GREEN

Safety Assessment of Retinol, Retinoic Acid, and Retinyl Esters as Used in Cosmetics

CIR EXPERT PANEL MEETING

JUNE 10-11, 2013

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Wilbur Johnson, Jr.
Senior Scientific Analyst
Date: May 17, 2013
Subject: Draft Report on Retinol and Retinyl Esters

In September of 2012, the Panel agreed to reopen the safety assessment of retinol and retinyl palmitate to consider new data (e.g., NTP photocarcinogenicity study results) and the possible addition of retinyl acetate, retinyl propionate, retinyl linoleate, retinyl oleate, retinyl rice branate, retinyl soyate, and retinyl tallate.

A recap of the history of CIR's review of retinol and retinyl palmitate indicates:

- In 1987, a CIR final safety assessment concluding that retinyl palmitate and retinol are safe as cosmetic ingredients in the present practices of use and concentration was published.
- In 2005, CIR confirmed that conclusion and did not reopen the report. The Panel did note an ongoing National Toxicology Program (NTP) photocarcinogenicity study on retinyl palmitate and agreed to review the findings when completed.

When the decision was made last September to reopen this safety assessment, the Panel made the point that the draft report should include a robust review of available photo co-mutagenicty and photo co-carcinogenicity data. Recognizing that retinoic acid was tested in the NTP study and its similarity to retinol and retinyl palmitate, the CIR staff added retinoic acid to this safety assessment. Revision of the safety assessment to include these data has been completed.

Included for your review is a copy of the draft report, the CIR report history, literature search strategy, ingredient data profile, 2013 FDA VCRP data, and minutes from the September 2012 Panel meeting. The draft report includes current use concentration data as well as the following data on retinyl propionate received from the Council:

- HRIPT on a face cream,
- 28-day ophthalmologic and dermatologic study on a moisturizer,
- Epiocular™ tissue equivalency assay on a face cream,
- human phototoxicity test on a face cream, and
- human photoallergy test on a face cream

These data are included in pdf files. Report comments (See pcpc1 pdf file) were also received from the Council.

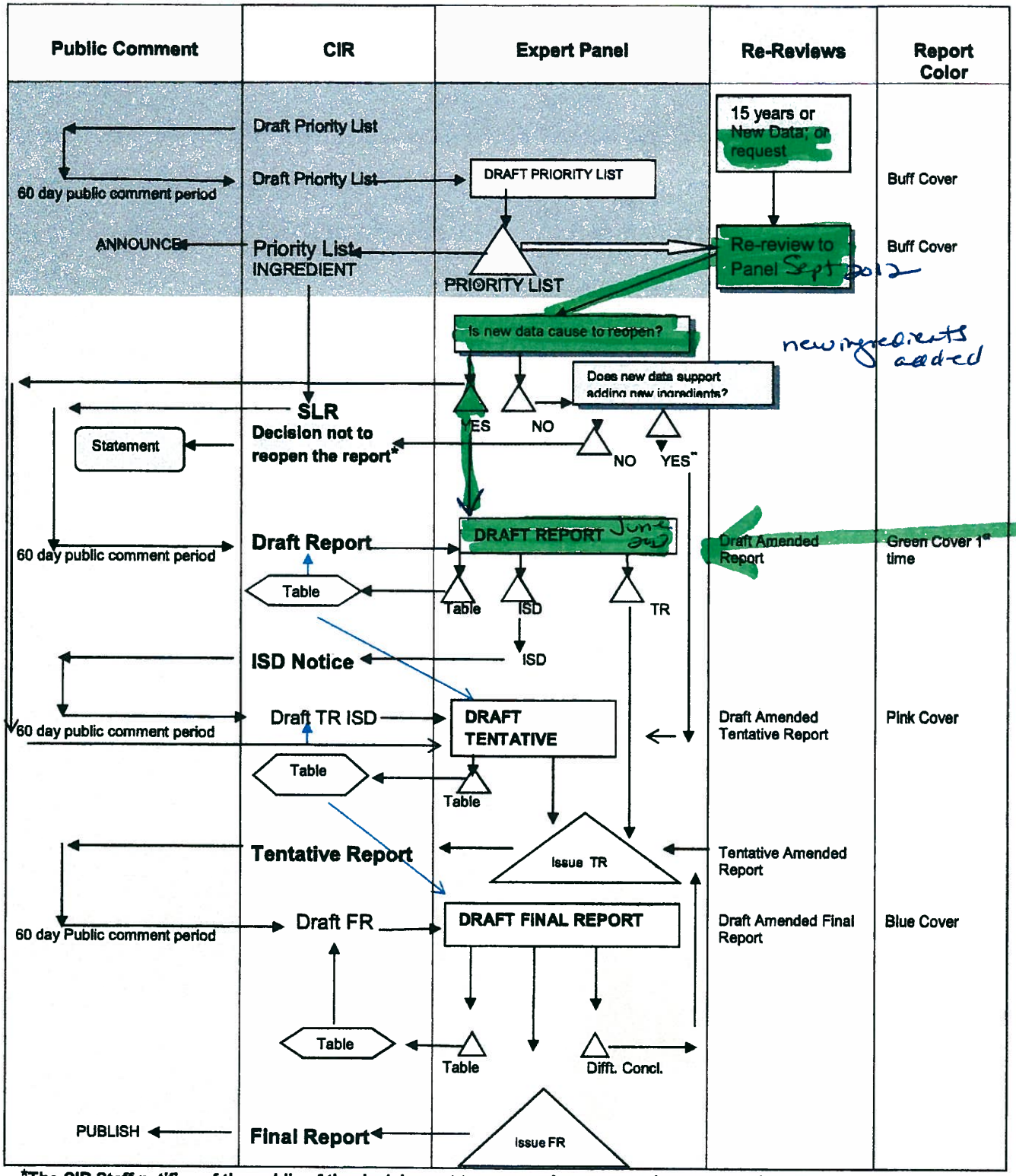
After reviewing the available data, the Panel needs to determine whether a tentative report should be issued or whether additional data are needed for completion of this safety assessment.

1101 17th Street, NW Suite 412, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(Email) cirinfo@cir-safety.org (Website) www.cir-safety.org

Retinyl Palmitate

June 2013

SAFETY ASSESSMENT FLOW CHART



*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

CIR History of:

Retinol and Retinyl Palmitate

A final report on the safety assessment of retinol and retinyl palmitate with the following conclusion was published in 1987: On the basis of the available animal and clinical data presented in this report, the CIR Expert Panel concludes that retinyl palmitate and retinol are safe as cosmetic ingredients in the present practices of use and concentration.

1st Re-review, Belsito and Marks Teams/Panel: June 13-14, 2005

The Panel confirmed its original conclusion and agreed that the final report on retinyl palmitate and retinol should not be reopened. This decision, published in 2008, was based on a review of published and unpublished data that became available after publication of the final report in 1987. The Panel stated its awareness of an ongoing National Toxicology Program (NTP) photococarcinogenicity study on retinyl palmitate and interest in reviewing the results upon study completion.

2nd Re-review, Belsito and Marks Teams/Panel: September 10-11, 2012

In response to a request from the Food and Drug Administration, a re-review document containing pertinent studies that entered the published literature since the Expert Panel's 2005 decision not to re-open the final report on retinol and retinyl palmitate was prepared for the Panel's review. Prior to development of this document, a letter from the Environmental Working Group (EWG) to the National Toxicology Program on the need to expedite the NTP photocarcinogenicity study on retinyl palmitate that was ongoing was made available by the Personal Care Products Council. The same is true for 2 letters to the editor of *Mutation Research* (summarized in the report text) relating to earlier published studies on the photogenotoxicity of retinyl palmitate. The letter from the EWG is being made available as an attachment for the Panel's review, along with comments on the completed NTP photocarcinogenicity study on retinyl palmitate from the EWG and Personal Care Products Council.

The Expert Panel agreed that the final report on retinyl palmitate and retinol should be re-opened, and that the re-opened safety assessment should include a robust review of available photo co-mutagenicty and photo co-carcinogenicity data.

3rd Review, Belsito and Marks Teams/Panel: June 10-11, 2013

The Draft Report has been revised to include current use concentration data as well as the following data on retinyl propionate received from the Council: HRIPT on a face cream, 28-day ophthalmologic and dermatologic study on a moisturizer, Epiocular™ tissue equivalency assay on a face cream, human phototoxicity test on a face cream, and human photoallergy test on a face cream.

Retinol and Retinyl Estders Check List for June, 2013. Analyst – Wilbur Johnson																				
			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
	Skin Penetration	Penetration Enhancement	ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
Retinol	X		X	X	X			X		X				X		X	X	X	X	X
Retinyl Palmitate	X		X					X		X		X	X	X		X	X	X	X	X
Retinoic Acid			X	X							X		X	X			X	X	X	X
Retinyl Acetate			X					X						X				X	X	
Retinyl Propionate												X		X		X				X
Retinyl Linoleate																				
Retinyl Oleate																				
Retinyl Rice Branate																				
Retinyl Soyate																				
Reinyl Tallate																				

Day 1 of the September 11-12, 2012 CIR Expert Panel Meeting – Dr. Belsito's Team

Retinol and Retinyl Palmitate

DR. BELSITO: Anything else? So, retinol and retinyl palmitate. This is buff.

To review or not review, that is the question. So, we looked at these in the 1980s. We re-reviewed in 2005, confirmed the original safety report. But we're aware of an ongoing NTP study for retinyl palmitate and retinoic acid, which has been published, stating that retinoic acid enhanced photo-carcinogenicity to the activity of UV, and of UVB; that retinyl palmitate enhanced photo-carcinogenic activity of UV and UVB.

And is that really true when you look at the data? And I gather that Curt and Paul were sent the full reports. I sort of scanned it very briefly.

I guess that I wasn't overwhelmed by the NTP data. However, what struck me is that the use concentration that we weren't given in the book, but it said under the "Cosmetic" section, that it was used up to 2.2 percent, which is a much higher level than we had looked at before. And that is -- someplace. "Use," "Cosmetic." So, in Panel Book, 11, it says "In a survey of 29 consumer cosmetic skin products labeled to contain retinoids, most products were found to contain either retinol or retinyl palmitate at concentrations of 2.2 percent." Whereas the data we had before was that those levels were significantly lower.

DR. ANDERSEN: So, where is this?

MR. JOHNSON: Yes, that statement doesn't represent current use concentration data from the industry, but it's from an earlier publication.

DR. BELSITO: I understand. But it -- the levels that you're giving us are much higher than the levels that we've been told that these are used at.

So, I mean, I think -- I mean, I'm assuming that came from Reference No. 5?

MR. JOHNSON: Yes.

DR. BELSITO: That's a 2009 reference, Wilbur. And, you know, when we last looked at this, the levels that we were looking at were a heck of a lot less. They were --

DR. SNYDER: .3,.5.

DR. BELSITO: Yes.

DR. SNYDER: How come Table 1 doesn't have any use concentrations?

DR. BELSITO: I don't have a clue. You know, they -- we weren't provided with current concentrations of use.

But, I mean, my point is that if, in fact, that report is correct, then regardless of what we think, we have 29 products that are being used at levels higher than we previously said "at the current concentrations of use."

Having said that, I think that studies on the photo-carcinogenicity of these products in mice are bogus, having spent three or four years doing mouse models of photo-carcinogenicity back in the early '90s, mouse skin does not behave as human skin, and you get a lot of warty-like papillomas, you get fibrosarcomas, because it's only two cell layers thick, and the light irradiates the muscle.

I mean, from a dermatologic standpoint, we treat people, particularly transplant patients with large numbers of skin cancers with oral retinoids. So, you know, in humans, they seem to have an anti-carcinogenic effect for skin cancers -- for UV-induced skin cancers. But, you guys read the full report.

DR. SNYDER: Yes, I would largely concur with your previous statement. I mean, I think that there's a lot of issues with this study. I think they've all been addressed in various formats in the information that we received.

I mean, we have a body of data that it has contradictory effects. Half the studies show either no effect or a protective effect. The other studies show maybe a weak association or something.

So, I guess from a standpoint of this individual NTP study, there is an expert panel that has been convened to review the data, independent panel, and they have not drafted their conclusion yet. So even considering that data set, I think we would want to see what that panel concludes in regard to their interpretation. I think there's 12 or 15 scientists that have been brought together to review that data.

DR. LIEBLER: Is that the one listed in the Wave II? Is that the one that Ray Novak is chairing?

DR. SNYDER: Jim Vaughan.

DR. LIEBLER: Right, yes. So they have not even drafted -- that we know of.

DR. ANDERSEN: The expert review (inaudible) --

DR. LORETZ: It's still being drafted.

DR. LIEBLER: Yes. It's a subcommittee of the Board of Scientific Counselors for NTP.

DR. SNYDER: So there's that issue. I don't think that the data -- for me, personally, I don't think the data -- I'm not impressed with the data that should make us change our opinion. (inaudible) Raised a different issue this morning about this higher use concentration that I think is not insignificant. I think we need to see where that's at.

But I guess, procedurally, we probably should -- we can proceed to try to get the report from the expert panel, or from whatever -- what did you call it?

DR. LIEBLER: It's the Board of Scientific Counselors subcommittee.

I think that would be helpful to us. My feeling was that -- to reopen to add the other ingredients. I didn't note the USE concentration increase, but even further supports my idea of reopening it.

DR. SNYDER: Procedurally reopen it.

DR. BELSITO: Okay.

DR. KLAASSEN: Where did you see this committee?

DR. SNYDER: It's in the -- it was in the NTP -- the full report for this thing is still draft form. And a component of that is the special committee that's been convened. And it says in there that it -- they're still awaiting their report. So -- and I went back in to NTP, and I still, I didn't see anything different about the availability of that report.

But it lists all of the participants in that.

DR. KLAASSEN: Okay. I didn't see that.

DR. ANDERSEN: But the suggestion to reopen would be based on reviewing use concentrations?

DR. BELSITO: To add seven ingredients, and to look at use concentrations.

DR. KLAASSEN: And, I think, also to look at that NTP report, see what this other committee (inaudible). Subcommittee.

DR. BELSITO: So, we're reopening -- or suggesting that we move the process to consider reopening.

DR. KLAASSEN: Right. Correct.

DR. BELSITO: Hopefully, have the final committee, NTP committee report, clarify the current concentrations of use, whether they've, in fact, increased to 2.2 percent, and to add these seven other --

DR. SNYDER: Consider adding those additional.

DR. BELSITO: And –

DR. KLAASSEN: (inaudible) All these things (inaudible) tricky.

DR. BELSITO: They certainly are.

Day 1 of the September 11-12, 2012 CIR Expert Panel Meeting – Dr. Marks' Team

Retinol and Retinyl Palmitate

Next in the Buff Book is Retinol and retinyl palmitate.

So this is -- these ingredients are to be looked again and considered whether or not we're going to open the document for review. In 1987, the CIR declared that these ingredients were safe. In 2005, it these were re-reviewed as safe but then we noted in that re-review that there was an NTP study ongoing and when that occurred and we had the results we would review those results and decide whether to reopen. So the first question is should we reopen based on the National Toxicology Program Data, which we got a wave on that. And obviously, I'm going to ask Tom about that. And then there is also the question of whether we should open to add seven more retinol esters.

So there are two issues. One, is there concern about the photocarcinogenesis of these ingredients? And based on the NTP study and reopen because of that? And is it a no- brainer to add -- reopen and add seven retinol esters? So Tom, do you want to start with the interpretation?

DR. SLAGA: Yeah, I would reopen it on both accounts.

DR. SHANK: So would I.

DR. MARKS: Okay.

MS. BRESLAWEC: I just want to point out that this is not a final study. It's still a draft study. It has not been issued in its final form.

DR. SLAGA: It's also the longest existing study in NTP's history.

MS. BRESLAWEC: Which could cause people to wonder why it hasn't been finalized. Just pointing that out.

DR. SHANK: But we also received several studies on photomutagenicity which were positive.

DR. SLAGA: Right. Right.

DR. SHANK: So my concern causing me to suggest we reopen it isn't based only on the NTP study but also the several photomutagenicity studies.

DR. SLAGA: And for clarification, it's really a photo co-carcinogenicity study, not a (inaudible).

DR. BERGFELD: Could you comment on the NTP study at all? The draft that you reviewed?

DR. SLAGA: Yeah, it's, actually, if Alan was here -- back in 2009, Alan Connie published a paper on creams the SKH-1 mouse as a vehicle, and all the vehicles from industry that he tried -- I'm giving a pre to the NTP. All of them increased UV or acted like a co- carcinogen to photocarcinogenicity. So there is a strong database which we don't even mention in here. We need to pull Alan Connie's paper 2009. I believe the first author is Lu, L-U.

MR. JOHNSON: Is it L-I-U?

DR. SLAGA: Because he did reformulate and took out a few things and made a cream that was not photo co- carcinogenic. Related to the NTP studies that obviously one of the big questions is that the cream control gave an enhancing effect but when they added the retinyl palmitate with it, it gave additional effects. And you could argue that it's possible the cream did something to the retinyl palmitate that gave a further effect, but it definitely, to me, there is a hint that there is something happening, and that is why I'd like to see, you know, more discussion of this. As Ron brought out, there is other data that actually says there is effect here. So I have some concerns.

DR. HILL: Do we know if any other those other retinol esters are in use? We don't -- we didn't try to --

MS. BRESLAWEC: We haven't looked..

DR. HILL: Okay.

DR. MARKS: So reopen. And the primary reason is to review the carcinogenicity of this mutagenicity of these ingredients?

DR. SLAGA: Well, to re-review all data since we looked at it back in 2005, be it photomutagenicity, photo co- carcinogenicity. It should be photo co-mutagenicity. To review because there is some other data that suggests that UV can actually change the structure and lead to oxides with some of these compounds.

DR. MARKS: So, Tom, again, to review the photomutagenicity and the photo co-carcinogenicity?

DR. HILL: Do we have to reopen if we think we're missing key pieces of information? Or can we actually table because we haven't done anything and find the additional data that we think we might not have captured and make sure it gets considered? And then meanwhile, we can survey and find out are these other esters being used. I mean, I don't know the rules of order here because I'm relatively new.

DR. SLAGA: Well, based on all the criticism of this study, it can be another year or two before all the dust settles before a peer review states precisely what happened.

DR. HILL: Because I know the environmental working group, you know, it's clear what their stance is, this surgeon, and we need to work on it. But for me the science here is extremely complex partly because these are endogenously present compounds and so we have binding proteins and endogenous processing and then there's the issue of, okay, if a particular individual is taking a high dose supplementation of Vitamin A already and then they add these on dermally, that might be a different circumstance than if they're not. So I was left with -- and I know Peter Fu personally, actually. He's a first rate guy in some of the chemistry parts of this, but to me the complexity was such that I couldn't reach a conclusion within the timeframe I had to do it and that's why I'm asking. I agree that the NTP study suggests there's a signal here but, gee, you're getting positive results on an ingredient that we looked at and saw no such before. It's a little strange.

DR. MARKS: So Ron, procedurally we can reopen, and after we review the data, decide we aren't going to reopen it and just handle the discussion. So this would be considered a re-review, updated information, but one in which we aren't reopening unless we decide to do it for the seven -- adding the seven retinol esters would warrant reopening that alone. If we had these ingredients, we'd reopen.

And so going on, Panel Book page 1, it's the one on the back side of the initial memo, are there any of these -- you notice Wilbur in red has put "no-brainer, add on; no- brainer, add on." So do these seven meet the retinol acetate, the propionate, the linoleate, the oleate, the rice brand. So now we have rice. Rice branate, soyate, which I assume is from soy but I'm not sure. And talate. Are these all no- brainers? Yes. Okay. So we would do those.

So Ron Hill, from a procedural point of view we would reopen this and just add those seven.

DR. BERGFELD: Could I add something?

DR. SHANK: I think there's a potential here that we may change the conclusion; therefore, that's why we should reopen. Adding on other ingredients is fine, but I think we really need to look at our conclusion based on what is new now.

DR. BERGFELD: I'd like to add on a practical point, in dermatology and cosmetics for photo aging and rejuvenation of skin, this group of chemicals are widely used in just over- the-counter products, not prescription products. And growing, is it very effective? And retinol is in almost everything now.

DR. MARKS: Well, I was struck on retinyl palmitate, when you look at the current frequency and concentration of use, there are over 2,000 uses. That's all on Panel Book 32.

MS. BRESLAWEC: A request that as part of the reopening it for re-review, that the CIR staff attempt to determine whether NTP is, in fact, repeating the study as has been rumored. Or what the status is of the draft.

DR. MARKS: Wilbur, did you hear that request?

MR. JOHNSON: I'm sorry.

DR. MARKS: Halyna, would you –

MS. BRESLAWEC: A request that the CIR staff determine, (1) when and if the draft will be finalized; and (2) if, in fact NTP is planning on redoing the study.

DR. MARKS: So we not only have that but we'll capture as Ron Shank mentioned, there are some photo mutagenicity.

DR. SHANK: There are several, and I think if only those studies were presented to the panel without the NTP study, we would ask to look at those studies and then if we find -- if we agree with their results, they are all positive for photomutagenicity, photo genotoxicity, we would then ask for a photocarcinogenicity study and we already have that. It may be flawed but before us it is also positive. So I think we really need to look at this very, very carefully.

DR. SLAGA: Just for a note, one of the reasons this study was extended so long is after Alan Connie found his results, he talked to NTP because those studies were underway and they had some confusion. So his cream showing an enhancing effect on photocarcinogenicity was one of the reasons. And NTP did not evaluate histopathologically the controls which I don't understand why they didn't do that.

MS. BRESLAWEC: It is essentially a study where the control -- a flawed study. I agree it may be the only study out there but it was significantly flawed.

DR. BERGFELD: I'd like to add that they did not read the dermatological literature before they did this study and created their control because --

DR. SLAGA: Alan Connie's study was published in 2009. This was started many years before that.

DR. BERGFELD: Well, I was just going to say anything that adds a lubricant to the top of the skin does enhance the UV excitement. We do that with psoriasis all the time to enhance the phototherapy.

DR. MARKS: So I'm not sure there if your conclusion is that when you add the retinol that you increase the carcinogenesis that indeed having a control showing some increase is expected. Now your end point is is there an enhancement of that or not? And this would suggest there's potentially an enhancement with these Retinols.

DR. SHANK: Well, there are. I have not read them myself but in our books, reference to studies where the retinol or retinyl palmitate actually reduced the UV-induced finding dimmers, which is the usual mechanism we think of inducing skin cancer by UV light. But, and that's a good thing. But the UV light also produced oxidate -- reactive oxidate -- reactive oxygen species, et cetera, which were, they suggest, pro-carcinogenic and mutagenic. So I think we really need to look at this very carefully.

DR. MARKS: Any other comments? So presumably tomorrow I will second a motion to reopen these ingredients to explore and clarify the more recent studies, including the NTP study on carcinogenicity of these ingredients or mutagenicity or genotoxicity, all of that, along with adding seven retinol esters, which are a bit of an aside. That's the lesser of the important issues.

DR. SHANK: Okay. Is this the time where we would ask for more data needs or do we wait until it's officially reopened?

DR. MARKS: Why don't we get it for the record now and then Ron Shank, I'll ask you tomorrow to note that.

DR. SHANK: Okay. I think if it's known, I'd like to know what the residual levels of retinol and retinyl palmitate were in the epidermis? What were those levels in the NTP study? Did they look at that? It was the UV -- the UV exposure was in the morning. The retinyl palmitate was applied in the afternoon. So if it's UV-induced photocarcinogenicity, the retinyl palmitate applied in the afternoon would still have to be there in the next morning's exposure. If they have any information on that, I would appreciate it.

And then I'd also like to know what are the normal levels of retinol in human epidermis. Is that known? And how much does cosmetic use of these compounds change that?

DR. MARKS: Any other comments? Thanks, Ron.

Retinol and Retinyl Palmitate

Then, looking at the retinol and retinyl palmatate re-review. Dr. Belsito?

DR. BELSITO: Yes, we looked at this data and there were a couple of things. We didn't really get this usual table on the concentration of use. However, on Page 11 of the Panel Book under cosmetic, the -- one, two, three, four -- fifth line down it says that most products were found to contain either retinol or retinyl palmitate at concentrations of 2.2 percent weight/weight, which is certainly higher than the prior concentrations we had looked at. So, if that is true, it alone would, I think, require that we re-open the document.

I think that other reasons to re-open it is certainly these two have received a large amount of attention and there are potentially other retinol products that could be added. So, I would vote to re-open it, to find out what the current concentrations of use are. Are they in fact up to 2 percent? And to add the other ingredients that can be added.

DR. MARKS: We second that motion to re-open. We wanted to do a robust review of the photomutogenicity of these compounds, the photocarcinogenicity, and the co- carcinogenicity. Tom, do you want to comment on these? Go ahead, Tom Slaga. And the geno tox.

DR. SLAGA: Yeah, there's really a large amount of new data that we have to scrutinize. And the way the photo co-mutogenicity and the photo co-carcinogenicity. And from the NTP report has a number of issues in it, even though there's a concern about the controls were positive with the claims, there is a publication now from Alan Connie's group that shows that a number of the creams used on humans actually increase the photo co-carcinogenicity alone, and so we need to really look at all of this in detail.

DR. MARKS: And certainly we agree to open -- to add the seven retinol esters. To alert industry, we would like to see if there is data needs relevant to residual levels of retinol and retinyl palmitate in the epidermis, would be helpful if there were actually -- we know the residual levels. And we'd also like to know what the normal levels of retinyl palmitate are in the epidermis.

DR. BERGFELD: Any other additive comments? We're going to vote to re-open this group of ingredients. Seeing none, I'll call the question. All those in favor of re- opening? Unanimous.

Safety Assessment of Retinol, Retinoic Acid, and Retinyl Esters as Used in Cosmetics

Status:	Draft Report for CIR Expert Panel Review
Release Date:	May17, 2013
Panel Meeting Date:	June10-11, 2013

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, Ph.D. James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Wilbur Johnson, Jr., M.S., Senior Scientific Analyst and Ivan Boyer, Ph.D., Toxicologist.

1101 17TH STREET, NW, SUITE 412 ◊ WASHINGTON, DC 20036-4702 ◊ PH 202.331.0651 ◊ FAX 202.331.0088 ◊ CIRINFO@CIR-SAFETY.ORG

Table of Contents

INTRODUCTION

A Cosmetic Ingredient Review (CIR) Final Report with the following conclusion was published 1987: On the basis of the available data presented in this report, the Expert Panel concludes that retinyl palmitate and retinol are safe as cosmetic ingredients in the present practices of use and concentration.[1]

Subsequently, at the June 13-14, 2005 CIR Expert Panel meeting, the Panel confirmed its original conclusion and agreed that the final report on retinyl palmitate and retinol should not be reopened. This decision, published in 2008, was based on a review of published and unpublished data that became available after publication of the final report in 1987.[2] In the discussion section that established the basis for confirming the original conclusion, the Panel noted an ongoing National Toxicology Program (NTP) photococarcinogenicity study on retinyl palmitate and expressed interest in reviewing the results upon study completion.

Public concern about the effects of retinyl palmitate in sunscreens have arisen. The Environmental Working Group (EWG)[3] asserted that almost half of the sunscreens on the market contain retinyl palmitate (RP), and, based in the EWG's interpretation of National Toxicology Program (NTP) study findings, EWG suggested that a health warning regarding the photocarcinogenic potential of sunscreens containing RP was appropriate. Media coverage has resulted in questions about the safety of sunscreens. The dermatology community has continued to support the need to use sunscreens.[4]

Thus, at the September 2012 Expert Panel meeting, the Panel deliberated on a re-review document that contained a summary of results from the 2011 NTP draft technical report on this study as well as other current data relating to the safety of retinol and retinyl palmitate in cosmetic products. After reviewing these data, the Panel determined that its safety assessment on retinol and retinyl palmitate should be reopened and also agreed that the new safety assessment should include the following additional ingredients: retinyl acetate, retinyl propionate, retinyl linoleate, retinyl oleate, retinyl rice branate, retinyl soyate, and retinyl tallate.

Recognizing that retinoic acid was tested in the NTP study and its similarity to retinol and retinyl palmitate, the CIR staff subsequently agreed to add retinoic acid to this safety assessment. The NTP photocarcinogenicity study was subsequently published in August of 2012, and the results are summarized in this safety assessment.

Physiological Role of Vitamin A and Metabolites

A review article updating the available information on the physiological role of vitamin A and its biologically active metabolites in the skin is available.[5] The pleiotropic effects of vitamin A are exerted mainly by one active metabolite, all-*trans* retinoic acid, which regulates the expression of a battery of target genes through several families of nuclear receptors, polymorphic retinoic acid response elements, and multiple coregulators.[6] They also involve extra nuclear and non-transcriptional effects, such as the activation of kinase cascades, that are integrated in the nucleus by means of phosphorylation of of several factors of retinoic acid signaling.

CHEMISTRY

Definition and Structure

The definitions of the retinoids reviewed in this safety assessment are included in Table 1. Structural formulas are included in Figure 1.

Photoreactivity

Retinol

Retinol and its esters have a broad absorption spectrum centered around an absorption maximum (≈ 325 nm) in the UV spectral region.[7] As a result, these retinoids can be efficiently photoexcited by sunlight in both the UVA and UVB spectral regions.

The exposure of retinol (in ethanol) to UVC light (at 254 nm) produced the following specific photoproducts: retinal, 5,6-epoxyretinol, 5,8-epoxyretinol, 13,14-epoxyretinol, and cleavage products.[8]

Retinyl Palmitate

When retinyl palmitate (in methanol) was exposed to monochromatic UVA (365 nm), the photodegradation products were as follows: anhydroretinol, palmitic acid, and 4,5-dihydro-5-methoxy-anhydroretinol.[9,10] Palmitic acid, anhydroretinol, and 2-butenyl palmitate are photooxidation products that resulted from the exposure of retinyl palmitate to UVC light (254 nm).[8] According to another source, in the presence of UVA light, retinyl palmitate (RP) decomposes into multiple products, including anhydroretinol (AR) and 5,6-epoxyretinyl palmitate (5,6-epoxy-RP).[11]

The photoirradiation of retinyl palmitate (RP) in ethanol using a UV lamp generating approximately equal levels of UVA and UVB light yielded the following photodecomposition products: 4-keto-RP, 11-ethoxy-12-hydroxy-RP, 13-ethoxy-14-hydroxy-RP, anhydroretinol (AR), and *trans*- and *cis*-15-ethoxy-AR.[12]

Retinoic Acid

In the presence of light from fluorescent lamps, the following products resulted from the photodegradation of retinoic acid: 13-*cis*-retinoic acid, 11-*cis*-retinoic acid, 11,13-*bis*-*cis*-retinoic acid, 9-*cis*-retinoic acid, 9,11-*bis*-*cis*-retinoic acid, all-*trans*-5,6-epoxyretinic acid, and 13-*cis*-5,6-epoxyretinoic acid.[13,14,15]

USE

Cosmetic

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, all of the following retinoids reviewed in this safety assessment function as skin conditioning agents in cosmetic products: retinyl palmitate, retinol, retinoic acid, retinyl acetate, retinyl propionate, retinyl linoleate, retinyl oleate, retinyl rice branate, retinyl soyate, and retinyl tallate.[16] In addition to this function, retinoic acid also functions as an antiacne agent and retinyl rice branate functions as an antioxidant. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2013 (Table 1), the following ingredients were being used in cosmetic products: retinyl palmitate, retinol, retinoic acid, retinyl acetate, retinyl propionate, retinyl linoleate, and retinyl tallate.[17] The results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2013, also included in Table 2, indicate that the following ingredients are being used in cosmetic products: retinyl palmitate, retinol, retinyl acetate, retinyl propionate, and retinyl linoleate.[18] Of the use concentrations reported, the highest maximum use concentration in leave-on or rinse-off products was reported for retinyl palmitate (1.97% in leave-on products; 1% in rinse-off products).

In an earlier survey of 29 consumer cosmetic skin products labeled to contain retinoids, most products were found to contain either retinol or retinyl palmitate at concentrations of 2.2% (w/w), while few products contained both ingredients. A number of products also contained cis isomers of retinol that could be quantitatively distinguished from the all-trans compound.[19]

Cosmetic products containing the retinoids reviewed in this safety assessment may be applied to the skin, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

Retinyl palmitate is used in hair sprays (pump sprays), face powders, foundation sprays, and body and hand sprays, and could possibly be inhaled. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm, compared with pump sprays.[20,21,22,23] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[20,21]

Noncosmetic

The Food and Drug Administration (FDA) has included retinyl palmitate and retinyl acetate on its list of direct food substances that are classified as generally recognized as safe (GRAS) (21 CFR: 184.1930).[24] These 2 retinoids are also

included on the FDA's list of nutrients/dietary supplements that are classified as GRAS (21 CFR: 582.5933 and 582.5636).[24] The FDA has also determined that available data on retinyl palmitate and retinyl acetate are inadequate to establish general recognition of safety and effectiveness of these retinoids as active ingredients in over-the-counter (OTC) drug products (21 CFR: 310.545).[25]

In response to a request from the European Commission, the Panel on Additives and Products or Substance used in Animal Feed (FEEDAP) was asked to provide a scientific opinion on the safety and efficacy of vitamin A (retinyl acetate, retinyl palmitate, and retinyl propionate) as an additive to feed and water for drinking for all animal species.[26] All consumer exposure calculations showed that liver is the only food of animal origin for which consumption poses a risk to the adult consmers. It was noted that this risk can be considerably reduced, but not eliminated, if the following levels proposed by the European Food Safety Authority (EFSA) for reduction of the maximum vitamin A content in feeding stuffs would be respected:

- Pigs: piglets (weaned or suckling): 16,000 IU/kg complete feed, pigs for fattening: 6,500 IU/kg complete feed and sows: 12,000 IU/kg complete feed.
- Poultry: chickens (including all minor poultry species) in the first 14 days of life and turkeys in the first 28 days of life: 20,000 IU/Kg complete feed. All poultry (for fattening, reared for laying, laying and breeding): 10,000 IU/kg complete feed.
- Milk replacers for all mammalian species: 25,000 IU/kg milk replacer
- Calves for rearing in the first 4 months of life, lambs ad kids for rearing in the first 2 months of life: 16,000 IU/kg complete feed. Cattle, sheep and goats for fattening: 10,000 IU/kg complete feed.
- Dairy cows: 200,000 IU vitamin A/cow per day

The FEEDAP opinion also stated that retinyl acetate, retinyl palmitate, and retinyl propionate are irritants to the skin and potential skin sensitizers. Additionally, it was noted that data on respiratory toxicity and on the levels of exposure of workers that would cause systemic or respiratory toxicity are not available; however, inhalation exposure of workers from handling certain formulations is likely. The FEEDAP recommended that protection measures against inhalation exposure for persons handling the additive be taken.

In 1982, FDA approved Accutane (isotretinoin, or 13-cis-retinoic acid) for use in the treatment of severe, recalcitrant nodular acne that is unresponsive to conventional therapy, including antibiotics.[27] Accutane can, however, be associated with serious adverse events, including birth defects. Following approval, it became evident that a formal risk management program would be needed due to the drug's harmful effects in pregnancy. In 2005, FDA approved a strengthened risk management plan for Accutane and generic isotretinoin, to make sure that women do not become pregnant while taking this medicine.[28]

TOXICOKINETICS

Based on the data presented below, it appears clear that the ingredients addressed in this safety assessment can penetrate the skin and distribute to tissues throughout the body. Less clear is the metabolic fate of these ingredients.

Absorption and Distribution

Dermal

Retinol

Retinol absorption from cosmetic formulations has been measured through excised human skin in diffusion cell studies.[29] Absorption through skin into the receptor fluid was 0.3% of the applied dose from a gel vehicle and 1.3% from an emulsion vehicle in 24-h studies.

The percutaneous absorption of retinol (vitamin A) from cosmetic formulations was studied to predict systemic absorption and to understand the significance of the skin reservoir in *in vitro* absorption studies.[30] Viable skin samples from fuzzy rats or human subjects were assembled in flow-through diffusion cells for *in vitro* absorption studies. In vitro studies using human skin and a gel or emulsion vehicle found 0.3% and 1.3% of the applied retinol, respectively, in the receptor fluid at 24 h. Levels of absorption in the receptor fluid increased over 72 h with the gel and emulsion vehicles. Using the gel vehicle, *in vitro* rat skin studies found 23% in skin and 6% in receptor fluid at 24 h, while 72-h studies found 18% in skin and

13% in receptor fluid. Thus, significant amounts of retinol remained in rat skin at 24 h and these amounts decreased by approximately 5% over 72 h, with proportional increases in the receptor fluid.

In vivo absorption studies using fuzzy rats were performed in glass metabolism cages for collection of urine, feces, and body content. Retinol (0.3%) formulations (hydroalcoholic gel and oil-in-water emulsion) containing ^{3}H-retinol were applied and absorption was measured at 24 h or 72 h. Results were reported as % of applied dose. The *in vivo* rat studies with the gel indicated 4% systemic absorption of retinol after 24 h, which did not increase at 72 h. Retinol remaining in rat skin was 18% and 13% of the applied dermal dose after 24 h and 72 h, respectively. Similar results were obtained using the oil-in-water vehicle.

The authors stated that the studies summarized above could be interpreted to indicate that retinol formed a reservoir in rat skin both *in vivo* and *in vitro*. Little additional retinol was bioavailable from the gel or emulsion 24 h after application. Comparison of the *in vitro* and *in vivo* results for rat skin indicates that the fraction of the applied retinol in the receptor fluid after 24 h *in vitro* was comparable to the fraction absorbed systemically through the skin after 24 h *in vivo*. Therefore, the authors concluded that the best single estimate of retinol systemic absorption from *in vitro* human skin studies is the 24-h receptor fluid value. However, the authors stated that the receptor fluid value from the 72-h extended study may be used in worst-case exposure estimates.[30]

Enhanced penetration of retinol was found from the dermal application of retinol in solid lipid nanoparticles incorporated into an oil-in-water cream, when compared to a conventional formulation.[31] Highest retinol concentrations were found in the stratum corneum and the upper viable epidermal layer. The penetration of retinyl palmitate was influenced even more by incorporation into the solid lipid nanoparticles.

A study was performed to deliver retinol topically and quantify the amount permeated in the stratum corneum and underlying skin. A static Franz-type diffusion cell was used, and porcine skin was clamped between the donor and receptor compartment. The stratum corneum faced the donor compartment, to which a 90% saturated solution of retinol in propylene glycol (200 µl) was added. The permeation experiment was carried out in triplicate. Treated skin was removed for analysis after 24 h. Retinol (10%) was retained in the stratum corneum and 90% permeated in the underlying skin.

Retinol and Retinyl Palmitate

The accumulation of retinyl palmitate and retinol was evaluated in the skin of SKH-1 mice (8 groups; 12 mice/sex/group) treated tropically with the following concentrations of retinyl palmitate (in cream) for 13 weeks (5 days/week): 0.1%, 0.5%, 1.0%, 5.0%, 10%, and 13%.[32] Additionally, 2 groups were untreated and treated with control cream, respectively. Because products containing retinyl palmitate are frequently applied to sun-exposed skin, and exposure to sunlight and UV light can alter endogenous concentrations of retinoids in the skin, mice in this study were also exposed to simulated solar light 5 days per week.

Retinyl palmitate diffused into the skin and was partially hydrolyzed to retinol. The levels of retinyl palmitate in the skin of mice administered retinyl palmitate cream were greater than control values. The levels of both retinyl palmitate and retinol increased with the application of higher concentrations of retinyl palmitate in the cream (statistically significant linear dose trends). The levels of retinyl palmitate and retinol in the stratum corneum, epidermis, and dermis of the untreated group, control cream-treated group, and 0.5% retinyl palmitate-treated group were determined. When compared to untreated mice and mice treated with the control cream, the levels of retinyl palmitate and retinol in the 0.5% retinyl palmitate group were substantially higher in all layers of the skin. In each of the 3 treatment groups, levels of both retinyl palmitate and retinol were significantly higher in the epidermis, lowest in the dermis, and somewhat intermediate in the stratum corneum ($P < 0.05$). The study results indicated that topically applied retinyl palmitate can elevate the concentrations of retinyl palmitate and retinol in the skin of SKH-1 mice.[32]

To evaluate the potential use of solid nanoparticles (SLN) in dermatology and cosmetics, glyceryl behenate SLN loaded with vitamin A (retinol and retinyl palmitate) and incorporated in a hydrogel and oil-in-water cream were tested for their influence on the penetration of these substances into porcine skin.[31] Conventional formulations served for comparison. Excised full thickness skin was mounted in Franz diffusion cells and the formulations were applied for 6 h and 24 h. High retinol concentrations were found in the upper skin layers following SLN preparations. The deeper regions showed only very low vitamin A levels. Because of a polymorphic transition of the lipid carrier with subsequent drug expulsion following the application to the skin, the drug localizing action appears to be limited for 6 h to 24 h. Best results were obtained with retinol SLN incorporated in the oil-in-water cream retarding drug expulsion. The penetration of the occlusion-sensitive drug retinyl palmitate was enhanced even more than that of retinol by SLN incorporation. In summary, enhanced absorption of retinol was found from SLN incorporated into an oil-in-water cream, when compared to a conventional formulation. Highest retinol

concentrations were found in the stratum corneum and the upper viable epidermal layer. The penetration of retinyl palmitate was elevated even more than that of retinol by incorporation into the SLN.

Three experiments were performed to determine the time course for accumulation and disappearance of retinyl palmitate and retinol in the stratified layers of skin from female SKH-1 mice that received single or repeated topical applications of creams containing 0.5% or 2% retinyl palmitate.[33] In the first experiment, 10-week old female SKH-1 mice (3 per group) received topical application of 2% retinyl palmitate cream (~ 75 µl) to the dorsal skin area between the base of the neck and the base of the tail. The cream was applied manually with a gloved finger to cover the entire dorsal region of the animal. Each application achieved approximately 2 mg cream/cm^2 of skin. Control animals received the vehicle cream only. At 1, 2, 3, or 6 days after cream application, the animals were killed and skin samples collected. The design of the second experiment was the same as that of the first, except that the animals (3 per group) received topical application of 0.5% retinyl palmitate cream. The animals were killed at 1, 2, 3, or 6 days after cream application and skin samples collected. In the third experiment, the protocol was similar to that of the first experiment, except that the mice received daily topical application of 2% retinyl palmitate cream (~75 µL) to the dorsal skin area, base of the neck and anterior to the base of the tail for 4 consecutive days. The animals were killed at 1, 3, 6, 11, or 18 days after the last cream application, and skin samples were collected. In all 3 experiments, samples of skin were separated into the stratum corneum, epidermis, and dermis.

Retinyl palmitate diffused rapidly into the stratum corneum and epidermal skin layers within 24 h following application of creams containing retinyl palmitate. Of the 3 skin layers, the highest level of retinyl palmitate and retinol per weight unit (ng/ml) at all time points was found in the epidermis. Levels of retinyl palmiate and retinol were lowest in the dermal layer and intermediate in the stratum corneum. The levels of retinyl palmitate and retinol in the separated skin layers and in the intact skin decreased with time, but levels of retinyl palmitate remained higher than control values for a period of up to 18 days. The application of retinyl palmitate to mouse skin elevated the normal physiological levels of retinyl palmitate and retinol in the skin.[33]

Levels of retinyl palmitate and retinol in the skin of female SKH-1 mice were investigated in another study. The animals received a standard HNIH-31 diet.[34] Retinoid levels were evaluated at ages ranging from 10 weeks to 68 weeks of age. The levels of retinyl palmitate and retinol were highest in the epidermis of 20-week-old mice, and decreased when the age increased to 60 and 68 weeks. The total amount of retinyl palmitate at 20 weeks of age was found to be 1.52 ng/mg skin, and decreased approximately 4-fold at 60 and 68 weeks of age. A similar trend was found for the effects of age on the levels of retinol.

Retinoic Acid

The percutaneous absorption of all-*trans*-[10,11-$^3H_2$]-retinoic acid was evaluated using 12 mature female virgin outbred Golden Syrian hamsters [Lak(LVG):SYR].[35] A single application of 17 µg/kg or 8.7 mg/kg (dissolved in acetone) to shaved dorsal skin resulted in rapid absorption and dose-dependent rates of elimination. Unchanged all-*trans* retinoic acid represented ≤ 4% of the total circulating radioactivity.

Airol® cream (0.05% tretinoin [all-*trans* retinoic acid]), plasma concentrations were compared after repeated dermal applications of the maximum dose that could be applied reliably and after oral administration of the highest non-teratogenic dose.[36] The test preparation was applied dermally in 2 equal portions to rats and rabbits at 2 g/animal/day (equivalent to a tretinoin dose of 3.7 mg/kg/day) and at 6 g/kg/day (equivalent to 3 mg tretinoin/kg/day), respectively. Following a single oral dose of 2 mg/kg, C_{max} and AUC for tretinoin in rat plasma were 285 ± 14.6 ng/ml and 595 ± 123 ng ·h/ml, respectively. Corresponding values for the rabbit were 78.4 ± 16.9 ng/ml and 126 ± 25.4 ng · h/ml. In both species, plasma concentrations of tretinoin after dermal application were consistently below the assay quantification limit (< 5 ng/ml and < 2 ng/ml for rat and rabbit, respectively), despite marked irritation of the skin. Therefore, repeated topical application of the test preparation produced plasma concentrations of tretinoin that were well below the plasma concentrations produced by a non-teratogenic oral dose of 2 mg/kg in the rat and rabbit.

Oral

Retinoic Acid

During the early primitive streak stage of development, pregnant Syrian Golden hamsters were given a single oral dose (35 µmol/kg) of [^{3}H]-all-*trans* retinoic acid, [^{3}H]-13-*cis*-retinoic acid, [^{3}H]-all-*trans*-4-oxo-retinoic acid, [^{3}H]-9-*cis*-retinal, or all [^{3}H]-*trans*-retinyl acetate.[37] The following tissues were sampled for radioactivity: brain, bladder, uterus, placenta, fetus, muscle, lung, heart, fat, adrenal, kidney and liver. The acidic retinoids-associated radioactivity was distributed to all of the tissues sampled, including the placenta and fetus. The largest and least accumulation were associated

with the liver (3.8 nmol eq./g tissue) and fat (0.9 nmol eq./g tissue), respectively. The other tissues sampled contained 2.5 to 3.0 nmol eq./g tissue. Retinal acetate- or 9-*cis*-retinal- radioactivity was concentrated in the liver and lung. In vivo, the all-*trans* retinoic acid was oxidized to all *trans*-4-oxo-retinoic acid and isomerized to 13-*cis*-retinoic acid; 13-*cis*-retinoic acid was oxidized to 13-*cis*-4-oxo-retinoic acid and isomerized to all-*trans* retinoic acid. Neither parent 9-*cis* retinal nor retinyl acetate was detected in maternal plasma. Maximum values for plasma concentrations of the parent retinoids were achieved within 60 min, and then followed exponential decay. When compared to all of the retinoids evaluated in this study, 13-*cis* retinoic acid covered the largest area under the plasma curve, had the slowest clearance, and the longest elimination of t1/2. Total plasma radioactivity, consisting of unidentified metabolites, remained elevated at 4 days post-dosing. It was noted that it was not possible to correlate maternal peak circulating concentrations of the parent retinoids, total radioactivity, plasma pharmacokinetic parameters or the total concentrations of residual radioactivity in fetal tissues with the different teratogenic potentices of the retinoids evaluated.

Retinyl Acetate

A study was performed to analyze the vitamin A content of liver and serum from 13 adult female African green vervet monkeys (*Chlorocebus aethiops*).[38] The monkeys were wild-caught and held in captivity for 2 years, during which they consumed a standard primate diet. Monkeys were fed lab diet containing 45 nmol (43 IU) vitamin A/g dry food (as retinyl acetate). Liver vitamin A concentration (mean ± 1 standard deviation) was 14.6 ± 2.3 µmol retinol/g liver. Retinyl palmitate accounted for most of the hepatic vitamin A (59% ± 2.5%). The serum retinol concentration (0.93 ± 0.21 µM) was not elevated.

Oral/Dermal

Human

Retinyl Palmitate and Retinol

Two groups of 14 female volunteers of child-bearing age were maintained on a vitamin A-poor diet and treated topically for 21 days with creams containing 0.30% retinol or 0.55% retinyl palmitate on approximately 3000 cm^2 of their body surface area, amounting to a total of approximately 30,000 IU vitamin A/subject/day.[39] Subsequently, after a 12-day wash-out period, the study groups received single oral doses of 10,000 IU or 30,000 IU retinyl palmitate, corresponding to the maximal European union (EU) allowance during pregnancy or three-times higher, respectively. Blood samples were collected over 24 h on study days -3 (pre-study), 1, 21 (first and last days of topical treatment), and 34 (oral administration) at 0, 1, 2, 4, 6, 12, 14-16 h and 24 h after treatment for determination of plasma concentrations of retinol and retinyl palmitate. On days 1 or 21 of topical treatment, no changes were measured in individual or group mean plasma C_{max}, $AUC_{0\text{-}24\ h}$, or other pharmacokinetic parameters of retinol or retinyl palmitate relative to pre-study data. In contrast, single oral doses of retinyl palmitate at 10,000 IU or 30,000 IU produced dose-related and sustained increases in *Cmax* and $AUC_{0\text{-}24\ h}$ values of plasma retinyl palmitate. The results of this study provide evidence that human topical exposure to retinol- or cosmetic creams containing retinyl palmitate at 30,000 IU/day and maximal use concentrations do not affect plasma levels of retinol or retinyl palmitate, whereas, single oral doses at 10,000 IU or 30,000 IU produce significant increases in plasma retinyl palmitate. Results relating to skin irritation and systemic toxicity are presented in the Skin irritation and Repeated Dose Toxicity sections of this report.

TOXICOLOGY

Acute Toxicity

Oral

Animal

Retinoic Acid

The teratogenicity of all-trans retinoic acid and retinyl acetate was evaluated using groups of 8 pregnant Sprague-Dawley rats.[40] The animals were fed a standard diet containing 14.4 nmol retinyl palmitate/g diet. Single equimolar oral doses (3.5 to 352 µmol/kg body weight) of the test substances were administered in corn oil (~ 250 µl) on day 8.5 of pregnancy. Control animals were dosed with corn oil only. Dams and fetuses were killed on day 19. Results relating to teratogenicity and fetal toxicity are included in the section on Reproductive and Developmental Toxicity. Oral dosing with

352 µmol/kg all-trans retinoic acid caused hair loss and hair discoloration in dams. At doses of ≥113 µmol/kg all-trans retinoic acid, the dams lost weight, but there were no overt signs of toxicity. No toxic effects of retinyl acetate on dams were reported.

Repeated Dose Toxicity

Inhalation

Animal

Retinoic Acid

In a study involving 12 male Hartley guinea pigs, a nebulizer was used to create an aerosol (mass mean diameter = 2.9 µ, a particle size expected to be respirable) of all-trans retinoic acid in solution for short-term inhalation exposure.[41] In addition to all-*trans* retinoic acid, other components (vehicle) of the aerosol were as follows: 25% PEG-35 castor oil, 0.01% butylated hydroxytoluene, and 10% phosphate-buffered saline. The animals received an estimated average all-trans retinoic acid dose of either 0.32 mg/kg (low dose, 1.4 mM), or 0.62 mg/kg (medium dose, 2.8 mM), or 1.26 mg/kg (high dose, 5.6 mM). Doses were administered for 6 consecutive days (20 min/day). Exposure resulted in an increase in all-trans retinoic acid levels in the lung, but not in the liver or plasma. Cellular lung levels of retinol, retinyl palmitate, and retinyl stearate appeared to have been unaffected (245.6 ± 1.7, 47.4 ± 3.4, and 132.8 ± 7.7 ng/g wet weight, respectively). Aerosol exposure also induced a dose-dependent protein expression of the cellular retinol-binding protein 1 (CRBP-1) in the lung. Additionally, aerosol exposure did not cause toxic side effects or tachyphylaxis throughout the study.

Oral

Animal

Retinol

Subclinical hypervitaminosis A in rats causes fragile bones. A study was performed to investigate possible mechanisms for vitamin A action.[42] Three groups of 15 mature female Sprague-Dawley rats were fed the following, respectively, for 12 weeks: standard diet containing 12 IU vitamin A per g pellet (control, C), or a standard diet supplemented with 120 IU (10 x C), or 600 IU (50 x C) vitamin A/g pellet. At the conclusion of the study, the concentrations of serum retinyl esters were elevated 4- and 20-fold in rats fed the supplemented diets. Although neither average food intake nor final body weights were significantly different among the groups, a dose-dependent reduction in serum levels of vitamins D and E, but not K, was found. In the 50 x C group, the length of the humerus was the same as in controls, but the diameter was reduced (- 4.1%, $p < 0.05$). Peripheral quantitative computed tomography (pQCT) at the diaphysis showed that bone mineral density (BMD) was unchanged and that periosteal circumference was decreased significantly (- 3.7%, $p < 0.05$). However, ash weight of the humerus was not affected. Because bone volume decreased, volumetric BMD, as measured by the bone ash method, increased (+ 2.5%, $p < 0.05$). It was concluded that vitamin A interference with other fat-soluble vitamins is a possible indirect mechanism of vitamin A action. Moreover, BMD measurements did not reveal early adverse skeletal changes induced by moderate excess vitamin A ingestion in rats.

Retinyl Palmitate

The consequences of acute and chronic vitamin A (retinyl palmitate) supplementation at therapeutic and excessive doses on the redox state of submitochondrial particles (SMP), isolated from adult rat cerebral cortex and cerebellum, were studied.[43] Groups of 5 adult male Wistar rats were used. The animals were treated once a day during 3 different periods: acutely (3 days or 7 days) or chronically (28 days). The 5 groups of animals were gavaged daily with one of the following: vehicle (0.15 M NaCl), and retinyl palmitate at 1000 IU/kg, 2500 IU/kg, 4500 IU/kg, and 9000 IU/kg. The lower 2 doses were described as therapeutic and, the other 2, excessive. All doses induced lipid peroxidation, protein carbonylation, and oxidation of protein thiol groups in cerebral cortex and cerebellum SMP. Furthermore, retinyl palmitate supplementation induced an increase in the superoxide ($O_2^{\bullet -}$) anion production, indicating an uncoupling in the electron transfer chain. In addition, locomotory and exploratory activity, which are associated with the cerebral cortex and cerebellum, were reduced by both acute and chronic retinyl palmitate supplementation. Retinyl palmitate induced a decrease in both locomotory and exploratory behavior. Together, these results show that vitamin A could be toxic at the subcellular level, inducing mitochondrial dysfunction and altering cerebral cortex and/or cerebellum-dependent behavior.

The effect of short-term vitamin A (retinyl palmitate) supplementation on the rat liver was studied using groups of 6 to 7 male Wistar rats (90 days old).[44] Groups were dosed orally (gavage; dose volume = 0.8 ml maximum) with one of the following: 0.15 M saline (control); 1,000 IU/kg/day; 2,500 IU/kg/day; 4,500 IU/kg/day; and 9,000 IU/kg/day. The animals were dosed once per day for 3 or 7 days. The animals were killed 24 h after the last dose; the liver was removed and homogenized, and liver mitochondria were isolated and studied. Increased liver peroxidation was observed in the liver of rats that received retinyl palmitate supplementation at 2,500; 4,500; or 9,000 IU/kg/day for 3 days (1.4- to 1.7-fold; $p < 0.01$). However, hepatic lipid peroxidation levels did not change after vitamin A supplementation for 7 days.

Increased (1.3- to 1.6-fold; $p < 0.01$) $O_2^{-\bullet}$ production in hepatic SMP of the rats that were treated with retinyl palmitate at 2,500; 4,500; or 9,000 IU/kg/day for 3 days was observed. Retinyl palmitate supplementation at any dose tested induced a 1.3- to 1.7-fold increase in $O_2^{-\bullet}$ production in hepatic SMP isolated from rats that were treated for 7 days ($p < 0.01$). Mitochondria that were isolated from the liver of rats that received vitamin A supplementation at 2,500; 4,500; or 9,000 IU/kg for 3 days presented higher lipid peroxidation levels when incubated for 10 minutes with buffer ($p < 0.05$). $CaCl_2$ (75 µM) induced a 2.5- to 2.9-fold increase of lipid peroxidation in liver mitochondria from animals that received retinyl palmitate supplementation at 2,500; 4,500; or 9,000 IU/kg for 3 days, when compared with mitochondria isolated from the liver of animals that received saline for 3 days ($p < 0.01$). Overall, the results of this study showed that mitochondria are a target of vitamin A-associated toxicity *in vivo*.[44]

A study was performed to compare electron flux and oxidative/nitrosative stress parameters on the heart among rats supplemented with vitamin A.[45] Adult male rats (strain not stated; 90 days old) were grouped (7 per group) and treated (by gavage; total volume = 0.8 ml) as follows for 28 days: vehicle (0.9% saline solution), and 1000, 2500, 4500, and 9000 IU retinyl palmitate (in saline)/kg/day. The heart was removed for analysis after 28 days. Electron flux and oxidative/nitrosative stress parameters were evaluated and statistics were conducted using Anova one-way, followed by Dunnet's *post hoc* test of significance. Retinyl palmitate supplementation induced an increase in the oxidation of lipids and proteins, and mitochondrial 3-nitrotyrosine content, an enzymatic imbalance (indicated by the increased superoxide dismutase (SOD)/catalase (CAT) ratio), and a decrease in electron transfer between respiratory chain complexes. These results suggest that vitamin A induces oxidative/nitrosative stress and mitochondrial impairment in the heart.

Retinyl Acetate

Groups of 25 female Sprague-Daweley rats (35 days old) were fed diets supplemented with (per kg diet) 125 or 250 mg retinyl acetate.[46] A third group was fed a basal diet only. Feeding was continued throughout the 180-day study. Dosing with 125 mg did not induce gross hepatotoxicity. However, dosing with 250 mg induced a low incidence of hepatic fibrosis in rats examined after 120 and 180 days of feeding.

The hemorrhagic toxicity of retinol acetate was evaluated using groups of 6 male Jcl:SD rats (4 weeks old). The animals were fed a laboratory ration for 1 week and then given an experimental diet containing 0.5% retinyl acetate for 7 days.[47] Control rats were fed basal diet. Retinyl acetate was classified as very toxic. Both food consumption and body weights were decreased throughout the experiment, which may have resulted from appetite depression. The following signs were observed: slight protrusion of the eyeballs, thinner pale body, rough hair, blepharoptosis, unsteady gait, epistaxis, and death. The pooling of abdominal fluid, diarrhea, and expansion of the intestine were reported at necropsy. Prothrombin and kaolin-activated partial thromboplastin time indices were reduced to 60% and 30%, respectively. A dietary level of 0.5% retinyl acetate appeared to have been too high for observation of a hemorrhagic effect, in that other toxic syndromes occurred and food intake was decreased.

A study was performed to analyze the vitamin A content of liver and serum from 13 adult female African green vervet monkeys (*Chlorocebus aethiops*).[38] The monkeys were wild-caught and held in captivity for 2 years, during which they consumed a standard primate diet. Monkeys were fed lab diet containing 45 nmol (43 IU) vitamin A/g dry food (as retinyl acetate). Results relating to distribution after feeding are included in the section on Toxicokinetics. Hypertrophy and hyperplasia of hepatic stellate cells were observed, which, in conjunction with elevated hepatic vitamin A, are evidence of toxicity.

Oral

Human

Retinyl Palmitate

One hundred and twenty-nine participants with severely sun-damaged skin on their lateral forearms were randomized to receive placebo or 25,000; 50,000; or 75,000 IU/day retinyl palmitate for 12 months.[48] While the primary study end points were clinical and laboratory safety of vitamin A (retinyl palmitate), toxicity information was reported. The measurement end points included quantitative karyometric image analysis and assessment of retinoid and rexinoid receptors in sun-damaged skin. There were no significant differences in expected clinical or blood toxicities between the groups of all participants randomized to placebo, 25,000 IU/day, 50,000 IU/day, and 75,000 IU/day. There was no evidence of a dose response for any of the following toxicities: alopecia, cheilitis, conjunctivitis, dry skin, peeling, epistaxis, headache, muscle stiffness, dysuria, exanthema, serum liver function tests (i.e., aspartate aminotransferase, alanine aminotransferase, and serum alkaline phosphatase and triglycerides). Because liver scans were only to be repeated in participants who experienced severe clinical or other signs of toxicity, there were no participants who underwent repeat liver scans during this clinical trial.

Karyometric features were computed from the basal cell layer of skin biopsies, and a total of 22,600 nuclei from 113 participants were examined, showing statistically significant, dose-response effects for retinyl palmitate at the 25,000 and 50,000 IU/day doses. These karyometric changes were associated with increases in retinoic acid receptors αand β, and retinoid X receptor α at the 50,000 IU/day retinyl palmitate dose. It was concluded that the the retinyl palmitate doses of 50,000 and 75,000 IU/day for 1 year proved to be safe and more efficacious than the 25,000 IU/day dose and can be recommended for future skin chemoprevention studies.[48]

Oral/Dermal

Human

Retinyl Palmitate and Retinol

Two groups of 14 female volunteers of child-bearing age were maintained on a vitamin A-poor diet and treated topically for 21 days with creams containing 0.30% retinol or 0.55% retinyl palmitate on approximately 3000 cm^2 of their body surface area, amounting to a total of approximately 30,000 IU vitamin A/subject/day.[39] Subsequently, after a 12-day wash-out period, the study groups received single oral doses of 10,000 IU or 30,000 IU retinyl palmitate. No objective adverse effects or individual complaints were recorded after the oral administration of retinyl palmitate at 10,000 IU or 30,000 IU. Additionally, no adverse systemic effects were observed. Results relating to skin irritation are included in that section of this report.

Ocular Irritation

Retinyl Propionate

Thirty-three subjects (18 to 55 years old) participated in a 28-day ophthalmologic and dermatologic safety evaluation of a facial moisturizer product containing 0.3% retinyl propionate (test product).[49] The subjects were instructed to apply the product twice daily throughout the duration of the study. For all ophthalmologic evaluations, average scores relating to the following indicated little to no change from baseline: eyelid swelling, palpebral conjunctiva, and bulbar conjunctiva. It was concluded that, overall, the test moisturizer was well-tolerated by the subjects. Results relating to skin irritation are included in that section below.

The EpiOcular™ human cell construct was used to assess the potential ocular irritancy of a face cream containing 0.3% retinyl propionate.[50] The MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay was used to assess cellular metabolism after exposure to the test substance for various exposure times. This assay measures the NAD(P)H-dependent microsomal enzyme reduction of MTT to a blue formazan precipitate. The duration of exposure resulting in a 50% decrease in MTT conversion in EpiOcular™ human cell constructs treated with the test substance (t_{50}), relative to controls, was determined. The face cream was not observed to reduce MTT directly in the absence of viable tissue, and a t_{50} of > 24 h was reported. The t_{50} for the positive control (0.3% Triton®-X-100) was 31 minutes.

Skin Irritation

Animal

Retinoic Acid

The effects of daily topical application of a therapeutic concentration of all-*trans* retinoic acid on epidermal thickness and dermal collagen and glycosaminoglycan (GAG) biosynthesis rates were studied during a period of 40 days.[51]

Each morning, approximately 0.1 ml all-*trans* retinoic acid (0.05% in ethanol) was applied by rubbing a cotton-soaked swab to the dorsal side of both ears of guinea pigs (number not stated). Control animals were treated with water. Beginning at 5 to 6 days, skin treated with all-*trans* retinoic acid became erythematous and scaly, both of which persisted throughout the experiment. The epidermis became thickened and hyperplastic, with marked psoriasiform histologic features.

A cream formulation containing all-*trans* retinoic acid (3.34 mM [0.1% all-trans retinoic acid] or 16.5 mM [0.5% all-trans retinoic acid]) was applied to dorsal skin of 6 castrated male pigs daily for 24 weeks.[52] Eight 2 x 2 cm test patch areas were established dorsally. Four patch areas were situated both to the left and right of the spine, with a 1.5-cm space interval. The designated cream was applied by rubbing an aliquot (0.005 to 0.01 g/cm^2) onto the patch area. The test protocol included a no-treatment placebo. Redness and scabbing were observed in areas treated with the all-*trans* retinoic acid creams. All areas treated with 0.1% or 0.5% all-*trans* retinoic acid creams appeared inflamed and sensitive.

Human

Retinyl Palmitate and Retinol

Two groups of 14 female volunteers of child-bearing age were maintained on a vitamin A-poor diet and treated topically for 21 days with creams containing 0.30% retinol or 0.55% retinyl palmitate on approximately 3000 cm^2 of their body surface area, amounting to a total of approximately 30,000 IU vitamin A/subject/day.[39] Subsequently, after a 12-day wash-out period, the study groups received single oral doses of 10,000 IU or 30,000 IU retinyl palmitate, corresponding to the maximal EU allowance during pregnancy or three-times the allowance, respectively. Transient mild (retinyl palmitate group) to moderate (retinol group) local irritation reactions on treatment sites were noted. After approximately 1 week of topical application, 13 of 28 study subjects had skin reactions (rash, itching) on treated sites.

In the retinol treatment group, skin reactions were observed in 9 or 14 subjects. For 1 subject with moderate to severe reactions, treatment was discontinued for 4 days. Although 2 of 14 subjects in the retinyl palmitate treatment group experienced itching, treatment was continued as scheduled. In all subjects, skin reactions stabilized and/or subsided after approximately 10 to 12 treatment days. One-hundred nine (total) adverse events in 25 subjects were reported. The severity of adverse events was rated by the medical investigator to be mild for 70 events, moderate for 37 events, and severe for 2 cases. For 44 adverse events, a possible or probable relationship to the treatment was considered, including itch, rash, or other dermal manifestations at the treatment sites. Additional study results are included at the end of the preceding section on Repeated Dose Toxicity.[39]

Retinol and Retinoic Acid

A randomized, double-blind study comparing topical retinol, retinoic acid, and a vehicle alone was performed.[53] Each retinoid or the vehicle was applied to buttock skin, and biopsies were taken at 0 h, 6 h, 24 h, and 96 h. When compared to the results for retinol (no or trace erythema), retinoic acid induced significant erythema. Retinoic acid also caused epidermal thickening when compared to retinol.

A prospective, 2-year multicenter study (double-blind, randomized, placebo-controlled) was performed to evaluate the safety and efficacy of tretinoin emollient cream 0.05% for the treatment of moderate to severe facial photodamage.[54] The study involved 204 subjects who applied the tretinoin emollient cream or a vehicle emollient cream (placebo) to the entire face once per day for up to 2 years. When compared to the placebo, application of the tretinoin cream resulted in significantly greater ($p < 0.05$) improvement in the following: clinical signs of photodamage (fine and coarse wrinkling, mottled hyperpigmentation, lentigines, and sallowness), overall photodamage severity, and the investigator's global assessment of clinical response . The results at histologic evaluation following application of tretinoin cream were as follows: no increase in keratinocytic or melanocytic atypia, dermal elastosis, or untoward effects on stratum corneum. Also, when compared to the placebo cream, there was a significant increase in facial procollagen 1C terminal, a marker for procollagen synthesis, at month 12 ($p = 0.0074$). This finding relating to facial procollagen synthesis resulted from immunohistochemistry studies conducted at 3 study centers.

The results from a study of 2 safety trials on isotretinoin (13-*cis*-retinoic acid) indicated that the most common adverse mucocutaneous side effects that patients complained about were: cheilitis, chapped lips, dry skin, redness or rash, peeling, dermatitis, itching, epistaxis, mucosal dryness, and dry or irritated eyes.[55]

Retinyl Propionate

The clinical and histological effects of retinyl propionate cream (0.15% retinyl propionate) on extrinsic skin photo-aging in man was assessed in a double-blind, randomized placebo-controlled study involving 80 subjects (mean age = 50 years; 11men, 69 women).[56] The subjects were randomly allocated to receive either retinyl propionate cream or its placebo base. The study period initially lasted 24 weeks (weeks 0 to 24), but was extended to 48 weeks (weeks 24 to 48) for those who agreed to continue. Subjects were instructed to apply a pea-sized amount of the cream, twice daily, to the face and dorsa of both forearms and hands. Of the 80 subjects, 75 completed the initial 24-week study period. Two of the 5 subjects who withdrew did so because of adverse cutaneous reactions (dermatitis of the face and forearms) to the retinyl propionate cream. One subject who withdrew also developed new actinic keratosis-like lesions on the forearm. The remaining 3 of 5 subjects who withdrew did so for reasons unrelated to application of the placebo or retinyl propionate cream. During the first 12-week treatment period, 10 subjects reacted to the placebo, and the reactions observed consisted of irritation, redness, and dryness or flaking of the face. These reactions, sometimes observed on the forearm as well, were also observed in 8 subjects tested with the retinyl propionate cream. Two of the 8 subjects withdrew from the study due to these reactioins to the retinyl propionate creram. Sixty subjects agreed to participate in the study for an additional 24weeks, and only 1 subject withdrew due to skin irritation. The authors noted that the overall findings in this study suggest that it is relatively unlikely that the retinyl propionate cream has an important influence on photoaging, but mild clinical improvements seem possible.

A facial moisturizer containing 0.5% retinyl propionate was evaluated in a 21-day cumulative irritation study involving 26 healthy subjects (18 to 65 years old).[57] The product (0.2 ml) was applied to a 2-cm x 2-cm occlusive patch, secured with hypoallergenic tape, for approximately 24 h per day. The distilled water negative control (0.2 ml) and the sodium lauryl sulfate (SLS, 0.2 ml) positive control were similarly applied. There was no evidence of significant irritation after 21-days of application of the moisturizer or distilled water. SLS was moderately irritating.

Thirty-three subjects (18 to 55 years old) participated in a 28-day ophthalmologic and dermatologic safety evaluation of a facial moisturizer product containing 0.3% retinyl propionate (test product).[49] The subjects were instructed to apply the product twice daily throughout the duration of the study. Two subjects had adverse reactions during the study. One subject had mild erythema and pruritus in the right temple area, lateral upper eyelid, and malar cheek after using the moisturizer for one week. The other subject experienced a severe sinus infection. Dermatological grades were indicative of little to no change in facial or neck skin (irritation and dryness) under the conditions of the test. It was concluded that, overall, the test moisturizer was well-tolerated by the subjects. Results relating to ocular irritation are included in that section above.

Phototoxicity and Photoallergenicity

Human

Retinoic Acid

Four prospective, randomized, and controlled trials involved healthy volunteers (≥ 18 years old) at 2 independent research facilities.[58] The 4 groups were described as follows: Trial A - (16 subjects: 13 females, 3 males), Trial B - (35 subjects: 20 females, 15 males). Trial C - (15 subjects: 12 females, 3 males), and Trial D - (53 subjects: 40 females, 13 males).

In each trial, the minimal erythema dose (MED) was determined using timed exposures to a fixed dose of full spectrum UVA + UVB energy (1 Med/h = 5.6 x 10-6 effective W/cm^2). In the phototoxicity trials (A and B), tretinoin 0.05% gel was applied under occlusion for 24 h using duplicate locations. The skin was examined for contact dermatitis at the end of the application period, after which the test article was reapplied. One of each pair of sites was exposed to ultraviolet radiation (Trial A: 10 x MED for UVA, followed by 0.5 MED for UVA + UVB; Trial B: 0.5 MED for UVA + UVB, followed by 10J/cm^2 of UVA), together with one control site. Skin sites were examined for phototoxic reactions at 24, 48, and 72 h (Trial A) and at 24 and 48 h (Trial B).

Trials C and D were photoallergenicity trials. In Trial C, the test article, tretinoin 0.05% gel, was applied in duplicate under occlusion for 24 h. Application was repeated twice weekly for 3 weeks (6 applications total) during the induction phase. The subjects returned to the testing center for patch removal, irradiation, and reaction evaluation. One of each pair of test article sites and an untreated control site were exposed to 10 x MED for UVA, followed by 0.5 x MED for UVA + UVB. In Trial D, sites were exposed to 3 x MED for UVA + UVB. Following a 14-day (Trial C) or 9- to 14-day non-treatment period, paired patches with test articles were applied to new sites for 24 h. During the challenge phase, 10 x MED for UVA was applied, followed by 0.5 MED of UVA + UVB (Trial C) , or 0.5 MED of UVA + UVB , followed by 10 J/cm^2 of UVA (Trial D). One of each pair of sites plus an untreated control site were irradiated (Trials C and D). Trial D did

not use a non-irradiated control site. Study results indicated that neither phototoxic nor photoallergic reactions occurred with tretinoin 0.05% in a gel formulation.

Retinyl Propionate

The phototoxicity of a face cream containing 0.4% retinyl propionate was evaluated using 10 healthy adult subjects (4 women, 6 men; 18 to 31 years old).[59] The subjects were fair-skinned individuals with Fitzpatrick skin types I, II, or III. The test procedure was described as a one-time 24-h semi-occlusive application of the product to duplicate sites on the lower back area, followed by exposure to UV radiation. Approximately 0.2 ml of the product was added to a semi-occlusive patch (2 x 2 cm) that remained in place for approximately 24 h. Patch removal was followed by immediate exposure to UVA (10 J/cm^2) and 0.5 MED of solar simulated radiation (SSR). The duplicate patch served as an unirradiated control. An adjacent skin site that was not treated was also exposed to UVA + SSR and served as an irradiated untreated control. Reactions were scored at 10 minutes post-irradiation and 24 h and 48 h later. No adverse experiences or unanticipated reactions were encountered or reported by any of the panelists. There also were no reactions that were suggestive of phototoxicity in any of the subjects. It was concluded that the face cream does not possess a detectable phototoxic potential in human skin.

The photoallergenicity of a face cream containing 0.4% retinyl propionate was evaluated using 28 healthy adult subjects (14 men, 14 women; 18 to 27 years old).[60] The subjects were fair-skinned individuals with Fitzpatrick skin types I, II, or III. The test protocol was described as a repeat insult patch test in which the test substance and ultraviolet radiation (solar simulated radiation) were administered to the same designated test sites on the mid or lower back area repeatedly over a 3-week period (6 induction exposures total). During induction, approximately 0.2 g of the product was added to each semi-occlusive patch (2 x 2 cm; patches in duplicate) that remained in place for 24 h. Patch removal was followed by exposure to 3 MED's from the xenon arc solar simulator. The site remained uncovered for 48 h post-exposure, after which the semi-occlusive patch was reapplied to the same site for 24 h. Patch removal was then followed by re-exposure to 3 MED's of solar simulated radiation. The challenge phase was initiated after a non-treatment period of 17 days. The product (0.2 g on semi-occlusive patch) was applied in duplicate to new designated skin sites (2 x 2 cm) on the opposite side of the lower back for 24 h. One of the sites was then irradiated with ½ an MED of SSR + UVA (4 J/cm^2). The duplicate patch remained unirradiated and served as a control treated site. An additional untreated normal skin site was also exposed to ½ MED + UVA (4 J/cm^2) and served as an irradiated, untreated control. Test sites were examined for reactions at 24 h, 48 h, and 72 h after UV exposure. No adverse experiences or unanticipated reactions were encountered or reported by any of the panelists. Following the challenge phase, no reactions were observed in any of the panelists at 24 h, 48 h, or 72 h post-exposure. It was concluded that the face cream does not possess a detectable photocontact-sensitizing potential in human skin.

Skin Sensitization

Human

Retinyl Propionate

The skin sensitization potential of a face cream containing 0.4% retinyl propionate was evaluated in a repeated insult patch test involving 102 normal subjects (between 18 and 74 years old).[61] Semi-occlusive patches (2 cm x 2 cm) containing the product were applied to the infrascapular area of the back (to right or left of midline) or to the upper arm. The patches were secured with hypoallergenic tape. The induction phased consisted of 9 consecutive 24 h applications of the product. The challenge phase was initiated after a 10- to 15-day non-treatment period. Challenge patches were applied to previously untreated sites for 24 h, after which reactions were scored at 24 h and 48 h post-removal. There was no evidence of sensitization reactions to the face cream in this study.

Modification of Allergic Sensitization/Immune Responses

Animal

Retinyl Palmitate

The role of vitamin A I n the diet on allergic sensitization during lactation and after weaning was investigated using an *in vivo* system for postnatal allergic sensitization in Balb/c mice.[62] Different diets (basal/vitamin A (as retinol equivalents); elimination/vitamin A (as retinyl palmitate supplemented) were fed to the dams throughout lactation and directly to the pups after weaning. The diets were defined as follows: basal diet (4.5 mg vitamin A [i.e., 4500 retinol equivalents]), VA-elimination diet (i.e., prepared using a vitamin A-free 'vitamin mix']), and vitamin A supplemented diet

(122,000 retinol equivalents as retinyl palmitate [i.e., 216 mg of retinyl palmitate/kg diet supplemented]). Allergic sensitization was induced with a single peritoneal ovalbumin (OVA) injection at day 28 after weaning. The phenotype of lymphocytes was analyzed by flow cytometry and functional data were obtained by analysis of IL-4/IFN-γ cytokine production and antibody production (OVA-specific IgG1 and IgE) in the offspring.

Vitamin A/retinyl palmitate supplementation during lactation and after weaning decreased CD3+, CD4+, CD8+, and B220+ populations in splenic lymphocytes and significantly enhanced IL-4 production and OVA-specific IgE measured after sensitization. In contrast, mice fed the vitamin A-elimination diet displayed no significant alteration of lymphocyte numbers and a slightly increased IL-4 production after sensitization. Thus, a single allergen injection during postnatal development induced allergic sensitization, the degree of which depended on the vitamin A content of the maternal diet during lactation and the diet of the pups after weaning. The authors suggested that these findings support a view that dietary vitamin A levels can play an important role determining the severity of the allergic sensitization.[62]

Retinol, Retinoic Acid, and Retinol Acetate

To determine whether retinoic acid could prevent inhibition of the mixed lymphocyte response (bovine lymphocytes) by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), cultures were incubated with TPA (10^{-8} M) and retinoic acid (0.1, 1.0, or 10 µM).[63] TPA alone depressed the mixed lymphocyte response, and addition of retinoic acid did not reverse this inhibition over the range of concentrations tested. However, retinoic acid alone (10 µM) inhibited the mixed lymphocyte response by ~75%. It also appeared that the combination of retinoic and TPA was more inhibitory than TPA alone. In other experiments, neither retinol (up to 100 µM) nor retinol acetate (up to 10 µM) reversed the inhibitory effects of TPA on the bovine mixed lymphocyte culture, and retinol alone was slightly inhibitory. Because retinoic acid alone depressed proliferation in the mixed lymphocyte response, the effects of retinoic acid on lymphocyte mitogenesis in response to the lectin phytohemagglutinin (PHA) were evaluated. At an optimal concentration of 0.1%, PHA caused an increase in [^{3}H]thymidine incorporation that amounted to 50 to 100-fold over the unstimulated cells. Over a concentration range of 0.1 to 10 µM, retinoic acid had little effect on this response. The effect of retinoic acid on DNA synthesis is summarized in the Genotoxicity section of this report.

A study was peformed to clarify the actioin of vitamin A on mucosal immunity associated with interleukin-5 (IL-5). The effects of retinyl acetate on mucosal IgA levels in IL-5 receptor α-chain knockout (IL-5Rα$^{-/-}$) mice was examined.[64] Daily supplementation of retinyl acetate (1 mg/mouse) increased Th2 cytokine levels and a number of their positive cells in the small intestinal mucosa of IL-5Rα$^{-/-}$ mice, as observed in wild-type or IL-5Rα$^{+/-}$ mice. Wild type and heterozygous mice increased the IgA level and a number of IgA-containing cells in the mucosa in response to the vitamin A treatment, but not in IL-5Rα$^{-/-}$ mice. Retinyl acetate increased anti-cholera toxin (CT) IgA levels in the mucosa of wild-type mice, improving their survival rate after exposure to 0.4 mg CT. However, retinyl acetate failed to induce resistance to CT toxicity in IL-5Rα$^{-/-}$ mice. The results of this study suggest that IL-5may play an important role in an action of vitamin A on the mucosal IgA system.

Human

Retinyl Palmitate

A study comparing the effect of vitamin A on cytokine secretion by mononuclear cells of adults and preterm newborns was performed.[65] Mononuclear cells (MC) from individuals of the 2 age groups were incubated with retinyl palmitate (0.5 to 50 µM) in the presence of phytohemagglutinin for assessing IL-2 and IFNγ production or LPS for assessing IL-1β, IL-1ra, IL-6, and IL-10 secretion. ELISA was used to test the level of cytokines in the supernatants. Retinyl palmitate *in vitro* inhibited the production of the anti-inflammatory cytokine IL-1ra by MC of preterm newborns and adults, but did not affect the secretion of the pro-inflammatory cytokines IL-1β, IL-6, and IL-10. Retinyl palmitate inhibited IL-10 secretion by cells from adults, but it did not significantly affect this function in cells from newborns, except when a supraphysiological concentration (50 µM) was tested. Additionally, retinyl palmitate stimulated the secretion of IL-2 by cells isolated from adults, but had no effect on those derived from premature neonates. The authors suggested that retinyl palmitate may affect the immune function of premature infants via inhibition of IL-1ra secretion.

A study was conducted to determine how retinol supplementation modified associations between gut-cytokine immune responses and the resolution of different diarrheal pathogen infections.[66] Stools were collected from 127 children (5 to 15 months old) enrolled in a randomized, placebo-controlled vitamin A supplementation trial. The children were screened for enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and *Giardia lamblia*. Fecal concentrations of the following were measured using an enzyme-linked immunosorbent assay: interleukin (IL)-6, IL-8, IL-4, IL-5, IL-10, monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Retinol-

supplemented children with fecal MCP-1 or IL-8 concentrations less than the median of detectable concentrations and IL-10 concentrations of at least median concentrations had longer durations of EPEC infection than did children in the placebo group. In supplemented children, detectable fecal TNF-α or IL-6 concentrations were associated with shorter ETEC infection durations, whereas, MCP-1 concentrations of at least the median were associated with longer infection durations. Children in this group who had Il-4, IL-5, or IFN-γ concentrations of at least median detectable concentrations had shorter durations of *G. lamblia* infections. It was concluded that the effect of supplementation on association between fecal cytokine concentrations and pathogen infection resolution depends on the role of inflammatory immune responses in resolving specific pathogen infections.

Arthritic Effect

Retinyl Acetate

Female C3H-A^{vy} mice (103 animals total) were used in a study evaluating retinyl acetate-induced arthritis.[67] The following groups were fed retinyl acetate in the diet daily: 83 mg/kg diet (29 mice), 41.5 mg/kg diet (31 mice), and 21.25 mg/kg diet (19 mice). Placebo beadlets were fed to the control group (24 mice). Each dose group was divided into subgroups, in which dosing was begun at the time of conception, at weaning (1 month), or at 3 months of age. The mice consumed a mean of 25 g of diet/week, corresponding to a daily retinyl acetate intake of approximately 300 µg, 150 µg, or 75 µg. The animals were killed after 3 to 16 months. Whole-body radiographs and histologic section of the hindlimbs were scored for the presence and severity of arthritis. When compared to placebo-treated mice, mice of all dose groups had a significantly higher incidence of arthritis. Histologic evidence of enthesopathic disease closely paralleled the radiographic changes, and ranged from small enthesophytes at tendinous and capsular insertions to complete periarticular bony bridging. Articular cartilage was not grossly affected. Both the incidence and severity of arthritis were significantly correlated with the total dose of retinyl acetate administered. Bony metaplasia was also observed.

Effect on Procollagen Expression

Retinoic Acid

All-*trans* retinoic acid (0.01%, 0.25%, or 0.05%) or its vehicle (70% ethanol, 30% polyethylene glycol, and 0.05% butylated hydroxytoluene) was applied to the buttock skin of elderly male subjects (mean age = 76 years).[68] Applications, under continuous occlusion, were made 3 times per week for 8 weeks. Biopsy specimens were obtained every 2 weeks and immunohistochemical analysis was performed to determine the levels of type I procollagen expression and inflammatory cell infiltration. Regardless of the concentration applied, topical all-*trans* retinoic acid increased type I procollagen expression in human skin after 2 weeks. Only 0.01% all-*trans* retinoic acid continuously increased type I procollagen expression for up to 8 weeks. Following 4 weeks of application, significant infiltrations of macrophages and neutrophils were observed in 0.025% and 0.05% all-*trans* retinoic acid-treated skin. Additionally, procollagen expression had returned to baseline after 4 weeks of application. It was concluded that excessive all-*trans* retinoic acid -induced inflammation might prevent collagen accumulation in aged skin despite the positive effect of all-*trans* retinoic acid on collagen production.

Case Reports

Retinol

A 25-year-old male patient had a history of increased vitamin A intake from a natural source, in addition to a high dose of vitamin A supplements.[69] He had supplemented his food intake with high doses of vitamin A (220,000 IU/day) and consumed steroidal anabolic drugs. The patient was diagnosed with chronic liver disease (attributed to increased vitamin A intake) with severe fibrosis, signs of portal hypertension, and marked hyperplasia of Ito cells. It was noted that chronic vitamin A toxicity may produce severe liver damage. The authors also noted that additional toxicity produced by anabolic intake could not be ruled out.

A 60-year-old male presented with symptoms of muscle soreness, alopecia, nail dystrophy, and ascites.[70] His clinical history revealed ingestion of large doses of vitamin A. He had been taking 500,000 units of vitamin A daily for 4 months, then 100,000 units monthly for 6 months. The patient continued to deteriorate with the development of refractory ascites, renal insufficiency, encephalopathy, and failure to thrive. Liver biopsy revealed the presence of Ito cells and vacuolated Kupffer cells, without the presence of cirrhosis.

Facial dermatitis developed in a 54-yer-old female, who had no history of allergy nor atopy, after using an antiwrinkle cream.[71] A use self-test of the product resulted in development of itchy erythema. Subsequent avoidance of the

product resulted in completely normal skin. Results of a repeated open application test (ROAT) of the cream indicated a positive reaction after 2 days (i.e., 5 applications). Test results for one of the ingredients, retinyl palmitate (in polycaprolactone [PCL]) were strongly positive (++ reaction). Subsequent patch tests yielded a + reaction for 5% retinyl palmitate in petrolatum, negative results for 5% PCL in petrolatum, and a ++ reaction for retinyl palmitate in PCL. When the patient performed an ROAT on 5% retinyl palmitate in petrolatum, a strongly infiltrated and itchy reaction (spreading to the forearm), appeared within 2 days (3 applications). Retinyl palmitate in PCL and 5% retinyl palmitate in petrolatum were both tested in consecutive controls. Retinyl palmitate in PCL yielded a doubtful reaction (+?) on day 3 in 1 of 25 control patients. Retinyl palmitate (5% in petrolatum) yielded a doubtful reaction (+?) on day 3 in 3 of 27 patients. All were negative on day 7.

In the following case report, intrahepatic cholestasis was caused by vitamin A intoxication. A 46-year-old patient consumed a shake and 2 multivitamin tablets for 12 years.[72] This equated to more than the recommended daily allowance for vitamin A consumption. Deranged liver function tests were consistent with a cholestatic process. Liver biopsy revealed features that were pathognomonic of vitamin A toxicity, without the usual fibrosis. When administration of the supplements ceased, both the jaundice and alkaline phosphatase levels normalized completely.

A case of acute hypervitaminosis A was reported.[73] An 18-year-old female presented with complaints of headache, vomiting, back pain, and diplopia after ingesting a high-dose vitamin A capsule (~ 10 million international units). The following signs were reported: bilateral papilloedema, slightly dilated pupils symmetrically (reaction to light noted), visual acuity of 6/60 (left eye) and 6/18 (right eye), and bilateral 6th cranial nerve palsy (more marked on the left side). MRI of brain and orbits were normal.

Retinoic Acid

Sudden redness and itching at application sites were reported for 3 patients after 1, 7, and 14 weeks of daily treatment with retinoic acid cream (0.05% retinoic acid), and patch tests were performed.[74] Test results were read after 72 h. The control groups were as follows: retinoic acid cream (25 subjects), retinoic acid (0.05%) in absolute alcohol (12 subjects), retinoic acid (0.005%) in absolute alcohol (10 subjects), retinoic acid (0.05%, without occlusion) in absolute alcohol (10 subjects), and absolute alcohol (10 subjects). Patch testing with the cream and with 0.05% retinoic acid in absolute alcohol caused strongly positive reactions in the patients, but only slight erythema in 3 control subjects. In that patch testing with 0.005% retinoic acid in absolute alcohol did not induce reactions in control subjects, but induced positive reactions in 3 patients, the authors noted the probability that these later reactions were due to contact allergy. At microsocpic examination of sites with positive reactions, intact epidermis and dense infiltration by mononuclear cells were reported.

Gangrenous cheilitis was associated with all-*trans* retinoic acid therapy for acute promyelocytic leukemia in a 67-year-old female patient.[75] The patient was started on induction treatment with all-*trans* retinoic acid (45 mg/m^2), which was discontinued on day 20. After treatment resumed on day 29, the gangrenous chelitis developed into black, painful eschars.

Retinyl Acetate

A 44-year-old male developed eczema while employed in industrial product ion of vitamins.[76] The job involved drying, sieving, and packing vitamin A (as retinyl acetate), and the eczema cleared after the patient changed jobs. Patch testing at concentrations (in petrolatum) of 0.1% to 10% revealed reactions ranging from + to ++ on days 2 and 3. Only ++ reactions were observed at concentrations ranging from 1% to 10%.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Animal

Oral

Retinol

Female Ibm RORO (spf) rats (20 rats [test] and 10 controls; 12 weeks old) were used to investigate whether high oral doses of vitamin A (retinol) caused fetal malformations and the extent at which retinyl esters are transferred from the mother to the fetuses.[77] Prior to mating, the control group was fed a basal diet that contained 4.5 x 10^3 RE/kg (1 retinol

equivalent [RE] = 1 µg retinol), and this diet was enriched with dry vitamin A palmitate type 500 (stabilized food industry product) containing 150.2 x 10^3 RE/g. Rats of both control and experimental groups had free access to food. In the experimental group, dietary vitamin A was stepwise increased to obtain retinyl ester concentrations of > 1525 nmol/ liter plasma before mating. Over an 8-month period prior to mating, vitamin A dosages of 15.2 x 10^3 RE/kg diet were fed for 40 days, 22.7 x 10^3 RE/kg diet were fed for 50 days, and the dose was increased to 45.5 x 10^3 RE for another 40 days and to 52.5 x 10^3 RE for 90 days. The mean body weights of experimental and control rats were not significantly different. All-*trans*, 13-*cis*, 4-oxo-all-*trans*, and 5,6-epoxy-all-*trans* retinoic acid vitamin A metabolites were determined in maternal and fetal plasma. Following high vitamin A intake, 4-oxo- and 5,6-epoxy retinoic acid concentrations were significantly higher in in the fetuses than in their mothers. Though high vitamin A intake by rat dams resulted in high maternal and fetal plasma concentrations of vitamin A and its metabolites, fetal malformations were not observed. The authors noted that this finding may be due to the fact that circulating retinyl esters are not teratogenic and that, after crossing the placental barrier, they are stored mainly in fetal liver.

Retinyl Palmitate

The effects of retinyl palmitate ingestion on sexual maturation and mammary gland development were determined in the context of a human food-based diet (whole food diet).[78] Female adolescent Sprague-Dawley rats randomized into 3 dietary groups (6 rats per group) were used. At 20 days of age (postnatal day 20 [p20]), female rats received either a whole-food diet with adequate levels of vitamin A, a diet with a 5.5-fold increase in vitamin A from fruits and vegetables (S diet), or a diet with a 6.2-fold increase in vitamin A provided as retinyl palmitate (RP diet). To determine the effect of dietary intervention on pubertal mammary gland development, rats were fed the experimental diets from p21 to p63 and had free access to food and distilled water. All rats were killed at p63.

To evaluate the effects of diet on early estrous cycles, defined as cycles within the first 2 weeks of sexual maturation, 12 rats per group received vaginal lavages daily from p43 through p50. To evaluate estrous cycles in more mature rats, 24 rats per dietary group were evaluated from p51 to p58. All rats were killed at p63.

The onset of vaginal opening was evaluated as a marker for sexual maturation. The age of vaginal opening onset was significantly delayed in rats fed the S diet ($p < 0.001$), when compared to those fed the adequate diet and the RP diet. The authors concluded that the S diet suppressed the onset of sexual maturation. The S diet also inhibited markers of mammary alveologenesis more than the RP diet. Furthermore, the authors concluded that these data demonstrate that the amount and source of vitamin A consumed by adolescent female rats can influence the onset of puberty and mammary gland alveolar development. Effects on mammary carcinogenesis included in this study are found in the Carcinogenicity section of this report.[78]

A study was performed to evaluate the effects of defined doses of retinyl palmitate at the critical time of limb morphogenesis in Swiss Webster albino mouse embryos.[79] Pregnant Swiss albino mice were administered retinyl palmitate (10,000 or 15,000 IU/kg, i.p.) on different days of pregnancy. The higher dose produced malformations in the forelimbs, by day 10, in 28.6% of mice and in the hindlimbs, by day 11, in 20.6% of the mice. Limb abnormalities (at both doses) were as follows: unilateral or bilateral micromelia (abnormally short) in the forelimbs and hindlimbs, shorter unilateral anterior and posterior limbs, limb malrotation, absence or malformation of fingers or toes, and an increased cleft between the metacarpal or metatarsal bones. Furthermore, 2 injections in one day with the lower dose resulted in more teratogenic effects than a single 15,000 IU/kg injection. Two injections of either dose on day 10 caused a greater incidence of embryo absorption.

The effects of retinyl palmitate supplementation, during gestation and lactation, on oxidative stress parameters of maternal and offspring tissues of female Wistar rats was studied.[80] Each group of pregnant female rats (except for one group of 7 rats), contained 6 animals. The respective groups received retinyl palmitate during pregnancy and lactation (21 days of gestation and 21 days of lactation) at oral (gavage) doses of 2500, 12,500, or 25,000 IU/kg/day. The maximum dose volume was 0.5 ml. The control group was dosed with saline. An increase in oxidative-damage markers in the reproductive tissues and plasma of dams was observed. In uteri, lipid peroxidation was increased at all doses ($p < 0.0001$). Also, the liver and kidney had significant alterations in glutathione-S-transferase (GST) activity. It was increased in the liver of dams and decreased in the kidneys of mothers and offspring. In pups, supplementation decreased the total antioxidant potential of the liver, along with decreased superoxide dismutase/catalase activity ratio in the kidney. The levels of lipoperoxidation were increased in male offspring, but decreased in female pups. Collectively, the results suggest that excessive vitamin A intake during gestation and lactation, at oral doses sufficient to produce signs of maternal toxicity, may cause adverse effects in the developing offspring.

A study was performed to investigate the effects of vitamin A supplementation in pregnant and nursing rats on maternal and offspring striatum and hippocampus.[81] Female Wistar rats (7 per group) were orally supplemented with retinyl palmitate (2500; 12,500; and 25,000 IU/kg/day) or saline (control) throughout pregnancy and nursing. A homing test was performed on offspring on postnatal days (PND) 5 and 10, and an open field test (OFT) was carried out on dams and offspring on PND 19 and 20. Redox parameters were evaluated at PND 21 for both dams and offspring. Supplementing Vitamin A during pregnancy and nursing increased the ratio of superoxide dismutase/catalase (SOD/CAT) ratio and oxidative damage in maternal and offspring striatum and hippocampus. Additionally, these effects were accompanied by behavioral alterations observed through the homing and OFT tests.

Retinol and Retinoic Acid

The teratogenicity of retinol, all-*trans*-retinoic acid, and 13-*cis*-retinoic acid (each in corn oil) was evaluated using groups of mature Wistar rats.[82] After mating, groups of pregnant females received the following single oral doses on gestation day 10: retinol (50-500 mg/kg; 5-10 rats/group), all *trans*-retinoic acid (20-100 mg/kg; 8-9 rats/group, and 13-*cis*-retinoic acid (50-1000 mg/kg; 6-8 rats/group). Twenty rats served as controls. A single oral dose of all-*trans* retinoic acid on day 10 *post coitum* produced consistently smaller fetuses, with a dose-dependent decrease in fetal weight and length. Dosing with all-*trans* retinoic acid did not cause an obvious effect on placental weight. A distinct dose-dependent increase in the percentage of fetuses with major defects was observed after dosing with all-*trans* retinoic acid. Most of the litters had similar defects, especially at high doses. The majority of defects at low doses (20and 30 mg/kg) were in the craniofacial region, and there was a high incidence of spina bifida and talipes (club foot) after dosing with 100 mg/kg. The average number of defects increased from one per fetus at the low dose (20 mg/kg) to approximately 8 malformations per fetus at the 100 mg/kg dose.

Dosing with 13-*cis*-retinoic acid produced similar defects, though a considerably higher dose was necessary to produce a significant incidence. There were no dose-related changes in fetal length or weight, even at the highest dose (1000 mg/kg). However, the number of malformations per fetus was increased at 1000 mg/kg. Dosing with retinol produced malformations that were similar to those produced after dosing with all-*trans*-retinoic acid or 13-*cis*-retinoic acid. However, at the 2 highest doses (200 and 500 mg/kg) the incidence pattern was different from that of all-*trans*-retinoic acid. Retinol produced a clear dose-dependent increase in the number of malformations per fetus. On day 10 in another experiment, all-*trans*-retinoic acid was administered intraperitoneally to female Wistar rats (after mating) at single doses of 5 mg/kg (2 rats), 25 mg/kg (5 rats) and 50 mg/kg (5 rats). The same 20 rats in the oral dosing experiment served as controls. Results indicated a range of malformations similar to those observed after oral dosing.[82]

In the same study, mated female New Zealand White rabbits received a single dose (10, 20, and 30 mg/kg; n= 6 per group) of all-*trans*-retinoic acid on gestation day 10. When compared to the 6 controls dosed with corn oil, neither dose produced a significant incidence of either external malformations or resorptions. A single oral dose of 13-*cis*-retinoic acid (10 or 20 mg/kg; n = 6 and 2, respectively) also did not produce external malformations.[82]

Retinyl Acetate and Retinoic Acid

Weanling male and female rats (number and strain not stated) were maintained on a vitamin A deficient diet, but supplemented daily with 5 µg retinoic acid or 1 µg retinyl acetate for 10 to 12 weeks.[83] The reproductive performance of these animals was then evaluated. The litters of rats fed the acid were stillborn, whereas, those of rats fed retinyl acetate were viable. When the same male and female rats were continued on their respective dietary regimen for a further period of 8 to 10 weeks, and mated, the female rats did not conceive after mating with males fed retinoic acid. However, the mating of females fed retinoic acid with males fed retinuyl acetate resulted in gestation resorption. Furthermore, both male and female rats initially made deficient in vitamin A and then given retinoic acid or retinyl acetate for 10 to 12 weeks were sterile. Female rats fed retinoic acid in the diet had a normal estrous cycle, but the testes of males treated similarly were reduced in size and had a considerable reduction in spermatozoal counts.

The effects of maternal administration of retinyl acetate on pup development and behavior were evaluated using groups of 14 pregnant nulliparous female Sprague-Dawley rats (≈ 13 weeks old)[84]. On gestation days 6 to 19, retinyl acetate was administered by oral gavage at doses of 25,000, 50,000, or 100,000 international units (IU)/kg/day. Significantly reduced live birth index, but few external abnormalities, were observed in male and female pups from dams dosed with 100,000 I.U./kg/day. Both 24- h and 48-h survival indices were also significantly reduced. The mean pup body weight gain at 100,000 I.U./kg/day was significantly reduced at days 1 to 3, 3 to 7, and 21 to 42. Pinna detachment and eye opening were significantly delayed in all male pups and in female pups from the 50,000 and 100,000 I.U./kg/day groups. Incisor eruption was significantly delayed in male and female pups from the 25,000 and 50,000 I.U./kg/day groups. The absence of

treatment-related effects was associated with the following: dam mean weight change, length of gestation, total litter size, surface righting, cliff avoidance, negative geotaxis, swimming development, open field activity, and discriminatory learning.

Both retinoic acid and retinyl acetate were evaluated for teratogenic activity in a study involving black-hooded rats (substrain of Long-Evans) and A/Jax mice (numbers of animals not stated).[85] Each test substance was administered orally on 2 or 3 days of gestation. Retinoic acid was administered to mice at doses of 1 mg, 2 mg, and 4 mg, and retinyl acetate was administered to mice at doses of 10 mg, 20 mg, and 40 mg. Retinoic acid was administered to rats at doses of 5 mg, 10 mg, and 20 mg, and retinyl acetate was administered to rats at a dose of 60 mg. The administration of retinoic acid (4 mg) on day 8, 9, or 10 of gestation was highly toxic to the fetuses. All but 1 of a total of 88 implantation sites resorbed in response to dosing with retinoic acid (4 mg), and the one that survived was severely malformed. The survivor had a shortened trunk, and extreme exophthalmos. The dose of retinyl acetate (administered on day 9) that had a comparable lethal effect on the fetuses was in the range of 40 mg, and this dose was toxic to the pregnant female as well.

Retinoic acid (2 mg), administered only on gestation day 9, was also highly teratogenic in mice, but did not have an extreme toxic effect on the fetuses. The resorption rate in this group was 70%. Seven animals in the first group of mice treated with retinoic acid (2 mg) on day 9 yielded 15 living fetuses with the following severe malformations: markedly shortened trunk, extreme exophthalmia, microcephalia, astomia, rudimentary or missing mandible, tail stump, no genital opening, malformed sternum and ribs, missing vertebrae, and some degree of agenesis of the hind limb. Spina bifida occulta in the sacral region was observed in 9 of 15 fetuses. The second group of mice (resorption rate = 32%) treated with retinoic acid (2 mg) on day 9 yielded 25 living fetuses. Of the 25 living fetuses, 11 (or 44%) had abnormalities such as cleft palate and some rib malformations. The dose of retinyl acetate (20 mg, administered on day 8 or 9) that produced a comparable pattern of malformations was also toxic to the mother, i.e., 4 of 14 treated females died. Dosing with retinyl acetate (20 mg) on day 9 produced 36% resorption, and 24 of 51 live fetuses had various malformations. A resorption rate of 24% was associated with the 1 mg dose of retinoic acid on day 8, compared to 16% on day 9. Cleft palate was the major abnormality observed after dosing with 1 mg retinoic acid, having occurred in 13 of 14 fetuses on day 8 ad 10 of 13 fetuses on day 9.[85]

Retinoic Acid was also a potent teratogen in the rat. Of 19 implants, 1 live fetus (severely malformed) was observed following dosing with 5 mg on days 9, 10, and 11. After daily dosing with 10 mg retinoic acid, 12 fetuses out of 112 implants survived. Twelve of the surviving fetuses had sacral spina bifida, 1 had exencephaly and spina bifida, and 1 had sirenomelia. When these results were compared with those for retinyl acetate administered over a similar treatment period, a 60 mg retinyl acetate dose caused a resorption rate of 54%, and 80% of the survivors had the following malformations: exencephaly (15%), cleft palate (80%), and ocular malformations (58%). After retinoic acid was administered daily on days 10 through 12, there was less lethality and similar teratogenicity (except for lower dose) when compared to the days 9 to 11 dosing period. At the lower daily dose of 5 mg, only 1 (marked exophthalmos) of 19 living fetuses was malformed. Following daily dosing with 10 mg retinoic acid, 3 of 37 implants were resorbed and the remaining 34 were malformed (many with cleft palate). Daily dosing of one female with 20 mg retinoic acid resulted in 7 of 10 implants resorbed. Collectively, the 3 survivors had exophthalmia, spina bifida, micrognathia, gastroschisis, and agnathia. Treatment with retinyl acetate (60 mg daily) on days 10, 11, and 12 produced a resorption rate of 22%, and 83% of the survivors had cleft palate and ocular malformations. The authors noted that the results of this study indicate that retinoic acid is teratogenic at doses much smaller than those that would be essential for retinyl acetate to produce malformations.[85]

The teratogenicity of all-trans retinoic acid and retinyl acetate was evaluated using groups of 8 pregnant Sprague-Dawley rats.[40] The animals were fed a standard diet containing 14.4 nmol retinyl palmitate/g diet. Single equimolar oral doses (3.5 to 352 µmol/kg body weight) of the test substances were administered in corn oil (~ 250 µl) on day 8.5 of pregnancy. It was noted that the dams dosed with 3.5 µmol/kg of either all-trans-retinoic acid or retinyl acetate were significantly younger and smaller than other animals used in the study. Control animals were dosed with corn oil only. Dams and fetuses were killed on day 19. Regarding the relative teratogenicity and embryolethality of the test substances, all-trans retinoic acid was more potent than retinyl acetate. The no-effect-levels were 3.5 µmol/kg body weight and 35 µmol/kg body weight for all-trans retinoic acid and retinyl acetate, respectively. All-trans retinoic acid caused 100% resorption, which decreased in a dose-dependent manner to 3.6% at 3.5 µmol/kg. All doses of retinyl acetate yielded similar resorption rates of ~ 4%.

Dosing with all-trans retinoic acid caused a significant increase in terata ($p \leq 0.0001$) at 113 µmol/kg. The terata were described as follows: exencephaly (75%), abdominal protrusion (17%), microcephaly (8%), and bulging crown (8%). At a dose of 352 µmol/kg retinyl acetate, terata were apparent and included eye abnormalities (25%) and bulging crowns (75%). Retinyl acetate also induced terata at a dose of 113 µmol/kg, primarily, slight cranial bulges. Terata were not observed at all-trans retinoic acid or retinyl acetate doses ≤ 35 µmol/kg. When skeletal changes were compared with the control group, all-trans retinoic acid was found to cause significant differences in the following: length of the mandible, rib,

radius, ulna, femur, and tibia bones. Dosing with retinyl acetate caused a slight change in length of the mandible. Results relating to acute oral toxicity in this study are included in that section of this report.[40]

The effect of 13-cis-retinoic acid and retinyl acetate on spermatogenesis was evaluated using groups of 30 adult male gerbils (*Gerbillus cheesemani*).[86] Animals of group 1 were injected i.p. with 13-cis-retinoic acid (6 mg) dissolved in 0.2 ml olive oil per 50 g body weight. Injections were made 3 times per week for 6 weeks. Group 2 animals were injected with retinyl acetate (6 mg) dissolved in 0.2 ml olive oil per 50 g body weight. A third group was injected with olive oil only and there was also an untreated control group. 13-cis-Retinoic acid induced almost complete cessation of spermatogenesis and produced alterations in the cytoplasm of Leydig cells. When compared to controls, no differences were observed in the testes of animals injected with retinyl acetate. However, retinyl acetate produced noticeable ultrastrucural changes in Leydig cells. At 12 weeks after the cessation of dosing, the changes observed were reversed.

Retinoic Acid

Oral dosing of 2 groups of 6 time-pregnant female Sprague-Dawley rats (100 and 250 mg/kg body weight all-trans retinoic acid [in corn oil], respectively) on gestation day 12 did not increase the malformation frequency or produce significant maternal or fetal toxic effects.[87] However, in the 100 g/kg group, one litter had a high frequency of neural tube and skeletal defects.

The teratogenicity of 13-*cis* retinoic acid was evaluated using groups of 10 mated female New Zealand White rabbits.[88] The groups were dosed orally (once daily) with 0, 3, or 15 mg/kg of the test substance on gestation days 8 to 11. Neither treatment-related embryotoxic nor teratogenic effects were observed at the 3 mg/kg/day dose level. However, treatment with 15 mg/kg/day significantly increased the rate of fetal resorptions (22%), and 13 of 68 surviving fetuses (16%) were malformed.

Both the embryotoxic and teratogenic potential of all-*trans* retinoic acid and 13-*cis* retinoic acid (both suspended in rapeseed oil) were evaluated using groups of Swiss hare rabbits.[89] The dose groups were described as follows: all-*trans* retinoic acid at doses of 0.7 mg/kg/day (12 rabbits), 2 mg/kg/day (13 rabbits), and 6 mg/kg/day (13 rabbits); 13-*cis* retinoic acid at doses of 3 mg/kg/day (13 rabbits), 7.5 mg/kg/day (12 rabbits), and 10 mg/kg/day (14 rabbits). The untreated control groups consisted of 11 rabbits (all-*trans* retinoic acid) and 2 groups of 11 and 12 rabbits, respectively (13-*cis* retinoic acid). The daily doses were administered orally on gestation days 6 to 18. There were no test substance-related signs of maternal toxicity in any all-*trans* retinoic acid dose group. At the 6 mg/kg daily dose, the resorption rate was significantly increased, and this was due mainly to 2 animals with complete resorption of all implants. Furthermore, teratogenicity was elicited, having affected 8.6% of the recovered specimen, when compared to the lower dose levels. Developmental effects were described as follows: visceral ectopia, skin erosions, acuadia, torsion of hindlimbs, and omphalocele. The incidence of skeletal findings was not sifgnificantly different from the norm. For all-trans retinoic acid, a dose of 6 mg/kg/day was considered the lowest teratogenic and embryotoxic dose detected in this study.

The lowest embryotoxic dose of 13-cis retinoic acid was determimed to be 7.5 mg/kg/day. A borderline effect was apparent at a dose of 3 mg/kg/day, due to 3 litters with an uncommonly high rate of resorption. The percentage of malformed fetuses (13.5%) and of litters with malformed specimen (50%) was significantly increased at a dose of 10/mg/kg/day. Teratogenicity was also observed at doses of 3 mg/kg/day (10% of examined fetuses) and 7.5 mg/kg/day (7.1% of examined fetuses). The pattern of defects was heterogeneous across the groups and included: anasarca, exencephaly, limb defects, brachycaudia, visceral ectopia, omphalocele, an umbilical knot, cleft palate, fusion of sternal elements, hemivertebra, and other minor skeletal anomalies.[89]

Transplacental pharmacokinetics of all-*trans*- and 13-*cis* retinoic were also studied. Pregnant rabbits were treated once daily on gestation days 7 to 12, and plasma and embryo samples were collected for HPLC analysis at various time intervals after the final dose. The main plasma metabolites of all-*trans*- and 13-*cis*-retinoic acid were all-*trans*-β-glucuronide and 13-cis -4-oxo-retinoic acid, respectively. The elimination of 13-*cis* retinoic acid and its metabolites from maternal plasma were much slower than of all-*trans* retinoic acid, resulting in accumulation of the 13-*cis*-isomers in plasma. All-*trans* retinoic acid and all-*trans*-oxo-retinoic acid were efficiently transferred to the rabbit embryo, reaching concentrations similar to the plasma levels. However, the 13-*cis* isomers reached the embryo to a lesser extent.[89]

Groups of 5 or 6 pregnant Jcl:ICR rats were dosed orally with all-*trans*-retinoic acid (in corn oil; doses ranging from 0.08 to 80 mg/kg) once on gestation day 10.5, 11.5, or 12.5 (vaginal plug = day 0).[90] Corn oil was administerd to control mice. The dams were killed on day 18.5 of gestation, and the fetuses were dissected. Fetal palates were observed under a dissecting microscope. The pattern alterations (defined as abnormalities) that were rare in control fetuses were

described as follows: shortness, fusion, maldirection, trirugal malalignment, modified cross, and cross. Though missing palatal ruga-8 was a common pattern alteration in vehicle controls, the incidence of this finding increased in a dose-dependent manner. Cleft palate was observed at doses $\geq$ 20 mg/kg. On each day of treatment, the total incidence of abnormal rugae increased from the dose of 0.3 or 0.6 mg/kg in a dose-dependent manner. Following dosing on day 11.5 or 12.5 of gestation, the incidence of supernumerary posterior to palatal ruga-3 increased in a dose-dependent manner. This increase was not observed on day 10.5. The authors noted that the results of this study indicate that the abnormalities of palatal ruga, missing ruga-8, and supernumerary posterior to ruga 3 are very sensitive indicators of all-*trans*-retinoic acid teratogenicity.

Retinyl Acetate

Pregnant female C57BL/6J mice (number not stated) were exposed to variable doses of retinyl acetate on gestation day 9, and the embryos were evaluated for changes in developing pharyngeal arch and pouch morphology, neural crest cell migration, and marker gene expression.[91] High (100 mg/kg) and moderate (50 and 25 mg/kg) doses of retinyl acetate caused significant craniofacial, cardiac outflow tract, and thymic abnormalities. Low doses of retinyl acetate (10 mg/kg) resulted in craniofacial and thymic abnormalities that were mild and of low penetrance. Exposed embryos had morphologic changes in the 2nd ad 3rd pharyngeal arches and pouches, changes in neural crest migration, abnormalities in cranial ganglia, and altered expression of the *Hoxa3* gene.

The teratogenicity of retinyl acetate was evaluated using groups of 12 to 15 female specific pathogen-free domestic cats (short-hair queens, 1 to 4 years old).[92] The female cats were given diets containing retinyl acetate concentrations of 6000, 306000, or 606000 retinol equivalents (RE)/kg diet (control, 306K, or 606K groups, respectively) for approximately 3 years. One retinol equivalent was defined as equal to 1 µg retinol = 3.3 international units (IU). Following mating, a total of 396 kittens was born in 97 litters. The pregnancy rate, number of kittens per gestation, and gestations per year were not significantly different among the treatment groups. Malformations occurred in the control (2 kittens), 306K (5 kittens), and 606K (11) dietary groups and included the following: cleft palate, cranioschisis, foreshortened mandible, stenotic colon, enlarged heart, and agenesis of the spinal cord and small intestine. The authors noted that the results of this study demonstrated that retinyl acetate at a concentration of 306000 retinol equivalents/kg diet has the potential for causing birth defects in kittens.

Ethanol was found to increase the incidence of cleft palate in random-bred Swiss white mice given retinyl acetate (3,400 or 5,100 IU) by stomach tube on day 12, when compared to retinyl-acetate mice given water.[93]

Dermal

Retinoic Acid

All-*trans* retinoic acid was administered topically to clipped interscapular skin (4 cm^2) of 48 virgin female hamsters. Three consecutive doses of 10.5 g/kg/day were administered during a critical stage of organogenesis (days 7, 8, and 9 of pregnancy).[35] Topical treatment with all-trans retinoic acid was associated with epidermal erythema and slight hyperplasia at the application site, but a significant teratogenic response was not observed.

Both the maternal and embryonic effects of all-*trans* retinoic acid after dermal administration to time-pregnant female Sprague-Dawley rats were evaluated.[87] One group of 7 rats and the remaining 2 groups of 6 rats received dermal applications of 25, 100, or 250 mg/kg body weight all-trans retinoic acid (in DMSO) on gestation days 11 through 14. Positive and negative control groups were dosed with ethylenethiourea (ETU) and DMSO, respectively. Beginning on day 15, the dams treated with all-*trans* retinoic acid had dermal lesions at the application site.

Significant maternal toxicity was reported. Most of the dams had vaginal bleeding and approximately 20% did not survive to day 19. When compared to the DMSO control group, maternal weight gain in animals treated with 25 mg/kg all-*trans* retinoic acid decreased by approximately 50%, and there was essentially no weight gain in the other 2 dose groups.

The decrease in average fetal weight was significant in these 2 dose groups (100 and 200 mg/kg), but the resorption and malformation frequencies were not significantly increased when compared to the control group Dermal application of the positive control significantly increased the frequency of skeletal anomalies, primarily tail defects. [87]

Parenteral

Retinoic Acid

The teratogenicity of all-*trans* retinoic acid was evaluated using groups of 10 pregnant female Wistar rats. All-trans retinoic acid (in corn oil) was administered i.p. at a dose of 50 mg/kg body weight from gestation day 8 to 10.[94] An untreated group and the group dosed i.p. with corn oil (vehicle) served as controls. Maternal deaths were not observed and there were no differences in food or water consumption between all-*trans* retinoic acid treated animals and controls. Additionally, maternal body weight gain during gestation was similar. Exposure to all-*trans* retinoic acid did not significantly alter the number of implantations or live fetuses per litter. The mean number of resorptions after dosing with all-trans retinoic acid was 2.5 ± 4.09, compared to 0.8 ± 1.32 (untreated control) and 0.4 ± 0.52 (vehicle control). Non-significant decreases in mean fetal body weight and length were also reported after dosing with all-*trans* retinoic acid. There were also no significant differences in placental weight and length. All-trans retinoic acid induced external, skeletal, and visceral malformations. The following malformations were observed: exencephaly, anophthalmia, tongue protrusion, exophthalmia, cleft palate, and cleft lip. The skeletal abnormalities induced by all-*trans* retinoic acid included acrania, micrognathia, extra ribs, reduced ossification of the skull, extralumbar vertebrae, and fusion of the lumbosacral vertebral arches. Visceral malformations included hydroureter and hydronephrosis.

Human

Oral

Retinol

A study examining the effects of vitamin A or beta carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh was performed.[95] The study involved pregnant women (13 to 45 years old) and their live-born infants to 12 weeks (84 days) postpartum. Five-hundred ninety-six community clusters (study sectors) were randomized for pregnant women to receive 7000 µg retinol equivalents (as retinyl palmitate), 42 mg of all-*trans* beta carotene, or placebo from the first trimester through 12 weeks postpartum. Married women (n = 125,257 total; 32,180 to 32,719 per group) underwent surveillance for pregnancy, ascertained by a history of amenorrhea and confirmed by a urine test. Groups were comparable across risk factors.

For the maternal mortality, neither of the supplemental groups was significantly different from the placebo groups. The numbers and all-cause, pregnancy-related mortality rates (per 100,000 pregnancies) were: 41 and 206 (95% confidence interval [CI] = 140-273) in the placebo group, 47 and 237 (95% CI = 166-309) in the vitamin A group, and 50 and 250 (95% CI = 177-323) in the beta carotene group. Relative risks of maternal mortality in the vitamin A and beta carotene groups were not statistically significantly different from controls (1.15; 95% CI = 0.75-1.76 and 1.21; 95% CI = 0.81-1.81 for the vitamin A and beta carotene groups, respectively).

There were 703 stillbirths in the placebo group, 665 in the vitamin A group, and 766 in the beta carotene group. Rates of stillbirths per 1000 births were: 47.9 (95% CI = 44.3 -51.5 [placebo]), 45.6 (95% CI = 42.1-49.2) [vitamin A]), and 51.8 (95% CI = 48.0-55.6 [beta carotene]). Relative risks of infants being still born were not statistically significantly different in the supplemented groups, compared to the placebo group (0.95; 95% CI = 0.85 -1.06 for the vitamin A group and 1.08; 95% CI = 0.97 to 1.21 for the beta carotene group). Infant mortality rates per 1000 births were: 68.1 (95% CI = 63.7-72.5 [placebo]), 65.0 (95% CI = 60.7-69.4 [vitamin A]), and 69.8 (95% CI = 65.4-72.3 [beta carotene]). It was concluded that, compared to the placebo, weekly vitamin A or beta carotene supplementation in pregnant women in Bangladesh did not reduce all-cause maternal, fetal, or infant mortality.[95]

Retinoic Acid

A large population-based study of pregnancies to women who were taking isotretinoin (13-*cis*-retinoic acid) during a 28-year period found that 84% of the patients that became pregnant elected to terminate the pregnancy. Of those pregnancies that were not electively terminated, estimates of spontaneous abortions ranged from 3% to 20%.[96,97] When children were followed for 7 years after birth, there was no incidence of further abnormalities.[97] Of those pregnancies that result in live births, approximately 48% to 82% of the children are healthy at birth.[98]

In Vitro

Retinoic Acid

The effects of retinoic acid on melanocytes from their generation through maturation were studied using a melanocyte differentiation inducement system of mouse embryonic stem cells (C57BL/6-derived).[99] Embryonic stem cells are pluripotent stem cells that are derived from the inner cell mass of early embryos, and these cells can differentiate into a variety of cell lines. The stem cells were seeded on ST2 cells and cultured to differentiate into melanocytes using a differentiation-inducing medium. Retinoic acid was added to the cultures at a final concentration of 1 to 100 nM. Study results indicated that retinoic acid had significantly different effects, depending on the stage of differentiation. Specifically, retinoic acid promoted differentiation in earlier stages, whereby embryonic stem cells become melanoblasts via neural crest cells. Retinoic acid inhibited differentiation in later stages, whereby melanoblasts become melanocytes.

Risk Assessment for Topical Application

Retinoic Acid

A risk assessment[100] of topical all-*trans*-retinoic acid was developed, and is stated as follows: If a daily dose of 20 g of 0.05% preparation (10 mg of all-*trans* retinoic acid) is applied to human skin for therapeutic or cosmetic purposes and a systemic bioavailability of 10% is assumed (probably overestimated), then 1 mg of all-*trans*-retinoic acid is expected to reach the central circulation daily. Assuming a body weight of 65 kg, the daily absorbed dose would be 0.015 mg/kg.

The author goes on to suggest that recent studies in the monkey have shown that all-*trans*-retinoic acid is a less potent teratogen when compared to 13-*cis*-retinoic acid. For example, while 5 mg/kg/day of 13-*cis*-retinoic acid were sufficient to yield a teratogenic response, daily doses of 10 mg/kg all-*trans*-retinoic acid had to be administered orally in this species to produce retinoic-specific defects.[101,102] The lower potency of the *trans*-isomer may find its explanation in its relatively rapid elimination rate: In all species examined, including the human, the elimination half-life of the *trans*-isomer was below 1 h. In contrast, the 13-*cis*-isomer is excreted much slower, and half-lives in the human as well as in larger animals, such as the monkey and rabbit, range from 10 to 30 h. Thus, the exposure to the 13-*cis*-retinoic acid and metabolites is much prolonged when compared to the all-*trans*-isomer. If these considerations hold true for the human, then the lowest teratogenic dose of all-*trans* retinoic acid in the human, which is not known today, would be expected to be more than 60-fold higher than the dose absorbed from daily administration of topical all-*trans*-retinoic acid.

In Vitro Study

Retinol

The developmental toxicity of retinol (in DMSO) was evaluated in the embryonic stem (ES)-D3 cell differentiation assay of the embryonic stem cell test.[103] The murine ES-D3 cell line was used, and this assay was performed to determine the test concentrations affecting ES-D3 cell differentiation into contracting cardiomyocytes. Exposure to the test substance, added from a 400-times concentrated stock solution in DMSO to culture medium, started at day 1 and lasted for 10 days. A test was considered valid when at least 21 out of 24 wells of the blank (non-exposed) cells and the solvent control plate contained contracting cardiomyocytes. Retinol caused a concentration-dependent decrease in ES-D3 cell differentiation in the 10^2 to 10^4 nM concentration range.

GENOTOXICITY

In Vitro-Bacterial Assays

Retinol, Retinoic Acid, and Retinyl Acetate

The genotoxicity of retinol, retinoic acid, and retinyl acetate were evaluated using the following *Salmonella typhimurium* strains: TA98, TA100, TA102, and TA1535.[104] Each retinoid was tested at doses ranging from 2.5 to 40 µg/plate both with and without metabolic activation. Neither of the 3 test substances induced toxicity, and there was no increase in the mutation frequency in any of the strains tested with or without metabolic activation.

In Vivo-Mammalian Cell Assays

Retinyl Palmitate

Positive photogenotoxic/photoclastogenic effects of retinyl palmitate in mouse lymphoma cells were reported in the following 2 studies.

L5178/$Tk^{+/-}$ mouse lymphoma cells were treated with several concentrations of retinyl palmitate either alone or in the presence of UVA light.[105] The treatment of cells with retinyl palmitate alone at concentrations of 25 to 100 µg/ml did not increase mutant frequencies over the negative control (i.e., no retinyl palmitate or UVA exposure). However, the treatment of cells with retinyl palmitate at concentrations of 1 to 25 µg/ml in the presence of UVA light (82.8 mJ/cm^2/min for 30 min) caused a dose-dependent mutation induction. The mean induced mutant frequency for treatment with 25 µg/ml in the presence of UVA light was approximately threefold higher than that for UVA alone (122×10^{-6}), suggesting potentiation by retinyl palmitate of the effects of UVA light exposure.

To elucidate the underlying mechanism of action, the mutants were examined for loss of heterozygosity (LOH) at 4 microsatellite loci spanning the entire chromosome 11, on which the Tk gene is located. The mutational spectrum for the RP + UVA treatment was significantly different from the negative control, but not significantly different from that of UVA exposure alone. Ninety-four percent of the mutants from combined retinyl palmitate + UVA treatment lost the Tk^+ allele, and 91% of the deleted sequences extended across more than 6 cM in chromosome length, indicating clastogenic events affecting a large segment of the chromosome. These results suggest that retinyl palmitate is photomutagenic in combination with UVA exposure in mouse lymphoma cells, with a clastogenic mode-of-action.[105]

In the presence of UVA light, retinyl palmitate (RP) decomposes into multiple products, including anhydroretinol (AR) and 5,6-epoxyretinyl palmitate (5,6-epoxy-RP). The photomutagenicity of AR and 5,6-epoxy-RP in L5178Y/$Tk^{+/-}$ mouse lymphoma cells was evaluated.[11] The treatment of cells with AR or 5,6-epoxy-RP alone at 10 and 25 µg/ml for 4 h did not yield a positive mutagenic response. However, because these concentrations did not induce a sufficient degree of cytotoxicity for the mouse lymphoma assay, it was not possible to determine whether these 2 compounds are mutagenic at concentrations approaching cytotoxic levels.

The treatment of cells with 1 to 25 µg/ml AR or 5,6-epoxy-RP in the presence of UVA light (315 to 400 nm) for 30 min (1.38 mW/cm^2) caused a potentiated photomutagenic effect. At 10 µg/ml (37.3 µM) AR in the presence of UVA light, the mutant frequency was approximately 3-fold higher than for UVA exposure alone. The mutant frequency for 5,6-epoxy-RP at a concentration of 25 µg/ml (46.3 µM) in the presence of UVA light was approximately 2-fold higher than for UVA exposure alone.[11]

To determine the underlying photomutagenic mechanism, the loss of heterozygosity (LOH) at 4 microsatellite loci spanning the entire chromosome 11 was examined for mutants induced by UVA light and either AR or 5,6-epoxy-RP exposure. Most mutants lost the $7k^+$ allele, and more than 70% of the chromosome damage extended across 38 cM of the chromosome. AR + UVA induced approximately twice as many mutants, with all 4 microsatellite markers lost from the chromosome 11 carrying the Tk^+ allele, compared to retinyl palmitate + UVA or 5,6-epoxy-RP + UVA treatments. These results suggest that 2 of retinyl palmitate's photodecomposition products are photomutagenic in mouse lymphoma cells, affecting a large segment of the chromosome. For detailed information on the photodecomposition of retinoids, 2 review articles on this subject have been published. [7,106]

In an earlier study, photoirradiation of anhydroretinol with UVA light in the presence of methyl linoleate generated lipid peroxidation products (i.e., methyl linoleate hydroperoxides) in an exposure-dependent manner.[107] In a subsequent study, electron spin resonance (ESR) spin-trap techniques were employed to explore the mechanism of lipid peroxidation initiation in such systems.[108] Irradiation of anhydroretinol with UVA in the presence of 2,2,6,6,-tetramethylpiperidine (TEMP), a specific probe for singlet oxygen, resulted in the formation of TEMP-O, demonstrating the formation of singlet oxygen under these conditions. During photoirradiation in the presence of 5,5-dimethyl N-oxide pyrroline (DMPO), a specific probe for superoxide, ESR signals for DMPO-OOH were formed, and these signals were quenched by superoxide dismutase. The involvement of singlet oxygen in the induction of lipid peroxidation was also evidenced by the inhibition of lipid peroxidation by sodium azide and the enhancement of lipid peroxidation by deuterium oxide. Overall, the results indicate that irradiation of anhydroretinol with UVA light generates reactive oxygen species, including singlet oxygen and superoxide, which mediate lipid peroxidation.

In another study, the same technique was used to determine whether or not irradiation of retinyl palmitate with UVA light produces reactive oxygen species.[109] Photoirradiation of retinyl palmitate in the presence of TEMP resulted in the formation of TEMPO. Both DMPO and 5-tert-butoxycarbonyl 5-methyl-1-pyrroline N-oxide (BMPO) are specific probes for superoxide. When photoirradiation of retinyl palmitate was performed in the presence of DMPO or BMPO, ESR signals for DMPO–$^{\bullet}$OOH or BMPO DMPO–$^{\bullet}$OOH were obtained. These results unambiguously confirmed the formation of superoxide radical anion. Consistent with free radical mechanism, there was a near complete and time-dependent photodecomposition of

retinyl palmitate and its photodecomposition products. ESR studies on the photoirradiation of 5,6-epoxy-RP and AR indicate that these compounds, as well as RP, can mediate free-radical formation and lipid peroxidation through the light-induced breakdown of RP.

The photogenotoxicity of retinyl palmitate, anhydroretinol (AR), and 5,6-epoxyretinyl palmitate (5,6-epoxy-RP) in human skin Jurkat T-cells was evaluated using the Comet assay.[110] This assay was used to assess light-induced fragmentation of cellular DNA, and was performed with and without UVA irradiation. Initially, a cell viability assay was performed, whereby fluorescein diacetate was added to light-irradiated cell suspensions. Light irradiation for up to 60 minutes did not significantly affect the viability of T cells. The viability of T cells, with and without light irradiation, in the presence of retinyl palmitate, AR, or 5,6-epoxy-RP at concentrations of 0, 50, 100, 150, and 200 µM was then determined. Cell death caused by these compounds was described as low in the absence of light (AR > 5,6-epoxy-RP > retinyl palmitate). With AR (100 µM) and 5,6-epoxy-RP (150 µM), 75% of the cells remained viable. With retinyl palmitate (200 µM), > 80% of the cells remained viable.

Cell death (photocytotoxicity in the presence of retinyl palmitate, AR, or 5,6-epoxy-RP in human skin Jurkat T-cells exposed to UVA (3.5 J/cm^2) and visible (6.3 J/cm^2) was much higher than without UVA or visible light exposure. For all 3 compounds, significant photocytotoxicity was observed for concentrations of 100 µM and greater. With 150 and 200 µM, the relative phototoxic potency of the retinoids conformed to the following order: retinyl palmitate = AR > 5,6-epoxy-RP. When treated with 100 µM of retinyl palmitate, 5,6-epoxy-RP, or AR, cell death was 39%, 22%, and 45%, respectively. These results indicate that retinyl palmitate, 5,6-epoxy-RP, and AR are photocytotoxic.

In the comet assasys for DNA fragmentation, the retinoid concentrations were 0 to 200 µM and the light exposure was 3.5 J/cm^2 (UVA light) or 6.13 J/cm^2 (visible). Photoirradiation of retinyl palmitate, AR, or 5,6-epoxy-RP, in ethanol, with UVA light produced UVA-induced DNA fragmentation in human skin Jurkat T-cells, but only when accompanied by significant levels of photocytotoxicity. There was a dose-response relationship between the levels of DNA fragmentation and the concentration (50, 100, 150, and 200 µM) of the retinoid used. In the presence of supercoiled ΦX174 phage DNA, retinyl palmitate, AR, and 5,6-epoxy-RP at concentrations of 0.1 and 1.0 mM in 10% ethanol were irradiated with UVA light at light doses of 7, 21, and 50 J/cm^2. Single strand breaks in supercoiled ΦX174 plasmid DNA were observed. At 50 J/cm^2, all 3 compounds yielded DNA-strand cleavage to significantly higher extents when compared to that of the control group. The retinoids in decreasing order of DNA-strand cleavage observed were AR > 5,6-epoxy-RP > retinyl palmitate ≥ control. No DNA cleavage was observed when either retinoid or light was absent. These results indicate that retinyl palmitate, AR, and 5,6-epoxy-RP can damage DNA in a cell free system and can be cytotoxic in cultured mammalian cells that are also exposed to light.[110]

The genotoxicity and photogenotoxicity of retinyl palmitate was evaluated using Chinese hamster ovary (CHO) cells in a standard chromosome-aberration test.[111] The procedure involved pre-irradiation (UVA irradiation followed by treatment with retinyl palmitate) or simultaneous irradiation (irradiation of cells in the presence of retinyl palmitate together). UVA irradiation was 350 or 700 mJ/cm^2, with the high UVA exposure (700 mJ/cm^2) selected to produce a small increase in the incidence of structural chromosome aberrations in cells in the absence of retinyl palmitate. Retinyl palmitate was tested up to concentrations exceeding its limit of solubility in the culture medium (i.e., ranging from 20 to 40 µg/mL).

No overt cytotoxicity was found in the dark or following irradiation. Treatment of the cells with retinyl palmitate in the dark as well as treatment under pre- or simultaneous irradiation conditions failed to produce biologically significant increases in the incidence of structural chromosome aberrations. The positive control substances 4-nitroquinolone and 8-methoxypsoralene caused significantly positive effects in the dark or under simultaneous irradiation, respectively. It was concluded that, under standard conditions for evaluating photogenotoxicity, retinyl palmitate had no *in vitro* genotoxicity or photogenotoxic potential and, therefore, that it is unlikely that retinyl palmitate would pose a local or systemic genotoxic or photogenotoxic risk.[111]

A letter to the editor addressing the preceding genotoxicity/photogenotoxicity study stated:[112]
"Dufour et al. conclude that application of retinyl palmitate to human skin posed no local or systemic genotoxic or photogenotoxic risk, based on a single *in vitro* genotoxicity test. This conclusion appears overstated; such a conclusion requires a full battery of *in vivo* and *in vitro* genotoxicity tests and perhaps even human studies. The authors inappropriately used results from one genotoxicity assay to refute results from various other genotoxicity assays that measure different genotoxic endpoints with varying sensitivities. We feel that it is important to bring these issues to the attention of the readers of *Mutation Research*."

A response to the aforementioned letter states:[113]

"In summary, our paper[111] and the papers by Mei et al.[11,105] exemplify the dilemmas facing genetic toxicologists in the current age. Different results can be obtained under different conditions, in different cells and using different culture media. This dilemma may be particularly relevant for in vitro photogenotoxicity tests. For example, a recent concept paper by the European Medicines Agency stated that "oversensitivity and the occurrence of pseudo-effects with in vitro models, in particular the mammalian cell test for photogenotoxicity, has become a major problem" and no longer recommends these tests for regulatory purposes. Nobody wants to see humans exposed to dangerous chemicals, but determining whether the positive results cause unnecessary alarm or whether the negative results cause unjustified complacency will continue to exercise the minds of the scientific community."

In a subsequent study involving many of the authors of the preceding study, results following the photoirradiation of retinyl palmitate (RP) in ethanol using a UV lamp generating approximately equal levels of UVA and UVB light were as follows:[12] The photodecomposition products identified were: 4-keto-RP, 11-ethoxy-12-hydroxy-RP, 13-ethoxy-14-hydroxy-RP, anhydroretinol (AR), and *trans*- and *cis*-15-ethoxy-AR. Photoirradiation of RP in the presence of a lipid, methyl linoleate, resulted in induction of lipid peroxidation. Lipid peroxidation was inhibited when sodium azide was present during photoirradiation, which suggests free radicals were formed. These results demonstrate that RP can mediate the UV light-induced free radical formation and induction of lipid peroxidation through the light-induced breakdown of RP.

Retinol

The photocytotoxicity and photomutagenicity of retinol were investigated using L5178Y/Tk$^{+/-}$ mouse lymphoma cells concomitantly exposed to retinol and UVA light.[114] The cells were treated with retinol at concentrations of 5 or 10 µg/ml for 4 h without UVA exposure, or treated with retinol (0.25 to 4 µg/ml) for 4 h and UVA exposure at 2.48 J/cm^2 during the first 30 minutes of the 4 h incubation period. In cells treated with retinol alone at 5 or 10 µg/ml in the absence of light, there was no increase in the mutant frequency (MF) in the *Tk* gene, and there was minimal cytotoxicity. However, treatment of cells with 1 to 4 µg/ml retinol in the presence of UVA (1.38 mW/cm^2 for 30 minutes) increased the MF in the *Tk* gene in a concentration-responsive manner and increased cytotoxicity. To elucidate the underlying mechanism of action, the mutational types of the *Tk* mutants were examined by determining their loss of heterozygosity (LOH) at four microsatellite loci spanning the entire length of chromosome 11, on which the *Tk* gene is located. The mutational spectrum for the retinol + UVA treatment was significantly different from those of the control and UVA exposure alone. More than 93% of the mutants in the retinol + UVA treated cells lost heterozygosity at the *Tk*1 locus, and the major types (58%) of mutations were LOHs extending to D11*Mit*42, an alternation involving approximately 6 cM of the chromosome. The main types of mutations in the control were non-LOH mutations. These results suggest that retinol is mutagenic in the presence of UVA light in mouse lymphoma cells through a clastogenic mode-of-action.

Retinoic Acid

At concentrations greater than 10 µM, retinoic acid depressed DNA synthesis in bovine mixed lymphocyte cultures, and the doses at which 50% inhibition occurred were 20, 35, 40, and 60 µM in 4 separate experiments.[63]

Retinyl Acetate

The genotoxicity of retinyl acetate was evaluated in a cytogenetic assay involving human (HE 2144) fibroblasts. The test substance was evaluated at concentrations up to 0.0656 mg/ml.[115] One control culture with solvent only was used for each series of experiments. The frequency of sister chromatid exchanges was scored on the basis of 20 to 30 metaphase spreads showing a different sister chromatid staining in all chromosomes. At the highest concentration tested, the value for sister chromatid changes per cell (10.04/cell) was twice the control value, and the value for chromatid breaks per cell 0.13/cell) exceeded the highest control values of chromatid breaks per cell.

Retinol, Retinoic Acid, and Retinyl Acetate

The effect of the following retinoids on sister chromatid exchange in exponentially growing V79 Chinese hamster cells was evaluated: all-*trans* retinol, all-*trans* retinoic acid, and all-*trans* retinyl acetate.[116] Each retinoid was evaluated at concentrations of 0.25 to 4 µg/ml, and there were no significant effects on sister chromatid exchange frequency when compared to untreated controls.

In Vivo/In Vitro

Retinyl Palmitate and Retinol

Retinyl palmitate, retinol, and retinoic acid, in oil-in-water creams at a concentration of 0.05%, were applied to the backs of adult Skh:hr-1 albino hairless mice, once per day for 3 days.[117] The application period was followed by exposure of the treated sites to UVB. The animals were then killed and the epidermis was removed for biochemical analysis. A431keratinocytes were incubated with retinol (2 µM) or retinyl palmitate (2 µM) in 1% ethanol for 24 h. Cell cultures were also incubated alone or in 1% ethanol only. At end of the 24-h incubation period, all cultures were exposed to UVB light and then incubated for 24 h. The action of retinyl palmitate and retinol on UVB-induced DNA damage and apoptosis in cultured A431 keratinocytes was analyzed. Topical retinol and retinyl palmitate significantly decreased (≈ 50%) the number of apoptotic cells as well as the formation of thymine dimers in the epidermis of mice exposed to acute UVB. However, neither of the 2 retinoids interfered with the apoptotic process in A431 keratinocytes exposed to UVB, whereas, DNA photodamage was decreased slightly in these cells in the presence of retinoids. It was concluded that the decrease in the numbers of UVB-induced apoptotic cells in hairless mouse epidermis following topical application of retinoids reflects a protection of DNA, which is a primary target of UVB irradiation, by a mechanism that is independent of the activation of retinoid nuclear receptors and does not involve the direct inhibition of apoptosis.

Modulation of Genotoxicity

Retinol, Retinoic Acid, and Retinyl Acetate

Retinol-, retinoic acid-, and retinyl acetate-induced alteration of mutation frequencies induced by carcinogens was studied using *Salmonella typhimurium* strains TA98, TA100, TA102, and TA1535.[104] Testing involved the following 7 carcinogens: aflatoxin B_1 (AFB), cyclophosphamide (CPP), 3-methylcholanthrene (MCA), benzo[*a*]pyrene (BP), benz[*a*]anthracene (BA), 9,10-dimethyl-1,2-benz[a]anthracene (DMBA), and mitomycin C (MMC). Retinol was evaluated at doses up to 40 µg/plate, and significantly reduced the number of His^+ revertants induced by AFB. It also reduced mutations induced by CPP or MCA, but not those induced by BP, BA, DMBA, or MMC. Additionally, the ability of retinol, retinoic acid and retinyl acetate to inhibit mutations caused by AFB and BP were studied and compared. All 3 retinoids caused a significant reduction in AFB-induced His+ revertants, in a dose-dependent manner. However, there was no effect on BP-induced revertants.

Irinotecan (CPT-11) is a common chemotherapeutic agent that can be genotoxic. A study was performed to evaluate the modulating effect of vitamins A (retinol), C, and E on the genotoxic activity of CPT-11 and to analyze the efficacy of DNA repair in lymphocytes (*in vitro*) of patients with diagnosed colorectal carcinoma and healthy individuals.[118] In healthy donors' cells, CPT-11 did not exert a strong genotoxic effect in the presence or absence of the vitamins. In turn, a statistically significant increase of DNA migration in the comet tails was noted in the patients' lymphocytes exposed to CPT-11. Vitamins A, C, and E in the incubation solutions acted synergistically to increase the level of DNA lesions in the cells exposed to CPT-11 *in vitro*. Analysis of the efficacy of DNA repair, performed after 2 h of post-incubation, showed a decrease in the percentage of DNA in the comet tails in all experimental samples.

Inhibition of DNA Synthesis

Retinyl Acetate

Female Sprague-Dawley rats were treated with either solvent (0.9% NaCl solution), 7,12-dimethylbenz(a)-anthracene, or 1-methyl-1-nitrosourea at 50 days of age.[119] The animals were also placed on either a placebo or retinyl acetate (1 mmol/kg diet) diet at 57 days of age. The incorporation of [^{3}H] thymidine into purified DNA isolated from mammary parenchymal cells was determined. Mammary cell DNA synthesis in animals teated with 7,12-dimethylbenz(a)anthracene or 1-methyl-1-nitrosourea was effectively inhibited by retinyl acetate.

The effect of retinyl acetate on DNA synthesis was studied using DNA from female Sprague-Dawley rat, mammary parenchymal cells.[119] The rats were injected i.v. with either solvent (saline), 7,12-dimethylbenz(α)anthracene (DMBA), or 1-methyl-1-nitrosourea (MNU) at 50 days of age and placed on either a placebo or retinyl acetate (1 mol/kg diet) diet at 57 days of age. [^{3}H]Thymidine incorporation into purified DNA was determined. Retinyl acetate effectively inhibited mammary cell DNA synthesis in both MNU- and DMBA-treated rats. However, inhibition of DNA synthesis was not observed in solvent-treated rats.

CARCINOGENICITY

Oral

Retinol and Retinyl Palmitate

The effects of retinyl palmitate ingestion on carcinogenesis were determined. Female adolescent Sprague-Dawley rats (total = 135), were randomized into 3 dietary groups (45 rats per group).[78] From p21 to p63 (i.e., the period of adolescent mammary-gland development), female rats received either a whole-food diet with adequate levels of vitamin A, a diet with a 5.5-fold increase in vitamin A from fruits and vegetables (S diet), or a diet with a 6.2-fold increase in vitamin A provided as retinyl palmitate (RP diet). Rats were injected with 50 mg 1-methyl-1-nitrosourea/kg body weight on p66. The rats were palpated twice per week for 6 months for the detection of mammary tumors. At 6 months post-carcinogen injection, latency and incidence of mammary tumors did not differ among dietary groups. However, compared with adolescent rats that consumed the adequate diet, consumption of S and RP diets reduced mammary cancer multiplicity (relative risk ~ 0.7, $P \leq 0.002$), and the reductions were associated with reductions in mammary gland alveolar development. More specifically, tumor multiplicity was reduced in rats fed either the S ($P = 0.0002$) or RP ($P = 0.002$) diet during adolescence. The authors concluded that these data demonstrate that the amount and source of vitamin A consumed by adolescent female rats can influence breast cancer risk. Effects on sexual maturation and mammary-gland development included in this study are found in the Reproductive and Developmental Toxicity section of this report.

Retinyl Acetate

Estrone- and progesterone-treated nulliparous and multiparous inbred GR/A female mice were fed retinyl acetate (82 mg/kg ration) daily, beginning at the onset of hormone treatment and for the duration of the study (13 to 16 weeks).[120] Control mice (65 mice) were fed gelatinized beadlets without retinyl acetate. A substantial increase in the incidence of mammary carcinomas was reported. In the first experiment, the mammary carcinoma incidence in nulliparous control and retinyl acetate-fed mice was 22/65 (34%) and 37/65 (57%) ($P < 0.05$), respectively. In the second experiment, these values were 27/48 (56%) and 37/48 (77%) ($P < 0.05$), respectively. The mammary carcinoma incidence in multiparous control and retinyl acetate-fed mice in experiment #1 was 13/30 (43%) and 23/30 (77%), respectively. In experiment #2, these values were 19/19 (100%) and 19/19 (100%), respectively. The authors interpreted these findings as a striking increase in the incidence of mammary carcinomas in hormonally treated GR/A female mice fed retinyl acetate.

The carcinogenicity of retinol acetate was evaluated using groups of 50 male and 50 female F344/DuCrj rats (specific pathogen free; 8 weeks old). Retinol acetate (0.25% or 0.125%, as gelatinized beadlets in distilled water) was administered in drinking water continuously for 104 weeks.[121] Control groups were given 0.25% placebo beadlets. All surviving animals were killed at 108 weeks. The survival rates were 72 to 84% and were sufficiently high for statistical comparison of all groups. Inhibition of body weight gain was marked in females of the high-dose group. Higher incidences of malignant pheochromocytomas, benign pheochromoctomas, and hyperplasias of the adrenal medulla were observed in the retinol acetate-treated groups. The combined incidences of tumors of the adrenal medulla in males and females of the high-dose groups and the incidence in females of the low-dose group were significantly higher than the incidence in the controls. Conversely, statistically significant decreases were found in the incidences of the mammary gland tumors in males of the high-dose group, of thyroid tumors in females of the high-dose group, and of clitoral gland tumors in females of both high- and low-dose groups. The authors concluded that retinol acetate given orally possesses the potential for increasing the incidence of pheochromocytomas in male and female F344 rats in a dose-related manner under the conditions of this bioassay.

A study was performed to determine the effect of long-term feeding of retinyl acetate on the initiation of mammary tumors induced by methylnitrosourea (MNU) or DMBA in female Sprague-Dawley rats (29 rats, 40 days old).[122] Retinyl acetate (328 g/kg) was added to the diet for 2 months (at which time supplementation was stopped) before administration of the carcinogens. Another group of rats (30 rats, 40 days old) received retinoid vehicle in the diet before carcinogen administration. This vehicle contained ethanol, trioctanoin, and dl-alpha-tocopherol. In the MNU model, a 50% increase in the number of mammary adenocarcinomas was observed in rats pretreated with retinyl acetate. In the DMBA model, pretreatment with retinyl acetate significantly increased the number of benign mammary tumors, but not mammary cancers. Furthermore, in the MNU model, pretreatment with the retinoid vehicle resulted in a 57% incidence (considered a relatively low incidence) of mammary cancers. In the DMBA model, pretreatment with the retinoid vehicle resulted in a 63% incidence of mammary cancers.

Photocarcinogenicity

Retinoic Acid

A cream containing a 0.3% mixture of retinoic acid was applied topically to the skin of mice, after which the application site was exposed to UV light.[123,124] The light source was a medium pressure mercury arc lamp that emitted UVB (1.38×10^3 mJ/cm^2) and some UVC. Albino hairless mice were irradiated with a strongly carcinogenic dose of UV radiation (from mercury lamp) for 10 months. Each irradiation was followed by topical application of 0.3% retinoic acid. A greater number of tumors developed in the retinoic acid treatment group when compared to animals exposed to UV light only. Retinoic acid (0.3%) was classified as toxic, in that one-third of the animals died during the first 4 months of the study.

When compared to the preceding study, opposite results following treatment with retinoic acid were presented in a subsequent study. Animals treated with 0.05% rertinoic acid had fewer tumors than untreated irradiated controls, and no effect was observed at retinoic acid concentrations of 0.025 and 0.005%.[125]

Retinoic acid was applied (0.001% to 0.3%) to the skin of mice for 2 weeks and then exposed to UV light.[126] Dosing resumed after UV light exposure. Enhancement of photcarcinogenicity was observed. In the same study, the topical application of retinoic acid to the skin of Skh-1 and Fü-Alb mice greatly enhanced the photocarcinogenicity that was induced by a moderate of simulated sunlight.[126] The skin of albino hairless mice was pre-treated with retinoic acid (0.01% and 0.001%, in methanol) for 2 weeks, and treatment was followed by 28 weeks of UV radiation (from xenon solar simulator) at mildly carcinogenic doses. Retinoic acid was applied after each irradiation. Exposure to UV light + each concentration of retinoic acid resulted in enhanced tumor formation. It was concluded that retinoic acid was a promoter of UV carcinogenesis

In another study, mice were dosed with UV for 6 weeks prior to application of retinoic acid (0.001% to 0.3%).[127] Enhancement of photocarcinogenicity was also observed in this study.

Hairless mice (lightly pigmented) were used in a phtocarcinogenicity study on retinoic acid.[128] These animals (hairless mice) were used instead of albino mice because, like humans, the hairless mice strain is capable of modest tanning. According to the first protocol, a strongly carcinogenic dose of UV light (from FS-20 sunlamps) was given for 30 weeks. Retinoic acid (0.001%) was applied after each irradiation, and was continued for an additional 15 weeks post-UV. In the second protocol, tumors were induced via 20 weeks of irradiation. Irradiation was followed by treatment with retinoic acid (0.01% and 0.001%). In both protocols, the UV dose used (~ 6 human MED) caused 100% tumor incidence in the UV controls by week 35. Study results indicated that retinoic acid had no effect on the following parameters: (1) time of appearance of the first tumors (latency); (2) total number of tumors; and (3) progression to larger tumors. Retinoic acid (0.01%) applied after tumor induction caused a significantly higher rate of regression in treated mice when compared to untreated controls.

Female Fü-Alb h/h mice were irradiated with simulated light (one-half erythemal dose per session), followed by treatment with retinoic acid.[129] Enhanced photocarcinogenicity in the presence of retinoic acid was found. Observations at microscopic examination indicated that treatment with retinoic acid without UV light caused a mild epidermal response, with slight epithelial hyperplasia in approximately 25% of mice treated with retinoic acid. Also, at microscopic examination, epithelial hyperplasia was observed in the skin of all mice exposed to UV light. Proliferation of the connective tissues and enlargement of the follicular cysts and fat cells were observed. The UV dose was delivered by a solar simulator, and was reported to be equal to half an MED. Multiple tumors developed. There were also areas of scarring that resulted from ulceration and dermal necrosis. At the end of the treatment period (week 28), groups dosed with retinoic acid (0.001% and 0.01%) showed a dose-dependent increase in the mean number of tumors of 53% and 104%, respectively. The authors noted that topical retinoic acid induced epithelial hyperplasia in non-irradiated controls. They also suggested that the higher incidence of macroscopic tumors in the retinoic acid-treated, UV-irradiated mice may have been related to the stimulation of epidermal cell proliferation.

Pigmented mice were pre-irradiated with a low dose (~1/2 human MED) of UV light (Westinghouse FS-20 sunlamps; 280 to 390 nm, with a peak at 313 nm) for 6 weeks.[130] Irradiation was followed by treatment with retinoic acid (0.01% in methanol) 3 times per week for 14 weeks. After 58 weeks, the only tumors observed were two 1 mm papillomas in the UV controls and three 1 mm papillomas in animlas treated with retinoic acid + UV. It was concluded that retinoic acid did not promote UV-induced carcinogenesis in mice that are able to produce melanin.

The effect of retinoic acid on photocarcinogenicity was evaluated using 2 animal models, Cryotithrix (crh, pigmented and albino) mice and sparsely haired albino fuzzy (fz) rats .[131] According to both models, the animals were exposed repeatedly to UV radiation (from long arc solar simulator) and retinoic acid (0.1 mg/mL in methanol) during a large portion of the animal's life. UV doses of 200 Robertson-Berger (RB) units were administered 3 times pre week for 40 weeks. UV doses were expressed in RB units, where a dose of 400 RB units $\approx$ 1 human erythemal dose when the source spectrum resembles sunlight. Some of the animals were not treated with retinoic acid. Retinoic significantly enhanced the photocarcinogenesis response in mice and rats.

A model of UV-induced skin carcinogenesis in Skh:HR-1 hairless albino mice and Skh:HR-2 hairless pigmented mice has been established.[132] Retinoic acid was applied at a concentration of 0.05%. The mice were initially exposed to 102 mJ/cm^2 UVB and 2040 mJ/cm^2 UVA per day, translated as 33% of the MED for these mouse strains with this light source. For the first 4 weeks, the exposures were increased by 20% per week, and remained at this level for the duration of the experiments. Irradiations were performed 5 days per week. Retinoic acid initially increased desquamation and caused mild inflammation, which subsided after a few weeks of treatment. In the absence of UV light, retinoic acid induced a light tan in the Skh:HR-2 mice, but not the Skh:HR-1 mice. A very dark tan developed in Skh:HR-2 mice treated with UV light and retinoic acid. Retinoic acid alone was not carcinogenic, but enhanced photocarcinogenesis. The light tan of Skh:HR-2 mice did not provide any protection from carcinogenesis. However, the dark tan that developed in the presence of retinoic acid + UV light provided some protection from carcinogenesis, but not enough to overcome the enhancing effect of retinoic acid.

Retinyl Palmitate

In 2000, retinyl palmitate was nominated by the U.S. FDA's Center for Food Safety and Applied Nutrition (CFSAN) and selected by the National Toxicology Program (NTP) as a high priority compound for phototoxicity and photocarcinogenicity studies at the National Center for Toxicological Research.[133] Accordingly, the principal objective of NTP's 1-year photocarcinogenesis study was to investigate the effects of topically applied skin cream containing retinol or retinyl palmitate on the photocarcinogenicity of simulated solar light or UV light in SkH-1 mice. While CIR normally summarizes the findings using our own words, in this case, excerpts from the abstract of the NTP study are concise and are quoted below in order to not add any interpretation to the NTP findings.[134]

> "Groups of 36 male and 36 female Crl:SKH-1 *(hr$^-$/hr$^-$)* hairless mice were irradiated 5 days per week (Monday through Friday) in the morning for 40 weeks with SSL at levels of 0.00, 6.85, or 13.70 mJ•CIE/cm^2 that were emitted from glass-filtered 6.5kW xenon arc lamps.[134] The mice received topical applications of control creams or creams containing 0.001% (w/w) retinoic acid or 0.1%, 0.5%, 1.0%, or 2.0% retinyl palmitate to the dorsal skin region in the afternoon of the same days of irradiance exposures. Separate groups of 36 female Crl:SKH-1 *(hr$^-$/hr$^-$)* hairless mice were irradiated with UV light emitted from fluorescent UVA or UVB lamps at a single level that was equivalent to the amount of UVA or UVB generated by SSL at a level of 13.70 mJ•CIE/cm^2 SSL, and received topical application of control cream or creams containing 1.0% retinyl palmitate or 0.001% retinoic acid. A 12-week observation period followed the 40-week treatment/exposure period. Additional groups of 36 male and 36 female mice received no cream and were exposed to 0.00, 6.85, 13.70, or 20.55 mJ•CIE/cm^2 SSL or to a single level of either UVA or UVB light (females only), equivalent to the amount of UVA or UVB generated by SSL at a level of 13.70 mJ•CIE/cm^2."
>
> "Mice that received no cream treatment and were exposed to increasing levels of SSL showed significant SSL exposure-dependent decreases in survival, earlier in-life onset of skin lesions, and significant SSL exposure-dependent increases in the incidences and multiplicities of in-life skin lesions, as well as in the incidences and multiplicities of histopathology determined squamous cell nonneoplastic skin lesions (hyperplasia and focal atypical hyperplasia) and neoplastic skin lesions (papilloma, carcinoma *in situ*, and/or carcinoma). Female mice that received no cream treatment and were exposed to UVA showed significant increases in survival, later onset of in-life skin lesions, and significantly decreased incidences and multiplicities of in-life skin lesions, when compared to female mice that received SSL at a level of 13.70 mJ•CIE/cm^2. Female mice that received no cream treatment and were exposed to UVB demonstrated significant decreases in survival and significant increases in the multiplicities of in-life skin lesions, when compared to female mice that received SSL at a level of 13.70 mJ•CIE/cm^2."
>
> "The control cream was composed of a base cream (85%, w/w) and diisopropyl adipate (15%, w/w). The topical treatment of mice with the control cream imparted significant effects, when compared with comparable measurements in mice that received no cream treatment and were exposed to the same level of SSL. Specifically, the exposure of mice to control cream resulted in decreased survival rates, earlier times to the onset of skin lesions, and increased incidences and multiplicities of in-life skin lesions and squamous cell neoplasms in both the absence and presence of SSL exposure and increased incidences and multiplicities of in-life skin lesions in female mice exposed to UVA."
>
> "The application of retinoic acid (0.001%, w/w) creams to mice significantly decreased survival, even in the absence of SSL exposure in male mice, when compared to mice that received the control cream and the same level of SSL. Significantly earlier in-life skin lesion onset and significantly increased multiplicities of skin lesions were observed

at each SSL level, including 0.00 mJ•CIE/cm^2, in male mice and in female mice exposed to 6.85 mJ•CIE/cm^2 SSL, UVA, or UVB. No histopathology was conducted on the retinoic acid cream-treated mice."

"Significant dose trend effects and earlier in-life skin lesion onsets were observed in mice that received the retinyl palmitate cream treatments in the presence of SSL, UVA, or UVB, compared with mice that received control cream treatment and the same level of irradiation. In mice exposed to SSL, there were significantly increased multiplicities of in-life skin lesions at retinyl palmitate doses of 0.1% to 1.0%. Significant dose-related trends were observed in the incidences of squamous cell carcinoma and/or squamous cell carcinoma *in situ* in female mice exposed to 6.85 mJ•CIE/cm^2 SSL. Significant retinyl palmitate dose-related increases were also observed in the multiplicities of squamous cell papilloma and in the combination of all squamous cell neoplasms."

The conclusions from this NTP study follow:

- "Under the conditions of these studies, the topical treatment of SKH-1 mice with the control cream resulted in earlier onsets of in-life skin lesions and higher incidences and multiplicities of in-life skin lesions in the absence and presence of SSL or UVA, and higher incidences and multiplicities of squamous cell neoplasms, when compared to untreated controls in the absence and presence of SSL."
- "Compared to the control cream, retinoic acid enhanced the photocarcinogenic activity of SSL and UVB in SKH-1 mice, based upon earlier onsets and increased multiplicities of in-life skin lesions."
- "Compared to the control cream, retinyl palmitate enhanced the photocarcinogenicity activity of SSL and UVB in SKH-1 mice, based upon earlier onsets and increased multiplicities of in-life skin lesions and increased incidences and multiplicities of squamous cell neoplasms."[134]

It appears likely that the composition of the cream vehicles used in NTP study will be a critical factor in the evaluation of the results. NTP used:

- 85% base cream plus 15% disiopropyl adipate to prepare the "control cream"
- 85% base cream plus 15% of a retinoic acid diisopropyl adipate solution to prepare a 0.01% retinoic acid "master batch cream"
- 85% base cream plus 2% retinyl palmitate and 13% diisopropyl adipate to prepare the retinyl palmitate "master batch cream"

The control and master batch creams were mixed together to prepare the "dose creams" to which the animals were exposed, except that the 2% retinyl palmitate master batch cream was used as the 2% retinyl palmitate dose cream. The base cream had the following composition:

- 70.02% deionized water
- 3.25% 96% glycerin
- 8.00% 2% Keltrol T solution
- 1.2% Veegum ultra
- 2.5% cetearyl alcohol
- 4.00% Eutanol G
- 0.80% dimethicone DC 200-100
- 2.40% Lipomulse 165
- 2.40% Brij 721 (Steareth-21)
- 4.00% Lipowax D
- 1.00% Germaben II
- 0.43% 10% solution of 85% phosphoric acid (q.s. pH to 3.5)

The NTP found that the control cream, alone, without any retinol or retinyl palmitate, caused substantial adverse effects in the control mice exposed to simulated sunlight, UVA, or UVB, as indicated by markedly reduced survivability and in-life skin lesion onset and elevated in-life skin lesion incidence and multiplicity. The already severe effects of the vehicle (control cream) were further aggravated by the presence of retinoic acid or retinyl palmitate in the dose creams.

The results indicate that the NTP study was significantly confounded and, thereby, seriously compromised by the effects of the "control cream" that was used as the vehicle. In contrast, no such vehicle effects were found in a similar NTP 1-year photocarcinogenesis study on glycolic and salicylic acids. The composition of the base cream in the study was:

- 59.65% deionized water
- 0.10% disodium EDTA
- 0.10% disodium EDTA
- 2.50% 96% glycerin
- 7.5% 2% Carbopol 981 solution
- 7.50% mineral oil 65/75
- 1.50% BRIJ 721
- 2.00% Stearic Acid XXX
- 0.25% cetearyl alcohol
- 2.50% octyl palmitate
- 1.00% Germaben II
- 0.50% 20% NaOH solution (q.s. pH to 7.0)

In this study, glycolic acid or salicylic acid was added to the base cream, which also served as the control cream, to produce the dose creams; No diisopropyl adipate was used in this study. Otherwise, there were few differences in the protocol of this NTP study compared to that of the retinoids.

Diisopropyl adipate is among the esters of dicarboxylic acids recently evaluated by the CIR Expert Panel and found to be safe in the present practices of use and concentrations. Maximum use concentrations were up to 8% in leave-on products in 2010. That assessment noted studies on human subjects showing that undiluted diisopropyl adipate was not irritating in 4-h patch tests and only moderately irritating in a 21-day cumulative irritancy test patch test. Formulations containing up to 20.75% diisopropyl adipate caused minimal to mild irritation but no sensitization in such tests. A photopatch study demonstrated that formulations containing up to 17.0% diisopropyl adipate were not phototoxic, primary irritants, or sensitizers.

These results indicate that the Crl:SKH-1 *(hr$^-$/hr$^-$)* hairless mice used in the NTP study of the retinoids are much more sensitive to the potential phototoxicity of diisopropyl adipate than human subjects. The striking sensitivity of these mice, compared to humans, indicates that the use of a vehicle containing 13% to 15% diisopropyl adipate in the NTP study severely limits the usability of the results to support the assessment of safety and risks associated with dermal exposure to retinol or retinyl palmitate in cosmetic products.

The overall conclusion in the NTP study is stated as follows: Treatment with the carrier cream increased the incidence of skin tumors in hairless mice, both in the presence and absence of synthetic solar light. Inclusion of retinoic acid or retinyl palmitate in the cream increased the number of tumors and decreased the time to appearance of tumors, compared to animals given just the carrier cream.[134]

An analysis regarding the photocarcinogenic potential of retinyl palmitate included the following perspective:[135]

> "A number of studies regarding retinyl palmitate has been published by the FDA. Of the 8 *in vitro* studies published by the FDA from 2002-2009, 4 demonstrated the generation of reactive oxygen species by retinyl palmitate when exposed to UVA radiation.[108,109,12,107] The generation of free radicals and their subsequent mutagenic potential have garnered understandable concern. However, when considered in the context of the antioxidant milieu found in human skin, the relevance of these findings becomes questionable. The capacity to quench reactive oxygen species is magnified by the complex network of antioxidants found in the normal human biochemical environment. In conjunction with both enzymatic antioxidants (e.g., catalase, peroxidase, superoxide dismutase, and glutathione reductase) and nonenzymatic antioxidants (e.g., vitamins C and E), vitamin A can neutralize harmful free radicals. In the isolative environment of laboratory study, however, cooperative interactions among other antioxidants are absent. As such, the protective properties of a single antioxidant are quickly depleted and may even become pro-oxidative when exposed to harmful stimuli. Furthermore, many antioxidants are inherently unstable if not properly formulated, preventing the full spectrum of enzymatic and nonenzymatic antioxidants from acting to reduce the pro-oxidative effects observed in these *in vitro* experiments."
>
> "To assess the carcinogenic potential of retinyl palmitate, the National Toxicology Program (NTP) conducted a large study using SKH-1 hairless mice. While the results from this study have not yet been published in a peer-reviewed forum, the preliminary data are available for review online.[136] In this study, SKH-1 hairless mice received 2 different concentrations of retinyl palmitate (0.1% and 0.5%), with controls receiving a vehicle control pH 7 cream. The animals were then irradiated with UV doses of 6.75 and 13.7 mJ/cm^2 and subsequently assessed for

photocarcinogenesis. In the groups irradiated with low-dose (6.75 mJ/cm^2) UV radiation, retinyl palmitate induced higher incidences of malignant lesions at concentrations of both 0.1% and 0.5%, when compared with the vehicle control pH 7 cream. However, only the group exposed to retinyl palmitate at a concentration of 0.5% showed a statistically significant increase. In the groups exposed to high-dose (13.5 mJ/cm^2) UV radiation, no statistically significant difference in the incidence of malignant lesions was observed between the vehicle control group and the group exposed to either 0.1% or 0.5% retinyl palmitate. Therefore, the study failed to demonstrate conclusively that the combination of retinyl palmitate and UV is photocarcinogenic. Of worthy note, the thinner epidermis of mice used in these studies allows for increased penetrance of UV radiation. Additionally, these mice are known to have a higher propensity to develop skin cancer. These intrinsic qualities suggest that data generated from these animal studies should be examined in context and caution should be exercised in extrapolating the relevance of these findings to humans."

"While published data on the photocarcinogenic potential of retinyl palmiate in humans are lacking, evidence from 40 years of use in clinical medicine provides a powerful basis from which to question the notion that retinyl palmitate in sunscreens is photocarcinogenic. Clinically, retinoids are used by dermatologists in two major areas of therapy. First, oral retinoids have been used with great success to prevent skin cancers in populations who are at high-risk, such as patients with xeroderma pigmentosum[137] and immunosuppressed patients (e.g. organ transplant)[138]. Second, dermatololgists commonly prescribe topical retinoids in the management of skin disorders such as acne, psoriasis, photoaging, cutaneous T-cell lymphoma, and a variety of other skin conditions. Among patients treated with topical or oral retinoids, no published data exist to date suggesting that these medications increase the risk of skin cancer."

"In conclusion, the available evidence from *in vitro* and animal studies fails to demonstrate convincing evidence indicating that retinyl palmitate imparts an increased risk of skin cancer. Furthermore, while no human data examining this relationship are available, decades of clinical observations support the notion that retinyl palmitate is safe for use in topical applications such as sunscreens."[135]

Co-carcinogenicity

Retinyl Acetate and Retinoic Acid

Male weanling hamsters were fed a commercial diet and received a series of 12 intatracheal instillations of 3 g benzo(α)pyrene.[139] Following the final instillation, the animals were randomly assigned to receive 100 µg (83 hamsters), 1600 µg (74 hamsters), or 3300 µg (later reduced to 2400 µg, 73 hamsters) retinyl acetate per week in divided intragastric doses. Hamsters in the 2400-µg dose group had a significantly higher incidence of respiratory tract tumors than those in the 100 µg dose group. Liver vitamin A stores increased in groups given 160 and 2400 µg retinyl acetate and corresponded to the oral administration of retinyl acetate.

The potential modifying effect of retinyl acetate on butylated hydroxylated anisole (BHA)-induced tumorigenesis (rat forestomach) was evaluated using 9 groups of male F344/DuCrj rats (specific pathogen-free, 5 weeks old).[99] Four groups (Groups 4, 5, 6, and 8), consisted of 10 rats each and the remaining groups consisted of the following: Group 1 (15 rats), Group 2 (20 rats), Group 3 (18 rats), Group 7 (20 rats), and Group 9 (9 rats). The animals were maintained on a diet containing 1% or 2% BHA by weight and, simultaneously, on drinking water supplemented with retinoic acid (0.05 to 0.25%) for 52 weeks. Marked hyperplastic changes of the forestomach epithelium were observed in all animals given 2% BHA. The co-administration of 0.25% retinoic acid significantly ($P < 0.05$) increased the incidence of forestomach tumors (squamous cell papilloma and carcinoma) to 60% (9/15; 2 rats with carcinoma), from 15% (3/20; 1 rat with carcinoma) in the group given retinoic acid-free water. In rats given 1% BHA, retinoic acid co-administered at a dose of 0.05, 0.1,0.2, or 0.25%, there was a dose-dependent enhancing effect on the development of BHA-induced epithelial hyperplasia. Tumors, all papillomas, were induced in 3 rats (17%) with 0.25% retinoic acid and in 1 rat (10%) with 0.05% retinoic acid co-administration. Retinoic acid alone did not induce hyperplastic changes in the forestomach. The authors suggested that these findings indicate that retinoic acid acted as a co-carcinogen in the BHA forestomach carcinogenesis of the rat.

Anticarcinogenicity

Animal

Retinyl Acetate

Male Syrian golden hamsters were fed a semisynthetic diet and given 12 weekly intratracheal instillations of benzo(α)pyrene (3 mg).[140] Intratracheal instillations were followed by intragastric administration of either 100, 1600, or 2400 µg retinyl acetate (each in 2 divided doses) per week. Half of the animals were housed conventionally and the other half was housed in laminar flow units. The 3 dose groups were defined as follows: 100 µg dose (57 hamsters - conventional housing; 52 hamsters - laminar-flow housing), 1600 µg dose (58 hamsters – conventional housing; 53 hamsters – laminar-flow housing), and 2400 µg dose (58 hamsters – conventional housing; 49 hamsters – laminar-flow housing). In hamsters housed conventionally, increased retinyl acetate intake was associated with an increased incidence of benign respiratory tract tumors. In all groups of hamsters housed in laminar flow units, there was a longer period to death with respiratory tract tumor than in conventionally housed hamsters. Additionally, increased retinyl acetate intake was associated with a somewhat lower incidence of respiratory tract tumors. Squamous papillomas of the forestomach were significantly reduced in all groups of hamsters that received high levels of retinyl acetate, regardless of housing.

The effect of feeding a pharmacological level of retinyl acetate on N-methyl-N-nitrosourea-induced mammary carcinogenesis was studied using female Sprague-Dawley rats.[141] At 50 days of age, the rats received N-methyl-N-nitrosourea (50 mg). At 7 days post-treatment with the carcinogen, groups of 25 rats were placed on laboratory chow diet supplemented with placebo or retinyl acetate (300 mg). The rats were palpated for the detection of mammary tumors twice per week, and the study was terminated 130 days after dosing with the carcinogen. When compared to the placebo group, treatment with retinyl acetate reduced the tumor incidence, lessened the average number of tumors per rat, and prolonged tumor latency.

The inhibition of mammary carcinogenesis following short-term dietary exposure to retinyl acetate was studied using groups of 20 weanling, virgin female Lewis rats.[142] The groups of rats were fed chow supplemented with 250 ppm retinyl acetate according to the following schedule: -2 to +1 weeks; +1 to +30 weeks; +1 to +12 weeks; +12 to +30 weeks; and -2 to +30 weeks. The control group received chow without retinyl acetate. Time 0 was the day of DMBA administration, on which the rats were given 20 mg DMBA (in 1 ml sesame oil) intragastrically. When compared to non-retinyl acetate treated controls at 30 weeks, the following groups fed retinyl acetate in the diet at various periods of time had a significant decrease in tumor multiplicity: -2 to +1 weeks; +1 to +30 weeks; +12 to +30 weeks; and -2 to +30 weeks. The greatest decrease in tumor multiplicity was observed in the longest-duration treatment group (-2 to +30 weeks). A nearly equal reduction was observed in the group subjected to short-term retinyl acetate exposure at the time of carciunogen availability (-2 to +1 weeks). Tumor development was temporary in the +1 to +12 weeks group, and the tumor values returned to control levels by week 30. The authors suggested that the results of this study indicate that retinyl acetate inhibition of mammary cancer is not limited to the late stage of the disease, because the retinoid was almost equally effective when administered for a short period of time during the time of carcinogen availability.

Groups of 55 virgin female C3H/He mice were fed a diet that included 0.1% N-4-(5-nitro-2-furyl)-2-thiazolyl formamide (FANFT, urinary bladder carcinogen) and retinyl acetate.[143] The first 4 groups received 300, 600, 1200, and 2400 IU of retinyl acetate per kg of feed plus FANFT, respectively. The fifth group served as the control and received 300 IU per kg of feed without FANFT. Bladders were removed and inspected for neoplasms after 45 weeks. At all doses administered, retinyl acetate significantly inhibited the formation of squamous cell tumors in the urinary bladder. It was noted that cannibalism resulted in the loss of 19 of 55 mice in the 300 IU group, and that this finding may warrant a change in this conclusion. Retinyl acetate also inhibited transitional cell carcinoma when administered in the amount of 600 IU per kg of feed. Urinary bladder tumors were not found in the control group.

Retinyl acetate was evaluated in a study to determine its efficacy in preventing mammary tumorigenesis in $C3H^{Vy}$ mice. After mating (males and females from same litter), the female offspring were distributed in groups of 5.[144] Each group was subdivided into 3 groups, based on the age of mice when the retinyl acetate diet was started (3 months of age, weaning age, or from the time of conception). For the diet started at the time of conception, mothers were fed retinyl acetate from the time of mating. The mice were fed a stock diet supplemented with retinyl acetate beadlets at concentraions of 83, 41, and 21 mg/kg diet. A stock diet supplemented with placebo beadlets served as the control. The mice were killed and necropsied after the first mammary tumor was observed or at 15 months of age (if no tumor development). There was no significant difference in the incidence of mammary tumors between control mice and mice fed retinyl acetate. The tumor incidence was 80% to 90% in all groups. The number of tumors per mouse and the tumor latency period were not influenced by retinyl acetate in the diet. However the following 2 unexpected observations were made: (1) A 70% hepatoma incidence was reported for control mice necropsied at 12 months of age or older; the incidences of this tumor were ≈ 11%, 17%, and 46% in mice fed 83, 41, and 21 mg retinyl acetate/kg diet, respectively. (2) Severe damage to most articulations was induced by retinyl acetate, even at the dose (21 mg/kg diet) that failed to cause any other signs of toxicity. The authors interpreted the results of this study to indicate that retinyl acetate was uanable to reduce the frequency or to retard the appearance of mammary carcinomas.

The cheek pouch epithelium of 65 Syrian golden hamsters was painted with 9,10-dimethyl 1,2-benzanthracene (DMBA) and different concentrations of retinyl acetate (up to 2%), both singly and in combination with DMBA.[145] A significant delay in tumor induction was observed in hamsters treated with DMBA in combination with a higher concentration (2%) of retinyl acetate. At lower concentrations (0.5% and 1%), neither inhibition nor a delay in tumor induction was observed.

The feeding of retinyl acetate (0.2 mM) to female $BD2F_1$ mice previously treated with a series of gastric intubations of DMBA did not significantly affect the incidence of mammary tumors.[146] Seventy-five mice fed retinyl acetate developed 31 mammary adenocarcinomas and 19 mammary adenoacanthomas (50 mammary tumors total). The 75 control control mice developed 22 mammary adenocarcinomas and 20 mammary adenoacanthomas. Treatment did not significantly affect body weight gains or mortality rates. The authors suggested that these results provided evidence that carcinogen-induced mouse mammary gland tumorigenesis *in vivo* is not influenced by hyperalimentation of a dietary retinoid.

The effects of retinyl acetate on the life span and the incidence of cryptogenic neoplasms in C3H/HeJ($^{+}$) male and femal mice was evaluated.[147] At 105 weeks, survival was reported as follows: untreated males (58%), untreated females (28%), treated males (39%), and treated females (14%). In treated groups, the average weight was 10 to 15% lower. The incidence of neoplasm-bearing mice and total neoplasms was 87% and 57%, respectively, in female controls. For treated females, these values were 93% and 55%, respectively. These values were 57% and 39%, respectively, in male controls and 50% and 38%, respectively, in treated males. For the most common neoplasms, i.e., neoplasms of the mammary gland and liver, there was no reduction of these neoplasms in treated mice. The numbers of ovarian neoplasms and lung adenomas were slightly lower. The authors concluded that, at best, retinyl acetate exerted only a slight inhibitory effect on the development of some types of cryptogenic neoplasms in mice.

Retinoic Acid

Available data suggest that the tumor-promotion stage of skin carcinogenesis involves at least 3 important steps:[148] (1) the induction of embryonic-looking cells (dark cells) in the epidermis; (2) an increased production of epidermal prostaglandins and polyamines; and (3) sustained proliferation of dark cells. Retinoic acid specifically inhibits step 2.

Albino hairless mice were irradiated with UV light (FS-20 bulbs; dose = 3 MED/exposure) 5 times per week for 4 weeks. Topical all-*trans* retinoic acid (≈ 0.001%, in acetone) was applied at 0, 1, 2, 3, and 4 h after each irradiation.[149] When compared to irradiated vehicle-treated controls, after 52 weeks, groups treated with retinoic acid were found to have fewer mice with tumors, fewer tumors per mouse, smaller tumor diameters, and slower growing tumors. Though the low tumor yields limited statistical evaluation, the reduction in tumor incidence was significant ($p = 0.05$).

Human

Retinyl Palmitate

A study was performed to determine whether retinyl palmitate alone or plus beta-carotene (BC) would be as effective as and less toxic than low-dose 13-cis retinoic acid (13cRA) in treating oral premalignant lesions and reducing the risk of oral cancer.[150] Initially, patients (167; ≥ 18 years) were stratified by dysplasia versus hyperplasia and randomly assigned to 13cRA (0.5 mg/kg/day orally for 1 year, followed by 0.25 mg/kg/day orally for 2 years) or beta-carotene (50 mg/kg/day orally) plus retinyl palmitate (25,000 U/day orally) for 3 years, and later (by protocol revision) to 13cRA or retinyl palmitate alone (25,000 U/day orally). However, after other randomized trials suggested an adverse effect of beta-carotene on lung cancer incidence/mortality, beta-carotene was dropped (i.e., the patients were randomly re-assigned to 13cRA or retinyl palmitate alone). The primary endpoint was oral premalignant clinical response at 3 months.

The 3-month clinical response rate of the combined BC plus retinyl palmitate and retinyl palmitate alone arm (32.5%) was not statistically equivalent to that of 13cRA (48.1%). The clinical response rate of retinyl palmitate alone (20%) was significantly lower than that of beta-carotene plus retinyl palmitate (42.9%; $P = 0.03$). Similar oral cancer-free survival rates were observed across all arms. There was no significant association between 3-month oral premalignant lesions response and subsequent oral cancer development ($P = 0.11$). Grades 2 and higher adverse events were more common in the 13cRA than other groups ($P < 0.0001$). It was concluded that this chemoprevention trial did not establish the equivalence of retinyl palmitate plus beta-carotene or retinyl palmitate alone with low dose 13cRA in reducing the long-term risk of oral cancer. Additionally, it was stated that 13cRA, beta-carotene plus retinyl palmitate, and retinyl palmitate alone cannot be recommended for chemoprevention.[150]

In Vitro

Retinal Acetate

The modulation of cell growth kitnetics and carcinogen-cellular interaction by β-retinyl acetate in mouse epidermal cell cultures was evaluated.[151] β-retinyl acetate was dissolved in DMSO as a 1 mg/ml solution. β-retinyl acetate altered the course of differentiation of the mouse Balb/c epidermal cells in culture, which resulted in a reduced rate of cell death. The extended life span of the cultures appeared to have been due to prolonged survival of cells and not an increased growth rate, considering that β-retinyl acetate inhibited the rate of cellular proliferation. This inhibition was observed only after completion of a full cell cycle in the presence of β-retinyl acetate. DNA repair in response to physical and chemical agents was quantitatively unaffected in the presence of β-retinyl acetate. Slightly decreased constitutive aryl hydrocarbon hydroxylase (AHH) activity was noted after exposure to β-retinyl acetate; however, the level of enzyme induced by bena[a]anthracene was strongly reduced to 20% of the controls. The binding of 7,12-dimethylbenz[a]anthracene to epidermal cell DNA was markedly decreased in the presence of β-retinyl acetate. Binding to cellular protein was significantly increased by β-retinyl acetate. The authors noted that the results of this study indicate that β-retinyl acetate may interfere with a number of cellular events that are believed to be involved in the process of chemical carcinogenesis. They also noted that the suppression of DNA synthetic rate and the reduction of carcinogen metabolism and binding to DNA could possibly impede the initiation steps of carcinogenesis.

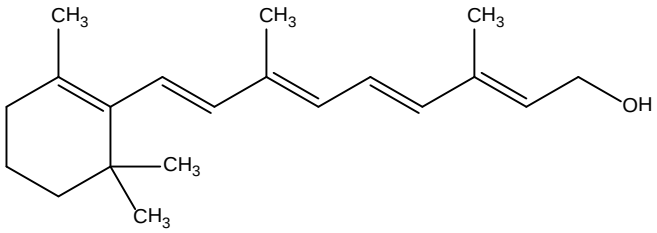
SUMMARY

The safety of the following retinoids in cosmetics is reviewed in this safety assessmnent: retinyl palmiate, retinol, retinoic acid, retinyl acetate, retinyl propionate, retinyl linoleate, retinyl oleate, retinyl rice branate, retinyl soyate, and retinyl tallate. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) combined with the results of an industry survey of ingredient use concentrations provided by the Personal Care Products Council, the majority of these ingredients were being used in cosmetic products. Of the use concentrations reported, the highest maximum use concentration in leave-on or rinse-off products was reported for retinyl palmitate (1.97% in leave-on products; 1% in rinse-off products). Retinyl palmitate and retinyl acetate are FDA-approved direct food additives that are generally recognized as safe.

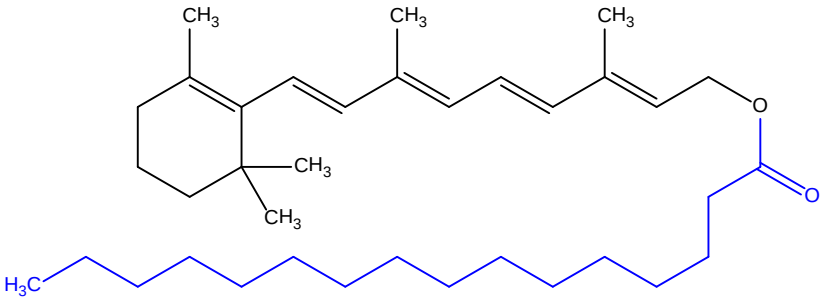
Retinoids readily penetrate the skin. Repeated inhalation exposure to retinoic acid in guinea pigs did not induce toxicity. However, repeated oral doses of retinol resulted in hypertrophy and hyperplasia of liver cells in monkeys, but no adverse skeletal changes in rats. Repeated oral doses of retinyl palmitate in rats were potentially toxic at the subcellular level. A facial moisturizer containing retinyl propionate did not induce significant ocular or skin irritation. There was no evidence of sensitization in subjects tested with a face cream containing retinyl propionate.

Retinol and its esters have an absorption maximum in the UV spectral region at approximately 325 nm, but retinoids were not found to be phototoxic or photoallergenic in human subjects. However, results were mixed regarding photocarcinogenic potential in the presence of UV light in animal studies. Retinoids were found to be reproductive toxicants in animal studies, and were also found to be anticarcinogenic as well as promoters of carcinogenicity/photocarcinogenicity.

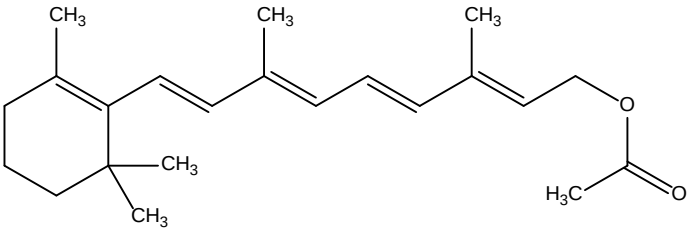
Figure 1: Strucutural formulas of some retinoids



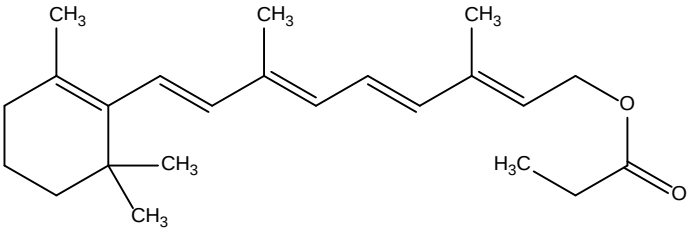
Retinol



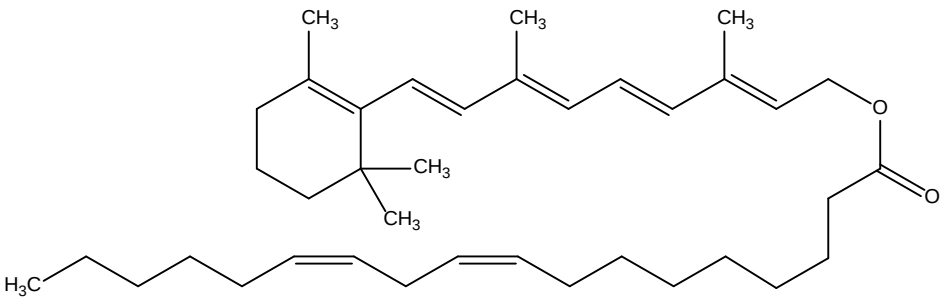
Retinyl Palmitate



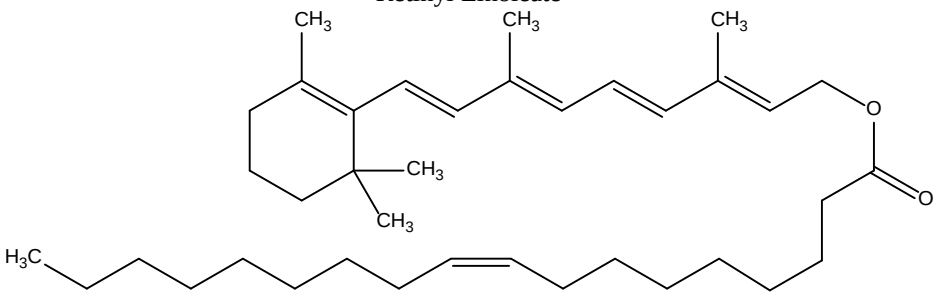
Retinyl Acetate



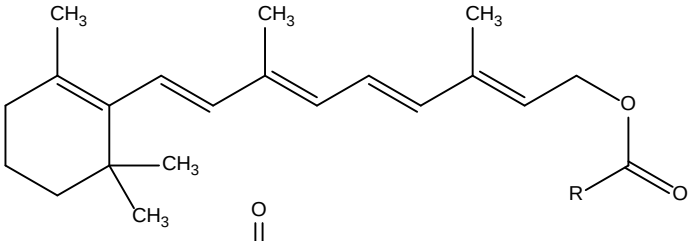
Retinyl Propionate

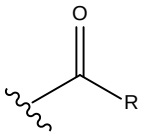


Retinyl Linoleate

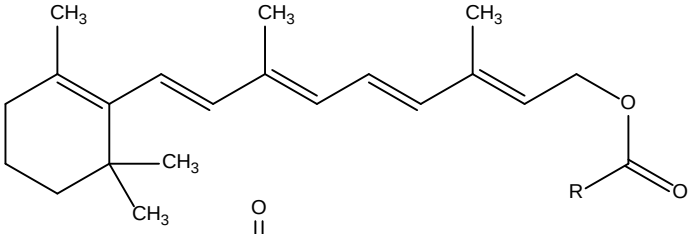


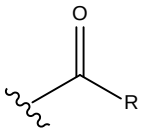
Retinyl Oleate



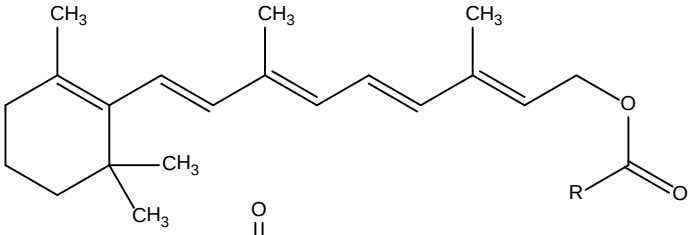
wherein  represent the ester formed from the mixture of fatty acids found in Oryza Sativa (Rice) Bran Oil

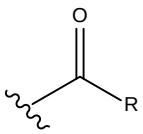
Retinyl Rice Branate



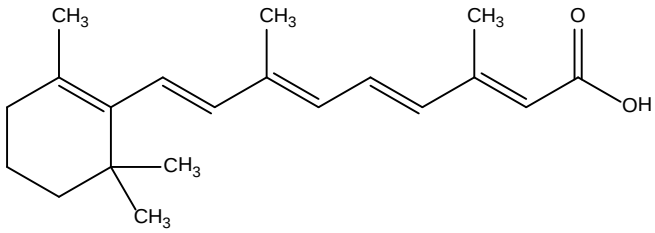
wherein  represent the ester formed from the mixture of fatty acids found in Soy Acid

Retinyl Soyate



wherein  represent the ester formed from the mixture of fatty acids found in Tall Oil Acid

Retinyl Tallate



Retinoic Acid

Table 1. Definitions and functions of the ingredients in this safety assessment.[16]

Ingredient, CAS No.	Definition	Function
Retinyl Palmitate [79-81-2]	Retinyl Palmitate is the ester of retinol and palmitic acid.	Skin-conditioning agents-miscellaneous
Retinol [11103-57-4;68-26-8]	Retinol is the organic compound that conforms to the formula: in Table 3.	Skin-conditioning agents-miscellaneous
Retinoic Acid [302-79-4]	Retinoic Acid is the organic compound that conforms to the formula in Table 3.	Antiacne agents
Retinyl Acetate [127-47-9]	Retinyl Acetate is the ester of retinol and acetic acid.	Skin-conditioning agents-miscellaneous
Retinyl Propionate [7069-42-3]	Retinyl propionate is the ester of retinol and propionic acid.	Skin-conditioning agents-miscellaneous
Retinyl Linoleate [631-89-0]	Retinyl linoleate is the ester of retinol and linoleic acid.	Skin-conditioning agents-miscellaneous
Retinyl Oleate [631-88-9]]	Retinyl oleate is the ester of retinol and oleic acid.	Skin-conditioning agents-miscellaneous
Retinyl Rice Branate	Retinyl Rice Branate is the product obtained by the reaction of retinol with rice bran acid.	Antioxidants; skin-conditioning agents-miscellaneous
Retinyl Soyate	Retinyl Soyate is the ester of retinol and soy acid.	Skin-conditioning agents-emollient; Skin-conditioning agents-miscellaneous
Retinyl Tallate	Retinyl Tallate is the ester of retinol and tall oil acid.	Skin-conditioning agents-miscellaneous

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure Provided in 2013.[17,18]

	Retinyl Palmitate		**Retinol**		**Retinoic Acid**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	182	0.01-0.5	18	0.0005-0.1	NR	NR
Incidental Ingestion	232	0.006-0.28	12	0.15	NR	NR
Incidental Inhalation- Sprays	85	0.0006-0.18	NR	NR	NR	NR
Incidental Inhalation- Powders	53	0.01-0.1	1	NR	NR	NR
Dermal Contact	1609	0.0000002-1.97	169	0.0005-1	4	NR
Deodorant (underarm)	1	NR	NR	NR	NR	NR
Hair - Non-Coloring	264	0.0001-0.5	3	0.1	NR	NR
Hair-Coloring	6	0.0011-0.02	NR	NR	NR	NR
Nail	32	0.01-0.1	4	0.01	NR	NR
Mucous Membrane	351	0.0000002-0.28	22	0.15	NR	NR
Baby Products	4	NR	1	NR	NR	NR
Duration of Use					NR	NR
Leave-On	1763	0.0001-1.97	173	0.0005-1	4	NR
Rinse off	383	0.00001-1	11	0.0005-0.01	NR	NR
Diluted for (bath) Use	15	0.0000002	4	NR	NR	NR
Totals/Conc. Range	2161	0.0000002-1.97	188	0.0005-1	4	NR
	Retinyl Acetate		**Retinyl Propionate**		**Retinyl Linoleate**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type						
Eye Area	5	0.11-0.12	2	0.1	6	0.1-0.11
Incidental Ingestion	1	0.06	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	1	NR	NR	NR	NR	NR
Dermal Contact	26	0.001-0.12	10	0.01-0.3	34	0.01-0.3
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	1	0.001-0.06	NR	0.045	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
Duration of Use						
Leave-On	27	0.05-0.12	9	0.01-0.3	34	0.01-0.3
Rinse off	NR	0.001-0.01	1	0.045-0.1	NR	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	27	0.001-0.12	10	0.01-0.3	34	0.01-0.3
	Retinyl Tallate					
	# of Uses	Conc. (%)				
Exposure Type						
Eye Area	1	NR				
Incidental Ingestion	NR	NR				
Incidental Inhalation- Sprays	NR	NR				
Incidental Inhalation- Powders	NR	NR				
Dermal Contact	1	NR				
Deodorant (underarm)	NR	NR				
Hair - Non-Coloring	NR	NR				
Hair-Coloring	NR	NR				
Nail	NR	NR				
Mucous Membrane	NR	NR				
Baby Products	NR	NR				
Duration of Use						
Leave-On	1	NR				
Rinse off	NR	NR				
Diluted for (bath) Use	1	NR				

Totals/Conc. Range	2	NR		

References

1. Elder, R. L. Final report on the safety assessment of retinyl palmitate and retinol. *JACT*. 1987;6(3):279-320.

2. Bergfeld, W. F. Belsito D. V. Klaassen C. D. Marks J. G. Jr. Shank R. C. Slaga T. J. Snyder P. W. and Andersen F. A. Annual review of cosmetic ingredient safety assessments: 2005/2006. Retinol and retinyl palmitate. *IJT*. 2008;27(1):120-127.

3. Environmental Working Group. http://www.ewg.org/2010sunscreen/. Date Accessed 7-28-2010.

4. Wang, S. Q., Dusza, S. W., and Lim, H. W. Safety of retinyl palmitate in sunscreens: a critical analysis. *J Am Acad.Dermatol.* 2010;63(5):903-906.

5. Fu, P. P., Xia, Q., Boudreau, M. D., Howard, P. C., Tolleson, W. H., and Wamer, W. G. Physiological role of retinyl palmitate in the skin. *Vitam.Horm.* 2007;75:223-256.

6. Al Tanoury, Z. Aleksandr P. and Rochette-Egly C. Vitamin A and retinoid signaling: genomic and non-genomic effects. *Journal of Lipid Research*. 2013;1-46.

7. Fu, P. P., Xia, Q., Yin, J. J., Cherng, S. H., Yan, J., Mei, N., Chen, T., Boudreau, M. D., Howard, P. C., and Wamer, W. G. Photodecomposition of vitamin A and photobiological implications for the skin. *Photochem.Photobiol.* 2007;83(2):409-424.

8. Crank, G. and Pardijanto M. S. Photooxidations and photosensitized oxidations of vitamin A and its palmitate ester. *J.Photochem.Photobiol.* 1995;85:93-100.

9. Lamb, L. E. Zaeba M. Plakoudas S. N. Sarna T. and Simon J. D. Retinyl palmitate and the blue-light-induced phototoxicity of human ocular lipofusacin. *Arch.Biochem.Biophys.* 2001;393:316-320.

10. Tatariunas, A. and Matsumoto S. A. A retinyl palmitate model of the phenomenon of the intrinsic fluorescence increase in ceroid-lipofuscin cytosomes. *Exp.Gerontol.* 2000;35:1327-1341.

11. Mei, N., Xia, Q., Chen, L., Moore, M. M., Chen, T., and Fu, P. P. Photomutagenicity of anhydroretinol and 5,6-epoxyretinyl palmitate in mouse lymphoma cells. *Chem.Res.Toxicol.* 2006;19(11):1435-1440.

12. Xia, Q., Yin, J. J., Wamer, W. G., Cherng, S. H., Boudreau, M. D., Howard, P. C., Yu, H., and Fu, P. P. Photoirradiation of retinyl palmitate in ethanol with ultraviolet light--formation of photodecomposition products, reactive oxygen species, and lipid peroxides. *Int.J Environ.Res.Public Health.* 2006;3(2):185-190.

13. Bempong, D. K. Honigberg I. L. and Meltzer N. M. Normal phase LC-MS determination of retinoic acid degradation products. *J.Pharm.Biomed.Anal.* 1995;13:285-291.

14. Murayama, A. Suzuki T. and Matsui M. Photoisomerization of retinoic acids under room light: a warning for cell biological study of geometrical isomers of retinoids. *J.Nutr.Sci.Vitaminol.* 1997;43:167-176.

15. Cahnman, H. J. A fast photoisomerization method for the preparation of tritium-labeled 9-cis-retinoic acid of high specific activity. *Anal.Biochem.* 1995;227:49-53.

16. Gottschalck, T. E. and Breslawec, H. P. International Cosmetic Ingredient Dictionary and Handbook. 14 *ed*. Washington, DC: Personal Care Products Council, 2012.

17. Food and Drug Administration (FDA). Information supplied to FDA by industry as part of the VCRP FDA database. 2013. Washington, D.C.: FDA.

18. Personal Care Products Council. Concentration of use by FDA product category. Retinol and retinyl esters. Unpublished data submitted by the Personal Care Products Council on 1-23-2013. 2013. pp.1-4.

19. Hubinger, J. C. Determination of retinol, retinyl palmitate, and retinoic acid in consumer cosmetic products. *J.Cosmet.Sci.* 2009;60(5):485-500.

20. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett*. 2011;205(2):97-104.

21. Bremmer HJ, Prud'homme de Lodder LCH, and van Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Date Accessed 8-24-2011. Report No. RIVM 320104001/2006. pp. 1-77.

22. Rothe H. Special aspects of cosmetic spray evaluation. 2011.

23. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing*. 2004;24-27.

24. Food and Drug Administration (FDA). Direct food additives. 21 CFR 184.1930; 582.5933; 582.5936. 2012.

25. Food and Drug Administration (FDA). Over-the-Counter (OTC) Drug Ingredients. 21 CFR 310.545. 2012.

26. Aquilina, G. Bach A. Bampidis V. et al. Scientific opinion on the safety and efficacy of vitamin A (retinyl acetate, retinyl palmitate, and retinyl propionate) as a feed additive for all animal species and categories. *EFSA Journal*. 2013;11(1):3037.

27. Food and Drug Administration (FDA). News & Events. Concerns regarding Accutane (isotretinoin). www.fda.gov. Date Accessed 5-14-2013.

28. Food and Drug Administration (FDA). Drugs. Inormation for healthcare professionals: Isotretinoin (marketed as Accutane). www.fda.gov. Date Accessed 5-14-2013.

29. Yourick, J. J. Jung C. t. and Bronaugh R. L. Percutaneous absorption of retinol in fuzzy rat (in vivo and in vitro) and human skin (in vitro) from cosmetic vehicles. *The Toxicologist*. 2006;90(S1):164.

30. Yourick, J. J. Jung C. t. and Bronaugh R. L. *In vitro* and *in vivo* percutaneous absorption of retinol from cosmetic formulations: Significance of the skin reservoir and prediction of systemic absorption. *Toxicol.Appl.Pharmacol.* 2008;231:117-121.

31. Jenning, V. Gysler A. Schafer-Korting M. et al. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. *Eur.J.Pharm.Biopharm.* 2000;49:211-218.

32. Yan, J., Xia, Q., Wamer, W. G., Boudreau, M. D., Warbritton, A., Howard, P. C., and Fu, P. P. Levels of retinyl palmitate and retinol in the skin of SKH-1 mice topically treated with retinyl palmitate and concomitant exposure to simulated solar light for thirteen weeks. *Toxicol Ind.Health.* 2007;23(10):581-589.

33. Yan, J., Wamer, W. G., Howard, P. C., Boudreau, M. D., and Fu, P. P. Levels of retinyl palmitate and retinol in the stratum corneum, epidermis, and dermis of female SKH-1 mice topically treated with retinyl palmitate. *Toxicol Ind.Health.* 2006;22(4):181-191.

34. Yan, J., Xia, Q., Webb, P., Warbritton, A. R., Wamer, W. G., Howard, P. C., Boudreau, M., and Fu, P. P. Levels of retinyl palmitate and retinol in stratum corneum, epidermis and dermis of SKH-1 mice. *Toxicol Ind.Health.* 2006;22.(3):103-112.

35. Willhite, C. C. Sharma R. P. Allen P. V. and Berry D. L. Percutaneous retinoid absorption and embryotoxicity. *J.Invest.Dermatol.* 1990;95(5):523-529.

36. Chou, R. Bürgin H. Schmitt G. and Eggers H. Absorption of tretinoin in rats and rabbits following oral and dermal application. *Arzneimittelforschung*. 1997;47(4):401-405.

37. Howard, W. B. Willhite C. C. Omaye S. T. and Sharma R. P. Comparative distribution, pharmacokinetics, and placental permeabilities of all-trans-retinoic acid, 13-cis-retinoic acid, all-trans-4-oxo-retinoic acid, retinyl acetate, and 9-cis-retinal in hamsters. *Arch.Toxicol.* 1989;63(2):112-120.

38. Mills, J. P. and Tanumihardjo S. A. Vitamin A toxicity in wild-caught African green vervet monkeys (*Chlorocebus aethiops)* after 2 years in captivity. *Comparative Medicine.* 2006;56(5):421-425.

39. Nohynek, G. J. Meuling W. J. A. Vaes W. H. J. Lawrence R. S. Shapiro S. Schulte S. Steiling W. Bausch J. Gerber E. Sasa H. and Nau H. Repeated topical treatment, in contrast to single oral doses, with vitamin A-containing preparations does not affect plasma concentrations of retinol, retinyl esters, or retinoic acids in female subjects of child-bearing age. *Toxicology Letters*. 2006;163(1):65-76.

40. Duitsman, P. K. and Olson J. A. Comparative embryolethality and teratogenicity of the all-trans isomers of retinoic acid, 3,4-didehydroretinyl acetate, and retinyl acetate in pegnant rats. *Teratology*. 1996;53(4):237-244.

41. Schäffer, M. W. Roy S. S. Mukhedrjee S. Ong D. E. and Das S. K. Uptake of all-trans retinoic acid-containing aerosol by inhalation to lungs in a guinea pig model system - A pilot study. *Exp.Lung Res.* 2010;36(10):593-601.

42. Lind, P. M. Johansson S. Rönn M. and Melhus H. Subclinical hypervitaminosis A in rat: Measurements of bone mineral density (BMD) do not reveal adverse skeletal changes. *Chemico-Biological Interactions*. 2006;159(1):73-80.

43. de Oliveira, M. R. and Moreira J. C. F. Acute and chronic vitamin A suplementation at therapeutic doses induces oxidative stress in submitochondrial particles isolated from cerebral cortex and cerebellum of adult rats. *Toxicology Letters*. 2007;173(3):145-150.

44. de Oliveira, M. R. Oliveira M. W. S. Lorenzi R. da Rocha R. F. and Moreira J. C. F. A Short-term vitamin A supplementation at therapeutic doses induces a pro-oxidative state in the hepatic environment and facilitates calcium-ion-induced oxidative stress in rat liver mitochondria independently from permeability transition pore formation. *Cell Biol.Toxicol.* 2009;25(6):545-560.

45. da Rocha, R. F., De Oliveira, M. R., Schonhofen, P., Schnorr, C. E., Dal, Pizzol F., and Moreira, J. C. Long-term vitamin A supplementation at therapeutic doses induces mitochondrial electrons transfer chain (METC) impairment and increased mitochondrial membrane-enriched fraction (MMEF) 3-nitrotyrosine on rat heart. *Free Radic.Res.* 2010;44(5):505-512.

46. McCormick, D. L. Hultin T. A. and Detrisac C. J. Potentiation of vitamin A hepatotoxicity by butylated hydroxytoluene. *Toxicol.Appl.Pharmacol.* 1987;90(1):1-9.

47. Takahashi, O. Hemorrhaic toxicity of a large dose of α–, β–, γ–, and δ–tocopherols, ubiquinone, β–carotene, retinol acetate, and L-ascorbic acid in the rat. *Fd.Chem.Toxic.* 1995;33(2):121-128.

48. Alberts, D. Ranger-Moore J. Einspahr J. Saboda K. Bozzo P. Liu Y. Xu X. C. Lotan R. Warneke J. Salasche S. Stratton S. Levine N. Goldman R. Islas M. Duckett L. Thompson D. Bartels P. and Foote J. Safety and efficacy of dose-intensive oral vitamin A in subjects with sun-damaged skin. *Clinical Cancer Research*. 2004;10(6):1875-1880.

49. North Cliff Consultants, Inc. A 28-day ophthalmologic and dermatologic safety evaluation of a moisturizer product containing 0.3% retinyl propionate. Unpublished data submitted by the Personal Care Products Council on 1-10-2013. 2013. pp.1-17.

50. Institute for in Vitro Sciences, Inc. Tissue equivalent assay with Epiocular™ cultures (face ceam containing 0.3% retinyl propionate). Unpublished data submitted by the Personal Care Products Council on 1-10-2013. 2008. pp.1-13.

51. Schiltz, J. R. Lanigan J. Nabial W. Petty B. and Birnbaum J. E. Retinoic acid induces cyclical changes in epidermal thickness and dermal collagen and glycosaminoglycan biosynthesis rates. *J.Invest.Dermatol.* 1986;87:663-667.

52. Gunning, D. B. Barua A. B. Myers R. K. Ueltschy A. Romans D. and Olson J. A. Comparative histologic effects of dialy topical application of creams containing all-trans-retinoic acid or all-trans-retinoyl ß-glucuronide on pig skin. *Skin.Pharmacol.Appl.Skin Physiol.* 2002;15(4):205-212.

53. Kang, S. Duell E. A. Fisher GJ et al. Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels of irritation. *J.Invest.Dermatol.* 1995;105:549-556.

54. Kang, S. Bergfeld W. Gottlieb et al. Long-term efficacy and safety of tretinoin emollient cream 0.05% in the treatment of photodamaged facial skin. *Am.J.Clin.Dermatol.* 2005;6(4):245-253.

55. Brelsford, M. and Beute T. C. Preventing and managing the side effects of isotretinoin. *Semin.Cutan.Med.Surg.* 2008;27(3):197-206.

56. Green, C. Orchard G. Cerio R. and Hawk J. L. M. A clinicopathological study of the effects of topical retinyl propionate cream in skin photoaging. *Clin.Exp.Dermatol.* 1998;23(4):162-167.

57. TKL Research, Inc. 21-day cumulative human irritation patch study (facial moisturizer containing 0.5% retinyl propionate). Unpublished data submitted by the Personal Care Products Council on 1-10-2013. 2005. pp.1-29.

58. Slade, H. B. Shroot B. Feldman S. R. Cargill D. I. and Stanfield J. Reappraising the phototoxicity of tretinoin: a report of four controlled clinical trials. *Photodermatol.Photoimmunol.Photomed.* 2009;25(3):146-152.

59. KGL, Inc. Human phototoxicity test of a topical product (face cream containing 0.4% retinyl propionate). Unpublished data submitted by the Personal Care Products Council on 1-10-2013. 2001. pp.1-15.

60. KGL, Inc. Human photoallergy test of a topical product (face cream containing 0.4% retinyl propionate). Unpublished data submitted by the Personal Care Products Council on 1-10-2013. 2001. pp.1-18.

61. TKL Research, Inc. Human repeated insult patch study (facial cream containing 0.4% retinyl propionate). Unpublished data submitted by the Personal Care Products on 1-10-2013. 2001. pp.1-27.

62. Rühl, R. Hänel A. Garcia A. L. Dahten A. Herz U. Schweigert F. J. and Worm M. Role of vitamin A elimination or supplementation diets during postnatal development on the allergic sensitization in mice. *Mol.Nutr.Food Res.* 2007;51:1173-1181.

63. Mastro, A. M. and Pepin K. G. The effects of retinoic acid and a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate, on lymphocyte proliferation. *Carcinogenesis.* 1982;3(4):409-413.

64. Nikawa, T. Ikemoto M. Kano M. Tokuoka K. Hirasaka K. Uehara S. Takatsu K. Rokutan K. and Kishi K. Impaired vitamin A-mediated mucosal IgA response in IL-5 receptor-knockout mice. *Biochem.Biophys.Res.Commun.* 2001;285(2):546-549.

65. Bessler, H. Wyshelesky G. Osovsky M. Prober V. and Sirota L. A comparison of the effect of vitamin A on cytokine secretion by mononuclear cells of preterm newborns and adults. *Neonatology*. 2007;91(3):196-202.

66. Long, K. Z. Santos I. S. Rosado J. L. Estrada-Gardia T. Haas M. Al Mamun A. Dupont H. L. and Nanthakumar N. N. Vitamin A supplementation modifies the association between mucosal innate and adaptive immune responses and resolution of enteric pathogen infections. *Am.J.Clin.Nutr.* 2011;93(3):578-585.

67. Boden, S. D. Labropoulos P. A. Ragsdale B. D. Gullino P. M. and Gerber L. H. Retinyl actate-induced arthritis in C3H-A^{vy} mice. *Arthritis Rheum.* 1989;32(5):625-633.

68. Yoon, H. S. Kim Y. K. and Chung J. H. High-concentration all-trans retinoic acid induces dermal inflammation and reduces the accumulation of type I procollagen in human skin in vivo. *Br.J.Dermatol.* 2011;165(3):669-672.

69. Castaño, G. Etchart C. and Sookoian S. Vitamin A toxicity in a physical culturist patient: A case report and review of the literature. *Annals of Hepatology*. 2006;5(4):293-295.

70. Cheruvattath, R. Orrego M. Gautam M. Byrne T. Alam S. Voltchenok M. Edwin M. Wilkens J. Williams J. W. and Vargas H. E. Vitamin A toxicity: When one a day doesn't keep the doctor away. *Liver Transplantation*. 2006;12(12):1888-1891.

71. Clemmensen, A., Thormann, J., and Andersen, K. E. Allergic contact dermatitis from retinyl palmitate in polycaprolactone. *Contact Dermatitis*. 2007;56(5):288-289.

72. Ramanathan, V. s. hensley G. French S. Eysselein V. Chung D. Reicher S. and Pham B. Hypervitaminosis A inducing intra-hepatic cholestasis - a rare case report. *Experimental and Molecular Pathology*. 2010;88(2):324-325.

73. Khasru, M. R. Yasmin R. Salek A. K. Khan K. H. Nath S. D. and Selim S. Acute hypervitaminosis A in a young lady. *Mymensingh Med.J.* 2010;19(2):294-298.

74. Lindgren, S. Groth O. and Molin L. Allergic contact response ro vitamin A acid. *Contact Dermatitis*. 1976;2(4):212-217.

75. Tanaka, M. Fukushima N. Itamura H. Urata C. Yokoo M. Ide M. Hisatomi T. Tomimasu R. Sueoka E. and Kimura S. Gangrenous cheilitis associated with all-trans rertinoic acid thexrapy for acute promyelocytic leukemia. *Int.J.Hematol.* 2010;91(1):132-135.

76. Heidenheim, M. and Jemec G. B. E. Occupational allergic contact dermatitis from vitamin A acetate. *Contact Dermatitis*. 1995;33:49.

77. Biesalski, H. K. Hemmes C. El Hanafy M. Weiser H. Zschaebitz H. and Stofft E. Long-term administration of high dose vitamin A to rats does not cause fetal malformations: macroscopic, skeletal and physicochmical findings. *J.Nutr.* 1996;126(4):973-983.

78. McDaniel, S. M., O'Neill, C., Metz, R. P., Tarbutton, E., Stacewicz-Sapuntzakis, M., Heimendinger, J., Wolfe, P., Thompson, H., and Schedin, P. Whole-food sources of vitamin A more effectively inhibit female rat sexual maturation, mammary gland

development, and mammary carcinogenesis than retinyl palmitate. *J Nutr.* 2007;137(6):1415-1422.

79. Rezaei, N., Hashemi Soteh, M. B., and Rahimi, F. Effects of limited doses of retinyl palmitate at the critical time of limb morphogenesis in mouse embryos. *Indian J Exp Biol.*. 2009;47(12):949-954.

80. Schnorr, C. E. Morrone M. D. S. Weber M. H. Lorenzi R. Behr G. A. and Moreira J. C. F. The effects of vitamin A supplementation to rats during gestation and lactation upon redox parameters: Increased oxidative stress and redox modulation in mothers and their offspring. *Food and Chemical Toxicology*. 2011;49(10):2645-2654.

81. Schnorr, C. E., da Silva, Morrone M., Simäes-Pires, A., da Rocha, R. F., Behr, G. A., and Moreira, J. C. Vitamin A supplementation in rats under pregnancy and nursing induces behavioral changes and oxidative stress upon striatum and hippocampus of dams and their offspring. *Brain Res.* 2011;1369:60-73.

82. Tember, E. A. Honeywell R. Buss N. E. and Renwick A. G. All-trans-retinoic acid in maternal plasma and teratogenicity in rats and rabbits. *Toxicol.Appl.Pharmacol.* 1996;141(2):456-472.

83. Juneja, H. S. Murthy S. K. and Ganguly J. Effect of retinoic acid on the reproductive performances of male and female rats. *Indian J.Exp.Biol.* 1964;2:153-154.

84. Kutz, S. A. Troise N. J. Cimprich R. E. Yearsley S. M. and Rugen P. J. Vitamin A acetate: A behavioral teratology study in rats. *Drug Chem.Toxicol.* 1989;12(3-4):259-275.

85. Kochhar, D. M. Teratogenic activity of retinoic acid. *Acta Pathol.Microbiol.Scand.* 1967;70:398-404.

86. Sadek, I. A. and Abdul-Mohsen M. H. Long-term admnistration of vitamin A and the process of spermatogenesis. *East.Mediterr.Health Journal*. 1999;5(1):123-129.

87. Seegmiller, R. E. Carter M. W. Ford W. H. and White R. D. Induction of maternal toxcity in the rat by dermal application of retinoic acid and its effect on fetal outcome. *Reprod.Toxicol.* 1990;4(4):277-281.

88. Eckhoff, C. Chari S. Kromka M. Staudner and H., Juhasz L. Rudiger H. and Agnish N. Teratogenicity and transplacental pharmacokinetics of 13-cis-retinoic acid in rabbits. *Toxicol.Appl.Pharmacol.* 1994;125(1):34-41.

89. Tzimas, G. Burgin H. Collins M. D. Hummler H. and Nau H. *Arch.Toxicol.* 1994;68(2):119-128.

90. Horie, S. and Yasuda M. Alterations in palatal ruga patterns in Jcl:ICR mouse fetuses from dams treated with all-*trans* retinoic acid. *Hiroshima J.Med.Sci.* 2001;50(1):17-25.

91. Mulder, g. b. Manley N. Grant J. Schmidt K. Zeng W. Eckhoff C. and Maggio-Price L. Effects of excess Vitamin A on development of cranial neural crest-derived structures: A neonatal and embryologic study. *Teratology*. 2000;62(4):214-226.

92. Freytag, T. L. Liu S. M. Rogers Q. R. and Morris J. G. Teratogenic effects of chronic ingestion of high levels of vitamin A in cats. *J.Anim.Physiol.Anim.Nutr.* 2003;87(1-2):42-51.

93. Lee, M. Potentiation of chemically induced cleft palate by ethanol ingestion during gestation in the mouse. *Teratog.Carcinog.Mutagen.* 1985;5(6):433-440.

94. Martínez-Angoa, A. Parra-Hernández E. Madrigal-Bujaidar E. Chamorro-Cevallos G. Carvajal-Sandoval G. and Zamudio-Cortes P. Reduction of all-trans retinoic acid-induced teratogenesis in the rat by glycine administration. *Birth Defects Res.A.* 2006;76(10):731-738.

95. West, K. P. Jr. Christian P. Labrique A. B. Rashid M. Shamim A. A. Klemm R. D. W. Massie A. B. Mehra S. Schulze K. J. Ali HY. Ullah B. Wu L. S. F. Katz J. Banu H. Akhter H. H. and Sommer A. Effects of vitamin A or beta carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh. *JAMA.* 2011;305(19):1986-1995.

96. Sladden, M. J. and Harman K. E. What is the chance of a normal pregnancy in a woman whose fetus has been exposed to isotretinoin? *Arch.Derm.* 2007;143:1187-1188.

97. Berard, A. Azoulay L. Koren G. et al. Isotretinoin, pregnancies, abortions, and birth defects. *Br.J.Clin.Pharm.* 2007;63:196-205.

98. Dai, W. S. LaBraico J. M. and Stern R. S. Epidemiology of isotretinoin exposure during pregnancy. *J.Am.Acad.Dermatol.* 1993;26:599-606.

99. Inoue, Y. Hasegawa S. Yamada T. Date Y. Mizutani H. Nakata S. Matsunaga K. and Akamatsu H. Bimodal effect of retinoic aciud on melanocyte differentiation identified by time-depoendent analysis. *Pigment Cell Melanoma Res.* 2012;25(3):299-311.

100. Nau, H. Embryotoxicity and teratogenicity of topical retinoic acid. *Skin Pharmacol.* 1993;6(1):35-44.

101. Hummler, H. Korte R. and Hendrickx A. G. Induction of malformations in the cynomolgus monkey with 13-cis retinoic acid. *Teratology.* 1990;42:263-272.

102. Hendrickx, A. G. and Hummler H. Teatogenicity of all-trans retinoic acid during early embryonic development in the cynomolgus monkey (*macaca fascicularis*). *Teratology.* 1992;45:65-74.

103. Louisse, J. Gönen S. Rietjens I. M. C. M. and Verwei M. Relative developmental toxicity potencies of retinoids in the embryonic stem cell test compared with their relative potencies in vivo and two other in vitro assays for developmental toxicity. *Toxicology Letters.* 2011;203(1):1-8.

104. Qin, S. and Huang C. C. Effect of retinoids on carcinogen-induced mutagenesis in Salmonella tester strains. *Mutation Research.* 1985;142(3):115-120.

105. Mei, N., Xia, Q., Chen, L., Moore, M. M., Fu, P. P., and Chen, T. Photomutagenicity of retinyl palmitate by ultraviolet a irradiation in mouse lymphoma cells. *Toxicol Sci.* 2005;88(1):142-149.

106. Fu, P. P. Cheng S. H. Coop L. Xia Q. Culp S. J. Tolleson W. H. Wamer W. G. and Howard P. C. Photoreaction, phototoxicity, and photocarcinogenicity of retinoids. *Journal of Environmental Science and Health*. 2003;C21(2):165-197.

107. Cherng, S. H., Xia, Q., Blankenship, L. R., Freeman, J. P., Wamer, W. G., Howard, P. C., and Fu, P. P. Photodecomposition of retinyl palmitate in ethanol by UVA light-formation of photodecomposition products, reactive oxygen species, and lipid peroxides. *Chem.Res.Toxicol.* 2005;18(2):129-138.

108. Yin, J. J. Xia Q. and Fu P. P. UVA photoirradiation of anhydroretinol-formation of singlet oxygen and superoxide. *Toxicol.Ind.Health*. 2007;23:625-631.

109. Xia, Q., Yin, J. J., Cherng, S. H., Wamer, W. G., Boudreau, M., Howard, P. C., and Fu, P. P. UVA photoirradiation of retinyl palmitate--formation of singlet oxygen and superoxide, and their role in induction of lipid peroxidation. *Toxicol Lett.* 2006;163(1):30-43.

110. Yan, J., Xia, Q., Cherng, S. H., Wamer, W. G., Howard, P. C., Yu, H., and Fu, P. P. Photo-induced DNA damage and photocytotoxicity of retinyl palmitate and its photodecomposition products. *Toxicol Ind.Health.* 2005;21(7-8):167-175.

111. Dufour, E. K. Whitwell J. Nohynek G. J. Kirkland D. and Toutain H. Retinyl palmitate is non-genotoxic in Chinese hanster ovary cells in the dark after pre-irradiation or simultaneous irradiation with UV light. *Mutation Research*. 2009;672(1):21-26.

112. Mei, N., Chen, T., Godar, D. E., and Moore, M. M. Letter to the editor. UVA-induced photomutagenicity of retinyl palmitate. *Mutat.Res.* 2009;677(1-2):105-108.

113. Dufour, E. K. Whitwell J. Nohynek G. J. Kirkland D. and Toutain H. Reply to the letter to the editor. *Mutat.Res.* 2009;677:107-108.

114. Mei, N. Hu J. Xia Q. Fu P. P. Moore M. M. and Chen T. Cytotoxicity and mutagenicity of retinol with ultraviolet A irradiation in mouse lymphoma cells. *Toxicology in Vitro*. 2010;24:439-444.

115. Sasaki, M. Sugimura K. Yoshida M. A. and Abe S. Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. *Senshokutai (Kromosoma)*. 1980;20:574-584.

116. Sirianni, S. R. Chen H. H. and Huang C. C. Effect of retinoids on plating efficiency, sister-chromatid exchange (SCE) and mitomycin-C induced SCE in cultured Chinese hamster cells. *Mutat.Res.* 1981;90:175-182.

117. Sorg, O. Tran C. Carraux P. Grand D. Hügin A. Didierjean L. and Saurat J. H. Spectral properties of topical retinoids prevent DNA damage and apoptosis after acute UV-B exposure in hairless mice. *Photochemistry and Photobiology*. 2005;81(4):830-836.

118. Kontek, R. Drozda R. Sliwinski M. and Grzegorczyk K. Genotoxicity of irinotecan and its modulation by vitamins A, C, and E in human lymphocytes from healthy individuals and cancer patients. *Toxicology in Vitro*. 2010;24(2):417-424.

119. Mehta, R. G. and Moon R. C. Inhibition of DNA synthesis by retinyl acetate during chemically induced mammary carcinogenesis. *Cancer Res.* 1980;40(4):1109-1111.

120. Welsch, C. W. Goodrich-Smith M. Brown C. K. and Crowe N. Enhancement by retinyl acetate of hormone-induced mammary tumorigenesis in female GR/A mice. *J.Natl.Cancder Inst.* 1981;67(4):935-938.

121. Kurokawa, Y. Hayashi Y. Maekawa A. takahashi M. and Kukubo T. High incidences of pheochomocyomas after long-term administration of retinol acetate to F344/DuCrj rats. *J.Natl.Cancer Inst.* 1985;74(3):715-723.

122. Grubbs, C. J. Eto I. Juliaa M. M. Hardin J. M. and Whitaker L. M. Effect of retinyl acetate and 4-hydroxyphenylretinamide on onitiation of chemically-induced mammary tumors. *Anticancer Res.* 1990;10(3):661-666.

123. Epstein, J. H. Chemicals and photocarcinogenesis. *Aust.J.Dermatol.* 1977;18:57-61.

124. Epstein, J. H. Effects of beta-carotene on ultraviolet-induced cancer formation in the hairless mouse skin. *Photochem.Photobiol.* 1977;25:211-213.

125. Kligman, L. H. Retinoic acid and photocarcinogenesis - a controversy. *Photodermatology*. 1981;4:88-101.

126. Forbes, P. D. Urbach F. and Davies R. E. Enhancement of experimental photocarcinogenesis by topical retinoic acid. *Cancer Lett.* 1979;7:85-90.

127. Forbes, P. D. Photocarcinogenesis: an overview. *J.Invest.Derm.* 1981;77:139-143.

128. Kligman, L. H. and Kligman A. M. Lack of enhancemernt of experimental photocarcinogenesis by topical retinoic acid. *Arch.Dermatol.Res.* 1981;270:453-462.

129. Hartmann, H. R. and Teelmann K. The influence of topical and oral retinoid treatment on photocarcinogenicity in hairless albino mice. Orfanos, C. E.Springer-Verlag; 1981:447-451.

130. Kligman, L. H. and Kligman A. M. Lack of enhancement of experimental photocarcinogenesis by retinoic acid. Orfanos, C. E. Braun-Falco O. Farber E. M. Grupper C. L. Polano M. K. and Schuppli R.Berlin: Springer-Verlag; 1981:411-415.

131. Davies, R. E. and Forbes P. D. Retinoids and photocarcinogenesis: a review. *J.Toxicol.-Cut.& Ocular Toxicology*. 1988;7:241-253.

132. Halliday, G. M. Robertson B. O. and Barnetson R. S. Topical retinoic acid enhances, and a dark tan protects, from subedemal solar-stimulated photocarcinogenesis. *J.Invest.Dermatol.* 2000;114:923-927.

133. Fu, P. P. Howard P. C. Culp S. G. Xia Q. Webb P. J. Blankenship L. R. Wamer W. G. and Bucher J. R. Do topically applied skin creams containing retinyl palmitate affect the photocarcinogenicity of simulated solar light? *J.Food Drug Anal.* 2002;10:262-268.

134. National Toxicology Program. NTP technical report of the photocarcinogenesis study of retinoic acid and retinyl palmitate in SKH-1 mice (simulated solar light and topical application study). http://ntp.niehs.gov. Date Accessed 4-26-2013.

135. Burnett, M. E. and Wang, S. Q. Current sunscreen controversies: a critical review. *Photodermatol.Photoimmunol.Photomed.* 2011;27(2):58-67.

136. Department of health and Human Services.National Toxicology Program (NTP). TR-568: technical report pathology tables and curves. http://ntp.niehs.nih.gov/index.cfm?objectid=555571BB-F1F6-975E-76F2BC5E369EB6F7. Date Accessed 8-17-2010.

137. Kraemer, K. H. et al. Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin. *New Engl.J.Med.* 1988;318:1633-1637.

138. McKenna, D. B. and Murphy G. M. Skin cancer chemoprophylaxis in renal transplant recipients: 5 years of experience using low-dose acitretin. *Br.J.Dermatol.* 1999;140:656-660.

139. Smith, D. M. Rogers A. E. herndon B. J. and Newberne P. M. Vitamin A (retinyl acetae) and benzo(α)pyrene-induced respiratory tract carcinogenesis in hanmsters fed a commercial diet. *Cancer Res.* 1975;35(1):11-16.

140. Smith, D. M. Rogers A. E. and Newberne P. M. Vitamin A and benzo(α)pyrene carcinogenesis in the respiratory tract of hamsters fed a semisynthetic diet. *Cancer Research*. 1975;35:1485-1488.

141. Thompson, H. J. Meeker L. D. and Becci P. J. Effect of combined selenium and retinyl acetate treatment on mammary carcinogenesis. *Cancer Res.* 1981;41(4):1413-1416.

142. McCormick, D. L. Burns F. J. and Albert R. E. Inhibition of rat mammary carcinogenesis by short dietary exposure to retinyl acetate. *Cancer Res.* 1980;40(4):1140-1143.

143. Dawson, W. D. Miller W. W. and Liles W. B. Retinyl acetate prophylaxis in cancer of the uinary bladder. *Invest.Urol.* 1979;16(5):376-377.

144. Maiorana, A. and Gullino P. M. Effect of retinyl acetate on the incidence of mammary carcinomas and hepatomas in mice. *J Natl.Cancer Inst.* 1980;64:655-663.

145. Kandarkar, S. V. Potdar P. D. and Sirsat S. M. Dose response effect of retinyl acetate on DMBA induced carcinogenesis in the hamster cheek pouch. *Neoplasma*. 1984;31(4):415-421.

146. Welsch, C. W. DeHoog J. V. and Moon R. C. Lack of an effect of dietary retinoids in chemical carcinogenesis of the mouse mammary gland: inverse relationship between mamary tumor cell anaplasia and retinoid efficacy. *Carcinogenesis*. 1984;5(10):1301-1304.

147. Stenbäck, F. Mu B. and Williams G. Retinyl acetate effects the life span and the incidence of cryptogenic neoplasms in C3H mice. *Nutr.Cancer*. 1987;10(3):119-128.

148. Slaga, T. J. Fischer S. M. Weeks C. E. and Klein-Szanto A. J. P. Multistage chemical carcinogenesis in mouse skin. *Curr.probl.Dermatol.* 1980;10:193-218.

149. Connor, M. J. Lowe N. J. Breeding J. H. and Chalet M. Inhibition of ultraviolet-B skin carcinogenesis by all trans-retinoic acid regimens that inhibit ornithine decarboxylase induction. *Cancer Res.* 1983;43:171-174.

150. Papadimitrakopoulou, V. A., Lee, J. J., William, W. N., Jr., Martin, J. W., Thomas, M., Kim, E. S., Khuri, F. R., Shin, D. M., Feng, L., Hong, W. K., and Lippman, S. M. Randomized trial of 13-cis retinoic acid compared with retinyl palmitate with or without beta-carotene in oral premalignancy. *J Clin.Oncol.* 2009;27(4):599-604.

151. Yuspa, S. H. Elgjo K. Morse M. A. and Wiebel F. J. Retinyl acetate modulation of cell growth kinetics and carcinogen-cellular interaction in mouse epidermal cell cultures. *Chem.-Biol.Interactions*. 1977;16(3):251-264.

2013 FDA VCRP Data

Retinol

01B - Baby Lotions, Oils, Powders, and Creams	1
02A - Bath Oils, Tablets, and Salts	4
03D - Eye Lotion	10
03G - Other Eye Makeup Preparations	8
05G - Tonics, Dressings, and Other Hair Grooming Aids	2
05I - Other Hair Preparations	1
07C - Foundations	2
07E - Lipstick	12
07F - Makeup Bases	1
07I - Other Makeup Preparations	3
08E - Nail Polish and Enamel	3
08G - Other Manicuring Preparations	1
10A - Bath Soaps and Detergents	6
12A - Cleansing	3
12C - Face and Neck (exc shave)	36
12D - Body and Hand (exc shave)	15
12F - Moisturizing	21
12G - Night	17
12H - Paste Masks (mud packs)	2
12I - Skin Fresheners	6
12J - Other Skin Care Preps	34
Total	**188**

Retinyl Palmitate

01B - Baby Lotions, Oils, Powders, and Creams	3
01C - Other Baby Products	1
02B - Bubble Baths	12
02D - Other Bath Preparations	3
03A - Eyebrow Pencil	12
03B - Eyeliner	15
03C - Eye Shadow	39
03D - Eye Lotion	49
03E - Eye Makeup Remover	2
03F - Mascara	18
03G - Other Eye Makeup Preparations	47
04A - Cologne and Toilet waters	4
04C - Powders (dusting and talcum, excluding aftershave talc)	1
04E - Other Fragrance Preparation	12
05A - Hair Conditioner	69
05B - Hair Spray (aerosol fixatives)	15
05C - Hair Straighteners	2
05E - Rinses (non-coloring)	1
05F - Shampoos (non-coloring)	70
05G - Tonics, Dressings, and Other Hair Grooming Aids	74

05H - Wave Sets	1
05I - Other Hair Preparations	32
06B - Hair Tints	1
06C - Hair Rinses (coloring)	1
06D - Hair Shampoos (coloring)	2
06H - Other Hair Coloring Preparation	2
07A - Blushers (all types)	43
07B - Face Powders	49
07C - Foundations	79
07D - Leg and Body Paints	2
07E - Lipstick	232
07F - Makeup Bases	13
07G - Rouges	3
07H - Makeup Fixatives	2
07I - Other Makeup Preparations	63
08A - Basecoats and Undercoats	4
08B - Cuticle Softeners	9
08C - Nail Creams and Lotions	5
08E - Nail Polish and Enamel	5
08G - Other Manicuring Preparations	9
10A - Bath Soaps and Detergents	68
10B - Deodorants (underarm)	1
10D - Feminine Deodorants	1
10E - Other Personal Cleanliness Products	35
11A - Aftershave Lotion	26
11E - Shaving Cream	4
11G - Other Shaving Preparation Products	6
12A - Cleansing	83
12B - Depilatories	5
12C - Face and Neck (exc shave)	200
12D - Body and Hand (exc shave)	144
12E - Foot Powders and Sprays	1
12F - Moisturizing	333
12G - Night	71
12H - Paste Masks (mud packs)	31
12I - Skin Fresheners	7
12J - Other Skin Care Preps	88
13A - Suntan Gels, Creams, and Liquids	3
13B - Indoor Tanning Preparations	40
13C - Other Suntan Preparations	8
Total	**2,161**

Retinoic Acid

12F - Moisturizing	1
12J - Other Skin Care Preps	3
Total	**4**

2013 FDA VCRP Data

Retinyl Acetate

03D - Eye Lotion	5
07B - Face Powders	1
07E - Lipstick	1
07I - Other Makeup Preparations	2
12C - Face and Neck (exc shave)	7
12D - Body and Hand (exc shave)	2
12F - Moisturizing	6
12G - Night	3
Total	**27**

Retinyl Propionate

03D - Eye Lotion	1
03G - Other Eye Makeup Preparations	1
12A - Cleansing	1
12C - Face and Neck (exc shave)	2
12F - Moisturizing	4
12G - Night	1
Total	**10**

Retinyl Linoleate

03C - Eye Shadow	2
03D - Eye Lotion	2
03G - Other Eye Makeup Preparations	2
07A - Blushers (all types)	1
12C - Face and Neck (exc shave)	9
12D - Body and Hand (exc shave)	3
12F - Moisturizing	3
12G - Night	5
12I - Skin Fresheners	1
12J - Other Skin Care Preps	6
Total	**34**

Retinyl Tallate

03D - Eye Lotion	1
Total	**1**

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: January 10, 2013

SUBJECT: Studies on Products Containing Retinyl Propionate

TKL Research, Inc. 2005. 21-Day cumulative human irritation patch study (facial moisturizer containing 0.5% Retinyl Propionate).

TKL Research, Inc. 2001. Human repeated insult patch study (face cream containing 0.4% Retinyl Propionate).

North Cliff Consultants, Inc. 2007. A 28-day ophthalmologic and dermatologic safety evaluation of a moisturizer product containing 0.3% Retinyl Propionate.

Institute for In Vitro Sciences, Inc. 2008. Tissue equivalent assay with Epiocular™ cultures (face cream containing 0.3% Retinyl Propionate).

KGL, Inc. 2001. Human phototoxicity test of a topical product (face cream containing 0.4% Retinyl Propionate).

KGL, Inc. 2001. Human photoallergy test of a topical product (face cream containing 0.4% Retinyl Propionate)

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

21- DAY CUMULATIVE HUMAN IRRITATION PATCH STUDY

Facial moisturizer containing 0.5 % Retinyl Propionate

TKL STUDY NO. [redacted]

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF REPORT:

July 13, 2005

TKL Research, Inc., 4 Forest Avenue, Paramus, NJ 07652 Tel (201) 587-0500 Fax (201) 587-0878 Email: info@tklresearch.com

TABLE OF CONTENTS

APPENDICES

SIGNATURES

Kathleen Georgeian
Kathleen Georgeian, Clinical Research Coordinator and Manager, Dermatologic Safety Testing

7/14/05
Date

Jonathan S. Dosik, MD
Principal Investigator

7/12/05
Date

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Research, Inc. (TKL) Corporate Quality Assurance Department and the report accurately reflects the raw data for this study.

Clinical research studies are performed by TKL in accordance with federal regulations and proposed guidelines for good clinical practices which include:

21 CFR Part 312,	Investigational New Drug Application
21 CFR Part 50,	Protection of Human Subjects
21 CFR Part 56,	Institutional Review Boards

Henry Bucci
Quality Assurance

7/13/05
Date

TITLE OF STUDY

21-Day Cumulative Human Irritation Patch Study

SPONSOR

[redacted]

[redacted]

STUDY MATERIALS

[redacted] 448-095 [redacted]

This is a facial moisturizer containing 0.5% retinyl propionate (CAS 7069-42-3)

Distilled Water
Sodium Lauryl Sulfate (SLS)
[redacted]

DATE STUDY INITIATED

June 1, 2004

DATE STUDY COMPLETED

June 22, 2004

DATE OF REPORT

July 13, 2005

INVESTIGATIVE PERSONNEL

Jonathan S. Dosik, MD
Dermatologist
Principal Investigator

Kathleen Georgeian
Clinical Research Coordinator
and Manager, Dermatologic Safety Testing

Tina Kelly
Assistant Manager, Dermatologic Safety Testing

CLINICAL SITE

TKL RESEARCH, INC.
1099 Wall Street West
Lyndhurst, NJ 07071

SUMMARY

Six study materials, [redacted]448-095, [redacted], were evaluated neat to determine their ability to cause irritation to the skin of normal volunteer subjects using an occlusive 21-day cumulative irritation patch study. Sodium lauryl sulfate (SLS), 0.2% w/v aqueous solution, served as a positive control and distilled water served as a negative control. Twenty-six subjects completed the study. The dermatologist was in attendance at the final evaluation.

This study determined the following irritation scores and associated classifications:

Study Material	Irritation Scores Total	Irritation Scores Normalized	Classification of Normalized Scores	C.I.I.
[redacted]				
[redacted]448-095	28.0	10.2	No significant irritation	.011
[redacted]				
Distilled water	51.0	18.6	No significant irritation	.023
SLS 0.2% w/v aqueous solution	946.5	344.5	Moderately irritating	.424
[redacted]				

1.0 OBJECTIVE

The study objective was to determine the ability of the study material to cause irritation 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 cause irritation. 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 product can be applied safely to human skin without significant risk of adverse reactions. Cutaneous irritation accounts for the majority of cases of contact dermatitis. Reactions consist of local inflammatory responses characterized by erythema and/or edema, or an erosive reaction characterized by local tissue destruction or necrosis. These reactions are due to direct damage to the epidermis cells and require no prior sensitization. No immunologic (allergic) mechanism is involved.

Cumulative irritancy patch evaluation can detect weak irritants, which require multiple applications to produce skin irritation. During and after first contacts with weak irritants, no visible skin alterations are observed. After repeat contact, the skin gradually becomes erythematous; drying and cracking occur; and later, oozing, crusting, and erosion may develop. An eczematous reaction with papules, vesicles, and edema may also develop. This evaluation procedure may also detect so-called "fatiguing substances" which are mild irritants that cause more strongly positive reactions with successive multiple skin exposures. The procedure employed is a modification of that described by Dr. B. M. Lanman[1] at the Joint Conference on Cosmetic Sciences, April 21-23, 1968 in Washington, D.C., and further modified by Phillips, et.al[2] and Berger, et.al[3].

3.0 STUDY DESIGN

3.1 STUDY POPULATION

A sufficient number of healthy subjects between the ages of 18 and 65 were enrolled to provide 25 completed subjects.

3.1.1 Exclusion Criteria

1. Use of immunosuppressive drug at time of enrollment and/or had received organ transplant.

2. Routine or frequent use of topical or systemic anti-inflammatory drug for a defined medical condition, eg, ibuprofen, corticosteroid, at time of enrollment. Aspirin use was not to exceed two tablets (650/mg total)/day.

3. Application within two weeks prior to study enrollment of any anti-inflammatory drug to skin area used in testing.

4. Clinically significant active dermatitis or skin disease anywhere on the body (excluding facial acne); skin cancer (or a history), at or near sites used in evaluation.

5. Insulin-dependent diabetes.

6. Asthma that required daily therapy or other chronic respiratory conditions.

7. Receiving allergy injections at time of enrollment, had received a final injection within the week prior to study enrollment or expected to begin injections during the study.

8. Treated for malignancy (of any kind) within the last six months.

9. Immune deficiency or autoimmune disease.

10. Bilateral mastectomy; mastectomy within the last year; axillary lymph nodes (both arms) removed for any reason.

11. Scars, moles or other blemishes/abnormalities within the study area which, in the supervisor's/designate's judgment would have interfered with grading/assessment of responses.

12. Erythema greater than Grade 1 (e.g., due to sunburn) in the study area.

13. Had a condition or was taking or had taken a medication which, in the Investigator's judgment, made the subject ineligible or placed the subject at undue risk. (If the potential subject was under the care of a physician, approval to participate may have been sought from that physician, at the Investigator's discretion and/or in accordance with regulatory requirements.)

14. Participating in another dermal clinical study of any kind or any clinical study which, in the judgment of the investigator, could have potentially affected responses in this study.

15. Had participated in a patch test within the last four weeks.

16. Had previously been dropped from a patch test due to tape irritation.

17. Unwilling or unable to give informed consent or to otherwise comply with protocol requirements.

3.1.3 Informed Consent

A properly executed informed consent document in compliance with FDA regulations (21 CFR 50) 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 IV).

3.2 DESCRIPTION OF STUDY

3.2.1 Outline of Study Procedures

The study extended over a 22-consecutive-day period with 21 product applications and evaluations. On Day 1, the study material and controls were applied to the back under conditions described in Section 3.3.4. Twenty-three (±1 hour) hours later the patches were removed. Twenty to 40 minutes after patch removal the sites were evaluated, the responses recorded, and identical patches applied to the same sites. This was repeated daily for a total of 21 days, including Saturdays and Sundays.

3.2.2 Study Flow Chart

Day	Activities
1	Obtained informed consent, reviewed completed medical screening form, applied patches
2-21	Staff removed patches, graded, applied patches
22	Staff removed patches, graded

3.2.3 Definitions Used for Grading Responses

Responses were graded using the following symbols.

Response	Numerical Equivalent
No apparent cutaneous involvement	0
Greater than 0, less than 1 (Faint, barely perceptible erythema OR slight dryness [glazed appearance])	0.5
Faint but definite erythema, no eruptions or broken skin OR no erythema but definite dryness; may have epidermal fissuring	1.0
Greater than 1, less than 2 (Well-define erythema OR faint erythema with definite dryness, may have epidermal fissuring)	1.5
Moderate erythema, may have a few papules OR deep fissures, moderate-to-severe erythema in the cracks	2.0
Greater than 2 less than 3 (Moderate erythema with barely perceptible edema OR severe erythema not involving a significant portion of the patch [halo effect around the edges], may have a few papules OR moderate-to-severe erythema	2.5
Severe erythema (beet redness), may have generalized papules OR moderate-to-severe erythema with slight edema (edges well defined by raising)	3.0
Greater than 3, less than 4 (Moderate-to-severe erythema with moderate edema(confined to patch area) OR moderate-to-severe erythema with isolated eschar formations or vesicles)	3.5
Generalized vesicles or eschar formations OR moderate-to-severe erythema and/or edema extending beyond the area of the patch.	4.0

The maximum obtainable individual score was a 4.0. Should a 2.0 reaction have occurred at any point during the study, further patch application on that subject would have been terminated with respect to the product involved. An "NP" symbol and a score of 2.0 would be assigned to all subsequent days.

An irritation score for each product was calculated by summing each individual's scores on each of 21 evaluation days and normalizing the data to 10 subjects. The normalized score is the total score divided by the total number of readings for all subjects and multiplied by 21 (the number of days) and by 10 (to normalize to 10 subjects).

The Cumulative Irritation Index (C.I.I.) was calculated by dividing the total score by the sums of the highest possible score multiplied by the number of subjects multiplied by the number of days.

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.

3.3 Study Material

3.3.1 Storage, Handling, and Documentation of Study Material

Receipt was documented in a general logbook that serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL Research, Inc. A sample of the study material was reserved and will be stored for a period of 6 months. At the conclusion of the clinical study, the remaining study material was discarded or returned and the disposition documented in the logbook. All information regarding the receipt, storage and disposition of the study material was also recorded on a Clinical Material Record form (see Appendix III) which is incorporated in this study report. All study material is kept in a locked product storage room accessible to clinical staff members only.

3.3.2 Nature of Study Material

[redacted]

Identification : [redacted]448-095 Moisturizer
Amount Applied : 0.2 mL

[redacted]

[redacted]

Special Instructions

Applied to patch pad no longer than 15 minutes prior to patch application.

The distilled water negative control and the SLS 0.2% positive control were applied in amounts of 0.2 mL.

3.3.3 Application of Study Material

Study material was applied to patches as instructed. Patches were applied in a randomized schedule to the infrascapular area of the back, either to the right or left of the midline.

3.3.4 Description of Patch Conditions

Material evaluated under occlusive patch conditions was applied to a 2-cm x 2-cm Webril pad attached to a non-porous, plastic film adhesive bandage (3M medical tape). The patch was secured with hypoallergenic tape (Micropore), as needed.

4.0 INTERPRETATION

To qualify as an "irritant", a substance should evoke inflammation on initial exposure (primary irritation) or on repeated exposure to an identical site (cumulative irritation). An irritant substance will cause dermatitis if it is permitted to act in sufficient concentration for a sufficient length of time. Irritant reactions may develop in all subjects, although individual susceptibility varies greatly.

The materials are classified as to irritancy in terms of the following scale, normalized to 10 subjects.

NORMALIZED SCORE	CLASSIFICATION
0 - 49	No significant irritation
50-199	Slightly irritating
200-449	Moderately irritating
450-630	Highly irritating

Substances in the high end of one classification may or may not be clinically different in irritation potential from materials in the low end of the next higher classification. The classification system should be considered useful for its comparative value for study substances that are relatively mild.

5.0 PROTOCOL

See Protocol - Appendix V.

6.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms (CRFs) are 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 is maintained either at a TKL facility in a secured room accessible only to TKL employees, or at an offsite location which provides 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 & DISCUSSION

Thirty-three subjects between the ages of 18 and 65 were enrolled and 26 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:		33
Number discontinued:		7
Voluntary withdrawal	3	
Protocol violation (2 subjects doing study at another facility) (1 subject was breastfeeding a child)	3	
Other (Subject scratched patch area)	1	
Number completed:		26

Source: Table 1, Appendix I

There were no adverse events reported.

The dermatologist was in attendance at the final evaluation.

A summary of response data is provided in Table 3, Appendix I. Individual dermatological response grades are provided in Data Listing 3 and residual readings in Data Listing 3A, Appendix II.

This study determined the following irritation scores and associated classifications:

Study Material	Irritation Scores Total	Irritation Scores Normalized	Classification of Normalized Scores	C.I.I.
[redacted]				
[redacted]448-095	28.0	10.2	No significant irritation	.011
[redacted]				
Distilled water	51.0	18.6	No significant irritation	.023
SLS 0.2% w/v aqueous solution	946.5	344.5	Moderately irritating	.424
[redacted]				

7.0 REFERENCES

1 B.M. Lanman, E.B. Elvers and C.J. Howard. "The Role of Human Patch Testing in a Product Development Program" Joint Conference on Cosmetic Sciences, The Toilet Goods Association, Washington, D.C., April 21-23, 1968.

2 L. Philips, M. Steinberg, H.I. Maibach and W.A. Akers. "Comparison of Rabbit and Human Skin Response to Certain Irritants". Toxicol. Appl. Pharmacol. 21.369, 1972.

3 R.S. Berger and J.P. Bowman. "A Reappraisal of the 21-day Cumulative Irritation Test in Man" J. Toxicol. - Cut. & Ocular Toxical. 1 (2). 109-115, 1982.

APPENDIX I

SUMMARY TABLES

TKL STUOY NO. [redacted]

TABLE 1: SUMMARY OF SUBJECT ENROLLMENT ANO OISPOSITION

	N (%)
SUBJECTS ENROLLED	33
SUBJECTS COMPLETEO ALL PHASES	26 (78.8)
TOTAL SUBJECTS OISCONTINUED	7 (21.2)
LOST TO FOLLOW-UP	3 (9.1)
PROTOCOL VIOLATION	3 (9.1)
OTHER REASONS	1 (3.0)

NOTE: ALL PERCENTAGES ARE RELATIVE TO TOTAL SUBJECTS ENROLLEO

SEE OATA LISTING 1 FOR FURTHER DETAIL

PROGRAM: OISPSMY.SAS/USES: FINAL/21JUL04:09:05:23/PRODUCT=R

TKL STUDY NO. [redacted]

TABLE 2: SUMMARY OF SUBJECT DEMOGRAPHICS

ALL ENROLLED SUBJECTS

AGE	
N (%) 18 TO 44	16 (48.5)
N (%) 45 TO 64	16 (48.5)
N (%) 65 AND UP	1 (3.0)
MEAN (SD)	42.6 (14.4)
MEDIAN	45.6
RANGE	18.6 TO 65.6
GENDER	
N (%) MALE	8 (24.2)
N (%) FEMALE	25 (75.8)
RACE	
N (%) CAUCASIAN	16 (48.5)
N (%) HISPANIC	16 (48.5)
N (%) OTHER	1 (3.0)

SEE DATA LISTING 2 FOR FURTHER DETAIL

PROGRAM: DEMOSMY.SAS/USES: DEMOGS/21JUL04:09:05:23/PRODUCT=R

[redacted]

TKL STUDY NO. [redacted]

TABLE 3: SUMMARY OF OERMATOLOGIC RESPONSE GRAOES
NUMBER OF SUBJECTS BY PRODUCT

PRODUCT= [redacted]448-095

	READING NO.																				
RESPONSE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0	32	28	28	28	28	28	27	27	26	26	25	25	25	25	25	21	22	21	21	21	21
.5	0	1	1	1	1	1	0	0	0	1	2	2	2	1	1	5	4	4	4	4	3
1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1	1	2
TOTAL EVALUABLE	32	29	29	29	29	29	27	27	27	27	27	27	27	27	27	27	26	26	26	26	26
NUMBER DISCONTINUEO	1	4	4	4	4	4	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTI8LE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT DEFINITE ERYTHEMA 2 = MODERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTI8LE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: SUMMARY.SAS/USES: RESPONSE, PROOLIST, FINAL/21JUL04:09:05:25/PRODUCT=R

TKL RESEARCH

TKL STUDY NO. [redacted]

TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES

NUMBER OF SUBJECTS BY PRODUCT

PRODUCT= DISTILLED WATER

	READING NO.																				
RESPONSE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0	31	28	28	27	28	28	26	26	25	22	22	22	22	19	19	18	20	21	21	21	21
.5	1	1	1	2	1	1	1	1	1	4	4	4	4	5	5	5	6	5	5	5	5
1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	4	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
TOTAL EVALUABLE	32	29	29	29	29	29	27	27	27	27	27	27	27	27	27	27	26	26	26	26	26
NUMBER DISCONTINUED	1	4	4	4	4	4	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTIBLE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT DEFINITE ERYTHEMA 2 = MODERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTIBLE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/21JUL04:09:05:25/PRODUCT=R

TKL RESEARCH

TKL STUDY NO. [redacted]

TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES

NUMBER OF SUBJECTS BY PRODUCT

PRODUCT= SLS 0.2% LOT# CS12470026

	READING NO.																				
RESPONSE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0	31	16	8	4	3	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.5	0	3	4	7	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	4	7	7	10	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.5	0	6	10	11	11	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	12	23	25	25	25	25	25	25	25	25	25	24	24	24	24	24
2.5	0	0	0	0	0	0	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
TOTAL EVALUABLE	32	29	29	29	29	29	27	27	27	27	27	27	27	27	27	27	26	26	26	26	26
NUMBER DISCONTINUEO	1	4	4	4	4	4	6	6	6	6	6	6	6	6	6	6	7	7	7	7	7

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTIBLE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT OEFINITE ERYTHEMA 2 = MODERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTIBLE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/21JUL04:09:05:25/PRODUCT=R

APPENDIX II

DATA LISTINGS

TKL STUDY NO. [redacted]

OATA LISTING 1: SUBJECT ENROLLMENT ANO DISPOSITION

SUBJECT NO.	STUDY DATES SCREENED	1ST APPLIC	ENDED	LAST READING #	COMPLETION STATUS	DAYS ON STUDY
1	06/01/04	06/01/04	06/22/04	21	C	22
2	06/01/04	06/01/04	06/22/04	21	C	22
3	06/01/04	06/01/04	06/22/04	21	C	22
4	06/01/04	06/01/04	06/22/04	21	C	22
5	06/01/04	06/01/04	06/22/04	21	C	22
6	06/01/04	06/01/04	06/22/04	21	C	22
7	06/01/04	06/01/04	06/22/04	21	C	22
8	06/01/04	06/01/04	06/22/04	21	C	22
9	06/01/04	06/01/04	06/22/04	21	C	22
10	06/01/04	06/01/04	06/22/04	21	C	22
11	06/01/04	06/01/04	06/22/04	21	C	22
12	06/01/04	06/01/04	06/22/04	21	C	22
13	06/01/04	06/01/04	06/22/04	21	C	22
14	06/01/04	06/01/04	06/22/04	21	C	22
15	06/01/04	06/01/04	06/22/04	21	C	22
16	06/01/04	06/01/04	06/22/04	21	C	22
17	06/01/04	06/01/04	06/22/04	21	C	22
18	06/01/04	06/01/04	06/22/04	21	C	22
19	06/01/04	06/01/04	06/22/04	21	C	22
20	06/01/04	06/01/04	06/22/04	21	C	22
21	06/01/04	06/01/04	06/18/04	16	O	18
22	06/01/04	06/01/04	06/22/04	21	C	22
23	06/01/04	06/01/04	06/22/04	21	C	22
24	06/01/04	06/01/04	06/08/04	6	V	8
25	06/01/04	06/01/04	06/08/04	6	V	8
26	06/01/04	06/01/04	06/22/04	21	C	22
27	06/01/04	06/01/04	06/22/04	21	C	22
28	06/01/04	06/01/04	06/22/04	21	C	22
29	06/01/04	06/01/04	06/22/04	21	C	22
30	06/01/04	06/01/04	06/02/04	0	V	2
31	06/01/04	06/01/04	06/03/04	1	L	3
32	06/01/04	06/01/04	06/03/04	1	L	3
33	06/01/04	06/01/04	06/02/04	1	L	2

KEY: 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/21JUL04:09:05:05

TKL STUDY NO. [redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

SUBJECT NO.	AGE	GENDER	RACE
1	46.9	FEMALE	HISPANIC
2	38.5	FEMALE	CAUCASIAN
3	44.7	FEMALE	CAUCASIAN
4	26.8	MALE	CAUCASIAN
5	64.1	FEMALE	CAUCASIAN
6	24.4	FEMALE	CAUCASIAN
7	46.7	FEMALE	CAUCASIAN
8	48.3	MALE	HISPANIC
9	51.8	FEMALE	CAUCASIAN
10	51.7	FEMALE	CAUCASIAN
11	65.6	FEMALE	CAUCASIAN
12	45.6	FEMALE	CAUCASIAN
13	32.3	MALE	CAUCASIAN
14	54.2	FEMALE	HISPANIC
15	25.0	FEMALE	HISPANIC
16	49.5	MALE	HISPANIC
17	18.6	FEMALE	CAUCASIAN
18	36.0	FEMALE	CAUCASIAN
19	54.5	MALE	HISPANIC
20	64.8	FEMALE	HISPANIC
21	60.3	MALE	HISPANIC
22	20.1	FEMALE	CAUCASIAN
23	20.0	FEMALE	CAUCASIAN
24	48.4	FEMALE	HISPANIC
25	52.1	FEMALE	HISPANIC
26	26.1	FEMALE	HISPANIC
27	63.8	FEMALE	CAUCASIAN
28	56.1	MALE	HISPANIC
29	21.6	FEMALE	WEST INDIAN
30	33.2	FEMALE	HISPANIC
31	34.2	MALE	HISPANIC
32	40.1	FEMALE	HISPANIC
33	38.9	FEMALE	HISPANIC

[redacted]

TKL STUDY NO. [redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted]448-095

SUBJECT NUMBER	READING NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	TOTAL SCORE
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
2	0	0	D	0	0	0	0	0	0	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	0	.5	0	0	0	0	0	0.5
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	.5	.5	.5	.5	.5	3.0
5	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0.0
7	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0.0
9	0	0	0	0	0	0	0	0	0	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	0	.5	.5	.5	.5	.5	1	3.5
11	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0.0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
14	0	0	0	0	0	0	0	0	0	0	.5	.5	.5	1	1	1	.5	.5	.5	.5	.5	7.0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTIBLE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT DEFINITE ERYTHEMA 2 = MODERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTIBLE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/21JUL04:09:05:07/PRODUCT=R

[redacted]

TKL STUDY NO. [redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted]448-095

SUBJECT NUMBER	READING NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	TOTAL SCORE
16	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	.5	.5	.5	.5	.5	.5	3.0
18	0	.5	.5	.5	.5	.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.5
19	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0.0
21	0	0	0	0	0	0	0	0	1	.5	.5	.5	.5	.5	.5	.5	X	X	X	X	X	4.5
22	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0.0
24	0	0	0	0	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
25	0	0	0	0	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
26	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0.0
28	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	1	1	1	1	4.0
30	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
31	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
32	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
33	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0

	ALL SUBJECTS	COMPLETED SUBJECTS ONLY
TOTAL SCORE	28.0	23.5
NORMALIZED TOTAL SCORE	10.2	9.0

C.I.I. = .011

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/21JUL04:09:05:07/PRODUCT=R

TKL RESEARCH
TKL STUDY NO. [redacted]
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= DISTILLED WATER

SUBJECT NUMBER	READING NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	TOTAL SCORE
1	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0.0
3	0	0	.5	.5	0	0	0	0	0	.5	.5	.5	.5	.5	.5	.5	0	0	0	0	0	4.5
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	0	0	0	0	0.5
5	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	.5	0	0	0	0	0	0	1.0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	.5	.5	.5	.5	.5	.5	.5	4.0
7	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0.0
9	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0.0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
12	0	0	0	.5	.5	.5	0	0	0	0	0	0	0	0	0	.5	0	0	0	0	0	2.0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	0	0	0	0	0	0.5
14	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	.5	.5	.5	.5	.5	5.5
15	0	0	0	0	0	0	.5	.5	.5	.5	.5	.5	.5	.5	.5	0	0	0	0	0	0	4.5

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTIBLE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT DEFINITE ERYTHEMA 2 = MOOERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTIBLE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/21JUL04:09:05:07/PROOUCT=R

TKL RESEARCH

TKL STUOY NO. [redacted]

OATA LISTING 3: OERMATOLOGIC RESPONSE GRADES

BY PROOUCT ANO SUBJECT

PRODUCT= OISTILLED WATER

SUBJECT	READING NO.																				TOTAL
NUM8ER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	SCORE
16	0	0	0	0	0	0	0	0	0	.5	.5	.5	.5	.5	.5	0	0	0	0	0	0	3.0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.5	0	0	0	0	0	0.5
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
19	0	0	0	0	0	0	0	0	0	.5	.5	.5	.5	0	0	0	0	0	0	0	0	2.0
20	0	0	0	0	0	0	0	0	1.5	1.5	1.5	1.5	1.5	1	1	1	.5	.5	.5	.5	.5	13.0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	X	X	X	X	0.0
22	0	.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
24	0	0	0	0	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
25	0	0	0	0	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
26	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	1	.5	.5	.5	.5	.5	3.5
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
29	.5	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	.5	.5	.5	.5	.5	6.0
30	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
31	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
32	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
33	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0

	ALL SUBJECTS	COMPLETED SUBJECTS ONLY
TOTAL SCORE	51.0	51.0
NORMALIZED TOTAL SCORE	18.6	19.6

C.I.I. = .023

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/21JUL04:09:05:07/PROOUCT=R

TKL RESEARCH
TKL STUDY NO. [redacted]
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= SLS 0.2% LOT# CS12470026

SUBJECT NUMBER	READING NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	TOTAL SCORE
1	0	0	1	1	1	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	34.5
2	0	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	34.0
3	0	0	0	0	.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	32.5
4	0	.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	37.0
5	0	0	.5	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	33.5
6	0	0	1	1	1	1	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	33.5
7	0	0	1	1	1	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	34.5
8	0	1	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	37.5
9	0	0	0	.5	1	1	.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	38.0
10	0	.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	37.0
11	0	0	.5	.5	.5	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	32.5
12	0	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	38.0
13	0	.5	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	36.5
14	0	0	0	0	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	28.0
15	0	0	.5	.5	1	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	41.0

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTIBLE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT DEFINITE ERYTHEMA 2 = MODERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTIBLE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/21JUL04:09:05:07/PRODUCT=R

TKL RESEARCH
TKL STUDY NO. [redacted]
DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= SLS 0.2% LOT# CS12470026

SUBJECT NUMBER	READING NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	TOTAL SCORE
16	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	36.0
17	1	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	39.0
18	0	1	1	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	37.0
19	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	36.0
20	0	0	0	.5	.5	.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	31.5
21	0	0	0	.5	.5	.5	2	2	2	2	2	2	2	2	2	2	X	X	X	X	X	21.5
22	0	1.5	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	37.5
23	0	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	38.0
24	0	0	0	0	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
25	0	0	0	0	0	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
26	0	0	.5	.5	.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	33.5
27	0	1.5	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	37.5
28	0	1.5	1.5	1.5	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	38.0
29	0	0	0	.5	1	1.5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	33.0
30	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
31	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
32	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0
33	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0.0

	ALL SUBJECTS	COMPLETED SUBJECTS ONLY
TOTAL SCORE	946.5	925.0
NORMALIZED TOTAL SCORE	344.5	355.8

C.I.I. = .424

PROGRAM: DETAIL.SAS/USES: RESPONSE, PRODLIST/21JUL04:09:05:07/PRODUCT=R

TKL RESEARCH
TKL STUDY NO. [redacted]
DATA LISTING 3A: RESIDUAL READINGS
BY PRODUCT AND SUBJECT

PRODUCT= SLS 0.2% LOT# CS12470026

SUBJECT NUMBER	READING NO. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1								2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1	1	1	1
2								2	2	2	2	2	1.5	1	1	1	1	1	1	0.5	0.5
3							2	2	2	2	2	1.5	1.5	1.5	1.5	1	1	1	1	0.5	0.5
4							2	2	2	2	1.5	1.5	1.5	1	1	1	1	1	1	1	1
5								2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1
6									2	2	2	2	2	2	2	2	1.5	1.5	1.5	1.5	1
7								2	2	2	2	2	1.5	1.5	1.5	1.5	1	1	1	1	1
8							2	2	2	2	1	1	1	1	1	1	1	1	1	1	1
9								2.5	2.5	2	2	2	2	2	2	2	2	1.5	1.5	1.5	1.5
10							2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
11								2	2	2	2	1.5	1.5	1.5	1.5	1.5	1	1	1	1	1
12							2	2	2	1.5	1.5	1.5	1	1	1	1	1	1	1	1	1
13								2	2	2	2	2	2	2	2	1.5	1	1	1	1	1
14									3	3	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
15								2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

KEY TO SYMBOLS:

0 = NO APPARENT CUTANEOUS INVOLVENT .5 FAINT, BARELY PERCEPTIBLE ERYTHEMA OR SLIGHT DRYNESS
1 = FAINT BUT DEFINITE ERYTHEMA 2 = MODERATE ERYTHEMA
2.5 MODERATE ERYTHEMA WITH BARELY PRECEPTIBLE EDEMA
3 = SEVERE ERYTHEMA

PROGRAM: RESID.SAS/USES: RESID, PRODLIST/21JUL04:09:05:17/PRODUCT=R

TKL RESEARCH
TKL STUDY ND. [redacted]
DATA LISTING 3A: RESIDUAL READINGS
BY PRODUCT AND SUBJECT

PRODUCT= SLS 0.2% LDT# CS12470026

SUBJECT	READING NO.																			
NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
16							2	2	2	2	2	2	2	1.5	1.5	1	1	1	1	1	1
17							2	2	2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
18							2	2	2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
19							2	2	2	2	2	2	2	2	1.5	1.5	1	1	1	1	1
20								2	2	2	2	2	2	1.5	1.5	1.5	1.5	1	1	1	1
21								2	2	2	2	2	2	2	2	1.5	X	X	X	X	X
22								2	2	2	2	2	2	2	1.5	1.5	1.5	1.5	1	1	1
23							2	1.5	1.5	1	1	1	1	1	1	1	1	1	1	1	1
26							2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1	1	1	1
27								2	2	2	2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5
28							2	2	2	2	2	2	2	1.5	1.5	1.5	1.5	1	1	1	1
29								2	2	2	2	2	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

PROGRAM: RESID.SAS/USES: RESID, PRODLIST/21JUL04:09:05:17/PRODUCT=R

HUMAN REPEATED INSULT PATCH STUDY

Face cream containing 0.4 % Retinyl Propionate

TKL STUDY NO. [redacted]

[redacted]

CONDUCTED FOR:

[redacted]

DATE OF REPORT:

March 1, 2001

TKL Research, Inc., 4 Forest Avenue, Paramus, NJ 07652 Tel (201) 587-0500 Fax (201) 587-0556 Email: info@tklresearch.com

TABLE OF CONTENTS

TITLE OF STUDY

Human Repeated Insult Patch Study

SPONSOR

[redacted]

STUDY MATERIAL

[redacted]24-001	Moisturizer	This is a face cream containing 0.4% retinyl propionate (CAS 7069-42-3).

DATE STUDY INITIATED

November 29, 2000

DATE STUDY COMPLETED

January 5, 2001

DATE OF REPORT

March 1, 2001

INVESTIGATIVE PERSONNEL

Alan H. Greenspan, MD
Dermatologist
Principal Investigator

Robert C. Reardon, PhD
Director of Operations

Kathleen Georgeian
Clinical Research Coordinator and
Manager, Dermatologic Safety Testing

Tina Kelly
Assistant Clinical Research Coordinator

Joanne Mruczek, RN
Senior Clinical Assistant

Evelyn Ferousis
Clinical Assistant

Karen Morales
Clinical Assistant

CLINICAL SITE

TKL RESEARCH, INC.
578 Driggs Avenue
Brooklyn, NY 11211

STATEMENT OF QUALITY ASSURANCE

This report has been reviewed by the TKL Quality Assurance Department and the report accurately reflects the raw data for this study.

Clinical research studies are performed by TKL Research, Inc. in accordance with federal regulations and proposed guidelines for good clinical practices which include:

21 CFR Part 312,	Investigational New Drug Application
21 CFR Part 50,	Protection of Human Subjects
21 CFR Part 56,	Institutional Review Boards

Quality Assurance Associate for Henry Briscoe

1 March 2001
Date

SUMMARY

One Moisturizer, [redacted]24-001, was evaluated neat to determine its ability to sensitize the skin of normal volunteer subjects using a semi-occlusive repeated insult patch study. One hundred two subjects completed the study.

Under the conditions employed in this study, there was no evidence of sensitization to [redacted]24-001.

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 volunteer subjects were screened and enrolled to ensure that 100 subjects completed the study.

3.1.1 Inclusion Criteria

Individuals were eligible for inclusion in the study if they:

1. were males or females, 18 years of age or older, 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 patch study Medical Screening form as well as a Medical/Personal History form; and

5. had read, understood and signed an informed consent agreement.

3.1.2 Exclusion Criteria

Individuals were excluded from participation in the study if they:

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 a child; and

5. had a known sensitivity to cosmetics, skin care products, or topical drugs as related to the material being evaluated.

3.1.3 Informed Consent

A properly executed informed consent document in compliance with FDA regulations (21 CFR 50) 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. A sample is included as Appendix IV.

3.2 Description of Study

3.2.1 Outline of Study Procedures

The study extended 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 evaluation sites while on the study or within two weeks of completing the study.

The Induction Phase consisted of 9 consecutive applications of the study material and subsequent evaluations of the patch application sites. Prior to application of the patches, the sites were outlined with a skin marker, e.g., 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 and sites were evaluated on the following Monday, i.e., 72 hours after patch application.*

*A Monday or Friday holiday may result in evaluation at 96 hours after patch application.

Following the ninth evaluation, the subjects were dismissed for a rest period of approximately 10-15 days.

Subjects who were absent once during the 3-week, 9-patch 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).

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 (i.e., 48 and 72 hours after application.

To be considered a completed case, a subject must have had 9 applications and no fewer than 8 subsequent readings during induction and 1 application and 2 readings during challenge. Only completed cases were used to assess sensitization.

Rechallenge was not required.

3.2.2 Definitions Used for Grading Responses

The symbols found in the data listings accompanying this report were used to express the response observed at the time of examination:

-	=	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 (i.e., reaction where study material was not in contact with the 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

3.2.3 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 STUDY MATERIAL

4.1 Storage, Handling, and Documentation of Study Material

Receipt of the material used in this study was documented in a general log book which serves as a permanent record of the receipt, storage, and disposition of all study material received by TKL Research, Inc. On the basis of information provided by the Sponsor, the study material was considered reasonably safe for evaluation on human subjects. After completion of the study, the remaining study material was returned to the Sponsor and the disposition documented in the log book. All information regarding the receipt, storage and disposition of the study material was also recorded on a Clinical Material Record form (see Appendix III) which is incorporated in this study report. All study material is kept in a locked product storage room accessible to clinical staff members only.

4.2 Nature of Study Material

Identification	:	[redacted]24-001 Moisturizer
Description	:	white cream
Quantity Provided	:	20 x 30 ml tube
Amount Applied	:	0.2 g
Expiration Date	:	5/01
Special Instructions	:	Applied to patch no longer than 30 minutes prior to patch application.

4.3 Application of Study Material

Study material was applied to patch as instructed and patch was 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 semi-occlusive patch conditions was applied to a 2 cm x 2 cm Webril pad. The pad was 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 an 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 PROTOCOL

See Protocol – Appendix V.

7.0 DOCUMENTATION AND RETENTION OF DATA

The case report forms were designed to identify each subject by subject number and/or subject entry number and, where appropriate, subject's initials, the study material evaluated and the reactions observed. Originals or copies of all case report forms, source documents, IRB documents (if required), correspondence, study reports, and all source data will be kept on hard-copy file for a minimum of five years from completion of the study. Storage is maintained either at a TKL Research, Inc. facility in a secured room accessible only to TKL employees, or at an offsite location which provides 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 Research, Inc.

8.0 RESULTS & DISCUSSION

One hundred nine subjects between the ages of 18 and 74 were enrolled and 102 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:		109
Number discontinued:		7
Lost to follow-up:	2	
Voluntary withdrawal:	5	
Number completed:		102

Source: Table 1, Appendix I

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.

9.0 CONCLUSION

Under the conditions employed in this study, there was no evidence of sensitization to [redacted]24-001.

10.0 REFERENCES

Kligman AM. The identification of contact allergens by human assay II. A critique of standard methods. J Invest Dermatol 1966; 47:369.

Kligman AM. The identification of contact allergens by human assay II. Factors influencing the induction and measurement of allergic contact dermatitis. J Invest Dermatol 1966; 47:375.

Hardy J. Allergy hypersensitivity in cosmetics. J Soc Cosmet Chem 1973; 24:423.

Marzulli FN, Maibach HI. Contact allergy: predictive testing in man. Contact Dermatitis 1976; 2:1.

Marzulli FN, Maibach HI. Effects of vehicles and elicitation concentration in contact dermatitis testing I: experimental contact sensitization in humans. Contact Dermatitis 1976; 2:325.

Marzulli FN, Maibach HI. Dermatotoxicology. 4th ed. New York:Hemisphere, 1991.

Fisher AA. 3rd ed. Contact Dermatitis. Philadelphia:Lea & Feiberger, 1986.

Shelanski HA, Shelanski MV. A new technique of human patch tests. Proc Sci Sect Toilet Goods Assoc 1953; 204:107-110.

Jordan WP, King SF. Related hypersensitivity in families. Contact Dermatitis 1977; 3:19-26.

Kligman AM, Epstein W. Updating the maximization test for identifying contact allergens. Contact Dermatitis 1975; 1:231-239.

Stotts, J. Planning, conduct and interpretation of human predictive sensitization patch tests. In: Drill VA, Lazar P, eds. Current Concepts In Cutaneous Toxicity. New York:Academic Press, 1980:41-53.

11.0 SIGNATURES

Kathleen Georgeian

Kathleen Georgeian, Clinical Research Coordinator and Manager, Dermatologic Safety Testing

2/27/01
Date

Alan H. Greenspan, MD
Principal Investigator

2/28/01
Date

RC Reardon

Robert C. Reardon, PhD
Director of Operations

2/27/01
Date

APPENDIX I

SUMMARY TABLES

TKL STUDY NO. [redacted]

TABLE 1: SUMMARY OF SUBJECT ENROLLMENT AND DISPOSITION

	n	(%)
Subjects enrolled	109	
Subjects completed all phases	102	(93.6)
Total subjects discontinued	7	(6.4)
Lost to follow-up	2	(1.8)
Voluntary withdrawal	5	(4.6)

Note: All percentages are relative to total subjects enrolled

See Data Listing 1 for further detail

Program: OISPSMY.SAS/USES: FINAL/19JAN01:10:10:22

TKL STUDY NO. [redacted]

TABLE 2: SUMMARY DF SUBJECT DEMOGRAPHICS

ALL ENROLLED SUBJECTS

Age	
n (%) 18 to 44	51 (46.8)
n (%) 45 to 64	48 (44.0)
n (%) 65 and up	10 (9.2)
Mean (SD)	45.0 (14.3)
Median	47.0
Range	18.0 to 74.0
Gender	
n (%) Male	17 (15.6)
n (%) Female	92 (84.4)
Race	
n (%) Caucasian	6 (5.5)
n (%) Hispanic	103 (94.5)

See Data Listing 2 for further detail

Program: DEMOSMY.SAS/USES: DEMOGS/19JAN01:10:10:22

[redacted]

TKL STUDY NO. [redacted]

TABLE 3: SUMMARY OF DERMATOLOGIC RESPONSE GRADES
NUMBER OF SUBJECTS BY PRODUCT

PRODUCT=[redacted]24-001

	Induction Reading									Make-	Challenge Phase		
Response	1	2	3	4	5	6	7	8	9	Up	48hr	72hr	96hr(*)
-	105	104	104	102	102	102	97	102	102	9	102	102	
Total evaluable	105	104	104	102	102	102	97	102	102	9	102	102	
Number absent	1	1	0	2	0	0	5	0	0		0	0	
Number discontinued	3	4	5	5	7	7	7	7	7		7	7	

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%

Program: SUMMARY.SAS/USES: RESPONSE, PRODLIST, FINAL/19JAN01:10:10:27

APPENDIX II

DATA LISTINGS

TKL STUDY NO. [redacted]

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	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
2	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	36
3	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
4	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
5	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
6	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
7	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
8	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
9	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
10	11/29/00	11/29/00		12/04/00	I0	L	6
11	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
12	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
13	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
14	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
15	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
16	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
17	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
18	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
19	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
20	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
21	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
22	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
23	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
24	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
25	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
26	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
27	11/29/00	11/29/00	01/02/01	01/08/01	C2	C	39
28	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
29	11/29/00	11/29/00		12/04/00	I1	S	6
30	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
31	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
32	11/29/00	11/29/00		12/11/00	I4	S	13
33	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
34	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
35	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
36	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
37	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
38	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
39	11/29/00	11/29/00	01/02/01	01/05/01	C2	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)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/19JAN01:10:10:06

TKL STUOY NO. [redacted]

DATA LISTING 1: SUBJECT ENROLLMENT AND OISPOSITION

Subject No.	Study Dates: Screened	1st Applic	Chall Applic	Ended	Last Reading #	Completion Status	Days on Study
40	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
41	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
42	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
43	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
44	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
45	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
46	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
47	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
48	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
49	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
50	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
51	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
52	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
53	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
54	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
55	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
56	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
57	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
58	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
59	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
60	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
61	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
62	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
63	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
64	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
65	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
66	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
67	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
68	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
69	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
70	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
71	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
72	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
73	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
74	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
75	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
76	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
77	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
78	11/29/00	11/29/00		12/11/00	I4	S	13

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/19JAN01:10:10:06

TKL STUDY NO. [redacted]

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	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
80	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
81	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
82	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
83	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
84	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
85	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
86	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
87	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
88	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
89	11/29/00	11/29/00		12/01/00	I0	S	3
90	11/29/00	11/29/00		12/01/00	I0	S	3
91	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
92	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
93	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
94	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
95	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
96	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
97	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
98	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
99	11/29/00	11/29/00		12/08/00	I2	L	10
100	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
101	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
102	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
103	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
104	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
105	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
106	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
107	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
108	11/29/00	11/29/00	01/02/01	01/05/01	C2	C	38
109	11/29/00	11/29/00	01/02/01	01/05/01	C2	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)

Program: DISPLIST.SAS/USES: DEMOGS, RESPONSE, FINAL/19JAN01:10:10:06

TKL STUDY NO. [redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
1	56.3	Male	Hispanic
2	42.3	Female	Hispanic
3	56.6	Male	Hispanic
4	33.8	Male	Hispanic
5	54.6	Female	Hispanic
6	40.1	Female	Hispanic
7	28.7	Female	Hispanic
8	55.1	Female	Hispanic
9	29.5	Female	Hispanic
10	40.4	Female	Hispanic
11	58.4	Female	Hispanic
12	32.4	Female	Hispanic
13	43.8	Male	Hispanic
14	40.2	Female	Hispanic
15	45.6	Male	Hispanic
16	80.6	Female	Hispanic
17	52.3	Female	Hispanic
18	53.0	Female	Hispanic
19	18.3	Female	Hispanic
20	28.4	Female	Hispanic
21	60.5	Female	Hispanic
22	30.5	Female	Hispanic
23	37.0	Female	Hispanic
24	19.2	Female	Hispanic
25	19.7	Female	Hispanic
26	53.7	Female	Hispanic
27	58.4	Female	Hispanic
28	32.7	Female	Hispanic
29	25.5	Female	Hispanic
30	44.4	Female	Hispanic
31	54.5	Female	Hispanic
32	41.3	Female	Hispanic
33	32.4	Female	Hispanic
34	51.6	Female	Hispanic
35	52.3	Male	Hispanic
36	58.7	Female	Hispanic
37	20.9	Female	Hispanic
38	23.5	Female	Hispanic
39	50.8	Female	Hispanic
40	18.2	Female	Hispanic

TKL STUDY NO. [redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
41	51.6	Female	Hispanic
42	51.3	Male	Hispanic
43	51.9	Female	Hispanic
44	35.1	Female	Hispanic
45	55.3	Male	Hispanic
46	40.9	Female	Hispanic
47	45.7	Female	Caucasian
48	64.1	Female	Hispanic
49	36.3	Female	Hispanic
50	50.1	Female	Hispanic
51	66.6	Female	Hispanic
52	26.3	Female	Hispanic
53	42.7	Female	Hispanic
54	47.9	Female	Caucasian
55	55.3	Female	Hispanic
56	65.0	Female	Hispanic
57	70.8	Female	Caucasian
58	57.6	Male	Caucasian
59	56.1	Female	Hispanic
60	72.8	Female	Caucasian
61	39.0	Female	Hispanic
62	42.1	Female	Hispanic
63	33.2	Female	Hispanic
64	20.0	Female	Hispanic
65	19.8	Female	Hispanic
66	34.0	Female	Hispanic
67	36.1	Female	Hispanic
68	39.3	Female	Hispanic
69	63.7	Female	Hispanic
70	18.0	Female	Hispanic
71	65.6	Female	Hispanic
72	47.1	Female	Hispanic
73	38.4	Female	Hispanic
74	19.2	Female	Hispanic
75	46.4	Female	Hispanic
76	65.0	Female	Hispanic
77	37.9	Female	Hispanic
78	34.4	Male	Hispanic
79	18.9	Female	Hispanic
80	43.5	Female	Hispanic

TKL STUDY NO. [redacted]
DATA LISTING 2: SUBJECT DEMOGRAPHICS

Subject No.	Age	Gender	Race
81	31.5	Female	Hispanic
82	74.0	Female	Caucasian
83	18.3	Male	Hispanic
84	43.6	Male	Hispanic
85	71.7	Female	Hispanic
86	57.2	Female	Hispanic
87	38.8	Female	Hispanic
88	55.7	Male	Hispanic
89	20.7	Female	Hispanic
90	49.1	Female	Hispanic
91	54.7	Female	Hispanic
92	47.2	Female	Hispanic
93	43.1	Male	Hispanic
94	66.2	Female	Hispanic
95	41.7	Male	Hispanic
96	48.6	Female	Hispanic
97	52.9	Female	Hispanic
98	56.1	Female	Hispanic
99	54.0	Female	Hispanic
100	41.5	Female	Hispanic
101	47.0	Female	Hispanic
102	48.5	Female	Hispanic
103	50.8	Female	Hispanic
104	57.4	Female	Hispanic
105	48.3	Female	Hispanic
106	57.4	Male	Hispanic
107	54.6	Male	Hispanic
108	51.9	Female	Hispanic
109	65.7	Female	Hispanic

[redacted]

TKL STUDY NO. [redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted]24-001

Subject	Induction Reading										Challenge Phase	
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr 96hr(*)
1	-	-	-	-	-	-	-	-	-		-	-
2	-	-	-	-	-	-	-	-	-		-	-
3	-	-	-	-	-	-	-	-	-		-	-
4	-	X	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-		-	-
6	-	-	-	-	-	-	-	-	-		-	-
7	-	-	-	-	-	-	-	-	-		-	-
8	-	-	-	-	-	-	-	-	-		-	-
9	-	-	-	-	-	-	-	-	-		-	-
10	X	X	X	X	X	X	X	X	X		X	X
11	-	-	-	-	-	-	-	-	-		-	-
12	-	-	-	-	-	-	-	-	-		-	-
13	-	-	-	-	-	-	-	-	-		-	-
14	-	-	-	-	-	-	-	-	-		-	-
15	-	-	-	X	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-		-	-
17	-	-	-	-	-	-	-	-	-		-	-
18	-	-	-	-	-	-	-	-	-		-	-
19	-	-	-	-	-	-	-	-	-		-	-
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, PRDDLIST/19JAN01:10:10:07

[redacted]

TKL STUDY NO. [redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted]24-001

Subject No.	Induction Reading 1	2	3	4	5	6	7	8	9	MU	Challenge Phase 48hr	72hr	96hr(*)
21	-	-	-	-	-	-	-	-	-		-	-	
22	-	-	-	-	-	-	-	-	-		-	-	
23	-	-	-	-	-	-	-	-	-		-	-	
24	-	-	-	-	-	-	-	-	-		-	-	
25	-	-	-	-	-	-	-	-	-		-	-	
26	-	-	-	-	-	-	-	-	-		-	-	
27	-	-	-	-	-	-	-	-	-		-	-	
28	-	-	-	-	-	-	-	-	-		-	-	
29	-	X	X	X	X	X	X	X	X		X	X	
30	-	-	-	-	-	-	-	-	-		-	-	
31	-	-	-	-	-	-	-	-	-		-	-	
32	-	-	-	-	X	X	X	X	X		X	X	
33	-	-	-	-	-	-	-	-	-		-	-	
34	-	-	-	-	-	-	-	-	-		-	-	
35	-	-	-	-	-	-	-	-	-		-	-	
36	-	-	-	-	-	-	-	-	-		-	-	
37	-	-	-	-	-	-	-	-	-		-	-	
38	-	-	-	-	-	-	-	-	-		-	-	
39	-	-	-	-	-	-	-	-	-		-	-	
40	-	-	-	-	-	-	-	-	-		-	-	
41	-	-	-	-	-	-	X	-	-	-	-	-	
42	-	-	-	-	-	-	X	-	-	-	-	-	
43	-	-	-	-	-	-	X	-	-	-	-	-	
44	-	-	-	-	-	-	-	-	-		-	-	
45	-	-	-	-	-	-	-	-	-		-	-	
46	-	-	-	-	-	-	-	-	-		-	-	
47	-	-	-	-	-	-	-	-	-		-	-	
48	-	-	-	-	-	-	-	-	-		-	-	
49	-	-	-	-	-	-	-	-	-		-	-	
50	-	-	-	-	-	-	-	-	-		-	-	
51	-	-	-	-	-	-	-	-	-		-	-	
52	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/19JAN01:10:10:07

[redacted]

TKL STUDY NO. [redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PRODUCT= [redacted]24-001

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	-	-	-	-	-	-	-	-	-		-	-	
60	-	-	-	-	-	-	-	-	-		-	-	
61	-	-	-	-	-	-	-	-	-		-	-	
62	-	-	-	-	-	-	-	-	-		-	-	
63	-	-	-	-	-	-	-	-	-		-	-	
64	-	-	-	-	-	-	-	-	-		-	-	
65	X	-	-	-	-	-	-	-	-	-	-	-	
66	-	-	-	-	-	-	-	-	-		-	-	
67	-	-	-	-	-	-	-	-	-		-	-	
68	-	-	-	-	-	-	-	-	-		-	-	
69	-	-	-	-	-	-	-	-	-		-	-	
70	-	-	-	-	-	-	-	-	-		-	-	
71	-	-	-	-	-	-	-	-	-		-	-	
72	-	-	-	-	-	-	-	-	-		-	-	
73	-	-	-	-	-	-	-	-	-		-	-	
74	-	-	-	-	-	-	-	-	-		-	-	
75	-	-	-	-	-	-	-	-	-		-	-	
76	-	-	-	-	-	-	-	-	-		-	-	
77	-	-	-	-	-	-	-	-	-		-	-	
78	-	-	-	-	X	X	X	X	X		X	X	
79	-	-	-	-	-	-	-	-	-		-	-	
80	-	-	-	-	-	-	-	-	-		-	-	
81	-	-	-	-	-	-	-	-	-		-	-	
82	-	-	-	-	-	-	-	-	-		-	-	
83	-	-	-	-	-	-	-	-	-		-	-	
84	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/19JAN01:10:10:07

[redacted]

TKL STUDY NO. [redacted]

DATA LISTING 3: DERMATOLOGIC RESPONSE GRADES
BY PRODUCT AND SUBJECT

PROOUCT= [redacted]24-001

Subject	Induction Reading										Challenge Phase		
No.	1	2	3	4	5	6	7	8	9	MU	48hr	72hr	96hr(*)
85	-	-	-	-	-	-	-	-	-		-	-	
86	-	-	-	-	-	-	-	-	-		-	-	
87	-	-	-	-	-	-	-	-	-		-	-	
88	-	-	-	-	-	-	-	-	-		-	-	
89	X	X	X	X	X	X	X	X	X		X	X	
90	X	X	X	X	X	X	X	X	X		X	X	
91	-	-	-	X	-	-	-	-	-	-	-	-	
92	-	-	-	-	-	-	-	-	-		-	-	
93	-	-	-	-	-	-	-	-	-		-	-	
94	-	-	-	-	-	-	-	-	-		-	-	
95	-	-	-	-	-	-	X	-	-	-	-	-	
96	-	-	-	-	-	-	-	-	-		-	-	
97	-	-	-	-	-	-	X	-	-	-	-	-	
98	-	-	-	-	-	-	-	-	-		-	-	
99	-	-	X	X	X	X	X	X	X		X	X	
100	-	-	-	-	-	-	-	-	-		-	-	
101	-	-	-	-	-	-	-	-	-		-	-	
102	-	-	-	-	-	-	-	-	-		-	-	
103	-	-	-	-	-	-	-	-	-		-	-	
104	-	-	-	-	-	-	-	-	-		-	-	
105	-	-	-	-	-	-	-	-	-		-	-	
106	-	-	-	-	-	-	-	-	-		-	-	
107	-	-	-	-	-	-	-	-	-		-	-	
108	-	-	-	-	-	-	-	-	-		-	-	
109	-	-	-	-	-	-	-	-	-		-	-	

(*) when required

Program: DETAIL.SAS/USES: RESPONSE, PRODLIST/19JAN01:10:10:07

NORTH CLIFF CONSULTANTS, INC.

STUDY [redacted]

A 28-DAY OPHTHALMOLOGIC AND DERMATOLOGIC SAFETY EVALUATION OF A MOISTURIZER PRODUCT

Facial moisturizer containing 0.3% Retinyl Propionate

FOR:

[redacted]

TEST MATERIAL IDENTIFICATION NUMBERS:

[redacted]448-093

NORTH CLIFF CONSULTANTS, INC
3747 WARSAW AVENUE
CINCINNATI, OHIO 45205

A 28-DAY OPHTHALMOLOGIC AND DERMATOLOGIC SAFETY EVALUATION OF A MOISTURIZER PRODUCT
[redacted]448-093 [redacted]
NCCI STUDY [redacted]

PURPOSE: The study was designed to evaluate the potential of a moisturizer product to induce dermal and/or ocular irritation when used a minimum of twice daily by female subjects. The primary measures of safety were product tolerance assessment based on ocular and dermal evaluations by an ophthalmologist and a dermatologist. Subjective ocular irritation potential (including stinging, burning, dryness and itching) and subject dermal irritation (including erythema and dryness) were also evaluated by each subject.

SPONSOR: [redacted]

SPONSOR REPRESENTATIVE: [redacted]

TESTING FACILITY: North Cliff Consultants, Inc.
3747 Warsaw Avenue
Cincinnati, OH 45205

PRINCIPAL INVESTIGATOR: Mary S. Bailey, Ph.D.

MEDICAL INVESTIGATOR-OPHTHALMOLOGIST: Kelly P. O'Neill, M.D.

MEDICAL INVESTIGATOR-DERMATOLOGIST: Everett Linn Jones, M.D.

STUDY PERSONNEL: **Study Coordinators:** Diane F. Fay
Maribeth Hueber

The list of North Cliff personnel involved in the study and the visitor's log are presented on Page 18.

REGULATORY STATUS: This study was conducted according to Good Clinical Practices (GCP) including written informed consent. An external review by an Institutional Review Board (IRB) was not requested.

STUDY DATES: February 4, 2005 - March 4, 2005

SAMPLE IDENTIFICATION: Moisturizer [redacted]448-093

This is a facial moisturizer containing 0.3% retinyl propionate (CAS 7069-42-3).

A 28-DAY OPHTHALMOLOGIC AND DERMATOLOGIC SAFETY EVALUATION OF A MOISTURIZER PRODUCT
[redacted]448-093 [redacted] [redacted]
NCCI STUDY [redacted]

TABLE OF CONTENTS

QUALITY ASSURANCE STATEMENT

STUDY NUMBER: [redacted]

TYPE OF STUDY: A 28-DAY OPHTHALMOLOGIC AND DERMATOLOGIC SAFETY EVALUATION OF A MOISTURIZER PRODUCT

SITE OF STUDY: NORTH CLIFF CONSULTANTS, Cincinnati, Ohio

In accordance with the Standard Operating Procedures of North Cliff Consultants, Inc., completed study records and the final report were audited.

Reviewed By:

Deanne M. Lind 3/6/07

Deanne M. Lind / Date
Quality Assurance

STUDY OVERVIEW

The study was conducted according to the Sponsor's protocol, A 28-Day Ophthalmologic and Dermatologic Safety Evaluation of a Moisturizer Product, approved and signed by the Principal Investigator on February 3, 2005, and the Medical Investigators on February 4, 2005. A copy of the protocol is presented in Attachment II.

Protocol Amendment Issue I, issued 04/04/05, approved and signed by the Investigator on April 6, 2005, provided for removing the statement concerning a minimum wear time of 8 hours each day; no minimum wear time per day was specified for the moisturizer product.

TEST SUBJECT ACCOUNTABILITY

Forty (40) healthy females, ages 18 through 55, who normally wear a moisturizer product, were screened for entrance into the study. Subjects were required to provide written informed consent and complete a Medical and Dermatological History Questionnaire. An ophthalmologist examined each prospective subject's eyes and a dermatologist evaluated the general condition of each subject's facial and neck skin.

Six (6) subjects were excused from study participation: two (2) subjects did not normally wear a moisturizer (<three times per week); one (1) subject presented with skin irritation >1 at the baseline evaluation; one (1) subject failed to wear her contact lenses to the baseline visit; one (1) subject was taking exclusionary medication; and one (1) subject declined participation.

Thirty-four (34) subjects were enrolled in the home use phase of the study. One (1) subject failed to complete the study: Subject #015 failed to return to the test facility for Visit 2 (02/18/05). (See Subject Termination Form, Page 17.) Thirty-three (33) subjects completed the study.

Originals of each subject's informed consent, medical questionnaire and data collection forms are located in the Investigator's study records. A record of each subject's visit to the laboratory for evaluation and product distribution/weight/return was maintained and is also located in the Investigator's study records.

STUDY PROCEDURE

Upon arrival at the test facility, prospective subjects provided written informed consent and completed a medical screener to assess their health status and other study inclusion/exclusion criteria. The ophthalmologist (Kelly P. O'Neill, M.D.) evaluated various ocular attributes of each subject and, if applicable, examined the general condition of their contact lenses; observations were recorded on Protocol Appendix C. The dermatologist (Everett Linn Jones, M.D.) evaluated the general condition of each subject's facial skin and neck skin for the presence of dryness and irritation; observations were recorded on Protocol Appendix D.

Qualifying subjects had their visual acuity checked via the Snellen Eye Chart and completed a Subjective Assessment Questionnaire assessing various subjective eye and skin attributes (i.e., stinging, burning, itching, dryness and redness). Subjects recorded their opinion on the Subjective Assessment Questionnaire (Protocol Appendix E).

Subjects then received the moisturizer test product to use as their only moisturizer for the duration of the 28-day use period; test facility personnel weighed each moisturizer product prior to distribution. Instructions on use of the test product and a daily diary for the next two-week period were also provided to each subject. Subjects were instructed to apply the moisturizer a minimum of two (2) times per day (morning and evening). Time(s) of application and removal were recorded on the daily diary.

STUDY PROCEDURE (Continued)

After using the test moisturizer for fourteen (14) days, the subjects returned to the test facility bringing their moisturizer product and completed diary. Test facility personnel weighed each moisturizer product and reviewed each subject's diary. Subjects completed the Subjective Assessment Questionnaire and recorded their opinions after using the test product for two (2) weeks. The ophthalmologist examined the eyes, and the dermatologist evaluated the facial and neck skin of each subject. Subjects received a new container of the moisturizer test product, and a diary for the next two-week period.

Subjects returned to the test facility after the second two-week period (Day 28), bringing their moisturizer product and completed diary. Test facility personnel weighed each moisturizer product and reviewed each subject's diary. Subjects had their visual acuity checked, then completed the Subjective Assessment Questionnaire and recorded their opinions after using the test product for the second two-week period. The ophthalmologist examined each subject's eyes and, if applicable, the condition of the subject's contact lenses; the dermatologist evaluated each subject's facial and neck skin. This completed the subject's participation in the study.

ADVERSE REACTIONS

Two subjects experienced adverse events during the conduct of the study. **Subject #033** experienced a sever sinus infection and was prescribed an antibiotic.

Subject #026 presented with mild erythema and pruritus in the right temple area, lateral upper lid and malar cheek after using the test moisturizer for one week. A consulting dermatologist (D. J. Blaney, M.D.) suggested the subject discontinue use of the test product temporarily for a one-week period and return for her scheduled Day 14 visit for evaluation.

Since no erythema was noted at the Day 14 visit, use of the test product was re-started; however, Subject #026 presented with mild erythema, dryness and papules within one week of re-starting product use. A test facility skin grader (D. R. Arnold) examined the subject, had the subject discontinue use of the test product and withdraw from the study.

Dr. E. L. Jones examined Subject #026 on Day 28 for follow-up. He concluded the subject experienced an irritation reaction, possibly related to the test product.

A copy of the Adverse Event Reporting Form for each subject is presented on Pages 14 through 16.

DEVIATIONS FROM THE PROTOCOL (By Subject)

Subject	Test Date (Day)	Description
013	02/17/05 (13)	Subject made only one application of the test product.
016	02/18/05 (14)	Subject made only one application of the test product.
021	03/02/05 (26)	Subject made only one application of the test product.
022	02/04/05 (0)	Subject made only one application of the test product.
and	02/12/05 (8)	" "
and	02/18/05 (14)	" "
and	02/23/05 (19)	" "
030	02/18/05 (14)	Subject arrived at the test facility after the ophthalmologist had left (subject was evaluated by the dermatologist).

DATA ANALYSES

Evaluations made by the ophthalmologist and dermatologist, and subjective assessments from each evaluation visit were transcribed into an Excel Spreadsheet and are presented in Tables I and II, respectively. Average Ophthalmologic and Dermatologic Evaluation and Subjective Assessment change from baseline is presented in Table III. Initial and final product weights, amount of product used by each subject, and the total number of uses of the product (obtained from each subject's bi-weekly diary) were also transcribed into an Excel Spreadsheet; this data is presented in Table IV.

Copies of each subject's bi-weekly diary were provided to the Study Sponsor following completion of the study.

RESULTS

Thirty-three (33) subjects completed the study, however, due to the reaction experienced by Subject #026, the subject's data was recorded but was not included in the final analyses (average of change from baseline).

Averages of the change from baseline for Ophthalmologic and Dermatologic Examination and Subjective Assessment of each subject's eyes and facial and neck skin is presented in the tables below and in Attachment I - Table III. A negative value indicates an increase in dryness, irritation (redness), etc., over the 28-day usage period.

OPHTHALMOLOGIC AND DERMATOLOGIC EVALUATIONS - AVERAGE CHANGE FROM BASELINE

EYELIDS - SWELLING		PALPEBRAL CONJUNCTIVA		BULBAR CONJUNCTIVA		FACIAL SKIN		NECK SKIN	
LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	IRRITATION	DRYNESS	IRRITATION	DRYNESS
-0.13	-0.06	0.03	0.03	-0.09	-0.09	0.17	0.02	-0.02	0.00

SUBJECTIVE ASSESSMENT - AVERAGE CHANGE FROM BASELINE

	STINGING	BURNING	ITCHING	DRYNESS	REDNESS	OTHER
FACE	-0.11	-0.09	-0.02	0.13	0.00	-0.05
NECK	0.00	0.00	0.00	0.02	0.00	0.00
EYES	0.00	0.02	-0.02	0.06	0.02	0.00

CONCLUSIONS

One subject (#026) experienced an adverse reaction that was possibly related to product use. The subject's reaction suggests an irritation reaction; details are presented in the subject's Adverse Event Reporting Form (Pages 14 and 15).

The overall results of this study indicate that the test moisturizer was well tolerated by the subjects. For all ophthalmologic attributes (eyelid swelling, palpebral conjunctiva and bulbar conjunctiva), average scores indicate little to no change from baseline. Dermatological grades indicate little to no change of facial or neck skin (irritation and dryness) under the conditions of the test.

Subjectively, average change from baseline scores demonstrate tolerance of the test moisturizer for various attributes of the face, neck and eyes (burning, stinging, itching, dryness, or redness).

Mary S. Bailey 3/16/07

Mary S. Bailey, Ph.D. / Date
Principal Investigator

OPHTHALMOLOGIST'S STATEMENT

At the conclusion of the study, I reviewed each subject's Ophthalmologic Examination Form and believe, based on my observations of each subject's eyes, and if applicable each subject's contact lenses, that the test product was well tolerated under the conditions of this test.

Kelly P. O'Neill MD 3/7/07

Kelly P. O'Neill, M.D. / Date
Medical Investigator - Ophthalmologist

DERMATOLOGIST'S STATEMENT

At the conclusion of the study, I reviewed each subject's Dermatologic Examination Form and believe, based on my observations of each subject's facial and neck skin, that the test product was well tolerated under the conditions of this test.

Everett Linn Jones MD 3-12-2007

Everett Linn Jones, M.D. / Date
Medical Investigator - Dermatologist

TEST MATERIAL INFORMATION:

Sponsor Identification Numbers: [redacted]448-093

Color: White

Physical Form: Lotion (Moisturizer)

Date Received: 02/01/05 (65 tubes)

Amount Received: See Attachment I - Table IV, Product Weight and Usage

Amount After Subject Dosing: See Attachment I - Table IV, Product Weight and Usage

Storage Conditions for Stock Test Substance: Refrigerated at lab; ambient temperature after dispensed to subjects

Product Usage Instructions: On Days 0, 14 and 28, subjects should wash/cleanse their face at least 60 minutes prior to evaluation. The subjects should come to the test facility with a clean face. If desired, lipstick may be worn on these evaluation days.
All subjects are required to use the moisturizer product a minimum of twice each day, preferably once in the morning and once in the evening. Subjects may apply the make-up they normally use following application of the moisturizer products. Subjects will be instructed to apply/remove the test product using their normal cosmetic regimen.

Stock Test Substance Disposition: All unused stock test substance will be returned to the Sponsor's sector after the final report has been issued. Include study number and sample code.

[redacted]

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

INVESTIGATOR PHYSICIANS' STUDY MONITORING RECORD

On 2/4/05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no _____ conditions observed on any of the participant test
(skin, eye, gynecological, etc.)
subjects which would be characterized as unexpected from participating in this type study.

☐ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) _____ _____ _____ _____ _____ _____ _____

Comments: _____

_____

_____

_____

_____

Kelly P O'Neill 2/4/05
(Investigator Physician's Signature / Date)

Kelly P O'Neill
(Investigator Physician's Printed Name)

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

INVESTIGATOR PHYSICIANS' STUDY MONITORING RECORD

On 02-04-05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no skin conditions observed on any of the participant test (skin, eye, gynecological, etc.) subjects which would be characterized as unexpected from participating in this type study.

☐ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) _____ _____ _____ _____ _____ _____ _____

Comments: _____

_____

_____

_____

_____

Everett Linn Jones, M.D. 02-04-05
(Investigator Physician's Signature / Date)

EVERETT LINN JONES, M.D.
(Investigator Physician's Printed Name)

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

CONSULTING PHYSICIANS' STUDY MONITORING RECORD

On 2/11/05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☐ There were no _____ conditions observed on any of the participant test subjects which would be characterized as unexpected from participating in this type study.
(skin, eye, gynecological, etc.)

☑ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) 026

Comments: After 4 days of use the subject began to experience mild pruritus + erythema at temple, lateral portion of rt upper eyelid (some edema there). The symptoms + signs increased over next few days c some burning when product was applied on 02/11/05 Dx: a contact dermatitis - what agent?

Donald J Blaney 2/11/05
(Consulting Physician's Signature / Date)

DONALD J BLANEY MD
(Consulting Physician's Printed Name)

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

INVESTIGATOR PHYSICIANS' STUDY MONITORING RECORD

On 2-18-05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no _____ conditions observed on any of the participant test subjects which would be characterized as unexpected from participating in this type study.
(skin, eye, gynecological, etc.)

☐ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) _____ _____ _____ _____ _____ _____ _____

Comments: _____

_____

_____

_____

_____

Kelly P O'Neill MD 2/18/05
(Investigator Physician's Signature / Date)

Kelly P O'Neill
(Investigator Physician's Printed Name)

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

INVESTIGATOR PHYSICIANS' STUDY MONITORING RECORD

On 02-18-05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no SKIN (skin, eye, gynecological, etc.) conditions observed on any of the participant test subjects which would be characterized as unexpected from participating in this type study.

☒ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) 026

Comments: Possible contact dermatitis from other source,

Everett Linn Jones, MD 2-18-05
(Investigator Physician's Signature / Date)

EVERETT LINN JONES, M.D
(Investigator Physician's Printed Name)

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

INVESTIGATOR PHYSICIANS' STUDY MONITORING RECORD

On 3-4-05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no _____ conditions observed on any of the participant test subjects which would be characterized as unexpected from participating in this type study.
(skin, eye, gynecological, etc.)

☐ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) _____ _____ _____ _____ _____ _____ _____

Comments: _____

_____

_____

_____

_____

K O'Neill MD 3/4/05
(Investigator Physician's Signature / Date)

Kelly P O'Neill
(Investigator Physician's Printed Name)

NORTH CLIFF CONSULTANTS, INC.

3747 WARSAW AVENUE
CINCINNATI, OHIO 45205
PHONE: (513) 251-4930
FAX: (513) 557-3732

6831 COLERAIN AVENUE
CINCINNATI, OHIO 45239
PHONE: (513) 245-5483
FAX: (513) 245-5485

INVESTIGATOR PHYSICIANS' STUDY MONITORING RECORD

On 3-4-05 (date), I attended Study # [redacted] as indicated in the study protocol. In my opinion, all aspects of the study protocol design and execution were being observed and adhered.

Observations during this monitoring visit were as follows:

☒ There were no SKIN (skin, eye, gynecological, etc.) conditions observed on any of the participant test subjects which would be characterized as unexpected from participating in this type study.

☐ The following test subjects exhibited responses which may or may not be related to exposure to one or more of the test products and specific comments regarding these observations follow:

Test Subject No.(s) _____ _____ _____ _____ _____ _____ _____

Comments: _____

_____

_____

_____

_____

Everett Linn Jones, MD 3-4-05
(Investigator Physician's Signature / Date)

EVERETT LINN JONES, M.D.
(Investigator Physician's Printed Name)

[redacted]

APPENDIX H

ADVERSE EVENT REPORTING FORM

14.

Subject Initials: JMM Subject #: 026

TO BE COMPLETED ONLY IF SUBJECT HAS AN ADVERSE EVENT (or reports a change in medication)

Date Reported: 02/11/05 Date of Onset: 02/08/05 Date Resolved: 2-18-05

Describe event: began c mild erythema & pruritus which increased over next several days. Involved area was limited to temple, rt lateral upper lid, malar cheek.

Severity: ☑ Mild ☐ Moderate ☐ Severe ☐ Serious

Treatment Management: ☐ No Change ☑ Treatment Temporarily Stopped: Stop- 2/11/05 Restart- 2-18-05

☐ Treatment Stopped Permanently

Outcome: *(check all that apply)* ☐ Continued on Study ☐ Consulted physician ☐ Medication taken (Complete below)

☐ Withdrawn from Study ☐ Hospitalized ☑ Other (explain)

Comments: Subject will D/C for 1 week, may re start product. Limited area is concerning but by history we can find no other contact likely

Was adverse event related to treatment or other study procedure(s)?

☐ Not related ☑ Possibly related ☐ Probably related ☐ Definitely related ☐ Relationship unknown

FOLLOW-UP ACTION TAKEN

Date	Action Taken	Comments
2-18-05	Resume treatment.	Itching has faded. Redness on right side has faded C-L-J

CONCOMITANT MEDICATION/RELATED MEDICATION

Medication	Total Daily Dose	Start Date mm/dd/yy	Stop Date mm/dd/yy	Indication
		/ /	/ /	
		/ /	/ /	
		/ /	/ /	

Comments:

Donald J Blaney MD.
consulting dermatologist
02/11/05

Everett Lein Jr 03-4-05
Investigator Date

[redacted]

APPENDIX H

ADVERSE EVENT REPORTING FORM

15.

Subject Initials: JMM Subject #: 026

TO BE COMPLETED ONLY IF SUBJECT HAS AN ADVERSE EVENT (or reports a change in medication)

Date Reported: 02/25/05 Date of Onset: 02/20/05 Date Resolved: 3-4-05

Describe event: mild erythema noted (R) malar cheek 02/20/05 / continued erythema with several "pimples" noted lower edge of cheek involvement 02/22/05 / c/o stinging immediately post-application lasting under 30 secs 02/24/05 pm & 02/25/05 am. / c/o itching @ present (✓) lower * page) DEA

Severity: ☑ Mild ☐ Moderate ☐ Severe ☐ Serious

Treatment Management: ☐ No Change ☑ Treatment Temporarily Stopped: Stop- 02/25/05 Restart- ____

☑ Treatment Stopped Permanently per 4/6/05 subject never restarted treatment

Outcome: *(check all that apply)* ☐ Continued on Study ☐ Consulted physician ☐ Medication taken (Complete below)

☑ Withdrawn from Study ☐ Hospitalized ☐ Other (explain)

Comments: subject will D/C product application x 1 week until next seen 03/04/05

Was adverse event related to treatment or other study procedure(s)?

☐ Not related ☑ Possibly related ☐ Probably related ☐ Definitely related ☐ Relationship unknown

FOLLOW-UP ACTION TAKEN

Date	Action Taken	Comments
3-4-05		Redness on right cheek has faded.

COMPLETE THIS SECTION FOR ANY ADVERSE EVENT MEDICATION

Medication	Total Daily Dose	Start Date mm/dd/yy	Stop Date mm/dd/yy	Indication
		/ /	/ /	
		/ /	/ /	
		/ /	/ /	

Comments:

*(R) temple exhibits erythema, dryness, minor papular involvement but appears to have possible dried serous drainage from 1 papule under subject's foundation DEA 02/25/05

Everett Levin Jr MD 3-4-05

Investigator Date

[redacted]

APPENDIX H

ADVERSE EVENT REPORTING FORM

Subject Initials: MAH Subject #: 033

TO BE COMPLETED ONLY IF SUBJECT HAS AN ADVERSE EVENT (or reports a change in medication)

Date Reported: 2-13-05 Date of Onset: 2-12-05 Date Resolved: 2-18-05

Describe event: severe sinus infection

Severity: ☐ Mild ☐ Moderate ☑ Severe ☐ Serious

Treatment Management: ☑ No Change ☐ Treatment Temporarily Stopped: Stop-_____ Restart-_____

☐ Treatment Stopped Permanently

Outcome: *(check all that apply)* ☐ Continued on Study ☑ Consulted physician ☑ Medication taken (Complete below)

☐ Withdrawn from Study ☐ Hospitalized ☐ Other (explain)

Comments: subj went to emergency room due to sinus infection.

Was adverse event related to treatment or other study procedure(s)?

☑ Not related ☐ Possibly related ☐ Probably related ☐ Definitely related ☐ Relationship unknown

FOLLOW-UP ACTION TAKEN

Date	Action Taken	Comments

COMPLETE THIS SECTION FOR ANY CONCOMITANT MEDICATIONS

Medication	Total Daily Dose	Start Date mm/dd/yy	Stop Date mm/dd/yy	Indication
Cephalexin	500 mg (3x)	2/12/05	2/18/05	sinus infection
		/ /	/ /	
		/ /	/ /	

Comments:

Everett Linn Jones, MD 3-4-05

Investigator Date

[redacted]

APPENDIX I

SUBJECT TERMINATION FORM

Subject Initials: ADV **Subject #:** 015 **Date:** 2.18.05

Date Subject Entered the Study: 02 / 04 / 05 (mm / dd / yy)

Date of Subject Withdrawal or Termination: 02 / 18 / 05 (mm / dd / yy)

Did subject drop out of study prior to study completion? ☑ YES ☐ NO

If yes, check reason for subject early withdrawal or termination:

- ☐ 1 = Adverse Event (documented on Appendix H)
- ☐ 2 = Lack of Compliance with Protocol (explain in comments section below)
- ☑ 3 = Personal Reasons (lack of transportation, work conflict, family problems, etc.)
- ☐ 4 = No Show, Lost to Follow-Up
- ☐ 5 = Other (specify): _____

Comments: Subject was called in to work and forgot about her scheduled appointment. Could not come in. Subj was asked to return product and did not. WS 3-4-05

Investigator or designee 02.18.05 Date

FINAL REPORT

Study Title

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Test Article

[redacted]448-093 Face cream containing 0.3% Retinyl Propionate

Authors

Greg Mun, B.A.
John W. Harbell, Ph.D.
Jennifer McDaniel, B.S.

Study Completion Date

14 March 2008

Performing Laboratory

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878

Study Number

[redacted]

[redacted]

Laboratory Project Number

[redacted]

TISSUE EQUIVALENT ASSAY
WITH EPIOCULAR™ CULTURES

SUMMARY

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50}		pH
			Preliminary (15-Mar-05)	Trial 1 (16-Mar-05)	
[redacted]	[redacted]448-093	Neat	> 16 hours	> 24 hours	5.0
Positive Control	0.3% Triton®-X-100	NA	34.4 minutes	31.0 minutes	NA

NA - Not Applicable

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The Tissue Equivalent Assay with EpiOcular™ Cultures of the test article, [redacted]448-093, was conducted in compliance with the U.S. FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the U.S. EPA GLP Standards 40 CFR 160 and 40 CFR 792, and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

The identity, strength, purity and composition or other characteristics to define the test or control articles have not been determined by the testing facility. The certificate of analysis was not provided by the Sponsor.

The stability of the test or control articles under the test conditions has not been determined by the testing facility and is not included in the final report.

Greg Mun, B.A.
Study Director

14 March 2008
Date

QUALITY ASSURANCE STATEMENT

Study Title: Tissue Equivalent Assay with EpiOcular Cultures

Study Number: [redacted]

Study Director: Greg Mun, B.A.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLP Standards (40 CFR 792 and 40 CFR 160) and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected and report dates of QA inspections of this study:

Phase Inspected	Audit Date(s)	Reported to Study Director	Reported to Management
Protocol and Initial Paperwork	15-Mar-05	15-Mar-05	15-Mar-05
Definitive Assay – Dosing of the test article (3 hour time point) and positive control)	16-Mar-05	16-Mar-05	17-Mar-05
Draft Report and Data	21-Apr-05	21-Apr-05	25-Apr-05
Final Report and Protocol Amendment	11-Mar-08	11-Mar-08	13-Mar-08

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Amanda K. Ulrey, RQAP-GLP
Quality Assurance

13-March-2008
Date

SIGNATURE PAGE

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

Initiation Date:	15 March 2005
Completion Date:	14 March 2008
Sponsor:	[redacted]
Sponsor's Representative:	[redacted]
Testing Facility:	Institute for In Vitro Sciences, Inc. 30 W. Watkins Mill Road, Suite 100 Gaithersburg, MD 20878
Archive Location:	Institute for In Vitro Sciences, Inc. Gaithersburg, MD 20878
Study Director:	14 March 2008 Greg Mun, B.A. Date

TEST ARTICLE RECEIPT

IIVS Test Article Number	Sponsor's Designation	Physical Description	Receipt Date	Storage Conditions*
[redacted]	[redacted]48-093	white cream	18-Mar-05	2° - 8°C

* - Protected from exposure to light

This is a face cream containing 0.3% retinyl propionate (CAS 7069-42-3).

TISSUE EQUIVALENT ASSAY WITH EPIOCULAR™ CULTURES

INTRODUCTION

The EpiOcular™ human cell construct (MatTek Corporation) was used to assess the potential ocular irritancy of the test article. The MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular metabolism after exposure to a test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (t_{50}).

The purpose of this study was to evaluate the potential toxicity of the test article, supplied by [redacted] as measured by the conversion of MTT by EpiOcular™ human cell constructs after exposure to a test article for various exposure times. The laboratory phase of the study was conducted from 15 March 2005 to 14 April 2005 at the Institute for In Vitro Sciences, Inc. After a time range finding assay, the test article was tested in a valid definitive assay to determine the time of exposure to a test article, which resulted in the t_{50} endpoint.

---

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

MATERIALS AND METHODS

Receipt of the EpiOcular™ Human Cell Construct Model

Upon receipt of the EpiOcular™ human cell construct model kit, the solutions were stored as indicated by the manufacturer. The EpiOcular™ human cell constructs were stored at 2-8°C until used. On the day of dosing an appropriate volume of EpiOcular™ human cell construct assay medium was removed and warmed to approximately 37°C. Nine-tenths mL of assay medium were aliquoted into the wells of six-well plates. The six-well plates were labeled to indicate test article and exposure time. The samples were inspected for air bubbles between the agarose gel and Millicell® insert prior to opening the sealed package. Cultures with air bubbles covering greater than 50% of the Millicell® area were not used. The 24-well shipping containers were removed from the plastic bag and their surfaces were disinfected with 70% ethanol. The EpiOcular™ human cell constructs were transferred aseptically into the 6-well plates. The EpiOcular™ human cell constructs were then incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for at least one hour. The medium was aspirated and 0.9 mL of fresh medium were added to each assay well below the EpiOcular™ human cell construct. The plates were returned to the incubator until treatment was initiated.

Assessment of Direct Test Article Reduction of MTT

The test article was added to a 1.0 mg/mL MTT (Sigma) solution in warm Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine (MTT Addition Media) to assess its ability to directly reduce MTT. Approximately 100 µL of the test article were added to 1 mL of the MTT solution and the mixture was incubated in the dark at 37°C for approximately one hour. If the MTT solution color turned blue/purple, the test article was presumed to have reduced the MTT. Water insoluble test materials may show direct reduction (darkening) only at the interface between the test article and the medium.

The test article, [redacted]448-093, was not observed to reduce MTT in the absence of viable cells.

Test Article Preparation

Prior to dosing, an aliquot of the test article, [redacted]448-093, was removed from its original container, placed into a 15 mL conical tube, and held at 2°-8°C. The test article was allowed to come to room temperature prior to dosing of the tissues.

pH Determination

The pH of the neat liquid test article, [redacted]448-093, was measured using pH paper (EMD Chemicals Inc.). The neat test article was applied to pH paper with 0-14 pH range in 1.0 pH unit increments. The neat test article was then applied to pH paper with a narrower range of 0-6 pH units with 0.5 pH unit increments, to obtain a more precise pH value. The pH value obtained from the narrower range pH paper is given in Table 1.

Time Range Finding Assay

A time range finding assay was performed to establish an appropriate exposure time range to be used in the definitive assay for the test article [redacted]448-093. Four exposure times of 1, 4, 8 and 16 hours were tested in the time range finding assay. One culture was treated per exposure time with 100 µL of the test article or control. The negative control (exposure time control), 100 µL of sterile, deionized water (Quality Biological), was exposed for 3 and 16 hours. The positive control, 100 µL of 0.3% Triton®-X-100 (Fisher), was exposed for 15 and 45 minutes (one culture per exposure time).

After the appropriate exposure time, the EpiOcular™ cultures were extensively rinsed with Calcium and Magnesium-Free Dulbecco's Phosphate Buffered Saline ($Ca^{++}Mg^{++}$-Free DPBS) and the wash medium was decanted. After rinsing, the tissues were transferred to 5 mL of Assay Medium for a 10 to 20 minute incubation at room temperature to remove any test article absorbed into the tissue. A 1.0 mg/mL solution of MTT in warm MTT Addition Media was prepared no more than 2 hours before use. Three-tenths mL of MTT solution were added to designated wells in a prelabeled 24-well plate. The EpiOcular™ constructs were transferred to the appropriate wells after rinsing with $Ca^{++}Mg^{++}$-Free DPBS. The plates were incubated at 37±1°C for approximately three hours in a humidified atmosphere of 5±1% CO_2 in air.

After the incubation period with MTT solution, the EpiOcular™ cultures were blotted on absorbent paper, cleared of excess liquid, and transferred to a prelabeled 24-well plate containing 2.0 mL of isopropanol in each designated well. The plates were sealed with parafilm and stored in the refrigerator (2-8°C) until the last exposure time was harvested. The plates were then shaken for at least two hours at room temperature.

At the end of the extraction period, the liquid within the Millicell® inserts was decanted into the well from which the Millicell® insert was taken. The extract solution was mixed and 200 µL were transferred to the appropriate wells of a 96-well plate. Two hundred µL of isopropanol were added to the two wells designated as the blanks. The absorbance at 550 nm (OD_{550}) of each well was measured with a Molecular Devices Vmax plate reader.

Definitive Assay

Based on the results of the time range finding assay, four exposure times were chosen for the definitive assay. The exposure times were chosen such that generally two exposure times were expected to result in survivals lower than 50% and two exposure times were expected to result in survivals greater than 50%. In general, the negative control exposure times were selected to fit the range of the test article or positive control exposure times. The negative control (100 µL of sterile, deionized water) was exposed for 15 minutes, 4, 8 and 24 hours. The positive control (100 µL of 0.3% Triton®-X-100) was exposed for 15 and 45 minutes. The procedures used to conduct the definitive assay were essentially the same as for the time range finding assay with the exception that duplicate cultures were dosed per exposure time.

Presentation of Data

The raw absorbance values were captured. The mean OD_{550} value of the blank control wells was calculated. The corrected mean OD_{550} of the exposure time controls was determined by subtracting the mean OD_{550} of the blank control from their mean OD_{550} values. The corrected

OD_{550} of the individual test article exposure times and the positive control exposure times was determined by subtracting the mean OD_{550} of the blank control from their OD_{550} values. All calculations were performed using an Excel spreadsheet. The following % of Control calculations were made:

$$\% \text{ of Control} = \frac{\text{corrected } OD_{550} \text{ of Test Article or Positive Control Exposure Time}}{\text{appropriate corrected mean } OD_{550} \text{ of Negative Control}} \times 100$$

Exposure time response curves were plotted with the % of Control on the ordinate and the test article or positive control exposure time on the abscissa. The t_{50} value was interpolated from each plot. To determine the t_{50}, the two consecutive points were selected, where one exposure time resulted in a relative survival greater than 50%, and one exposure time resulted in less than 50% survival. Two select points were used to determine the slope and the y-intercept for the equation $y=m(x) + b$. Finally, to determine the t_{50}, the equation was solved for $y=50$. If all of the exposure time points show greater than 50% survival, the t_{50} value is presented as greater than the maximum exposure time.

Criteria for a Valid Test

The assay results were accepted if the positive control, 0.3% Triton®-X-100, resulted in a t_{50} value within two standard deviations of the historical mean (updated every three months). The corrected mean OD_{550} value for the minimum negative control exposure time was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 240 minutes).

RESULTS AND DISCUSSION

Time Range Finding Assay

A time range finding assay was performed, consisting of four exposure times of 1, 4, 8 and 16 hours for the test article, [redacted]448-093, supplied by [redacted] The exposure time response curves are included in Appendix B. Based upon the results of the time range finding assay, four exposure times were selected for the test article for the definitive assay (see Materials and Methods). The t_{50} results for the time range finding assay are presented in Table 1, under "Preliminary". Finally, the test article, [redacted]448-093, was not observed to reduce MTT directly in the absence of viable tissue.

Definitive Assay

The EpiOcular™ cultures were treated in duplicate with the test article, [redacted]448-093, at four exposure times of 8, 16, 20 and 24 hours. The negative control was exposed in duplicate for 15 minutes, 4, 8 and 24 hours. Table 1 summarizes the t_{50} results of the definitive Tissue Equivalent Assay With EpiOcular™ Cultures for the test article and the positive control, 0.3% Triton®-X-100, under "Trial 1". The exposure time response curves are included in Appendix B. Since the positive control fell within two standard deviations of the historical mean (15.1 – 38.8 minutes), and the corrected mean OD_{550} value for the minimum negative control exposure time (1.930) was within 20% of the corrected mean OD_{550} value for the maximum negative control exposure time (up to 240 minutes) (1.891), the assay results were accepted.

Table 1

IIVS Test Article Number	Sponsor's Designation	Conc.	t_{50}		pH
			Preliminary (15-Mar-05)	Trial 1 (16-Mar-05)	
[redacted]	[redacted]448-093	Neat	> 16 hours	> 24 hours	5.0
Positive Control	0.3% Triton®-X-100	NA	34.4 minutes	31.0 minutes	NA

NA - Not Applicable

01C002

KGL, INC. (Ivy Laboratories)

FINAL REPORT - February 12, 2001

KGL Protocol: [redacted]

Sample Coded: [redacted]24-001

University City Science Center
3401 Market Street - Suite 226 and Suite 232
Philadelphia, PA 19104-3355 (USA)

Telephone: [215] 387-8400
FAX: [215] 387-1046

[redacted]

Title: Human Phototoxicity Test of A Topical Product

Sponsor: [redacted]

Face cream containing 0.4 % Retinyl Propionate

Principal Investigator: Kays Kaidbey, M.D. (Board Certified Dermatologist)

Testing Facility: Ivy Laboratories (KGL, INC.)
University City Science Center
3401 Market Street
Suite 226 and Suite 232
Philadelphia, PA 19104-3355
(Phone: 215-387-8400)

Sponsor Study: [redacted]

Final Report Date: February 12, 2001

Kays Kaidbey, M.D. (Dermatology)
Principal Investigator

February 12, 2001
Date

"The names of Ivy Laboratories (KGL, INC.), any officer, employee, or collaborating scientist are not to be used for any advertising, promotional or sale purposes without the written consent of Ivy Laboratories."

FINAL REPORT

STUDY TITLE:

Human Phototoxicity Test of a Topical Product

KGL PROTOCOL:

Ivy Laboratories - KGL Protocol [redacted]

STUDY GUIDELINES:

All procedures were conducted in compliance with the regulations of the Food and Drug Administration (FDA) ([21 CFR 50, 56, 312) ICH-GCP Consolidated Guidelines, May 9, 1997 Federal Register) and in accordance with KGL's Standard Operating Procedures (SOP's).

OBJECTIVE:

The purpose of this test was to determine the phototoxic potential of a topically applied test material in human volunteers.

DESIGN RATIONALE:

The procedure involved a one-time 24-hour semi-occlusive application of the test material to duplicate sites on the lower back area followed by a single

exposure to UV radiation. The duplicate site served as an unirradiated control. The evaluator was blinded as to the identity of the test product.

STUDY SPONSOR:

[redacted]

SPONSOR STUDY:

[redacted]

TESTING FACILITY:

Ivy Laboratories (KGL INC.)

University City Science Center

3401 Market Street - Suite 226

Philadelphia, PA 19104-3355

INFORMED SUBJECT CONSENT:

After the protocol, reasons for the study, possible associated risks and potential benefits or risks of the treatment had been completely explained, a signed,

informed consent was obtained from each volunteer prior to the start of the study. Copies of all consent forms are on file at Ivy Laboratories (KGL, INC.). A copy of the blanket consent form used in this study is enclosed in Appendix A. Each subject was assigned a permanent identification number and completed a Medical History Form. These forms are also on file at Ivy Laboratories.

PRINCIPAL INVESTIGATOR:

Kays Kaidbey, M.D. (Board Certified Dermatologist)

Medical Director, KGL, INC.

Telephone: (215) 387-7009 or (215) 387-8400 (Ivy Laboratories)

FAX: (215) 387-1046

KGL ADMINISTRATIVE STRUCTURE:

Emelia Yardley (Receptionist/Initial Screening)

Linda E. Lowe (Medical Records/Initial Screening)

Carolyn Lindsay (Technician/Patcher)

Robert D. Moye (Expert Grader)

John B. Chicchi (Quality Assurance)

CONDUCTION DATES:

This study was conducted between January 8, 2001 through January 11, 2001.

TEST MATERIAL(S):

The test material used in this study was a preparation coded [redacted]24-001 supplied by the sponsor and identified as a Moisturizer. The material was tested at full strength as supplied.

This is a face cream containing 0.4% retinyl propionate (CAS 7069-42-3).

TEST PRODUCT ACCOUNTABILITY AND STORAGE:

All test samples and materials were received in good condition by our Quality Assurance Department. The test materials and quantities were checked against the protocol for (1) amount (2) product number or code (3) material container etc. The materials were individually listed on a special sheet signed by the receiver, and the laboratory supervisor. All test samples were stored at ambient conditions in an inaccessible location under the supervision of the investigator.

DISPOSITION OF REMAINING CLINICAL SUPPLIES:

All remaining test materials will be disposed of in accordance with applicable governmental regulations following submission and acceptance of the final written report by the Sponsor.

PANEL COMPOSITION:

Ten (10) healthy, Caucasian, adult volunteers between the ages of 18 and 65 years were recruited for this study (see Demographic Data). These were fair

skin individuals with skin types I, II, or III defined as follows (Federal Register 43: 38260, 1978):

Type I - Always burns easily; never tans (sensitive)

Type II - Always burns easily; tans minimally (sensitive)

Type III - Burns moderately; tans gradually (light brown)

None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics. The criteria for inclusion were as outlined in the sponsor's protocol on page 5. The criteria for exclusion were as outlined in the sponsor's protocol on pages 5, 6 and 7 and included the following:

1 - History of hypersensitivity and photosensitive dermatoses

2 - History of drug hypersensitivity or recurrent dermatological diseases

3 - Pregnancy or breastfeeding mothers

4 - Scars, moles or other blemishes over the back which can interfere with the study

5 - Recent sunburn

6 - Subjects receiving systemic or topical drugs or medications

7 - Other medical conditions considered by the investigator as sound reasons for disqualification from enrollment into the study.

SUBJECT ASSIGNMENT:

Volunteer subjects were screened and selected as described above and assigned a study number. The initials of each subject accepted into the study were recorded sequentially as they were enrolled.

RECORDING OF DATA:

The case report forms (CRF's) for this study were provided by both the Sponsor and the Investigator. All case report forms were completed in actual time, during each subject's visit. Copies of the CRF's will be retained by the investigator along with the original signed informed consent forms.

HANDLING OF STUDY DOCUMENTS:

All study related documents, case report forms (CRF's), original informed subject consent forms and any data generated were kept under secure lock in the technician's office for the duration of the study.

TEST SITE:

The test site was the mid or lower back. The test site was inspected prior to test product application to ensure that the skin was normal in appearance and free of irritation or other blemishes.

METHOD[1,2]:

The lower midback served as the testing site. The individual Minimal Erythema Dose (MED) was first determined on the back by giving a series of exposures to solar simulated radiation (SSR) from the solar simulator[3] in 25% dose increments. The MED was recorded 16-26 hours later as the smallest dose required to produce minimally visible uniform erythema. Approximately 0.2ml of the test agent was delivered to duplicate squares of non-woven cotton cloth (Webril, Curity) measuring 2x2cm using a 1ml plastic tuberculin syringe no longer than 30 minutes prior to application. The loaded Webril pads were then applied to the designated test sites on the mid or lower back, covered by and held securely with Scanpor® tape thus providing semi-occlusive conditions. Twenty-four (24±2) hours later, one patch was removed and the test site immediately exposed to 10.0J/cm^2 of UVA and to 0.5 MED of solar simulated radiation (SSR). The two wavebands were isolated by filtration from the solar simulator as described below under "light source". The duplicate patch served as an unirradiated control. An adjacent skin site which was not treated was also exposed to UVA + SSR as outlined above and served as an irradiated untreated control. Reactions were graded 10 minutes after irradiation and again twenty-four (24) and forty-eight (48) hours later. All scores were recorded on the Case Record Forms.

APPRAISAL OF RESPONSES:

A phototoxic material will produce either a wheal-and-flare response immediately after exposure, or intense erythema and edema twenty-four (24) and forty-eight (48) hours later. The presence of whealing or erythema and flare 10 minutes after irradiation was recorded. Delayed erythema and edema were evaluated 24 hours and 48 hours after exposure using a six-point scale as follows:

0 = negative, no response

± = equivocal reaction, barely perceptible erythema with no clearly defined border

1 = mild but definite erythema with clearly defined border

2 = moderate clearly defined erythema

3 = strong erythema or edema

4 = bulla or vesiculation

LIGHT SOURCE:

This was a 150-watt compact xenon arc source (Solar Light Company, see reference #3) equipped with a UV-reflecting dichroic mirror, a 1mm thick Schott WG320 filter and a 1mm thick UG5 filter to produce a solar simulated (SSR) waveband extending from 290 to 410nm. This SSR waveband was used to determine the individual MED. A 1mm thick Schott WG-345 filter was then added to eliminate the UVB component (290-320nm). The resultant spectrum was a broad continuous band in the UVA region extending from 320 to 400nm. Total irradiance at skin level was measured with a calibrated Eppley Thermopile.

The total SSR intensity at skin level averaged 97.5mW/cm^2. The UVA irradiance averaged 67.5mW/cm^2. The size of the irradiated field on the skin surface was approximately a 1cm diameter circle.

ADVERSE EXPERIENCES:

No adverse experiences or unanticipated reactions were encountered or reported by any of the panelists.

CONCOMITANT MEDICATIONS/ILLNESSES:

There were no concomitant illnesses reported by any of the subjects in this study, and none reported taking any concomitant medication other then females who were using contraceptives for birth control.

RESULTS:

Ten (10) healthy, adult Caucasian volunteers were screened for this study. All ten subjects qualified and satisfied the inclusion criteria and were enrolled in this study after signing the informed consent. There were 4 females and 6 males ranging in age from 18 to 31 years. The demography is shown in Table 1. There were no protocol deviations. No side effects or unexpected reactions of any kind were observed in any of the panelists and all ten subjects completed the investigation as outlined in the sponsor's protocol issued March 16, 1999.

The results of UV exposures are summarized in Tables 2 to 4. No reactions suggestive of phototoxicity were seen in any of the ten test panelists.

CONCLUSION:

Under the presently described test conditions, the test product coded [redacted]**24-001** does not possess a detectable phototoxic potential in human skin.

REFERENCES:

1 Kaidbey, KH and Kligman, AM: Identification of topical photosensitizing agents in humans. J. Invest. Dermatol., 70: 149-151, 1978.

2 Kaidbey, KH and Kligman, AM: Identification of contact photosensitizers by human assay. In "Current concepts in cutaneous toxicity", edited by V.A. Drill and P. Lazar. Academic Press, Inc., pp. 55-68, New York, NY, 1980.

3 Berger, D.S.: Specification and design of solar ultraviolet simulators. J. Invest. Dermatol., 53: 192-199, 1969.

TABLE 1

DEMOGRAPHIC DATA

Subject Number:	Subject Initials:	Age:	Sex:	Race:	Skin type:
01	RLH	18	M	C	III
02	JFK	31	M	C	III
03	CPC	22	M	C	III
04	RAB	22	F	C	III
05	VMS	22	F	C	III
06	BTB	18	M	C	III
07	AKB	19	M	C	III
08	KLV	19	F	C	III
09	WLM	19	F	C	III
10	WFB	22	M	C	III

TABLE 2

PHOTOTOXICITY BIOASSAY TESTING RESULTS

INDIVIDUAL EVALUATIONS 10 MINUTES POST EXPOSURE

Sample: [redacted]24-001

Subject Number	Unirradiated Site	Irradiated Site	UV Irradiated Untreated Control Site
001	0	0	0
002	0	0	0
003	0	0	0
004	0	0	0
005	0	0	0
006	0	0	0
007	0	0	0
008	0	0	0
009	0	0	0
010	0	0	0

GRADING SCALE

0 = negative, no response
± = equivocal reaction, barely perceptible erythema with no clearly defined border
1 = mild but definite erythema with clearly defined border
2 = moderate clearly defined erythema
3 = strong erythema or edema
4 = bulla or vesiculation

TABLE 3

PHOTOTOXICITY BIOASSAY TESTING RESULTS

INDIVIDUAL EVALUATIONS 24 HOURS POST EXPOSURE

Sample: [redacted]24-001

Subject Number	Unirradiated Site	Irradiated Site	UV Irradiated Untreated Control Site
001	0	0	0
002	0	0	0
003	0	0	0
004	0	0	0
005	0	0	0
006	0	0	0
007	0	0	0
008	0	0	0
009	0	0	0
010	0	0	0

GRADING SCALE

0 = negative, no response
± = equivocal reaction, barely perceptible erythema with no clearly defined border
1 = mild but definite erythema with clearly defined border
2 = moderate clearly defined erythema
3 = strong erythema or edema
4 = bulla or vesiculation

TABLE 4

PHOTOTOXICITY BIOASSAY TESTING RESULTS

INDIVIDUAL EVALUATIONS 48 HOURS POST EXPOSURE

Sample: [redacted]24-001

Subject Number	Unirradiated Site	Irradiated Site	UV Irradiated Untreated Control Site
001	0	0	0
002	0	0	0
003	0	0	0
004	0	0	0
005	0	0	0
006	0	0	0
007	0	0	0
008	0	0	0
009	0	0	0
010	0	0	0

GRADING SCALE

0 = negative, no response
± = equivocal reaction, barely perceptible erythema with no clearly defined border
1 = mild but definite erythema with clearly defined border
2 = moderate clearly defined erythema
3 = strong erythema or edema
4 = bulla or vesiculation

01C001

KGL, INC. (Ivy Laboratories)

FINAL REPORT - April 16, 2001

KGL Protocol: [redacted]

[redacted]

Sample Coded: [redacted]24-001

University City Science Center
3401 Market Street - Suite 226 and Suite 232
Philadelphia, PA 19104-3355 (USA)

Telephone: [215] 387-8400
FAX: [215] 387-1046

[redacted]

Title: Human Photoallergy Test of A Topical Product

Sponsor: [redacted]

Face cream containing 0.4% Retinyl Propionate

Principal Investigator: Kays Kaidbey, M.D. (Board Certified Dermatologist)

Testing Facility: Ivy Laboratories (KGL, INC.)
University City Science Center
3401 Market Street
Suite 226 and Suite 232
Philadelphia, PA 19104-3355
(Phone: 215-387-8400)

Sponsor Study: [redacted]

Final Report Date: April 16, 2001

Kays Kaidbey

Kays Kaidbey, M.D. (Dermatology)
Principal Investigator

April 16, 2001
Date

"The names of Ivy Laboratories (KGL, INC.), any officer, employee, or collaborating scientist are not to be used for any advertising, promotional or sale purposes without the written consent of Ivy Laboratories."

FINAL REPORT

STUDY TITLE:

Human Photoallergy Test of a Topical Product

KGL PROTOCOL:

Ivy Laboratories - KGL Protocol [redacted]

STUDY GUIDELINES:

All procedures were conducted in compliance with the regulations of the Food and Drug Administration (FDA) ([21 CFR 50, 56, 312) ICH-GCP Consolidated Guidelines, May 9, 1997 Federal Register) and in accordance with KGL's Standard Operating Procedures (SOP's).

OBJECTIVE:

The objective of this study was to determine the photosensitization (photocontact allergenicity) potential of a topically applied test material (facial moisturizer) in human volunteers by means of the Photocontact Allergenicity Test (see references #1 and #2).

DESIGN RATIONALE:

This was a repeat insult patch test wherein test materials and ultraviolet radiation (solar simulated radiation) were administered to the same designated

test sites over the mid or lower back area repeatedly for a total of six (6) induction exposures over a 3 week period followed by a challenge phase after a rest period of at least 14 days. The evaluator was blinded as to the identity of the test product.

STUDY SPONSOR:

[redacted]

SPONSOR STUDY:

[redacted]

PRINCIPAL INVESTIGATOR:

Kays Kaidbey, M.D. (Board Certified Dermatologist)

Medical Director, KGL, INC.

Telephone: (215) 387-7009 (direct)

(215) 387-8400 (Ivy Laboratories)

FAX: (215) 387-1046

TESTING FACILITY:

Ivy Laboratories (KGL, INC.)

3401 Market Street - Suite 226

Philadelphia, PA 19104-3355

Phone: (215-387-8400)

FAX: (215-387-1046)

KGL ADMINISTRATIVE STRUCTURE:

Emelia Yardley (Receptionist/Initial Screening)

Linda E. Lowe (Medical Records/Database)

Madeline Billings (Patcher)

Robert D. Moye (Expert Grader)

John B. Chicchi (Quality Assurance)

CONDUCTION DATES:

This study was conducted from January 8, 2001 through February 16, 2001.

INFORMED CONSENT:

Signed, informed subject consent was obtained from each volunteer prior to the start of the study, after the protocol, reasons for the study, possible associated risks and potential benefits or risks of the treatment had been completely

explained. Copies of all consent forms are on file at Ivy Laboratories (KGL, INC.). A copy of the blanket informed consent used in this study is enclosed in Appendix A. Each subject was assigned a permanent identification number and completed a Medical History Form. These forms are also on file at Ivy Laboratories.

TEST MATERIALS:

The test sample used in this study was supplied by the sponsor and was coded [redacted]24-001 (Moisturizer). The material was tested at full strength as supplied.

This is a face cream containing 0.4% retintyl propionate (CAS 7069-42-3).

TEST PRODUCT ACCOUNTABILITY:

All test samples and materials were received in good condition by our Quality Assurance Department. The test materials and quantities were checked against the protocol for (1) amount (2) product number or code (3) material container etc. The materials were individually listed on a special sheet signed by the receiver, the laboratory supervisor and the investigator (physician). All test materials were stored at ambient conditions in an inaccessible location under the supervision of the investigator.

DISPOSITION OF REMAINING CLINICAL SUPPLIES:

All remaining test materials will be disposed of in accordance with applicable governmental regulations following submission and acceptance of the final written report by the Sponsor.

PANEL COMPOSITION:

Healthy, Caucasian, adult volunteers over the age of 18 years were recruited for this study. These were fair skin individuals with skin types I, II, or III defined as follows (Federal Register 43: 38260, 1978):

Type I - Always burns easily; never tans (sensitive)
Type II - Always burns easily; tans minimally (sensitive)
Type III - Burns moderately; tans gradually

None of the subjects had a medical or dermatological illness and none were sensitive to sunlight or to topical preparations and/or cosmetics. The criteria for inclusion were as outlined in the sponsor's protocol on page 6. The criteria for exclusion were as outlined in the sponsor's protocol on pages 7 and 8 and included the following:

1 - History of sun hypersensitivity and photosensitive dermatoses

2 - History of drug hypersensitivity or recurrent dermatological diseases

3 - Pregnancy or mothers who are breastfeeding

4 - Scars, moles or other blemishes over the back which can interfere with the study

5 - Recent sunburn

6 - Subjects receiving systemic or topical drugs or medications, including potential photosensitizers within the previous 4 weeks

7 - Other medical conditions considered by the investigator as sound reasons for disqualification from enrollment into the study.

SUBJECT ASSIGNMENT:

Volunteer subjects were screened and selected as described above and assigned a study number. The initials of each subject accepted into the study were recorded sequentially as they were enrolled.

RECORDING OF DATA:

The case report forms (CRF's) for this study were provided by both the Sponsor and the Investigator. All case report forms were completed in actual time, during each subject's visit. All scores were recorded on the Case Report Forms. Copies of the CRF's will be retained by the investigator along with the original signed informed consent forms.

HANDLING OF STUDY DOCUMENTS:

All study related documents, case report forms (CRF's), original informed subject consent forms and any data generated were kept under secure lock in the technician's office for the duration of the study.

TEST SITE:

The test site was the mid or lower back. The test site was inspected prior to test product application to ensure that the skin was normal in appearance and free of irritation or other blemishes.

METHOD[1,2]:

Test patches were applied to the lower back of each subject. The entire test was composed of three distinct phases: (1) Pre-testing phase (2) Induction phase and (3) Challenge phase.

(1) PRE-TESTING PHASE:

After signing an informed consent form (on Day 1), the Minimal Erythema Dose (MED) of each subject was determined by exposing one side of the midback to a series of exposures (1cm diameter circular areas) in 25% increments from the xenon arc solar simulator, the details of which are listed below. The subject's MED is the shortest exposure time that produces a minimally visible faint erythema 20 to 24 hours later.

(2) INDUCTION PHASE:

Approximately 0.2gms of the test material was applied to a 2x2cm square of non-absorbing cotton cloth (Webril) no longer than 30 minutes prior to application to

to the designated test site. The loaded pad was then applied to one side of the lower back and fastened to the skin with Scanpor tape, thus providing a semi-occlusive patch. The patch was left in place for twenty-four (24) hours. At the end of that period, the patch was removed and the site wiped off with dry gauze and exposed to three minimal erythema doses (MED's) from the xenon arc solar simulator. The site was then left open for a forty-eight (48) hour period, after which the subjects returned to the testing facility and the patch was reapplied to the same designated test site under a semi-occlusive dressing as previously outlined. Twenty-four (24) hours later, the patch was removed and the site re-exposed to 3 MED's of solar simulated radiation. This sequence was repeated to the same test site twice weekly for a total of three weeks (total of 6 exposures).

(3) CHALLENGE:

Seventeen (17) days following the last induction dose, the subjects returned to the testing facility for a single challenge exposure. The test material was applied as previously specified (0.2gms) in duplicate to new designated skin sites measuring 2 x 2cm on the opposite side of the lower back, under a semi-occlusive dressing for a period of approximately 24 hours. One patch was then removed and any excess test material wiped off with dry gauze. The site was then irradiated with 1/2 an MED of solar simulated radiation (SSR) plus 4J/cm^2 of UVA which was obtained by filtering the beam from the solar simulator to

eliminate short (UVB) wavelengths (see Light Source). The duplicate patch remained unirradiated and served as a control treated site. One additional untreated normal skin site was also exposed to ½ MED plus 4J/cm^2 of UVA and served as an irradiated, untreated control.

EVALUATION OF SKIN REACTIONS:

All test sites were examined for reactions at 24, 48 and 72 hours following exposure of the sites to UV radiation. Each subject reported back to the testing facility at all three time points to have the responses appraised by an evaluator other than the person applying the test product, and who was unaware of the nature of the test substance.

Skin reactions were scored according to the following scale:

Erythema (redness):

0 : No visible erythema

1 : Mild erythema (faint pink to definite pink)

2 : Moderate erythema (definite redness)

3 : Severe erythema (very intense redness)

E = Edema

P = Papules

V = Vesicles

B = Bullae

Other response characteristics were graded as follows:

S = Spreading reaction - evidence of the reaction beyond the pad area.

W = Weeping - evidence of release of fluid from a vesicular or bullous reaction.

LIGHT SOURCE[3]:

This was a 150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and a 1mm thick Schott WG-320 filter to produce simulation of the solar spectrum (290nm-400nm). A 1mm thick UG5 filter was added to remove reflected heat and remaining visible radiation. Total irradiance at skin level was measured with a calibrated Eppley Thermopile. The size of the irradiated field was approximately a 1-cm diameter circle. UVA was obtained from this same source by passing the beam through a 1mm Schott WG345 filter (Schott Glass Technologies). This provided a continuous spectrum between 320 and 420nm with a peak between 360-370nm. Total irradiance (UVB + UVA) at skin level was 60.0mW/cm^2. The UVA intensity was 30.0mW/cm^2.

ADVERSE EXPERIENCES:

No adverse experiences or unanticipated reactions related to the test product were reported by any of the panelists in this study.

CONCOMITANT MEDICATIONS/ILLNESSES:

Other than females on birth control, one subject #13 (initials ALG, a female) developed an ear infection on January 24, 2001 for which her physician prescribed Amoxicillin 500mgs, three times a day for ten days. Amoxicillin was not related to the product under investigation.

RESULTS:

Thirty-two (32) healthy, adult Caucasian volunteers were screened for this study. Of the 32 screened, four subjects were not interested in participating. The remaining twenty-eight (28) subjects who qualified and who satisfied the inclusion criteria were enrolled in this study after signing the informed consent. There were 14 females and 14 males ranging in age from 18 to 27 years. The demography is shown in Table 1. Three subjects #07, #16 and #20 were dropped from the study because they failed to return to the laboratory for their scheduled visits and were lost to follow-up. The remaining 25 subjects completed this investigation as outlined in the sponsor's protocol issued March 16, 1999. There were no protocol deviations. No side effects or unexpected reactions of any kind were observed in any of the panelists.

Following the challenge phase, no reactions were seen in any of the panelists at either 24, 48 or 72 hours post exposure. The results of the challenge are summarized in the enclosed tables (Tables 2 through 5).

CONCLUSIONS:

Under the presently described test conditions, the test material coded [redacted]24-001 does not possess a detectable photocontact-sensitizing potential in human skin.

REFERENCES

(1) Kaidbey, KH and Kligman AM: Photomaximization test for identifying photoallergic contact sensitizers. Contact Dermatitis, 6: 161-169, 1980.

(2) Kaidbey, KH and Kligman AM: Identification of contact photosensitizers by human assay. In "Current concepts in cutaneous toxicity, edited by V.A. Drill and P. Lazar. Academic Press Inc., pp. 55-68, 1980

(3) Berger DS: Specification and design of solar ultraviolet simulators. J.Invest.Dermtol. 53: 192-199, 1969.

TABLE 1

DEMOGRAPHIC DATA

Subject Number:	Subject Initials:	Age:	Sex:	Race:
01	A.D.H.	18	M	C
02	K.A.C.	19	F	C
03	J.M.M.	21	F	C
04	L.M.S.	19	M	C
05	S.R.H.	20	F	C
06	D.E.S.	23	M	C
07	A.M.A.	20	M	C
08	C.E.H.	18	F	C
09	E.T.C.	18	F	C
10	D.M.K.	18	F	C
11	L.E.J.	18	F	C
12	D.M.B.	18	F	C
13	A.L.G.	18	F	C
14	M.E.W.	22	M	C
15	S.M.P.	18	F	C
16	M.B.R.	27	M	C
17	J.M.C.	22	M	C
18	J.P.B.	19	M	C
19	K.L.V.	21	F	C
20	D.M.A.	20	M	C
21	N.E.M.	18	F	C
22	S.L.F.	21	F	C
23	G.R.M.	19	M	C
24	D. – O.	18	M	C
25	C.R.D.	18	M	C
26	K.D.L.	18	M	C
27	M.A.S.	20	M	C
28	D.M.O.	18	F	C

TABLE 2

RESULTS OF PHOTOMAXIMIZATION TESTING

Untreated Irradiated Control Site

Subject Number:	24 Hours	48 Hours	72 Hours
01	0	0	0
02	0	0	0
03	0	0	0
04	0	0	0
05	0	0	0
06	0	0	0
07	Dropped from study		
08	0	0	0
09	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	Dropped from study		
17	0	0	0
18	0	0	0
19	0	0	0
20	Dropped from study		
21	0	0	0
22	0	0	0
23	0	0	0
24	0	0	0
25	0	0	0
26	0	0	0
27	0	0	0
28	0	0	0

GRADING SCALE:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesiculo-bullous reaction)

TABLE 3

RESULTS OF PHOTOMAXIMIZATION TESTING (24 Hour Grading)

Sample: [redacted]24-001

Subject Number:	Unirradiated Control	UVA Irradiated
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	Dropped from study	
08	0	0
09	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	Dropped from study	
17	0	0
18	0	0
19	0	0
20	Dropped from study	
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0
28	0	0

GRADING SCALE:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesiculo-bullous reaction)

TABLE 4

RESULTS OF PHOTOMAXIMIZATION TESTING (48 Hour Grading)

Sample: [redacted]24-001

Subject Number:	Unirradiated Control	UVA Irradiated
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	Dropped from study	
08	0	0
09	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	Dropped from study	
17	0	0
18	0	0
19	0	0
20	Dropped from study	
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0
28	0	0

GRADING SCALE:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesiculo-bullous reaction)

TABLE 5

RESULTS OF PHOTOMAXIMIZATION TESTING (72 Hour Grading)

Sample: [redacted]24-001

Subject Number:	Unirradiated Control	UVA Irradiated
01	0	0
02	0	0
03	0	0
04	0	0
05	0	0
06	0	0
07	Dropped from study	
08	0	0
09	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	Dropped from study	
17	0	0
18	0	0
19	0	0
20	Dropped from study	
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0
28	0	0

GRADING SCALE:

0 = Not sensitized
1 = Mild sensitization (viz. erythema and a little edema)
2 = Moderate sensitization (erythema with infiltration, spreading reaction beyond the borders of the patch, with or without vesiculation)
3 = Strong sensitization (large vesiculo-bullous reaction)

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: January 23, 2013

SUBJECT: Concentration of Use by FDA Product Category: Retinol and Retinyl Esters

.

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 by FDA Product Category*

Retinol
Retinyl Palmitate
Retinyl Acetate
Retinyl Linoleate
Retinyl Oleate
Retinyl Propionate
Retinyl Rice Branate
Retinyl Soyate
Retinyl Tallate

Ingredient	FDA Code†	Product Category	Maximum Concentration of Use
Retinol	03C	Eye shadow	0.05%
Retinol	03D	Eye lotion	0.003-0.1%
Retinol	03E	Eye makeup remover	0.0005%
Retinol	05G	Tonics, dressings and other hair grooming aids	0.1%
Retinol	07C	Foundations	0.001-0.005%
Retinol	07E	Lipstick	0.15%
Retinol	08E	Nail polish and enamel	0.01%
Retinol	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01%
Retinol	12C	Face and neck products not spray	0.005-1%
Retinol	12D	Body and hand products not spray	0.0005-0.3%
Retinol	12F	Moisturizing products not spray	0.08%
Retinol	12G	Night products not spray	0.005-0.1%
Retinyl Palmitate	02B	Bubble baths	0.0000002%
Retinyl Palmitate	03A	Eyebrow pencil	0.05%
Retinyl Palmitate	03B	Eyeliner	0.03-0.1%
Retinyl Palmitate	03C	Eye shadow	0.1%
Retinyl Palmitate	03D	Eye lotion	0.01-0.14%
Retinyl Palmitate	03E	Eye makeup remover	0.01%

Retinyl Palmitate	03F	Mascara	0.01-0.5%
Retinyl Palmitate	03G	Other eye makeup preparations	0.01-0.056%
Retinyl Palmitate	04A	Colognes and toilet waters	0.1%
Retinyl Palmitate	04C	Powders (dusting and talcum)	0.1%
Retinyl Palmitate	04E	Other fragrance preparations	0.02%
Retinyl Palmitate	05A	Hair conditioners	0.0001-0.01%
Retinyl Palmitate	05B	Hair sprays pump spray	 0.002-0.0025%
Retinyl Palmitate	05F	Shampoos (noncoloring)	0.0001-0.5%
Retinyl Palmitate	05G	Tonics, dressings and other hair grooming aids	0.005-0.05%
Retinyl Palmitate	05I	Other hair preparations (noncoloring)	0.002%
Retinyl Palmitate	06A	Hair dyes and colors (all types requiring caution statement and patch test)	0.02%
Retinyl Palmitate	06B	Hair tints	0.0011-0.005%
Retinyl Palmitate	06C	Hair rinses (coloring)	0.02%
Retinyl Palmitate	07A	Blushers (all types)	0.01-0.2%
Retinyl Palmitate	07B	Face powders	0.01-0.1%
Retinyl Palmitate	07C	Foundations spray	0.0045-0.5% 0.006%
Retinyl Palmitate	07D	Leg and body paints	0.001-0.06%
Retinyl Palmitate	07E	Lipstick	0.006-0.28%
Retinyl Palmitate	07I	Other makeup preparations	0.003-0.15%
Retinyl Palmitate	08A	Basecoats and undercoats (manicuring preparations)	0.01%
Retinyl Palmitate	08B	Cuticle softeners	0.1%
Retinyl Palmitate	08C	Nail creams and lotions	0.01-0.1%
Retinyl Palmitate	08E	Nail polish and enamel	0.01%
Retinyl Palmitate	08G	Other manicuring preparations	0.061%
Retinyl Palmitate	09A	Dentifrices	0.15%

Retinyl Palmitate	10A	Bath soaps and detergents	0.0001-0.05%
Retinyl Palmitate	10D	Feminine hygiene deodorants powder	 0.0006%
Retinyl Palmitate	10E	Other personal cleanliness products	0.01-0.05%
Retinyl Palmitate	11A	Aftershave lotion	0.00044-0.056%
Retinyl Palmitate	11E	Shaving cream (aerosol, brushless and lather)	0.00001-0.1%
Retinyl Palmitate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0001-1%
Retinyl Palmitate	12B	Depilatories	0.014%
Retinyl Palmitate	12C	Face and neck products not spray	 0.0001-0.21%
Retinyl Palmitate	12D	Body and hand products not spray spray	 0.0001-1.97% 0.015%
Retinyl Palmitate	12F	Moisturizing products not spray	 0.05-0.25%
Retinyl Palmitate	12G	Night products not spray	 0.0075-0.31%
Retinyl Palmitate	12H	Paste masks and mud packs	0.00013-0.044%
Retinyl Palmitate	12J	Other skin care preparations	0.0001-0.6%
Retinyl Palmitate	13A	Suntan gels, creams and liquids not spray	 0.006-0.1%
Retinyl Palmitate	13B	Indoor tanning preparations	0.1-0.18%
Retinyl Acetate	0.3D	Eye lotion	0.11-0.12%
Retinyl Acetate	07E	Lipstick	0.06%
Retinyl Acetate	10A	Bath soaps and detergents	0.001%
Retinyl Acetate	10E	Other personal cleanliness products	0.01%
Retinyl Acetate	12C	Face and neck products not spray	 0.05-0.11%
Retinyl Acetate	12F	Moisturizing products not spray	 0.11%
Retinyl Linoleate	03C	Eye shadow	0.1%

Retinyl Linoleate	03D	Eye lotion	0.11%
Retinyl Linoleate	07A	Blushers (all types)	0.1%
Retinyl Linoleate	07C	Foundations	0.05%
Retinyl Linoleate	07F	Makeup bases	0.01%
Retinyl Linoleate	12C	Face and neck products not spray	0.2%
Retinyl Linoleate	12D	Body and hand products not spray	0.3%
Retinyl Linoleate	12G	Night products not spray	0.1-0.12%
Retinyl Linoleate	12J	Other skin care products	0.01-0.1%
Retinyl Propionate	03D	Eye lotion	0.1%
Retinyl Propionate	10A	Bath soaps and detergents	0.045%
Retinyl Propionate	12A	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.1%
Retinyl Propionate	12C	Face and neck products not spray	0.3%
Retinyl Propionate	12G	Night products not spray	0.01%

*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.
†Product category codes used by FDA

Information collected in 2012
Table prepared January 23, 2013

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: September 6, 2012

SUBJECT: Comments on the Draft Report on the Retinol and Retinyl Palmitate Prepared for the September 10-11, 2012 CIR Expert Panel Meeting

Key Issue

Throughout the report it should be made clear that the NTP photocarcinogenicity study is still a draft report. It would be helpful for CIR staff to contact NTP to determine if they have any estimate of when the NTP report will be finalized. A CIR report citing the NTP study should not be published until the NTP report is final (as the draft NTP report says "not for attribution)". The NTP did not study retinol as incorrectly stated on p.15 in the paragraph introducing the study, the study was on retinoic acid and Retinyl Palmitate.

Additional Comments

Memo - The safety of ingredients added to reports during the re-review process should be supported by the data already in the report. If the CIR Expert Panel determines that data are needed to support the additional ingredients, the ingredients should not be added to the report.

Memo, last paragraph - "vitamin D" should be "vitamin A"

p.2-3, references 10 and 11 - Reference 10 (cited on p.2) is an abstract of the study more completely described in reference 11 (cited on p.3). It is not necessary to present this study twice in the Percutaneous Absorption and Distribution section.

p.3 - From the information presented in the draft CIR report, it is not clear that reference 13 really studied the "accumulation" of Retinyl Palmitate and Retinol in the skin of SKH-1 mice. Were measurements made only after 13 weeks of exposure? If so, it is not known if the levels in the skin increased over time, or if they reached a plateau shortly after the exposure began.

p.4 - Rather than saying a review exists, it might be helpful to briefly mention the authors conclusions, as this review (reference 16) includes at least some of the group that conducted the NTP photocarcinogenicity study.

p.5 - The word "gavage" is not a verb

p.5 - At what dose was Retinyl Palmitate associated with decreases in locomotory and exploratory behavior (reference 19)?

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

p.6 - The human oral Retinyl Palmitate study (reference 22) did not look at the toxicity of Retinyl Palmitate. It should not be in a "Toxicity" section, or the heading of the section should be changed to "Repeated Dose Exposure".

p.6 - Reference 23 compares dermal absorption to oral absorption. This reference needs to be moved to the "Percutaneous Absorption and Distribution" section.

p.7-8 - Although human cells were used, reference 25 should be presented under an "*In Vitro*" subheading rather than "Human".

p.8 - Please give some indication of the vitamin A status of the children studied in reference 26. Were these well-nourished children? If not, this reference is not relevant to assessing the safety of Retinol in cosmetic products.

p.8 - The following sentence is not complete: "The symptoms after orthotopic liver transplantation."

p.9 - In the description of reference 33, please be more specific when describing the gestation days of treatment. Rather than stating that treatment was on "different days of pregnancy", please state the specific gestation days on which the mice were treated. The meaning of treatment described as "by day 10" and "by day 11" is not clear. Were the mice treated on just day 10 and just day 11? On which gestation day did 2 injections of the lower dose cause more teratogenic effects than a single higher dose?

p.10 - At what doses were effects on homing and OFT tests observed (reference 35)?

p.10 - Was the vitamin A status of the women in the study (reference 36) in rural Bangladesh stated? If these women were vitamin A deficient, this study is not relevant to assessing the safety of Retinol in cosmetic products.

p.12 - The conclusion that 5,6-epoxy-RP is photocytotoxic does not seem to be supported by the information provided in the summary in the CIR report. In the second complete paragraph on p.12 it states that in the absence of light, 5,6-epoxy-RP at 150 μM, 75% of the cells were viable (so 25% were not viable). In the third complete paragraph it says with light exposure (the type of exposure is not clear), cell death was 22% at 100 μM 5,6-epoxy-RP. This does not appear to be significantly different from exposure with no light.

p.13 - The study to which "the preceding study" refers is not clear.

p.14 - In reference 32, what was the diet given to the rats after day p63?

p.14 - Please indicate what the authors of reference 53 concluded.

p.15 - In the NTP photocarcinogenicity study, how many hours/day were the mice irradiated?