour patch on a virgin site.
Skin Grading: Induction: 24 hours after patch removal.
Challenge: 24 hours, 72 hours and 96 hours (if needed) after patch removal.

Investigator

Richard R. Eisenberg, M.D.
Board Certified Dermatologist

Number of subjects

Two hundred twenty-four (224) subjects enrolled/
Two hundred and eight (208) subjects completed

Panel description

Male and female subjects ages 18 to 70 years.

Conclusion

Non-sensitizing	☑	Non-irritating	☑
Sensitizing	☐	Irritation acceptable (normal) for product type	☐
		Irritation higher than normal for product type	☐
Additional data needed	☐		

Signature

Richard Eisenberg 8/12/03

Richard R. Eisenberg, M.D. **Date**
Board Certified Dermatologist

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT:

ATTENTION: Christine K. Wood
Clinical Research Associate
Department of Biological Sciences

TEST: Exclusive Repeated Insult Patch Test
Protocol No.: 1.01

TEST MATERIAL: U03192.07 1001449G

EXPERIMENT REFERENCE NUMBER: C03-0542.07

Richard R. Eisenberg, M.D.
Board Certified Dermatologist

Michael Traudt, Ph.D.
Director, Clinical Evaluations

Robert W. Shanahan, Ph.D.
Principal Investigator

Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor any member of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

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Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study No.: C03-0542.07

The objective of the Quality Assurance Unit (QAU) is to monitor the conduct and reporting of clinical laboratory studies. These studies have been performed with adherence to ICH Guideline E6 for Good Clinical Practice and requirements provided for in 21 CFR parts 50 and 56 and in accordance to standard operating procedures and applicable protocols. The QAU maintains copies of study protocols and standard operating procedures and has inspected this study on the date(s) listed below. The findings of these inspections have been reported to management and the Study Director. All materials and data pertinent to this study will be stored in the Archive Facility at 70 New Dutch Lane, Fairfield, New Jersey, 07004, unless specified otherwise, in writing by the Sponsor.

Date(s) of inspection:
July 8, 2003
August 6, 2003
August 11, 2003
August 12, 2003
August 15, 2003

Senior personnel involved:

Richard Hettenbach, M.A. - Senior Director of Regulatory Affairs & Quality Assurance

Marie Terlizzese, M.S.
Quality Assurance Associate

The representative signature of the Quality Assurance Unit signifies that this study has been performed in accordance with standard operating procedures and study protocol as well as government regulations regarding such procedures and protocols.

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: Two hundred twenty-four (224) qualified subjects, male and female, ranging in age from 18 to 70 years, were selected for this evaluation. Two hundred eight (208) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, ages 18 - 70.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: U03192.07 1001449G

Study Schedule:

Panel #	Initiation Date	Proposed Completion Date	Actual Completion Date
20030264	June 18, 2003	July 25, 2003	August 7, 2003

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 ml of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing*. This was then applied to the appropriate treatment site to form a semi-occluded patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period. It was noted that due to a holiday weekend which occurred during the Induction Phase, subjects who required a makeup day experienced a delay between applications.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications are discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Tuesday and Thursday removal, and forty-eight hours following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic twenty-four and seventy-two hours post-application.

*Manufactured by TruMed Technologies, Inc., Burnsville, MN

Evaluation Key:

0 = No visible skin reaction
+ = Barely perceptible or spotty erythema
1 = Mild erythema covering most of the test site
2 = Moderate erythema, possible presence of mild edema
3 = Marked erythema, possible edema
4 = Severe erythema, possible edema, vesiculation, bullae and/or ulceration

Results:

The results of each participant are appended (Table 1).

Observations remained within normal limits throughout the test interval.

Summary:

Under the conditions of this study, test material, U03192.07 1001449G, did not indicate a potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20030264

Individual Results

U03192.07 -- 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0[m]	0	0	0	0	---DNC---	
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor
DNC = Did not complete study

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
29	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	+	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	----------DID NOT COMPLETE STUDY----------											
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	2[A]	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0[m]	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	----------DID NOT COMPLETE STUDY----------											
56	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor
A = Changed to adjacent site

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	----------DID NOT COMPLETE STUDY----------										
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	----------DID NOT COMPLETE STUDY----------								
72	0	0	0	0	----------DID NOT COMPLETE STUDY----------							
73	0	----------DID NOT COMPLETE STUDY----------										
74	0	0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0	0	0
76	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	----------DID NOT COMPLETE STUDY----------							
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
113	0	0	0	0	0	0	0	0	0	0	0	0
114	0	0	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0	0
123	0	0	0	0	0	0	0	0	0	0	0	0
124	0	0	0	0	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0	0	0	0	0
126	0	0	0	0	0	0	0	0	0	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0	0
128	0	0	0	0	0	0	0	0	0	0	0	0
129	0	0	0	0	0	0	0	0	0	0	0	0
130	0	0	0	0	0	0	0	0	0	0	0	0
131	0	0	0	0	0	0	0	0	0	0	0	0
132	0	0	0	0	0	0	0	0	0	0	0	0
133	0	0	0	0	0	0	0	0	0	0	0	0
134	0	0	0	0	0	0	0	0	0	0	0	0
135	0	0	0	----------DID NOT COMPLETE STUDY----------								
136	0	0	0	0	0	0	0	0	0	0	0	0
137	0	0	0	0	0	0	0	0	0	0	0	0
138	0	0	0	0	0	0	0	0	0	0	0	0
139	0	0	0	0	0	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
141	0	0	0	0	0	----------DID NOT COMPLETE STUDY----------						
142	0	0	0	0	0	0	0	0	0	0	0	0
143	0	0	0	0	0	0	0	0	0	0	0	0
144	0	0	0	0	0	0	0	0	0	0	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0
146	0	0	0	0	0	0	0	0	0	0	0	0
147	0	0	0	0	0	0	0	0	0	0	0	0
148	0	0	0	0	0	0	0	0	0	0	0	0
149	0	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0	0	0	0	0	0
151	0	0	0	0	0	0	0	0	0	0	0	0
152	0	0	0	0	0	0	0	0	0	0	0	0
153	0	0	0	0	0	0	-------DID NOT COMPLETE STUDY--------					
154	0	0	0	0	0	0	-------DID NOT COMPLETE STUDY--------					
155	0	0	0	0	0	0	0	0	0	0	0	0
156	0	0	0	0	0	0	0	0	0	0	0	0
157	0	0	0	0	0	0	0	0	0	0	0	0
158	0	0	0	0	0	0	0	0	0	0	0	0
159	0	0	0	0	0	0	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0	0	0	0	0
161	0	0	0	0	0	0	0	0	0	0	0	0
162	0	0	0	0	0	0	0	0	0	0	0	0
163	0	0	0	0	0	0	0	0	0	0	0	0
164	0	0	0	0	0	0	0	0	0	0	0	0
165	0	0	0	0	0	0	0	0	0	0	0	0
166	0	0	0	0	----------DID NOT COMPLETE STUDY----------							
167	0	0	0	0	0	0	0	0	0	0	0	0
168	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
169	0	0	0	0	0	0	0	0	0	0	0	0
170	0	0	0	0	0	0	0	0	0	0	0	0
171	0	0	0	0	0	0	0	0	0	0	0	0
172	0	0	0	0	0	0	0	0	0	0	0	0
173	0	0	0	0	0	0	0	0	0	-	0	0
174	0	0	0	0	0	0	0	0	0	0	0	0
175	0	0	0	0	0	0	0	0[m]	0	0	0	0
176	0	0	0	0	0	0	0	0	0	0	0	0
177	0	0	0	0	0	0	0	0	0	0	0	0
178	0	0	0	0	0	0	0	0	0	0	0	0
179	0	0	0	0	0	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0	0	0	0	0	0
181	0	0	0	0	0	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0	0	0	0
183	0	0	0	0	0	0	0	0	0	0	0	0
184	0	0	0	0	0	0	0	0	0	0	0	0
185	0	0	0	0	0	0	0	0	0	0	0	0
186	0	0	0	0	0	0	0	0	0	0	0	0
187	0	0	0	0	0	0	0	0	0	0	0	0
188	0	0	0	0	0	0	0	0	0	0	0	0
189	0	0	0	0	0	0	0	0	0	0	0	0
190	0	0	0	0	0	0	0	0	0	0	0	0
191	0	0	0	0	0	0	0	0	0	0	0	0
192	0	0	0	0	0	0	0	0	0	0	0	0
193	0	0	0	0	0	0	0	0	0	0	0	0
194	0	0	0	0	0	0	0	0	0	0	0	0
195	0	0	0	0	0	0	0	0	0	0	0	0
196	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch
m = Additional makeup day granted at the discretion of the clinic supervisor
- = Observation omitted. Subject unable to report as scheduled due to an emergency.

Table 1
(continued)
Panel #20030264

Individual Results

U03192.07 1001449G

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
197	0	0	0	0	0	0	0	0	0	0	0	0
198	0	0	0	0	0	0	0	0	0	0	0	0
199	0	0	0	0	0	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0	0	0	0	0	0
201	0	0	0	0	0	0	0	0	0	0	0	0
202	0	0	0	0	0	0	0	0	0	0	0	0
203	0	0	0	0	0	0	0	0	0	0	0	0
204	0	0	0	0	0	0	0	0	0	0	0	0
205	0	0	0	0	0	0	0	0	0	0	0	0
206	0	0	0	0	0	0	0	0	0	0	0	0
207	0	0	0	0	0	0	0	0	0	0	0	0
208	0	0	----------DID NOT COMPLETE STUDY----------									
209	0	0	----------DID NOT COMPLETE STUDY----------									
210	0	0	0	0	0	0	0	0	0	0	0	0
211	0	0	0	0	0	0	0	0	0	0	0	0
212	----------DID NOT COMPLETE STUDY----------											
213	0	0	0	0	0	0	0	0	0	0	0	0
214	0	0	0	0	0	0	0	0	0	0	0	0
215	0	0	0	0	0	0	0	0	0	0	0	0
216	0	0	0	0	0	0	0	0	0	0	0	0
217	0	0	0	0	0	0	0	0	0	0	0	0
218	0	0	0	0	0	0	0	0	0	0	0	0
219	0	0	0	0	0	0	0	0	0	0	0	0
220	0	0	0	0	0	0	0	0	0	0	0	0
221	0	0	0	0	0	0	0	0	0	0	0	0
222	0	0	0	0	0	0	0	0	0	0	0	0
223	0	0	0	0	0	0	0	0	0	0	0	0
224	0	0	0	0	0	0	0	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch

Table 2
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
1	DW	50	F
2	AK	59	M
3	VP	56	F
4	JE	53	M
5	AK	63	F
6	JH	44	F
7	GM	58	F
8	GA	27	M
9	JG	70	F
10	JO	59	F
11	RO	61	M
12	HN	35	F
13	LC	32	F
14	AD	23	F
15	EG	53	F
16	KS	47	F
17	BB	58	M
18	MT	35	F
19	PA	50	F
20	RC	51	M
21	JO	35	F
22	KF	22	F
23	MT	40	F
24	NI	51	F
25	MM	62	F
26	GM	63	M
27	YH	42	F
28	MC	41	F

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
29	SS	25	F
30	IJ	37	F
31	LR	44	F
32	EG	42	F
33	BS	56	F
34	JN	44	F
35	EM	36	F
36	MG	53	F
37	DG	22	M
38	EV	35	M
39	CH	31	F
40	RR	49	M
41	CT	32	F
42	KH	33	F
43	DM	51	F
44	VP	58	F
45	MB	68	F
46	DW	45	F
47	IF	36	F
48	AS	59	F
49	AB	55	F
50	NT	54	F
51	DM	51	F
52	DD	56	F
53	PL	57	F
54	MM	37	F
55	SB	22	F
56	VM	44	M

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
57	HM	49	M
58	CA	46	M
59	JC	47	M
60	ER	52	M
61	RS	36	F
62	NS	26	F
63	PC	18	M
64	AT	34	F
65	LT	35	F
66	ST	37	M
67	TC	36	F
68	JH	53	F
69	LP	55	F
70	DB	24	F
71	EM	23	M
72	TM	21	F
73	KM	23	F
74	YC	20	F
75	TD	43	F
76	JS	31	M
77	MB	20	F
78	MR	22	F
79	ML	52	F
80	RM	62	M
81	FS	51	M
82	MA	56	F
83	SD	57	F
84	RB	38	M

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
85	SR	48	F
86	NK	49	F
87	LK	20	F
88	PP	45	M
89	CS	30	F
90	DG	34	F
91	CW	66	F
92	JC	25	M
93	NM	25	F
94	TZ	48	F
95	FH	39	M
96	AM	56	F
97	KR	36	F
98	SR	34	F
99	SK	46	F
100	JD	45	M
101	KP	48	F
102	CL	59	F
103	PO	34	F
104	CT	37	M
105	AT	60	M
106	MT	52	F
107	LS	45	M
108	AD	34	F
109	BB	64	F
110	EF	49	F
111	AM	42	F
112	JP	67	M

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
113	MS	70	F
114	CM	56	F
115	VS	29	F
116	HP	65	F
117	JS	68	F
118	CL	68	F
119	MB	56	F
120	CG	65	F
121	MP	51	M
122	IM	20	F
123	CE	42	F
124	JM	64	F
125	MA	38	F
126	EC	26	F
127	GH	52	F
128	DG	50	F
129	KB	43	M
130	CC	21	F
131	CP	27	M
132	PG	40	F
133	AI	55	F
134	SY	64	F
135	GP	64	F
136	SS	25	M
137	QA	49	F
138	RZ	64	F
139	RO	65	F
140	AT	59	F

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
141	DA	21	F
142	LM	40	F
143	JL	67	F
144	ST	46	M
145	MM	56	M
146	AS	37	F
147	JT	37	M
148	PT	67	M
149	WT	64	F
150	BM	61	F
151	DR	43	F
152	LM	45	F
153	SM	52	F
154	DD	66	M
155	EM	43	F
156	JW	50	F
157	PT	42	F
158	DP	41	F
159	PP	68	F
160	SF	67	F
161	BM	63	F
162	SC	54	F
163	SD	23	F
164	ET	20	F
165	AA	43	F
166	KB	33	F
167	DS	64	F
168	JN	60	F

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
169	FN	69	F
170	RC	58	M
171	LC	49	F
172	VL	48	M
173	RA	43	M
174	GT	33	F
175	TS	23	M
176	DK	24	M
177	LB	66	F
178	AD	58	F
179	AC	37	F
180	GD	26	F
181	JB	55	F
182	JR	25	F
183	AL	43	F
184	CB	41	F
185	MD	35	F
186	EG	50	F
187	AA	44	M
188	BB	61	F
189	SD	46	F
190	RH	35	F
191	AK	24	F
192	BC	22	F
193	TO	68	M
194	MO	68	F
195	DZ	39	F
196	MZ	38	M

Table 2
(continued)
Panel #20030264

Subject Data

Subject Number	Initials	Age	Sex
197	RB	57	F
198	CT	39	F
199	JJ	47	M
200	DG	64	F
201	DB	59	M
202	NP	23	M
203	FP	64	F
204	NJ	47	F
205	CM	51	F
206	JF	40	M
207	DB	43	M
208	JM	20	F
209	MM	44	M
210	WG	70	M
211	CW	53	F
212	CS	42	F
213	BL	41	F
214	EM	56	F
215	CB	60	F
216	JA	35	M
217	LL	51	F
218	PM	51	F
219	LT	37	F
220	SL	24	F
221	AB	35	M
222	GH	38	M
223	GS	62	M
224	CN	27	F

TKL RESEARCH

HUMAN REPEATED INSULT PATCH TEST
SUMMARY
Formula N° 740017 15

Face lotion containing 2% Stearyl Benzoate

Study N° (TKL Research, Inc.) DS111309/100310
Study N°) DT036360
Report N° DS111309/100310-1

Study Objective: The main objective of this study is to confirm that the application of the product under the test conditions does not induce delayed contact allergic responses.
Secondarily the potential irritant effect of the product may be evaluated during the induction phase of the protocol.

Method: Study under dermatological supervision.
Induction: Occlusive patches applied on the back, 3 times each week for 3 weeks (total of nine induction patches). Patches applied for 24 hours.
Rest phase: 2 weeks.
Challenge: one 24-hour occlusive patch on a virgin site.
Skin Grading: Induction: 24 hours after patch removal.
Challenge: 24 hours, 48 hours, and 72 hours (if needed), after patch removal.

Investigator: Jonathan S. Dosik, MD (Dermatology)

Number of subjects: 200 required. 206 completed.

Panel description: Male and female subjects ages 18 to 70 years.

Conclusion:

Non-sensitizing	■	Non-irritating	■
Sensitizing	☐	Irritation acceptable (normal) for product type	☐
		Irritation higher than normal for product type	☐
Additional data needed	☐		

Signatures:

Kathleen Georgeian 3/3/10
Kathleen Georgeian **Date**
Director, Dermatologic Safety Testing
TKL Research, Inc.

3/2/10
Jonathan S. Dosik, MD **Date**
Principal Investigator
Dermatologist
TKL Research, Inc.

TKL RESEARCH

REPEATED INSULT PATCH TEST

TKL STUDY NO. DS111309/100310-1

NO. DT036360

CONDUCTED FOR:

DATE OF ISSUE:

March 5, 2010

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APPENDICES

I SUMMARY TABLES
II DATA LISTINGS
III INFORMED CONSENT DOCUMENTS

SIGNATURES

This study was conducted in compliance with the requirements of the protocol and TKL's Standard Operating Procedures, and in the spirit of GCP ICH Topic E6.[1] The report accurately reflects the raw data for this study.

Jonathan S. Dosik, MD
Dermatologist
Principal Investigator

3/2/10
Date

Kathleen Georgeian
Director, Dermatologic Safety Testing

3/3/10
Date

Michelle Medina
Clinical Research Coordinator

3/3/10
Date

STATEMENT OF QUALITY CONTROL

The Quality Control Unit of the Dermatological Safety Department conducted a 100% review of all study-related documents. The protocol was reviewed prior to the start of the study, and the medical screening forms and informed consent documents were reviewed in-process of the study. The regulatory binder and study data were reviewed post-study to ensure accuracy. The study report was reviewed and accurately reflects the data for this study.

[1] ICH Topic E6 "Note for guidance on Good Clinical Practices (CPMP/ICH/135/95)" – ICH Harmonised Tripartite Guideline for Good Clinical Practices having reached Step 5 of the ICH Process at the ICH Steering Committee meeting on 1 May 1996.

TITLE OF STUDY

Repeated Insult Patch Test

SPONSOR

STUDY MATERIAL

740017 15 Face Cream

DATE STUDY INITIATED

December 28, 2009

DATE STUDY COMPLETED

February 12, 2010

DATE OF ISSUE

March 5, 2010

INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD - Dermatologist
Principal Investigator

Kathleen Georgeian
Director, Dermatologic Safety Testing

Michelle Medina
Clinical Research Coordinator

CLINICAL SITE

TKL RESEARCH, INC.
4 Forest Avenue
Paramus, NJ 07652

SUMMARY

One (1) study material, Formula No. 740017 15, was evaluated neat to determine its ability to sensitize the skin of volunteer subjects with normal skin using an occlusive repeated insult patch study. Two hundred six (206) subjects completed the study.

Under the conditions employed in this study, Formula No. 740017 15 was found to be non-irritating and there was no evidence of sensitization.

1.0 OBJECTIVE

The objective of this study was to determine the ability of the study material to cause sensitization by repeated topical applications to the skin of humans under controlled patch study conditions.

2.0 RATIONALE

Substances that come into contact with human skin need to be evaluated for their propensity to irritate and/or sensitize. Once an appropriate pre-clinical safety evaluation has been performed, a reproducible, standardized, quantitative patch evaluation procedure must be used to demonstrate that a particular material can be applied safely to human skin without significant risk of adverse reactions. The method herein employed is generally accepted for such a purpose.

Repeated insult patch evaluation is a modified predictive patch study that can detect weak sensitizers that require multiple applications to induce a cell-mediated (Type IV) immune response sufficient to cause an allergic reaction. Irritant reactions may also be detected using this evaluation method, although this is not the primary purpose of this procedure. Results are interpreted according to interpretive criteria based upon published works, as well as the clinical experience of TKL Research, Inc. These interpretive criteria are periodically reviewed and amended as new information becomes available.

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of subjects were enrolled to provide 200 completed subjects. In the absence of any sensitization reactions in this sample size (200 evaluable subjects), a 95% upper confidence bound on the population rate of sensitization would be 1.5%.

3.1.1 Inclusion Criteria

Individuals eligible for inclusion in the study were those who:

1. were males or females, 18 to 70 years of age, in general good health;
2. were free of any systemic or dermatologic disorder which, in the opinion of the investigative personnel, would have interfered with the study results or increased the risk of adverse events;
3. were of any skin type or race, providing the skin pigmentation would allow discernment of erythema;
4. had completed a medical screening procedure; and
5. had read, understood, and signed an informed consent agreement.

3.1.2 Exclusion Criteria

Individuals excluded from participation in the study were those who:

1. had any visible skin disease at the study site which, in the opinion of the investigative personnel, would have interfered with the evaluation;

2. were receiving systemic or topical drugs or medication which, in the opinion of the investigative personnel, would have interfered with the study results;
3. had psoriasis and/or active atopic dermatitis/eczema;
4. were females who were pregnant, planning to become pregnant during the study, or breast-feeding;
5. had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated; and/or
6. were participating in another study or had been recruited to participate in another study concurrently.

3.1.3 Informed Consent

A properly executed informed consent document was obtained from each subject prior to entering the study. The signed informed consent document is maintained in the study file. In addition, the subject was provided with a copy of the informed consent document (see Appendix III).

3.2 DESCRIPTION OF STUDY

3.2.1 Outline of Study Procedures

Subjects participated in the study over a 6-week period involving 3 phases: (1) Induction, (2) Rest, and (3) Challenge. Prior to study entry, the subjects were screened to assure that they met the inclusion/exclusion criteria. Informed consent was obtained. Each subject was provided with a schedule of the study activities. All subjects were told to avoid wetting the patches and were asked not to engage in activities that caused excessive perspiration. They were instructed to notify the staff if they experienced any discomfort beyond mild itching or observed any adverse changes at the patch sites, while on the study or within 2 weeks of completing the study.

The Induction Phase consisted of 9 applications of the study material and subsequent evaluations of the patch sites. Prior to application of the patches, the sites were outlined with a skin marker, eg, gentian violet. The subjects were required to remove the patches approximately 24 hours after application. They returned to the facility at 48-hour intervals to have the sites evaluated and identical patches applied to the same sites. Patches applied on Friday were removed by subjects after 24 hours. The sites were evaluated on the following Monday, ie, 72 hours after patch application.[2] Following the ninth evaluation, the subjects were dismissed for a rest period of approximately 10-15 days.

Subjects who were absent once during the induction phase received a make-up (MU) patch at the last induction visit. The MU applications were graded 48 hours later at the MU visit, or were recorded as N9G (no ninth grading).

[2] A Monday or Friday holiday could result in evaluation at 96 hours after patch application.

The Challenge Phase was initiated during the sixth week of the study. Identical patches were applied to sites previously unexposed to the study material. The patches were removed by subjects after 24 hours and the sites graded after additional 24-hour and 48-hour periods (ie, 48 and 72 hours after application). Rechallenge was performed whenever there was evidence of possible sensitization.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during induction, and a single application and 2 readings during challenge. Only completed cases were used to assess sensitization.

3.2.2 Study Flow Chart

WEEK 1

DAY	ACTIVITIES
1*	Staff obtained informed consent, reviewed completed medical screening form, applied patches
2	Subject removed patches
3	Staff graded sites, applied patches
4	Subject removed patches
5	Staff graded sites, applied patches
6	Subject removed patches

WEEK 2

DAY	ACTIVITIES
1	Staff graded sites, applied patches
2-6	Same as Week 1

WEEK 3

DAY	ACTIVITIES
1-6	Same as Week 2

WEEK 4

DAY	ACTIVITIES
1	Staff graded sites; applied make-up (MU) induction patches, if required
2	Subject removed MU patches
3	Staff graded MU induction sites at MU visit
2-7	Rest period

WEEK 5

DAY	ACTIVITIES
1-7	Rest period

* Study flow starting with Week 1, Day 1, was altered when enrollment occurred on Wednesday or Friday. Study flow could be altered if a holiday occurred during the study.

WEEK 6

DAY	ACTIVITIES
1	Staff applied patches
2	Subject removed patches
3	Staff graded sites
4	Staff graded sites

3.2.3 Definitions Used for Grading Responses

The symbols found in the scoring scales below were used to express the response observed at the time of examination:

SYMBOL		REACTION
-	=	No reaction
?	=	Minimal or doubtful response, slightly different from surrounding normal skin
+	=	Definite erythema, no edema
++	=	Definite erythema, definite edema
+++	=	Definite erythema, definite edema and vesiculation

SPECIAL NOTATIONS

E	=	Marked/severe erythema
S	=	Spreading of reaction beyond patch site (ie, reaction where material did not contact skin)
p	=	Papular response > 50%
pv	=	Papulovesicular response > 50%
D	=	Damage to epidermis: oozing, crusting and/or superficial erosions
I	=	Itching
X	=	Subject absent
PD	=	Patch dislodged
NA	=	Not applied
NP	=	Not patched (due to reaction achieved)
N9G	=	No ninth grading

3.2.4 Evaluation of Responses

All responses were graded by a trained dermatologic evaluator meeting TKL's strict certification requirements to standardize the assignment of response grades.

4.0 NATURE OF STUDY MATERIAL

4.1 STUDY MATERIAL SPECIFICATIONS

Identification : 740017 15 Face Cream
Amount Applied : 0.2 g

4.2 STORAGE, HANDLING, AND DOCUMENTATION OF STUDY MATERIAL

Receipt of the material used in this study was documented in a general logbook, which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL. On the basis of information provided by the sponsor, the study material was considered reasonably safe for evaluation on human subjects. A sample of the study material was reserved and will be stored for a period of 6 months. All study material was kept in a locked product storage room accessible to clinical staff members only. At the conclusion of the clinical study, the remaining study material was discarded or returned to the sponsor and the disposition documented in the logbook.

4.3 APPLICATION OF STUDY MATERIAL

All study material was supplied by the sponsor. Material was applied in an amount proportionate to the patch type or as requested by the sponsor, generally 0.2 mL or g or an amount sufficient to cover the 2 cm x 2 cm patch. The patches were applied to the infrascapular area of the back, either to the right or left of the midline, or to the upper arm.

4.4 DESCRIPTION OF PATCH CONDITIONS

Material evaluated under occlusive patch conditions is applied to a 2 cm x 2 cm Webril pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patches are secured with hypoallergenic tape (Micropore), as needed.

Material evaluated under semi-occlusive patch conditions is applied to a 2 cm x 2 cm Webril pad. The pads are affixed to the skin with hypoallergenic tape (Micropore).

5.0 INTERPRETATION

Sensitization is characterized by an acute allergic contact dermatitis. Typical sensitization reactions begin with an immunologic response in the dermis resulting in erythema, edema formation, and secondary epidermal damage (vesiculation), sometimes extending beyond the patch site and often accompanied by itching. Sensitization reactions tend to be delayed. The reaction typically becomes evident between 24 and 48 hours, peaks at 48-72 hours and subsequently subsides. The reaction is often greater at 72 hours than at 48 hours. The severity of the reaction is generally greater during the challenge phase of a Repeated Insult Patch Test (RIPT) than that seen during induction.

Irritant reactions are characterized as a non-immunologic, localized, superficial, exudative, inflammatory response of the skin due to an externally applied material. The typical initial reaction does not develop much edema or vesiculation but results in scaling, drying, cracking, oozing, crusting, and erosions. The reaction is usually sharply delineated, not spreading beyond the patch site. Irritant reactions are typically evident by 24 hours and diminish over the next 48-72 hours. Removal of the offending agent results in gradual improvement of the epidermal damage. The

reaction seen at 72 hours is, therefore, less severe than that seen at 48 hours. Finally, the severity of the reaction experienced in the challenge phase is generally similar to that seen during induction.

If the results of the study indicate the likelihood of sensitization, the recommended practice is to rechallenge the subjects who have demonstrated sensitization-like reactions to confirm that these reactions are, indeed, associated with the product. Our preferred rechallenge procedure involves the application of the product to naïve sites, under both occlusive and semi-occlusive patch conditions. Use of the semi-occlusive patch condition helps to differentiate irritant and sensitization reactions. Generally speaking, if a product is a sensitizer it will produce a similar reaction under both occlusion and semi-occlusion. Whereas, if the product has caused an irritant reaction, the reactions will be less pronounced under the semi-occlusive condition.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) were designed to identify each subject by subject number and initials, and to record demographics, examination results, adverse events, and end of study status. Originals or copies of all CRFs, correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of 5 years from completion of the study. Storage was maintained either at a TKL facility in a secured room accessible only to TKL employees, or at an offsite location which provided a secure environment with burglar/fire alarm systems, camera detection and controlled temperature and humidity. Documentation will be available for the sponsor's review on the premises of TKL.

7.0 RESULTS AND DISCUSSION

Two hundred twenty-five (225) subjects between the ages of 18 and 70 were enrolled and 206 subjects completed the study (see Tables 1 and 2 in Appendix I and Data Listings 1 and 2 in Appendix II).

The following table summarizes subject enrollment and disposition.

Number enrolled:		225
Number discontinued:		19
Lost to follow-up:	10	
Voluntary withdrawal:	9	
Number completed:		206

Source: Table 1, Appendix I

There were no adverse events reported on either study.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3, Appendix II.

8.0 CONCLUSION

Under the conditions employed in this study, Formula No. 740017 15 was found to be non-irritating and there was no evidence of sensitization.

9.0 REFERENCES

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APPENDIX I

SUMMARY TABLES

Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	112
Subjects completed induction phase	107 (95.5)
Subjects completed all phases	105 (93.8)
Total subjects discontinued	7 (6.3)
Lost to follow-up	6 (5.4)
Voluntary withdrawal	1 (0.9)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

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Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	32 (28.6)
N (%) 45 to 64	62 (55.4)
N (%) 65 and up	18 (16.1)
Mean (SD)	50.4 (13.1)
Median	52.1
Range	18.5 to 70.9
Gender	
N (%) Male	26 (23.2)
N (%) Female	86 (76.8)
Race	
Asian	1 (0.9)
Black	11 (9.8)
Caucasian	85 (75.9)
Hispanic	14 (12.5)
Other	1 (0.9)

See data listing 2 for further detail.

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Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = 740017 15 DT036360

	Induction Reading										Challenge Phase		
Response	1	2	3	4	5	6	7	8	9	Make Up	48hr	72hr	96hr(*)
-	112	110	105	107	103	106	105	105	104	20	106	105	
Total evaluable	112	110	105	107	103	106	105	105	104	20	106	105	
Number absent	0	0	5	2	6	2	3	3	3		0	0	
Number discontinued	0	2	2	3	3	4	4	4	5		6	7	

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=107)

Response	n(%) Subjects
-	107 (100.0%)

(*) when required

Key to Symbols:
- = No reaction ? = Minimal or doubtful response, slightly / different from surrounding normal skin
+ = Definite erythema, no edema ++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50%

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Table 1: Summary of Subject Enrollment and Disposition

	N (%)
Subjects enrolled	113
Subjects completed induction phase	102 (90.3)
Subjects completed all phases	101 (89.4)
Total subjects discontinued	12 (10.6)
Lost to follow-up	4 (3.5)
Voluntary withdrawal	8 (7.1)

Note: All percentages are relative to total subjects enrolled.

See data listing 1 for further detail.

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Table 2: Summary of Subject Demographics
All Enrolled Subjects

Age	
N (%) 18 to 44	37 (32.7)
N (%) 45 to 64	68 (60.2)
N (%) 65 and up	8 (7.1)
Mean (SD)	47.7 (13.1)
Median	50.5
Range	19.8 to 70.1
Gender	
N (%) Male	35 (31.0)
N (%) Female	78 (69.0)
Race	
Asian	4 (3.5)
Black	9 (8.0)
Caucasian	86 (76.1)
Hispanic	14 (12.4)

See data listing 2 for further detail.

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Table 3: Summary of Dermatologic Response Grades
Number of Subjects by Product

Product = 740017 15 DT036360

	Induction Reading										Challenge Phase		
Response	1	2	3	4	5	6	7	8	9	Make Up	72hr	96hr	120hr(*)
-	110	106	103	101	102	98	102	100	98	12	101	101	
Total evaluable	110	106	103	101	102	98	102	100	98	12	101	101	
Number absent	0	0	0	2	1	5	1	3	4		0	0	
Number discontinued	3	7	10	10	10	10	10	10	11		12	12	

Maximum Elicited Response During Induction
All Subjects Completing Induction (N=102)

Response	n(%) Subjects
-	102 (100.0%)

(*) when required

Key to Symbols:
- = No reaction ? = Minimal or doubtful response, slightly / different from surrounding normal skin
+ = Definite erythema, no edema ++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50%

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APPENDIX II

DATA LISTINGS

Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
001	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
002	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
003	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
004	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
005	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
006	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
007	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
008	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
009	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
010	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
011	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
012	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
013	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
014	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
015	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
016	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
017	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
018	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
019	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
020	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
021	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
022	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
023	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
024	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
025	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
026	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
027	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
028	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
029	12/28/09	12/28/09	--	01/08/10	13	L	12
030	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
031	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
032	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
033	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
034	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
035	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
036	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
037	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
038	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
039	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
040	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
041	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
042	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
043	12/28/09	12/28/09	--	01/04/10	I1	L	8
044	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
045	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
046	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
047	12/28/09	12/28/09	--	01/04/10	I1	L	8
048	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
049	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
050	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
051	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
052	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
053	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
054	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
055	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
056	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
057	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
058	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
059	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
060	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
061	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
062	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
063	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
064	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
065	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
066	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
067	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
068	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
069	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
070	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
071	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
072	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
073	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
074	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
075	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
076	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
077	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
078	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
079	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
080	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
081	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
082	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
083	12/28/09	12/28/09	--	02/01/10	I9	S	36
084	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
085	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
086	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
087	12/28/09	12/28/09	02/01/10	02/04/10	C1	L	39
088	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
089	12/28/09	12/28/09	--	01/18/10	I8	L	22
090	12/28/09	12/28/09	--	01/13/10	I5	L	17
091	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
092	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
093	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
094	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
095	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
096	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
097	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
098	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
099	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
100	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
101	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
102	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
103	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
104	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
105	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
106	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
107	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
108	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
109	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
110	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
111	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39
112	12/28/09	12/28/09	02/01/10	02/04/10	C	C	39

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
001	70.7	Male	Caucasian
002	54.7	Female	Caucasian
003	67.4	Male	Caucasian
004	70.1	Male	Caucasian
005	63.0	Female	Caucasian
006	63.2	Female	Black
007	61.3	Female	Black
008	67.3	Female	Caucasian
009	55.6	Male	Black
010	49.2	Female	Caucasian
011	52.0	Female	Caucasian
012	66.9	Female	Caucasian
013	61.1	Female	Caucasian
014	22.3	Female	Caucasian
015	69.8	Female	Caucasian
016	38.6	Female	Caucasian
017	66.7	Male	Caucasian
018	33.9	Female	Black
019	52.6	Female	Caucasian
020	50.6	Female	Caucasian
021	43.3	Female	Hispanic
022	36.0	Male	Hispanic
023	52.8	Female	Caucasian
024	54.2	Male	Caucasian
025	55.3	Female	Caucasian
026	46.3	Female	Caucasian
027	48.7	Female	Hispanic
028	65.3	Female	Caucasian
029	49.3	Female	Caucasian
030	46.3	Female	Caucasian
031	42.4	Female	Caucasian
032	56.6	Male	Caucasian
033	55.7	Male	Hispanic
034	65.9	Female	Caucasian
035	51.8	Female	Caucasian
036	56.2	Male	Caucasian
037	50.3	Male	Caucasian

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
038	49.5	Female	Caucasian
039	53.5	Male	Caucasian
040	60.8	Female	Caucasian
041	58.3	Female	Caucasian
042	40.3	Female	Caucasian
043	45.0	Female	Hispanic
044	68.1	Female	Caucasian
045	52.8	Female	Caucasian
046	48.8	Female	Caucasian
047	27.1	Female	Caucasian
048	26.4	Female	Caucasian
049	40.4	Female	Caucasian
050	54.2	Male	Caucasian
051	67.6	Female	Caucasian
052	67.6	Male	Caucasian
053	65.1	Female	Caucasian
054	68.0	Male	Caucasian
055	52.5	Male	Black
056	70.9	Female	Caucasian
057	59.0	Male	Caucasian
058	63.1	Female	Caucasian
059	61.1	Female	Caucasian
060	44.8	Female	Hispanic
061	64.2	Female	Caucasian
062	43.0	Female	Caucasian
063	58.7	Female	Caucasian
064	52.1	Female	Caucasian
065	55.9	Female	Caucasian
066	58.5	Female	Hispanic
067	36.4	Female	Asian
068	55.5	Female	Black
069	63.2	Female	Caucasian
070	67.1	Male	Caucasian
071	59.6	Female	Caucasian
072	47.6	Female	Caucasian
073	48.7	Female	Caucasian
074	37.4	Female	Caucasian

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
075	19.6	Male	Caucasian
076	45.9	Female	Caucasian
077	47.7	Female	Caucasian
078	53.8	Male	Caucasian
079	19.9	Female	Caucasian
080	25.6	Female	Caucasian
081	38.4	Female	Caucasian
082	36.7	Female	Caucasian
083	40.5	Female	Caucasian
084	48.5	Female	Black
085	63.7	Male	Hispanic
086	46.8	Female	Hispanic
087	50.8	Female	Black
088	22.5	Male	Caucasian
089	54.3	Female	Black
090	49.5	Female	Caucasian
091	44.4	Female	Caucasian
092	31.4	Male	Caucasian
093	54.4	Female	Black
094	40.8	Female	Hispanic
095	67.0	Male	Caucasian
096	33.2	Female	Hispanic
097	50.5	Male	Hispanic
098	53.6	Female	Caucasian
099	58.2	Female	Caucasian
100	39.7	Female	Caucasian
101	66.7	Female	Caucasian
102	49.7	Female	Hispanic
103	18.5	Male	Caucasian
104	31.5	Female	Black
105	50.2	Female	Caucasian
106	22.5	Female	Caucasian
107	28.9	Female	Hispanic
108	61.2	Female	Caucasian
109	42.7	Female	Caucasian
110	24.6	Female	Caucasian
111	45.5	Female	Caucasian
112	55.8	Female	BI-RACIAL

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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
001	-	-	-	-	-	-	-	-	-		-	-	
002	-	-	-	-	-	-	-	-	-		-	-	
003	-	-	-	-	-	-	-	-	-		-	-	
004	-	-	-	-	-	-	-	-	-		-	-	
005	-	-	-	-	-	-	-	-	-		-	-	
006	-	-	-	-	-	-	-	-	-		-	-	
007	-	-	-	-	-	-	-	-	-		-	-	
008	-	-	-	-	-	-	-	-	-		-	-	
009	-	-	-	-	X	-	-	-	-	-	-	-	
010	-	-	-	-	-	-	-	-	-		-	-	
011	-	-	-	-	-	-	-	-	-		-	-	
012	-	-	-	-	-	-	-	-	-		-	-	
013	-	-	-	-	-	-	-	-	-		-	-	
014	-	-	-	-	X	-	-	-	-	-	-	-	
015	-	-	-	-	-	-	-	-	-		-	-	
016	-	-	-	-	-	-	-	-	-		-	-	
017	-	-	-	-	-	-	-	-	-		-	-	
018	-	-	-	-	-	-	-	-	N9G		-	-	
019	-	-	-	-	-	-	-	-	-		-	-	
020	-	-	-	-	-	-	-	-	-		-	-	
021	-	-	X	-	-	-	-	-	-	-	-	-	
022	-	-	-	X	-	-	-	-	-	-	-	-	
023	-	-	-	-	-	-	-	-	-		-	-	

Key to Symbols:
- = No reaction ? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema ++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
N9G = No ninth grading NA = Not applied NP = Not patched due to reaction achieved
X = Reading not performed due to missed visit or subject discontinuation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50% NR=Data not recorded
MU = Make-up reading for missed induction visit

(*) When required
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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
024	-	-	-	-	-	-	-	-	-		-	-	
025	-	-	-	-	-	-	-	-	-		-	-	
026	-	-	-	-	-	-	-	-	-		-	-	
027	-	-	-	-	-	-	-	-	-		-	-	
028	-	-	-	-	-	-	-	-	-		-	-	
029	-	-	-	X	X	X	X	X	X		X	X	
030	-	-	-	-	-	-	-	-	-		-	-	
031	-	-	-	-	-	-	-	-	-		-	-	
032	-	-	-	-	-	-	-	-	-		-	-	
033	-	-	-	-	-	X	-	-	-	-	-	-	
034	-	-	-	-	-	-	-	X	-	-	-	-	
035	-	-	-	-	-	-	-	-	-		-	-	
036	-	-	-	-	-	-	-	-	-		-	-	
037	-	-	-	-	-	-	-	-	-		-	-	
038	-	-	-	-	-	-	-	-	-		-	-	
039	-	-	-	-	-	-	-	-	-		-	-	
040	-	-	-	-	-	-	-	-	-		-	-	
041	-	-	-	-	-	-	-	-	-		-	-	
042	-	-	-	-	-	-	-	-	-		-	-	
043	-	X	X	X	X	X	X	X	X		X	X	
044	-	-	-	-	-	-	-	-	-		-	-	
045	-	-	-	-	-	-	-	-	-		-	-	
046	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
Generated on 02/08/10:11:13 by DETAIL.SAS/USES: RESPONSE, PRODLIST

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Cballenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72br	96br(*)
047	-	X	X	X	X	X	X	X	X		X	X	
048	-	-	-	-	-	-	-	-	-		-	-	
049	-	-	-	-	-	X	-	-	-	-	-	-	
050	-	-	-	-	-	-	X	-	-	-	-	-	
051	-	-	-	-	-	-	-	-	-		-	-	
052	-	-	-	-	-	-	-	-	-		-	-	
053	-	-	-	-	-	-	-	-	-		-	-	
054	-	-	-	-	-	-	-	-	-		-	-	
055	-	-	-	-	-	-	-	-	-		-	-	
056	-	-	-	-	-	-	-	-	-		-	-	
057	-	-	-	-	-	-	-	-	-		-	-	
058	-	-	-	-	-	-	-	-	-		-	-	
059	-	-	-	-	-	-	-	-	-		-	-	
060	-	-	-	-	-	-	-	-	-		-	-	
061	-	-	-	-	-	-	-	-	-		-	-	
062	-	-	-	-	-	-	-	-	-		-	-	
063	-	-	-	-	-	-	-	-	-		-	-	
064	-	-	-	-	-	-	-	-	-		-	-	
065	-	-	-	-	-	-	-	-	-		-	-	
066	-	-	-	-	-	-	-	-	-		-	-	
067	-	-	-	-	-	-	-	-	-		-	-	
068	-	-	-	-	-	-	-	-	-		-	-	
069	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
Generated on 02/08/10:11:13 by DETAIL.SAS/USES: RESPONSE, PRODLIST

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
070	-	-	-	-	-	-	-	-	-		-	-	
071	-	-	-	-	-	-	X	-	-	-	-	-	
072	-	-	-	-	-	-	-	-	-		-	-	
073	-	-	-	-	-	-	-	-	-		-	-	
074	-	-	-	-	-	-	-	-	-		-	-	
075	-	-	-	-	-	-	-	-	-		-	-	
076	-	-	-	-	-	-	-	-	-		-	-	
077	-	-	-	-	-	-	-	-	-		-	-	
078	-	-	-	-	-	-	-	-	N9G		-	-	
079	-	-	X	-	-	-	-	-	-	-	-	-	
080	-	-	-	-	-	-	-	-	-		-	-	
081	-	-	-	-	-	-	-	X	-	-	-	-	
082	-	-	-	-	-	-	-	-	-		-	-	
083	-	-	-	-	-	-	-	-	-		X	X	
084	-	-	-	-	-	-	-	-	-		-	-	
085	-	-	-	-	-	-	-	-	-		-	-	
086	-	-	-	-	-	-	-	-	-		-	-	
087	-	-	-	-	-	-	-	-	-		-	X	
088	-	-	-	X	-	-	-	-	-	-	-	-	
089	-	-	-	-	-	-	X	-	X		X	X	
090	-	-	-	-	-	X	X	X	X		X	X	
091	-	-	-	-	-	-	-	-	-		-	-	
092	-	-	X	-	-	-	-	-	-	-	-	-	

(*) When required
Generated on 02/08/10:11:13 by DETAIL.SAS/USES: RESPONSE, PRODLIST

Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
093	-	-	-	-	-	-	-	-	-		-	-	
094	-	-	-	-	-	-	-	-	-		-	-	
095	-	-	-	-	X	-	-	-	-	-	-	-	
096	-	-	-	-	-	-	-	-	-		-	-	
097	-	-	-	-	-	-	-	-	-		-	-	
098	-	-	X	-	-	-	-	-	-	-	-	-	
099	-	-	-	-	-	-	-	-	-		-	-	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	X	-	-	-	-	
103	-	-	X	-	-	-	-	-	-	-	-	-	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	-	-	-	-	-	-	-	N9G		-	-	
109	-	-	-	-	X	-	-	-	-	-	-	-	
110	-	-	-	-	X	-	-	-	-	-	-	-	
111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	-	-	X	-	-	-	-	-	-	-	

(*) When required
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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
001	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
002	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
003	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
004	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
005	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
006	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
007	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
008	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
009	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
010	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
011	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
012	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
013	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
014	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
015	01/04/10	01/04/10	--	01/06/10	I1	S	3
016	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
017	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
018	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
019	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
020	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
021	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
022	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
023	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
024	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
025	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
026	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
027	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
028	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
029	01/04/10	01/04/10	--	01/27/10	I8	S	24
030	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
031	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
032	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
033	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
034	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
035	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
036	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
037	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
038	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
039	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
040	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
041	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
042	01/04/10	01/04/10	--	01/08/10	I1	S	5
043	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
044	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
045	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
046	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
047	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
048	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
049	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
050	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
051	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
052	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
053	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
054	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
055	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
056	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
057	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
058	01/04/10	01/04/10	--	01/06/10	I0	S	3
059	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
060	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
061	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
062	01/04/10	01/04/10	--	01/06/10	I0	S	3

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
063	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
064	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
065	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
066	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
067	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
068	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
069	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
070	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
071	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
072	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
073	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
074	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
075	01/04/10	01/04/10	--	01/08/10	I2	S	5
076	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
077	01/04/10	01/04/10	--	01/13/10	I2	L	10
078	01/04/10	01/04/10	--	01/13/10	I2	L	10
079	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
080	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
081	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
082	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
083	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
084	01/04/10	01/04/10	--	01/08/10	I1	S	5
085	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
086	01/04/10	01/04/10	--	01/06/10	I0	S	3
087	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
088	01/04/10	01/04/10	--	02/08/10	I9	L	36
089	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
090	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
091	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
092	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
093	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 1: Subject Enrollment and Disposition

	Study Dates						
Subject No.	Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days in Study
094	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
095	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
096	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
097	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
098	01/04/10	01/04/10	--	01/11/10	I1	L	8
099	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
100	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
101	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
102	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
103	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
104	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
105	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
106	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
107	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
108	01/04/10	01/04/10	02/08/10	02/12/10	C	C	40
109	01/06/10	01/06/10	02/08/10	02/12/10	C	C	38
110	01/06/10	01/06/10	02/08/10	02/12/10	C	C	38
111	01/06/10	01/06/10	02/08/10	02/12/10	C	C	38
112	01/06/10	01/06/10	02/08/10	02/12/10	C	C	38
113	01/06/10	01/06/10	02/08/10	02/12/10	C	C	38

Key:
Last Reading # (I=Induction Phase, C=Challenge Phase)
Completion Status (C=Completed, L=Lost to follow-up, S=Voluntary withdrawal, V=Protocol violation, AE=Adverse event, O=Other)

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
001	45.7	Female	Caucasian
002	53.1	Male	Caucasian
003	53.7	Female	Caucasian
004	44.7	Female	Caucasian
005	65.5	Male	Caucasian
006	31.3	Female	Hispanic
007	38.7	Female	Caucasian
008	68.1	Male	Caucasian
009	57.0	Female	Caucasian
010	70.1	Male	Caucasian
011	60.2	Male	Caucasian
012	55.8	Female	Black
013	50.6	Male	Black
014	67.0	Female	Caucasian
015	45.4	Female	Caucasian
016	55.1	Female	Black
017	43.2	Female	Black
018	56.3	Male	Black
019	28.8	Male	Caucasian
020	53.4	Female	Caucasian
021	35.5	Male	Caucasian
022	67.0	Male	Caucasian
023	49.1	Female	Caucasian
024	53.6	Male	Caucasian
025	57.5	Female	Caucasian
026	51.0	Female	Caucasian
027	22.6	Female	Caucasian
028	57.2	Male	Black
029	53.8	Female	Caucasian
030	58.7	Female	Caucasian
031	45.7	Male	Caucasian
032	45.6	Female	Caucasian
033	24.6	Female	Hispanic
034	52.3	Female	Caucasian
035	67.7	Female	Caucasian
036	66.1	Female	Caucasian
037	45.5	Female	Black

Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
038	30.9	Male	Caucasian
039	31.0	Female	Caucasian
040	53.1	Female	Caucasian
041	54.7	Male	Caucasian
042	36.4	Female	Hispanic
043	59.6	Female	Caucasian
044	53.9	Female	Caucasian
045	53.0	Female	Caucasian
046	62.7	Female	Caucasian
047	52.4	Female	Caucasian
048	47.8	Female	Caucasian
049	47.6	Male	Caucasian
050	21.3	Male	Caucasian
051	56.0	Female	Caucasian
052	57.4	Female	Caucasian
053	39.8	Female	Caucasian
054	43.7	Female	Hispanic
055	62.0	Female	Caucasian
056	57.1	Female	Caucasian
057	54.8	Male	Caucasian
058	64.2	Male	Hispanic
059	47.6	Female	Caucasian
060	38.1	Male	Caucasian
061	49.3	Female	Caucasian
062	59.1	Female	Caucasian
063	60.0	Female	Caucasian
064	42.4	Female	Caucasian
065	46.3	Male	Black
066	56.1	Female	Caucasian
067	65.4	Female	Caucasian
068	64.2	Female	Caucasian
069	48.8	Female	Caucasian
070	45.3	Female	Asian
071	52.0	Female	Caucasian
072	22.1	Female	Caucasian
073	52.5	Female	Caucasian
074	61.9	Female	Caucasian

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Data Listing 2: Subject Demographics

Subject No.	Age	Gender	Race
075	32.0	Female	Caucasian
076	56.6	Female	Caucasian
077	26.8	Female	Hispanic
078	27.7	Male	Hispanic
079	53.9	Male	Caucasian
080	58.7	Female	Caucasian
081	62.2	Male	Caucasian
082	36.8	Male	Asian
083	63.0	Female	Caucasian
084	22.1	Male	Caucasian
085	49.9	Female	Caucasian
086	38.2	Female	Asian
087	19.8	Female	Hispanic
088	21.7	Male	Hispanic
089	24.7	Female	Caucasian
090	44.6	Female	Caucasian
091	23.2	Female	Black
092	33.7	Male	Caucasian
093	49.4	Female	Caucasian
094	31.9	Female	Caucasian
095	53.2	Female	Caucasian
096	62.9	Female	Caucasian
097	27.4	Male	Caucasian
098	33.2	Female	Hispanic
099	57.8	Female	Caucasian
100	59.1	Male	Caucasian
101	56.4	Female	Caucasian
102	64.1	Male	Caucasian
103	46.2	Male	Caucasian
104	46.1	Female	Caucasian
105	25.7	Female	Hispanic
106	27.9	Male	Hispanic
107	50.5	Female	Caucasian
108	51.9	Female	Caucasian
109	46.4	Female	Caucasian
110	30.2	Male	Hispanic
111	27.8	Male	Hispanic
112	44.9	Female	Caucasian
113	49.7	Female	Asian

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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	72hr	96hr	120hr(*)
001	-	-	-	-	-	-	-	-	-		-	-	
002	-	-	-	-	-	X	-	-	-	-	-	-	
003	-	-	-	-	-	-	-	-	-		-	-	
004	-	-	-	-	-	-	-	-	-		-	-	
005	-	-	-	-	-	-	-	-	-		-	-	
006	-	-	-	-	-	X	-	-	-	-	-	-	
007	-	-	-	-	-	-	-	-	-		-	-	
008	-	-	-	-	-	-	-	-	-		-	-	
009	-	-	-	-	-	-	-	-	-		-	-	
010	-	-	-	-	-	-	-	-	-		-	-	
011	-	-	-	-	-	-	-	-	-		-	-	
012	-	-	-	-	-	-	-	-	-		-	-	
013	-	-	-	-	-	-	-	-	-		-	-	
014	-	-	-	-	-	-	-	-	-		-	-	
015	-	X	X	X	X	X	X	X	X		X	X	
016	-	-	-	-	-	-	-	-	-		-	-	
017	-	-	-	-	-	-	-	-	-		-	-	
018	-	-	-	-	-	-	-	-	-		-	-	
019	-	-	-	-	-	-	-	-	-		-	-	
020	-	-	-	-	-	-	-	-	-		-	-	
021	-	-	-	-	-	-	-	-	-		-	-	
022	-	-	-	-	-	-	-	-	-		-	-	
023	-	-	-	-	-	-	-	X	-	-	-	-	

Key to Symbols:
- = No reaction ? = Minimal or doubtful response, slightly different from surrounding normal skin
+ = Definite erythema, no edema ++ = Definite erythema, definite edema
+++ = Definite erythema, definite edema and vesiculation
N9G = No ninth grading NA = Not applied NP = Not patched due to reaction achieved
X = Reading not performed due to missed visit or subject discontinuation
D = Damage to epidermis: oozing, crusting and/or superficial erosions
p = Papular response >50% NR=Data not recorded
MU = Make-up reading for missed induction visit

(*) When required
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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	72hr	96hr	120hr(*)
024	-	-	-	-	-	-	-	-	-		-	-	
025	-	-	-	-	-	-	-	-	-		-	-	
026	-	-	-	-	-	-	-	-	-		-	-	
027	-	-	-	-	-	-	-	X	-	-	-	-	
028	-	-	-	-	-	-	-	-	-		-	-	
029	-	-	-	-	-	-	-	-	X		X	X	
030	-	-	-	-	-	-	-	-	-		-	-	
031	-	-	-	-	-	-	-	-	-		-	-	
032	-	-	-	-	X	-	-	-	-	-	-	-	
033	-	-	-	-	-	-	-	-	-		-	-	
034	-	-	-	-	-	-	-	-	-		-	-	
035	-	-	-	-	-	-	-	-	-		-	-	
036	-	-	-	-	-	X	-	-	-	-	-	-	
037	-	-	-	-	-	-	-	-	-		-	-	
038	-	-	-	-	-	-	-	-	-		-	-	
039	-	-	-	-	-	-	-	-	-		-	-	
040	-	-	-	-	-	-	-	X	-	-	-	-	
041	-	-	-	-	-	-	-	-	-		-	-	
042	-	X	X	X	X	X	X	X	X		X	X	
043	-	-	-	-	-	-	-	-	-		-	-	
044	-	-	-	-	-	-	-	-	-		-	-	
045	-	-	-	-	-	-	-	-	-		-	-	
046	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	72hr	96hr	120hr(*)
047	-	-	-	-	-	-	-	-	-		-	-	
048	-	-	-	-	-	-	-	-	-		-	-	
049	-	-	-	-	-	-	-	-	-		-	-	
050	-	-	-	-	-	-	-	-	-		-	-	
051	-	-	-	-	-	-	-	-	-		-	-	
052	-	-	-	-	-	-	-	-	-		-	-	
053	-	-	-	-	-	-	-	-	-		-	-	
054	-	-	-	X	-	-	-	-	-	-	-	-	
055	-	-	-	-	-	-	-	-	-		-	-	
056	-	-	-	-	-	-	-	-	-		-	-	
057	-	-	-	-	-	-	-	-	-		-	-	
058	X	X	X	X	X	X	X	X	X		X	X	
059	-	-	-	-	-	-	-	-	-		-	-	
060	-	-	-	-	-	-	-	-	-		-	-	
061	-	-	-	-	-	-	-	-	-		-	-	
062	X	X	X	X	X	X	X	X	X		X	X	
063	-	-	-	-	-	-	-	-	-		-	-	
064	-	-	-	-	-	-	-	-	-		-	-	
065	-	-	-	-	-	-	-	-	-		-	-	
066	-	-	-	-	-	-	-	-	-		-	-	
067	-	-	-	-	-	-	-	-	-		-	-	
068	-	-	-	-	-	-	-	-	-		-	-	
069	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	72hr	96hr	120hr(*)
070	-	-	-	-	-	-	-	-	-		-	-	
071	-	-	-	-	-	-	-	-	-		-	-	
072	-	-	-	-	-	-	-	-	-		-	-	
073	-	-	-	-	-	-	-	-	-		-	-	
074	-	-	-	-	-	-	-	-	-		-	-	
075	-	-	X	X	X	X	X	X	X		X	X	
076	-	-	-	-	-	-	-	-	-		-	-	
077	-	-	X	X	X	X	X	X	X		X	X	
078	-	-	X	X	X	X	X	X	X		X	X	
079	-	-	-	-	-	-	-	-	N9G		-	-	
080	-	-	-	-	-	X	-	-	-	-	-	-	
081	-	-	-	-	-	-	-	-	-		-	-	
082	-	-	-	-	-	-	-	-	-		-	-	
083	-	-	-	-	-	-	-	-	-		-	-	
084	-	X	X	X	X	X	X	X	X		X	X	
085	-	-	-	-	-	-	-	-	-		-	-	
086	X	X	X	X	X	X	X	X	X		X	X	
087	-	-	-	-	-	-	-	-	-		-	-	
088	-	-	-	-	-	X	-	-	-	-	X	X	
089	-	-	-	-	-	-	-	-	-		-	-	
090	-	-	-	-	-	-	-	-	N9G	-	-	-	
091	-	-	-	-	-	-	-	-	-		-	-	
092	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
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Data Listing 3: Dermatologic Response Grades
By Product and Subject

Product = 740017 15 DT036360

Subject No.	Induction Reading										Challenge Phase		
	1	2	3	4	5	6	7	8	9	MU	72hr	96hr	120hr(*)
093	-	-	-	-	-	-	-	-	-		-	-	
094	-	-	-	-	-	-	-	-	-		-	-	
095	-	-	-	-	-	-	-	-	-		-	-	
096	-	-	-	-	-	-	-	-	-		-	-	
097	-	-	-	X	-	-	-	-	-	-	-	-	
098	-	X	X	X	X	X	X	X	X		X	X	
099	-	-	-	-	-	-	-	-	-		-	-	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	-	-		-	-	
103	-	-	-	-	-	-	-	-	-		-	-	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	-	-	-	-	N9G		-	-	
106	-	-	-	-	-	-	-	-	N9G		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
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111	-	-	-	-	-	-	-	-	-		-	-	
112	-	-	-	-	-	-	-	-	-		-	-	
113	-	-	-	-	-	-	-	-	-		-	-	

(*) When required
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APPENDIX III

INFORMED CONSENT DOCUMENTS

TKL RESEARCH, INC. 4 FOREST AVENUE PARAMUS, NJ 07652

INFORMED CONSENT

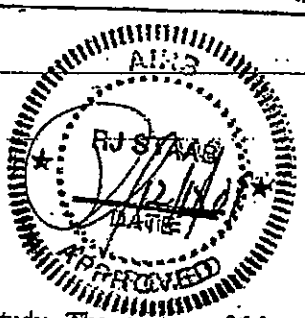
REPEATED INSULT PATCH STUDY

STUDY NO.: DS 111309

Study Doctor: Jonathan S. Dosik
TKL Research Incorporated
4 Forest Avenue, Paramus, New Jersey 07652

Telephone Number: 201-587-0500

After Office Hours: 201-587-0505



PURPOSE

You are invited to participate in this RIPT (Repeated Insult Patch Test) research study. The purpose of this research is to determine if the study materials being tested can be applied to human skin without causing an allergic reaction. The study will involve a minimum of 100 participants.

STUDY MATERIALS

The study materials being tested include or may be components of a liquid gas, adhesive, topical liquid, cosmetics, skin care products, shampoos, shower gel/body wash, antiperspirants/deodorants, fragrances, soaps, sunscreens, chapstick, fibers, insect repellents, antimicrobials, and/or any other materials which are intended for and/or may come into contact with human skin. Also included is SLS (sodium lauryl sulfate, a soap solution) which is used as a positive control (an agent that will cause a reaction).

The cosmetic/inactive ingredients of the study materials are commonly used in industry for topical products and have history of safe use in our product portfolio. The active ingredients of the study materials are approved at the respective concentrations per the US FDA monograph for topical liquid actives.

STUDY DURATION

This study consists of 13 visits (14 visits, if required) over 6 weeks, most visits lasting approximately 10 minutes. You will receive a schedule of visit dates and instructions.

PROCEDURE

Before you can start the study, the study staff will speak about any questions you may have. You will be asked to read and sign this form stating that you understand the study procedures. The study staff will begin screening you to see if you meet all study entrance requirements. This study consists of three phases, which include Induction, Rest and Challenge which are explained below.

Each patch received during this study will contain one cosmetic study product. However, more than one patch will be applied with several different cosmetic study products (up to 16). The dose of the study product will be about 0.2mL, covering a 2cm by 2cm area. Two of the products will be applied to a 25mm circular area. You will wear the study product and 2cm x 2cm patch(s) as well as the 25mm circular patch(s) on your back.

Induction: The first three weeks of the study are called the induction phase. During the induction phase you will report to TKL Research on Mondays, Wednesdays and Fridays. At each visit study staff will apply up to 16 patches to your back. Each patch will be removed 24 and/or 48 hours after application and new patch(s) will be applied at each visit. Your skin will be examined before any study product is applied. The patch(s) applied on Monday and Wednesday will remain on your back for 24 to 48 hours. Patches applied on Friday will be removed on Saturday if 24 hour patch and 48 hour patch(s) applied on Friday will remain in place for 72 hours until you return to TKL Research on the following Monday for patch removal. At each of these induction visits, a clinical evaluator will examine your back to see if you are reacting to any of the products. If you have a strong reaction at the study site (where the study product is applied), the study product will not be applied to that site, but may be applied to another site. The induction period consists of 10 visits.

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Rest: During week four of the study, you will begin a rest period during which study product will not be applied to your back and you will not have to report to TKL Research. This rest period will last through weeks four and five.

Challenge: After the rest period is over and week six begins (the final week of the study), you will receive the same products applied on a new area of the back. The study products (with patches) will be put on the part of your back that has not received study product before. During this phase of the study, you will have to return to TKL Research for three more visits. The first visit during the challenge phase you will have your back evaluated and identical patches re-applied. You will return to TKL Research 48 hours after initial challenge patch application for patch removal and skin evaluation. Finally you will return to TKL for your final visit, 72 hour after initial challenge patch application, for your final evaluation. If the study doctor/staff determines that it is necessary to make additional evaluations, due to reactions, you will be asked to come back for an additional visit.

If you are a female of childbearing potential (i.e., not surgically sterile or have not experienced menopause), you must agree to prevent pregnancy throughout this study by using at least one form of accepted birth control [e.g., oral/injectable/transdermal contraceptive pill, IUD, condom/diaphragm with spermicide, abstinence (no sexual intercourse)].

If you are breastfeeding a child, you will not be permitted to participate in this study. Pregnancy and breastfeeding are prohibited to prevent any unforeseen risk to an unborn child or breast-feeding child.

HJ STAAB
DATE
APPROVED

SUBJECT REQUIREMENTS

You must agree to make all your scheduled visits to TKL Research. You must not apply products such as creams, lotions and moisturizers on or near the test sites. You must avoid sun exposure or the use of tanning beds on your back (including the rest period). You must agree to refrain from swimming during the course of the study. You must agree to minimize water exposure on the patch area while showering or bathing by taking a low tub bath or frontal shower. You will receive written instructions for this study.

POTENTIAL RISKS

Some of the materials may be irritating under certain conditions but the degree of irritation is not expected to be greater than that described below. Individuals participating in this study may experience side effects such as redness, swelling, itching, cracking, peeling, or in rare cases, small blisters or sores. Reactions usually occur only where the study materials or patch materials (such as the patch tape adhesive) touch the skin. On rare occasions, the reactions may spread beyond the patch. A reaction may result in localized lightening or darkening of the skin, which may persist in an occasional individual. Reactions may be due to either skin irritation or allergy to either study materials or patch materials (e.g., patch tape adhesive). It may be necessary to do additional application (rechallenge) to determine if an allergic reaction has occurred. If you should prove to be allergic, you can expect to react to this material if you encounter it at a later date. Whenever possible, you will be informed as to the identity of the material in order that you may avoid contact with it in the future.

COMPENSATION FOR INJURY

For any significant reactions that may occur as a direct result of your participation in this study, appropriate and reasonable medical treatment will be provided by TKL Research, Inc. at no cost to you to resolve the immediate problem. Provision of such medical care is not an admission of legal liability or responsibility for the condition being treated. If such reactions occur, TKL personnel should be contacted immediately at (201) 587-0505 day, night or weekends. Extended medical care will not be provided.

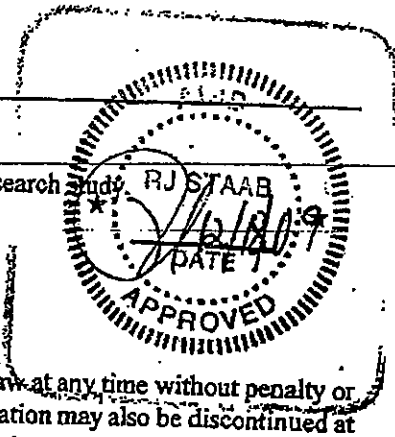
SIGNIFICANT NEW FINDINGS

You will be informed of any significant new findings that may affect your willingness to continue your participation.

INFORMED CONSENT

REPEATED INSULT PATCH STUDY

STUDY NO.: DS 111309



POTENTIAL BENEFITS

There is no personal benefit other than the satisfaction of participation in a clinical research study.

ALTERNATIVE TREATMENT

Since this study is for research only, the only alternative is for you not to participate.

WITHDRAWAL FROM STUDY

Participation in the study is voluntary and you may refuse to participate or may withdraw at any time without penalty or loss of benefits, other than financial, to which you are otherwise entitled. Your participation may also be discontinued at any time without your consent by the study doctor, the Institutional Review Board (IRB) (a committee that reviews studies to help ensure that the rights and welfare of the participants are protected and that the study is carried out in an ethical manner), the Food and Drug Administration (FDA), or the study sponsor(s) (the company(ies) that makes the product(s) being evaluated). If you fail to comply with study procedures, your participation may be terminated.

COST

Your participation in the study will not incur any cost to you.

FINANCIAL INCENTIVE

You will be paid a sum of $115 upon completion of this study. If in the judgment of the investigating personnel, it is best to discontinue your participation in this study due to an adverse experience or severe reaction you will be paid in full for your participation. If you drop out of your own accord for personal reasons beyond your control you will be paid proportionately ($8.85 per visit). If you are dismissed for refusal to obey rules or follow instructions you will not be paid.

CONFIDENTIALITY AND AUTHORIZATION

Reports prepared by TKL Research will utilize statistical information only and at no time will your name be used. A federal regulation called the "Health Insurance Portability and Accountability Act"(HIPAA), which went into effect on April 14, 2003, describes how your personal health information may be used, disclosed and made accessible to you. This privacy rule is designed to protect the confidentiality of your personal health information. The following information describes how the HIPAA rule applies to you and your rights.

This study can be performed only by collecting and using your personal health information. Your study records will be kept as confidential as possible under local, state and federal laws. Personnel from the following organizations may examine your study records: the sponsor, personnel associated with this study, regulatory agencies, such as the Food and Drug Administration (FDA) or Environmental Protection Agency (EPA), and the Allendale Institutional Review Board (IRB), a committee that has reviewed this study to help ensure that your rights and welfare as a research participant are protected and that the study is carried out in an ethical manner. Because of the number of individuals who may see your records, absolute confidentiality cannot be guaranteed.

Personal health information that may be used and disclosed includes that which is obtained to determine your eligibility to participate and that which is collected from the procedures that are carried out. It may identify you by name, address, telephone number, Social Security Number, study number, date of birth or other identifiers. Once the information is disclosed, it is possible that it may be re-disclosed, at which time it is no longer protected by federal regulations but may be by state laws.

If the final study data are prepared for publication and other reports, your identity will not be revealed. Under these federal privacy regulations, you have the right to see and copy any of the information gathered about you, until your study records are no longer kept by the study doctor. However, your records may not be available until the study has been completed.

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REPEATED INSULT PATCH STUDY

STUDY NO.: DS 111309

You may, by written notice to the study doctor, cancel your authorization to use or disclose your personal information at any time. If you withdraw your authorization, the information collected to that time may still be used to preserve the scientific integrity of the study.

By signing this consent form, you authorize these uses and disclosures of your personal information. If you do not authorize these uses and disclosures, you will not be able to participate in the study.

WHO TO CALL

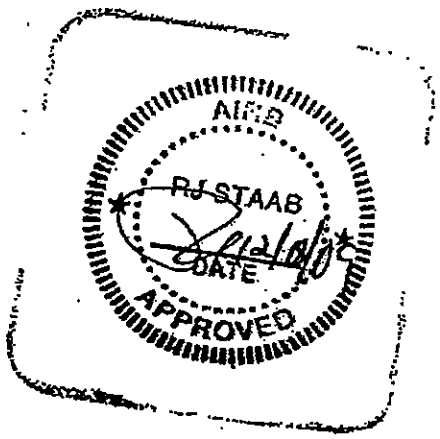
Additional information regarding this research is available either before or during the course of this study. If you have any questions or research related side effect or injury, you may contact Michelle Medina, Asst. Mananger Dermatologic Safety at (201) 587-0505,e xt 2230. You may contact the Allendale Institutional Review Board, Old Lyme, Connecticut, (860)434 5872, if you have a question about your rights as a research subject. Review of this research study by Allendale IRB is not an endorsement of the study or its outcome.

A signed copy of this consent form will be given to you.

I have read the information and description of this research study given in this consent form. I have been informed of the risks and benefits, and all my questions have been answered to my satisfaction. I voluntarily consent to participate. By signing this form I have not given up any of my legal rights which I would otherwise have as a research subject.

ENTRY NO.	PRINT NAME	SIGNATURE	DATE

SIGNATURE OF WITNESS DATE



TKL RESEARCH, INC. 4-FOREST AVENUE PARAMUS, NJ 07652

INFORMED CONSENT

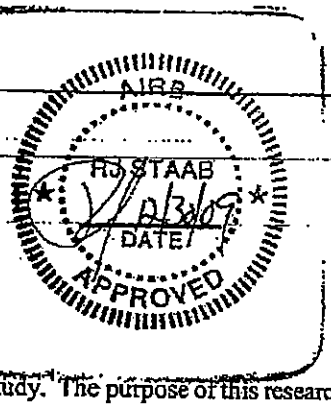
REPEATED INSULT PATCH STUDY

STUDY NO.: DS100310

Study Doctor: Jonathan S. Dosik
TKL Research Incorporated
4 Forest Avenue, Paramus, New Jersey 07652

Telephone Number: 201-587-0500

After Office Hours: 201-587-0505



PURPOSE

You are invited to participate in this RIPT (Repeated Insult Patch Test) research study. The purpose of this research is to determine if the study materials being tested can be applied to human skin without causing an allergic reaction. The study will involve a minimum of 100 participants.

STUDY MATERIALS

The study materials being tested include or may be components of a sunscreen, adhesive, topical liquid, cosmetics, skin care products, shampoos, shower gel/body wash, antiperspirants/deodorants, fragrances, soaps, sunscreens, chapstick, fibers, insect repellents, antimicrobials, and/or any other materials which are intended for and/or may come into contact with human skin. Also included is SLS (sodium lauryl sulfate, a soap solution) which is used as a positive control (an agent that will cause a reaction).

The cosmetic/inactive ingredients of the study materials are commonly used in industry for topical products and have history of safe use in our product portfolio. The active ingredients of the study materials are approved at the respective concentrations per the US FDA monograph for topical liquid actives.

STUDY DURATION

This study consists of 13 visits (14 visits, if required) over 6 weeks, most visits lasting approximately 10 minutes. You will receive a schedule of visit dates and instructions.

PROCEDURE

Before you can start the study, the study staff will speak about any questions you may have. You will be asked to read and sign this form stating that you understand the study procedures. The study staff will begin screening you to see if you meet all study entrance requirements. This study consists of three phases, which include Induction, Rest and Challenge which are explained below.

Each patch received during this study will contain one cosmetic study product. However, more than one patch will be applied with several different cosmetic study products (up to 16). The dose of the study product will be about 0.2mL, covering a 2cm by 2cm area. You will wear the study product and 2cm x 2cm patch(s) on your back.

Induction: The first three weeks of the study are called the induction phase. During the induction phase you will report to TKL Research on Mondays, Wednesdays and Fridays. At each visit study staff will apply up to 16 patches to your back. Each patch will be removed 24 and/or 48 hours after application and new patch(s) will be applied at each visit. Your skin will be examined before any study product is applied. The patch(s) applied on Monday and Wednesday will remain on your back for 24 to 48 hours. Patches applied on Friday will be removed on Saturday if 24 hour patch and 48 hour patch(s) applied on Friday will remain in place for 72 hours until you return to TKL Research on the following Monday for patch removal. At each of these induction visits, a clinical evaluator will examine your back to see if you are reacting to any of the products. If you have a strong reaction at the study site (where the study product is applied), the study product will not be applied to that site, but may be applied to another site. The induction period consists of 10 visits.

TKL RESEARCH, INC. 4 FOREST AVENUE PARAMUS, NJ 07652

INFORMED CONSENT

REPEATED INSULT PATCH STUDY

STUDY NO.: DS100310

Rest: During week four of the study, you will begin a rest period during which study product will not be applied to your back and you will not have to report to TKL Research. This rest period will last through weeks four and five.

Challenge: After the rest period is over and week six begins (the final week of the study), you will receive the same products applied on a new area of the back. The study products (with patches) will be put on the part of your back that has not received study product before. During this phase of the study, you will have to return to TKL Research for three more visits. The first visit during the challenge phase you will have your back evaluated and identical patches re-applied. You will return to TKL Research 48 hours after initial challenge patch application for patch removal and skin evaluation. Finally you will return to TKL for your final visit, 72 hour after initial challenge patch application, for your final evaluation. If the study doctor/staff determines that it is necessary to make additional evaluations, due to reactions, you will be asked to come back for an additional visit.

If you are a female of childbearing potential (i.e., not surgically sterile or have not experienced menopause), you must agree to prevent pregnancy throughout this study by using at least one form of accepted birth control [e.g., oral injectable/transdermal contraceptive pill, IUD, condom/diaphragm with spermicide, abstinence (no sexual intercourse)].

If you are breastfeeding a child, you will not be permitted to participate in this study. Pregnancy and breast-feeding are prohibited to prevent any unforeseen risk to an unborn child or breast-feeding child.

RJSTAAB 12/30/09 DATE APPROVED

SUBJECT REQUIREMENTS

You must agree to make all your scheduled visits to TKL Research. You must not apply products such as creams, lotions and moisturizers on or near the test sites. You must avoid sun exposure or the use of tanning beds on your back (including the rest period). You must agree to refrain from swimming during the course of the study. You must agree to minimize water exposure on the patch area while showering or bathing by taking a low tub bath or frontal shower. You will receive written instructions for this study.

POTENTIAL RISKS

Some of the materials may be irritating under certain conditions but the degree of irritation is not expected to be greater than that described below. Individuals participating in this study may experience side effects such as redness, swelling, itching, cracking, peeling, or in rare cases, small blisters or sores. Reactions usually occur only where the study materials or patch materials (such as the patch tape adhesive) touch the skin. On rare occasions, the reactions may spread beyond the patch. A reaction may result in localized lightening or darkening of the skin, which may persist in an occasional individual. Reactions may be due to either skin irritation or allergy to either study materials or patch materials (e.g., patch tape adhesive). It may be necessary to do additional application (rechallenge) to determine if an allergic reaction has occurred. If you should prove to be allergic, you can expect to react to this material if you encounter it at a later date. Whenever possible, you will be informed as to the identity of the material in order that you may avoid contact with it in the future.

COMPENSATION FOR INJURY

For any significant reactions that may occur as a direct result of your participation in this study, appropriate and reasonable medical treatment will be provided by TKL Research, Inc. at no cost to you to resolve the immediate problem. Provision of such medical care is not an admission of legal liability or responsibility for the condition being treated. If such reactions occur, TKL personnel should be contacted immediately at (201) 587-0505 day, night or weekends. Extended medical care will not be provided.

SIGNIFICANT NEW FINDINGS

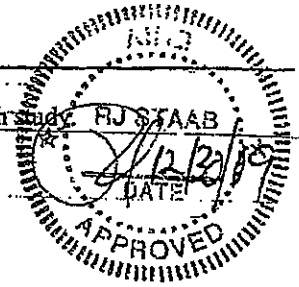
You will be informed of any significant new findings that may affect your willingness to continue your participation.

TKL RESEARCH, INC. 4 FOREST AVENUE PARAMUS, NJ 07652

INFORMED CONSENT

REPEATED INSULT PATCH STUDY

STUDY NO.: DS100310



POTENTIAL BENEFITS

There is no personal benefit other than the satisfaction of participation in a clinical research study.

ALTERNATIVE TREATMENT

Since this study is for research only, the only alternative is for you not to participate.

WITHDRAWAL FROM STUDY

Participation in the study is voluntary and you may refuse to participate or may withdraw at any time without penalty or loss of benefits, other than financial, to which you are otherwise entitled. Your participation may also be discontinued at any time without your consent by the study doctor, the Institutional Review Board (IRB) (a committee that reviews studies to help ensure that the rights and welfare of the participants are protected and that the study is carried out in an ethical manner), the Food and Drug Administration (FDA), or the study sponsor(s) (the company(ies) that makes the product(s) being evaluated). If you fail to comply with study procedures, your participation may be terminated.

COST

Your participation in the study will not incur any cost to you.

FINANCIAL INCENTIVE

You will be paid a sum of $115 upon completion of this study. If in the judgment of the investigating personnel, it is best to discontinue your participation in this study due to an adverse experience or severe reaction you will be paid in full for your participation. If you drop out of your own accord for personal reasons beyond your control you will be paid proportionately ($8.21 per visit). If you are dismissed for refusal to obey rules or follow instructions you will not be paid.

CONFIDENTIALITY AND AUTHORIZATION

Reports prepared by TKL Research will utilize statistical information only and at no time will your name be used. A federal regulation called the "Health Insurance Portability and Accountability Act"(HIPAA), which went into effect on April 14, 2003, describes how your personal health information may be used, disclosed and made accessible to you. This privacy rule is designed to protect the confidentiality of your personal health information. The following information describes how the HIPAA rule applies to you and your rights.

This study can be performed only by collecting and using your personal health information. Your study records will be kept as confidential as possible under local, state and federal laws. Personnel from the following organizations may examine your study records: the sponsor, personnel associated with this study, regulatory agencies, such as the Food and Drug Administration (FDA) or Environmental Protection Agency (EPA), and the Allendale Institutional Review Board (IRB), a committee that has reviewed this study to help ensure that your rights and welfare as a research participant are protected and that the study is carried out in an ethical manner. Because of the number of individuals who may see your records, absolute confidentiality cannot be guaranteed.

Personal health information that may be used and disclosed includes that which is obtained to determine your eligibility to participate and that which is collected from the procedures that are carried out. It may identify you by name, address, telephone number, Social Security Number, study number, date of birth or other identifiers. Once the information is disclosed, it is possible that it may be re-disclosed, at which time it is no longer protected by federal regulations but may be by state laws.

If the final study data are prepared for publication and other reports, your identity will not be revealed. Under these federal privacy regulations, you have the right to see and copy any of the information gathered about you, until your study records are no longer kept by the study doctor. However, your records may not be available until the study has been completed. There is no expiration date for this authorization.

TKL RESEARCH, INC. 4 FOREST AVENUE PARAMUS, NJ 07652

INFORMED CONSENT

REPEATED INSULT PATCH STUDY

STUDY NO.: DS100310

You may, by written notice to the study doctor, cancel your authorization to use or disclose your personal information at any time. If you withdraw your authorization, the information collected to that time may still be used to preserve the scientific integrity of the study.

By signing this consent form, you authorize these uses and disclosures of your personal information. If you do not authorize these uses and disclosures, you will not be able to participate in the study.

WHO TO CALL

Additional information regarding this research is available either before or during the course of this study. If you have any questions or research related side effect or injury, you may contact Michelle Medina, Asst. Mananger Dermatologic Safety at (201) 587-0505,e xt 2230. You may contact the Allendale Institutional Review Board, Old Lyme, Connecticut, (860)434 5872, if you have a question about your rights as a research subject. Review of this research study by Allendale IRB is not an endorsement of the study or its outcome.

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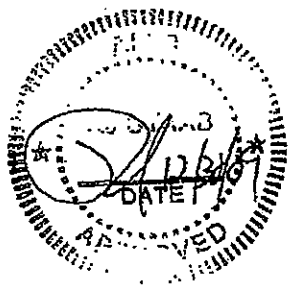
ENTRY NO. PRINT NAME SIGNATURE DATE

SIGNATURE OF WITNESS DATE



Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 24, 2010

SUBJECT: HRIPTs on Products Containing C12-15 Alkyl Benzoate

Clinical Research Laboratories, Inc. 2008. Repeated insult patch test of a concealer containing 4.2% C12-15 Alkyl Benzoate. CRL Study Number: CRL38208-4.

Consumer Product Testing Co. 2010. Repeated insult patch test of a hand creme containing 0.07398% C12-15 Alkyl Benzoate. Experiment Reference Number: C10-0807-04.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Clinical
Research
Laboratories, Inc.

Final Report

Repeated Insult Patch Test

CLIENT:

ATTENTION: containing 4.2% C12-15 Alkyl Benzoate

TEST MATERIAL: **Concealer MU31-84-1,**

CRL STUDY NUMBER: **CRL38208-4**

AUTHORIZED SIGNATURES:

Bruce E. Kanengiser, M.D.
President/Medical Director

Michael J. Muscatiello, Ph.D.
Executive Vice President/COO

George J. Neumaier, M.D.
Diplomate American Board of Dermatology

REPORT DATE: **May 30, 2008**

371 Hoes Lane • Piscataway, NJ 08854 • (732) 981-1616 • FAX (732) 981-0520

Clinical Research Laboratories, Inc.

Good Clinical Practice
Quality Assurance Audit Statement

Clinical Study Number: CRL38208-4

Start Date: March 31, 2008

Completion Date: May 9, 2008

The clinical study listed above was conducted in accordance with Clinical Research Laboratories, Inc. Standard Operating Procedures, which incorporate the principles of Good Clinical Practice defined by applicable guidelines and regulations established by U.S. Regulatory Agencies. The conduct of the study was monitored for compliance, and the associated records, including source documents or raw data, were reviewed for documentation practices and accuracy by a Project Manager/Study Director and/or a Quality Assurance Representative. Standard Quality Assurance audit procedures for this final report and study related documents were conducted, as indicated below.

Signature of QA Auditor

5/30/08
Date

FINAL REPORT

REPEATED INSULT PATCH TEST

PURPOSE

The purpose of this study was to determine the dermal irritation and sensitization potential of a test material.

INVESTIGATIVE SITE

Clinical Research Laboratories, Inc.
371 Hoes Lane
Piscataway, New Jersey 08854
732-981-1616

TEST MATERIAL

The following test material was provided by and was received by Clinical Research Laboratories, Inc. on March 21, 2008:

Test Material	Test Condition	Patch Type
Concealer (1 1	Test as received	Semi-occlusive*

The test material was coded with the following CRL identification number:

CRL38208-4

STUDY DATES

This study was initiated on March 31, 2008 and was completed on May 9, 2008.

* Semi-occlusive Strip (TruMed Technologies Inc., Burnsville, Minnesota)

PANEL SELECTION

Each subject was assigned a permanent CRL identification number. All subjects signed an Informed Consent Form in compliance with 21 CFR Part 50: "Protection of Human Subjects" and a HIPAA Authorization Form in compliance with 45 CFR Parts 160 and 164. All subjects completed a Subject Profile/Medical History Form provided by Clinical Research Laboratories, Inc. prior to the study (Subject Demographics - Appendix I). Subjects who met the following criteria were impaneled:

- Male and female panelists between the ages of 18 and 70;
- Subjects who have completed a Panelist Profile/Medical History;
- Subjects who are in general good health as determined by a Panelist Profile/Medical History;
- Subjects who do not exhibit any skin diseases that might be confused with a skin reaction from the test material;
- Subjects willing to sign an Informed Consent Form in conformance with 21 CFR Part 50: "Protection of Human Subjects";
- Subjects who have completed a HIPAA Authorization Form in conformance with 45 CFR Parts 160 and 164;
- Females who are not pregnant or lactating;
- Subjects who demonstrate dependability and intelligence in following directions;
- Subjects who are not currently using any systemic or topical corticosteroids, anti-inflammatory drugs or antihistamines.

TEST METHOD

Prior to the application of the patch, the test area was wiped with 70% isopropyl alcohol and allowed to dry. The test material, which was prepared as described in the Test Material section of the report, was applied to the upper back (between the scapulae) and was allowed to remain in direct skin contact for a period of 24 hours.

TEST METHOD (Continued)

Patches were applied to the same site on Monday, Wednesday, and Friday for a total of 9 applications during the Induction Period. This schedule may have been modified to allow for missed visits or holidays. If a subject was unable to report on an assigned test date, the test material was applied on 2 consecutive days during the Induction Phase and/or a makeup day was added at the end of the Induction Phase.

The sites were graded by a CRL technician for dermal irritation 24 hours after removal of the patches by the subjects on Tuesday and Thursday and 48 hours after removal of the patches on Saturday, unless the patching schedule was altered as described above.

The sites were graded according to the following scoring system:

Dermal Scoring Scale

0 No visible skin reaction
± Barely perceptible erythema
1+ Mild erythema
2+ Well defined erythema
3+ Erythema and edema
4+ Erythema and edema with vesiculation

If a "2+" reaction or greater occurred, the test material was applied to an adjacent virgin site. If a "2+" reaction or greater occurred on the new site, the subject was not patched again during the Induction Phase but was challenged on the appropriate day of the study. At the discretion of the Study Director, patch sites with scores less than a "2+" may have been changed.

Following approximately a 2-week rest period, the challenge patches were applied to previously untreated test sites on the back. After 24 hours, the patches were removed by a CRL technician and the test sites were evaluated for dermal reactions. The test sites were re-evaluated at 48 and 72 hours. Subjects exhibiting reactions during the Challenge Phase of the study may have been asked to return for a 96-hour reading.

RESULTS

This study was initiated with 112 subjects. Four subjects discontinued study participation for reasons unrelated to the test material. A total of 108 subjects completed the study.

Individual dermal scores recorded during the Induction and Challenge Phases appear in Table I.

CONCLUSION

Based on the test population of 108 subjects and under the conditions of this study, the test material identified as [redacted] 78-1.1.1 did not demonstrate a clinically significant potential for eliciting dermal irritation or sensitization.

RETENTION

Test materials and all original forms of this study will be retained by Clinical Research Laboratories, Inc. as specified in CRL Standard Operating Procedures 30.6 and 30.6C, unless designated otherwise by the Sponsor.

TABLE I

Summary of Dermal Scores

Test Material:	Concealer

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	Discontinued											
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	±	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	±	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Concealer

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	±	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Concealer

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	±	0	0	0	0	0
66	0	0	0	0	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	±	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0	0
74	0	Discontinued										
75	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Concealer

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
76	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	±	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0	0
90	Discontinued											
91	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0

TABLE I
(Continued)

Summary of Dermal Scores

Test Material:	Concealer

Subject Number	**Induction Scores**									**Challenge Scores**		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**24 Hour**	**48 Hour**	**72 Hour**
101	Discontinued											
102	0	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	0	0	0	0	0
112	0	0	0	0	0	0	0	0	0	0	0	0

Appendix I

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
1	MB	18220	57	F
2	BF	00054	51	F
3	DC	12376	48	F
4	AS	01584	46	F
5	PG	13984	43	F
6	RP	16060	21	F
7	JT	09713	53	F
8	CL	14668	60	F
9	CK	09605	63	F
10	PK	18678	54	M
11	SB	13858	64	F
12	JM	07179	44	F
13	LA	16749	46	F
14	AM	18842	24	M
15	MP	16022	66	F
16	PA	02727	65	F
17	SM	12510	24	F
18	NS	16253	69	F
19	KH	15618	55	F
20	JR	08854	43	F
21	CM	03444	67	F
22	KC	13388	60	F
23	NF	14449	47	F
24	KM	14519	37	F
25	HS	07067	49	F
26	AC	14858	39	F
27	PM	18953	54	F
28	MM	15443	43	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
29	MP	03505	46	F
30	NK	17220	40	F
31	MB	03650	60	F
32	AS	16567	32	F
33	AA	18956	23	F
34	PN	03277	47	F
35	AR	18777	65	M
36	KM	08600	46	F
37	ED	17217	65	F
38	MH	01132	66	F
39	IA	10338	62	F
40	JP	15332	30	F
41	DW	10884	43	F
42	BL	18894	26	M
43	WW	16548	35	F
44	RH	18256	69	M
45	JC	06977	47	F
46	LL	10701	57	F
47	SH	18975	50	F
48	PM	09260	49	F
49	DM	18979	40	F
50	JV	18978	39	F
51	AC	05637	57	F
52	LA	15065	63	F
53	HP	17132	58	F
54	SC	18974	21	F
55	JB	12170	60	F
56	VJ	04247	62	F

Appendix I
(Continued)

Subject Demographics

Subject Number	Subject Initials	CRL ID #	Age	Sex
57	TP	07612	40	F
58	JW	08904	45	F
59	ET	09473	65	F
60	DP	08438	48	F
61	CS	14389	66	F
62	KT	06277	49	F
63	PM	03457	67	F
64	SA	18981	38	F
65	EW	18412	43	F
66	KW	14133	34	F
67	LF	15023	68	F
68	LL	07031	48	F
69	CS	18814	18	F
70	JK	14141	64	F
71	RK	14142	66	M
72	FA	18719	50	F
73	SS	18791	26	M
74	MC	13494	55	F
75	RB	08241	26	F
76	MV	02437	58	F
77	JL	13249	25	F
78	MP	18840	22	M
79	DS	15209	45	M
80	KC	12260	32	F
81	TR	13698	38	F
82	SI	18989	40	F
83	NP	17123	40	F
84	JG	18387	21	F

Subject Number	Subject Initials	CRL ID #	Age	Sex
85	HJ	18846	38	F
86	NO	01162	57	F
87	TM	13580	26	F
88	MZ	00237	52	F
89	MH	11093	46	F
90	CN	18252	51	F
91	BG	14700	20	F
92	TR	18931	34	F
93	AG	17450	28	F
94	CW	18095	43	F
95	SF	07438	29	F
96	AB	18851	20	F
97	BC	10128	54	F
98	LT	04909	55	F
99	BC	10444	57	F
100	CP	09254	38	F
101	LT	06804	35	F
102	RB	05376	56	M
103	MG	14013	35	F
104	SJ	13519	54	F
105	JB	14759	53	F
106	LF	18999	26	F
107	RS	09790	54	F
108	CN	19001	18	F
109	IV	10514	53	F
110	CT	19004	24	F
111	SN	16751	41	M
112	DH	19006	36	F

Consumer Product Testing Co.

EST. 1975

FINAL REPORT

CLIENT:

ATTENTION: _ist

TEST: Repeated Insult Patch Test
Protocol No.: 1.01

TEST MATERIAL: Hand Crème –
Containing 0.07398 % C12-15 Alkyl Benzoate

EXPERIMENT REFERENCE NUMBER: C10-0807.04

Reviewed by: Richard R. Eisenberg, M.D.
Medical Director
Board Certified Dermatologist

Approved by: Michael Caswell, Ph.D., C.C.R.C., C.C.R.A.
Director, Clinical Evaluations

Approved by: Joy Frank, R.N.
Executive Vice President, Clinical Evaluations

This report is submitted for the exclusive use of the person, partnership, or corporation to whom it is addressed, and neither the report nor the name of these Laboratories nor any member of its staff, may be used in connection with the advertising or sale of any product or process without written authorization.

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234

Consumer Product Testing Co.
EST. 1975

QUALITY ASSURANCE UNIT STATEMENT

Study Number: C10-0807.04

The Consumer Product Testing Company, Incorporated (CPTC) Quality Assurance Unit (QAU) is responsible for monitoring the conduct, content and reporting of all clinical laboratory studies that are conducted at CPTC.

This study has been conducted in accordance with ICH Guideline E6 for *Good Clinical Practice*, the requirements of 21 CFR Parts 50 and 56, other applicable regulations, CPTC Standard Operating Procedures, and the approved Study Protocol.

The CPTC QAU has reviewed all data, records, and documents relating to this study and also this Final Report. The following QAU representative signature certifies that all data, records, and documents relating to this study and also this Final Report have been reviewed and are deemed to be acceptable, and the study conforms to all of the requirements as indicated above.

Quality Assurance Representative

6/6/10
Date

70 New Dutch Lane • Fairfield, New Jersey 07004-2514 • (973) 808-7111 • Fax (973) 808-7234
Clinical • Toxicology • Analytical Chemistry • Microbiology

Objective: To determine by repetitive epidermal contact the potential of a test material to induce primary or cumulative irritation and/or allergic contact sensitization.

Participants: Fifty-eight (58) qualified subjects, male and female, ranging in age from 16 to 76 years, were selected for this evaluation. Forty-nine (49) subjects completed this study. The remaining subjects discontinued their participation for various reasons, none of which were related to the application of the test material.

Inclusion Criteria:

a. Male and female subjects, age 16[a] and over.
b. Absence of any visible skin disease which might be confused with a skin reaction from the test material.
c. Prohibition of use of topical or systemic steroids and/or antihistamines for at least seven days prior to study initiation.
d. Completion of a Medical History form and the understanding and signing of an Informed Consent form.
e. Considered reliable and capable of following directions.

Exclusion Criteria:

a. Ill health.
b. Under a doctor's care or taking medication(s) which could influence the outcome of the study.
c. Females who are pregnant or nursing.
d. A history of adverse reactions to cosmetics or other personal care products.

Test Material: Hand Crème –

Study Schedule:

Panel #	Initiation Date	Completion Date
20100091	February 24, 2010	April 8, 2010

[a]With parental or guardian consent

Methodology:

The upper back between the scapulae served as the treatment area. Approximately 0.2 g of the test material, or an amount sufficient to cover the contact surface, was applied to the 1" x 1" absorbent pad portion of a clear adhesive dressing and allowed to volatilize for several minutes. This was then applied to the appropriate treatment site to form a semi-occlusive patch.

Induction Phase:

Patches were applied three (3) times per week (e.g., Monday, Wednesday, and Friday) for a total of nine (9) applications. The site was marked to ensure the continuity of patch application. Following supervised removal and scoring of the first Induction patch, participants were instructed to remove all subsequent Induction patches at home, twenty-four hours after application. The evaluation of this site was made again just prior to re-application. If a participant was unable to report for an assigned test day, one (1) makeup day was permitted. This day was added to the Induction period. Inclement weather, which occurred within the first induction exposure, prevented many subjects from reporting to the testing facility. They were instructed to remove the patches and report on the following day. Inclement weather occurred again during the Induction Phase. Subjects reported on the next available test day.

With the exception of the first supervised Induction Patch reading, if any test site exhibited a moderate (2-level) reaction during the Induction Phase, application was moved to an adjacent area. Applications were discontinued for the remainder of this test phase, if a moderate (2-level) reaction was observed on this new test site. Applications would also be discontinued if marked (3-level) or severe (4-level) reactivity was noted.

Rest periods consisted of twenty-four hours following each Tuesday and Thursday removal, and forty-eight hours following each Saturday removal.

Challenge Phase:

Approximately two (2) weeks after the final Induction patch application, a Challenge patch was applied to a virgin test site adjacent to the original Induction patch site, following the same procedure described for Induction. The patch was removed and the site scored at the clinic twenty-four and seventy-two hours post-application.

Methodology (continued):

Evaluation Criteria (Erythema and additional Dermal Sequelae):

0	=	No visible skin reaction	E	=	Edema
0.5	=	Barely perceptible	D	=	Dryness
1	=	Mild	S	=	Staining
2	=	Moderate	P	=	Papules
3	=	Marked	V	=	Vesicles
4	=	Severe	B	=	Bullae
			U	=	Ulceration
			Sp	=	Spreading

Erythema was scored numerically according to this key. If present, additional Dermal Sequelae were indicated by the appropriate letter code and a numerical value for severity.

Results:

The results of each participant are appended (Table 1).

Observations remained within normal limits throughout the test interval.

Subject demographics are presented in Table 2.

Summary:

Under the conditions of this study, test material, Hand Crème – , did not indicate a potential for dermal irritation or allergic contact sensitization.

Table 1
Panel #20100091

Individual Results

Hand Crème – I

Subject Number	Induction Phase									Virgin Challenge Site	
	24*hr	1	2	3	4	5	6	7	8	9	24*hr	72 hr
1	0	0[W]	DID NOT COMPLETE STUDY									
2	0	0[W]	0	0	0	0	0	0	0	0	0	0
3	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
4	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
5	-	0[W]	0	0	0	0	0	0	0	0	0	0
6	-	0[W]	0	0	0	0	0	0	0	0	0	0
7	-	0[W]	0	0	0	DID NOT COMPLETE STUDY						
8	0	0[W]	0	0	0	0	0	0	0	0	0	0
9	0	0[W]	0	0	0	0	0	0	0	0	0	0
10	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
11	-	0[W]	0	0	0	0	0	0	0	0	0	0
12	0	0[W]	0	0	0	0	0	0	0	0	0	0
13	0	0[W]	0	0	0	0	0	0	0	0	0	0
14	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
15	-	0[W]	0	0	0	0	0	0	DID NOT COMPLETE STUDY			
16	0	0[W]	0	0	0	0	0	0	0	0	0	0
17	0	0[W]	0	0	0	0	0	0	0	0	0	0
18	-	0[W]	0	0	0	0	0	0	0	0	0	0
19	-	0[W]	0	0	DID NOT COMPLETE STUDY							
20	0	0[W]	0	0	0	0	0	0	0	0	0	0
21	0	0[W]	0	0	0	0	0	0	0	0	0	0
22	0	0[W]	0	0	0	0	0	0	0	0	0	0
23	0	0[W]	0	0	0	0	0	0	0	0	0	0
24	DID NOT COMPLETE STUDY											
25	0	0[W]	0	0	0	0	0	0	0	0	0	0
26	0	0[W]	0	0	0	0	0	0	0	0	0	0
27	-	0[W]	0	0	0	0	0	0	0	0	0	0
28	-	0[W]	0	0	0	0	0	0	0	0	0	0
29	DID NOT COMPLETE STUDY											

24* = Supervised removal of 1st Induction and Challenge Patch
W = Inclement weather. Subject unable to report as scheduled
- = Subject not present for supervised removal

Table 1
(continued)
Panel #20100091

Individual Results

Hand Crème – 2-4

Subject Number	24*hr	Induction Phase 1	2	3	4	5	6	7	8	9	Virgin Challenge Site 24*hr	72 hr
30	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
31	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
32	-	0[W]	0	0	0	0	0	0[W]	0[W]	0	0	0
33	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
34	0	0[W]	0	0	0	0	0	0	0	DNC		
35	-	0[W]	0	0	0	0	0	0	0	0	0	0
36	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
37	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
38	-	0[W]	0	0	0	0	0	0	0	0	0	0
39	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
40	-	0[W]	0	0	0	0	0	0[W]	0	0	0	0
41	-	0.5[W]	0.5	0	0	0	0[W]	0[W]	0	0	0	0
42	0	0[W]	0	0	0	0	0	0[W]	0	0	0	0
43	DID NOT COMPLETE STUDY											
44	-	0[W]	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0[W]	0	0	0	0	0
46	0	0	0	0	0	0	0[W]	0	0	0	0	0
47	0	0	0	0	0	0	0[W]	0	0	0	0	0
48	0	0	0	0	0	0	0[W]	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0	0
50	0	DID NOT COMPLETE STUDY										
51	0	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0[W]	0[W]	0	0	0	0
57	0	0	0	0	0	0	0[W]	0[W]	0	0	0	0
58	0	0	0	0	0	0	0[W]	0	0	0	0	0

24* = Supervised removal of 1st Induction and Challenge Patch
W = Inclement weather. Subject unable to report as scheduled
- = Subject not present for supervised removal
DNC = Did not complete study

Table 2
Panel #20100091

Subject Demographics

Subject Number	Initials	Age	Sex
1	CMS	30	F
2	JMM	33	F
3	GMG	59	F
4	RM	46	M
5	AV	66	M
6	AJS	22	F
7	AMW	19	F
8	JLP	25	F
9	DMD	22	M
10	JMG	35	F
11	CAM	21	F
12	GSB	19	M
13	RAS	61	F
14	LV	31	F
15	JAS	40	M
16	MRZ	41	F
17	MNM	42	F
18	GMV	27	M
19	DEC	19	M
20	MDN	34	F
21	EAF	55	F
22	BAM	63	F
23	MRB	19	M
24	MB	62	F
25	JLM	34	F
26	TJG	22	F
27	DJB	54	F
28	SS	62	M
29	MCS	27	M

Table 2
(continued)
Panel #20100091

Subject Demographics

Subject Number	Initials	Age	Sex
30	CJC	28	M
31	JAH	59	F
32	CS	25	F
33	TOK	25	M
34	MB	58	F
35	VVL	16	F
36	SLK	30	F
37	TDK	32	F
38	MMH	49	F
39	KAD	56	F
40	TJD	57	M
41	DPB	32	M
42	MAC	60	F
43	GIA	19	F
44	CMG	27	F
45	BMM	45	F
46	NPM	47	M
47	HC	32	M
48	RJD	54	M
49	LSG	60	F
50	LAB	44	F
51	JL	68	F
52	MML	42	F
53	GDS	41	F
54	SPM	41	F
55	YV	21	F
56	JPM	76	M
57	DJM	66	M
58	ELL	63	F

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D. 8/23/10
Industry Liaison to the CIR Expert Panel

DATE: August 23, 2010

SUBJECT: Comments on the Draft Alkyl Benzoate Report Prepared for the August 30-31, 2010 CIR Expert Panel Meeting

p.1 - The Benzoic Acid and Sodium Benzoate conclusion should be updated based on the discussion of the CIR Expert Panel at the June, 2010 meeting. Isopropyl Alcohol and Propyl Alcohol have a tentative conclusion of safe as used.

p.1-2 - The UV absorption spectrum on C12-14 Alkyl Benzoate provided by Cognis (in the Council submission of July 9, 2010) should be mentioned in the Chemistry Section.

p.2, p.10, Table 3 - In the description of the use information, it is not clear what is meant by the product categories in parenthesis. It appears that they apply to the whole range of concentrations rather than the highest concentration. The use information needs to be updated for the revised concentration of use information provided on July 8, 2010 - the reported use of Behenyl Benzoate was a mistake and was removed from the revised concentration of use information. The use of Behenyl Benzoate needs to be removed from the text (it is not included in Table 3).

p.3 - In the Short-Term Toxicity section, please provide the media in which Benzoic Acid and Sodium Benzoate were given. Were the concentrations of >1% in the diet or drinking water?

p.3 - What doses of which compound were used in the neurobiological study in rats and mice?

p.4 - For all developmental toxicity studies, the specific days of gestation the pregnant animals were treated should be stated.

p.5 - Is it possible to obtain the primary reference for the Methyl Benzoate study cited to a 1970 review? What route, e.g., gavage, diet, was used in this study? What was the duration of this study? What type of tumors were observed?

p.5 - What was the duration of the carcinogenicity of the non-oxidative hair dye that included Benzoic Acid?

p.5 - Were the studies of Benzoic Acid really clinical studies (subjects were specifically treated with Benzoic Acid), or were they case reports of accidental exposures?

p.5, 6 - In the Dermal Irritation section and in the Summary, what is meant by "the benzoates"? Have these reactions been reported for alkyl benzoates, or is this referring to salts of benzoic acid?

p.5 - Please change "HIRPTs" to "HRIPTs"

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

p.6 - What was the total number of subjects in the 4 studies regarding the sensitization potential of Benzoic Acid?

p.6 - 1% is a concentration rather than a dose. Is this a dietary or drinking water concentration? Is the 4% concentration of Sodium Benzoate used in the reproductive and developmental toxicity study a dietary or drinking water concentration?

p.9, Table 2 - The title of this Table needs to be corrected, these are benzoate ingredients not acetate ingredients. The density of C12-15 Alkyl Benzoate provided by Cognis (July 9, 2010 submission) should be added to this table.

p.10, Table 3 - To be consistent with the FDA product categories, please change "Infant" to "Baby".