Safety Assessment of Tocopherols and Tocotrienols as Used in Cosmetics

Status:	Draft Final Amended Report for Panel Review
Release Date:	February 21, 2014
Panel Meeting Date:	March 17-18, 2014

The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This re-review was prepared by Monice M. Fiume, Senior Scientific Analyst/Writer, and Bart A. Heldreth, Ph.D., Chemist.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume MMF
Senior Scientific Analyst/Writer
Date: February 21, 2014
Subject: Re-Review of Tocopherols and Tocotrienols as Used in Cosmetics

Enclosed is the Draft Final Amended Report on the Safety Assessment of Tocopherols and Tocotrienols as Used in Cosmetics. There are 14 ingredients included in this re-review. At the December 2013 meeting, the Panel re-opened the safety assessment on tocopherols to add five ingredients, and subsequently concluded all of these ingredients are safe as used.

The Council had commented that the report should acknowledge that there are studies on vitamin E supplementation with unfavorable results. An Epidemiology section has been added to the report that refers to the fact that the health benefits of vitamin E are still being debated, but those studies are not relevant to the cosmetic use of tocopherols and tocotrienols. The Discussion addresses this issue as well.

Additionally, information on tocopherol regulatory proteins has been incorporated, as requested by the Panel. This information is indicated by vertical lines in the margins. Information from a few additional papers has also been added to the report, and this new information is also indicated by vertical lines.

A letter was received, in response to the Panel's statement during its December discussion, that sensitization is rarely observed with tocopherol. The Council stated that it has been brought to their attention that recently d,l-α-tocopherol tested positive in an animal sensitization study, and a study summary should be available on the ECHA website sometime in late 2014. This letter is included with this report submittal.

Comments on the Tentative Amended Report were received from the Personal Care Products Council, and these comments have been addressed.

The Panel should be prepared to issue a Final Amended Report with a safe as used conclusion.

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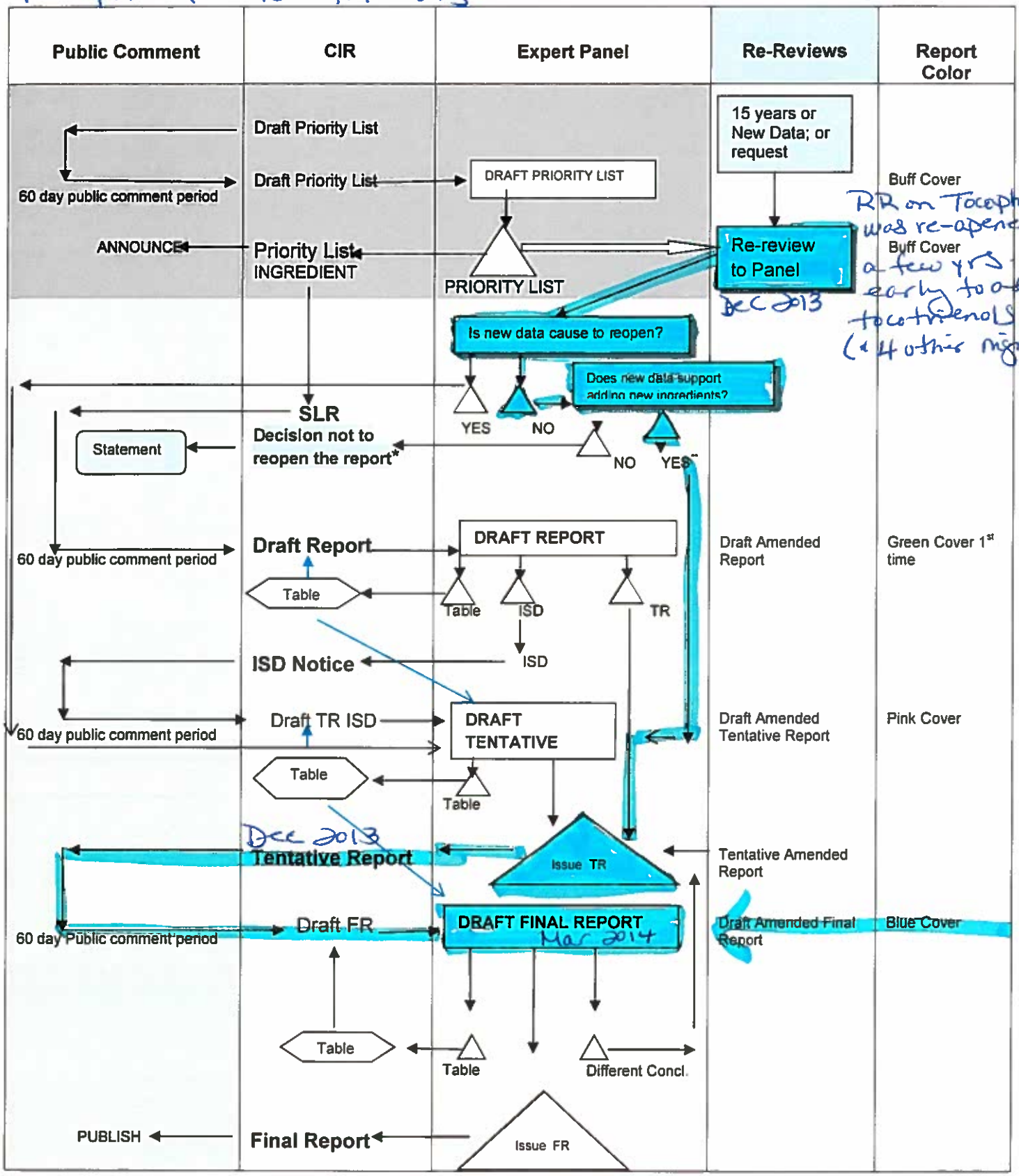
SAFETY ASSESSMENT FLOW CHART

Tocopherols & Tocotrienols — Mar 2014



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Tocopherols Re-Review History

December 9-10, 2013: Re-Review

Tocotrienols is scheduled for review in 2013. In preparing that review, we recalled that the tocopherols were reviewed in 2002. Tocotrienols and tocopherol together comprise vitamin E. Subsequently, although it has not been 15 years since the tocopherols were reviewed, it is proposed that tocopherols be re-reviewed, and tocotrienols be included in that re-review.

Original Report Ingredients:
tocopherol
tocopheryl acetate
tocopheryl linoleate
tocopheryl linoleate/oleate
tocopheryl nicotinate
tocopheryl succinate
dioleyl tocopheryl methylsilanol
potassium ascorbyl tocopheryl phosphate
tocophersolan

Proposed Add-ons:
tocotrienols
ascorbyl tocopheryl acetate
ascorbyl tocopheryl maleate
tocopheryl phosphate
sodium tocopheryl phosphate

The Panel found in appropriate to include the proposed add-ons in the re-review, and issued a Tentative Amended Report with a safe as used conclusion.

March 17-18, 2014: draft Final Amended Report

The Panel was informed that a letter was received, in response to the Panel's statement during its December discussion, that sensitization is rarely observed with tocopherol. The Council stated that it has been brought to their attention that recently d,l-α-tocopherol tested positive in an animal sensitization study, and a study summary should be available on the ECHA website sometime in late 2014.

Tocopherols/Tocotrienols* – Mar 2014 – Monice Fiume																	
	Reported Use	**Method of Mfg**	**Toxicokinetics**	**Dermal Penetration**	**Animal Tox – Acute, Dermal**	**Animal Tox – Acute, Oral**	**Animal Tox, Acute, Inhalation**	**Animal Tox – Rptd Dose, Derm**	**Animal Tox, Rptd Dose, Oral**	**Animal Tox – Rptd Dose, Inhal**	**Repro/Dev Tox**	**Genotox**	**Carciniogenicity**	**Photocard**	**Dermal Irr/Sens**	**Phototoxicity**	**Ocular Irritation**
ORIGINAL REPORT																	
Tocopherol	X	X	X	X		X			X		X	X	X	X	X		X
Tocopheryl Acetate	X	X	X		X	X			X		X	X	X	X	X	X	X
Tocopheryl Linoleate	X	X													X		
Tocopheryl Linoleate/Oleate	X																
Tocopheryl Nicotinate	X	X				X	X								X		X
Tocopheryl Succinate	X	X				X						X		X			
Dioleyl Tocopheryl Methylsilanol	X	X				X						X			X		X
Potassium Ascorbyl Tocopheryl Phosphate	X	X															
Tocophersolan	X	X				X			X		X				X		X
RE-REVIEW																	
Tocopherol	X		X	X								X	X	X			
Tocopheryl Acetate	X		X	X	X	X			X			X			X	X	X
Tocopheryl Linoleate	X																
Tocopheryl Linoleate/Oleate	X																
Tocopheryl Nicotinate	X																
Tocopheryl Succinate	X											X	X				
Dioleyl Tocopheryl Methylsilanol	X																
Potassium Ascorbyl Tocopheryl Phosphate	X																
Tocophersolan	X								X								
Tocotrienols	X	X	X	X					X				X				
Ascorbyl Tocopheryl Acetate																	
Ascorbyl Tocopheryl Maleate	X																
Tocopheryl Phosphate			X		X	X			X			X			X		X
Sodium Tocopheryl Phosphate	X																

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

TOCOPHEROL RR SEARCH STRATEGY AND RESULTS

New Ingredients

tocotrienols – 6829-55-6/per SciFinder; for toctrienol – mixture of alpha-, gamma-, and delta-
ascorbyl tocopheryl acetate – no CAS no
ascorbyl tocopheryl maleate - no CAS no.
tocopheryl phosphate – CAS No.: 425429-22-7 – complex mixture of tocopherol and phosphoric acid
sodium tocopheryl phosphate - no CAS No.

Online Database – 10-10-13
Tocotrienols and all others – no regulatory/no REACH

PubMed Search – 10-3-13
tocotrienols OR (tocopheryl AND ((ascorbyl AND (acetate OR maleate)) OR (sodium AND phosphate))) – 718 hits

SciFinder substance search – 10/11/2013
Nothing on tocotrienols or the others
Hit on tocotrienols – 512 references

ChemPortal – 10/11/13
nothing

NTP – 10/8/13
nothing

OECD – 10/8/13
nothing

EU - 10/10/13
All listed, with no limitations

Existing Ingredients
tocopherol – CAS Nos: 54-28-4; 16698-35-4; 10191-41-0; 119-13-1; 1406-18-4; 1406-66-2; 2074-53-5; 59-02-9; 7616-22-0
consists of alpha, beta, gamma, and/or delta
tocopheryl acetate – CAS Nos: 52225-20-4; 58-95-7; 7695-91-2
tocopheryl linoleate – CAS No.: 36148-84-2
tocopheryl linoleate/oleate - no CAS No.
tocopheryl nicotinate – CAS Nos.: 16676-75-8; 43119-47-7; 51898-34-1; 86362-36-9
tocopheryl succinate – CAS Nos.: 17407-37-3; 4345-03-3
dioleyl tocopheryl methylsilanol - no CAS No.
potassium ascorbyl tocopheryl phosphate - no CAS No.
tocophersolan – CAS No.: 30999-06-5; 9002-96-4

Online Database – 10/10/13
Tocopherol; Tocopherol Succinate – nothing new since original rpt
Tocopherol Acetate – check REACH
All others – no regulatory/no REACH

PubMed Search:
tocopherol OR (tocopheryl AND (acetate OR linoleate OR oleate OR nicotinate OR succinate OR (dioleyl AND methylsilanol) OR (potassium AND ascorbyl AND phosphate))) OR tocophersolan – from 1998 on
AND (SENSITIZ* OR SENSITIS* OR PHOTOTOX* OR IRRITA* OR CARCINOGEN* OR MUTAGEN* OR TOXIC* OR PHOTOSENS* OR (TUMOR AND (PROMOT* OR INITIAT*))) - 1937 hits

SciFinder:
tocopherol/1998 on/refined by doc –

others from original rpt/1998 on/refined by doc – 2319

60 papers ordered for tocotrienols & tocopherol using PubMed

ChemPortal – 10/11/13
nothing

NTP – 10/8/13
tocopheryl acetate – short-term oral; could only access results

OECD – 10/8/13
nothing

EU - 10/10/13
Tocopherol Acetate – SCCP 2001 opinion paper
All listed, with no limitations

REACH – 10/10/13
Tocopheryl acetate - http://apps.echa.europa.eu/registered/data/dossiers/DISS-9ebd4bee-d585-2070-e044-00144f67d031/DISS-9ebd4bee-d585-2070-e044-00144f67d031_DISS-9ebd4bee-d585-2070-e044-00144f67d031.html

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Additional references were obtained as the document was prepared based on the references cited in existing papers.

FULL PANEL – DEC 9 2013 - TOCOPHEROL

DR. BERGFELD: So at last we're moving on to the re-reviews and the vitamin E products, Dr. Marks.

DR. MARKS: So this is a re-review of the tocopherols a/k/a vitamin E components. And in 2002, the panel published a safety assessment that nine ingredients were safe. That's on the memo from Monice. Today we're making a decision whether to reopen the safety assessment with the purpose of adding the tocotrienols. And our team felt that this was a no-brainer and we could reopen and add the tocotrienols with a safe conclusion.

DR. BELSITO: Second.

DR. BERGFELD: Okay. Ron Hill?

DR. HILL: That's all right.

DR. BERGFELD: You're voting for it already?

DR. HILL: No, I (inaudible).

DR. BERGFELD: I'm going to now. Any comments?

DR. BELSITO: Well, we need the inhalation boilerplate. And although these are derived from plants, this is one where we didn't think we needed any of the botanical boilerplate. We did want some of the sections of anti-carcinogenicity moved to just the carcinogenicity section. And apparently there's some epidemiologic data out there in humans suggesting that consumption does not prevent and in some cases enhances, according to Carol, the incidence of cancer. So we wanted all of that brought into the carcinogenicity section. Discussed a little bit that, again, we don't think it's relevant to these as used in cosmetics, but just so that anyone reading this is aware that we were aware of these reports. And that was basically it, safe as used inhalation boilerplate, no botanical, and a little bit more extensive carcinogenicity summaries.

DR. BERGFELD: This is in the comment area. Anything else. Dr. Marks?

DR. MARKS: Yeah, I felt, obviously, since I made the motion, we could move on with a safe conclusion. But I did know that it'd be nice to have HRIPT data 5.4 percent. Previously it had been the leave-on products had been at 2 percent in '99 and now we have 5.4 percent. So it's double the concentration, but clearly the clinical experience, the data from the North American group, would support that. Even though it's over double the concentration it still should be safe.

DR. BELSITO: We patch tested 100 percent.

DR. MARKS: Yeah, I know.

DR. BERGFELD: Did you feel that you wanted to put that in the discussion of this document? If you're going to reopen it, you're going to have a discussion. You're going to bring that in?

DR. MARKS: Yeah. What do you think, Don?

DR. BELSITO: We could take a look at the discussion and I can -- you know, I may have -- and I did this document before Thanksgiving. I may have actually looked at the old document and there may be data there that supported it because I didn't even flag that as an issue or I just thought it was a non-issue because we don't see it clinically. So, you know, we can, I think, formulate the discussion. We're reopening it, we're going to see all of the data.

DR. BERGFELD: Okay.

DR. MARKS: Yeah, I didn't see evidence to support the higher concentration, but I agree with you, Don. What was this morning for the North American group? We tested something like 4,800 patch test patients and only had a dozen who were sensitive. And that was, as you said, patch testing of need, but that's a little different than the HRIPT.

DR. BERGFELD: Okay. So the motion is to reopen. Ron, do you have some other comment?

DR. HILL: Yeah, I do.

DR. BERGFELD: Okay.

DR. HILL: So let me preface by saying not that I have any profound safety concerns at this point, but I'm going to do some additional -- there's a group in our shop where I work that's been working on tocotrienols for very many years. And, in fact, there's an additional chemistry group that's making analogs of that.

What I can say from that is these aren't no- brainer additions to me because vitamin E, as you get it, while it will have some tocotrienols in there, they're very minor constituent amounts. But the biochemistry of tocotrienols is actually quite disparate from tocopherols. And so, like I say, I'll be asking some questions and doing a little bit deeper digging, but there's some question as to whether these are, in fact, no-brainer additions if people are proposing to you tocotrienols as appearing in basically high concentration ingredients in contrast to the vitamin E sources in this country which have very low levels of tocotrienol.

DR. BERGFELD: Well, we're going to reopen this if we vote to that and we can take that up as an issue. And certainly the chemists can be pre-warned to look at that. So I'm going to call the question to reopen. All those in favor of reopening, please raise your hand. Thank you. And on the phone, our three amigos?

DR. BELSITO: But we said reopen with a safe as used conclusion, so we're going as a tentative final with this report.

DR. BERGFELD: Well, we're still going to get (inaudible).

DR. BELSITO: Right, I understand.

DR. SHANK: Reopen.

DR. BERGFELD: Open, okay. Dan? Tom?

DR. SLAGA: Reopen, Tom.

DR. BERGFELD: Okay. Dan's gone? Well, I will assume Dan says yes.

Belsito Team - Dec 9 2013

DR. BELSITO: Okay, so next are the tocopherols, so basically we're adding in some ingredients here and looking at whether this was sound grouping, and did we get everything right, and are we having any issues with these? So, I guess the first thing is Dan to comment on the grouping. Are you happy and also are we missing any ingredients from the cosmetic dictionary that we should be adding in here?

DR. LIEBLER: Yeah, I think it's done. All the things that are in the report at this point are fine with me. There's some very creative ways of hooking tocopherols together with other molecules, but even despite its structural diversity, I don't think that there's any reason that these don't all belong together. There are essentially ways of incorporating either free or sterified tocopherols, and they're all okay with me. What I can't speak to is whether or not there are any other ingredients in the dictionary that should be included that aren't, but as long as they are tocopherol derivatives, they probably should be at least a third.

DR. BELSITO: Okay. So, am I understanding that you thought there were some additional ingredients that could be added, or you were comfortable with what was added and move on?

DR. LIEBLER: Oh, I'm comfortable with what was added, and I'm unaware of any others, but there could be. I just don't -- I didn't look in the dictionary to try and find out.

DR. BELSITO: Okay. So, if we're comfortable with the ingredients, then my feeling looking at it is safe as used inhalation boilerplate, and although these are plant- derived, they're specific ingredients, and I didn't think we needed the botanical boilerplate for this particular group. But we'll toss that out for further conversation.

DR. LIEBLER: Yeah, I don't think we need it either. These are -- unlike the botanicals which are usually aqueous extracts or not very purified mixtures, these are actually highly enriched lipid products that -- the ones that are from plant sources that are pretty highly purified, and then a large percentage of what's being used is produced synthetically. So, yeah, I don't think we need the plant (inaudible) boilerplate on these.

DR. BELSITO: Okay. Kurt, Paul, comments, additions to the document?

DR. SNYDER: I was just going to ask Dan and Kurt to comment on the -- we're adding the tocotrienols, and they do have an increased absorption compared to the parent document when it was first reviewed, and was that significant enough to be of concern?

DR. LIEBLER: I don't think so. One thing I pointed out is -- it wasn't covered in the report is that I used to the vitamin E field for quite a while, and I remembered that it was some literature, probably in the mid- 90s, on tocopherol-transfer protein and their role in packaging tocopherol into lipoproteins for distribution, so I think a lot of the difference between tocopherol and tocotrienols has to do with the fact that the tocopherols are selectively taken -- are handled by these tocopherol bindings proteins, and the tocotrienols apparently are not. So, this has more to do with the in vivo kinetics of the compounds and distribution into tissues and then possibly some of the oral data, but for topically applied materials I don't think there are really big issues here. In general, these are very modestly absorbed, very modestly metabolized.

DR. SNYDER: I was --

DR. LIEBLER: I don't --

DR. KLAASEN: Good.

DR. BELSITO: Yes, Halyna.

DR. BRESLAWEC: Yeah, there's a section in the report entitled Anti-Carcinogenicity.

DR. BELSITO: What page you on?

DR. BRESLAWEC: I have a PDF. It's page 22 of the PDF. It's just the term anti-carcinogenicity suggests a curative affect, and we would prefer if that term not be used and all that information just be included under carcinogenicity or whatever or taken out, or if you're going to discuss the epidemiological studies that show, I think, an opposite effect.

MS. EISENMANN: The epi-studies are showing an opposite effect. People taking vitamin E are not having reduced cancer incidents. They're having -- some studies are coming out greater cancer incidents, so I thought it was a little -- might be imbalanced to just show the animal studies they're showing as an anti-carcinogen, or as when you have people taking high levels of supplements, of antioxidant supplements, they're not seeing a benefit.

DR. BELSITO: So, you would propose that these animal studies either be deleted completely or just moved to the carcinogenicity study?

DR. BRESLAWEC: Our concern is that the terms anti-carcinogenicity and the data summarized on here might suggest a curative effect --

DR. BELSITO: Right.

DR. BRESLAWEC: -- and that is something we would not like. So, it either needs to be presented with full data and balanced out with the epidemiology studies or just put the whole section under carcinogenicity which is a standard format for these types of reports.

DR. BELSITO: So, move it to carcinogenicity and then where would you -- would you keep the epi-studies as separate or move those to carcinogenicity as well?

DR. BRESLAWEC: I'm not sure the epi-studies are in the report.

DR. BELSITO: They're not.

DR. KLAASEN: They probably should be.

DR. BELSITO: And move those into the carcinogenicity section as well?

MS. FIUME: So, the oral studies on vitamin E supplements, human studies on vitamin E supplement?

DR. BRESLAWEC: Again, the panel can choose a variety of ways of dealing with it. It's just that our concern is that it not be presented as a curative effect.

DR. BELSITO: Okay, so any other comments? Yes, Rachel?

MS. WEINTRAUB: I have a question about the language in the discussion on page 11 of the PDF. In talking about the tumor- promoting ability, the last sentence states the general experience of the panel is that to tocopherol is not a tumor-promoter. Instead of the language "general experience," should it be "based on the data presented?"

DR. BELSITO: You're on page 11?

MS. WEINTRAUB: Yes, sorry. Eleven, it's the third paragraph under the discussion.

DR. BELSITO: But that's the discussion from the original report, so we really can't change that.

MS. WEINTRAUB: Oh, okay. Is it? I thought it was not the original report.

DR. BELSITO: Well, if you're on page 11, that's the introduction and the discussion portion of that page is the discussion from the original report on tocopherols.

MS. WEINTRAUB: Oh, okay. Yes, it is in italics.

SPEAKER: (off mic)

MS. WEINTRAUB: Oh, okay. My apologies then. And then the other thing is just more substantive in terms of whether -- I thought in that paragraph I wanted to make sure that the panel was comfortable with the language in the document about sort of the tumor promotion and the language clarifying that it was one particular study that seems to be inconsistent with others.

DR. BELSITO: It's, again, from the original discussion. So, you said "Of concern to the panel however was one study on myacine in which tocopherol acted as a complete tumor promoter. The procedures used in this study were different from most tumor-promotion studies and results have not been repeated." Is that the --

MS. WEINTRAUB: Yeah, I mean, it says it a number of times throughout the report. Most of it is in italics language, but wanted to make sure generally that the panel was comfortable with how this discussion and with the data presented on this issue.

DR. BELSITO: I think if we add the anti- carcinogenicity data under the carcinogenicity section, we add in the epi-studies, basically what we have is to craft a new discussion. I think that discussion has not yet been crafted, so, we can go back and add language. I mean, so, I think what we need to do is we need to give Monice here if we're going out as (inaudible) tentative final with the safe-as- used and inhalation boilerplate, don't need a botanical because these are actually pure ingredients even though they're derived from plants, except do we need to say anything about heavy metals and pesticides?

DR. KLAASEN: No.

DR. BELSITO: No.

DR. SYNDER: Well, I think with this stuff that we're basically saying we want to re-open, we want to add the other ingredients, and then we'll proceed from there, but with the caveat that the discussion will be captured, hopefully, in the new craft of the discussion and summary statements.

DR. BELSITO: Right, I mean, but we're re-opening and at this point are we, I mean -- we've reopened before and gone in with the safe-as-used and move this along, so that's what I'm suggesting. And then we need to give Monice some idea of what are the points of the discussion that we need to bring up.

What I'm hearing from Rachel is she was concerned about the language, the carcinogenicity language in the original report, and I think that what we need to do is draft or give her some idea of the language we would like to see to address -- I mean, the only real issue that I'm seeing anyone raise are these anti-carcinogenicity, carcinogenicity, that one study, the human epi-studies that I wasn't aware of that suggested that it might not have a protective effect, and since I haven't seen them, it's a little hard for me to get an idea how to craft that language. But we need to wordsmith that out, but that would be done in the discussion anyway, which presumably wouldn't substantially change the document, so we could move this along and go tentative-final, safe-as-used, inhalation boilerplate, and draft a discussion which is going to really just focus on the carcinogenicity issues that were raised --

DR. LIEBLER: Right, I agree with that. I think there's actually a very large literature. Monice has probably got much of it in the -- on mild skin carcinogenesis studies. The large majority of what was done indicates that tocopherols inhibit tumorigenesis. There's that one study that Monice has pulled out from page 21, PDF

to 22, at length, but I think that that could be shrunk down quite a bit and incorporated in a much-reduced form. The bulk of the literature indicates that tocopherols are inhibitory toward use whether photo or chemical carcinogenicity.

There's also a very large literature on oral supplements of vitamin E and randomized perspective trials as well as a whole lot of other epidemiology. I don't know that we need to talk about it much at all. It would be perhaps good to cite a review or two to indicate that -- to mention this and indicate that the panel's aware of it, but it's not really relevant to our evaluation.

MS. FIUME: Thank you, Dr. Liebler, because I did look at the concentrations of use, and I believe 3 percent or something like that is the oral concentration of tocopherol and tocopherol acetate that incidental ingestion with around 5 percent is the highest concentration used, so it wouldn't be anything close to oral ingestion of --

DR. LIEBLER: Correct.

MS. FIUME: -- vitamin E supplements.

DR. LIEBLER: Right. Maybe Tom Slaga could comment on this one study with the (inaudible) promotion results, but I think it was widely considered to be an artifact at the time it was published, and no one's reproduced it.

DR. BELSITO: Well, I think, I mean, this isn't a 15-year review. I believe that Tom was on the panel when we looked at vitamin E. That was only what -- five -- it wasn't that long ago. I mean, the whole reason for opening tocopherol at this point is to add in tocotrienol which was on our high-priority list for this year, and given the relationship to the tocopherols, it was just let's open the whole thing and get it over with since a lot of the supporting data will come from the tocopherol document. So, I think Tom has already weighed in on that issue in the original report.

MS. FIUME: And Dr. Belsito, actually it was 2002 it was reviewed, so tocopherols weren't that far away, so it seemed to make sense to combine them.

DR. BELSITO: Right. Okay, anything else?

MS. WEINTRAUB: I just wanted to clarify. So, under photocarcinogenicity on page 21, the language - there's a study about increased tumor multiplicity. It's not in italics, so my assumption is that is a new study, and this supported a trend toward increased tumor multiplicity.

DR. BELSITO: So, you're talking about the tumor promotion. Which study is this? Is it at the bottom of the page or is it --

MS. WEINTRAUB: No, it's the only study --

DR. BELSITO: The photocarcinogenicity study?

MS. WEINTRAUB: Yes.

DR. BELSITO: Okay.

MS. WEINTRAUB: So, does this change the sort of one-off nature of the study that was mentioned in the previous report? Because it states there was a 14.9 percent increase in tumors reported.

DR. BELSITO: It wasn't statistically significant, and one of the issues with mouse skin studies and UVB and carcinogenicity is that the minute you put on any vehicle that slicks down the stratum corneum, you actually increase the absorption of light, so that you might think you're getting the same dose of light, but what is actually getting down into the epidermis -- and in many of these mouse studies actually you see more fibrous sarcomas that you never see from UVB because their skin is so thin they're actually radiating the dermis and deeper tissue.

It wasn't statistically significant, and I think that the addition of vitamin E to Surgilube is probably going to allow for greater -- it's going to smooth out the stratum corneum in these mice even more and actually allow for a slightly higher level of UV to be getting into the skin causing these effects. That, I don't think it bothered me.

DR. SYNDER: It didn't raise my concern because it was not statistically significant, so.

MS. FIUME: But is that something that should be addressed in the discussion so it doesn't look like it was overlooked? And just say that the results were not statistically significant?

DR. BELSITO: I think the whole issue in the discussion are some of the -- we have this large body of evidence that suggests that tocopherols actually may be, you know, as anti-oxidants, protective, but there is data out there that -- there's some epi-data. There's this study, but the overwhelming sense of the panel is that those aren't issues because -- and furthermore at levels that are used in cosmetic products, we're not concerned, although there are pretty high levels used. Right? What's the highest leave- on we have?

MS. FIUME: Tocopherol acetate is used up to 36 percent --

DR. BELSITO: Thirty-six percent.

MS. FIUME: -- in cuticle softeners.

DR. BELSITO: Yeah.

DR. LIEBLER: Monice?

MS. FIUME: Yes?

DR. LIEBLER: What's this study with female rabbi's? (Laughter)

MS. FIUME: Oops. I just wanted to see if anybody would catch it. What page is that on, Dr. Liebler?

DR. LIEBLER: Bottom of page PDF 18, last paragraph.

MS. FIUME: Can you tell me the report section because I'm actually in my Word document right now?

DR. LIEBLER: Toxicological studies, dermal, with tocopherol phosphates.

MS. FIUME: Thank you.

DR. LIEBLER: You need to specify orthodoxy reforms. (Laughter)

MS. WEINTRAUB: It's the sentence with Footnote 46.

MS. FIUME: Thank you. Oh, there it is.

DR. BELSITO: Okay, anything else on the tocopherols. Okay, so we're reopening safe-as-used. You've got a sense of how to craft the discussion around some of the contradictory evidence on whether it's protective or not protective in terms of carcinogenicity, and the inhalation boilerplate, and since it is plant derived, maybe just a brief sentence to get in the fact that these are purified ingredients. We were not concerned about the usual heavy metals, pesticides, et cetera, that might be found in the plants they're derived from.

Marks Team – Dec 9 2013

DR. MARKS: If not, we move onto the tocopherols. And these ingredients all comprise what is a group known as vitamin E. And so, this is a scheduled re-review of tocopherols. In 2002, the Panel reviewed 10 as listed on a memo, 10 ingredients, finding a safe conclusion. And in this re-review, we want to reconfirm the safety of those ingredients, and then it was suggested to add the tocotrienols to this group. And those four ingredients, again, are listed in the memo from Monice dated November the 15th.

So two questions. Reopen to add on the tocotrienols? If yes, is it a no-brainer? And it appears to be a no-brainer from my point of view.

DR. BERGFELD: I agree.

DR. SLAGA: Yeah.

DR. MARKS: So I would reopen those --

DR. SLAGA: And I agree, too. And it still would have the same conclusion.

DR. MARKS: So, yeah. And Ron Shank says, "Not much toxicology on the tocotrienols, but ample data on the tocopherols, which can be used as a read-across. Reopen to include the new ingredients, and the conclusion is the same." Let me see. I said in my comments, I didn't say it was necessary. I just said it would be good to have an HRIPT at 5.4 percent. If we reopen it, the use of tocopherol and leave-ons, it's increased by over 100 percent. It's gone from two percent to 5.4 percent.

And we don't have an HRIPT at that concentration, but again, when you look at the clinical data on it, it's got to be safe because we weren't endemics of allergy to tocopherol at that concentration of 5.4. And as far as the add-ons, the tocopherol phosphate, we have good irritation and sensitization data, which is "safe."

DR. BERGFELD: In your patch testing, what is the routine for vitamin E?

DR. MARKS: We used to patch test with it, and the number of individuals with sensitivity was so low, we dropped it. And, Monice, you have that data in here. I forget, it was thousands of individuals, and it was just a couple dozen maybe that were sensitive to tocopherol.

Now, you know, what were we testing with that? We were testing tocopherol. What was in it, which portions of it? I'm not sure we know actually as the North American group. Where is it? I have it highlighted somewhere here. Not a photoallergen. The ECHA, not a sensitizer. That's the tocopherol acetate. Local lymph node, that's with the tocopherol phosphate. Somewhere in here you had the North American group quoted.

DR. BERGFELD: I'm wondering if it's in the original.

DR. MARKS: Maybe it was the original.

DR. BERGFELD: -- don't you think, from the original point, the summary statement?

DR. MARKS: Yeah. Let me see. So I would reopen and "safe." And, Jay, if you had for the 524, that would be nice. If you don't, unless Don wants it, I think we could move forward.

MS. FIUME: Dr. Bergfeld, it is summarized in the original report. That's where it is. Dr. Marks, under the human dermal irritation and sensitization summary for the original report on tocopherols.

DR. MARKS: What page is that? You can just give me the numbers. It was like 4,000, wasn't it, that was in that review in that article that were tested, and maybe 24 were positive. I forget the exact numbers. It was large.

MS. FIUME: Yeah. I don't have the exact numbers in the original.

DR. MARKS: Okay.

DR. BERGFELD: Did you have the REACH number? I just was looking for it. I didn't see it. Was it under --

MS. FIUME: The summary data was under dermal irritation and sensitization of humans, but I don't have the numbers there. I'm just going to look and see if there were any original reports.

DR. BERGFELD: Here it is, non-human from the original report in rabbits.

MS. FIUME: Are you on page 24?

DR. BERGFELD: I am on 24.

MS. FIUME: Under the human --

DR. BERGFELD: Under human, okay. Human, oh, there it is. It's italicized. Okay.

DR. ANSELL: But it doesn't have --

DR. MARKS: No. Okay. At any rate, we'll move forward. So, Tom, we'll move forward to -- let me see, who presents this? It's me. I'll move to reopen to add the tocotrienols. Am I saying that right, "tocotrienols?" And with the conclusion "safe."

DR. SLAGA: Great.

DR. BERGFELD: Could I go back to this page 23? In the summary it says, "1992 results in a large of number of outbreaks in creams containing tocopherol. Positive patches were seed." I suspect that's leading. That makes you think it's really a sensitizer. You probably need to add that it's rare or whatever term you've used in the original there.

DR. MARKS: Which page are you --

DR. BERGFELD: That's 23 under "human." It's italicized. It's right on the top.

DR. MARKS: Twenty-three. Was that the one where it was composed of multiple ingredients?

DR. BERGFELD: North American contact -- yeah, that's the one that you suggested that it was probably due to something else. But you also said that there were only 23 out of 45 --

DR. MARKS: Well, I'm not sure. I was doing that out of memory. I wasn't doing that out of --

DR. BERGFELD: But this sounds more, whatever.

DR. MARKS: So 23 --

DR. BERGFELD: It's 23 at the top under "humans."

DR. MARKS: "Human," from the original report. Oh, yeah. To me it was pretty -- they say, however, the outbreaks were thought to be due to a metabolite or contaminant of the product. So to me, that's directly out. That's why I wasn't concerned about that Swiss outbreak. And in the original report, it was felt to be safe. So I think it's fine.

DR. BERGFELD: You didn't want to put "rare" or something in front of that just to qualify it a little bit more?

DR. MARKS: No. I guess, Monice, what you could do is get the original from the North America. I don't know where I got those numbers. Maybe it was another ingredient.

MS. FIUME: No, that's right. It was between 1985 and 1989, 4,887 patients patches with five percent. Twelve percent were allergic. Twelve patients were thought to have an allergic reaction. Two were irritated, and two were questioned.

DR. MARKS: Yeah, okay. I doubled it. I put it in the 20s. So it's 4,800, and only 12 were felt to be allergic. And these, of course, are highly selected patients because everybody we --

DR. BERGFELD: How did you put the 20 against 4,000?

DR. MARKS: Twelve.

DR. BERGFELD: No, I don't mean that. Incidental, rare?

DR. MARKS: Rare.

DR. BERGFELD: Yeah. I just think that if you add that, it would make more sense.

DR. MARKS: And if we wanted to even be more illustrative, the North America group deleted this as an ingredient to patch test in their screen because it was such a rare reaction.

DR. BERGFELD: That's a discussion.

DR. MARKS: Yeah, that would be discussed. Okay. So we will -- let me see. Let me go back. We will reopen with the adding tocotrienols with the conclusion of "safe as used."

DR. ANSELL: And we had an editorial comment. We've added a section called anticarcinogenicity.

DR. BERGFELD: Oh, that's nice.

DR. ANSELL: Well, it shows reduction in tumor incidence.

DR. BERGFELD: All these medical (inaudible) doesn't do any of that.

SPEAKER: That was our comment that there should be some mention of that rather than just having all the -- leaving it sounding like it's got that anticarcinogenesis activity.

DR. ANSELL: Yeah. First of all, anticarcinogenicity is a claim, not an effect per se.

DR. BERGFELD: Okay. So we'd like to have both elements that these are carcinogenicity studies, which have specific results, and we'd like to mention --

SPEAKER: Epidemiology data that unfortunately doesn't confirm.

DR. MARKS: Did you hear that, Tom? Did you hear these comments?

DR. SLAGA: Yeah, I heard it, but, you know, we have in a number of reports, anticarcinogenicity. And to me, you know, what that signifies is carcinogenesis study, but the chemical tocopherol, what have you, would have an anti-effect which is in support of the carcinogenicity study, too.

DR. BERGFELD: So where would you put that? Where would you put that anticarcinogenetic activity, in the discussion as a --

DR. SLAGA: Couldn't it be just with the carcinogenesis studies because the controls usually have to have tocopherol or what have you as a control. And if it shows negative, that means it's not carcinogenetic.

DR. ANSELL: Right. I think it is a carcinogenicity result, you know. To put it down as an anti-carcinogen, I just think, as a heading is inappropriate. But we also --

DR. SLAGA: Well, it could be under carcinogenesis, and maybe that's where it should be as a sub under that. Then it doesn't really sound as a separate type of -- we have added that, you know, the writer to put that in a number of extracts from plants. We'd have to go back and re-amend a lot of reports.

DR. BERGFELD: So are you nixing what Jay suggests, or what are you doing with it? He wants another title, another topic title.

DR. SLAGA: I would put it as a sub-cat under carcinogenesis.

DR. MARKS: Does that sound good to you, Jay?

MS. FIUME: That's actually what it is right now.

DR. MARKS: That's what it is. You just want to get rid of the heading "anti-carcinogenic." Is that it?

DR. ANSELL: Pretty much.

DR. BERGFELD: What do you want to call it?

DR. MARKS: It's just under that called --

DR. ANSELL: Yeah.

DR. MARKS: Either it's pro-carcin or there is anti-carcin. And that would be under the heading, and you'd just read it rather than highlight it as anti- carcinogenetic. Is that right?

DR. ANSELL: Yeah. It's a result.

DR. MARKS: I think it's just editorial.

DR. SLAGA: Right.

DR. BERGFELD: We don't normally add editorials to studies.

DR. MARKS: No, no, it's the heading. To me it's an editorial change. So, Tom, I'll let you make the final determination. Are you fine with just -- yeah. So, Tom, I think you're fine with that then, just put it under the carcinogenic heading, and that's just another paragraph highlighting as anti-carcinogenetic effect.

DR. ANSELL: Which would be a part of the discussion as to whether we conclude it's carcinogenetic or not.

DR. MARKS: Yeah, I think we're in agreement, Jay, with what you suggest. Is that right, Tom?

DR. SLAGA: Yeah.

DR. MARKS: Yeah, okay. Any other comments?

DR. ANSELL: Well, the second comment is that there's epi data, which we think is relevant to this whole discussion, too.

DR. MARKS: Epidemiologic data on carcinogenesis or what?

DR. ANSELL: This presumption that it's anti- carcinogenetic.

DR. MARKS: Yeah, okay. Okay, fine.

DR. BERGFELD: Where you put that?

DR. ANSELL: Do we have --

DR. BERGFELD: I didn't see it.

DR. ANSELL: Don't we have epi studies?

DR. BERGFELD: I haven't seen it.

MS. FIUME: No, because they are mostly on pure vitamin E, oral supplementation of pure vitamin E, which I did not think were relevant to the cosmetic safety because the incidental ingestion of tocopherol and tocopherol acetate is no higher than two percent for tocopherol and three percent for tocopherol acetate. And those are generally undiluted vitamin E. If the team would like, I can find the summery review, but I don't know how much in depth you would like those studies to go.

SPEAKER: I don't think we were thinking in depth at all, just some mention so it's not left -- that the animal data is just kind of left unchallenged.

DR. MARKS: Okay. Have we got that settled?

DR. BERGFELD: With the --

DR. MARKS: Carcinogenesis, good. Tom, that okay with you?

DR. SLAGA: That's okay.

DR. MARKS: Okay. So tomorrow I will move we'll reopen, and we'll add to the tocotrienols with a "safe" conclusion, and note these editorial comments. Tomorrow if you want to make them, Jay, you may or we'll just assume they're going to occur. I mean, it doesn't change the intent of the document.

DR. ANSELL: No.

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Safety Assessment of Tocopherols and Tocotrienols as Used in Cosmetics

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ABSTRACT

The Expert Panel assessed the safety of 14 tocopherols and tocotrienols, and concluded these ingredients are safe as used in cosmetics. The tocopherols are reported to function in cosmetics as antioxidants or skin conditioning agents; in contrast, tocotrienols is not reported to function as an anti-oxidant in cosmetics, but as a light stabilizer, oral care agent, or skin conditioning agent. The Panel reviewed the new and existing animal and clinical data to determine the safety of these ingredients in determining safety, and found it appropriate to extrapolate the existing information to conclude on the safety of all the tocopherols and tocotrienols.

INTRODUCTION

In 2002, the Panel published a review on tocopherol, the component most commonly associated with vitamin E. Although it has not been 15 years since the report on the tocopherols was published, the Panel opened a re-review of the tocopherols to include tocotrienols (a 2013 priority), as well as a few additional tocopherols that were not a part of the original report. In the original safety assessment of tocopherols, the Panel concluded these ingredients are safe as used in cosmetics:[1]

tocopherol
tocopheryl acetate
tocopheryl linoleate
tocopheryl linoleate/oleate
tocopheryl nicotinate
tocopheryl succinate
dioleyl tocopheryl methylsilanol
potassium ascorbyl tocopheryl phosphate
tocophersolan

The Panel stated the following cosmetic ingredients should be included in this safety assessment:

tocotrienols
ascorbyl tocopheryl acetate
ascorbyl tocopheryl maleate
tocopheryl phosphate
sodium tocopheryl phosphate

All relevant published literature that has become available since the CIR safety assessment was issued on tocopherols, and all relevant literature on tocotrienols and the tocopherols that are being proposed for addition to this report, are presented in this review. For the purpose of the review, relevant literature refers to that which applies to the safety of tocopherols and tocotrienols as used in cosmetics. Health claims have been made for vitamin E; that information is not relevant to the safety of vitamin E in cosmetics, and therefore, the studies investigating those claims are not included in this review.

Tocopherol and tocopheryl acetate are generally recognized as safe (GRAS) food ingredients. Because systemic exposure via cosmetic use is not expected to exceed the levels found safe for food use, oral toxicity data on tocopherol and tocopheryl acetate are not included in this review.

Some of the ingredients included in this report are ascorbyl-containing compounds. In 2005, the Panel concluded that L-ascorbic acid, ascorbyl phosphates, and ascorbates are safe as used in cosmetics.[2]

Excerpts from the 2002 report summary on tocopherols are included in each appropriate report section, and are indicated by *italicized text*.

CHEMISTRY

Definition and Structure

Tocopherols and tocotrienols are amphiphilic lipids that share in common a substituted chromanol core.[3] Together, the tocopherols and tocotrienols comprise vitamin E, differing structurally by substitution on the polar, chromanol core by either a lipophilic saturated, phytyl side chain, or by an unsaturated, isoprenoid (geranylgeranyl) side chain, respectively. Tocopherols have three stereocenters (*) which are all of the *R*-configuration (D-form) when obtained from natural sources. Synthetically produced tocopherols, however, are commonly racemic mixtures.[4] Tocotrienols have one stereocenter and three double bonds, which are in the *R*-configuration and are all *trans*-geometries, respectively, when obtained from natural sources. Synthetically produced tocotrienols, however, can potentially have any combination of stereochemistry and double bond geometries, or mixtures thereof.

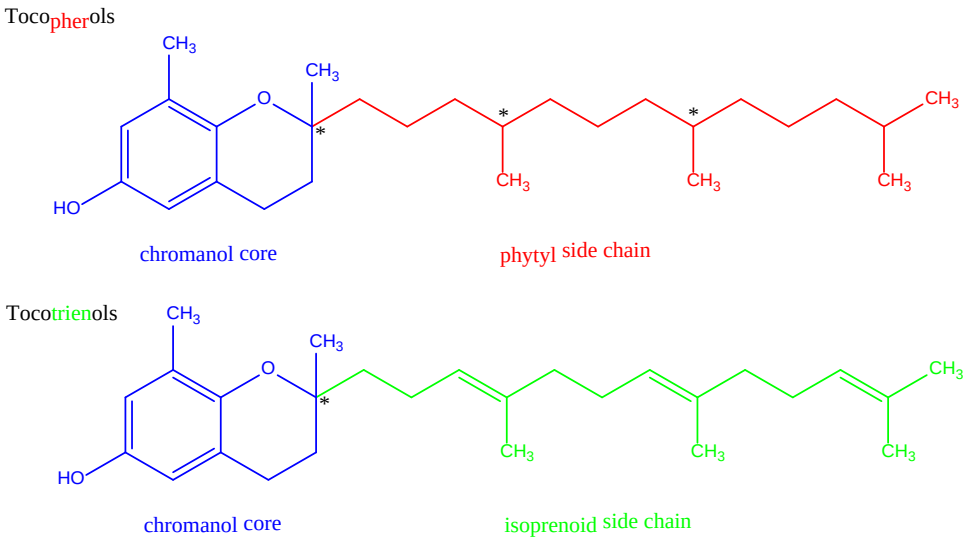


Figure 1. Tocopherols and Tocotrienols

In addition to possible stereochemistries and double bond geometries, vitamin E is comprised of eight naturally occurring structural analogs: four tocopherols (α-, β-, γ-, and δ-analogs) and four tocotrienols (α-, β-, γ-, and δ-analogs).[4] These analogs differ structurally by the presence and placement of additional methyl groups around the aromatic ring of the chromanol core. All four of the tocopherol analogs are listed in the definition for Tocopherol. According to the definition for the cosmetic ingredient, however, tocotrienols only contains the α-, γ-, and δ-analogs (i.e., no β-tocotrienol).

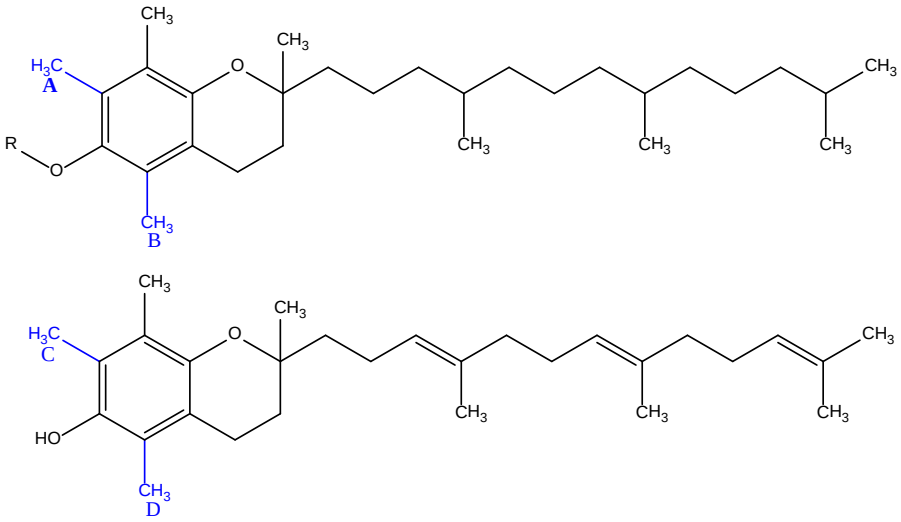


Figure 2. α-, β-, γ-, and δ-analogs
-wherein methyl groups A and B are present in the case of α-tocopherol, only A for β-tocopherol, only B for γ-tocopherol, and neither for δ-tocopherol
-wherein methyl groups C and D are present in the case of α-tocotrienol, only C for β-tocotrienol, only D for γ- tocotrienol, and neither for δ- tocotrienol
-wherein R is hydrogen or, in the case of the tocopheryl ingredients, constitutes, together with the attached oxygen, an ester

Further substituted tocopheryl ingredients, including tocopheryl acetate, tocopheryl linoleate, tocopheryl linoleate/oleate, tocopheryl nicotinate, tocopheryl succinate, dioleyl tocopheryl methylsilanol, potassium ascorbyl tocopheryl phosphate, tocophersolan, ascorbyl tocopheryl acetate, ascorbyl tocopheryl maleate, tocopheryl phosphate, and sodium tocopheryl phosphate, involve ester linkages of the corresponding acid to the free alcohol of tocopherol (e.g., tocopheryl nicotinate is the tocopheryl ester of nicotinic acid).

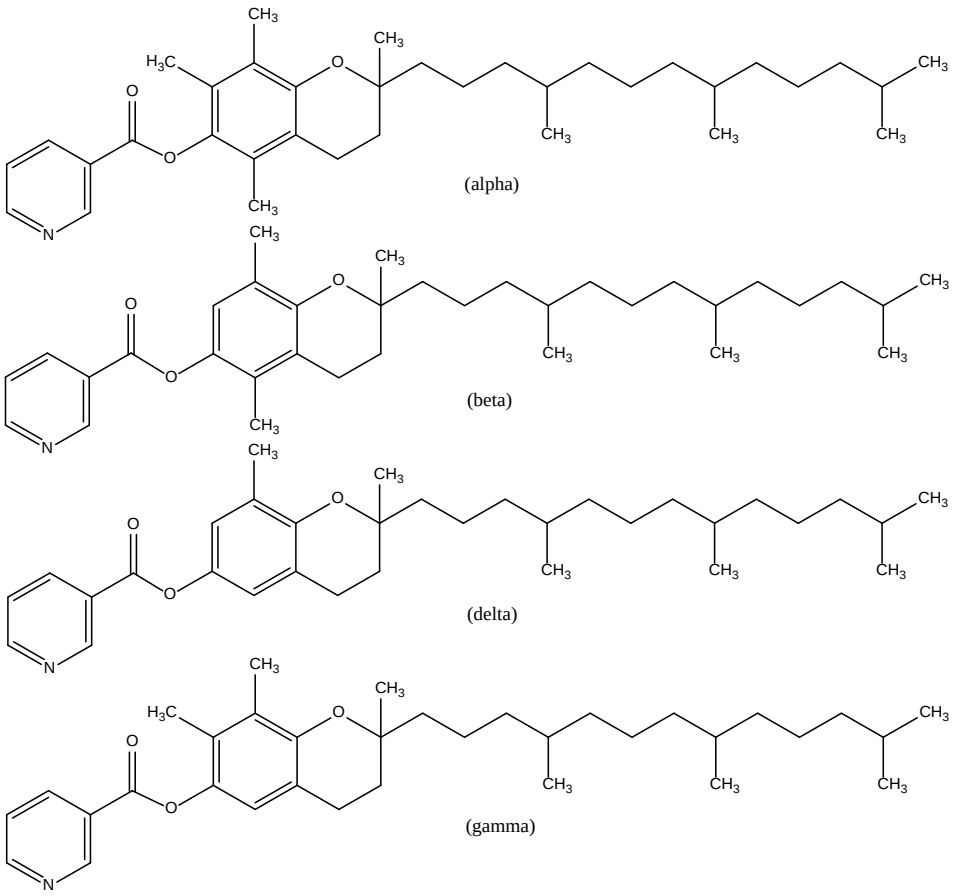


Figure 3. Tocopheryl Nicotinate.

The definitions and structures of each ingredient are found in Table 1.

Physical and Chemical Properties

α-, β-, γ- and δ-Tocopherols and tocotrienols are lipophilic molecules.[5] Tocopherol is readily oxidized upon exposure to atomospheric conditions or light, but the oxidation propensity varies among α-, β-, γ- and δ- analogs, because of differences in oxidation potential and reactivity with molecular oxygen. Limited physical and chemical properties data are provided in Table 2.

Method of Manufacture

From the original report on Tocopherols

Tocopherol is isolated on a commercial scale from vegetable oils. It is produced synthetically by condensing isophytol with tri-, di-, or monomethylhydroquinone; when produced synthetically, racemic mixtures of eight stereoisomers are formed. Tocopheryl acetate, linoleate/oleate, and nicotinate are prepared by the esterification of tocopheryl with the respective acid. Tocopheryl succinate is obtained by the vacuum steam distillation and succinylation of edible vegetable oil products; it can also be prepared by treating α-tocopherol with succinic acid anhydride in pyridine. Potassium ascorbyl tocopheryl phosphate is manufactured using a phosphate diester linkage of vitamin E and vitamin C, formulated as a potassium salt. Tocophersolan is prepared from crystalline d-α-tocopheryl succinate by esterification of the acid group with polyethylene glycol.[1]

Tocotrienols can be isolated from a number of plant sources, including *Vitis vinifera* and *Avena sativa*.[3] The extraction method with the highest efficiency is Soxhlet extraction using hexane. Tocotrienols can also be synthesized via a multitude of synthetic pathways, most of which involve multi-step processes to alkenylate the chromanol core.[6]

Natural Occurrence

From the original report on Tocopherols

Tocopherol occurs naturally in four forms, i.e., d-α-, d-β-, d-γ-, and d-δ-. α-, β-, γ-, and δ-tocotrienols are also found in nature; these compounds differ from tocopherol only in that the side chain is saturated in tocopherol and unsaturated in tocotrienol. Tocopherol is found largely in plant materials; it is also found in vegetable fats and oils, dairy products, meat, eggs, cereals, and nuts.[1]

Tocopherols and tocotrienols are synthesized by photosynthetic organisms, and because this synthesis is accomplished stereo-specifically via enzymes, the resulting tocopherols always possess the same stereochemical configurations, with the R configuration at C-2 (ring-sidechain junction) and the sidechain methyl substituents.[7-10] Tocotrienols are present in seeds, fruits, and latex. Tocopherols are the exclusive form of vitamin E in leaves of plants and the seeds of most dicots; tocotrienols are found in the seed endosperm of a limited number of dicots. Tocotrienols are the primary form of vitamin E found in the seed endosperm of most monocots, including wheat, rice, and barley, and tocotrienols are also found in coconut oil, cocoa butter, and soybeans.

Natural sources of tocopherols include sunflower, peanut, walnut, sesame, and olive oils; tocotrienols are not found in these oils.[9] γ-Tocopherol is often the most prevalent form of vitamin E in plant seeds and the products derived from them; corn, soybean and sesame oil and walnuts, pecans, and peanuts are good sources of γ-tocopherol.[11] Tocopheryl phosphate has been identified in various seeds and nuts, dairy products, green vegetables, fruits, and cereals.[12]

Palm oil is one of the richest sources of tocotrienols; tocotrienols compose 70% of the vitamin E in palm oil.[9] Crude palm oil extracted from the fruits of *Elaeis guineensis* contains up to 800 mg/kg tocotrienols.[8] Different analogs are more abundant in some plants than others; for example, rice bran oil is a major source of γ-, but not α-, tocotrienol.[7] Some other examples of plants containing significant amounts of tocotrienols are provided in Table 3.

Ultraviolet Absorbance

From the original report on Tocopherols

Tocopherol, tocopheryl acetate, and tocopheryl succinate absorb in the ultraviolet B (UVB) range. Reported absorption maxima are 292-295 nm (in ethanol) for tocopherol, 284 nm (in ethanol) and 285.5 nm (in cyclohexane) for tocopheryl acetate, and 286 nm (in ethanol) for tocopheryl succinate.[1]

USE

Cosmetic

Most of the tocopherols are reported to function in cosmetics as antioxidants or skin conditioning agents.[13] In contrast, tocotrienols is not reported to function in cosmetics as an antioxidant, but instead as a light stabilizer, oral care agent, or skin conditioning agent (Table 1.) The Food and Drug Administration (FDA) collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). VCRP data obtained from the FDA in 2013 report that the frequency of use increased considerably for both tocopherol and tocopheryl acetate. The reported use of tocopherol increased from 1072 (1998 data) to 6175 uses (2013 data), and the reported use of tocopheryl acetate increased from 1322 (1998 data) to 8960 uses (2013 data).[14] For both of these ingredients, the 2013 VCRP data includes uses under more than one name. Tocopherol has 6003 reported uses as tocopherol, 122 reported uses as tocopherol *d*-alpha, and 50 reported uses tocopherol *dl*-alpha. Tocopheryl acetate has 8433 reported uses as tocopheryl acetate and 527 reported uses as tocopheryl acetate *dl*-alpha. Both of these ingredients are used in almost every product category. Tocopheryl linoleate also increased in use frequency since the time of the original assessment, from 103 to 279 formulations; the use of the other tocopherols that were included in the original safety assessment did not change much.

The use concentration of tocopherol, but not of tocopheryl acetate, has increased since the original assessment. According to the survey conducted by the Personal Care Products Council (Council) in 2013, the concentration of use of tocopherol in leave-on products increased from 2% in 1999 to 5.4% in 2013.[15] Tocopheryl acetate continued to have the highest reported use concentration; it is used at up to 36% in leave-on formulations, and that use is in cuticle softeners. The original safety assessment reported that tocopheryl acetate was used at 100% in “vitamin E oil”.

Ascorbyl tocopherol acetate and tocopheryl phosphate are not reported to be used according to VCRP data and the Council survey.[14]

The current and historical frequency and concentration of use data for the previously-reviewed tocopherols are provided in Table 4. Table 5 provides the frequency and concentration of use data for the ingredients that are proposed as add-ons to this report. The tocopherols are used in baby product formulations (0.1% tocopheryl acetate in baby lotions, oils, and creams) and formulations that can be incidentally ingested (3% tocopheryl acetate in lipsticks).

Several of the tocopherols are used in products that can possibly be inhaled. For example, tocopheryl acetate is used at up to 5% in foot powders and sprays and up to 0.2% in aerosol hair spray formulations, and tocopherol is used at up to 1% in pump hair spray formulations. 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 <10 μm compared with pump sprays.[16,17] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[18,19] Tocopheryl acetate is used at up to 0.1% in aerosol deodorants, and there is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[18] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

All tocopherols named in this report are listed in the European Union inventory of cosmetic ingredients.[20] α-Tocopherol acetate is the most common form of vitamin E used in commercial sunscreen and skin care products.[21]

Non-Cosmetic

α-Tocopherol and α-tocopheryl acetate are GRAS food ingredients when used as a nutrient, and α-tocopherol is GRAS as a chemical preservative in food when used in accordance with good manufacturing practices. (21CFR182.8890; 21CFR182.8892; 21CFR182.8390) The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a group allowable daily intake (ADI) of 0.15-2 mg/kg for *dl*-α-tocopherol and *d*-α-tocopherol, concentrate, singly or in combination.[22]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

From the original report on Tocopherols

In a dermal absorption study using human subjects, tocopheryl acetate was substantially absorbed in the skin, but systemic availability was not observed. Also, conversion to tocopherol was not seen. In a study using rats, approximately 6% of the applied dose penetrated into the epidermis after 5 days. Most studies found that some tocopheryl acetate was converted to tocopherol. Irradiation of the animals dosed with tocopheryl acetate resulted in a significant increase in the amount of tocopheryl acetate found in the skin as compared to non-irradiated animals, and irradiation significantly increased the amount of tocopherol found in the skin of test animals. Tocopherol was "more efficiently" absorbed from w/o than o/w emulsions. The liposomal form increased absorption from o/w emulsions but did not have an effect on w/o emulsions.[1]

In oral ingestion studies with human subjects, administration of tocopherol and tocopheryl acetate resulted in increased tocopherol concentration; the mean serum concentration peaked at approximately 7-8 h following dosing. In one study, d-*α-tocopherol had a greater bioavailability than dl-α-tocopheryl. In an oral study using rats, tocopheryl acetate generally had a greater uptake than tocopheryl nicotinate. The liver was the principal storage site, but the adrenal glands had the greatest uptake. Most of the radioactivity was recovered as tocopheryl quinone, but in a few tissues, such as the adrenal glands, it was recovered mostly as tocopherol. In another study using rats that were fed tocopheryl acetate for up to 14 wks, a linear relationship was found between time and tissue concentration of tocopherol. In mice that were fed tocopheryl acetate and irradiated, a dose-dependent increase was seen for tocopherol in the ventral skin compared to controls, whereas irradiation decreased dorsal skin tocopherol concentrations.*[1]

Oral

The structural differences between tocopherol and tocotrienols result in a difference in the penetration of these compounds into tissues. The presence of three unsaturated bonds in the carbon sidechain allows tocotrienols to penetrate tissues with saturated fatty layers, such as the brain and the liver, more readily than tocopherol, which has a fully saturated side chain.[8,9]

Many studies have been conducted on the distribution of tocopherols and tocotrienols with oral administration, and the studies demonstrate that tocopherols distribute to almost all tissues in the body, and the distribution and metabolism varies among the tissues. α-Tocopherol is the predominant form of vitamin E in human and animal tissues, and it has the highest oral bioavailability, and *RRR*-α-tocopherol (natural vitamin E) has approximately twice the systemic availability of synthetic tocopherol (*all-rac*-tocopherol). Humans also have significantly greater plasma and tissue concentrations of α- than γ-tocopherol, and supplementation with α-tocopherol actually decreases plasma and tissue γ-tocopherol. The tocotrienols are not as prevalent in the body as the tocopherols, and oral absorption of the tocotrienols has been reported to be incomplete. One study reported that α-tocopherol out-competes α-tocotrienols for transport to systems in the body when these ingredients are co-supplemented. Orally administered tocopherols and tocotrienols are distributed to the skin and adipose tissue.[23-34]

The distribution and intracellular trafficking of vitamin E may be modulated by tocopherol regulatory proteins; three have been identified that specifically bind tocopherols.[34] Tocopherol transfer protein (TTP) is a cytosolic lipid-binding and transfer protein that has been found in rat brain, spleen, lung, and kidney, in some human brains, and in the mouse uterus. Tocopherol-associated protein (TAP), also a cytosolic lipid-binding and transfer protein, is found in bovine and human tissues; the greatest levels in human tissues were in the adult liver. The third protein, tocopherol-binding protein (TBP), is found in rat liver and heart, rabbit heart, bovine heart, and human placenta. Only TTP has been shown to influence plasma and tissue α-tocopherol concentrations. Relative affinities of purified TTP for α-tocopherol, as determined by competition between vitamin E analogs for transfer between liposomes and membranes *in vitro*, were 100% for RRR-α-tocopherol, 38% for β-tocopherol, 9% for γ-tocopherol, 2% for δ-tocopherol, 12% for α-tocotrienol, and 11% for SRR-α-tocopherol.

Tocopherols and tocotrienols were found in the skin of female hairless mice fed a chow containing 29.7 mg α-tocopherol, 10.3 mg γ-tocopherol , 3.1 mg α-tocotrienol, and 7.4 mg γ-tocotrienol per kg chow.[35] However, the distribution in the chow was different than that found in the skin; approximately 58% of the vitamin E in the chow was α-tocopherol and 20% was tocotrienols, but approximately 89% of the vitamin E found in the skin was α-tocopherol.

A procedure was developed that allowed for the simultaneous determination of tocopherols and tocotrienols, and the presence of these cogeners in tissues of the hairless mouse was determined.[36] The skin contained mostly α-tocopherol (5.4 nmol/g; 85.6%), but α-tocotrienol (0.24 nmol/g; 3.4%) and γ-tocotrienol (0.76 nmol/g; 10.4%) were also found. Tocotrienols were

also identified in the skin + subcutis, but to a lesser extent. The brain contained primarily α-tocopherol (5.4 nmol/g; 99.8%), and it contained no tocotrienols. The heart, kidneys, and liver also contained primarily α-tocopherol, but some tocotrienols were present, and the highest amount of α-tocopherol was found in the heart (24.2 nmol/kg; 98.1%).

Groups of 10 female CF-1 mice were fed a basal diet (containing 75 IU/kg vitamin E) or basal diet supplemented with 0.3% a γ-tocopherol-rich mixture of tocopherols for 4 wks.[37] One gram of the mixture contained 900 mg total tocopherols with 13.0% of α-tocopherol, 1.5% β-tocopherol, 56.8% γ-tocopherol, and 24.3% δ-tocopherol. Pooled urine and fecal samples were collected for each group during the last 4 days of the study, and at study termination, blood and liver samples were collected. High pressure liquid chromatography/electrochemical detection (HPLC/EC) and liquid chromatography-electrospray ionization/ mass spectrometry (LC-ESI-MS) were used to analyze the major metabolites of the different tocopherols and tocotrienols in the samples. The major route of excretion was fecal; 18 tocopherol-derived metabolites and α-, γ-, and δ-tocopherol were identified in the fecal samples of the mice fed the tocopherol-diet. Short-chain degradation metabolites, particularly γ- and δ-carboxyethyl hydroxychromans and carboxymethylbutyl hydroxychromans were detected in urine, serum, and liver samples, and tocopherols were detected in serum and liver samples. The majority of the urinary metabolites were excreted as glucuronide conjugates.

The researchers also examined the metabolite profile of pooled samples of feces, blood, and urine from a study in which male CF-1 mice were fed a basal diet or the basal diet supplemented with 0.17% of a tocotrienol preparation for 21 wks; the preparation was a 65% oil suspension that contained 20.2% α-tocotrienol, 4.0% β-tocotrienol, 16.1% γ-tocotrienol, 24.3% δ-tocotrienol, 14.8% α-tocopherol, and 3.1% γ-tocopherol.[37] Twenty-four tocotrienol-derived side-chain degradation metabolites were identified in fecal samples, as were parent α-, γ-, and δ-tocotrienols; more short-chain than long-chain tocotrienols-derived metabolites were present in the feces. Short-chain degradation metabolites, particularly carboxyethyl hydroxychromans, carboxymethylbutyl hydroxychromans, and carboxymethylhexenyl hydroxychromans were detected in urine.

The metabolism of a tocopherol supplement was evaluated in humans.[37] Two male subjects were given six softgels containing γ-tocopherol-rich dietary supplements at 0 h, and six more softgels at 10 h. Each softgel contained 200 mg β-tocopherol, 78 mg δ-tocopherol, 133 mg α-tocopherol, and 2 mg tocotrienols. Fecal and urine samples were collected at 0, 12, 24, and 48 h, and blood samples were taken at 0 and 12 h. At 24 h, almost all the side-chain degradation products that were identified in mouse fecal samples (described previously) were found in human fecal samples, and the metabolite concentrations increased over time. γ-Tocopherol-derived metabolites were more prominent than δ- and α-derived metabolites at 24 h. The major metabolites found in the urine were carboxyethyl hydroxychromans and carboxymethylbutyl hydroxychromans.

Eight-day pregnant rats were dosed by gavage with 1 g/kg tocotrienol-rich fraction (1.23 mmol α-tocotrienol and 0.94 mmol tocopherol per g; isolated from palm oil) suspended in vitamin-E deficient corn oil for nine days.[38] The rats were killed on day 17 of gestation, and tissues were collected from the dams and the neonates. In the maternal brain, the α-tocopherol content and α-tocotrienol content changed by 0.1-fold and 5-fold, respectively. In the fetal brain, α-tocotrienol content increased more than 20-fold. Subsequent testing with lower doses of tocotrienols (5 mg/kg bw) over a longer time period (not specified) supported the finding that dietary tocotrienol was distributed to the brain.

Tocopherol Depletion by UV in the Skin

Tocopherol

Female C3H/HeN Tac mice were treated topically with 5.3 µmol *RRR*-α-tocopherol or with 500 nmol of [^{14}C]-α-tocopherol and 4800 nmol α-tocopherol, in 50 µl acetone, 15 min prior to ultraviolet B (UVB) irradiation; additional groups of mice were treated with [^{14}C]-α-tocopherol or α-tocopherol, in 50 µl acetone or dimethyl sulfoxide (DMSO) for 3 h prior to irradiation.[39] The dorsal skin was shaved, and the animals were irradiated with 2.6-2.9 J/m^2/s for 1 h (10 kJ/m^2) using Westinghouse FS-20 UV lamps. Approximately 80% of the lamp output was in the UVB range (290-320 nm). The researchers noted that the AIN 76 chow used in the study contained *d,l*-α-tocopheryl acetate. Depletion of α-tocopherol in the skin by UVB was quantified following application of 5.3 µM synthetic d_3-α-tocopherol in acetone or DMSO 3 h prior to irradiation. A 1-h exposure to UVB substantially depleted the level of d_3-α-tocopherol in both treatment groups, and approximately 40-60% of the applied α-tocopherol in the epidermis was consumed by UVB treatment.

With the 15-min tocopherol exposure prior to UV, the researchers found that α-tocopherol was most likely not absorbed into the epidermis prior to irradiation, and that photooxidation occurred on the skin surface. The vehicle that was used did not have an effect on the depletion of topically applied tocopherol, but it did have an effect on the products of photooxidation; more polar products occurred when DMSO was used as the vehicle.

Tocopherol Acetate

The hydrolysis of α-tocopheryl acetate to α-tocopherol was determined in non-irradiated and irradiated mouse skin.[40] Without UV, deuterium-labeled (d_3-) α-tocopheryl acetate in an inert cream (50 mg of a 5% cream, 5.3 µmol d_3-α-tocopheryl acetate) was applied to the shaved dorsal skin of female C3H/HeN Tac mice. With UV, either shaved mice were irradiated for 60 min with a total dose of 13 kJ/m^2 UVB, and then treated with 5.3 µmol d_3-α-tocopheryl acetate in either the inert cream, or, d_3-α-tocopheryl acetate was applied 15 min before UVB irradiation. All animals were killed 1, 3, 6, 24, or 48-h

after dosing, and the epidermis was isolated. At 24 and 48-h post-dosing with d_3-α-tocopheryl acetate in non-irradiated mice, d_3-α-tocopherol increased up to 10 times that of endogenous α-tocopherol. Application of d_3-α-tocopheryl acetate 60 min after irradiation resulted in a 40-fold increase in epidermal α-tocopherol. In both cases, hydrolysis was <1% of the total applied dose. Hydrolysis of α-tocopheryl acetate increased with increased UVB dose. Application of d_3-α-tocopheryl acetate prior to irradiation eliminated hydrolysis.

A dose-response effect of UVB on d_3-α-tocopheryl acetate hydrolysis was then examined. Shaved mice were exposed to UVB for 60, 90, or 120 min, corresponding to 13, 19.5, or 26 kJ/m^2, respectively, in a single dose or in multiple 30-min/day doses, and the mice were treated with 5.3 µmol d_3-α-tocopheryl acetate in the inert cream. The animals were killed 24 h after dosing, and the epidermis was isolated. UVB exposure, as a 30-min multiple day dose, potentiated UVB-induced hydrolysis of d_3-α-tocopheryl acetate to d_3-α-tocopherol.

The researchers also examined the effect of rate of absorption into the skin on hydrolysis by applying 5.3 µmol d_3-α-tocopheryl acetate in 50 µl DMSO, following the same procedure described earlier. In non-irradiated mouse skin, hydrolysis was maximal at 3-h post-treatment with d_3-α-tocopheryl acetate in DMSO, which was earlier than what was observed using the inert cream, suggesting that hydrolysis was limited by absorption in these mice. The researchers commented that although maximal absorption occurred earlier than when d_3-α-tocopheryl acetate was applied in an inert cream, the maximal capacity for hydrolysis was the same (i.e., ≈80 pmol d_3-α-tocopherol/mg epidermis). In mice irradiated for 1 h prior to dosing, a time-dependent increase in hydrolysis was observed at 6 ad 24-h post-treatment, which was similar to the response observed with the inert cream, suggesting that absorption is not the limiting factor in UVB-induced hydrolysis of d_3-α-tocopheryl acetate. These conclusions were supported by the fact that a 10-fold greater dose of d_3-α-tocopheryl acetate resulted in a 4-6 fold increase in d_3-α-tocopherol levels in non-irradiated mice, but only a 2-3 fold increase in irritated mice. Further testing by the researchers led to the suggestion that the esterase activity at the surface of the skin was capable of converting d_3-α-tocopheryl acetate to d_3-α-tocopherol.

Tocopherol Phosphate

One study found that following UV exposure, reduction of endogenous α-tocopherol in the skin was not inhibited by pre-treatment with α-tocopheryl phosphate.[41] Pretreatment of SKH-1 mouse skin cultures with 0.5% α-tocopheryl-6-*O*-phosphate produced a two- to three-times greater amount of endogenous α-tocopherol than that found in control skin, and the UV-induced reduction in cutaneous α-tocopherol was inhibited.

Dermal Penetration

In Vitro

The effect of vehicle on the dermal penetration of α-tocopherol was determined in micro-Yucatan pig skin.[42] The dermal penetration of 1% tocopherol was evaluated in several vehicles, and α-tocopherol permeated into the receptor fluid regardless of the vehicle used. The greatest permeation found in viable skin + the receptor was from an emulsion vehicle that contained 10% isopropyl myristate; 12.24% of the applied dose was found in the viable skin and receptor fluid. The lowest penetration, 6.47% applied dose, was observed with an alcoholic vehicle that was 96% SD alcohol.

The researchers also examined the kinetics of permeation and metabolism of α-tocopheryl acetate across micro-Yucatan pig skin.[43] Formulations containing a 5% tocopheryl acetate solution in isopropyl myristate, a 5% tocopheryl acetate emulsion, and a 1% solution of α-tocopherol in isopropyl myristate were studied, and permeation was measured at 2, 6, 12, and 24 h. Any presence of tocopheryl acetate in the receptor fluid was below the limit of detection. Tocopheryl acetate was converted to tocopherol within the viable skin tissue.

In vitro skin penetration data on tocopheryl acetate were obtained from a robust summary of data submitted to the European Chemical Agency (ECHA) by a private source as part of the REACH chemical registration process. These data are available on ECHA's website.[44] The *in vitro* skin absorption study, performed according to OECD Guideline 428, indicated that tocopheryl acetate in an alpha-hydroxy acid (AHA) vehicle (composition not defined further) penetrated into and through intact and stripped pig skin, and the total skin penetration rates were time- and formulation-dependent; the differences were not statistically significant. First, intact skin was exposed to 6 mg/cm^2 of 5% [^{3}H]tocopheryl acetate cream (300 µg active substance/ cm^2) in one of three AHA vehicles for 1, 6, or 18 h. After 1 h, 1.1-1.3% of the dose (based on penetration in the stratum corneum, remaining skin tissues layers, and chamber liquid) was absorbed. After 6 h, absorption was 2.6-4.2%, and after 18 h, 3.1-4.2% of the dose absorbed. Using stripped skin (stratum corneum removed), higher absorption rates through the remaining skin tissue was observed for all three formulations.

The dermal penetration of tocopheryl acetate in human skin samples was measured using four different vehicles, and the bio-conversion of tocopheryl acetate to tocopherol was determined.[5] Tocopheryl acetate was (1) delivered in a triglyceride oil (caprylic/capric acid triglyceride); (2) surfactant-solubilized in water (PPG-26-buteth-26 and PEG-40 hydrogenated castor oil); (3) encapsulated in soybean phosphatidylcholine liposomes; or (4) encapsulated in nanotopes™. The final concentration of tocopheryl acetate was 2%. A dose of 11 mg/cm^2 of each test formulation (corresponding to 220 µg/cm^2 tocopheryl acetate) was applied to skin samples under occlusive and non-occlusive conditions. The distribution of tocopherol acetate and tocopherol at the skin surface, in the horny layers, and in the underlying skin was determined after 8 h.

The recovery rate of tocopheryl acetate + tocopherol (vitamin E_{total}) exceeded 90% in each experiment. Tocopheryl acetate in the triglyceride oil was only deposited at the skin surface and in the horny layers. With the other three formulations, differences were found with occlusive versus non-occlusive application; more vitamin E_{total} was found in the underlying skin with the non-occlusive application. The conversion to free tocopherol only occurred in the underlying skin layer, and the percent bioconversion was greater with occlusive application. The distribution of vitamin E_{total} and the bioconversion of tocopheryl acetate to tocopherol from each formulation are summarized in Table 6.

An *in vitro* study using human cadaver skin was conducted to determine the absorption of tocopheryl acetate from various vehicles.[45] Modified Franz diffuser cells, and 95% degassed ethanol, as opposed to modified phosphate-buffered saline, was used in the receiver compartment. Sampling times ranged from 2-48 h. Tocopheryl acetate, as a 5% solution, was prepared in ethanol, isopropyl myristate, and mineral oil; 5% gel formulations were prepared in a 1% or 3% water-soluble polymer. Isopropyl myristate was the only vehicle that statistically significantly increased permeation through the skin. The calculated permeabilities (using a mathematical model) of the 5% tocopheryl acetate formulations were 1.0×10^{-4} cm/h with ethanol; 1.1×10^{-2} cm/h with isopropyl myristate; 1.4×10^{-4} cm/h with mineral oil; 2.1×10^{-4} cm/h with the 1% water-soluble polymer gel; and 4.7×10^{-4} cm/h with the 3% water-soluble polymer gel.

In Vivo

Tocopherol

Dermal application of 5 mg/cm^2 α-tocopherol to the backs of female hairless SKH1 mice for 24 h resulted in a 62-fold increase of α-tocopherol in the epidermis and a 22-fold increase in the dermis.[46]

Tocopheryl Acetate

An ointment containing 2% or 20% tocopheryl acetate was applied to the backs of five male Wistar rats for 24 h, and a 2 cm-diameter skin punch was then taken to determine the content of vitamin E in the skin.[47] (The ointment contained 15% squalane.) Application of 20% tocopheryl acetate, but not 2%, resulted in a statistically significant increase of vitamin E in the skin. Vitamin E increased by 2.9 µg/g skin with the 20% ointment, but only by 0.8 µg/g skin with the 2% ointment.

Tocopherols and Tocotrienols

A 5% w/v solution containing a tocotrienol-rich palm oil fraction (TRF) in polyethylene glycol (PEG)-400 was applied to the skin of four female hairless mice.[35] The distribution of four analogs in the TRF was approximately 35% α-tocopherol; approximately 10% γ-tocopherol; approximately 25% α-tocotrienols; and approximately 30% γ-tocotrienols. Four test sites were marked using polypropylene plastic rings, and 20 µl of the test mixture were applied to the area marked by two of the rings and 20 µl of PEG-400 were applied to the other two areas. After 2 h, the excess substance on the treated area was removed, first by rinsing three times with ethanol/water (95:5) and then twice with water alone. Half of the test sites were shielded, and the skin on the back of each animal was then irradiated for 29 min using an Oriel 1000-W solar simulator with an output of 2.8 mW/cm^2 of UVA and UVB light; this corresponded to 3 MED. The animals were then killed.

Baseline values of each analog in PEG-400-treated skin were determined first; approximately 8.5 nmol/g skin α-tocopherol, approximately 3 nmol/g skin γ-tocopherol, approximately 7.5 nmol/g skin α-tocotrienols, and approximately 7 nmol/g skin γ-tocotrienols were present in the skin. Dermal application of the TRF resulted in a 28-fold increase in α-tocopherol, an 80-fold increase in α-tocotrienol, a 130-fold increase in γ-tocopherol, and a 51-fold increase in γ-tocotrienol in the skin. The percent distribution of each of the analogs in the TRF mixture was compared with its percent distribution in skin. The percent distribution of each analog that penetrated the skin was significantly different from its distribution in the mixture; higher percentages of α-tocopherol, similar amounts of γ-tocopherol, and lower percentages of α- and γ-tocotrienols were found in the skin, compared to the distribution in the TRF mixture.

In the skin from the UV-irradiated sites, approximately 40% of the vitamin E remained in the TRF-treated skin; there were no significant differences in the degree of destruction of the various analogs. Approximately 80% of vitamin E remained in the PEG-treated skin. The researchers examined the effect of UV-irradiation on the breakdown of the analogs in the TRF solution itself; 86% α-tocopherol, 83% γ-tocopherol, 83% α-tocotrienol, and 84% γ-tocotrienol remained after irradiation.

The distribution of tocopherol and tocotrienol in the skin was also examined.[48] Forty µl of a 5% (w/v) solution α-tocopherol, α-tocotrienol, or γ-tocotrienol in PEG-400 was applied to the backs of SKH-1 hairless mice for 0.5, 1, 2, or 4 h using a 2 cm^2 polypropylene plastic ring, and the animals were killed after the skin was rinsed. Four animals were used per group. The concentration of each test article was measured in the skin layers. Application of all three analogs significantly increased the concentrations of vitamin E in the skin; application of the 5% α-tocopherol solution resulted in a 200- to 2000-fold increase in skin α-tocopherol content. The concentration of each analog in the skin increased with time, but the relationship between the analogs and the content of each skin layer did not change. When expressed per µ, the highest concentrations of vitamin E was found in the uppermost layer of the stratum corneum (5 µm), and the concentration of α-tocopherol was statistically significantly greater than that of α- or γ-tocotrienol. When the thickness of each skin layer was considered, the lower skin layers contained appreciable amounts of vitamin E. The largest fraction of vitamin E in the skin following topical application was found in the deeper subcutaneous layers; the papillary dermis (31-130 µm) and the dermis (131-530 µm) contained the major portions of vitamin E if the total amount of each analog in each of the layers was summed and expressed as a

percentage per its respective total. At 0.5 h, significant increases in vitamin E were found in the subcutaneous fat, suggesting rapid penetration of vitamin E through the skin.

Antioxidant Properties

From the original report on Tocopherols

Tocopherol is the major lipid-soluble chain-breaking antioxidant of membranes and an important cellular protectant against oxidative damage. It exerts antioxidant effects by trapping peroxyl radicals. Some researchers found that tocopherol was depleted from the skin upon exposure to UV light; they postulated that other antioxidants that can recycle tocopherol can also be depleted.[1]

Tocotrienols are reported to exert antioxidant effects by scavenging chain-propagating peroxyl radicals.[9]

Miscellaneous Effects

From the original report on Tocopherols

α-Tocopherol is a good inhibitor of nitrosation because its phenol ring is fully substituted. α-Tocopherol might inhibit formation of skin nitrosating agents from NO_2, but it did not inhibit nitrosamine production from skin nitrosating agents. Tocopheryl acetate altered the cellular response of rats to nitrite; it prevented nitrite-related mortality and the decrease in glutathione S-transferase activity.[1]

TOXICOLOGICAL STUDIES

Single Dose (Acute) Toxicity

From the original report on Tocopherols

In rats, the dermal LD_{50} is >3 g/kg for tocopherol and >2 g/kg for tocophersolan. The oral LD_{50} of tocopherol, tocopheryl acetate, tocopheryl nicotinate, tocopheryl succinate, and tocophersolan are greater than 4, 16, 10, 7, and 7 g/kg, respectively, in rats. In mice, the oral LD_{50} of tocopherol is >25 ml/kg and of tocopheryl acetate is >4 g/kg.[1]

Dermal

Tocopheryl Acetate

According to robust summary data submitted to ECHA, the dermal LD_{50} of tocopheryl acetate is > 3 g/kg bw in albino rats.[44] Five animals per group were dosed with 1 or 3 g/kg bw undiluted tocopheryl acetate in vegetable oil under an occlusive patch for 24 h. Slight erythema was observed 24-48 h after exposure. Slight abrasion was observed in one low-dose female, two high-dose females, and two high-dose males.

Tocopheryl Phosphate

The acute dermal toxicity of mixed tocopheryl phosphates (MTP) was determined in New Zealand rabbits; the dermal LD_{50} was greater than 1130 mg/kg bw MTP in female rabbits.[49] MTP is a mixture of α-tocopheryl phosphate and α-di-tocopheryl phosphate, and is produced by phosphorylating d-α-tocopherol with P_4O_{10}; the final composition typically contained α-tocopheryl phosphate, α-di-tocopheryl phosphate, and α-tocopherol in the ratio of 1:0.49:0.08 (w/w/w). (Unless indicated otherwise, this is the composition of MTP tested in various studies that are summarized throughout this report.) An aq. gel containing 1130 mg/kg bw MTP (918 mg/kg bw α-tocopherol equivalents) was applied to the clipped dorsal skin of five male and five female rabbits for 24 h using surgical gauze. The test site was washed upon patch removal, and evaluated for irritation at 24 h, 7 days, and 14 days. At 24 h, slight to well-defined erythema was observed in 4/5 males and all females, and slight to moderate edema was observed in 2/5 males and all females. Signs of irritation were not observed at days 7 and 14. All animals survived until study termination; no gross findings were observed at necropsy.

Oral

Tocopheryl Phosphate

The oral LD_{50} of MTP is >1130 mg/kg bw in Wistar rats.[49] Groups of five male and five female were dosed by gavage with 1130 mg/kg bw MTP (918 mg/kg bw α-tocopherol equivalents) in distilled water, and killed after 14 days. All animal survived until study termination.

Repeated Dose Toxicity

From the original report on Tocopherols

In rats, tocopherol was not toxic in a 60-day study and tocopheryl acetate was not toxic in an 8-wk feeding study. In a 90-day study, 7 of 10 male rats dosed orally with 2000 mg/kg d-α-tocopherol died in 9 to 11 wks because of internal hemorrhage; other signs of toxicity were observed in a dose-dependent manner. Rats fed ≤2% tocophersolan for 3 mos did not have any treatment-related effects. In a 2-yr study in which rats were fed ≤2000 mg/kg/day dl-α-tocopheryl acetate and supplemented with vitamin K, no significant treatment-related effects were observed. High doses of tocopherol and tocopheryl acetate have hemorrhagic activity. [1]

Oral

Tocophersolan

Groups of five male Sprague-Dawley rats were dosed by gavage with an aqueous solution of 0.5, 1, or 2 g/k g tocophersolan once daily for 5 days, and the animals were then killed.[50] No signs of toxicity were observed, and all animals survived until

study termination. Heart, kidney, liver, and spleen weights were not affected by dosing with tocophersolan, and there were no dose-dependent or statistically significant changes in hematology, clinical chemistry, or urinalysis parameters.

Tocopheryl Phosphate

Groups of 10 male and 10 female Sprague-Dawley rats were fed a diet containing 0, 1, 3, or 5% MTP for 90 days; this MTP was composed of 72% α-tocopheryl phosphate and α-ditocopheryl phosphate, 13% α-tocopherol, 6% water, 7% phosphoric acid, and 2% other. [12] No clinical signs of toxicity, adverse effects on body weights, or test article-related mortality were reported. There were some statistically significant changes in hematology and clinical chemistry parameters, but generally these changes were dose-dependent and not considered toxicologically significant. No statistically significant, dose-dependent changes in organ weight were observed compared to controls. There were no gross changes observed at necropsy; changes in relative heart and epididymal organ weight were considered incidental. The only significant finding was the presence of foreign material in sinusoidal macrophages associated with mild inflammatory changes in the mesenteric lymph nodes in male and female treated animals that occurred in a dose-dependent manner; the foreign material was identified as tocopheryl phosphate. In the mid- and high-dose groups, but not the low-dose groups, the foreign material often had a crystalline appearance. Foreign material was also present in the small intestines of the mid- and high-dose animals. The NOAEL was 1% MTP in the diet.

Three repeated-dose oral toxicity studies of MTP were performed in rats, and no test article-related toxicity was observed.[49] The animals were dosed by gavage for 28 days in each of the studies. In the first study, 10-11 Sprague-Dawley rats/gender/group were dosed with 0, 368, 735, or 982 mg/kg bw/day MTP in a medium-chain triglyceride vehicle (corresponding to 0, 45.9, 229.5, and 458.8 mg/kg bw/day tocopherol equivalents, respectively). In the second study, groups of 4-5 Sprague-Dawley rats/gender/group were dosed with 0 or 869 mg/kg bw/day MTP (corresponding to 00 and 642 mg/kg bw/day tocopherol equivalents, respectively). And in the third study, groups of 5 Wistar rats/gender/group were dosed with 0, 56.5, 282.5 or 565 mg/kg bw/day MTP (corresponding to 0, 45.9, 229.5, and 458.8 mg/kg bw/day tocopherol equivalents, respectively). The animals were killed on day 29 in the first and third studies, and after a 14-day recovery period in the second study. No test-article related mortality, no signs of toxicity, and no microscopic or gross lesions were observed in any of the studies.

Tocotrienols

Groups of 10 male and 10 female F344/DuCrj rats were fed a diet containing 0, 0.19, 0.75 or 3% of a tocotrienols preparation for 13 wks; the preparation consisted of 21.4% α-tocotrienol, 3.5% β-tocotrienol, 36.5% γ-tocotrienol, 8.6% δ-tocotrienol, 20.5% α-tocopherol, 0.7% β-tocopherol, 1.0% γ-tocopherol, and 0.5% δ-tocopherol.[51] Another group was fed a diet containing 0.69% α-tocopherol. No remarkable changes in general appearance and no morality was reported. Body weight gain was decreased in the males fed the 3% tocotrienols diet as compared to the other groups, but feed consumption was similar to the other groups. Relative adrenal glands to body weights were dose-dependently increased in all treated males, and lung weights were statistically significantly decreased in all treated females. In the 3% tocotrienols group, statistically significant increases were observed in brain, heart, liver, kidneys, and testes weights of males and liver and spleen weights of females and statistically significant decreases in ovary and uterus weights were observed in females. Statistically significant changes were noted in various hematological parameters, and most of these changes were thought to have little toxicological significance; however, the statistically significant decrease in platelets in males, but not females, in a dose-dependent manner was thought to be a physiological response. Several hematology parameters were statistically significantly decreased in females of the 0.75 and 3% tocotrienols group. Effects on several serum biochemistry values also were considered of little toxicological significance. The NOAEL was 0.19% tocotrienols preparation in the diet; a no-observable effect level (NOEL) could not be determined.

Ocular Irritation

From the original report on Tocopherols

In ocular irritation studies, tocopherol was non-irritating in some tests and was a minimal or very slight ocular irritant in others. Tocopheryl acetate, tocopheryl nicotinate, and a mixture of dioleyl tocopheryl methylsilanol and oleic acid were not irritating to rabbit eyes. Tocophersolan was a slight ocular irritant. [1]

Tocopheryl Acetate

According to robust summary data submitted to ECHA, tocopheryl acetate was not irritating to rabbit eyes in one study, but it produced weak to moderate conjunctival irritation in another study.[44] Undiluted tocopheryl acetate was instilled into the conjunctival sac of three Vienna White rabbits, and the eyes were not rinsed. The eyes were scored at 1, 24, 48, and 72 h after instillation. Slight irritation was observed at 1-48 h, and the eyes were normal at 72 h.

In a modified Draize test, the same protocol was followed as above, and undiluted dl-α-tocopherol was instilled into the eyes of six rabbits; again, the eyes were not rinsed. Weak to moderate conjunctival irritation (i.e., redness) was observed, which subsided by day 7. No corneal changes were reported.

Tocopheryl Phosphate

MTP was not irritating to rabbit eyes.[49] One-tenth ml of an aq. gel containing 47.5 mg MTP (38.4 mg/kg bw α-tocopherol equivalents) was instilled into the conjunctival sac of one eye of one male and two female New Zealand rabbits; the contralateral eye served as a control. The eyes were scored for irritation 1, 24, 48, and 72 h after dosing.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

From the original report on Tocopherols

Oral administration of tocopherol ((up to 75 mg /day in the diet), tocopheryl succinate, and tocophersolan did not have reproductive or developmental effects in rats, and tocopheryl acetate (≤ 1.6 g/kg/day) generally did not have any reproductive or developmental effects in rabbits, hamsters, rats, or mice. Tocopherol and tocopheryl acetate had some effect on reducing the number of malformations observed in neonates from diabetic dams. Tocopherol did not have an effect on zinc deficiency-induced teratogenicity. In some studies, tocopheryl acetate potentiated the embryo-lethal effects of cortisone acetate. Tocopheryl succinate reduced some reproductive effects, but not all, induced by TCDD. [1]

GENOTOXICITY

From the original report on Tocopherols

Tocopherol, tocopheryl acetate, tocopheryl succinate, and a mixture of dioleyl tocopheryl methylsilanol and oleic acid were generally not mutagenic. The only effects observed were a dose-dependent increased elution rate of DNA in alkali in a DNA strand breakage assay and 50% inhibition in the incorporation of [^{3}H]-thymidine in a thymidine incorporation assay with tocopherol. Tocopherol has some anti-mutagenic activity and was able to modulate some mutagenic effects. Tocopheryl succinate also had some mutagenicity modulatory activity. Tocopherol and tocopheryl succinate generally did not affect UV-induced mutagenicity. [1]

In Vitro

Tocopherol

According to robust summary data submitted to ECHA, tocopherol was not genotoxic in a mammalian cell assay.[44] The genotoxic potential of d-γ-tocopherol (92.6% pure) was evaluated in a mammalian cell assay. Chinese hamster ovary (CHO) cells were exposed to 2.9 and 14.6 µg/ml (6.8 and 34 µM, respectively) d-γ-tocopherol for 5 h without metabolic activation.

Tocopheryl Acetate

According to robust summary data submitted to ECHA, tocopheryl acetate was not genotoxic in Ames tests or a chromosomal aberration assay.[44] Two Ames tests were performed; the first was performed with *Salmonella typhimurium* TA1535, TA97, TA98, TA100, and TA102, with and without metabolic activation, at test concentrations of 0, 50, 158, 500, 1580, and 5000 µg/plate *all-rac*- α-tocopheryl acetate in ethanol. The second was performed using *S. typhimurium* TA1535, TA1537, TA98, and TA100 at concentrations of 0, 20, 100, 500, 2500, and 5000 µg/plate tocopheryl acetate in DMSO, with and without metabolic activation. Vehicle and appropriate positive controls were used in each study. Tocopheryl acetate was not mutagenic.

In the chromosomal aberration assay, human peripheral lymphocytes were exposed to dl-α-tocopheryl acetate/*all-rac*-α-tocopheryl acetate in 0.5% ethanol, with and without metabolic activation. Without metabolic activation, cells were exposed to 200, 600, and 1800 µg/ml for 24 h or 75, 300, or 1200 µg/ml for 3 h. With metabolic activation, cells were exposed to 200, 600, and 1800 µg/ml for 5 h or 75, 300, or 1200 µg/ml for 3 h. A negative (vehicle) control and appropriate positive controls were used. Tocopheryl acetate was not genotoxic.

Tocopheryl Phosphate

MTP was not mutagenic in the Ames test nor genotoxic in a chromosomal aberration assay.[49] Two Ames test were conducted with *S. typhimurium* TA98, TA100, TA1535, ad TA1537 and *Escherichia coli* WP2 uvrA. The test concentrations were 0.9-2825 µg/plate aq. MTP (0.73-2294 µg tocopherol equivalents/plate) in the first study, and 88.3-2825 µg/plate MTP (71.7-2294 µg tocopherol equivalents/plate) in the second study. The tests were conducted with and without metabolic activation, and positive and negative controls gave valid results.

Two assays for chromosomal aberrations were conducted using CHO cells; the test article was suspended in 1% carboxymethyl cellulose. Concentrations of 13.4-40.8 and 15.5-37.9 µg/plate MTP (10.9-33.1 and 12.6-30.8 µg tocopherol equivalents/plate, respectively) were tested with metabolic activation, and concentrations of 26.1-40.8 and 3.7-67.8µg/plate and MTP (21.2-33.8 and 3.0-55.1 µg tocopherol equivalents/plate, respectively) were tested without metabolic activation in the two studies. The only difference in protocol between the two studies was that, in the second study without metabolic activation, the cells were exposed continuously for 20 h, as opposed to a 3-h exposure and 17-h recovery period.

Tocopheryl Succinate

Tocopheryl succinate was weakly positive in a sister chromatid exchange assay in the presence of metabolic activation, and was negative for genotoxicity in a chromosome aberration assay.[52] Two trials were performed. In the first trial, concentrations of 5, 7, 10, and 50 µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and doses of 30, 100, 300, and 1000 µg/ml were tested with metabolic activation. In the second trial, concentrations of 15.1, 19.95, 25.2, and

30.2µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and concentrations of 202, 298.2, 396, and 497µg/ml were tested with metabolic activation. Vehicle (DMSO) and appropriate positive controls were used.

In the chromosomal aberration assay, D-α-tocopheryl succinate concentrations of 39.8, 49.8, 60, and 75 µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and doses of 400, 450, and 500 µg/ml were tested with metabolic activation.[53] In the second trial, concentrations of 24.9, 30.1, and 35 µg/ml D-α-tocopheryl succinate were tested without metabolic activation, and concentrations 4100, 5000,and 6000 µg/ml were tested with metabolic activation. Vehicle (DMSO) and appropriate positive controls were used.

CARCINOGENICITY

From the original report on Tocopherols

Tocopheryl acetate (≤ 2 g/kg/day) was not carcinogenic in a dietary study. Neoplasms developed in animals injected subcutaneously with tocopherol or tocopheryl acetate and soya oil; however, neoplasms were not seen in animals dosed with tocopherol or tocopheryl acetate only. Mixed results were found when studying the effects of tocopherol, tocopheryl acetate, and tocopheryl succinate on the modulation of carcinogenic effects of other agents. In most cases, there was an inhibition of the effect of the other agent; in some cases, no effect was seen. However, in one study, tocopherol acted as a complete tumor promoter, with an efficiency approaching a standard tumor promoter, the same promoter whose activity was inhibited by tocopherol in other studies. In a modulation study, tocopherol reduced spontaneous pulmonary tumorigenesis in A/J mice. [1]

In a dermal study in mice, tocopherol reduced photocarcinogenesis. However, dermally applied tocopheryl acetate and tocopheryl succinate were reported to enhance photocarcinogenesis. After oral administration, tocopherol appeared to reduce UV-induced lesions. Orally administered tocopheryl acetate reduced the incidence of skin cancer, but toxicity was observed.[1]

Oral

Tocopherol

Female Sprague-Dawley rats were given a single intraperitoneal (i.p.) injection of 50 mg/kg bw *N*-methyl-*N*-nitrosourea, and one week later, the animals were fed AIN-93M diet alone or supplemented with 0.1, 0.3,or 0.5% mixed tocopherols (57% γ-, 24% δ-, 13% α-, and approximately 0.5% β-tocopherol) for 9 wks, and the animals were then killed.[54] After 9 wks of supplementation with tocopherols, serum γ- and δ-tocopherol levels were statistically significantly increased. A dose-dependent inhibition in tumor growth was observed, and a statistically significant decrease in tumor burden and tumor multiplicity was reported at necropsy. Tumor burden was inhibited by 80% in the highest dose group. The researchers reported that γ- and δ-tocopherol, but not α-tocopherol, activated PPAR-γ.

A mixed tocopherol diet statistically significantly reduced azoxymethane (AOM)-induced colonic aberrant crypt foci in male F344 rats.[55] Male F344 rats were fed a modified AIN-76A diet at 5 wks of age, and then, at age 7 wks, were given subcutaneous (s.c.) injections of 15 mg/kg bw AOM once weekly for 2 wks. One day after the second injection of AOM, groups of animals were maintained on the AIN-76A diet alone or supplemented with 1000 ppm mixed tocopherols; the mixed tocopherols consisted of 58% γ-tocopherol, 21% δ-tocopherol, and 12% α-tocopherol. All animals were killed 8 wks after the second AOM injection. AOM-induced colonic aberrant crypt foci was reduced approximately 55% in the mixed tocopherol-fed rats, as compared to the controls.

Tocopheryl Succinate

The effect of tocopheryl succinate on benzo(*a*)pyrene (BaP)-induced forestomach tumors was studied in Kunming mice.[56] Thirty female mice were used per group. Negative and vehicle (corn oil) control groups were dosed with 1 g/kg bw succinic acid by gavage four times per wk for 4 wks. The vehicle controls and all other mice were dosed with 1 mg/animal B(a)P, two times per wk for 4 wks. The test groups were dosed with 1.25 or 2.5 g/kg bw tocopheryl succinate by gavage four times per wk, or with 20 mg/kg bw tocopheryl succinate via i.p. injection two times for wk, for 4 wks. The positive control group was dosed with B(a)P only. The animals were killed 11, 16, or 29 wks after the first dose of B(a)P.

The incidence of forestomach tumors was 100% in the positive controls, and there was an average of 5.4 tumors/mouse at wk 29. Similar results were observed in the vehicle-control group. With tocopheryl succinate, tumors were seen in only 81.8% and 76.9% of the mice dosed orally with 1.25 and 2.5 g/kg bw tocopheryl succinate, respectively, and only 50% of the mice dosed i.p. with tocopheryl succinate. The number of tumors per mouse was inhibited by 68.5% with 1.25 g/kg bw oral, 70.4% with 2.5 g/kg bw oral, and 79.6% with 20 mg/kg bw i.p. tocopheryl succinate. Tocopheryl succinate significantly affected total tumor volume per mouse. Total tumor volume per mouse was decreased by 63.5% and 81.1% with 1.25 and 2.5 g/kg bw tocopheryl succinate, respectively. With i.p. administration, total tumor volume was decreased by 84.1%.

Tocotrienols

Groups of 10 female FVB/N HER-2/neu transgenic female mice were dosed by gavage, three times per week, with 0, 50, or 100 mg/kg tocotrienols (10% γ- and 90% δ-) in olive oil to determine the effect of tocotrienols on the development of mammary tumors.[57] The appearance of tumors was significantly delayed in high-dose animals, and a statistically significant decrease in wk-26 tumor volume was also observed in this group. There were no statistically significant differences in the

incidence of metastasis or the kinetics of tumor incidence on tumor volume and multiplicity between the treated and control groups.

Photocarcinogenicity

Tocopherol

Female Skh-1 mice were exposed dorsally to 2250 J/m^2 UVB (equivalent to 1 MED) three times per week for 10 wks, and the animals were then dosed topically with vehicle (Surgilube®; n=20) or 5 mg *d*-α-tocopherol in vehicle for 15 wks with no additional UVB.[58] Topical treatment with tocopherol resulted in a trend toward increased tumor multiplicity, with 14.9% more tumors reported with tocopherol than with vehicle alone. A non-statistically significant increase in tumor burden (20.7%) was observed following treatment with tocopherol when compared to vehicle only. Tumor growth rate was also increased. However, fewer tumors were malignant in animals dosed with tocopherol than in the controls.

Tumor Promotion

Dermal

Tocopherol

The ability of dermally-applied *dl*-α-tocopherol to induce tumor promotion was studied in groups of 24-27 female SENCAR mice.[59] Four promotion protocols were used; the effects of tocopherol with or without vitamin C were determined with each protocol. In each case, the test compounds were applied to dorsal skin that was clipped free of hair. The vehicles were acetone for tocopherol and 75% acetone/25% water for vitamin C. Each protocol and the corresponding groups are described:

- Protocol A: a single topical application of 10 nmol of the tumor initiator 7,12-dimethylbenz(a)anthracene (DMBA) was applied; 1 wk later, the proposed tumor promoter was applied topically 2x/wk. Test groups were exposed to 80 µmol tocopherol, with and without 80 µmol vitamin C.
- Protocol B: a single topical application of 10 nmol DMBA; 1 wk later, the proposed promoter was applied topically 2x/wk. Test group were exposed to 8 or 80 µmol tocopherol, with and without 80 µmol vitamin C.
- Protocol C: tocopherol was applied topically 2x/wk for 4 wks; a single topical application of 10 nmol DMBA followed; 1 wk later, 4 µg of the weak tumor promoter mezerein was applied topically 2x/wk. The test group was exposed to 80 µmol tocopherol.
- Protocol D: the proposed promoter or the promoter and β-radiation were applied topically 2x/wk for 4 wks; a single topical application of 10 nmol DMBA followed; 1 wk later, 4 µg mezerein was applied topically 2x/wk. Test groups were exposed to 8 µmol tocopherol without vitamin C and to 80 µmol tocopherol with and without 80 µmol vitamin C.

Protocols A and C determined the number of tumors/animal (tumor multiplicity) 98 days after initiation with DMBA; protocols B and D determined tumor multiplicity 153 days after initiation with DMBA. With protocol A and B, tocopherol caused a statistically significant increase in tumors. Using protocol B, the low concentration of tocopherol did not produce tumors, with or without vitamin C.

Following protocols C and D, application of tocopherol prior to initiation with DMBA and then promotion with mezerein statistically significantly increased the tumor multiplicity. The increase in tumor multiplicity compared to controls was greater when measured after 98 days than after 153 days. β-Radiation amplified the effect of high-dose tocopherol; however, using protocol D, vitamin C did not. Tumor multiplicity in groups exposed to β-radiation → DMBA → mezerein, 8 µmol tocopherol → DMBA → mezerein, or tocopherol, 8 µmol + β-radiation → DMBA → mezerein was not statistically significantly different from the group exposed to DMBA → mezerein. Also, tumor multiplicity in the group exposed to tocopherol, 80 µmol + Vitamin C, 80 µmol → DMBA → mezerein was not significantly different from the group exposed to 80 µmol tocopherol → DMBA → mezerein. Pretreatment with tocopherol prior to DMBA reduced tumor latency.

Anti-Proliferative Effects/Pro-Apoptotic Effects

The effect of α- and γ-tocopherol and α- and γ-tocotrienols on proliferation and apoptosis was examined in rat normal hepatocyte (RLN-10) and hepatoma (dRLh-84) cells.[60] γ-Tocotrienols had the greatest effect in rat hepatoma cells; 50-100 µM γ-tocotrienols strongly decreased cell number in a dose-dependent manner after 24 h; γ-tocotrienols strongly suppressed proliferation of dRLh-84 cells, and 25 mM γ-tocotrienols induced DNA fragmentation in dRLh-84 cells. α-Tocotrienols also exerted effects, but to a lesser extent. α- and γ-Tocopherol did not affect cell number, proliferation, or DNA fragmentation. The researchers stated that the results suggested that caspase-8 activity was involved in the induction of apoptosis by tocotrienols.

DERMAL EFFECTS

Dermal Irritation and Sensitization

Non-Human

From the original report on Tocopherols

Tocopherol, 1%, was a weak primary skin irritant in rabbits in one study, and it was a weak cumulative irritant in guinea pigs in another study. Cosmetic formulations containing 2% dl-tocopherol, 12% vitamin E in wheat germ, and 32% mixed tocopherols in a wheat germ and vegetable oil base had mean cumulative irritation scores of 31, 7, and 12 (maximum possible score of 64), respectively, in rabbits. Tocopheryl acetate and tocopheryl nicotinate were generally not irritating to rabbit skin. A single dose of a mixture of dioleyl tocopheryl methylsilanol and oleic acid was not irritating to rabbits, but slight erythema was observed following multiple applications. The same was observed with 75% tocophersolan in guinea pigs.[1]

A mixture containing <0.1% tocopherol was not a sensitizer in an open epicutaneous test, whereas "higher concentrations" of tocopheryl acetate can cause sensitization in this test. However, tocopheryl acetate was not sensitizing in a guinea pig maximization test. Tocophersolan was not a sensitizer in a Buehler test.[1]

Tocopheryl Acetate

According to robust summary data submitted to ECHA, tocopheryl acetate is not irritating to rabbit skin.[44] In a study in which a 2.5 cm^2 semi-occlusive patch containing 0.5 ml undiluted tocopheryl acetate was applied to a shaved area on the back or the flank of two male and one female Vienna White rabbits, no erythema or edema was observed. The test sites were scored 30-60 min after patch removal and at 24, 48, and 72 h after application. In a similar study using six New Zealand white rabbits, application of an occlusive patch containing 0.5 ml tocopheryl acetate to intact and abraded skin did not result in erythema or edema, and the primary irritation index was 0.

Tocopheryl Phosphate

MTP was not a dermal irritant in New Zealand rabbits.[49] A dose of 0.5 g/site of an aq. gel containing 88-101 mg/kg bw MTP (38.4 71-82 mg/kg bw α-tocopherol equivalents) was applied to a 10 cm^2 area of clipped dorsal skin of one male and two female rabbits. The semi-occlusive patch was removed after 4 h, and the test site was scored for irritation at 1, 24, 48, and 72 h after patch removal. The only observation was a barely perceptible erythema observed in the male at 60 min.

A local lymph node assay was performed to evaluate the sensitization potential of MTP, and no evidence of sensitization was observed.[49] Groups of five female CBA/J mice were dosed with 25 µl of 5, 10, or 25% MTP in reverse osmosis water (corresponding to 1.13, 2.26, or 5.65 mg MTP, respectively); the test article was applied to the dorsal aspect of the ear daily for 3 consecutive days. On day 6, the animals were given a single intravenous injection of [H^3]thymidine, and then killed 5 h after the injection. Several negative controls and a positive control (25% hexylcinnamaldehyde in acetone/olive oil) were used.

Human

From the original report on Tocopherols

Tocopherol and tocopheryl acetate were not irritants or sensitizers in clinical studies. Patients patch-tested by the North American Contact Dermatitis Group rarely reacted to tocopherol. A cosmetic line containing tocopheryl acetate introduced in Switzerland in 1992 resulted in a large number of outbreaks; positive patch tests with tocopheryl linoleate were seen. However, the outbreaks were thought to be due to a metabolite or contamination of the product. Tocopheryl nicotinate was not an irritant or a sensitizer. Case reports exist for tocopherol- and tocopheryl acetate-containing products.

Tocopheryl Acetate

A cuticle softener containing 36% tocopheryl acetate was essentially non-irritating in clinical testing.[61] A 24-h single-insult occlusive patch test was conducted in 19 subjects. One subject had a + reaction, and the primary irritation index was 0.03.

According to robust summary data submitted to ECHA, dl-α-tocopheryl acetate is not a sensitizer in humans.[44] During induction, 203 subjects were exposed to undiluted tocopheryl acetate; 10 applications were made over a 2-wk period. The challenge was performed after a 2-wk non-treatment period, and the test substance was applied once daily for 3 days. The mean irritation index after induction was 0.076/subject; none of the subjects showed a higher irritation grade than 1. No positive reactions were reported after challenge.

Contact Allergy – Case Reports

Two cases were reported of reactions following use of products containing tocopheryl acetate.[62] In the first case report, eczematous dermatitis occurred on the eyelids and neck of a subject with xerotic skin after 4 wks of use; the oil was reported as pure vitamin E oil. Patch testing with an Italian baseline series and the oil resulted in a positive reaction to the oil only; a ++ reaction was observed on day 2 and +++ on day 3. A strong positive reaction was observed on day 3 in a repeated open application test (ROAT) on the volar forearm using the subject's own product. Negative results were obtained in follow-up testing 1 month later; various concentrations and brands of tocopheryl acetate were used. Additionally, negative results were obtained in a ROAT using a new sample of neat vitamin E oil.

Similar results were reported in the second case study. Eyelid and periocular dermatitis resulted from the use of the vitamin E acetate lipogel used by the patient; patch testing with the Italian baseline series and the subject's own product resulted in

positive results with the product only (+ on day 2 and ++ on day 3). ROATs performed on the volar forearm with the subject's product gave a positive result on day 4. However, additional patch tests performed with the active ingredient and excipients in the lipogel and various concentrations and brands of tocopheryl acetate produced negative results. Negative results were also observed 1 month later in patch tests and ROATs performed using the vitamin E acetate lipogel.

Phototoxicity and Photoallergenicity

From the original report on Tocopherols

Tocopheryl acetate, 0.2 ml applied under an occlusive patch for 24 h prior to irradiation, was not phototoxic in a study in 11 subjects.[1]

Non-Human

Tocopheryl Acetate

According to robust summary data submitted to ECHA, dl-α-tocopheryl acetate was not photoallergenic in Himalayan guinea pigs.[44] Undiluted tocopheryl acetate was used at induction, and concentrations of 5, 50, 75, and 100% were used at challenge. For induction, undiluted test material was applied epicutaneously to an 8 cm^2 area on 20 guinea pigs. (Intradermal injections of Freund's Complete Adjuvant (FCA) were used during induction.) The test sites were then exposed to 1.8 J/cm^2 UVB and 10 J/cm^2 UVA. This procedure was repeated four times within 2 wks of the induction phase. A group of 10 control animals were treated with FCA only. The challenge was conducted 3 wks after the start of induction; the animals were exposed epicutaneously on both flanks with the test article at concentrations of 100%, 75%, 50% and 25% in ethanol. The treated sites were then either exposed to 10 J/cm UVA or were not exposed to irradiation. The test sites were scored 24, 48 and 72 hrs after the challenge exposure.

A slight erythematous skin reaction was observed in two of the 20 test animals following challenge; no consistent or significant differences were detected between the irradiated and non-irradiated test sites, and the reactions were not clearly dependent on the test article concentration. It was stated that the reactions most likely resulted from cutaneous hyperirritability. No reactions were observed in the control group.

Photoprotective Effects

From the original report on Tocopherols

Tocopherol was generally found to inhibit UVB-induced lipid peroxidation. Dermal application of tocopheryl acetate also decreased lipid peroxidation, but oral administration was reported not have an effect. Both single and multiple applications of tocopherol inhibited 8-MOP photobinding to DNA/RNA and protein. A single application of tocopheryl acetate did not affect photobinding, but multiple applications protected..[1]

Application of tocopherol both prior to and after irradiation increased the MED. Tocopheryl acetate application after irradiation resulted in decreased skin thickness. In a study in which subjects were given a supplement containing tocopherol and ascorbic acid, a significant increase in MED was observed as compared to controls.[1]

Non-human and human studies have been conducted to examine whether tocopherols have a photoprotective effect. Generally, the non-human studies have assessed the protection against oxidative damage in the skin. The human studies examined phototoxic effects and changes in MED. These studies are summarized in Table 7.

Effect on Irritated Skin

Non-Human

Tocopheryl Acetate

The effect of tocopheryl acetate ointment containing 15% squalane on allergic contact dermatitis was investigated in male Wistar rats.[47] Allergic contact dermatitis was induced with 2,4-dinitrochlorobenzene (DNCB)-acetone, and 0.1 g tocopheryl acetate ointment was applied to the test site after the initiation of inflammation. The ointment was applied using a 2 cm diameter film, and the film was covered with gauze and a bandage. The tocopheryl acetate ointment inhibited allergic contact dermatitis in a dose-dependent manner, with significant inhibition of erythema observed with 20-40% tocopheryl acetate. An ointment with 2-10% tocopheryl acetate had an inhibitory effect on erythema. The inhibition was confirmed microscopically in keratinocytes from skin samples taken from the treated area.

The researchers then examined the effect of the tocopheryl acetate ointment on DNCB-acetone-induced irritant contact dermatitis on the backs of male Wistar rats; the ointment was applied in the same manner as described previously. The ointment with 20% tocopheryl acetate significantly reduced erythema,

The researchers also examined the effect of the ointment on irritant contact dermatitis in mice. Irritant contact dermatitis was induced in male ddY mice by applying phorbol 12-myristate 13-acetate (PMA)-acetone to the ears of each animal; 20 mg of the tocopheryl acetate ointment was then applied to both sides of the ear. The 20% tocopheryl acetate ointment significantly reduced ear swelling.

Tocotrienols

Tocotrienols reduced allergic dermatitis in mice.[63] Allergic dermatitis was induced in male NC/Nga mice using picryl chloride, with and without administration of 1 mg/day/animal tocotrienols. Scratching behavior, dermal thickening, and serum histamine levels were statistically significantly reduced by tocotrienols administration. Subsequent studies concluded that tocotrienols significantly suppressed degranulation of mast cells and significantly reduced histamine release, and it also suppressed protein kinase C activity.

Effect on Barrier Function of Damaged Skin

Non-Human

Tocopheryl Acetate

Skin barrier function in male Wistar rats was damaged using a detergent and DNCB, and the effect of tocopheryl acetate on the damaged skin was evaluated.[47] The damaged test site was covered for 18 h with 0.1 g tocopheryl acetate ointment; ointments containing 2-40% tocopheryl acetate were used. Ointment containing 2% tocopheryl acetate had little effect on damaged skin. However, concentrations of 5-40% tocopheryl acetate statistically significantly reduced the increase in transepidermal water loss; the maximum effect was observed with 20% tocopheryl acetate. The 20% ointment also statistically significantly decreased the erythema intensity.

EPIDEMIOLOGY

Health claims exist in the literature regarding vitamin E supplementation; however, the benefits of vitamin E supplements are still being debated. The original CIR safety assessment on tocopherol referred to numerous studies in which tocopherol appeared to have protective effect in carcinogenicity modulation studies.[1] However, as an example of possible negative effect of vitamin E, dietary supplementation with α-tocopherol was reported to significantly increase the risk of prostate cancer among healthy men in the Selenium and Vitamin E Cancer Prevention Trial.[64] While the CIR recognizes that many articles on the effects of vitamin E supplementation can be found in the published literature, these articles are not included in this safety assessment because they are not relevant to the cosmetic use of the tocopherols.

SUMMARY

This re-review addresses the safety of tocopherols and tocotrienols as used in cosmetics. In 2002, the Panel published a review on tocopherol, concluding these ingredients are safe as used in cosmetics. Tocopherol is the component most commonly associated with vitamin E. However, tocotrienols is also a component of vitamin E, so it is appropriate to develop a report that includes all of these ingredients. This summary includes only information that has become available since the CIR safety assessment was issued on tocopherols, and all information on the tocotrienols and the tocopherols that have been added to this report.

Most of the tocopherols are reported to function in cosmetics as antioxidants or skin conditioning agents; tocotrienols is not reported to function as an antioxidant, instead it is listed as functioning as a light stabilizer, oral care agent, or skin conditioning agent. VCRP data obtained from the FDA in 2013 report that the frequency of use increased considerably for both tocopherol and tocopheryl acetate. The reported use of tocopherol increased from 1072 (1998 data) to 6175 uses (2013 data), and the reported use of tocopheryl acetate increased from 1322 (1998 data) to 8960 uses (2013 data). The use concentration of tocopherol, but not of tocopheryl acetate, has increased since the original assessment. According to the survey conducted by the Council in 2013, the concentration of use of tocopherol in leave-on products increased from 2% in 1999 to 5.4% in 2013. Tocotrienols is used in 433 formulations, with a reported maximum leave-on concentration reported of 0.12%.

Tocopherols and tocotrienols are distributed throughout the body, and the distribution and metabolism varies among the tissues. Tocopherol is the predominant form of vitamin E in human and animal tissues, and it has the highest bioavailability; natural vitamin E has approximately twice the systemic availability of synthetic tocopherol. The distribution and intracellular trafficking of vitamin E may be modulated by tocopherol regulatory proteins, but only one of the proteins, tocopherol transfer protein, has been shown to influence plasma and tissue α-tocopherol concentrations.

The structural differences between tocopherol and tocotrienols result in a difference in the penetration of these compounds into tissues. The presence of three unsaturated bonds in the carbon side chain allows tocotrienols to penetrate tissues with saturated fatty layers, such as the brain and the liver, more readily than tocopherol, which has a saturated carbon side chain. However, the tocotrienols are not as prevalent in the body as the tocopherols, and oral absorption of the tocotrienols has been reported to be incomplete. Orally administered tocopherols and tocotrienols are distributed in the skin and adipose tissue. Dermally applied tocopheryl acetate is hydrolyzed to tocopherol upon exposure to UV. Dermally-applied tocopherols do penetrate the skin.

Toxicity of dermally-applied (single-dose) tocopheryl acetate and tocopheryl phosphate and orally-administered tocophersolan (repeated dose) or tocopheryl phosphate (single and repeated-dose) is not remarkable. In rats fed a diet containing ≤3% tocotrienols for 13 wks, a statistically significant decrease in platelets in males, but not females, in a dose-dependent manner was thought to be a physiological response.

Undiluted tocopheryl acetate was not irritating to rabbit eyes in one study, but it produced weak to moderate conjunctival irritation in another study. Undiluted mixed tocopheryl phosphates (MTP) was not irritating to rabbit eyes.

Numerous genotoxicity studies were conducted with tocopherol, tocopheryl acetate, MTP, and tocopheryl succinate. The only remarkable result was a weak positive result for tocopheryl succinate in a sister chromatid exchange assay in the presence of metabolic activation.

Topical treatment of Skh-1 mice with 5 mg d-α-tocopherol for 15 wks, following 10 wks of UVB irradiation, resulted in a trend toward increased tumor multiplicity in females compared to those mice exposed to vehicle only. A non-statistically significant increase in tumor burden was observed following treatment with tocopherol compared to controls. However, fewer tumors were malignant in animals dosed with tocopherol than in the controls. Mixed results were observed in a tumor promotion study; higher doses of tocopherol increased tumor multiplicity, and greater increases were seen after 98 days than after 153 days. In oral studies, tocopheryl acetate and tocopheryl succinate tended to decrease tumors in rodents. Tocotrienols delayed the onset of the appearance of tumors.

Undiluted tocopheryl acetate and an aq. gel containing 88-101 mg/kg bw MTP were not irritating to rabbit skin. No evidence of sensitization was observed in a local lymph node assay with MTP. Tocopheryl acetate did not have photoallergenic effects in guinea pigs. A cuticle softener containing 36% tocopheryl acetate was essentially non-irritating in a single-insult occlusive patch test in 19 subjects. Tocopheryl acetate is not a sensitizer in humans.

Tocopherol, tocopheryl acetate, and tocopheryl phosphate had some photoprotective effects in mice. Tocopherol and tocopheryl acetate were shown to have a photoprotective effect in humans. Tocopheryl acetate inhibited contact dermatitis in rats and reduced erythema, and tocotrienols reduced allergic dermatitis in mice.

The CIR recognizes that many articles on the effects of vitamin E supplementation can be found in the published literature; however, these articles are not included in this safety assessment because they are not relevant to the cosmetic use of the tocopherols.

DISCUSSION

The Expert Panel determined that the 2002 safety assessment on tocopherols should be expanded to include tocotrienols (a 2013 priority), as well as a few additional tocopherols. Although data were not available on all of the ingredients, the Panel found the existing data on the tocopherols are sufficient to support the safety of this entire family of ingredients.

The Panel noted that the current reported maximum use concentration of tocopherol (i.e., 5.4%) is higher than what was reported in the original assessment (i.e., 2%), and that irritation and sensitization data at these higher concentrations are not available. The Panel discussed the issues of irritation and sensitization during both the original and current review of tocopherols, and in both instances, determined that irritation and sensitization of the ingredients included in this review were not of concern. In the 2002 safety assessment, the Panel was initially concerned with possible irritation and sensitization because of a large number of outbreaks reported in Switzerland with the release of a new line of cosmetics that contained tocopheryl linoleate; however, the researchers thought the outbreaks were due to either a contaminant or a metabolite. Other safety data in the 2002 report indicated that tocopherol was not an irritant or a sensitizer. Irritation and sensitization data that became available since the 2002 review report was issued report that tocopheryl acetate is not an irritant or a sensitizer, and tocopheryl phosphate is not a sensitizer. Additionally, the Panel commented that dermal reactions to tocopherol are rare, and that the North American Contact Dermatitis Group deleted this ingredient from its standard testing because of the extremely low incidence of reactions.

During the original review of tocopherols, the Panel did carefully consider that the tumor promoting ability of tocopherol, tocopheryl acetate, and tocopheryl succinate had been extensively studied. In most studies, tocopherol is reported to inhibit tumor promotion, and studies published since the original tocopherol report seem to support this conclusion. The general experience of the Panel is that tocopherol is not a tumor promoter. Additionally, photocarcinogenicity testing with tocopherol did not raise any concerns for the Panel.

The Panel also noted that they are aware of epidemiology studies that have been conducted with vitamin E, and results of these studies are inconclusive; positive and negative effects have been observed with the use of vitamin E supplements. After acknowledging the availability of vitamin E supplement studies with mixed results, the Panel stated that the systemic exposure of vitamin E supplementation is much higher than that expected during cosmetic use; therefore, any negative conclusions made during these studies did not cause concern for the cosmetic use of these ingredients.

Natural tocopherols and tocotrienols are plant-derived ingredients. However, these ingredients are specific, purified, highly-enriched lipid compounds, and often the tocopherols are produced synthetically. For these reasons, the usual concerns expressed during the discussion of botanical ingredients did not apply to the ingredients in this report.

Finally, the Panel discussed the issue of incidental inhalation exposure to tocopherols. The Panel stated that although there were no inhalation data available, the tocopherols are used at relatively low concentrations in products that could incidentally be inhaled, e.g., tocopheryl acetate is used at up to 5% in foot powders and sprays and up to 0.2% in aerosol hair spray for-

mulations, and tocopherol is used at up to 1% in pump hair spray formulations. The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at http://www.cir-safety.org/cir-findings.

CONCLUSION

The CIR Expert Panel concluded that the following 14 ingredients are safe in the present practices of use and concentration in cosmetics described in this safety assessment:

ascorbyl tocopheryl acetate*
ascorbyl tocopheryl maleate
dioleyl tocopheryl methylsilanol
potassium ascorbyl tocopheryl phosphate
sodium tocopheryl phosphate
tocopherol
tocophersolan
tocopheryl acetate
tocopheryl linoleate
tocopheryl linoleate/oleate
tocopheryl nicotinate
tocopheryl phosphate*
tocopheryl succinate
tocotrienols

*Not reported to be in current use. If these ingredients were to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.

TABLES

Table1. Definitions, Structures, and Functions [13]

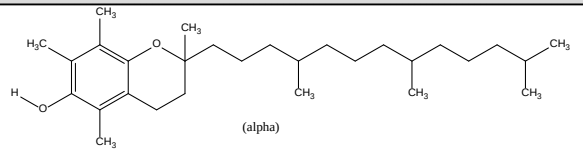
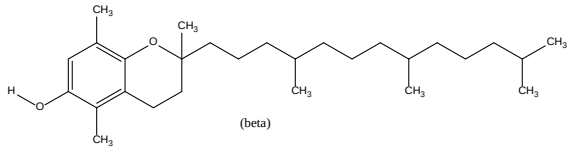
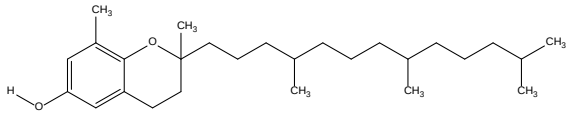
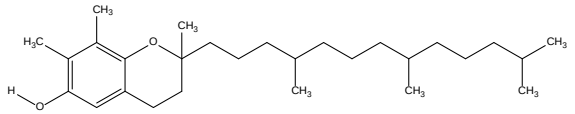
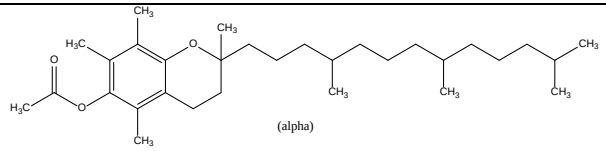
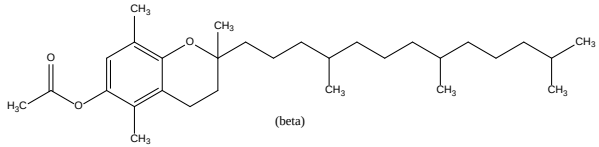
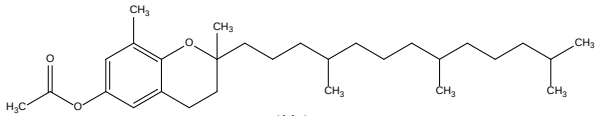
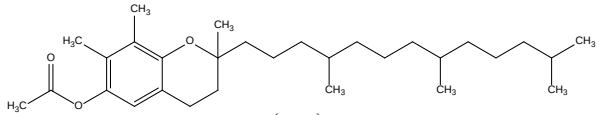
Ingredient/CAS No.	Definition	Structure**	Function(s)
Ingredients in Original Safety Assessment			
Tocopherol 54-28-4 (gamma): 16698-35-4 (beta); 10191-41-0 (DL-); 2074-53-5 (DL-); 59-02-9 (D-); 119-13-1; 1406-18-4; 1406-66-2; 7616-22-0	consists of alpha-tocopherol, beta-tocopherol, delta-tocopherol and/or gamma-tocopherol and conforms to the formulae:	 (alpha)  (beta)  (delta)  (gamma)	antioxidant; fragrance ingredient; skin-conditioning agent - miscellaneous; skin-conditioning agent - occlusive
Tocopheryl Acetate 52225-20-4; 58-95-7; 7695-91-2	the ester of tocopherol and acetic acid	 (alpha)  (beta)  (delta)  (gamma)	antioxidant; skin-conditioning agent – miscellaneous

Table1. Definitions, Structures, and Functions [13]

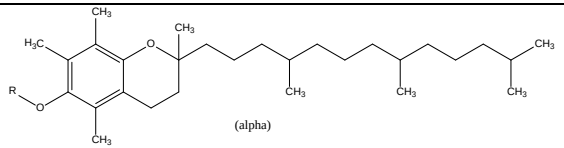
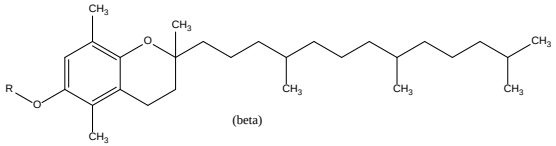
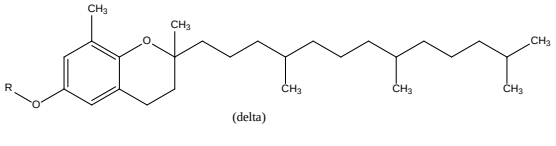
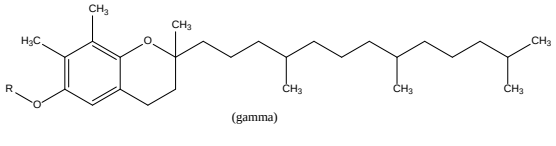
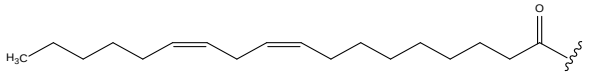
Ingredient/CAS No.	Definition	Structure**	Function(s)
Tocopheryl Linoleate 36148-84-2	the ester of tocopherol and linoleic acid	 (alpha)  (beta)  (delta)  (gamma) wherein R is 	antioxidant; skin-conditioning agent – miscellaneous

Table1. Definitions, Structures, and Functions [13]

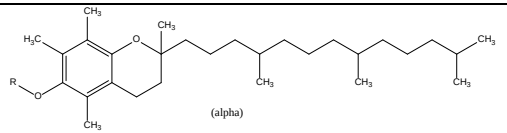
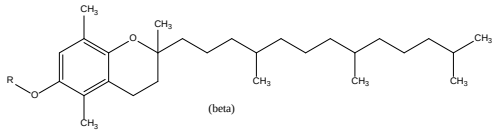
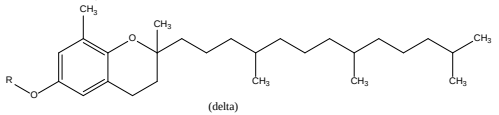
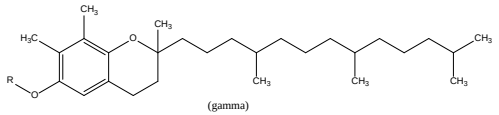
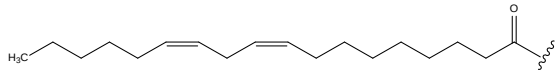
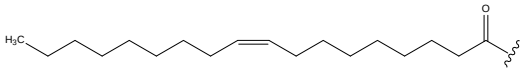
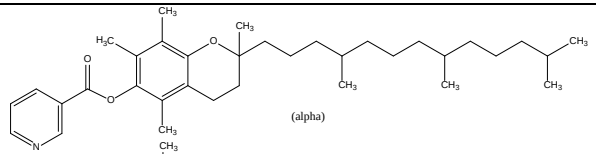
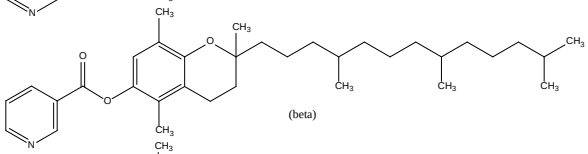
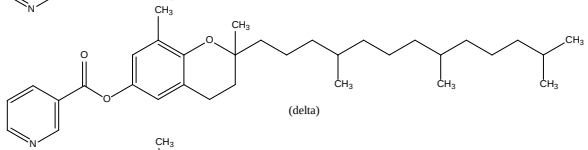
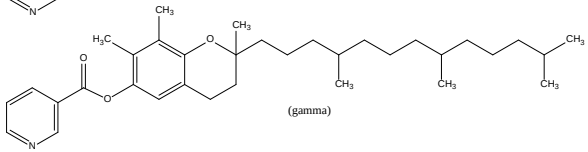
Ingredient/CAS No.	Definition	Structure**	Function(s)
Tocopheryl Linoleate/Oleate	the ester of tocopherol and a mixture of linoleic acid and oleic acid	 (alpha)  (beta)  (delta)  (gamma) wherein R is  or 	antioxidant; skin-conditioning agent – miscellaneous
Tocopheryl Nicotinate 16676-75-8; 43119-47-7; 51989-34-1; 86362-36-9	the ester of tocopherol and nicotinic acid	 (alpha)  (beta)  (delta)  (gamma)	antioxidant; skin-conditioning agent – miscellaneous

Table1. Definitions, Structures, and Functions [13]

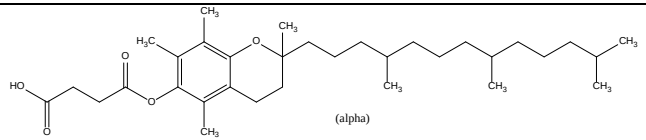
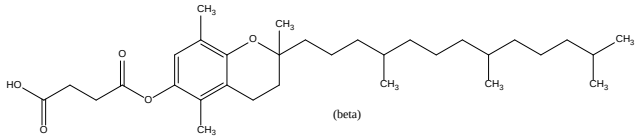
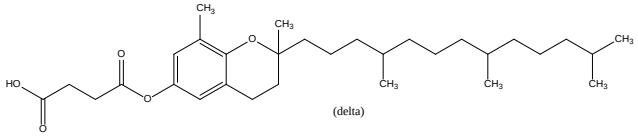
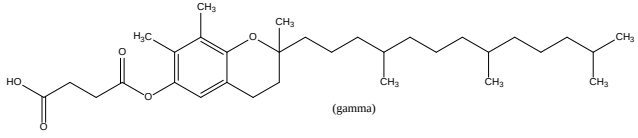
Ingredient/CAS No.	Definition	Structure**	Function(s)
Tocopheryl Succinate 17407-373-; 4345-03-3	the ester of tocopherol and succinic acid	 (alpha)  (beta)  (delta)  (gamma)	antioxidant

Table1. Definitions, Structures, and Functions [13]

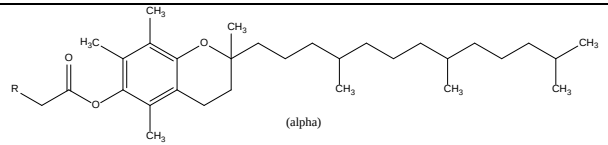
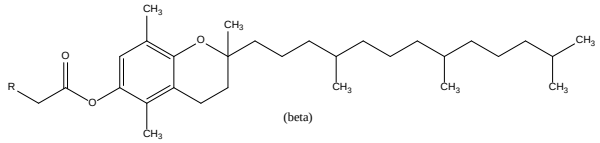
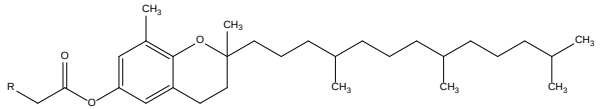
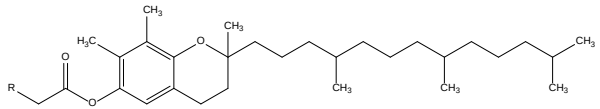
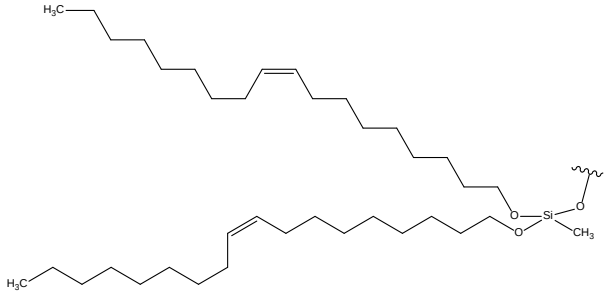
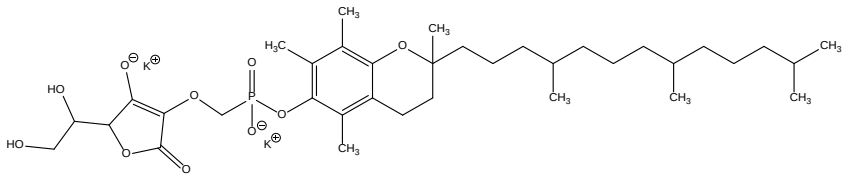
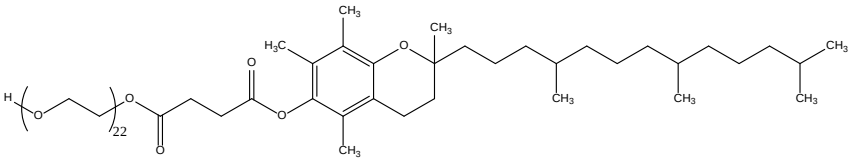
Ingredient/CAS No.	Definition	Structure**	Function(s)
Dioleyl Tocopheryl Methylsilanol	the dioleyl ether of tocopheryl acetate monoether with methylsilanetriol	 (alpha)  (beta)  (delta)  (gamma) wherein R is 	antioxidant; skin-conditioning agent – miscellaneous
Potassium Ascorbyl Tocopheryl Phosphate	the organic compound that conforms to the formula. *The potassium salt of ascorbyl tocopheryl phosphate.*		antioxidant
Tocophersolan 30999-06-5 9002-96-4	the organic compound that conforms to the formula. *The PEG-22 ester of tocopheryl succinate.*		antioxidants

Table1. Definitions, Structures, and Functions [13]

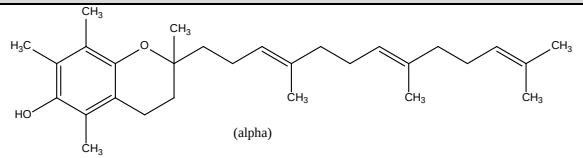
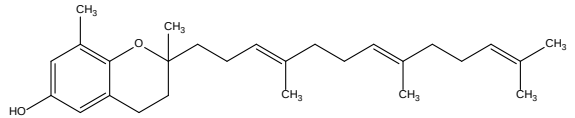
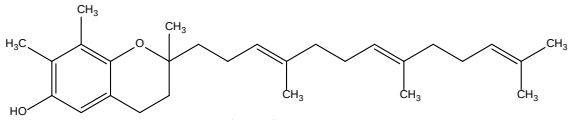
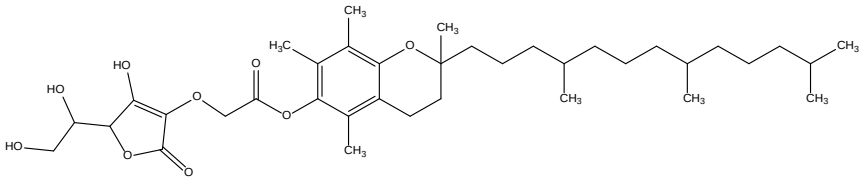
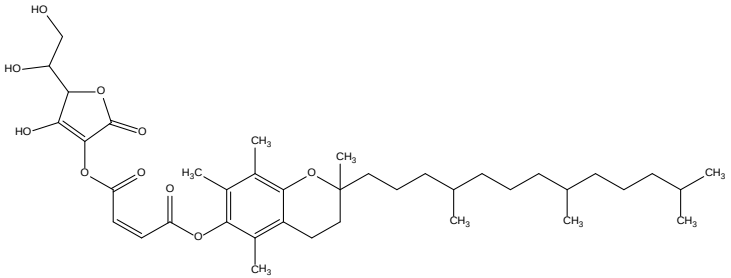
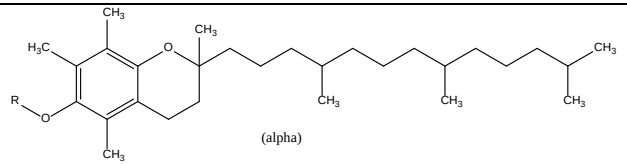
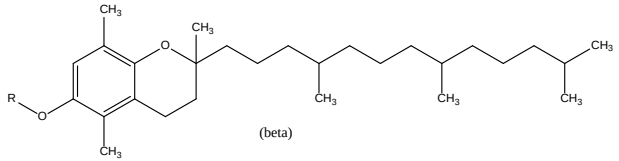
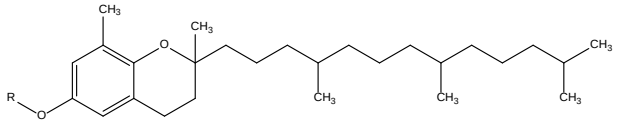
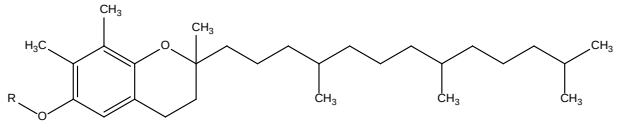
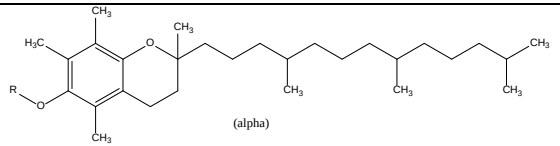
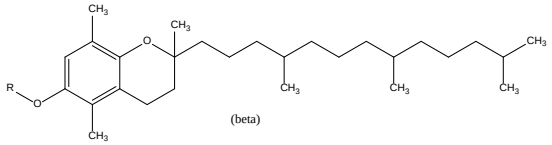
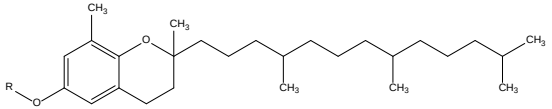
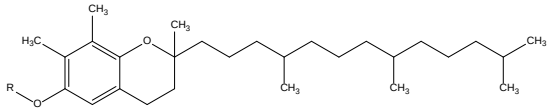
Ingredient/CAS No.	Definition	Structure**	Function(s)
Ingredients Being Considered for Inclusion in Re-Review			
Tocotrienols	a mixture of alpha-, gamma- and delta-tocotrienols which conform to the following formulae:	 (alpha)  (delta)  (gamma)	light stabilizer; oral care agent; skin-conditioning agent - miscellaneous
Ascorbyl Tocopheryl Acetate	the organic compound that conforms to the formula. *The ascorbyl tocopheryl derivative with a glycolate linkage.*		skin conditioning agent - miscellaneous
Ascorbyl Tocopheryl Maleate	the organic compound that conforms to the formula. *The ascorbyl tocopheryl derivative with a maleate linkage.*		antioxidant; skin-conditioning agent – emollient

Table1. Definitions, Structures, and Functions [13]

Ingredient/CAS No.	Definition	Structure**	Function(s)
Tocopheryl Phosphate 425429-22-7	a complex mixture of tocopherol and phosphoric acid. *A complex mixture of tocopheryl phosphate esters.*	 (alpha)  (beta)  (delta)  (gamma) wherein R is the residue of phosphoric acid or other tocopheryl phosphate moieties	hair conditioning agent; surfactant - cleansing agent; surfactant - emulsifying agent
Sodium Tocopheryl Phosphate	the sodium salt of a complex mixture of esters of phosphoric acid and tocopherol. *The sodium salt of a complex mixture of tocopheryl phosphate esters.*	 (alpha)  (beta)  (delta)  (gamma) wherein R is the residue of sodium salt of phosphoric acid or other tocopheryl phosphate moieties	antioxidant; emulsion stabilizer; reducing agent; skin-conditioning agent - miscellaneous; surfactant - emulsifying agent; viscosity increasing agent - aqueous

**these structures were generated by CIR

Table 2. Physical and chemical properties

Property	Description	Reference
	Tocopherol	
molecular weight	α-tocopherol: 430.7 β-tocopherol: 416.7 γ-tocopherol :416.7 δ-tocopherol: 402.7	36
λ_{max}	α- tocopherol: 292 nm β- tocopherol: 296 nm γ- tocopherol: 298 nm δ- tocopherol: 298 nm	36
stability	readily oxidized upon exposure to atmospheric conditions or light	5
	Tocopheryl Acetate	
molecular weight	430.71	65
	Tocophersolan	
physical appearance and form	white to light brown, waxy solid	66
melting point	37-41° C	66
solubility	miscible in water	66
specific gravity	1.06 (at 45° C)	66
	Tocopheryl Phosphate	
solubility	water soluble	67
	Tocotrienols	
molecular weight	α-tocotrienol: 424.7 β- tocotrienol: 410.7 γ- tocotrienol: 410.7 δ- tocotrienol: 396.7	36
λ_{max}	α- tocotrienol: 292 nm β- tocotrienol: 295 nm γ- tocotrienol: 298 nm δ- tocotrienol: 292 nm	36

Table 3. Plants containing tocotrienols (µg/g) [3]

Plant Species	Tissue	α-	β-	γ-	δ-
Pinaceae	bark	n.d.	n.d.	n.d.	700
Pinus nigra pallasiana pyramidata	bark	n.d.	n.d.	n.d.	1600
Cinnamosma fragrans	bark	n.d.	n.d.	n.d.	700
Cinnamosma macrocarpa	bark	n.d.	n.d.	n.d.	1600
Bixa orellana	seed	18.7	1.84	534.7	977.9
Hevea brasiliesis.	latex	522.4	below detection	196.7	1869.6
Aesculus hippocastanum	seed	97	n.d.	626	336
Rosmarinus officinalis	seed	560.5	300.3	109.4	trace
Delphinium ajacis	seed	566	153	trace	n.d.
Litchi chinensis	seed	below detection	below detection	3.37	488
Elaeis guineensis	fruit	4-193	0-234	0-526	0-123
Cocos nucifera	seed	104.0	26.0	36.2	204
Zea mays	seed; young seedling	0-239	0.450	0-20	0-709

n.d. – none detected

Table 4. Current and historical frequency and concentration of use of tocopherols and tocotrienols according to duration and exposure

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2013[14]	**1998[1]**	**2013[15]**	**1999[1]**	**2013[14]**	**1998[1]**	**2013[15]**	**1999[1]**
	Tocopherol,[#]**				**Tocopheryl Acetate****			
Totals*	**6175**	**1072**	**0.0000009-5.4**	**0.001-2**	**8960**	**1322**	**0.00000001-36**	**0.001-36**
Duration of Use								
Leave-On	*4944*	*940*	*0.000003-5.4*	*0.001-2*	*7068*	*1024*	*0.00000001-36*	*0.001-36*
Rinse-Off	*1172*	*130*	*0.0000009-3*	*0.001*	*1827*	*268*	*0.000075-10*	*0.0001-25*
Diluted for (Bath) Use	*59*	*2*	*0.05-1*	*0.01-0.8*	*65*	*30*	*0.001-0.1*	*0.05-0.1*
Exposure Type								
Eye Area	919	121	0.00002-3	0.02-0.6	887	77	0.000045-4.9	0.01-1
Incidental Ingestion	778	260	0.0005-2	0.05-0.9	887	85	0.0035-3	0.1-3
Incidental Inhalation-Spray	89 5[c]; 1912[a,c]	8 3[c]	0.000003-1 aerosol: 0.00002-0.0057 pump:0.0001-1	0.001-0.5	163 2[c]; 3438[a,c]	48 3[c]	0.00000001- 0.5 aerosol: 0.00000001- 0.2 pump: 0.000001-0.2 0.5-5[c]	0.02-0.1
Incidental Inhalation-Powder	140 5[c]; 812[b,c]	56 3[c]	0.0005-0.02	0.02-0.6	216 2[c]; 1338[b,c]	29 3[c]; 10[b]	0.008-1.1 0.5-5[c]	0.02-0.1
Dermal Contact	4718	654	0.0000009-5.4	0.001-2	6682	907	0.000045-5	0.0001-25
Deodorant (underarm)	21[a]	2	not spray: 0.000015-0.8 aerosol: 0.0001-0.0005 pump: 0.0006	0.050.0	31[a]	NR	not spray: 0.1-0.5 aerosol: 0.001-0.1	0.2
Hair - Non-Coloring	400	87	0.000003-0.25	0.001-0.6	1027	252	0.00000001- 10	0.001-0.3
Hair-Coloring	165	9	0.0001-0.1	0.001	79	1	0.00013-0.05	0.001
Nail	32	3	0.00001-0.2	0.05-0.3	133	61	0.002-36	0.01-36
Mucous Membrane	1312	304	0.0000009-3	0.01-0.9	1620	185	0.0003-3	0.05-3
Baby Products	20	2	0.0002	1	63	9	0.001-0.1	0.001-0.1
	Tocopheryl Linoleate				**Tocopheryl Linoleate/Oleate**			
Totals*	**103**	**279**	**0.0001-1**	**0.1-2**	**2**	**NR**	**0.05-0.1**	**NR**
Duration of Use								
Leave-On	*99*	*273*	*0.0001-1*	*0.1-2*	*2*	*NR*	*0.05-1*	*NR*
Rinse-Off	*4*	*4*	*0.001-0.003*	*2*	*NR*	*NR*	*0.05*	*NR*
Diluted for (Bath) Use	*NR*	*2*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	12	64	0.05-0.2	0.1-2	NR	NR	NR	NR
Incidental Ingestion	9	33	0.05-0.5	0.1-2	1	NR	NR	NR
Incidental Inhalation-Spray	49[a,b]	NR	aerosol: 0.01	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	10 16[b,c]	31	0.1	0.3-2	NR	NR	NR	NR
Dermal Contact	75	231	0.001-1	0.1-2	NR	NR	0.05-1	NR
Deodorant (underarm)	NR	NR	NR	NR	1	NR	NR	NR
Hair - Non-Coloring	2	3	0.0001-0.01	NR	NR	NR	NR	NR
Hair-Coloring	NR	28	NR	NR	NR	NR	NR	NR
Nail	8	11	0.01	NR	NR	NR	NR	NR
Mucous Membrane	10	36	0.05-0.5	0.1-2	1	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Tocopheryl Nicotinate				**Tocopheryl Succinate**			
Totals*	**39**	**3**	**0.001**	**0.0001-1**	**8**	**4[#]**	**0.00001-0.038**	**NR**
Duration of Use								
Leave-On	*37*	*1*	*0.001*	*0.01-0.2*	*8*	*#*	*0.00001-0.038*	*NR*
Rinse-Off	*2*	*2*	*0.1-1*	*0.0001-1*	*NR*	*#*	*0.00001- 0.0001*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*#*	*NR*	*NR*
Exposure Type								
Eye Area	7	NR	0.13	NR	1	#	0.00001-0.038	NR
Incidental Ingestion	11	NR	0.06-0.6	NR	NR	#	0.00008	NR
Incidental Inhalation-Spray	12[a,c]	NR	0.001-0.1	NR	NR	#	0.00001[a]	NR
Incidental Inhalation-Powder	4[b,c]	NR	NR	NR	NR	#	NR	NR
Dermal Contact	21	NR	0.05-1	0.1	8	#	0.00001-0.038	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	#	NR	NR
Hair - Non-Coloring	7	3	0.001-0.2	0.0001-1	NR	#	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	#	NR	NR
Nail	NR	NR	NR	NR	NR	#	NR	NR
Mucous Membrane	NR	NR	0.06-0.6	NR	NR	#	0.00008- 0.0001	NR
Baby Products	NR	NR	NR	NR	NR	#	NR	NR

Table 4. Current and historical frequency and concentration of use of tocopherols and tocotrienols according to duration and exposure

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2013[14]	**1998[1]**	**2013[15]**	**1999[1]**	**2013[14]**	**1998[1]**	**2013[15]**	**1999[1]**
	Dioleyl Tocopheryl Methylsilanol				**Potassium Ascorbyl Tocopheryl Phosphate**			
Totals*	**7**	**12**	**0.014**	**NR**	**37**	**15**	**0.01-0.2**	**0.02**
Duration of Use								
Leave-On	*7*	*12*	*0.014*	*NR*	*37*	*13*	*0.01-0.2*	*0.02*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*NR*	*2*	*0.01*	*0.02*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	4	1	0.01-0.03	NR
Incidental Ingestion	5	4	0.014	NR	NR	NR	0.02	0.02
Incidental Inhalation-Spray	NR	NR	NR	NR	1 28[a,c]	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	10[b,c]	NR	0.01	NR
Dermal Contact	2	3	NR	NR	37	15	0.01-0.1	0.02
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	0.01-0.2	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	5	NR	NR	NR	NR	NR	NR
Mucous Membrane	5	4	0.014	NR	NR	NR	0.02	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	Tocophersolan							
Totals*	**37**	**2**	**NR**	**0.05-0.2**				
Duration of Use								
Leave-On	*37*	*1*	*NR*	*0.05-0.2*				
Rinse-Off	*NR*	*1*	*NR*	*NR*				
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*				
Exposure Type								
Eye Area	1	NR	NR	NR				
Incidental Ingestion	1	NR	NR	NR				
Incidental Inhalation-Spray	2	NR	NR	NR				
Incidental Inhalation-Powder	NR	NR	NR	NR				
Dermal Contact	34	2	NR	0.05-2				
Deodorant (underarm)	NR	NR	NR	NR				
Hair - Non-Coloring	2	NR	NR	NR				
Hair-Coloring	NR	NR	NR	NR				
Nail	NR	NR	NR	NR				
Mucous Membrane	1	NR	NR	NR				
Baby Products	NR	NR	NR	NR				

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses
**total VCRP data include listings under multiple names
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.
[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[#] in the 1998 data, some uses are for a tradename/mixture and not defined; for tocopherol, 50 uses are not defined; for tocopheryl succinate, 4 uses are not defined
NR – no reported use

Table 5. Frequency and concentration of use according to duration and type of exposure – new ingredients

	# of Uses[14]	*Max Conc of Use (%)*[15]	*# of Uses*[14]	*Max Conc of Use (%)*[15]	*# of Uses*[14]	*Max Conc of Use (%)*[15]
	Tocotrienols		**Ascorbyl Tocopheryl Maleate**		**Sodium Tocopheryl Phosphate**	
Totals*	**433**	**0.0015-0.12**	**38**	**NS**	**10**	**NR**
Duration of Use						
Leave-On	*182*	*0.015-0.12*	*36*	*NS*	*7*	*NR*
Rinse-Off	*240*	*0.0015*	*2*	*NS*	*3*	*NR*
Diluted for (Bath) Use	*11*	*NR*	*NR*	*NS*	*NR*	*NR*
Exposure Type						
Eye Area	6	0.019-0.039	9	NS	2	NR
Incidental Ingestion	61	0.016-0.019	1	NS	NR	NR
Incidental Inhalation-Spray	74[a,c]	NR	24[a,b]	NS	4[a,c]	NR
Incidental Inhalation-Powder	26[b,c]	NR	NR	NS	NR	NR
Dermal Contact	360	0.0015-0.12	37	NS	10	NR
Deodorant (underarm)	NR	NR	NR	NS	NR	NR
Hair - Non-Coloring	9	0.015	NR	NS	NR	NR
Hair-Coloring	NR	NR	NR	NS	NR	NR
Nail	3	NR	NR	NS	NR	NR
Mucous Membrane	297	0.0015-0.019	1	NS	NR	NR
Baby Products	NR	NR	NR	NS	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] It is possible these products are powders, but it is not specified whether the reported uses are powders.
[c] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, there fore the information is captured in both categories
NR – no reported use
NS – not yet surveyed

Table 6. Tocopherol and tocopheryl acetate recovery (mol %) and % bioconversion tocopheryl acetate to tocopherol in human skin *in vitro*

		Surface		**Horny Layer**		**Underlying Skin**[5]	
Formulation	**Vitamin E form**	**Non-Occlusive**	**Occlusive**	**Non-Occlusive**	**Occlusive**	**Non-Occlusive**	**Occlusive**
in triglyceride oil	tocopherol+acetate	79.2	74.2	12.6	17.0	0.4	--
	tocopherol	1.2	1.2	0.1	0.1	--	--
surfactant-solubilized in water	tocopherol+acetate	43.2	48.4	20.7	27.6	26.6	16.6
	tocopherol	1.3	1.4	0.9	1.3	9.3	8.1
	% conversion					*34.8*	*48.9*
soybean phosphatidylcholine liposomes	tocopherol+acetate	30.6	40.6	23.6	33.0	36.8	20.2
	tocopherol	1.3	1.9	1.3	1.7	14.5	10.5
	% conversion					*39.4*	*51.2*
nanotope	tocopherol+acetate	24.5	29.5	24.0	31.1	45.3	31.7
	tocopherol	1.12	1.5	1.1	1.5	19.5	15.69
	% conversion					*43.1*	*49.2*

Table 7. Photoprotective Effects

Test Article	Test Population	Dose/Conc Tested	UV Exposure/Source	Protocol	Results	Reference
			In Vitro			
α-tocopherol-6-*O*-phosphate	skin cultures from female hairless SKH mice	0-2% in distilled water	- 10-40 kJ/m^2 for 3-10 min -DNA-FIX apparatus (290-380 nm)	-skin cultures were exposed for up to 3 h -the effect of α-tocopheryl acetate in DMSO pretreatment was also evaluated	-TBARS were statistically significant reduced by tocopheryl phosphate; less of an effect was observed with tocopheryl acetate -tocopheryl phosphate and acetate decreased SBCs at 1, but not 20-40, kJ/m^2	[41]
			Non-Human			
			Topical			
TRF; contains tocopherols and tocotrienols; in PEG-400	female hairless mice	5%; 20 µl applied	- exposed for 29 min; 3 MED equiv. - Oriel 1000-W simulator, 2.8 mW/cm^2 UVA and UVB (290-400 nm)	- using 1 cm^2 polypropylene rings, TRF and PEG-400 were each applied to 2 sites for 2 h - half the sites were irradiated	- the concentrations of all forms of vitamin E were statistically significantly decreased in PEG-400 treated skin after irradiation, but the amounts of vitamin E statistically significantly increased in irradiated TRF-treated skin	[35]
α-tocopherol	female hairless SKH1 mice	5 mg/cm^2	- 25 J/cm^2 UVA + UVB; 10 MED equiv. -solar simulator model 14S (Solar Light Co.)	- tocopherol was applied on the backs and sides of the mouse 24 h prior to UV	- increased superoxide dismutase, reduced glutathione levels, and statistically significantly reduced the formation of epidermal lipid hydroperoxides, indicating protection of cutaneous tissue against oxidative damage	[46]
α-, γ-, or δ-tocopherol α-tocopheryl acetate - neutral cream vehicle	4 female C3H/HeNTac mice/group	50 mg of 1or 5% dispersions	- 3.6-3.7 J/m^2/s for 60 min -6 Westinghouse FS20 lamps; 80% of the output in UVB range	- the cream was applied to the shaved backs of animals 15 min before irradiation	- vitamin E compounds in a 5% dispersion inhibited thymidine dimer formation in epidermal DNA - α-tocopherol had the greatest effect; statistically significant decreases were observe with γ-, or δ-tocopherol, but the decrease with α-tocopheryl acetate was not statistically significant	[68]
RRR-α-tocopherol RRR-α-tocopheryl succinate	15 female Skh:2 hairless pigmented dark-eyed mice/group	5%	- UV exposure was increased gradually until reaching the maintenance dose of 0.24 J/cm^2/exposure 24 wks of exposure, for a total exposure of ~15 J/cm^2 - 4 Westinghouse FS40 bulbs; 265-440 nm	- 100 µl of an o/w emulsion of standard cosmetic base vehicle were applied 3x/wk for 1 wk prior to UV exposure and until study termination	-no toxic effects - both forms protected against blistering; α-tocopherol was most effective (73% of mice did not blister with α-tocopherol; 33% did not blister with the succinate) - both protected against pigmentation; tocopherol was more protective -both forms protected against tumors compared to untreated mice -time to tumor developmental was significantly delayed with tocopherol	[69]
tocopheryl acetate in acetone	hairless SKH-1 mice	2.6% solution (5.2 mg/0.2 ml)	- 90 mJ/cm^2 (1 MED); single dose of UV	- tocopheryl acetate was applied dermally either 30 min before or 30 min after UV - the effect on various reaction in the skin were measured	- cyclobutane pyrimidine dimers repair was increased 1.5- and 2-fold by tocopheryl acetate before and after UV, respectively - DNA synthesis was increased, but not in a statistically significant manner	[70]

Table 7. Photoprotective Effects

Test Article	Test Population	Dose/Conc Tested	UV Exposure/Source	Protocol	Results	Reference
tocopheryl acetate in acetone	hairless SKH-1 mice, 20/group	2.6% solution (5.2 mg/0.2 ml)	- initial dose of 90 mJ/cm^2 -dose was increased by 25% weekly until reaching 275 mJ/cm^2	- dermal application of tocopheryl acetate 30 min before or 30min after UV -mice were treated with maximum UV dose for 24 wks	tocopheryl acetate delayed tumor formation and yield until wk 20; by wk 24, tumor incidence was comparable for all groups	[70]
			Oral			
RRR-α-tocopheryl acetate	15 female Skh:2 hairless pigmented dark-eyed mice/group	62.5 IU/kg chow; oral intake of 0.276 IU/day	- maintenance dose: 0.24 J/cm^2/exposure 24 wks of exposure, for a total exposure of ~15 J/cm^2 - 4 Westinghouse FS40 bulbs; 265-440 nm		-no toxic effects -some protection against blistering (27% of mice did not blister) - protected against pigmentation - protected against tumors compared to untreated mice -time to tumor developmental was significantly delayed	[69]
tocopherols and tocotrienols	8 female hairless HR-1 mice/group	1.) vitamin E-free 2) 50 mg/kg α-tocopherol 3.) 229 mg/kg T-mix (see below)	- 180 mJ/cm^2 1x/day for 7 days - UVM-28 UV lamp	- animals were fed their respective diets for 6 wks - at 6 wks, half the animals were killed; half continued on their test diet and were irradiated	- sunburn was greatest in the vitamin E-free group, followed by the α-tocopherol group; weak sunburn was seen with T-mix - no significant differences in TEWL or skin hydration between the groups	[71]
tocopherols and tocotrienols	9 female hairless HR-1 mice/group	as above	- 180 mJ/cm^2 2x/wk	- all mice dosed topically with 390 nmol/0.1 ml acetone - 1 wk later, each group was started on its respective test diet and were irradiated	- incidence of papillomas appeared in wk 7 - α-tocopherol suppressed the incidence of papillomas in the final 4 wks - t-mix clearly suppressed the incidence of papillomas at wk 10	[71]
			Human			
			Topical			
10% tocopherols, 0.3% tocotrienols, and other cosmetic ingredients	30 subjects	2 mg/cm^2	- mean MED was 326.67 mJ/cm^2 - radiation dose was 2x the MED	- the test formulation was applied to a 2 cm^2 area of skin on the buttocks, and the site was irradiated 30 min after application of the formulation - test sites were evaluated immediately and 6 and 24 h after UVB exposure - an untreated and vehicle-, irradiated, controls were used	Phototoxic reactions were statistically significantly decreased at the test site than at the untreated site	[72]
o/w formulation containing 2.5% tocopheryl acetate and 5% vitamin C in 20% NEO-PCL autoemulsionable (cetearyl alcohol, decyl oleate, sodium cetearyl sulfate, sodium methylparaben, sodium propylparaben, and water) and 5% aq. propylene glycol	20 Caucasian subjects with Fitzpatrick's phototype II, II-III, or III			- subjects were exposed to 6 increasing doses of SSR (Oriel 16s – 300 W solar simulator; erythemal doses of 14.5-45.0 mJ/cm^2) on the back - effect on the cutaneous response was examined by applying the test formulation 20 min before or 0.5, 3, 6, 9, and 12 h after irradiation	- application prior to irradiation produced a 36.9% increase in MED - application after irradiation produced a 19.8% increase in MED - application of the vehicle only 20 min before and 0.5, 3, 6, 9,and 12 h after irradiation did not have a statistically significant effect on MED	[73]

Abbreviations: MED – minimal erythema dose; SBC – sunburn cells; SSR – simulated solar radiation; T-mix – consist of 21.8% α-tocopherol, 1.0% γ-tocopherol, 23.4% α-tocotrienol, 37.4% γ-tocotrienol; TBARS – thiobarbituric acid reactive substances; TEWL – transepidermal water loss; TRF – tocotrienol-rich fraction

REFERENCES

1. Andersen FA (ed). Final report on the safety assessment of tocopherol, tocopheryl acetate, tocopheryl linoleate, tocopheryl linoleate/oleate, tocopheryl nicotinate, tocopheryl succinate, dioleyl tocopheryl methylsilanol, potassium ascorbyl tocopherol phosphate, and tocophersolan. *International Journal of Toxicology*. 2002;21(Suppl 3):51-116.

2. Andersen FA (ed). Final report of the safety assessment of *L*-ascorbic acid, calcium ascorbage, magnesium ascorbate, magnesium ascorby phosphate, sodium ascorbate, and sodium ascorbyl phosphate as used in cosmetics. *Int J toxicol*. 2005;24(Suppl 2):51-111.

3. Harinantenaina L. Tocotrienols in Plants: Sources and importance. Chapter: 4. Watson RR and Preedy VR. In: *Tocotrienols. Vitamin E Beyond Tocopherols*. Boca Ratopn, FL: CRC Press; 2009:43-60.

4. Weber SU, Lodge JK, Saliou C, and Packer L. Antioxidants. Chapter: 28. Barel AO, Paye M, and Maibach HI. In: *Handbook of Cosmetic Science and Technology*. 3 ed. New York, NY: Informa Healthcare; 2009:302-303.

5. Baschong W, Artmann C, Hueglin D, and Roeding J. Direct evidence for bioconversion of vitamin E acetate into vitamin E: An *ex vivo* study in viable human skin. *J Cosmet Sci*. 2001;52(3):155-161.

6. Netscher T. Synthesis of vitamin E. *Vitamin Horm*. 2007;76:155-202.

7. Sen CK, Khanna S, Rink C, and Roy S. Tocotrienols: The emerging face of natural vitamin E. *Vitam Horm*. 2007;76:203-261.

8. Sen CK, Khanna S, and Roy S. Tocotrienols: Vitamin E beyond tocopherols. *Life Sci*. 2006;78(18):2088-2098.

9. Wong RS and Radhakrishnan AK. Tocotrienol research: past into present. *Nutr Rev*. 2012;70(9):483-490.

10. Jensen SK and Lauridsen C. Alpha-tocopherol stereoisomers. *Vitamin Horm*. 2007;76:281-308.

11. Jiang Q, Christen S, Shigenaga MK, and Ames BN. γ-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am J Clin Nutr*. 2001;74:714-722.

12. Gianello R, Hall WC, Kennepohl E, Libinaki R, and Ogru E. Subchronic oral toxicity study of mixed tocopheryl phosphates in rats. *Int J toxicol*. 2007;26(5):475-490.

13. Gottschalck TE and Breslawec H. International Cosmetic Ingredient Dictionary and Handbook. Washington, DC: Personal Care Products Council, 2012.

14. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2013. Dated Jan 15.

15. Personal Care Products Council. 10-30-2013. Concentration of Use by FDA Product Category: Tocopherol and Related Ingredients. Unpublished data submitted by Personal Care Products Council. 10 pages.

16. Johnsen MA. The influence of particle size. *Spray Technology and Marketing*. 2004;14(11):24-27.

17. Rothe H. Special Aspects of Cosmetic Spray Evalulation. 9-26-2011. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, D.C.

18. Bremmer HJ, Prud'homme de Lodder LCH, and Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 2006. Report No. RIVM 320104001/2006. pp. 1-77.

19. 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.

20. European Commission. CosIng database; following Cosmetic Regulation No. 1223/2009. http://ec.europa.eu/consumers/cosmetics/cosing/. Date Accessed 9-23-2013.

21. Scientific Committee on Consumer Products and Non-Food Products (SCCNFP). Opinion of the Scientific Committee on Consumer Products and Non-Food Products intended for consumers concerning the use of alpha-tocopherol acetate in cosmetic products. http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out148_en.pdf. Date Accessed 10-10-2013.

22. Joint FAO/WHO Expert Committee on Food Additives (JECFA). dl-α-Tocopherol. http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/. Date Accessed 10-20-2013.

23. Ikeda S, Niwa T, and Yamashita K. Selective uptake of dietary tocotrienols into rat skin. *J Nutr Sci Vitaminol (Tokyo)*. 2000;46(3):141-143.

24. Ikeda S, Tohoyama T, Yoshimura H, Hamamura K, Abe K, and Yamashita K. Dietary α-tocopherol decreases α–tocotrienol but not γ–tocotrienol concentration in rats. *J Nutr*. 2003;133:428-434.

25. Okabe M, Oji M, Ikeda I, Tachibana H, and Yamada K. Tocotrienol levels in various tissues of Sprague-Dawley rats after intragastric administration of tocotrienols. *Bioscience, Biotechnology, and Biochemistry*. 2002;66(8):1768-1771.

26. Patel V, Khanna S, Roy S, Ezziddin O, and Sen CK. Natural vitamin E alpha-tocotrienol: retention in vital organs in response to long-term oral supplementation and withdrawal. *Free Radic Res*. 2006;40(7):763-771.

27. Shibata A, Nakagawa K, Shirakawa H, Kormann AW, Kawakami Y, Takashima R, Ohashi A, Sato S, Ohsaki Y, Kimura F, Kimura T, Tsuduki T, Komai M, and Nakamura H. Physiological effects and tissue distribution from large doses of tocotrienol in rats. *Biosci Biotechnol Biochem*. 2012;76(9):1805-1808.

28. Uchida T, Nomura S, Ichikawa T, Abe C, and Ikeda S. Tissue distribution of vitamin E metabolites in rats after oral adminstration of tocopherol or tocotrienol. *J Nutr Sci Vitaminol*. 2011;57:326-332.

29. Uchida T, Nomura S, Sakuma E, Hanzawa F, and Ikeda S. α-Tocopherol does not Accelerate Depletion of γ-Tocopherol and Tocotrienol or Excretion of their Metabolites in Rats. *Lipids*. 2013;8(7):687-695.

30. Weiser H, Riss G, and Kormann AW. Biodiscrimination of the eight α-tocopherol stereoisomers results in preferential accumulation of the four 2*R* forms in tissues and plasma or rats. *J Nutr*. 1996;126:2539-2549.

31. Yap SP, Yuen KH, and Lim AB. Influence of route of administration on the absorption and disposition of alpha-, gamma- and delta-tocotrienols in rats. *J Pharm Pharmacol*. 2003;55(1):53-58.

32. Burton GW, Traber MG, Acuff RV, Walters DN, Kayden H, Hughes L, and Ingold KU. Human plasma and tissue α-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitam E. *Am J Clin Nutr*. 1998;67:669-684.

33. Khanna S, Patel V, Rink C, Roy S, and Sen CK. Delivery of orally supplemented α-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. *Free Radic Biol Med*. 2005;39(10):1310-1319.

34. Blatt DH, Leonard SW, and Traber MG. Vitamin E kinetics and the function of tocopherol regulatory proteins. *Nutrition*. 2001;17(10):799-805.

35. Weber C, Podda M, Rallis M, Thiele JJ, Traber MG, and Packer L. Efficacy of topically applied tocopherols and tocotrienols in protection of murine skin from oxidative damage induced by UV-irradiation. *Free Radic Biol Med*. 1997;22(5):761-769.

36. Podda M, Weber C, Traber MG, and Packer L. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols, and ubiquinones. *J Lipid Res*. 1996;37:893-901.

37. Zhao Y, Lee M-J, Cheung C, Ju J-H, Chen Y-K, Liu B, Hu L-Q, and Yang CS. Analysis of multiple metabolites of tocopherols and tocotrienols in mice and humans. *J Agric Food Chem*. 2010;58(8):4844-4852.

38. Sen CK, Khanna S, and Roy S. Tocotrienol: The natural vitamin E to defend the nervous system? *Ann NY Acad Sci*. 2004;1031:127-142.

39. Kramer-Stickland KA, Krol ES, and Liebler DC. UV-B-Induced photooxidation of vitamin E in mouse skin. *Chen Res Toxicol*. 1999;12(2):187-191.

40. Kramer-Stickland KA and Liebler DC. Effect of UVB on hydrolysis of a-tocopherol acetate to a-tocopherol in mouse skin. *Journal of Investigative Dermatology*. 1998;111(2):302-307.

41. Nakayama S, Katoh EM, Tsuzuki T, and Kobayashi S. Protective effect of α-tocopherol-6-*O*-phosphate against ultraviolet B-induced damage in cultured mouse skin. *J Invest Dermatol*. 2003;121:406-411.

42. Rangaranajan M and Zatz JL. Effect of formulation on the topical delivery of alpha-tocopherol. *J Cosmet Sci*. 2003;54(2):161-174.

43. Rangaranajan M and Zatz JL. Kinetics of permeation and metabolism of alpha-tocopherol and alpha-tocopheryl acetate in micro-Yucatan pig skin. *J Cosmet Sci*. 2001;52(1):35-50.

44. European Chemicals Agency. 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyldecyl)-2H-benzopyran-6-yl acetate. http://echa.europa.eu. Date Accessed 10-10-2013.

45. Mahamongkol H, Bellantone RA, Stagni G, and Plakogiannis FM. Permeation study of five formulations of alpha-tocopherol acetate through human cadaver skin. *J Cosmet Sci*. 2005;56(2):91-103.

46. Lopez-Torres M, Thiele JJ, Shindo Y, Han D, and Packer L. Topical application of alpha-tocopherol modulates that antioxidant network and diminishes ultraviolet-induced oxidative damage in murine skin. *Br J Dermatol*. 1998;138(2):207-215.

47. Kuriyama K, Shimizu T, Horiguchi T, Watabe M, and Abe Y. Vitamin E ointment at high dose levels suppresses contact dermatitis in rats by stabilizing keratinocytes. *Inflamm Res*. 2002;51(10):483-489.

48. Traber MG, Rallis M, Podda M, Weber C, and Maibach HI. Penetration and distribution of alpha-tocopherol, alpha- or gamma-tocotrienols applied individually onto murine skin. *Lipids*. 1998;33:87-91.

49. Libinaki R, Ogru E, Gianello R, Bolton L, and Geytenbeek S. Evaluation of the safety of mixed tocopheryl phosphates (MTP) -- a formulation of alpha-tocopheryl phosphate plus alpha-di-tocopheryl phosphate. *Food Chem Toxicol*. 2006;44(7):916-932.

50. Gopinathan S, O'Neill E, Rodriguez LA, Champ R, Phillips M, Nouraldeen A, Wendt M, Wilson AGE, and Kramer JA. In vivo toxicology of excipients commonly employed in drug discovery in rats. *Journal of Pharmacological and Toxicological Methods*. 2013;68(2):284-295.

51. Nakamura H, Furukawa F, Nishikawa A, Miyauchi M, Son H-Y, Imazawa T, and Hirose M. Oral toxicity of a tocotrienol preparation in rats. *Food Chem Toxicol*. 2001;39(8):799-805.

52. National Toxicology Program (NTP). *In vitro* cytogenetics - sister chromatid exchanges. D-alpha-tocoperyl succinate. http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invitrosce.scedata&study_no=185103&cas_no=4345%2D03%2D3&endpointlist=SCE. Date Accessed 10-11-2013.

53. National Toxicology Program (NTP). *In vitro* cytogenetics assay - chromosome aberrations. D-alpha tocopheyrl succinate. http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invitroca.cadata&study_no=185103&cas_no=4345%2D03%2D3&endpointlist=CAB. Date Accessed 10-11-2013.

54. Lee HJ, Ju J, Pauyl S, So J-Y, DeCastro A, Smolarek A, Lee M-J, Yang CS, Newmark HL, and Suh N. Mixed tocopherols prevent mammary tumorigenesis by inhibiting estrogen action and activating PPAR-γ. *Clin Cancer Res*. 2009;15(12):4242-4249.

55. Newmark HL, Huang M-T, and Reddy BS. Mixed tocopherols inhibit azoxymethane-induced aberrant crypt foci in rats. *Nutrition and Cancer*. 2006;56(1):82-85.

56. Wu K, Shan YJ, Zhao Y, Yu JW, and Liu BH. Inhibitory effects of RRR-α-tocpheyrl succinate on benzo(a)pyrene (B(a)P)-induced forestomach carcinogenesis in female mice. *World J Gastroenterol*. 2001;7(1):60-65.

57. Pierpaoli E, Viola V, Barucca A, Orlando F, Galli F, and Provinciali M. Effect of annatto-tocotrienols supplementation on the development of mammary tumors in HER-2/neu transgenic mice. *Carcinogenesis*. 2013;34(6):1352-1360.

58. Burns EM, Tober KL, Rigenback JA, Kusewitt DF, Young GS, and Oberyszyn TM. Differential effects of topical vitamin E and C E ferulic® treatments on ultraviolet light B-induced cutaneous tumor development n Skh-1 mice. *PLoS ONE*. 2013;8(5):1-9.

59. Mitchel REJ and McCann RA. Skin tumor promotion by Vitamin E in mice: amplification by ionizing radiation and Vitamin C. *Cancer Detect Prev*. 2003;27(2):102-108.

60. Sakai M, Okabe M, Yamasaki M, Tachibana H, and Yamada K. Induction of apoptosis by tocotrienol in rat hepatoma dRLh-84 cells. *Anticancer Research*. 2004;24:1683-1688.

61. Anonymous. 1996. Clinical evaluation report: Human patch test of a nail oil - cuticle softener containing 36% Tocopheryl Acetate. Unpublished data submitted by Personal Care Products Council. 1 pages.

62. Corazza M, Minghetti S, Borghi A, Bianchi A, and Virgili A. Vitamin E contact allergy: a controversial subject. *Dermatitis*. 2012;23(4):167-169.

63. Tsuduki T, Kuriyama K, Nakagawa K, and Miyazawa T. Tocotrienol (unsaturated vitamin E) suppresses degranulation of mast cells and reduces allergic dermatitis in mice. *J Oleo Sci*. 2013;62(10):825-834.

64. Klein EA, Thompson IM Jr, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM, Karp DD, Liever MM, Walther PJ, Klotz L, PArsons JK, Chin JL, Darke AK, Lippman SM, Goodman GE, Meyskens FL Jr, and Baker LH. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E cancer prevention trial (SELECT). *JAMA*. 2011;306(14):1549-1556.

65. Merck & Co., Inc. The Merck Index. http://themerckindex.cambridgesoft.com/themerckindex/Forms/Home/ContentArea/Home.aspx. Date Accessed 4-8-2011.

66. Handbook of Pharmaceutical Excipients. 6th *ed*. Pharmaceutical Press, 2009.

67. Kato E and Takahashi N. Improvement by sodium dl-a-tocopheryl-6-O-phosphate treatment of moisture-retaining ability in stratum corneum through increased ceramide levels. *Bioorg Med chem*. 2012;20(12):3837-3842.

68. McVean M and Liebler DC. Prevention of DNA photodamage by vitamin E compounds and sunscreens: roles of ultraviolet absorbance and cellular uptake. *MolCarcinog*. 1999;24(3):169-176.

69. Burke KE, Clive J, Combs GF Jr, Commisso J, Keen CL, and Nakamura RM. Effects of topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. *Nutr Cancer*. 2000;38(1):87-97.

70. Berton TR, Conti CJ, Mitchell DL, Aldaz CM, Lubet RA, and Fischer SM. The effect of vitamin E acetate on ultraviolet-induced mouse skin carcinogenesis. *Mol Carcinog*. 1998;23(3):175-184.

71. Yamada Y, Obayashi M, Ishikawa T, Kiso Y, Ono Y, and Yamashita K. Dietary tocotrienols reducts UVB-induced skin damage and sesamin enhnaces tocotrienol effects in hairless mice. *J Nutr Sci Vitaminol*. 2008;54:117-123.

72. Pedrelli VF, Lauriola MM, and Pigatto PD. Clinical evaluation of photoprotective effect by a topical antioxidants combination (tocopherols and tocotrienols). *J Eur Acad Dermatol Venereol*. 2012;26(11):1449-1453.

73. Aguilera J, de Gálvez MV, Sánchez C, and Herrera-Ceballos E. Changes in photoinduced cutaneous erythema with topical application of a combination of vitamins C and E before and after UV exposure. *J Dermatol Sci*. 2012;66(3):216-220.

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TOCOPHEROL	12E - Foot Powders and Sprays	5
TOCOPHEROL	12F - Moisturizing	778
TOCOPHEROL	12G - Night	132
TOCOPHEROL	12H - Paste Masks (mud packs)	51
TOCOPHEROL	12I - Skin Fresheners	14
TOCOPHEROL	12J - Other Skin Care Preps	213
TOCOPHEROL	13A - Suntan Gels, Creams, and Liquids	15
TOCOPHEROL	13B - Indoor Tanning Preparations	56

TOCOPHEROL	13C - Other Suntan Preparations	9
TOCOPHEROL, D-ALPHA-	02B - Bubble Baths	1
TOCOPHEROL, D-ALPHA-	02D - Other Bath Preparations	3
TOCOPHEROL, D-ALPHA-	03A - Eyebrow Pencil	1
TOCOPHEROL, D-ALPHA-	03B - Eyeliner	6
TOCOPHEROL, D-ALPHA-	03C - Eye Shadow	4
TOCOPHEROL, D-ALPHA-	03D - Eye Lotion	1
TOCOPHEROL, D-ALPHA-	03G - Other Eye Makeup Preparations	4
TOCOPHEROL, D-ALPHA-	05F - Shampoos (non-coloring)	1
TOCOPHEROL, D-ALPHA-	05G - Tonics, Dressings, and Other Hair Grooming	1
TOCOPHEROL, D-ALPHA-	05I - Other Hair Preparations	3
TOCOPHEROL, D-ALPHA-	07A - Blushers (all types)	1
TOCOPHEROL, D-ALPHA-	07B - Face Powders	3
TOCOPHEROL, D-ALPHA-	07C - Foundations	5
TOCOPHEROL, D-ALPHA-	07E - Lipstick	10
TOCOPHEROL, D-ALPHA-	07I - Other Makeup Preparations	3
TOCOPHEROL, D-ALPHA-	08F - Nail Polish and Enamel Removers	1
TOCOPHEROL, D-ALPHA-	10A - Bath Soaps and Detergents	1
TOCOPHEROL, D-ALPHA-	11A - Aftershave Lotion	1
TOCOPHEROL, D-ALPHA-	12A - Cleansing	4
TOCOPHEROL, D-ALPHA-	12C - Face and Neck (exc shave)	20
TOCOPHEROL, D-ALPHA-	12D - Body and Hand (exc shave)	11
TOCOPHEROL, D-ALPHA-	12F - Moisturizing	21
TOCOPHEROL, D-ALPHA-	12G - Night	2
TOCOPHEROL, D-ALPHA-	12H - Paste Masks (mud packs)	1
TOCOPHEROL, D-ALPHA-	12I - Skin Fresheners	4
TOCOPHEROL, D-ALPHA-	12J - Other Skin Care Preps	7
TOCOPHEROL, D-ALPHA-	13B - Indoor Tanning Preparations	1
TOCOPHEROL, D-ALPHA-	13C - Other Suntan Preparations	1
TOCOPHEROL, DL-ALPHA	03D - Eye Lotion	1
TOCOPHEROL, DL-ALPHA	03G - Other Eye Makeup Preparations	1
TOCOPHEROL, DL-ALPHA	05A - Hair Conditioner	2
TOCOPHEROL, DL-ALPHA	07C - Foundations	3
TOCOPHEROL, DL-ALPHA	07I - Other Makeup Preparations	3
TOCOPHEROL, DL-ALPHA	12A - Cleansing	1
TOCOPHEROL, DL-ALPHA	12C - Face and Neck (exc shave)	4
TOCOPHEROL, DL-ALPHA	12D - Body and Hand (exc shave)	7
TOCOPHEROL, DL-ALPHA	12F - Moisturizing	9
TOCOPHEROL, DL-ALPHA	12G - Night	4
TOCOPHEROL, DL-ALPHA	12I - Skin Fresheners	10
TOCOPHEROL, DL-ALPHA	12J - Other Skin Care Preps	2
TOCOPHEROL, DL-ALPHA	13B - Indoor Tanning Preparations	1
TOCOPHEROL, DL-ALPHA	13C - Other Suntan Preparations	2
TOCOPHERYL ACETATE	01A - Baby Shampoos	4
TOCOPHERYL ACETATE	01B - Baby Lotions, Oils, Powders, and Creams	38
TOCOPHERYL ACETATE	01C - Other Baby Products	20
TOCOPHERYL ACETATE	02A - Bath Oils, Tablets, and Salts	14
TOCOPHERYL ACETATE	02B - Bubble Baths	22
TOCOPHERYL ACETATE	02D - Other Bath Preparations	19
TOCOPHERYL ACETATE	03A - Eyebrow Pencil	15
TOCOPHERYL ACETATE	03B - Eyeliner	74
TOCOPHERYL ACETATE	03C - Eye Shadow	322
TOCOPHERYL ACETATE	03D - Eye Lotion	138
TOCOPHERYL ACETATE	03E - Eye Makeup Remover	8
TOCOPHERYL ACETATE	03F - Mascara	139
TOCOPHERYL ACETATE	03G - Other Eye Makeup Preparations	152
TOCOPHERYL ACETATE	04A - Cologne and Toilet waters	15
TOCOPHERYL ACETATE	04B - Perfumes	4
TOCOPHERYL ACETATE	04C - Powders (dusting and talcum, excluding afte	7
TOCOPHERYL ACETATE	04E - Other Fragrance Preparation	67
TOCOPHERYL ACETATE	05A - Hair Conditioner	323

TOCOPHERYL ACETATE	05B - Hair Spray (aerosol fixatives)	74
TOCOPHERYL ACETATE	05C - Hair Straighteners	3
TOCOPHERYL ACETATE	05E - Rinses (non-coloring)	3
TOCOPHERYL ACETATE	05F - Shampoos (non-coloring)	250
TOCOPHERYL ACETATE	05G - Tonics, Dressings, and Other Hair Grooming	234
TOCOPHERYL ACETATE	05H - Wave Sets	6
TOCOPHERYL ACETATE	05I - Other Hair Preparations	116
TOCOPHERYL ACETATE	06A - Hair Dyes and Colors (all types requiring cau	60
TOCOPHERYL ACETATE	06B - Hair Tints	2
TOCOPHERYL ACETATE	06C - Hair Rinses (coloring)	7
TOCOPHERYL ACETATE	06D - Hair Shampoos (coloring)	5
TOCOPHERYL ACETATE	06H - Other Hair Coloring Preparation	5
TOCOPHERYL ACETATE	07A - Blushers (all types)	130
TOCOPHERYL ACETATE	07B - Face Powders	197
TOCOPHERYL ACETATE	07C - Foundations	214
TOCOPHERYL ACETATE	07D - Leg and Body Paints	6
TOCOPHERYL ACETATE	07E - Lipstick	840
TOCOPHERYL ACETATE	07F - Makeup Bases	57
TOCOPHERYL ACETATE	07G - Rouges	58
TOCOPHERYL ACETATE	07H - Makeup Fixatives	13
TOCOPHERYL ACETATE	07I - Other Makeup Preparations	202
TOCOPHERYL ACETATE	08A - Basecoats and Undercoats	15
TOCOPHERYL ACETATE	08B - Cuticle Softeners	19
TOCOPHERYL ACETATE	08C - Nail Creams and Lotions	1
TOCOPHERYL ACETATE	08D - Nail Extenders	1
TOCOPHERYL ACETATE	08E - Nail Polish and Enamel	14
TOCOPHERYL ACETATE	08F - Nail Polish and Enamel Removers	7
TOCOPHERYL ACETATE	08G - Other Manicuring Preparations	23
TOCOPHERYL ACETATE	10A - Bath Soaps and Detergents	382
TOCOPHERYL ACETATE	10B - Deodorants (underarm)	29
TOCOPHERYL ACETATE	10D - Feminine Deodorants	9
TOCOPHERYL ACETATE	10E - Other Personal Cleanliness Products	244
TOCOPHERYL ACETATE	11A - Aftershave Lotion	93
TOCOPHERYL ACETATE	11D - Preshave Lotions (all types)	5
TOCOPHERYL ACETATE	11E - Shaving Cream	40
TOCOPHERYL ACETATE	11F - Shaving Soap	7
TOCOPHERYL ACETATE	11G - Other Shaving Preparation Products	40
TOCOPHERYL ACETATE	12A - Cleansing	243
TOCOPHERYL ACETATE	12B - Depilatories	7
TOCOPHERYL ACETATE	12C - Face and Neck (exc shave)	574
TOCOPHERYL ACETATE	12D - Body and Hand (exc shave)	644
TOCOPHERYL ACETATE	12E - Foot Powders and Sprays	2
TOCOPHERYL ACETATE	12F - Moisturizing	1,437
TOCOPHERYL ACETATE	12G - Night	189
TOCOPHERYL ACETATE	12H - Paste Masks (mud packs)	80
TOCOPHERYL ACETATE	12I - Skin Fresheners	26
TOCOPHERYL ACETATE	12J - Other Skin Care Preps	295
TOCOPHERYL ACETATE	13A - Suntan Gels, Creams, and Liquids	35
TOCOPHERYL ACETATE	13B - Indoor Tanning Preparations	96
TOCOPHERYL ACETATE	13C - Other Suntan Preparations	13
TOCOPHERYL ACETATE, DL-ALPHA	01C - Other Baby Products	1
TOCOPHERYL ACETATE, DL-ALPHA	02A - Bath Oils, Tablets, and Salts	7
TOCOPHERYL ACETATE, DL-ALPHA	02D - Other Bath Preparations	3
TOCOPHERYL ACETATE, DL-ALPHA	03B - Eyeliner	7
TOCOPHERYL ACETATE, DL-ALPHA	03C - Eye Shadow	6
TOCOPHERYL ACETATE, DL-ALPHA	03D - Eye Lotion	4
TOCOPHERYL ACETATE, DL-ALPHA	03E - Eye Makeup Remover	1
TOCOPHERYL ACETATE, DL-ALPHA	03F - Mascara	18
TOCOPHERYL ACETATE, DL-ALPHA	03G - Other Eye Makeup Preparations	3
TOCOPHERYL ACETATE, DL-ALPHA	04B - Perfumes	1
TOCOPHERYL ACETATE, DL-ALPHA	05A - Hair Conditioner	2
TOCOPHERYL ACETATE, DL-ALPHA	05B - Hair Spray (aerosol fixatives)	2
TOCOPHERYL ACETATE, DL-ALPHA	05C - Hair Straighteners	1

TOCOPHERYL ACETATE, DL-ALPHA	05F - Shampoos (non-coloring)	4
TOCOPHERYL ACETATE, DL-ALPHA	05G - Tonics, Dressings, and Other Hair Groomin	3
TOCOPHERYL ACETATE, DL-ALPHA	05I - Other Hair Preparations	2
TOCOPHERYL ACETATE, DL-ALPHA	07B - Face Powders	12
TOCOPHERYL ACETATE, DL-ALPHA	07C - Foundations	7
TOCOPHERYL ACETATE, DL-ALPHA	07E - Lipstick	47
TOCOPHERYL ACETATE, DL-ALPHA	07F - Makeup Bases	2
TOCOPHERYL ACETATE, DL-ALPHA	07G - Rouges	1
TOCOPHERYL ACETATE, DL-ALPHA	07I - Other Makeup Preparations	26
TOCOPHERYL ACETATE, DL-ALPHA	08A - Basecoats and Undercoats	5
TOCOPHERYL ACETATE, DL-ALPHA	08B - Cuticle Softeners	8
TOCOPHERYL ACETATE, DL-ALPHA	08C - Nail Creams and Lotions	3
TOCOPHERYL ACETATE, DL-ALPHA	08E - Nail Polish and Enamel	15
TOCOPHERYL ACETATE, DL-ALPHA	08F - Nail Polish and Enamel Removers	5
TOCOPHERYL ACETATE, DL-ALPHA	08G - Other Manicuring Preparations	17
TOCOPHERYL ACETATE, DL-ALPHA	10A - Bath Soaps and Detergents	10
TOCOPHERYL ACETATE, DL-ALPHA	10B - Deodorants (underarm)	2
TOCOPHERYL ACETATE, DL-ALPHA	10D - Feminine Deodorants	1
TOCOPHERYL ACETATE, DL-ALPHA	10E - Other Personal Cleanliness Products	22
TOCOPHERYL ACETATE, DL-ALPHA	11A - Aftershave Lotion	11
TOCOPHERYL ACETATE, DL-ALPHA	11E - Shaving Cream	4
TOCOPHERYL ACETATE, DL-ALPHA	12A - Cleansing	23
TOCOPHERYL ACETATE, DL-ALPHA	12B - Depilatories	19
TOCOPHERYL ACETATE, DL-ALPHA	12C - Face and Neck (exc shave)	27
TOCOPHERYL ACETATE, DL-ALPHA	12D - Body and Hand (exc shave)	45
TOCOPHERYL ACETATE, DL-ALPHA	12F - Moisturizing	75
TOCOPHERYL ACETATE, DL-ALPHA	12G - Night	14
TOCOPHERYL ACETATE, DL-ALPHA	12H - Paste Masks (mud packs)	5
TOCOPHERYL ACETATE, DL-ALPHA	12I - Skin Fresheners	10
TOCOPHERYL ACETATE, DL-ALPHA	12J - Other Skin Care Preps	32
TOCOPHERYL ACETATE, DL-ALPHA	13A - Suntan Gels, Creams, and Liquids	3
TOCOPHERYL ACETATE, DL-ALPHA	13B - Indoor Tanning Preparations	10
TOCOPHERYL ACETATE, DL-ALPHA	13C - Other Suntan Preparations	1
TOCOPHERYL LINOLEATE	03D - Eye Lotion	3
TOCOPHERYL LINOLEATE	03F - Mascara	9
TOCOPHERYL LINOLEATE	05F - Shampoos (non-coloring)	1
TOCOPHERYL LINOLEATE	05G - Tonics, Dressings, and Other Hair Groomin	1
TOCOPHERYL LINOLEATE	07B - Face Powders	10
TOCOPHERYL LINOLEATE	07C - Foundations	4
TOCOPHERYL LINOLEATE	07E - Lipstick	9
TOCOPHERYL LINOLEATE	07I - Other Makeup Preparations	3
TOCOPHERYL LINOLEATE	08C - Nail Creams and Lotions	1
TOCOPHERYL LINOLEATE	08E - Nail Polish and Enamel	7
TOCOPHERYL LINOLEATE	10A - Bath Soaps and Detergents	1
TOCOPHERYL LINOLEATE	12A - Cleansing	2
TOCOPHERYL LINOLEATE	12C - Face and Neck (exc shave)	11
TOCOPHERYL LINOLEATE	12D - Body and Hand (exc shave)	5
TOCOPHERYL LINOLEATE	12F - Moisturizing	17
TOCOPHERYL LINOLEATE	12G - Night	7
TOCOPHERYL LINOLEATE	12I - Skin Fresheners	1
TOCOPHERYL LINOLEATE	12J - Other Skin Care Preps	4
TOCOPHERYL LINOLEATE	13B - Indoor Tanning Preparations	3
TOCOPHERYL LINOLEATE	13C - Other Suntan Preparations	4
TOCOPHERYL LINOLEATE/OLEATE	07E - Lipstick	1
TOCOPHERYL LINOLEATE/OLEATE	12C - Face and Neck (exc shave)	1
TOCOPHERYL NICOTINATE	03B - Eyeliner	5
TOCOPHERYL NICOTINATE	03D - Eye Lotion	2

TOCOPHERYL NICOTINATE	05A - Hair Conditioner	1
TOCOPHERYL NICOTINATE	05G - Tonics, Dressings, and Other Hair Grooming	4
TOCOPHERYL NICOTINATE	05I - Other Hair Preparations	2
TOCOPHERYL NICOTINATE	07E - Lipstick	11
TOCOPHERYL NICOTINATE	07F - Makeup Bases	1
TOCOPHERYL NICOTINATE	07I - Other Makeup Preparations	1
TOCOPHERYL NICOTINATE	12A - Cleansing	1
TOCOPHERYL NICOTINATE	12C - Face and Neck (exc shave)	2
TOCOPHERYL NICOTINATE	12D - Body and Hand (exc shave)	2
TOCOPHERYL NICOTINATE	12F - Moisturizing	3
TOCOPHERYL NICOTINATE	12H - Paste Masks (mud packs)	1
TOCOPHERYL NICOTINATE	12J - Other Skin Care Preps	2
TOCOPHERYL NICOTINATE	13B - Indoor Tanning Preparations	1
TOCOPHERYL SUCCINATE	03G - Other Eye Makeup Preparations	1
TOCOPHERYL SUCCINATE	12C - Face and Neck (exc shave)	1
TOCOPHERYL SUCCINATE	12D - Body and Hand (exc shave)	1
TOCOPHERYL SUCCINATE	12F - Moisturizing	3
TOCOPHERYL SUCCINATE	12J - Other Skin Care Preps	2
DIOLEYL TOCOPHERYL METHYLSILANOL	07E - Lipstick	5
DIOLEYL TOCOPHERYL METHYLSILANOL	08A - Basecoats and Undercoats	1
DIOLEYL TOCOPHERYL METHYLSILANOL	12C - Face and Neck (exc shave)	1
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	03D - Eye Lotion	4
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	04E - Other Fragrance Preparation	1
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	07I - Other Makeup Preparations	1
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	12C - Face and Neck (exc shave)	7
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	12D - Body and Hand (exc shave)	3
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	12F - Moisturizing	16
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	12G - Night	1
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	12I - Skin Fresheners	1
POTASSIUM ASCORBYL TOCOPHERYL PHOSPHATE	12J - Other Skin Care Preps	3
TOCOPHERSOLAN	03D - Eye Lotion	1
TOCOPHERSOLAN	05I - Other Hair Preparations	2
TOCOPHERSOLAN	07E - Lipstick	1
TOCOPHERSOLAN	08C - Nail Creams and Lotions	1
TOCOPHERSOLAN	12C - Face and Neck (exc shave)	20
TOCOPHERSOLAN	12D - Body and Hand (exc shave)	3
TOCOPHERSOLAN	12F - Moisturizing	7
TOCOPHERSOLAN	12J - Other Skin Care Preps	1
TOCOPHERSOLAN	13C - Other Suntan Preparations	1
TOCOTRIENOLS	02B - Bubble Baths	7
TOCOTRIENOLS	02D - Other Bath Preparations	4
TOCOTRIENOLS	03D - Eye Lotion	4
TOCOTRIENOLS	03G - Other Eye Makeup Preparations	2
TOCOTRIENOLS	05A - Hair Conditioner	4
TOCOTRIENOLS	05F - Shampoos (non-coloring)	3
TOCOTRIENOLS	05G - Tonics, Dressings, and Other Hair Grooming	1
TOCOTRIENOLS	05I - Other Hair Preparations	1
TOCOTRIENOLS	07E - Lipstick	61
TOCOTRIENOLS	07I - Other Makeup Preparations	23
TOCOTRIENOLS	08B - Cuticle Softeners	1

TOCOTRIENOLS	08C - Nail Creams and Lotions	2
TOCOTRIENOLS	10A - Bath Soaps and Detergents	11
TOCOTRIENOLS	10E - Other Personal Cleanliness Products	214
TOCOTRIENOLS	11G - Other Shaving Preparation Products	1
TOCOTRIENOLS	12A - Cleansing	6
TOCOTRIENOLS	12C - Face and Neck (exc shave)	14
TOCOTRIENOLS	12D - Body and Hand (exc shave)	12
TOCOTRIENOLS	12F - Moisturizing	44
TOCOTRIENOLS	12G - Night	2
TOCOTRIENOLS	12H - Paste Masks (mud packs)	1
TOCOTRIENOLS	12J - Other Skin Care Preps	14
TOCOTRIENOLS	13C - Other Suntan Preparations	1

Ascorbyl Tocopheryl Acetate		0

ASCORBYL TOCOPHERYL MALEATE	03D - Eye Lotion	7
ASCORBYL TOCOPHERYL MALEATE	03G - Other Eye Makeup Preparations	2
ASCORBYL TOCOPHERYL MALEATE	07E - Lipstick	1
ASCORBYL TOCOPHERYL MALEATE	11G - Other Shaving Preparation Products	1
ASCORBYL TOCOPHERYL MALEATE	12F - Moisturizing	15
ASCORBYL TOCOPHERYL MALEATE	12G - Night	9
ASCORBYL TOCOPHERYL MALEATE	12H - Paste Masks (mud packs)	1
ASCORBYL TOCOPHERYL MALEATE	12J - Other Skin Care Preps	2

SODIUM TOCOPHERYL PHOSPHATE	03D - Eye Lotion	1
SODIUM TOCOPHERYL PHOSPHATE	03G - Other Eye Makeup Preparations	1
SODIUM TOCOPHERYL PHOSPHATE	11D - Preshave Lotions (all types)	1
SODIUM TOCOPHERYL PHOSPHATE	11G - Other Shaving Preparation Products	2
SODIUM TOCOPHERYL PHOSPHATE	12C - Face and Neck (exc shave)	3
SODIUM TOCOPHERYL PHOSPHATE	12G - Night	1
SODIUM TOCOPHERYL PHOSPHATE	12J - Other Skin Care Preps	1

Tocopheyrl Phosphate		0

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Final Report on the Safety Assessment of Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan[1]

Tocopherol and its several ester and ether derivatives all function as antioxidants in cosmetic formulations; they also have other functions, such as skin conditioning. Tocopheryl Acetate, Tocopherol, and Tocopheryl Linoleate are used in 2673 formulations, generally at concentrations of up to 36%, 5%, and 2%, respectively, although Tocopheryl Acetate is 100% of vitamin E oil. Tocophersolan, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, and Potassium Ascorbyl Tocopheryl Phosphate, combined, are used in 36 formulations at concentrations lower than those reported for the frequently used ingredients. Tocopherol may be isolated from vegetable oils or synthesized using isophytol and methylhydroquinone. Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, and Tocopheryl Succinate all were absorbed in human skin. In rat skin, Tocopheryl Acetate is hydrolyzed to Tocopherol. Tocopherol is a natural component of cell membranes thought to protect against oxidative damage. Tocopherol, Tocopheryl Acetate, and Tocopheryl Succinate each were reported to protect against ultraviolet radiation–induced skin damage. These ingredients are generally not toxic in animal feeding studies, although very high doses (≥2 g/kg/day) have hemorrhagic activity. These ingredients are generally not irritating or sensitizing to skin or irritating to eyes, although a Tocopheryl Acetate did produce sensitization in one animal test, and Tocophersolan was a slight eye irritant in an animal test. Reproductive and developmental toxicity tests in animals using Tocopherol, Tocopheryl Acetate, Tocopheryl Succinate, and Tocophersolan were all negative or showed some effect of reducing toxicity. Tocopherol, Tocopheryl Acetate, Tocopheryl Succinate, and Dioleyl Tocopheryl Methylsilanol were almost uniformly negative. These ingredients exhibit antimutagenic activity consistent with their antioxidant properties. Tocopherol was not carcinogenic. The ability of Tocopherol, Tocopheryl Acetate, and Tocopheryl Succinate to modulate the carcinogenic effect of other agents (e.g., tumor promotion) has been extensively studied. One study showing tumor promotion in mice may be discounted as not reproducible and not consistent with the large volume of data suggesting that the antioxidant properties of these agents protect against tumor induction. Specifically, the frequent use of Tocopherol as a negative control in other tumor promotion studies suggests that Tocopherol is not a tumor promoter. Tocopherol has been shown to reduce the photocarcinogenic effect of ultraviolet radiation in mice. Similar studies with Tocopheryl Acetate and Tocopheryl Succinate, however, demonstrated some enhancement of photocarcinogenesis, although the effect was not dose related. In clinical studies, Tocopherol, Tocopheryl Acetate, and Tocopheryl Nicotinate were not irritants or sensitizers. A report of a large number of positive patch-tests to Tocopheryl Linoleate in one cosmetic product were considered to result from a contaminant or metabolite. The Cosmetic Ingredient Review Expert Panel considered that these data provide an adequate basis on which to conclude that Tocopherol, Tocophersolan, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, and Potassium Ascorbyl Tocopheryl Phosphate are safe as used in cosmetic formulations. Although there were no inhalation toxicity data, these ingredients are used at such low concentrations in hair sprays that no inhalation toxicity risk was considered likely. Because methylhydroquinone is used in the chemical synthesis of Tocopherol, there was concern that hydroquinone may be present as an impurity. In such cases, residual levels of hydroquinone would be expected to be limited to those achieved by good manufacturing practices.

INTRODUCTION

This report reviews the safety of Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan. All of these ingredients function as antioxidants, and most function as skin-conditioning agents (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1999a–f; Wenninger and McEwen 1997). Because these ingredients all have Tocopherol in their structure and function in a similar manner as cosmetic ingredients, they have been grouped with the expectation that safety test data on one will be applicable to all.

Vitamin E, a fat-soluble vitamin, designates a group of compounds that have qualitatively the biological activity of α-tocopherol. Biological activity of a vitamin nature is expressed

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Monice Zondlo Fiume, former Scientific Analyst. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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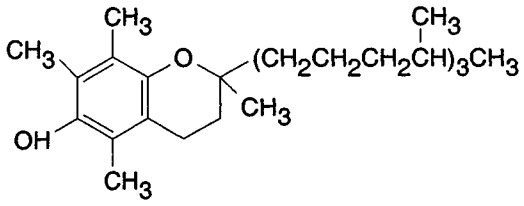
by α-, β-, γ-, and δ-tocopherol, each of which can exist in various stereoisomeric forms (Vanderveen and Vanderveen 1990). Vitamin E is also comprised of α-, β-, γ-, and δ-tocotrienol (Schmidt and Nikoleit 1993); tocopherols differ from tocotrienols only in the side chain, which is saturated in tocopherols and unsaturated in tocotrienols (Vanderveen and Vanderveen 1990). Both the tocopherols and the tocotrienols occur in nature. α-Tocopherol is the most important member because of activity and occurrence. In the esters of α-tocopherol, the reactive hydroxyl group is protected and these ingredients are more stable (Papas 1993).

CHEMISTRY

Definition and Structure

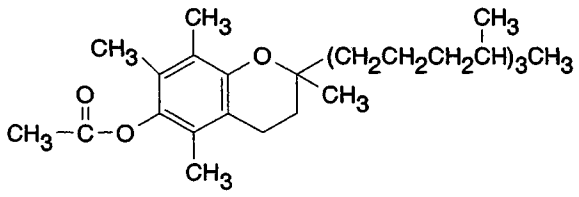
Tocopherol—CAS nos. 59-02-9 (*d*-alpha), 1406-18-4, 10191-41-0 (Wenninger and McEwen 1997), and 2074-53-5 (DL-α) (National Academy of Science [NAS] 1996), a 6-chromal derivative (Nachbar and Korting 1995)—is the organic compound that conforms to the following formula (Wenninger and McEwen 1997):



Tocopherol is also known as Vitamin E; Natural Vitamin E; D-Alpha Tocopherol; DL-α-Tocopherol; Mixed Tocopherols (Wenninger and McEwen 1997); α-Tocopherol (Lewis 1993a; Budavari 1989); *d*-α-Tocopherol; *dl*-α-Tocopherol; (*R*,*R*,*R*)-α-Tocopherol; (2*R*,4′*R*,8′*R*)-α-Tocopherol (Lewis 1993a); 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2H-1-Benzopyran-6-ol (Wenninger and McEwen 1997; Budavari 1989); 2H-1-Benzopyran-6-ol, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)- (Wenninger and McEwen 1997); *dl*-2,5,7,8-Tetramethyl-2-(4′,8′,12′-Trimethyltridecyl)-6-Chromanol (CTFA 1999a); 2,5,7,8-Tetramethyl-2-(4′,8′,12′-Trimethyltridecyl)-6-Chromanol (Budavari 1989); 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-6-Chromanol (Vanderveen and Vanderveen 1990); *dl*-2,5,7,8-Tetramethyl-2-(4′,8′,12′-Trimethyltridecyl)-6-Chromanol (Lewis 1993b); 5,7,8-Trimethyltocol (Lewis 1993a; Lide 1993; Vanderveen and Vanderveen 1990; Budavari 1989); Racemic 5,7,8-Trimethyltocol, and all-*rac*-alpha Tocopherol (BASF 1994a).

International agreement specifies that natural α-tocopherol should be designated as *RRR*-α-tocopherol and the synthetic form as all-*rac*-α-tocopherol (Papas 1993). However, the natural and synthetic forms are often designated as *d*- and *dl*-α-tocopherol, respectively, in commercial use and in published literature.

Tocopheryl Acetate (CAS nos. 7695-91-2 [Wenninger and McEwen 1997]; 58-95-7 (*d*-α-) [NAS 1996]) is the ester of Tocopherol (q.v.) and acetic acid (Wenninger and McEwen 1997) that conforms to the following formula (CTFA, personal correspondence):

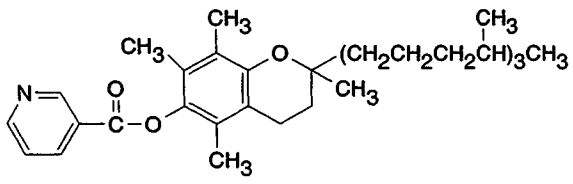


Tocopheryl Acetate is also known as D-Alpha Tocopheryl Acetate; DL-Alpha Tocopheryl Acetate; D-α-Tocopheryl Acetate; DL-α-Tocopheryl Acetate; Vitamin E Acetate; 2H-1-Benzopyran-6-ol, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-, Acetate; (Wenninger and McEwen 1997); 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2H-1-Benzopyran-6-ol Acetate (Wenninger and McEwen 1997; Hoffmann-LaRoche 1995; Budavari 1989); α-Tocopherol Acetate; α-Tocopheryl Acetate (Budavari 1989) all-*rac* alpha-Tocopherol Acetic Acid Ester; 5,7,8-Trimethyltocol Acetate (BASF 1993a); *dl*-2,5,7,8-Tetramethyl-6-Acetoxy-2-(4′,8′,12′-Trimethyltridecyl) Chromane (CTFA 1999b); 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-6-Chromanol Acetate (Hoffmann-LaRoche 1995; Budavari 1989; Roche 1994); and (2*RS*, 4′*RS*, 8′*RS*)-2,5,7,8-Tetramethyl-2-(4′,8′,12′-Trimethyltridecyl)-6-Chromanyl-Acetat (Hoffmann-LaRoche 1996).

Tocopheryl Linoleate (CAS no. 36148-84-2) is the ester of Tocopherol (q.v.) and linoleic acid (q.v.) (Wenninger and McEwen 1997). Tocopheryl Linoleate is also known as D-Alpha Tocopheryl Linoleate; DL-Alpha Tocopheryl Linoleate; DL-α-Tocopheryl Linoleate; Vitamin E Linoleate; and 9,12-Octadecadienoic Acid, 3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2H-1-Benzopyran-6-yl Ester.

Tocopheryl Linoleate/Oleate (no CAS no. found) is the ester of Tocopherol (q.v.) and a mixture of linoleic acid (q.v.) and oleic acid (q.v.) (Wenninger and McEwen 1997). Tocopheryl Linoleate/Oleate is also known as DL-α-Tocopheryl Linoleate/Oleate.

Tocopheryl Nicotinate (CAS nos. 16676-75-8, 51898-34-1) is the ester of Tocopherol (q.v.) and nicotinic acid (Wenninger and McEwen 1997) that conforms to the following formula (CTFA, personal correspondence):



Tocopheryl Nicotinate is also known as D-Alpha Tocopheryl Nicotinate; DL-Alpha Tocopheryl Nicotinate; DL-α-Tocopheryl Nicotinate; Vitamin E Nicotinate; 3-Pyridinecarboxylic Acid,

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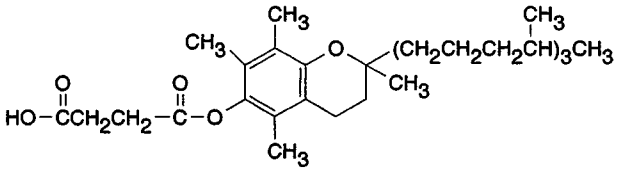
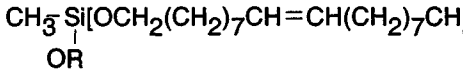
3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2H-1-Benzopyran-6-yl Ester (Wenninger and McEwen 1997); and all-*rac*-alpha-Tocopherol Nicotinate (BASF 1994b).

Tocopheryl Succinate (CAS no. 4345-03-3) is the ester of Tocopherol (q.v.) and succinic acid (Wenninger and McEwen 1997) that conforms to the following formula (CTFA, personal correspondence):



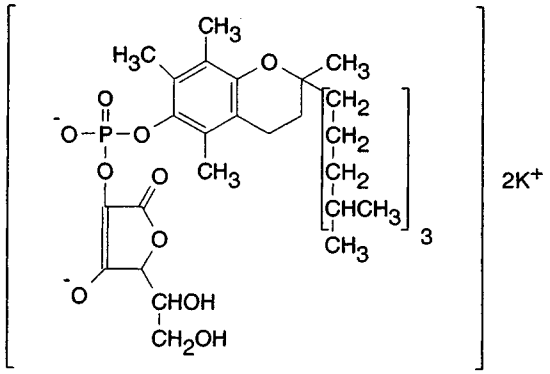
Tocopheryl Succinate is also known as D-Alpha Tocopheryl Succinate; DL-Alpha Tocopheryl Succinate; Vitamin E Succinate; Butanedioic Acid, Mono[3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2Hgl-Benzopyran-6-yl] Ester (Wenninger and McEwen 1997); α-Tocopheryl Acid Succinate; and Vitamin E Acid Succinate (Budavari 1989).

Dioleyl Tocopheryl Methylsilanol (CAS nos. 143-28-2; 130986-04-8 [Exsymol 1988]) is the dioleyl ether of Tocopheryl Acetate (q.v.) monoether with methysilanetriol (Wenninger and McEwen 1997) that conforms to the formula (Exsymol 1988):



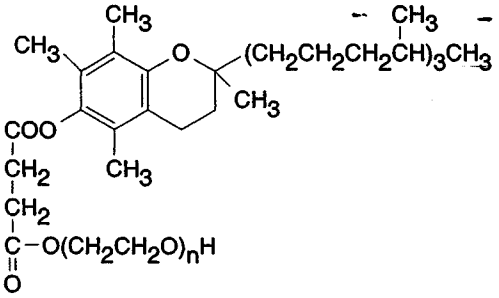
R = Tocopheryl Acetate

Potassium Ascorbyl Tocopheryl Phosphate (no CAS no. found) is the compound that conforms to the formula (Wenninger and McEwen 1997):



Potassium Ascorbyl Tocopheryl Phosphate is also known as L-Ascorbic Acid 2-[3,4-Dihydro-2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)-2H-1-Benzopyran-6-yl Hydrogen Phosphate] Potassium Salt (CTFA 1999c).

Tocophersolan (CAS no. 30999-06-5) is the compound that conforms to the formula (Wenninger and McEwen 1997):



Where n has an average value of 22.

Tocophersolan is also known as α-Tocopheryl Polyethylene Glycol 1000 Succinate. This ingredient is a water-soluble form of vitamin E (Krasavage and Terhaar 1977).

Physical And Chemical Properties

Physical and chemical properties are described in Table 1.

Manufacture and Production

Tocopherol is isolated on a commercial scale from vegetable oils, usually by molecular distillation, extraction with organic solvents, or by absorption chromatography (Vanderveen and Vanderveen 1990). Tocopherol is prepared synthetically by condensing isophytol with tri-, di-, or monomethylhydroquinone (Papas 1993). When produced synthetically, racemic mixtures of eight stereoisomers are formed.

Tocopheryl Acetate is prepared by the esterification of *dl*-α-Tocopherol with acetic acid (CTFA 1999b).

Tocopheryl Linoleate/Oleate is prepared by the esterification of Tocopherol and a mixture of linoleic and oleic acids (CTFA 1999d).

Tocopheryl Nicotinate is prepared by the esterification of Tocopherol and nicotinic acid (CTFA 1999e).

Tocopheryl Succinate is obtained by the vacuum steam distillation and succinylation of edible vegetable oil products (NAS 1996). Tocopheryl Succinate can also be prepared by treating α-Tocopherol with succinic anhydride in pyridine (Budavari 1989).

Dioleyl Tocopheryl Methylsilanol—no information was available on the manufacture of this ingredient. A manufacturer reported that 1 kg of a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol contains 5.0 g monomethylsilanetriol, of which is 1.5 g silicon, 0.5 g Tocopheryl Acetate, and 1000 g oleic acid qsp (Exsymol 1988).

Potassium Ascorbyl Tocopheryl Phosphate is manufactured using a phosphate diester linkage of vitamin E and vitamin C, formulated as a potassium salt (CTFA 1999c).

Tocophersolan is prepared from crystalline *d*-α-Tocopheryl Succinate by esterification of the acid group with polyethylene glycol (Krasavage and Terhaar 1977).

Natural Occurrence

Tocopherol, which occurs in nature in four forms, i.e., *d*-α-, *d*-β-, *d*-γ-, and *d*-δ- (Papas 1993), is found largely in plant

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TABLE 1
Physical and chemical properties

Property	Description	Reference
	Tocopherol	
Physical characteristics	Viscous, oily liquid with a weak odor	CTFA 1999a
	Viscous yellow to reddish almost odorless clear oil	BASF 1998a
	Transparent, brownish-red, viscous oil with a characteristic odor	Henkel 1996
	Yellow to amber, nearly odorless, clear, viscous oil	NAS 1996
	Light brown liquid with a mild, oily odor	BASF 1995
	Red to reddish-brown viscous liquid with a slight, mild odor	Papas 1993
	Slightly viscous, pale yellow oil	Lide 1993; Budavari 1989
	Viscous, yellow to reddish brown practically odorless clear oil	BASF 1994a
	Red, nearly odorless, viscous oil (*d*-α-Tocopherol)	*21 CFR 184.1890*
	Pale yellow viscous oil (*dl*-α-Tocopherol)	
Molecular formula	$C_{29}H_{50}O_2$	Wenninger and McEwen 1997
Molecular weight (Da)	430.7	BASF 1994a
	430.71	Lide 1993
	430.69	Budavari 1989
	(α-) 430.72	Papas 1993
	(β-) 416.69	
	(γ-) 416.69	
	(δ-) 402.67	
Melting point	3°C	BASF 1995
	2.5°C–3.5°C	Lide 1993; Budavari 1989
Boiling point	200°C–220°C (0.1 mm Hg)	CTFA 1999a
	350°C	BASF 1995
	210°C (0.1 mm Hg); decomposes at 350°C	Lide 1993
	200°C–220°C (0.1 mm Hg)	Budavari 1989
Solubility	Freely soluble in ether, chloroform, acetone, and vegetable oils; soluble in dehydrated alcohol; practically insoluble in water	CTFA 1999a
	Freely soluble in alcohol; miscible with acetone, chloroform, ether, fats, and vegetable oil; insoluble in water	NAS 1996
	Sparingly soluble in water; soluble in many organic solvents	BASF 1995
	Soluble in hydrocarbons, chlorinated hydrocarbons, alcohols, fats, and oils; insoluble in water	BASF 1994a
	Soluble in alcohol, ether, and acetone	Lide 1993
	Practically insoluble in water	Lewis 1993a
	Freely soluble in oils, fats, acetone, alcohol, chloroform, ether, other fat solvents	Lewis 1993a; Budavari 1989
Density	0.947–0.958 (25°C)	CTFA 1999a
	0.947–0.958 (d_4^{20})	BASF 1994a, 1998a
	0.935 (20°C)	BASF 1995
	0.950 (d_4^{25})	Budavari 1989
Index of refraction	1.503–1.507 (25°C)	CTFA 1999a
	1.503–1.507 (n_D^{20})	BASF 1994a, 1998a
	1.5045 (n_D^{25})	Budavari 1989
	1.503–1.507 (20°C)	FDA 1998a
Specific rotation	+0.65° (alcohol) ($[\alpha]_D^{25}$)	Lide 1993
	−3.0° (benzene) ($[\alpha]_{5461}^{25}$)	Budavari 1989
	+0.32° (alcohol) ($[\alpha]_{5461}^{25}$)	
	Synthetic Tocopherol is optically inactive	Schmidt and Nikoleit 1993

(Continued)

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TABLE 1
Physical and chemical properties *(Continued)*

Property	Description	Reference
Acid value	1.0 max	Henkel 1996
	2.0 max	BASF 1994a
Flash point	Approximately 260°C	BASF 1995
Ignition temperature	Approximately 280°C	BASF 1995
Stability/reactivity	Sensitive to oxygen and light	CTFA 1999a
	Unstable to oxidizing agents; darkens on exposure to air and light	BASF 1994a, 1998a
	Sensitive to light, oxygen, alkalies, and oxidizing agents	Henkel 1996
	Not affected by high temperatures or acids; decomposes in UV light; oxidized in the presence of iron salts or rancid fats	Vanderveen and Vanderveen 1990
	Stable to heat and alkalies in the absence of oxygen; not affected by acids up to 100°C; slowly oxidized by atmospheric oxygen, rapidly by ferric and silver salts	Budavari 1989
	Oxidizes and darkens in air and on exposure to light	FDA 1998a
	Tocopheryl Acetate	
Physical characteristics	Yellowish-brown viscous, oily liquid	CTFA 1999b
	Light yellow practically odorless viscous oil	BASF 1993a, 1998b
	Light yellow almost odorless oily liquid	BASF 1996a
	Colorless to yellow or greenish-yellow, nearly odorless, clear viscous oil	NAS 1996
	Slightly yellow, clear, practically odorless viscous oil	Hoffmann-LaRoche 1995
	Clear yellowish viscous almost odorless oil	Henkel 1994
	Clear yellow nearly odorless liquid	Roche 1994
	Pale yellow viscous liquid	Budavari 1989
Molecular formula	$C_{31}H_{52}O_3$	Wenninger and McEwen 1997
Molecular weight (Da)	472.73	SpecChem* 1998a; Hoffmann-LaRoche 1995; Budavari 1989
	472.75	NAS 1996; Lide 1993
	472.76	Roche 1994; Hoffmann-LaRoche 1995
	472.8	BASF 1993a
Melting point	−27.5°C	CTFA 1999b; SpecChem* 1998a; Hoffmann-LaRoche 1995; Roche 1994
Boiling point	Decomposes	SpecChem* 1998a
	224°C (0.3 mm Hg)	CTFA 1999b; Budavari 1989
	>300°C	BASF 1996a
	443°C (750 mm Hg)	Hoffmann-LaRoche 1995
	>200°C	Roche 1994
	184°C (0.01 mm Hg)	Lide 1993; Budavari 1989
Solubility	Freely soluble in organic solvents; practically insoluble in water	CTFA 1999b
	Soluble in many organic solvents; sparingly soluble in water	BASF 1996a
	Freely soluble in alcohol; miscible with acetone, chloroform, ether, and vegetable oils; insoluble in water	NAS 1996
	"Well soluble" in ethanol, acetone, diethyl ether, chloroform, and vegetable oil; practically insoluble in water	Hoffmann-LaRoche 1995

(Continued on next page)

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TABLE 1
Physical and chemical properties *(Continued)*

Property	Description	Reference
	Soluble in ethanol; miscible with acetone, ether, chloroform, and vegetable oils; insoluble in water	Roche 1994
	Soluble in hydrocarbons, alcohols, fats, and oils; insoluble in water	BASF 1993a
	Soluble in ether, acetone, and chloroform	Lide 1993
	Freely soluble in acetone, chloroform, and ether; less readily soluble in alcohol; practically insoluble in water	Budavari 1989
Specific gravity	0.95 (25/25°C)	CTFA 1999b; Roche 1994
Density	0.947–0.966 (25°C)	CTFA 1999b
	0.9533 (water = 1)	SpecChem* 1998a
	0.952–0.966 (20°C)	BASF 1993a, 1996a, 1998b; Hoffmann-LaRoche 1995
	0.932 (60°C)	BASF 1996a
	0.9533 (21.5°C)	Lide 1993
Index of refraction	1.49–1.51 (25°C)	CTFA 1999b
	1.494–1.498 (n_D^{20})	BASF 1993a, 1998b; Hoffmann-LaRoche 1995
	1.495–1.497 (n_D^{20})	Lide 1993
	1.4950–1.4972 (n_D^{20})	Budavari 1989
Specific rotation (*d*-form)	≥+24°	Henkel 1994
	+0.25° ($[\alpha]_D^{25}$) (c = 10 in chloroform)	Budavari 1989
	+3.2° ($[\alpha]_D^{25}$) (in ethanol)	
Acid value	1.0 max	Henkel 1994
	2.0 max	Hoffmann-LaRoche 1995; BASF 1993a
Octanol/water partition coefficient	12.2	BASF 1996a
Flash point	210°C	BASF 1996a
	243°C	Hoffmann-LaRoche 1995
Ignition temperature	303°C	BASF 1996a
	320°C	Hoffmann-LaRoche 1995
Stability/reactivity	Resistant to heat and oxygen; sensitive to alkalis and strong oxidizing agents	CTFA 1999b; BASF 1993a, 1998b
	Combustible	SpecChem* 1998a
	Fairly stable to heat and air, but sensitive to light and alkalies	Hoffmann-LaRoche 1995
	Resistant against light and oxygen; unstable in the presence of alkalis and oxidizing agents; may crystallize if stored at temperatures <4°C	Henkel 1994
	Unstable in the presence of alkali	NAS 1996
	Tocopheryl Linoleate	
Molecular formula	$C_{47}H_{80}O_3$	Wenninger and McEwen 1997
	Tocopheryl Linoleate/Oleate	
Physical characteristics	Oily liquid with a faint odor	CTFA 1999d
Solubility	Insoluble in water	CTFA 1999d
Reactivity	Prone to oxidation	CTFA 1999d
	Tocopheryl Nicotinate	
Physical characteristics	Yellow to orange-yellow liquid or solid with a faint odor	CTFA 1999e
	Yellow solid-liquid with a mild odor	BASF 1996b
	Viscous, yellow oil which crystallizes at room temperature; lipophillic	BASF 1994b

(Continued)

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TABLE 1
Physical and chemical properties *(Continued)*

Property	Description	Reference
Molecular formula	$C_{35}H_{53}NO_3$	Wenninger and McEwen 1997
Molecular weight (Da)	535.8	BASF 1994b
Melting point	45°C	CTFA 1999e
	47°C	BASF 1996b
Solubility	Soluble in ethyl alcohol, acetone, chloroform, dimethylformamide (DMF), and vegetable oils; insoluble in water	CTFA 1999e
	Soluble in many organic solvents; sparingly soluble in water	BASF 1996b
	Soluble in organic solvents, fats, and oils; insoluble in water	BASF 1995
Loss on drying	≤0.5% (2 h at 105°C)	BASF 1994b
Density	0.97 (50°C)	BASF 1996b
Flash point	265°C	BASF 1996b
Ignition temperature	330°C	BASF 1996b
Stability	Stable	CTFA 1999e
	Not resistant to strong acids and alkalis	BASF 1994b
	Tocopheryl Succinate	
Physical characteristics	White to yellowish solid with a characteristic odor	CTFA 1999f
	Solid	SpecChem* 1998b
	White to off-white crystalline powder with little or no taste or odor	NAS 1996
	White powder	Budavari 1989
Molecular formula	$C_{33}H_{54}O_5$	Wenninger and McEwen 1997
Molecular weight (Da)	530.87	SpecChem* 1998b
	530.79	NAS 1996
	530.76	Budavari 1989
Melting point	70°C–75°C	CTFA 1999f
	75°C	SpecChem* 1998b
	Approximately 75°C	NAS 1996
	76°C–77°C	Budavari 1989
Boiling point	Decomposes	CTFA 1999f; SpecChem* 1998b
Solubility	Soluble in ethyl alcohol (20°C); insoluble in water (20°C)	CTFA 1999f
	Very soluble in chloroform; soluble in acetone, alcohol, ether, and vegetable oils; insoluble in water	NAS 1996
	Soluble in alcohol, ether, acetone, or vegetable oils; slightly soluble in alcohol solutions; insoluble in water	Vanderveen and Vanderveen 1990
	Practically insoluble in water	Budavari 1989
Specific rotation *D-α* form	> +24° ($[\alpha]_D^{25}$)	NAS 1996
Stability/reactivity	Combustible	SpecChem* 1998b
	Stable in air; unstable to alkali and heat	NAS 1996
	Dioleyl Tocopheryl Methylsilanol (mixture with oleyl alcohol)	
Physical characteristics	Slightly yellow, limpid liquid with an oily and viscous aspect and a slightly aromatic odor	Exsymol 1988 viscous aspect
Solubility	Miscible with most fatty compounds and ethyl alcohol; not miscible with water	Exsymol 1988
Density	Approximately 0.85 (20°C)	Exsymol 1988
Refractive index	Approximately 1.45 (20°C)	Exsymol 1988
Saponification index	<2	Exsymol 1988

(Continued on next page)

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TABLE 1
Physical and chemical properties *(Continued)*

Property	Description	Reference
	Potassium Ascorbyl Tocopheryl Phosphate	
Physical characteristics	White to yellowish white with a faint characteristic odor; hygroscopic	CTFA 1999c
Solubility	Soluble in water; sparingly soluble in methyl alcohol; practically insoluble in ethyl alcohol, acetone, ethyl acetate, ether, chloroform, and tetrahydrofuran; insoluble in glacial acetic acid	CTFA 1999c
Stability	Stable against heat and light	CTFA 1999c
	Tocophersolan	
Physical characteristics	Pale yellow, practically tasteless waxy substance	Krasavage and Terhaar 1977
Molecular formula	$C_{33}H_{54}O_5 \cdot (C_2H_4O)_n$ (average value of n = 22)	Wenninger and McEwen 1997
Average molecular weight (Da)	1000	Krasavage and Terhaar 1977
Solubility	Forms a clear solution in water at concentrations of $\leq$20%	Krasavage and Terhaar 1977
Stability	Stable in air; solutions do not hydrolyze under normal handling and storage	Krasavage and Terhaar 1977
	Stable under normal handling and storage conditions	Madhavi and Salunkhe 1996

*Spectrum Chemical Manufacturing Corporation.

materials (Budavari 1989); the typical concentration in mixed Tocopherol products is 10% to 20%, 1% to 3%, 55% to 66%, and 18% to 33% α-, β-, γ-, and δ-Tocopherol, respectively (Papas 1993). α-, β-, γ-, and δ-Tocotrienol, which differ from Tocopherol only in the side chain, are also found in nature; the side chain is saturated in Tocopherol and unsaturated in tocotrienol. Tocopherol is found in vegetable fats and oils, dairy products, meat, eggs, cereals, nuts, and leafy green and yellow vegetables (Vanderveen and Vanderveen 1990). The general range of tocopherols found in nature is 0.01 to 200 mg tocopherols per 100 g food (Tomassi and Silano 1986). Vegetable oils, such as wheat germ, cottonseed, corn, soybean, and sunflower oil, have 50 to 300 mg Tocopherol/100 g. Peanut, olive, and coconut oil and cabbage, spinach, and asparagus have 5 to 10 mg Tocopherol/100 g. Eggs, butter, cheese, meat, other vegetables, fruit, and cereals have 0.5 to 5 mg Tocopherol/100 g. Fish oils are low or devoid of Tocopherol (Tsallas, Molgat, and Jeejeebhoy 1986). The relative proportions of α-, β-, γ-, and δ-tocopherol vary with plant source, and the tocopherol content depends on the season, plant maturation stage, time and methods of harvesting, method of storage, and genetic factors (Tomassi and Silano 1986).

Analytical Methods

Tocopherol has been determined in cosmetic formulations using gas-liquid chromatography (GLC); recovery of Tocopherol ranged from 91% to 102% (Sheppard and Stutsman 1977). Tocopherol has been determined using GLC coupled with a visible-light detector, high-performance liquid chromatography (HPLC) using an ultraviolet (UV) detector (Vanderveen and Vanderveen 1990), reverse-phase HPLC (Kagan et al. 1992), HPLC with fluorescence detection (FDA 1998b), reverse-phase HPLC with electrochemical detection, UV-based detection (Liebler et al. 1996), and the Emmerie-Engel reaction (FDA 1998c). Tocopherol has been determined in serum using liquid chromatography (DeLeenheer, De Bevere, and Claeys 1979), a liquid-chromatographic separation with wavelength-programmed UV/visible absorbance and amperometeric electrochemical detection (MacCrehan and Schönberger 1987), and HPLC (Kock et al. 1997); in tissue samples using a negative-ion chemical ionization mass-spectrometry method (McClure and Liebler 1995) and a stable-isotope dilution capillary gas chromatography-mass spectrometry assay (Liebler et al. 1996); and in human red blood cells using HPLC with UV detection (Moyano et al. 1997).

Tocopheryl Acetate was determined in cosmetic formulations using GLC; recovery of Tocopheryl Acetate ranged from 93% to 103% (Sheppard and Stutsman 1977). Tocopheryl Acetate was determined in a water-based multivitamin mixture using solid-phase extraction and reverse-phase HPLC (Savolainen et al. 1988).

Impurities/Composition

Tocopherol. Food-grade Tocopherol, as DL-α-Tocopherol, must consist of not less than 96% and not more than 102% $C_{29}H_{50}O_2$, and heavy metals (as Pb) must be <10 mg/kg (NAS 1996). Food-grade D-α-Tocopherol concentrate must consist of

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not less than 40.0% of total tocopherols, of which not less than 95.0% consists of D-α-Tocopherol ($C_{29}H_{50}O_2$), and heavy metals (as Pb) must be <10 mg/kg.

BASF (1994a) reported that food- and United States Pharmacopeia (USP)-grade Tocopherol contains ≤20 mg/kg heavy metals (USP), ≤3 mg/kg arsenic, ≤10 mg/kg lead, ≤25 mg/kg zinc, ≤50 mg/kg zinc + copper, and ≤0.1% sulfate ash. Tocopherol is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, residual reactants, or other substances (BASF 1998c). Stabilizers are not added to Tocopherol during or after manufacturing.

Tocopherol, an oil of natural origin, contains low quantities of other vegetable oil constituents due to the raw materials (Henkel 1996).

Tocopheryl Acetate. Food-grade Tocopheryl Acetate, as DL-α- and D-α-Tocopheryl Acetate, must consist of not less than 96% and not more than 102% $C_{31}H_{52}O_3$, and heavy metals (as Pb) must be <10 mg/kg (NAS 1996). USP-grade Tocopheryl Acetate must contain a minimum of 919 mg/g (1250 IE units) *RRR*-α-Tocopheryl Acetate (Henkel 1994).

Tocopheryl Acetate contains ≤1% free Tocopherol, ≤20 ppm heavy metals, ≤10 ppm lead, ≤0.1% sulfate ash (Hoffmann-LaRoche 1995; BASF 1993a), and ≤3 ppm arsenic; it meets USP requirements for organic volatile impurities (Hoffmann-LaRoche 1995). Tocopheryl Acetate is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, or other substances (BASF 1998d). Stabilizers are not added to Tocopheryl Acetate during or after manufacturing.

Tocopherol Acetate, an oil that is a protected form of naturally occurring vitamin E, contains low quantities of other vegetable oil constituents due to the raw material (CTFA 1999b; Henkel 1994).

Tocopheryl Linoleate. The purity of Tocopherol used as the raw material in manufacturing Tocopheryl Linoleate was demonstrated using HPLC (Ennagram 1999). More than 97% of the material appears under a sharp peak.

Tocopheryl Linoleate/Oleate. Tocopheryl Linoleate/Oleate is composed of >50% *dl*-α-Tocopheryl Linoleate, >25% *dl*-α-Tocopheryl Oleate, and >21% other *dl*-α-Tocopherol esters (CTFA 1999d).

Tocopheryl Nicotinate. Tocopheryl Nicotinate contains ≤0.1% free nicotinic acid (BASF 1994b) and ≤20 ppm heavy metals (BASF 1996c). Tocopheryl Nicotinate is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, or other substances. Stabilizers are not added to Tocopheryl Nicotinate during or after manufacturing.

Tocopheryl Succinate. Food-grade Tocopheryl Succinate must consist of not less than 96% and not more than 102% $C_{33}H_{54}O_5$, and heavy metals (as Pb) must be <10 mg/kg (NAS 1996). It is not known what residual concentrations of pyridine might be present if Tocopheryl Succinate is prepared by treating α-Tocopherol with succinic anhydride in pyridine as described in the previous section.

Dioleyl Tocopheryl Methylsilanol. One manufacturer reported that a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol does not contain preservatives (Exsymol 1998).

Tocophersolan. Eastman Chemical Co. (1999) specifies that Tocophersolan is to have a monoester content of 70% to 80%, and their historical records indicate an average monoester concentration of 74.7%. The major impurities include approximately 10% Tocophersolan diester, 10% free polyethyleneglycol (PEG), and 1% free α-Tocopherol. Minor impurities include succinic acid ester of Tocophersolan monoester, free succinic acid, propionate ester of Tocophersolan, ethyl ester of Tocopheryl Succinate, methyl ester of Tocopheryl Succinate, and α-Tocopheryl Succinate (raw material).

Ultraviolet Absorbance

Tocopherol, Tocopheryl Acetate, and Tocopheryl Succinate absorb in the UVB range. The reported absorption maxima (in ethanol) are 292 (BASF 1994a), 294 (Budavari 1989), and 295 (Kagan et al. 1992) nm for Tocopherol; 284 (in ethanol) (BASF 1993a) and 285.5 (in cyclohexane) nm for Tocopheryl Acetate; and 286 nm (in ethanol) for Tocopheryl Succinate (Budavari 1989).

USE

Cosmetic

The ingredients reviewed in this report all function as antioxidants, but have other functions (CTFA 1999a–f; Wenninger and McEwen 1997) as shown in Table 2.

Product formulation data submitted by the Food and Drug Administration (FDA) in 1998, based on industry data, reported that Tocopherol was present in a total of 1072 cosmetic formulations, Tocopheryl Acetate was present in 1322 formulations, Tocopheryl Linoleate was present in 279 formulations, Tocopherol Nicotinate was present in 3 formulations, Tocopheryl Succinate was present in 4 formulations, Dioleyl Tocopheryl Methylsilanol was present in 12 formulations, Potassium Ascorbyl Tocopheryl Phosphate was present in 15 formulations, and Tocophersolan was present in 2 formulations (FDA 1998d) (Table 3).

Concentration of use values are no longer reported to the FDA by the cosmetics industry (FDA 1992). Data submitted to CTFA reported that Tocopherol was used at concentrations of ≤5% (CTFA 1999 g; Henkel 1998), Tocopheryl Acetate was used at concentrations of ≤36% (and at 100% in vitamin E oil), Tocopheryl Linoleate was used at concentrations of ≤2%, Tocopheryl Nicotinate was used at concentrations of ≤1% (CTFA 1999 g), with recommended concentrations of use of 0.1% to 1.0% (BASF 1994b), Dioleyl Tocopheryl Methylsilanol and oleyl alcohol (0.05% Tocopheryl Acetate, 0.5% mono methylsilanetriol, remainder oleic alcohol) were recommended for use at concentrations of 3% to 6% (Exsymol 1998),

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TABLE 2
Ingredient functions (CTFA 1999a–f; Wenninger and McEwen 1997)

Ingredient	Function(s)
Tocopherol	Antioxidant; humectant; skin protectant; skin-conditioning agent—humectant; skin-conditioning agent—occlusive; skin-conditioning agent—emollient; skin-conditioning agent—miscellaneous
Tocopheryl Acetate	Antioxidant; humectant; skin protectant; skin-conditioning agent—humectant; skin-conditioning agent—emollient; skin-conditioning agent—miscellaneous
Tocopheryl Linoleate	Antioxidant; skin-conditioning agent—miscellaneous
Tocopheryl Linoleate/Oleate	Antioxidant; skin-conditioning agent—emollient; skin-conditioning agent—miscellaneous
Tocopheryl Nicotinate	Antioxidant; skin-conditioning agent—emollient; skin-conditioning agent—miscellaneous; oral health care drugs
Tocopheryl Succinate	Antioxidant; humectant; skin protectant; skin-conditioning agent—humectant; skin-conditioning agent—emollient
Dioleyl Tocopheryl Methylsilanol	Antioxidant; skin-conditioning agent—miscellaneous
Potassium Ascorbyl Tocopheryl Phosphate	Antioxidant; anti-dandruff agent
Tocophersolan	Antioxidant

Potassium Ascorbyl Tocopheryl Phosphate was used at concentrations of 0.02%, and Tocophersolan was used at concentrations of ≤0.2% (CTFA 1999g) (Table 4).

International

Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, and Tocopheryl Nicotinate are listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category* (CLS) (Rempe and Santucci 1997). Tocopherol, as Natural Vitamin E, which conforms to the standards of the *Japanese Cosmetic Ingredients Codex* (JCIC), has precedent for use without restriction in all CLS categories, whereas Tocopherol, as DL-α-Tocopherol, which conforms to the specifications of the *Japanese Standards of Cosmetic Ingredients* (JSCI), has precedent for use without restriction in the CLS categories, cleansing preparations and nail makeup preparations and for use at ≤1% in all other CLS categories (hair care, treatment, makeup, fragrant, suntan/sunscreen, eyeliner, lip, oral, and bath preparations). Tocopheryl Acetate, as D-α-Tocopheryl Acetate, which conforms to the standards of the JCIC, has precedent for use at ≤1% in all CLS categories except eyeliner, lip, and oral preparations, in which it is not used, whereas Tocopheryl Acetate, as DL-α-Tocopheryl Acetate, which conforms to the specifications of the JSCI, has precedent for use without restriction in the CLS categories, cleansing preparations and nail makeup preparations and for use at ≤1% in all other CLS categories. Tocopheryl Linoleate, as DL-α-Tocopheryl Linoleate, which conforms to the standards of the JCIC, has precedent for use without restriction in the CLS categories, cleansing preparations and nail makeup preparations and at ≤1% in all other CLS categories except eyeliner and bath preparations, in which it is not used. Tocopheryl Nicotinate as DL-α-Tocopheryl Nicotinate, which conforms to the specifications of the JSCI, has precedent for use without restriction in the CLS categories, cleansing preparations and nail makeup preparations and at ≤1% in all other CLS categories except eyeliner preparations, in which it is not used. Other restrictions for α-Tocopherol and the α-tocopheryl ingredients include ≤5% as DL-α-Tocopherol.

Tocopherol, Tocophersolan, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, and Potassium Ascorbyl Tocopheryl Phosphate do not appear in Annex II (list of substances that must not form part of the composition of cosmetic products) or Annex III (list of substances that cosmetic products must not contain except subject to the restrictions and conditions laid down) of the *Cosmetics Directive of the European Union* (European Economic Community 1995).

Noncosmetic

Tocopherol has functional use in foods as a nutrient, dietary supplement, and antioxidant (NAS 1996). Tocopherols are generally recognized as safe (GRAS) for use in foods as chemical preservatives (*21 Code of Federal Regulations [CFR] 182.3890*) and tocopherols and Tocopheryl Acetate are GRAS in foods as nutrients (*21CFR182.8890; 182.8892*). *d*-α-Tocopherol and *dl*-α-Tocopherol, when meeting the specifications of the *Food Chemicals Codex*, are GRAS for use as inhibitors of nitrosamine formation and in pump-cured bacon at concentrations not to exceed good manufacturing practice (*21 CFR 184.1890*). In foods, tocopherols, especially mixed tocopherols, are effective antioxidants for lard, tallow, poultry fat, and other animal products (Papas 1993). Recommended use concentrations are 100 to 300 ppm of the fat or oil portion of foods; amounts >500 ppm

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TABLE 3
Product formulation data (FDA 1998d)

Product category	Total no. formulations in category	Total no. of formulations containing ingredient							
		Tcphrl*	TAcet*	TLin*	TNic*	TSucc*	DTM*	PATP*	Tsolan*
Baby shampoos	21		1						
Baby lotions, oils, powders, and creams	53		7						
Other baby products	29	2	1						
Bath oils, tablets, and salts	124	2	10	1					
Bubble baths	200		5	1					
Bath capsules	<4								
Other bath preparations	159	3	15						
Eyebrow pencil	91	5	2						
Eyeliner	514	6	9						
Eye shadow	506	79	26	56				1	
Eye lotion	18	3	4	2					
Eye makeup remover	84	1	1						
Mascara	167	9	16	2					
Other eye makeup preparations	120	18	19	4					
Colognes and toilet waters	656		5						
Perfumes	195	1							
Powders	247	3	15						
Other fragrance preparations	148	1	19						
Hair conditioners	636	37	73		1				
Hair sprays (aerosol fixatives)	261	6	24						
Permanent waves	192	1	1						
Rinses (noncoloring)	40		3						
Shampoos (noncoloring)	860	19	81	1	1				
Tonics, dressings, and other hair-grooming aids	549	17	36	2	1				
Other hair preparations	276	7	33						
Hair rinses (coloring)	33	1							
Hair bleaches	113	2	1						
Other hair-coloring preparations	59	6							
Blushers (all types)	238	57	44	28					
Face powders	250	53	14	31					
Foundations	287	76	53	19					
Lipstick	790	260	85	33			4		
Makeup bases	132	17	7	7					
Rouges	12	1	1	1					
Makeup fixatives	11	2	2						
Other makeup preparations	135	24	17	3					
Basecoats and undercoats	48		15	2					
Cuticle softeners	19	1	5						
Nail creams and lotions	17		4	2					
Nail polish and enamel	80		17	3			5		
Nail polish and enamel removers	34		3						
Other manicuring preparations	61	2	17	3					
Bath soaps and detergents	385	21	31	1					
Deodorants (underarm)	250	2							
Other personal cleanliness products	291	4	7						

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TABLE 3
Product formulation data (FDA 1998d) *(Continued)*

Product category	Total no. formulations in category	Total no. of formulations containing ingredient							
		Tcphrl*	TAcet*	TLin*	TNic*	TSucc*	DTM*	PATP*	Tsolan*
Aftershave lotion	216	3	18	2					
Preshave lotions (all types)	14		2						
Shaving cream	139	4	7						
Other shaving preparation products	60		6						
Cleansing preparations	653	14	35	1					1
Depilatories	28	3	1						
Face and neck preparations (excluding shaving)	263	23	48	9					
Body and hand preparations (excluding shaving)	796	44	96	6					
Foot powders and sprays	35	3	3						
Moisturizing preparations	769	73	169	30				7	1
Night preparations	188	18	34	4					
Paste masks (mud packs)	255	17	15	1				2	
Skin fresheners	184	3	5	1					
Other skin care preparations	692	50	93	16			3	4	
Suntan gels, creams, and liquids	136	10	33					1	
Indoor tanning preparations	62	5	19	1					
Other suntan preparations	38	3	9	6					
No. of uses under tradenames/mixtures		50				4			
1998 total		**1072**	**1322**	**279**	**3**	**4**	**12**	**15**	**2**

*Tcphrl = Tocopherol; TAcet = Tocopheryl Acetate; TLin = Tocopheryl Linoleate; TNic = Tocopheryl Nicotinate; TSucc = Tocopheryl Succinate; DTM = Dioleyl Tocopheryl Methylsilanol; PATP = Potassium Ascorbyl Tocopheryl Phosphate; Tsolan = Tocophersolan.

are not recommended because of a potential pro-oxidant effect. Additionally, Tocopherol is used in meat curing as a nitrosamine blocker (Lewis 1993b).

As a nutrient, the relationship between International Units (IU) (or the equivalent USP units) and the weight of Tocopherol is: 1 mg DL-α-Tocopherol = 1.1 IU (Vanderveen and Vanderveen 1990). According to the guidelines determined by the FDA, the US recommended daily allowances of vitamin E for labeling purposes are 5 IU for infants, 10 IU for children under 4, and 30 IU for adults, children over 4, and pregnant or lactating women. According to the Food and Nutrition Board of the National Research Council, the recommended dietary allowance for daily vitamin E are 4 to 6 IU for infants, 5 to 7 IU for children, and 12 to 15 IU for adults. The usual, prophylactic dose of Vitamin E Preparation (a form of α-Tocopherol that includes *d*- or *dl*-α-Tocopherol; *d*- or *dl*-α-Tocopheryl Acetate; and *d*- or *dl*-α-Tocopheryl Succinate) is 5 to 30 IU, and the therapeutic dosage is 4 to 5 times the recommended daily allowance (RDA) or 1 IU/kg/day (NAS 1996).

Tocopheryl Acetate and Tocopheryl Succinate both have functional use in foods as a nutrient and a dietary supplement (NAS 1996). Label claims should be based on 1 mg DL-α-Tocopheryl Acetate = 1 IU, 1 mg D-α-Tocopheryl Acetate = 1.36 IU, and 1 mg D-α-Tocopheryl Succinate = 1.21 IU, respectively. Tocopheryl Acetate also has use in pharmaceutical applications for preparations with a lipophilic base and for aqueous preparations (BASF 1993a).

Tocophersolan is used as a dietary food supplement and it is used in medicine (function not specified) (Lewis 1993b). Tocophersolan provides 260 mg of *d*-α-Tocopherol per gram (387 IU) (Krasavage and Terhaar 1977).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

General

The major route of absorption of Tocopherol is the lymphatic system. Tocopherol esters are partially hydrolyzed by intestinal esterases before they are absorbed and partially absorbed as intact esters. Tocopherols are not re-esterified during the absorption process. The most functional absorptive surface is generally located in the medial small intestine. From the lymphatic system, Tocopherols pass into the systemic circulation where they are

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TABLE 4
Concentration of use as a function of product type (CTFA 1999g)

	Concentration of use (%)					
Product category	Tocopherol	Tocopheryl Acetate	Tocopheryl Linoleate	Tocopheryl Nicotinate	Postassium Ascorbyl Tocopheryl Phosphate	Tocophersolan
Other baby products	1					
Baby lotions, oils, powders, and creams		0.001–0.1				
Bath oils, tablets, and salts	0.01	0.05				
Bubble baths		0.05–0.1				
Bath capsules		0.1				
Other bath preparations	0.8					
Eyebrow pencil	0.04–0.3	0.02–0.2				
Eyeliner	0.02–0.5	0.02–1.5				
Eye shadow	0.02–0.5	0.02–0.8	0.6–2			
Eye lotion		0.05–1				
Mascara	0.1–0.2	0.01–0.1	0.1			
Other eye makeup preparations	0.03–0.6	0.2				
Colognes and toilet waters		0.02–0.1				
Perfumes	0.04					
Powders (dusting and talcum (excluding aftershave talcum)		0.1				
Other fragrance preparations	0.5	0.02				
Hair conditioners	0.1–0.3	0.001–0.1		0.1–1		
Hair sprays (aerosol fixatives)	0.001	0.02				
Shampoos (noncoloring)	0.01–0.05	0.001–0.3		0.0001–1		
Tonics, dressings, and other hair-grooming aids	0.001–0.6	0.01–0.2		0.01		
Other hair preparations (noncoloring)				0.2		
Hair dyes and colors (all types requiring caution statement and patch test)	0.001	0.001				
Blushers (all types)	0.02–0.6	0.02–0.2	0.1			
Face powders	0.02–0.6	0.02–0.1	0.3–2			
Foundations	0.02–0.8	0.02–0.8	0.1–2	0.1	0.02	
Leg and body paints	0.3					
Lipstick	0.05–0.9	0.1–3	0.1–2		0.02	
Makeup bases	0.4	0.1–0.4				
Rouges		0.1				
Makeup fixatives	0.1	0.1–0.2				
Other makeup preparations	0.3–0.8	0.05–0.7	0.5			
Basecoats and undercoats (manicuring preparations)	0.05	0.02–0.4				
Cuticle softeners		0.05–0.1				
Nail creams and lotions	0.05	0.3–36				
Nail polish and enamel	0.3	0.02–0.1				
Nail polish and enamel removers		0.01–0.1				
Other manicuring preparations	0.3	0.02–0.1				
Bath soaps and detergents	0.02–0.2	0.001–0.6				
Deodorants (underarm)	0.05	0.2				
Other personal cleanliness products	0.03–0.4					

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TABLE 4
Concentration of use as a function of product type (CTFA 1999g) *(Continued)*

Product category	Concentration of use (%)					
	Tocopherol	Tocopheryl Acetate	Tocopheryl Linoleate	Tocopheryl Nicotinate	Postassium Ascorbyl Tocopheryl Phosphate	Tocophersolan
Aftershave lotion	0.2	0.02–0.2				
Shaving cream (aerosol/brushless/lather)	0.6	0.05–0.5	2			
Skin cleansing preparations (cold creams/ cleansing lotions/liquids/pads)	0.03	0.0001–25				
Depilatories	0.1	0.1				
Face and neck creams/lotions/powders/ sprays (excluding shaving preparations)	0.03–2	0.003–6	0.1–0.5		0.02	
Body and hand creams/lotions/powders/ sprays (excluding shaving preparations)	0.02–2	0.001–25	0.3	0.1	0.02	
Foot powders and sprays		0.1				
Moisturizing creams/lotions/powders/ sprays	0.05–2	0.1–25	0.1–2		0.02	0.2
Night creams/lotions/powders/sprays (excluding shaving preparations)	0.05	0.05–8			0.02	
Paste masks (mud packs)		0.03–0.3			0.02	
Skin fresheners	0.05	0.01–0.1				0.05
Other skin care preparations		0.001–6				
Suntan gels, creams, and liquids	0.001–0.3	0.05–1	2		0.02	
Indoor tanning preparations	0.3	0.01–0.3			0.02	
Other suntan preparations	0.01	0.5				

bound to nonspecific lipoproteins (Tomassi and Silano 1986). α-Tocopherol generally accounts for 85% to 95% of the total circulating Tocopherols (Weber, Bendich, and Machlin 1997). In the blood, Tocopherol is carried primarily in the low-density lipoprotein fraction (Tsallas, Molgat, and Jeejeebhoy 1986). From the general circulation, tocopherols are taken up by the liver and by virtually all extrahepatic tissues and concentrated in the membrane-containing structures of the cells. Tocopherol is stored mainly in adipose tissue and removed when the fat is mobilized (Vanderveen and Vanderveen 1990); liver and muscle contain the next largest stores (Tsallas, Molgat, and Jeejeebhoy 1986). Actual Tocopherol concentrations are greatest in platelets and the adrenal glands, pituitary gland, and testes. In general, mobilization of tocopherols from tissues is very low (Tomassi and Silano 1986). Excess intake of Tocopherol results mostly in the conversion to water-soluble lactone, esterification to glucuronic acid, and excretion in the urine (Tsallas, Molgat, and Jeejeebhoy 1986).

The endogenous concentration of Tocopherol in the skin is approximately 1.0 nmol/g (Kagan et al. 1992). Blood concentrations in healthy adults, children, infants, and premature infants are approximately 1, 0.8, 0.4, and 0.24 mg/dl, respectively (Tsallas, Molgat, and Jeejeebhoy 1986).

Dermal

Eleven subjects, eight males and three females, that had "at least three discrete clinically diagnosable keratoses" on their forearms were used in a study to determine whether topically applied Tocopheryl Acetate was substantially absorbed into skin and whether it converted into free Tocopherol (Alberts et al. 1996). Tocopheryl Acetate was supplied in a vehicle cream at a concentration of 125 mg/g. The subjects applied the cream to their forearms twice daily for 3 months. Clinical evaluation, blood sampling, and 3-mm punch biopsies were performed prior to and at the end of treatment. The mean concentrations found in skin, using pooled biopsy samples of two to three subjects, and plasma are given in Table 5.

Tocopheryl Acetate was not detected in three of the four pooled skin samples prior to application; a concentration of 23.6 μg/g tissue determined in one pooled skin sample was assumed to be related to prior use of a sunscreen containing Tocopheryl Acetate. The researchers stated that absorption of Tocopheryl Acetate was substantial, but systemic availability was not observed. Also, systemic biotransformation to its unesterified form did not occur.

dl-α-Tocopheryl-[1-5-methyl-^{14}C]-Acetate (3 μCi/mg) in 0.5% benzene solution was sprayed topically on 14 skin samples

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TABLE 5
Mean Tocopherol and Tocopheryl Acetate concentrations in skin and plasma (Alberts et al. 1996)

	In skin (μg/g)			In plasma (μg/g)	
Time	Tocopheryl Acetate	α-Tocopherol	γ-Tocopherol	Tocopheryl Acetate	α-Tocopherol
Baseline	5.9 ± 1.8	38.9 ± 17.9	6.0 ± 3.9	2.1 ± 0.9	12.7 ± 6.3
3 months	256.3 ± 195.5	36.3 ± 20.9	4.4 ± 2.3	2.5 ± 1.3	13.3 ± 6.1

from the heads of seven patients, and the application site was rapidly dried and the area occluded (Kamimura and Matsuzawa 1968). Penetration was determined after 4, 6, and 24 hours using autoradiography. After 4 hours, large amounts of autoradiographic silver grains were present in the horny layer of the epidermis, and some were found in the pilary canal and the secretory portion and excretory duct of the sebaceous gland. After 6 hours, large amounts of grains were present in the horny and "prickle" layers of the epidermis, the inner and outer root sheaths, the secretory portion of the sebaceous gland, and the periductal tissue of the sweat gland, and some were found in the pilary canal, the hair papilla, the excretory duct of the sebaceous gland, the periglandular tissue of the sweat gland, blood vessels, and intercellular septum. At 24 hours, large amounts of grains were present in the outer root sheath, hair papilla, and periglandular and periductal tissue of the sweat gland, and some were found in the horny and prickle layers of the epidermis, pilary canal, inner root sheath, the secretory portion and excretory duct of the sebaceous gland, and intercellular septum.

The dermal absorption and distribution of [^{14}C]-Tocopherol (specific activity 85.2 μCi/μM) was determined in human skin grafted onto male athymic nude mice (Klain 1989). A chemical dose of 10 μg Tocopherol, 1.5 μCi, was applied to a 1.45-cm^2 circular graft area on each mouse. Groups of six mice were killed 1, 2, 4, 8, and 24 hours after application. Six mice were placed in metabolic cages where expired air and urine were collected for 24 hours. One hour after dosing, the greatest amount of radioactivity was present in mouse skin, followed by muscle, liver, blood, lungs, adipose tissue, spleen, and kidneys. A linear increase with time in tissue radioactivity was observed. The skin grafts were "highly radioactive" after 4 and 24 hours. Four hours after dosing, the greatest amount of radioactivity in the skin grafts was in the epidermis; after 24 hours, it was in the dermis. No radioactivity was found in expired air. After 24 hours, 0.2% of the radioactivity was excreted in the urine.

The penetration of 5% α-Tocopherol (0.12 M), Tocopheryl Acetate (0.11 M), Tocopheryl Linoleate (0.07 M), and Tocopheryl Succinate (0.09 M) was determined using three human abdominal skin samples and the penetration of 5% α-Tocopherol was determined using three Skh:HR-1 mouse dorsal skin samples (Bissett, Chatterjee, and Hannon 1990). The skin samples were mounted in Franz-type diffusion cells, with 0.785 cm^2 skin surface area exposed. The average cumulative penetration through human skin for Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, and Tocopheryl Succinate was 14, 79, 51, and 48 μg/cm^2, respectively, after 2 hours and 227, 840, 823, and 637 μg/cm^2, respectively, after 24 hours. Using mouse skin, the average cumulative penetration of Tocopherol was 50 and 628 μg/cm^2 after 2 and 24 hours, respectively. Penetration through mouse skin was approximately three times greater than that through human skin.

In a skin penetration study, "only low amounts" of Tocopheryl Acetate penetrated through intact pig skin (Hoffmann-LaRoche 1995). (Details not provided.)

The quantitative penetration of 5% vitamin E in ethanol through rat skin was reported by Djerassi, Machlin, and Nocka (1986). The reported amount of material recovered (details not provided) from the skin surface, horny layer, other skin tissue layers, and chamber liquid after 1, 6, and 16 hours is presented in Table 6.

Groups of male Wistar rats were used to determine the penetration of Tocopherol and Tocopheryl Acetate (Beijersbergen van Henegouwen, Junginger, and de Vries 1995). One group of 10 rats was used to determine the horizontal transport into and the penetration through the epidermis. Tocopherol and Tocopheryl Acetate, both 2.5% in ethanol, were applied to a shaved

TABLE 6
Recovered Tocopherol in a skin penetration study (Djerassi, Machlin, and Nocka 1986)

	Skin surface (residual material)		Horny layer		Other skin tissue layers		Chamber liquid	
Time	μg/cm^2	%	μg/cm^2	%	μg/cm^2	%	μg/cm^2	%
1 hour	256.31	85.4	24.90	8.3	18.71	6.2	0.08	—
6 hours	162.83	54.3	21.29	7.1	115.68	38.6	0.20	0.1
16 hours	144.52	48.2	32.20	10.7	122.80	40.9	0.48	0.2

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site approximately 12 cm in diameter on the left side of the back of each rat (35 μl/cm^2), whereas ethanol only was applied to a shaved site on the right side. Two rats were killed 5 hours after dosing, and skin samples were taken from the test and control sites. This procedure was repeated over the next 4 days. Recovery of Tocopherol and Tocopheryl Acetate was equal. Tocopherol and Tocopheryl Acetate were similar in regard to penetration and horizontal migration. On day 1, 2.6% ± 2.21% and 2.7% ± 2.31% of the Tocopherol and Tocopheryl Acetate, respectively, was transported horizontally into the epidermis. On day 5, these values were 6.0% ± 1.57% and 5.8% ± 1.91%, respectively.

The amount of Tocopherol and Tocopheryl Acetate in the epidermis after a single dose or repeated applications of 0.25% Tocopheryl Acetate was also determined (Beijersbergen van Henegouwen, Junginger, and de Vries 1995). For the single dose, 0.25% Tocopheryl Acetate in ethanol (35 μl/cm^2) was applied to shaved sites approximately 20 cm in diameter on the backs of five rats. One animal was killed hourly after application, and skin samples were taken from the test site and an untreated site on the back. Five hours after pretreatment with ethanol, 17 ± 4.9 ng/mg Tocopherol was found in both the stratum corneum and viable layer. For the daily applications, 0.25% Tocopheryl Acetate in ethanol (35 μl/cm^2) was applied to a shaved site approximately 12 cm in diameter on the left side of the back of 10 rats; ethanol was applied to the right side. Two animals were killed 5 hours after dosing, and skin samples were taken from the test and control sites. This procedure was repeated over the next 4 days. The amounts measured are reported in Table 7.

The researchers determined that the amount of endogenous Tocopherol was 17 ± 4.9 ng/mg. A measurable amount of Tocopherol was not formed by hydrolysis after a single application of Tocopheryl Acetate. With repeated applications of Tocopheryl Acetate, a small but steady increase was observed in the amount of Tocopherol in the skin of treated and control sites.

D-α-[5-methyl-^{14}C]-Tocopheryl Acetate was applied to a 2 cm diameter site on the back of Skh-1 mice (Trevithick and Mitton 1993). Tocopheryl Acetate, 2.88 μCi in nonradioactive Tocopheryl Acetate or 2.41 μCi in a medium-chain triglyceride cosmetic base prepared from fractionated coconut oil, was applied for 24 hours; collars were used to prevent grooming. The doses applied were approximately 3.0 mg/cm^2 [^{14}C]-Tocopheryl Acetate in nonradioactive Tocopheryl Acetate and 0.80 mg/cm^2 in cosmetic base. After 24 hours, the sites were wiped using hexane to remove unabsorbed [^{14}C]-Tocopheryl Acetate. The skin at the site of application and two areas of untreated skin were excised.

Using liquid scintillation counting, 1.7% of [^{14}C]-Tocopheryl Acetate applied in nonradioactive Tocopheryl Acetate and 11.5% applied in the cosmetic base were removed by washing the treated site with hexane. It appeared that the application sites were not groomed by the animals. The results are summarized in Table 8. Radioactive label in the skin of irradiated test animals was significantly greater than that found in the skin of the nonirradiated test animals. The researchers stated that the results indicated that "topically applied α-tocopheryl acetate is bioconverted to the active vitamin and free radical scavenger (α-tocopheryl) within skin tissue." Also, that the results suggest that "UV-B irradiation increases both the absorption and bioconversion of α-tocopheryl acetate to α-tocopherol in the skin of hairless mice."

Mice were used to examine the conversion of Tocopheryl Acetate to Tocopherol in skin (Kramer and Liebler 1996). d^3-Tocopheryl Acetate, 5.3 μmol, was applied to the skin in an inert cream. Epidermal samples were taken prior to application and 6, 24, and 48 hours after dosing, and the amount of d^3-Tocopherol was four-, ten-, and eightfold greater, respectively,

TABLE 7

Tocopherol and Tocopheryl Acetate recovered in the skin (Beijersbergen van Henegouwen, Junginger, and de Vries 1995)

	Total epidermis (ng/mg)		Viable layer (ng/mg)		Control epidermis (ng/mg)	
Time	Tocopherol	Tocopheryl Acetate	Tocopherol	Tocopheryl Acetate	Tocopherol	Tocopheryl Acetate
			Single dose			
1 hour	14 ± 1	1160 ± 100	16 ± 1	290 ± 30		
2 hours	15 ± 4	830 ± 290	22 ± 8	230 ± 25		
3 hours	16 ± 3	1290 ± 110	16 ± 1	240 ± 30		
4 hours	17 ± 5	1230 ± 220	17 ± 1	320 ± 40		
5 hours	16 ± 2	1920 ± 330	24 ± 6	540 ± 300		
			Daily application			
Day 1	16 ± 2.1	1240 ± 180	33 ± 6.3	380 ± 70	12 ± 0.8	18 ± 5.8
Day 2	22 ± 5.2	2510 ± 50	47 ± 8.1	640 ± 125	19 ± 4.6	25 ± 7.9
Day 3	28 ± 1.9	3330 ± 110	66 ± 8.9	1400 ± 130	18 ± 1.7	47 ± 3.1
Day 4	35 ± 5.8	3930 ± 830	80 ± 12.3	1000 ± 250	19 ± 3.3	67 ± 5.4
Day 5	42 ± 4.3	4040 ± 600	102 ± 19.7	1510 ± 160	20 ± 2.6	149 ± 7.5

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TABLE 8
Recovery of Tocopherol and conversion of Tocopheryl Acetate (Trevithick and Mitton 1993)

Vehicle	Site	Total ^{14}C- (Ci/min/mg skin)	α-Tocopherol (ng/mg skin)	γ-Tocopherol (ng/mg skin)	Converted (%)
Tocopheryl Acetate	Treated	2.6 ± 0.8	91 ± 11	1.8 ± 0.3	4.5
Tocopheryl Acetate	Untreated	0.43 ± 0.1	19.9 ± 6.1	0.43 ± 0.07	4.99
Cosmetic base	Treated	4.6 ± 0.3	13.0 ± 0.2	0.38 ± 0.01	6.0 ± 0.4
Cosmetic base	Untreated	1.1 ± 0.4	14.1 ± 0.9	0.4 ± 0.03	6.01 ± 0.65

when compared to baseline values. However, the absolute amount of d^3-Tocopheryl Acetate hydrolyzed was <1% of the total dose. In mice that were irradiated with 2.5 J/m^2/sec UVB for 1 hour prior to application of d^3-Tocopheryl Acetate, the conversion to d^3-Tocopherol increased three to fourfold at each sample period. The researchers stated that "this suggests that UV-B exposure triggers increased conversion of [Tocopheryl Acetate] to the active antioxidant [Tocopherol], which enhances the ability of topical [Tocopheryl Acetate] to confer protection against UV-B induced oxidative damage."

The dermal penetration of radioactive and non-radioactive Tocopherol was determined in vitro using full-thickness abdominal skin from a female hairless mouse (Tojo and Lee 1989). The penetration profile was determined using HPLC and liquid scintillation counting. Radioactive Tocopherol penetrated quickly, whereas a 48-hour lag time was observed with nonradioactive Tocopherol. The researchers postulated that the lag time in penetration assayed by HPLC was due to bioconversion of Tocopherol in the skin.

The absorption of Tocopherol (vitamin E) from water-in-oil (w/o) and oil-in-water (o/w) emulsions, both bound and not bound by liposomes, was studied (Szulc et al. 1995). In ointments in which Tocopherol was not bound by liposomes, "empty" liposomes were added to the ointment. "Conventional ointments" containing Tocopherol were used as reference preparations. A dose of 0.8 g of each ointment was spread on a 10-cm^2 surface of the interior forearm of 30 subjects for 6 hours. The amount of Tocopherol absorbed was calculated on the basis of the difference between the initial content of the sample and the amount present after exposure. The amount of Tocopherol absorbed from w/o emulsions containing Tocopherol (w/o), Tocopherol in liposome form (L—w/o), and Tocopherol and empty liposomes (LP—w/o) was 50.1%, 42.7%, and 49.4%, respectively. The amount of Tocopherol absorbed from o/w emulsions containing Tocopherol (o/w), Tocopherol in liposome form (L—o/w), and Tocopherol and empty liposomes (LP—o/w) was 23.3%, 39.7%, and 22.1%, respectively. Statistically, absorption from w/o was greater than o/w, from w/o was greater than L—w/o, and from L—o/w was greater than o/w. In examining the amount of Tocopherol absorbed by each individual, a range of 31% to 70%, 21% to 60%, and 31% to 80% was absorbed from the w/o, L—w/o, and LP—w/o emulsions, respectively, and of 0% to 40%, 21% to 60%, and 0% to 50% was absorbed from the o/w, L—o/w, and LP—o/w emulsions, respectively. The researchers concluded that Tocopherol was absorbed "more efficiently" from a w/o ointment rather than from a o/w ointment, and that the liposome form of Tocopherol increased absorption from an o/w emulsion, but had "virtually no effect" on absorption from a w/o ointment.

Oral

The serum concentration of Tocopherol was determined after ingestion of *d*-Tocopherol, *d*-Tocopheryl Acetate, *d*-Tocopheryl Acetate plus pectin, *dl*-Tocopheryl Acetate in soft gelatin capsules, and *d*-Tocopheryl Succinate in hard gelatin capsules (Horwitt et al. 1984). Each capsule contained 400 IU, which corresponded to 268 mg of *d*-Tocopherol, 294 mg *d*-Tocopheryl Acetate, 312 mg *d*-Tocopheryl Acetate with pectin, 400 mg *dl*-Tocopheryl Acetate, and 330 mg *d*-Tocopheryl Succinate. Twenty subjects, 11 males and 9 females, were given two capsules (800 IU) of one Tocopherol preparation with 100 ml of whole milk; the milk was used to provide fat for the formation of chylomicrons. After a 2-week nontreatment period, a different preparation was given; this was repeated until all five were administered. Blood samples were taken prior to dosing and at 8, 24, and 48 hours after dosing.

Large variations in the mean serum concentrations of Tocopherol were observed. Some of the variations could have been due to differences in total serum lipids, which ranged from 334 to 1195 mg/dl. The peak mean serum concentration of Tocopherol was observed after 8 hours with *d*-Tocopherol (1.71 mg/dl compared to baseline of 0.95 mg/dl), and *d*-Tocopheryl Acetate with pectin (1.61 mg/dl compared to baseline of 0.95 mg/dl) and after 24 hours with *d*-Tocopheryl Acetate (1.49 mg/dl compared to baseline of 0.92 mg/dl), *dl*-Tocopheryl Acetate (1.29 mg/dl compared to baseline of 0.93 mg/dl), and *d*-Tocopheryl Succinate (1.32 mg/dl compared to baseline of 0.96 mg/dl). After 24 hours, a statistically significant difference in the percentage increase of Tocopherol above baseline was observed between *d*-Tocopherol, which resulted in a 71% increase, *d*-Tocopheryl Acetate, which resulted in a 61% increase, and *d*-Tocopheryl Acetate with pectin, which resulted in a 62% increase, compared to *dl*-Tocopheryl Acetate, which produced a 32% increase. Because of the large individual variations, the differences between *d*-Tocopherol, *d*-Tocopheryl Acetate, and *d*-Tocopheryl Acetate with pectin were not statistically significant. The researchers

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compared their results in humans to those obtained in the rat fetal-resorption tests and stated that "animal assay data do not correlate with data from studies of absorption and retention in serum of α-tocopherols ingested by humans"; the rat assays underestimate the potency of free tocopherol.

The oral absorption of a single 500 IU dose of water soluble D-α-Tocopherol in a soft-gelatin capsule with 200 ml of water was determined in five fasted male and five fasted female subjects (Bateman and Uccellini 1985). Blood samples were drawn at various intervals for 24 hours. All values were determined after subtracting endogenous Tocopherol (12.0 ± 1.05 μmol/l). One subject was a "very bad absorber" and one was a "very good absorber"; however, the data from all 10 subjects was used. Mean plasma concentrations peaked at approximately 7 hours. The following were determined (abbreviations not defined): V/F = 14.3 l; $t_{1/2}$ = 21.3 h; K_{abs} = 0.87/h; K_{elim} = 0.0032/h; T_{lag} = 1.92 h; AUC_0^∞ = 433 μmol/l; and KV/F = 160 ml/min.

The pharmacokinetics and bioavailability of *d-* and *dl-α*-Tocopherol were compared in a double-blind randomized crossover study using 12 male subjects (Ferslew et al. 1993). The subjects were fed a diet that contained 30 mg α-Tocopherol for 5 days prior to dosing. Tocopherol was administered as two 400 mg soft-gelatin capsules, and 200 ml of fluid was given with dosing and 2 and 4 hours after administration. Blood samples were taken 3 days, 1 day, and just prior to dosing and at various intervals after dosing. The second Tocopherol formulation was given using the same protocol following a 10-day nontreatment period. The pharmacokinetic parameter values, determined using difference data with each subject's own baseline minimum, were determined for plasma and red blood cells (RBCs) and are summarized in Table 9.

Because of an "extreme degree" in variation in the data between subjects, a statistically significant difference was not found between values obtained with *d-* and *dl-α*-Tocopherol for many of the parameters measured. Mean plasma α-Tocopherol concentrations were greater for the *d-* versus the *dl*-preparation 24 to 96 hours after administration; this difference was statistically significant only at 24 hours. Area under the curve (AUC) was statistically significantly increased with *d-* compared to *dl-α*-Tocopherol. The RBC mean α-Tocopherol concentration was statistically increased with administration of *d-* versus *dl-α*-Tocopherol at 24 to 96 hours after dosing, and C_{max} and AUC were significantly greater with *d-α*-Tocopherol. *d-α*-Tocopherol had a greater bioavailability than *dl-α*-Tocopherol.

Seven subjects were given *dl-α*-Tocopheryl Acetate, ascorbic acid, or Tocopheryl Acetate and ascorbic acid combined orally for 30 days (Jeng et al. 1996). Blood samples were taken 3 days before and just prior to dosing and on days 4, 7, 14, 21, and 35 after dosing. After administration of Tocopheryl Acetate, the plasma concentration of vitamin E (assumed to be Tocopherol) was statistically significantly increased on days 14 and 21, and the increase was greater on day 21. The plasma malondialdehyde (MDA) concentration was significantly decreased on day 14. In the subjects given Tocopheryl Acetate and ascorbic acid combined, the plasma vitamin E concentration was statistically significantly increased on days 4, 7, 14, and 21, and the greatest increase was observed on day 7, after which it declined. The plasma MDA was statistically significantly decreased on days 7 and 14, and the greatest decrease was observed on day 7.

Groups of four rhesus monkeys were fed a semipurified diet for 188 weeks; the diet of three of the four groups was supplemented with 5, 50, or 500 mg/kg *dl*-Tocopheryl Acetate (Machlin and Gabriel 1982). The plasma Tocopherol concentrations were determined every 1 to 2 months. For the animals fed control feed or diet supplemented with 5 mg/kg Tocopheryl Acetate, plasma Tocopherol concentrations decreased to "almost negligible values" in 50 weeks. In the animals given 50 mg/kg Tocopheryl Acetate, the plasma Tocopherol concentrations remained constant throughout the study. In those given 500 mg/kg, a relatively rapid increase in plasma Tocopherol concentration was observed by week 6, followed by a slow but continuous increase throughout the remainder of the study; the slope from 6 to 188 weeks was statistically significant.

Groups of 10 fasted female albino rats were dosed by stomach tube with 4 ml of an emulsion containing ethanolic solutions of either 2 mg *dl-α*-Tocopheryl Acetate and 50 μCi *dl-α*-Tocopheryl-[1′,2′-$^3H_2$]-Acetate or 2.3 mg *dl-α*-Tocopheryl Nicotinate and 50 μCi *dl*-3,4-$^3H_2$-α-Tocopheryl Nicotinate (Gallo-Torres et al. 1971). Two animals per group were killed 3, 6, 12, 24, or 48 hours after dosing.

TABLE 9

Pharmacokinetic values for Tocopherol in plasma and RBCs (Ferslew et al. 1993)

	K_α* (h^{-1})	$t_{1/2\alpha}$* (h)	β* (h^{-1})	$t_{1/2\beta}$* (h)	lagtime (h)	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-96}* (μg · h/ml)
				Plasma				
d-	0.419 ± 0.112	2.8 ± 0.5	0.015 ± 0.003	80.6 ± 19.2	2.9 ± 0.6	19.3 ± 0.9	13.5 ± 1.4	649 ± 69.5
dl-	0.489 ± 0.131	3.0 ± 0.8	0.019 ± 0.004	73.2 ± 20.6	3.5 ± 0.4	19.7 ± 1.8	12.6 ± 2.1	423 ± 69.4
				RBC				
d-	0.458 ± 0.156	3.3 ± 1.0	0.005 ± 0.001	223.4 ± 56.8		4.8 ± 0.2	15.8 ± 2.7	376 ± 16.9
dl-	0.683 ± 0.295	1.8 ± 0.8	0.006 ± 0.001	180.6 ± 56.8		4.0 ± 0.2	14.5 ± 3.2	305 ± 12.8

*K_α = absorption constant; $t^*_{1/2\alpha}$ = half-life of absorption; β = elimination constant; $t^*_{1/2\beta}$ = elimination half-life; AUC = area under the α-Tocopherol concentration-time profile.

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Uptake of radioactivity was greater with Tocopheryl Acetate than Tocopheryl Nicotinate at all time periods except for 12 hours after dosing. The liver was the principal storage site of Tocopherol, but the adrenal glands had the greatest accumulation of radioactivity (expressed as dpm/g wet tissue). In both test groups, most of the radioactivity in tissue was recovered as tocopheryl quinone; in a few tissues, it was recovered mostly as Tocopherol. Twelve hours after the animals were given Tocopheryl Acetate, 47% to 86% of the radioactivity recovered in the blood, liver, spleen, kidneys, heart, ovaries, adipose tissue, and skeletal muscle was recovered as tocopheryl quinone, and 79% of the radioactivity recovered in the adrenal glands was as Tocopherol. After 24 hours, 82% of the radioactivity recovered in the adrenal glands and 66% recovered in skeletal muscle was as Tocopherol; in each of the remaining examined tissues, 52% to 83% of the radioactivity was recovered as tocopheryl quinone. After 12 hours in the animals given Tocopheryl Nicotinate, 51% to 84% of the radioactivity recovered in the blood, liver, kidneys, heart, ovaries, and adipose tissue was recovered as tocopheryl quinone, whereas 61%, 74%, and 58% of the radioactivity recovered in the spleen, adrenal glands, and skeletal muscle, respectively, was Tocopherol. After 24 hours, 90% of the radioactivity recovered in the adrenal glands was as Tocopherol; in each of the remaining examined tissues, 54% to 79% of the radioactivity was recovered as tocopheryl quinone.

Lister hooded rats were used to compare the absorption of Tocopherol in three different vehicles (Kelleher et al. 1972). Paired groups of six nonfasted animals were given 0.2 ml of 3 μCi *dl*-[5-methyl-^{3}H]-α-Tocopherol in arachis oil and in Tween 80 emulsion or 0.2 ml in arachis oil and in an absolute alcohol suspension. Feces, but not urine, were collected for 7 days, and the animals were then killed. Absorption was determined by measuring fecal excretion of unabsorbed Tocopherol.

In comparing arachis oil and Tween 80, the total dose administered to each animal was 9.8×10^6 and 9.0×10^6 cpm, respectively. The mean percentage of Tocopherol absorbed was not statistically significantly different between the groups; 70.1% was absorbed with arachis oil and 75.5% was absorbed with Tween 80. However, the total radioactivity in the liver and kidneys was statistically significantly different between the two groups. In the animals dosed using arachis oil, the total hepatic and total renal radioactivity was 4.5×10^3 and 0.8×10^3 cpm, respectively. Using Tween 80, the amounts were 91.1×10^3 and 25.6×10^3 cpm, respectively.

In comparing arachis oil and alcohol, the total dose administered to each animal was 2.77×10^6 and 2.7×10^6 cpm, respectively. The mean percentage of Tocopherol absorbed and the amount of radioactivity recovered in the kidneys were not statistically significant between the groups. Total absorption was 60.3% with arachis oil and 64.2% with the alcohol suspension, respectively, and total renal radioactivity was 4.3×10^3 and 7.0×10^3 cpm, respectively. However, the total radioactivity in the liver was statistically significantly different between the two groups. Total hepatic radioactivity was 21.7×10^3 and 31.3×10^3 cpm using arachis oil and alcohol, respectively.

Kelleher et al. (1972) also compared the absorption after a small (40 μg) and a large (20 mg) dose of radioactive *dl*-α-Tocopherol in arachis oil using two groups of six Lister hooded rats. In one group, the animals were dosed with 5.15×10^6 cpm in the small dose, untreated for 7 days while feces were collected, and then dosed with 3.49×10^6 cpm in the large dose. In the second group, the animals were dosed with 5.21×10^6 cpm in the large dose, untreated for 7 days while feces were collected, and then dosed with 5.22×10^6 cpm in the small dose. Feces were collected for 7 days after each second dose. In both groups, absorption was statistically greater after dosing with 40 μg Tocopherol as compared to dosing with 20 mg. In the first group, the mean absorption was 67.5% and 31.5% for the 40-μg and 20-mg doses, respectively. In the second group, these values were 57.1% and 30.1%, respectively. The researchers noted that "even though the percentage absorption was less with the larger dose a very much greater net quantity of α-tocopherol was absorbed."

Mature female Sprague-Dawley rats were fed a diet supplemented with 1000 or 10,000 mg/kg *dl*-Tocopheryl Acetate; mature animals were used to reduce the influence of tissue growth on Tocopherol concentration (Machlin and Gabriel 1982). Groups of six animals given 1000 mg/kg Tocopheryl Acetate were killed prior to dosing and at weeks 1, 4, 9, and 14. After 14 weeks of dosing, all animals were fed basal chow, and groups of six animals were killed after 1, 2, or 4 weeks. Groups of four animals given 10,000 mg/kg Tocopheryl Acetate were killed prior to dosing and at weeks 1, 2, 4, 8, 14, and 20. After 8 weeks of dosing, three groups of four animals were given basal chow and killed after 1, 2, or 4 weeks.

Generally, a linear relationship was found between time and tissue concentrations of Tocopherol. The same pattern of uptake was observed with both doses, but the rate of accumulation and the maximal concentrations reached were "consistently and considerably" greater in the high-dose groups. The relative increase in the Tocopherol content was greatest in the liver, and the accumulation rate of Tocopherol was greatest in liver and adipose tissue and lowest in muscle and brain. The rate of depletion of Tocopherol was more rapid than the rate of accumulation from all tissues except adipose tissue. With the exception of a more rapid rate of depletion from plasma for the high dose compared to the low dose, the rates of depletion were similar for both doses.

In a photocarcinogenicity study (described later), female C3H/HeN mice were fed chow containing 100 or 200 IU/kg *d*-α-Tocopheryl Acetate or basal chow for 37 weeks; the animals were irradiated with UVB during weeks 4 to 17 and 20 to 21 of the study (Gerrish and Gensler 1993). Dorsal and ventral skin was removed from three to six mice per group, and the concentration of α-Tocopherol was determined. A dose-dependent increase in the concentration of α-Tocopherol in the skin was reported for the ventral skin of dorsally irradiated mice; the ventral skin concentration of Tocopherol was statistically

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significantly increased in mice fed 200 IU/kg Tocopheryl Acetate compared to those given basal chow. Irradiation had a tendency to deplete the dorsal skin concentrations of α-Tocopherol.

Antioxidant Properties

The primary function of Tocopherol is to retard cellular lipid oxidation (Tsallas, Molgat, and Jeejeebhoy 1986). Tocopherol interdigitates with phospholipids, cholesterol, and triglycerides in the membranous parts of cells (Vanderveen and Vanderveen 1990). Tocopherol is the major lipid-soluble chain-breaking antioxidant of membranes (Kagan et al. 1992), and it is an important cellular protectant against oxidative damage (Liebler et al. 1996). It exerts antioxidant effects by trapping peroxyl radicals. One source states that α-Tocopherol is the most potent biological antioxidant, followed by β- = γ- > δ-Tocopherol (Papas 1993), whereas another states that δ-Tocopherol has the greatest antioxidant power (Vanderveen and Vanderveen 1990). However, Papas (1993) did report that in food systems, γ-, δ-, and β-Tocopherol have more antioxidant activity than α-Tocopherol. Esters of α-Tocopherol, such as acetate and succinate, do not function as antioxidants in foods because the active hydroxyl group is protected.

According to Papas (1993), Tocopherols have antioxidant activity based on the "tocopherol-tocopherylquinone redox system." A hydrogen is released to form an α-tocopherol free radical, the release of a second hydrogen forms methyl-tocopherylquinone (unstable), and the methyl-tocopherylquinone is converted to α-tocopherylquinone. (See Figure 1.) Another possibility is that the Tocopherol radical can be reduced back to Tocopherol by ascorbate or glutathione (Bjørneboe, Bjørneboe, and Drevon 1990).

Jurkiewicz, Bissett, and Buettner (1995) examined the antioxidant capabilities of *dl*-α-Tocopherol and α-Tocopheryl Acetate using groups of 10 female albino hairless Skh:HR-1 mice. Tocopherol and Tocopheryl Acetate, 5% in isopropanol, were applied dermally to the animals, with coverage of approximately 2 mg/cm^2, for 3 weeks. The dorsal skin of the animals was then removed and ascorbate radical measurement and spin trapping were performed with UV exposure.

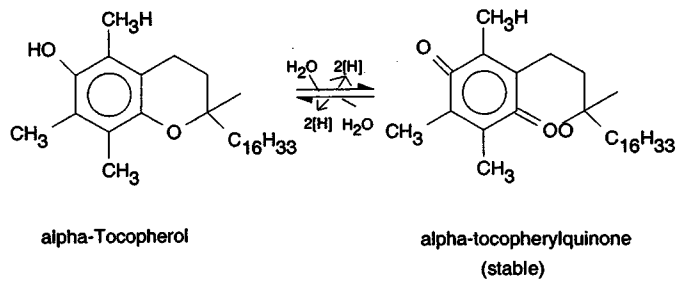

FIGURE 1
Tocopherol-tocopherylquinone redox system.

Tocopherol appeared to increase the ascorbate free radical ($Asc^{\cdot -}$) in the presence of radiation in a nonstatistically significant manner; this indicated that Tocopherol could possibly "act as a weak photoreactive agent." Tocopheryl Acetate did not enhance or protect against UV-induced $Asc^{\cdot -}$ formation. The $Asc^{\cdot -}$ electron paramagnetic resonance (EPR) levels were significantly greater in UV-exposed Tocopherol-treated samples compared to vehicle levels. Tocopheryl Acetate did not have an effect on $Asc^{\cdot -}$ EPR levels. Tocopherol enhanced the α-[4-pyridyl 1-oxide]-*N*-*tert*-butyl nitrone (POBN) signal as compared to controls during UV irradiation in a nonstatistically significant manner. Tocopheryl Acetate did not affect UV-induced formation of POBN radical adducts.

Methanol-water dispersions, liposomes, and skin homogenates containing Tocopherol were illuminated with UV light that was similar to solar UV light in the presence and absence of endogenous or exogenous reductants to determine whether Tocopherol in the skin absorbed UV light, generating the tocopheroxyl radical, and depleted other antioxidants via its own recycling (Kagan et al. 1992). The UV light source, which was 30 cm from the sample, was a solar simulator that had a wavelength range of 295 to 400 nm. The power density of the light at the sample surface in the spectral region of 310 to 400 nm was 1.5 mW/cm^2 and it decreased to 10% of this value at 295 nm.

Illumination of Tocopherol-containing methanol-water suspensions and dioleoylphosphatidylcholine (DOPC) liposomes resulted in the immediate appearance of the tocopheroxyl radical electron spin resonance (ESR) signal; the signal was not present in the dark. Partial or complete filtering of wavelengths in the absorption spectrum of Tocopherol during illumination of liposomes partially or completely eliminated the tocopheroxyl radical ESR signal. The generation of the tocopheroxyl radical was also examined using homogenates of hairless mouse skin, and similar results were obtained.

It was then determined by illuminating DOPC liposomes containing Tocopherol in the presence and absence of ascorbate that the tocopheroxyl radicals induced by UV light could deplete other antioxidants. UV illumination of Tocopherol in liposomes stimulated oxidation of ascorbate in a concentration-dependent manner. Reducing antioxidants can donate electrons to α-tocopheroxyl radicals, recycling Tocopherol. This interaction does not occur with antioxidants that do not have reducing potency, such as β-carotene. Again, this was also examined using homogenates of hairless mouse skin and similar results were obtained.

The researchers found that Tocopherol was depleted upon illumination with UV light because of its antioxidant properties and that it can be directly destroyed because the tocopheroxyl radical is generated by solar UV light. They postulated that "dual depletion of this major skin antioxidant implies that, after solar illumination, the skin is highly vulnerable to oxidative stress. Also, because antioxidants like ascorbate and dihydrolipoate can recycle vitamin E [Tocopherol], they can become depleted by solar UV illumination. Such depletion leaves the skin susceptible

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to free radical attack of vital cellular targets and may actually facilitate tumor initiation and promotion as well as other UV-induced lesions of the skin."

Effect on Lipid Peroxidation

Tocopherol. The effect of Tocopherol on lipid peroxidation was examined in UVB-irradiated human skin fibroblasts (Kondo et al. 1990). Fibroblasts in the growing phase, which were obtained from the elbow, back, inguinal area, or upper arm of five healthy subjects, were irradiated in the presence of phosphate-buffered saline with UVB obtained with polychromatic light from a two-tube sun lamp (Toshiba FL 20SE), wavelength range of 280 to 320 nm and maximum at 305 nm, at a dose of 500 J/m^2. Immediately after irradiation, a medium containing 100 μg/ml *dl*-α-Tocopherol was added to the cultures. Irradiated and unirradiated cells were collected after 48 hours, and MDA was measured. MDA increased following UVB irradiation in the absence, but not in the presence, of Tocopherol; the difference was statistically significant. The authors concluded that these findings indicate that "*dl*-α-tocopherol inhibits UVB-induced lipid peroxidation."

Epidermal tissue from groups of 10 hairless HRO-hr/hr mice was used to determine the effect of vitamin E (assumed to be Tocopherol) on polyamine and oxygenated free-radical metabolism (Khettab et al. 1988). Repeated applications of 100 μl of an emulsion containing 0.25% Tocopherol were applied dermally 1 hour before and 1 and 2 hours after irradiation. The animals were given a dose of 3.2 J/cm^2 UVA and UVB from a Suntest lamp (280 to 400 nm); this dose was three times the minimum erythema dose (MED). Tocopherol reduced MDA production 60% compared to animals that were irradiated but had no emulsion applied. Tocopherol also statistically significantly reduced polyamine metabolism; specifically, the amount of putrescine and spermidine in the epidermis was significantly less than that observed in irradiated controls.

Tocopheryl Acetate. Female hairless SKH-1 mice were fed a diet containing <2, 60, or 600 IU *dl*-α-Tocopheryl Acetate/kg diet for 3 weeks before irradiation to determine the effect on lipid peroxidation and thymidine incorporation (Record et al. 1991). The animals were irradiated by a single exposure of UV light, with 0.36 J/cm^2/min UVA and 0.12 J/cm^2/min UVB. The animals acted as their own controls, as one side of the animal was covered during irradiation. The animals were given an intraperitoneal (IP) injection of [methyl-^{3}H]-thymidine after irradiation and were killed 1 hour later. High dietary intake of Tocopheryl Acetate did not alter lipid peroxidation. High concentrations of Tocopheryl Acetate restored the incorporation of thymidine to values similar to those of controls.

The effect of topical applications of 5% Tocopheryl Acetate on MDA (a breakdown product of lipid peroxides) in the skin was examined (Djerassi, Machlin, and Nocka 1986). Although details were not provided, MDA reportedly was decreased by 40% to 80% of the control value of irradiated hairless mice.

Effect on Photobinding

The effect of single and multiple applications of Tocopherol and Tocopheryl Acetate on the photobinding of 8-methoxypsoralen (8-MOP) and chlorpromazine (CPZ) to epidermal DNA, protein, and lipids was examined using female albino Wistar rats (Schoonderwoerd et al. 1991). For the single application, *dl*-α-Tocopherol or *dl*-α-Tocopheryl Acetate in ethanol, 35 μl/cm^2, was applied to the shaved left side of the back of each animal and ethanol was applied to the shaved right side. After 20 minutes, a solution of 8-[methyl-^{3}H]-MOP or [^{3}H]-CPZ in ethanol (35 μl/cm^2; 2.27 $\times$ 10^{-3} M; 1.33 μCi/ml) was applied to both the test and control sites. Groups of three animals were dosed with 0.00001 to 0.001 M Tocopherol/8-MOP and 0.01 to 0.05 M Tocopheryl Acetate/8-MOP and groups of five animals were dosed with 0.01, 0.05, or 0.1 M Tocopherol/8-MOP, 0.1 M Tocopheryl Acetate/8-MOP, 0.1 M Tocopherol/CPZ, and 0.1 M Tocopheryl Acetate/CPT. Twenty minutes after application of 8-MOP or CPZ, the animals were irradiated for 1 hour using five Philips TL 80/10 R lamps, spectral region 345 to 410 nm with the peak at 370 nm, which were placed above the metabolism cages. (Number of animals per cage not specified.) For the multiple applications, 35 μl/cm^2 Tocopherol or Tocopheryl Acetate, 0.005 M, was applied to the backs of five animals daily for 4 days. Ethanol was applied to the backs of animals in the control group. Five hours after the last application, 35 μl/cm^2 8-MOP or CPZ in ethanol, 2.27 $\times$ 10^{-3} M, was applied to the back of each animal. Twenty minutes later, the rats were irradiated as described previously. Two animals were dosed comparably but not irradiated.

A single application of 0.01 to 0.1 M Tocopherol inhibited 8-MOP photobinding to DNA/RNA and protein, whereas it enhanced photobinding of 8-MOP to lipids. Tocopheryl Acetate did not significantly affect photobinding of 8-MOP, and neither Tocopherol nor Tocopheryl Acetate protected against CPZ photobinding. With multiple applications, Tocopherol and Tocopheryl Acetate protected against photobinding of 8-MOP to DNA/RNA and proteins, but not to lipids. Again, CPZ photobinding was not affected.

Beijersbergen van Henegouwen, Junginger, and de Vries (1995) also examined the effect of Tocopherol and Tocopheryl Acetate on photobinding of 8-MOP using groups of six Wistar rats. *d*-α-Tocopherol or *d*-α-Tocopheryl Acetate, 0.25%, was applied to a shaved site of the left side of the back of each animal and ethanol was applied to a shaved site on the right side; applications were made once daily for 5 days. Five hours after the last application, 8-[methyl-^{3}H]-MOP in ethanol (0.7 ml; 1.33 μCi/ml) was applied to the two areas. Fifteen minutes after application of 8-MOP, the animals were exposed to UVA for 1 hour (216 kJ/m^2). Philips TL 80/10R lamps, with a spectral region of 345 to 410 nm and an overall light intensity of 60 W/m^2, were used. The animals were then killed; the epidermis and the dermis were separated. The epidermis was separated into a protein-containing and a lipid-containing fraction. The photobinding of 8-MOP was also determined after single applications of 0.002%, 0.005%, 0.01%, and 0.25% Tocopherol and

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Tocopheryl Acetate. A single application of Tocopheryl Acetate did not affect photobinding, but some protection was observed after 5 days of dosing. Single and multiple applications of Tocopherol were also protective.

Effect on Erythema

Tocopherol. The effect of Tocopherol on UV-induced erythema was determined using rabbits (Roshchupkin, Pistsov, and Potapenko 1979). *dl*-α-Tocopherol in ethanol (0.1 ml/cm^2) was applied to rabbit skin 1 hour before or 2 minutes, 1, 5, or 10 hours after irradiation; surface concentration was 10^{-10} mol/cm^2. The UV source was a super-high-pressure mercury vapor lamp SVD-120QA with two liquid filters; the length of irradiation was 12 to 60 seconds.

Tocopherol application 1 hour before irradiation (five animals) increased the MED 140% of the control value. Tocopherol application 2 minutes after irradiation increased the MED 173% (four animals) and 154% (nine animals). Applications 1, 5, and 10 hours after irradiation increased the MED 158% (three animals), 112% (nine animals), and 111% (nine animals) of the control value, respectively.

Tocopheryl Acetate. The effect of Tocopheryl Acetate on UV-induced erythema was also determined using rabbits (Roshchupkin, Pistsov, and Potapenko 1979). α-Tocopheryl Acetate in ethanol (0.1 ml/cm^2) was applied to rabbit skin 1 hour before or 2 minutes after irradiation; surface concentration was 10^{-10} mol/cm^2. The UV source was a super-high-pressure mercury vapor lamp SVD-120QA with two liquid filters; the length of irradiation was 12 to 60 seconds. Tocopheryl Acetate did not significantly affect the MED.

Hairless skh-1 mice were exposed to UV radiation, and the effect of Tocopheryl Acetate on erythema was observed (Trevithick et al. 1992). The animals were exposed to a dose of 0.115 to 0.23 J/cm^2 UVB, and *d*-α-Tocopheryl Acetate was applied after exposure. Tocopheryl Acetate reduced the erythema index by 40% to 55% with a dose of 0.115 J/cm^2. In two experiments using magnetic resonance imaging, skin thickness was significantly reduced 29% and 54% at 24 hours and 26% and 61% at 48 hours.

Hairless male skh-1 mice were used to determine the effect of Tocopheryl Acetate on irradiation-induced erythema using skin thickness as an indicator (Trevithick et al. 1993). The animals were exposed to a total UVB dose of 0.115 J/cm^2 (3.3 to 3.8 times the MED) using four Phillips TL40W/12 tubes. The animals served as their own controls, as half of each animal's back was covered. At specified times after irradiation, D-α-Tocopheryl Acetate was applied to half of the back of each animal. With application of Tocopheryl Acetate at 0 to 8 hours after irradiation, skin thickness increased less than that observed without Tocopheryl Acetate.

Immunologic Effects

Tocopherol. The effect of Tocopherol on cell-mediated immunity was examined in a randomized double-blind placebo-controlled study performed using 88 healthy elderly subjects (Meydani et al. 1997). The test subjects were given capsules containing 60, 200, or 800 mg *dl*-α-Tocopherol in soybean oil daily for 235 days; the control group was given capsules containing soybean oil only.

Baseline serum Tocopherol concentrations were similar for all four groups. Serum Tocopherol concentrations were statistically significantly increased in a dose-dependent manner in all three test groups compared to controls at months 1 and 4. Administration of Tocopherol did not alter the percentage of T cells, B cells, or T-helper or T-cytotoxic cells. In examining the results of delayed-type hypersensitivity (DTH) skin response, a significant increase in diameter of induration was observed after 128 days for the test groups as compared to baseline; the diameter of induration was "representative of the current status of cell-mediated immunity." No change was observed in the placebo group. When expressed as median percent change, subjects of the 200-mg/day group had a statistically significant increase in DTH (65%) as compared to controls (17%). The median percent changes in the 60- and 800-mg/day groups (41% and 49%, respectively), were not significantly greater than the control group. Dosing with Tocopherol did not affect total nonspecific immunoglobulin concentrations when compared to baseline values. Antibody titers against hepatitis B vaccine increased significantly in the 200- and 800-mg/day groups, but no significant effect was observed on antibody response to diphtheria vaccine. A significant increase in the geometric mean of antibody titer in response to tetanus toxoid was observed in the 200-mg/day group. The researchers concluded that Tocopherol supplementation enhanced indices of T cell–mediated function, and that this enhancement was not associated with any adverse effects.

Groups of 26 specific pathogen–free female C3H/HeNTac (H-2^k; MTV$^-$) mice, housed five per cage, were dosed dermally with 25 mg *dl*-α-Tocopherol in 200 μl acetone three times per week; dosing was initiated 3 weeks prior to the start of irradiation and continued throughout the experiment (Gensler and Magdaleno 1991). To avoid oxidation, Tocopherol was applied 30 minutes after irradiation. The animals were irradiated with 6.0 J/m^2/sec UVB from a bank of six FS40 Westinghouse fluorescent sunlamps at a distance of 20 cm for 30 minutes per day, 5 days per week, for 12 weeks. Control mice were treated with solvent. One organ equivalent of splenocytes from irradiated mice was injected intravenously (IV) into 24 normal recipients that were then challenged with the syngeneic, antigenic, UV-induced tumor cell line UVM12. The capacity of splenocytes from UV-irradiated mice to inhibit normal rejection of UVM12 was abrogated. The proportion of mice with tumors was statistically lower in mice receiving splenocytes from irradiated mice treated with Tocopherol compared to those from irradiated control mice.

Gensler and Magdaleno (1991) also characterized the phenotypes of the splenocytes used in the passive transfer. The splenocytes were incubated with monoclonal antibodies specific

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for leukocyte differentiation or activation antigens. In irradiated control mice, the proportion of splenocytes that reacted positively for Ia antigen was decreased and the proportions that reacted positively for Mac-1 and Mac-2 were increased. The decrease in Ia^+ splenocytes was prevented by Tocopherol. Irradiation resulted in an increase in the proportion of interleukin-2 (IL-2) receptor–positive splenocytes relative to unirradiated controls; application of Tocopherol did not influence the UV-induced decrease in $IL\text{-}2R^+$ splenocytes. Tocopherol did significantly increase the proportion of splenocytes from irradiated mice that reacted positively for Lyt-2 and L3T4 antigens.

A group of two male Sprague-Dawley rats was dosed with 0.5 ml of 100 mg/kg α-Tocopherol in 75% ethanol by gavage once daily for 5 days in order to evaluate its effect on macrophage and monocyte activation (Bulger et al. 1997). Two groups of controls were used; one group was given 0.5 ml saline and one was given 0.5 ml ethanol. This study was repeated three times for a total of six animals per group. All animals were killed 24 hours after the last dose, and peritoneal macrophages and whole blood were harvested. The macrophages were stimulated with lipopolysaccharide (LPS) and tumor necrosis factor (TNF) production was measured after 18 hours; whole blood was stimulated with 10 μg/ml LPS and TNF production was measured after 3 and 6 hours. Serum uptake of α-Tocopherol was increased. In peritoneal macrophages, TNF production was increased in ethanol controls compared to saline controls. Tocopherol statistically significantly inhibited TNF production when compared to the ethanol controls, but not when compared to saline controls. In whole blood, TNF production was greatest at 3 hours. The TNF response was statistically decreased compared to saline control values at both 3 and 6 hours. The ethanol control group had a non-statistically significant increase in TNF response compared to the saline controls.

Groups of five "old" (12-week-old) male F344 rats were fed a normal (50 mg/kg diet) or "high vitamin E" diet (585 mg/kg diet; assumed to be Tocopherol) for 12 months in order to determine whether vitamin E supplementation improved cellular immune function that decreases with aging (Sakai and Moriguchi 1997). Body weights and feed consumption were measured weekly. "Young" (8-week-old) male F344 rats were used as the controls. The animals were killed after termination of dosing, and cellular immune function was examined. The proliferation of splenic lymphocytes to phytohemagglutinin (PHA) and concanavalin A (Con A) was determined.

Body weights and feed consumption were similar for the two groups of old animals. Body weights of old animals were statistically greater than those of young animals; feed consumption data for young animals were not given. Plasma vitamin E concentrations were statistically lower in old animals fed a normal diet compared to old animals fed the high vitamin E diet and to young animals. Spleen weights were slightly but statistically decreased in both groups of old animals compared to the young controls. The number of splenocytes was statistically decreased in old animals fed a normal diet as compared to young animals. No significant difference in splenocyte number was observed between old animals fed a high vitamin E diet and young animals.

Splenocytes were isolated and incubated with PHA or Con A for 72 hours. Proliferation of whole splenocytes with PHA or Con A was statistically less in old animals fed a normal diet compared to the young controls. In old animals fed the high vitamin E diet, splenocyte proliferation was slightly less with PHA and similar with Con A when compared to young animals. Macrophages were isolated from splenocytes of young and old animals and then cultured with splenic lymphocytes of young animals with Con A stimulation for 72 hours. Splenic lymphocyte proliferation was statistically increased by incubation with macrophages from young animals. Macrophages from old animals fed the normal diet did not have an effect on proliferation of splenic lymphocytes, but macrophages from old animals fed the high vitamin E diet significantly enhanced the proliferation of splenic lymphocytes almost as much as the macrophages from young rats. Splenic lymphocytes isolated from young and old animals were cultured with macrophages isolated from young animals with Con A stimulation for 72 hours. No significant difference in splenic lymphocyte proliferation was observed between old animals fed the control diet and the young animals, whereas the responsiveness of splenic lymphocytes to macrophages was statistically increased in old animals fed the high vitamin E diet as compared to the other two groups. IL-2 activity was determined in splenocytes cultured with Con A for 48 hours. IL-2 production was statistically decreased in old animals fed the normal diet compared to the young animals, whereas IL-2 production was significantly increased in old animals fed the high vitamin E diet.

The effect of Tocopherol on the distribution of oral mucosal Langerhans cells was examined using groups of male and female golden hamsters (Schwartz et al. 1985). The left buccal pouch mucosa of two groups of 11 animals was painted three times per week with 0.5% 7,12-dimethylbenz(a)anthracene (DMBA) in heavy mineral oil. One of the DMBA groups was also given 10 mg DL-α-Tocopherol in peanut oil (four drops of a 50-mg/ml solution) twice weekly on days alternate to DMBA. A group of nine animals was given Tocopherol only, and in a control group of six animals, the buccal mucosa was painted with mineral oil. The animals were killed after 8 weeks.

When compared to controls, administration of Tocopherol resulted in a significant decrease of focal aggregates of Langerhans cells in the buccal epidermis, but not in the number of interfocally distributed Langerhans cells. A statistically significant decrease in the number of focal aggregates and the number of interfocally distributed Langerhans cells was observed in the DMBA-only test group compared to controls. Administration of Tocopherol with DMBA produced a significant increase in Langerhans cell density as compared to the animals given DMBA only. Morphologically, the Langerhans cells in the animals given DMBA only had a loss of the normal dendritic network and a shrinking of the cell body following DMBA application; the administration

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of Tocopherol in conjunction with DMBA resulted in "much less pronounced effects on the morphology of Langerhans cells."

The induction of TNF-α by α-Tocopherol was examined in male Syrian hamsters; the right buccal pouches of the animals were painted three times weekly with DMBA in heavy mineral oil for 14 weeks (Shklar and Schwartz 1988). After 14 weeks, a group of 20 animals was given a 0.1-ml injection of 1.9 mg/ml Tocopherol twice weekly for 4 weeks. A sham-injected control group was dosed with minimal essential medium; an untreated control group (that had tumors initiated with DMBA) was also used. The animals were killed after 18 weeks on study.

Tumors were observed in all animals by week 14. After 4 weeks of administration of Tocopherol, notable tumor regression was seen, whereas the control animals had numerous, moderately sized papillary tumors. At microscopic examination of tumors of the test animals, areas of degeneration and connective tissue were found beneath the tumors that were "densely infiltrated with macrophages and lymphocytes." Greater than 50% of the cells in each histologic field from the buccal pouches of the animals dosed with Tocopherol were positive for TNF-α; the positive cells were primarily macrophages that occurred in large amounts adjacent to the regressing epidermoid carcinomas and within tumors where degeneration of the tumor cells was apparent. Minimal TNF-α was observed in control animals.

Tocopheryl Acetate. The effect of Tocopheryl Acetate on cell-mediated immunity was examined in a double-blind, placebo-controlled study using 32 elderly subjects (Meydani et al. 1990). Five male and 13 female subjects were given two capsules containing 400 mg *dl*-α-Tocopheryl Acetate in soybean oil for 30 days; 4 male and 10 female control subjects were given capsules containing only soybean oil. Blood and urine samples were taken prior to and at the end of testing.

Plasma α-Tocopherol concentrations increased threefold in the test group. Positive DTH responses were recorded at 24 and 48 hours for each antigen. The cumulative score and antigen score were significantly increased in the test group, and the percent change in the test group was significantly greater than that in the controls. A significant increased in Con A–stimulated mitogenic response was observed in the test group, but no significant change was observed in response to PHA or *Staphylococcus aureus* Cowan I. IL-2 formation in response to Con A was significantly increased, and the percent change was significantly greater in the test group compared to the controls. A positive correlation was found between changes in IL-2 concentration and changes in plasma α-Tocopherol concentration. No significant change in serum immunoglobulin concentrations was observed. A significant decrease in PHA-stimulated prostaglandin E_2 (PGE_2) formation was observed in test subjects, and the percent decrease was significantly greater than in the control group. Tocopheryl Acetate improved immune responsiveness.

Cytokine production was examined in a single-blind study after supplementation with *dl*-α-Tocopheryl Acetate (Jeng et al. 1996). A group of 10 subjects were given 400 mg Tocopheryl Acetate daily for 28 days. Three other groups of 10 subjects were given 1 g ascorbic acid, Tocopheryl Acetate + ascorbic acid, or a placebo. Blood samples were taken prior to dosing and on days 14, 28, and 35.

Administration of Tocopheryl Acetate or Tocopheryl Acetate + ascorbic acid resulted in a significant increase in the plasma concentration of α-Tocopherol on days 14 and 28, with the greater increase seen on day 14. On day 35, the concentrations were returning to baseline values. Plasma MDA was significantly reduced on day 14 when compared to the placebo group. On day 14, production of IL-1β and TNF-α by peripheral blood mononuclear cells (PBMCs) were increased 1.5- and 1.26-fold, respectively; this increase was significant compared to baseline, to the group supplemented with ascorbic acid, and to the group given the placebo. The group given Tocopheryl Acetate + ascorbic acid had greater IL-1β and TNF-α production. IL-6 production was not significantly increased in any of the groups.

The ability of Tocopheryl Acetate to prevent UV-induced antigenic tumor susceptibility was determined using the UVM12 cell line (Gensler et al. 1996). Groups of 30 shaved specific pathogen–free female BALB/cAnNTacfBR (H-2^d) mice were dosed dermally with 12.5, 25, or 50 mg *dl*-α-Tocopheryl Acetate in 0.2 ml acetone three times per week; dosing was initiated 3 weeks prior to the start of irradiation and continued throughout the experiment. (During dosing, Tocopheryl Acetate was applied 30 minutes after irradiation.) The animals were irradiated with 6.44 J/m^2/sec UVB from a bank of six unfiltered FS40 Westinghouse fluorescent sunlamps that emit a continuous spectrum from 270 to 390 nm, with a peak emission of 313 nm (75% of the output was in the range of 280 to 320 nm), at a distance of 20 cm for 30 minutes per day, 5 days per week, for 12 weeks. Tumor challenges consisted of intradermal injection in the flanks of mice with 5×10^5 tumor cells in 100 μl of phosphate-buffered saline. UV-induced susceptibility was not prevented with any dose of Tocopheryl Acetate tested. Tumor incidence was significantly increased in UV-irradiated groups compared to non-irradiated groups. Tocopheryl Acetate, 25 mg/kg, enhanced the growth of injected UVM12 cells.

A group of two male Sprague-Dawley rats was dosed with 0.5 ml of 100 mg/kg α-Tocopheryl Acetate in 75% ethanol by gavage once daily for 5 days in order to evaluate its effect on macrophage and monocyte activation (Bulger et al. 1997). Two groups of controls were used; one group was given 0.5 ml saline and one was given 0.5 ml ethanol. This study was repeated three times for a total of six animals per group. All animals were killed 24 hours after the last dose, and peritoneal macrophages and whole blood were harvested. The macrophages were stimulated with LPS and TNF production was measured after 18 hours; whole blood was stimulated with 10 μg/ml LPS and TNF production was measured after 3 and 6 hours.

Gastritis was observed in one animal. Serum uptake of α-Tocopherol was increased. In peritoneal macrophages, TNF production was increased in ethanol controls compared to saline controls. Tocopheryl Acetate significantly inhibited TNF

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production when compared to the ethanol controls, but not when compared to saline controls. In whole blood, TNF production was greatest at 3 hours. The TNF response was significantly decreased compared to saline control values at both 3 and 6 hours. The ethanol control group had a nonstatistically significant increase in TNF response compared to the saline controls.

Groups of six SL mice were fed a diet containing 0, 20, or 200 mg α-Tocopheryl Acetate/kg feed for 50 days prior to and 36 days after being immunized to determine the influence of Tocopherol esters on antibody production using a hapten-carrier conjugate as an antigen (Tanaka, Fujiwara, and Torisu 1979). The primary immunization consisted of an IV injection of 0.2 ml of a 25% *v/v* suspension of hamster erythrocytes (HRBC) in saline. Twenty-eight days after the primary immunization, the animals were given an IV injection of 0.2 ml of a 25% 2,4,6-trinitrophenyl (TNP)-HRBC suspension. Hemagglutinin titres to HRBC were measured prior to immunization and on days 4, 8, and 14 after and hemagglutinin titres to TNP were measured on days 28, 32, and 36 after primary immunization. Compared to animals fed a diet containing no Tocopherol, significant increases were observed in anti-HRBC titres on days 4, 8, and 14 in animals fed 200 mg Tocopheryl Acetate/kg feed and in antibody response to TNP on days 32 and 36 in animals fed 200 mg Tocopheryl Acetate.

The same procedure was repeated with groups of eight SL mice and compared to results obtained in HRBC-nonprimed mice given control chow or feed with Tocopheryl Acetate. Nonprimed mice were injected with TNP-HRBC at the same time primed mice were given the injection. TNP antibody titres were again measured 32 and 36 days after the primary immunization. No statistically significant difference in antibody response to TNP was observed in nonprimed mice. A significant, dose-dependent increase was observed in primed mice.

Tanaka, Fujiwara, and Torisu (1979) also fed groups of 12 DDD mice 20 or 200 mg Tocopheryl Acetate/kg feed or chow without Tocopherol for 50 days and then immunized the animals with HRBC while being supplemented. Antibody titres were measured on days 4, 7, 14, and 21. Anti-HRBC hemagglutinin and 2-mercaptoethanol–resistant antibody titres were significantly increased on day 7 for both groups given Tocopheryl Acetate when compared to control animals. Supplementation with Tocopheryl Acetate seemed to augment in a nonstatistically significant manner primary immunoglobulin M (IgM) and later IgG antibody; the researchers postulated that Tocopheryl Acetate "facilitated the shift of antibody from IgM to IgG."

Spleen cells from DDD mice fed 200 mg/kg Tocopheryl Acetate, a Tocopherol-deficient diet, or normal chow and primed with HRBC or TNP–bovine serum albumin (BSA) were transferred by Tanaka, Fujiwara, and Torisu (1979) 7 or 28 days after priming, respectively, into groups of eight 600 rad–irradiated syngeneic mice. The recipient mice were immunized with TNP-HRBC simultaneously with the cell transfer. TNP antibody titres were measured 4, 7, 14, and 21 days after immunization with TNP-HRBC. The TNP antibody titres were statistically significantly increased in mice given Tocopheryl Acetate as compared to those fed a Tocopherol-deficient diet on days 7 and 14. The researchers stated that the results indicated that "the activity of helper cells (T cells) was enhanced by treatment with [Tocopheryl Acetate] supplementation."

PBMCs were cultured with *dl*-α-Tocopheryl Acetate and stimulated with LPS for 24 hours (Jeng et al. 1996). PBMCs were also cultured with ascorbic acid and Tocopheryl Acetate + ascorbic acid combined. TNF-α production was significantly increased in all test cultures. IL-1β production was not affected. Production of PGE_2 was significantly decreased by Tocopheryl Acetate and Tocopheryl Acetate + ascorbic acid combined, with Tocopheryl Acetate producing the greater reduction.

Tocopheryl Nicotinate. Groups of six SL mice were fed a basal diet or one containing 226 mg α-Tocopheryl Nicotinate/kg feed for 50 days prior to and 36 days after being immunized to determine the influence of Tocopherol esters on antibody production using a hapten-carrier conjugate as an antigen (Tanaka, Fujiwara, and Torisu 1979). The primary immunization consisted of an IV injection of 0.2 ml of a 25% *v/v* suspension of HRBC in saline. Twenty-eight days after the primary immunization, the animals were given an IV injection of 0.2 ml of a 25% TNP-HRBC suspension. Hemagglutinin titres to HRBC were measured prior to immunization and on days 4, 8, and 14 after immunization and hemagglutinin titres to TNP were measured on days 28, 32, and 36 after primary immunization. Compared to animals fed a diet containing no Tocopherol, statistically significant increases were observed in anti-HRBC titres on day 4 and in antibody response to TNP on day 32 in animals fed Tocopheryl Nicotinate.

The same procedure was repeated with groups of eight SL mice and compared to results obtained in HRBC-nonprimed mice given control chow or feed with Tocopheryl Nicotinate. Nonprimed mice were injected with TNP-HRBC at the same time primed mice were given the injection. TNP antibody titres were again measured 32 and 36 days after the primary immunization. No statistically significant difference in antibody response to TNP was observed in nonprimed mice; the animals fed Tocopheryl Nicotinate had less anti-TNP antibody than the animals fed no Tocopherol. A significant, dose-dependent increase was observed in primed mice.

Tocopheryl Succinate. The ability of Tocopheryl Succinate to prevent UV-induced antigenic tumor susceptibility was determined using the UVM12 cell line (Gensler et al. 1996). Groups of 30 shaved specific pathogen–free female BALB/cAnNTacfBR ($H\text{-}2^d$) mice were dosed dermally with 2.5, 12.5, or 25 mg *dl*-α-Tocopheryl Succinate in 0.2 ml acetone three times per week; dosing was initiated 3 weeks prior to the start of irradiation and continued throughout the experiment. During dosing, Tocopheryl Succinate was applied 30 minutes after irradiation. The animals were irradiated with 6.44 J/m^2/sec UVB from a bank of six unfiltered FS40 Westinghouse fluorescent sunlamps that emit a continuous spectrum from 270 to 390 nm, with a peak emission of 313 nm (75% of the output was in the range of 280 to

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320 nm), at a distance of 20 cm for 30 minutes per day, 5 days per week, for 12 weeks. Tumor challenges consisted of intradermal injection in the flanks of mice with 5×10^5 tumor cells in 100 μl of phosphate-buffered saline. UV-induced susceptibility was not prevented with any dose of Tocopheryl Succinate tested. Tumor incidence was statistically significantly increased in UV-irradiated groups compared to nonirradiated groups. Tocopheryl Succinate, 25 mg/kg, enhanced the growth of injected UVM12 cells.

Two male Sprague-Dawley rats were dosed with 0.5 ml of 100 mg/kg α-Tocopheryl Succinate in 75% ethanol by gavage once daily for 5 days in order to evaluate its effect on macrophage and monocyte activation (Bulger et al. 1997). Two groups of controls were used; one group was given 0.5 ml saline and one was given 0.5 ml ethanol. This study was repeated three times for a total of six animals per group. All animals were killed 24 hours after the last dose, and peritoneal macrophages and whole blood were harvested. The macrophages were stimulated with LPS and TNF production was measured after 18 hours; whole blood was stimulated with 10 μg/ml LPS and TNF production was measured after 3 and 6 hours.

The death of one animal was not compound-related. Gastritis was observed in one animal. Serum uptake of α-Tocopherol was increased approximately twofold over controls. In peritoneal macrophages, TNF production was increased in ethanol controls compared to saline controls. Tocopheryl Succinate significantly inhibited TNF production when compared to the ethanol controls, but not when compared to saline controls. In whole blood, TNF production was greatest at 3 hours. The TNF response was significantly decreased compared to saline control values at both 3 and 6 hours. The ethanol control group had an increase in TNF response compared to the saline controls, but the increase was not statistically significant.

Anti-Inflammatory Activity

The anti-inflammatory activity of *d*-α-Tocopherol was examined using male Holtzman rats (Stuyvesant and Jolley 1967). A subcutaneous injection of an adjuvant containing *Mycobacterium butyricum* was made in the tail of each animal. By day 19, 18 of the 21 control animals had grade 4 lesions (confluent involvement of one or more extremity of two or more times the normal diameter), 1 had a grade 3 lesion (>1 cm often becoming confluent but not yet involving the entire extremity), and 1 had a grade 2 lesion ($\geq$4 lesions that are $\leq$1 cm in diameter); only 1 control animal had no apparent lesions. A group of 24 rats was given IP injections of 0.6 ml of Tocopherol once a week, starting on the day of administration of the adjuvant. By day 19, 7 of the 24 test animals did not develop lesions, 6 had grade 1 lesions (<4 lesions that are <3 mm in diameter), 7 had grade 2 lesions, 1 had grade 3 lesions, and 3 had grade 4 lesions. In a group of six animals in which the first dose of Tocopherol was administered on day 5, one animal had grade 1 lesions, two had grade 2 lesions, two had grade 3 lesions, and one had grade 4 lesions. When the first dose of Tocopherol was not administered to a group of six animals until day 9, four animals had grade 3 lesions and two animals had grade 4 lesions.

Effect on Nitroso Compound Formation

Tocopherol. α-Tocopherol is a good inhibitor of nitrosation because its phenol ring is fully substituted (Mirvish 1986). α-Tocopherol reduced NO_2 to NO in organic solvents and lipids, and its emulsions in water reduce nitrite to NO; these reactions have been observed with α-Tocopherol, but not Tocopheryl Acetate. Also, Tocopherol was a strong inhibitor of nitrosamide production from N-butylacetamide and NO_2 and its dimer N_2O_4. α-Tocopherol might inhibit formation of skin nitrosating agents from NO_2, but it did not inhibit nitrosamine production from skin nitrosating agents.

The use of Tocopherol as a nitrite scavenger for the prevention of nitrosamine formation was examined in a model system (Kamm et al. 1977; Mergens et al. 1978). A water-dispersible form of Tocopherol was prepared and spray-dried to yield a free-flowing solid that contained 33% by weight α-Tocopherol. In evaluating the effect of 8.0 mM α-Tocopherol powder on 7.0 mM nitrite as a function of pH in an aqueous suspension, it was found that as the pH was increased, the reaction of Tocopherol and nitrite slowed; at pH 2 or 3, the reaction proceeded quite rapidly, whereas at pH 5, less than 5% of the initial nitrite disappeared in 1 hour. Spray-dried, water-dispersible formulations of 8.0 mM α- and γ-Tocopherol were effective nitrite scavengers in simulated gastric fluid (pH 1.3); α-tocopherolquinone, an oxidation product of Tocopherol, was unreactive toward nitrite. α-Tocopherol also inhibited aminopyrene nitrite–induced hepatotoxicity in male Sprague-Dawley rats. Additionally, it reduced the amount of nitrosodimethylamine formed in cigarette smoke and nitrosopyrrolidine formed in bacon.

Tocopherol Acetate. In a short-term study (described in 'Animal Toxicology'), administration of *dl*-α-Tocopheryl Acetate with nitrite altered the cellular responses of rats to nitrite (Chow et al. 1984). Supplementation with Tocopheryl Acetate prevented nitrite-related mortality, liver, muscular, and blood effects, and the decrease in glutathione *S*-transferase activity. Nitrite potentiated Tocopherol deficiency in rats.

Cytotoxicity

Tocopherol. The concentration of Tocopherol needed to produce a 50% reduction in viable cell number (ID_{50}) was determined using KB cells of human epidermoid carcinoma (Mochida, Goto, and Saito 1985). After 72 hours, the ID_{50} value of *dl*-α-Tocopherol was 110 μg/ml.

The effect of Tocopherol on morphological changes and growth inhibition was determined using mouse melanoma (B-16) and fibroblast cells (L-cells) in culture (Prasad and Edwards-Prasad 1982). *dl*-α-Tocopherol diluted in specialized solvent and then ethanol did not affect either growth or morphology.

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Tocopherol Acetate. Prasad and Edwards-Prasad (1982) also determined the effect of Tocopherol Acetate on morphological changes and growth inhibition of mouse melanoma (B-16) and fibroblast cells (L-cells) in culture. Exposure to *dl*-α-Tocopherol Acetate diluted in specialized solvent and then water did not affect either growth or morphology.

Erythrocytes from fasted male Wistar rats were incubated with 0.6, 6, 60, or 600 μg/ml *dl*-α-Tocopheryl Acetate in ethanol (Díez-Marques et al. 1986). A dose-dependent increase in osmotic fragility was observed. Preincubation of erythrocytes with Tocopheryl Acetate prior to incubation with phenylhydrazine hydrochloride (PHH) did not modify the increased osmotic fragility induced by PHH; the only protective effect observed was that of 6 μg/ml on the osmotic fragility induced by 0.001 M PHH. Preincubation with Tocopheryl Acetate prior to incubation with hydrogen peroxide potentiated the toxic effect of hydrogen peroxide on osmotic fragility.

Tocopherol Nicotinate. The effect of Tocopheryl Nicotinate on morphological changes and growth inhibition was determined using mouse melanoma (B-16) and fibroblast cells (L-cells) in culture (Prasad and Edwards-Prasad 1982). *dl*-α-Tocopherol Nicotinate diluted in ethanol did not affect growth or morphology.

Tocopheryl Succinate. The cytotoxicity of Tocopheryl Succinate to human carcinoma SCC-25 squamous carcinoma cells was determined under various conditions (Schwartz et al. 1992). Exposure of the cells to 500 μM *dl*-α-Tocopheryl Succinate for 1 hour at normal pH (7.4) killed 30% and 45% of normally oxygenated and hypoxic cells, respectively. Exposure for 6 hours killed 90% and 63% of normally oxygenated and hypoxic cells, respectively. At a pH of 6.45, cytotoxicity was decreased with normally oxygenated but not hypoxic cells. Exposure to 70 μM Tocopheryl Succinate resulted in a 15% and 26% increase in the superoxide dismutase activities and in the nonprotein sulfhydryl content in the cells. The glutathione *S*-transferase activity in SCC-25 cells was increased by 40% to 45% over controls after exposure to Tocopheryl Succinate. [^{3}H]-Thymidine incorporation was significantly reduced after SCC-25 cells were exposed for 2 hours to 70 μM Tocopheryl Succinate; exposure of normal human epidermal keratinocytes to Tocopheryl Succinate resulted in increased [^{3}H]-thymidine incorporation.

Schwartz and Shklar (1992) cultured seven different human malignant cell lines with Tocopheryl Succinate to determine the cytotoxic effect. α-Tocopheryl Succinate was cultured with two oral carcinoma cell lines, SCC-25 and SQ-38, two breast cell lines, MCF-7 and ZR75, two lung carcinomas, SK-MES and CALV-3, and one malignant melanoma, A375. Normal human keratinocytes and breast cells were also cultured with Tocopheryl Succinate. Compared to untreated tumor cells, treatment of tumor cells with Tocopheryl Succinate resulted in decreased viability. Morphological changes were observed. [^{3}H]-Thymidine proliferation and succinic dehydrogenase activity were quantitatively reduced compared to normal cells. The researchers concluded that Tocopheryl Succinate can substantiate a selective cytotoxic effect on human tumor cell growth.

The effect of Tocopheryl Succinate on morphological changes and growth inhibition was examined using mouse melanoma cells (B-16) and fibroblasts (L-cells) (Prasad and Edwards-Prasad 1982). *d*-α-Tocopheryl Succinate in ethanol produced dose-dependent changes in the morphology of melanoma cells; these changes were not reversible when Tocopheryl Succinate was removed from the medium. Significant morphological changes were not observed with mouse fibroblasts, except for those due to toxicity at high concentrations. Tocopheryl Succinate also inhibited the growth of melanoma cells in a dose-dependent manner. Cell growth was determined using protein content, number of viable cells, or colony forming ability. The concentrations of Tocopheryl Succinate needed to inhibit cell growth by 50% were reported. If protein content was measured, the concentrations of Tocopheryl Succinate needed to inhibit cell growth by 50% for melanoma cells was 6 μg/ml and for fibroblasts, 10 μg/ml. If the number of viable melanoma cells was determined, the 50% growth inhibition level was 5.5 μg/ml in serum and 1.2 μg/ml in serum-free medium. When the determination used colony formation, 50% growth inhibition for fibroblasts grown in serum was 5.7 μg/ml. In examining the effect of Tocopheryl Succinate on melanin content, it was determined that melanoma cells treated with Tocopheryl Succinate contained approximately twice as much melanin than those found in untreated culture.

Mouse melanoma (B-16), mouse neuroblastoma (NBP_2), and rat glioma (C-6) cells were cultured with *d*- and *dl*-α-Tocopheryl Succinate to determine the effect of each form on cell growth and morphology (Rama and Prasad 1983). Growth inhibition was assayed using colony formation with melanoma cells and using cell number per dish for neuroblastoma and glioma cells. Both forms of Tocopheryl Succinate were equally effective in inhibiting cell growth of mouse melanoma, mouse neuroblastoma, and rat glioma cells. Morphological changes were observed with all cell types. The researchers compared the results using Tocopheryl Succinate to those obtained with other known antioxidants and found that "the effects of [Tocopheryl Succinate] on tumor cells are mediated, in part, by antioxidant mechanisms."

The effect of Tocopheryl Succinate on the radiation response of mouse neuroblastoma (NBP_2) and mouse fibroblast (L-cells) cells in culture was also examined (Sarria and Prasad 1984). *dl*-α-Tocopheryl Succinate in ethanol was added to the cell cultures just prior to ^{60}Co γ-irradiation. Cells were irradiated at a dose rate of 23 rad/min. Tocopheryl Succinate was added to the cultures 2 days after irradiation. Irradiated controls were supplemented with ethanol and sodium succinate or were untreated; sham-irradiated controls were treated similarly. γ-Irradiation and Tocopheryl Succinate inhibited the growth of mouse neuroblastoma cells in culture. The growth of fibroblast cells was also reduced, but the fibroblasts were less sensitive to γ-irradiation and Tocopheryl Succinate than the neuroblastoma cells. Tocopheryl Succinate enhanced the growth

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inhibitory effect of γ-irradiation on mouse neuroblastoma cells, but it did not enhance the effect of γ-irradiation on mouse fibroblasts in culture.

Protection Against UV-Induced Cytotoxicity

Tocopherol. The protective effect of Tocopherol on UVB-induced damage to human skin fibroblasts was examined in a colony-forming assay (Kondo et al. 1990). Skin fibroblasts, which were obtained from the elbow, back, inguinal area, or upper arm of five normal subjects, were irradiated with UVB obtained with polychromatic light from a two-tube fluorescent sun lamp (Toshiba FL 20SE), wavelength range of 280 to 320 nm and maximum at 305 nm; the irradiation was 0.2 mW/cm^2 at a distance of 10 cm. Doses of 250 to 1000 J/m^2 were tested. *dl*-α-Tocopherol in ethanol, 10, 100, or 1000 μg/ml, was added to the cell cultures immediately after irradiation. Control cultures were supplemented with ethanol. A protective effect was observed with 100 and 1000 μg/ml Tocopherol. The surviving fraction was statistically significantly increased with 100 and 1000 μg/ml of Tocopherol at all UVB doses and with 10 μg/ml Tocopherol at UVB doses of 250, 750, and 1000 J/m^2.

Tocopheryl Succinate. The effect of Tocopheryl Succinate on UVB-induced cytotoxicity was examined in a colony-forming assay using Chinese hamster V-79 cells (Sugiyama et al. 1992). The cells were pretreated for 24 hours with 25 μM α-Tocopheryl Succinate and then irradiated with UVB light from four Toshiba FL 20SE-30 lamps, wavelength range of 280 to 360 nm with maximum at 305 nm; UVB doses of 200, 400, and 600 J/m^2 were tested. Control cells were cultured with dimethyl sulfoxide (DMSO) only. Pretreatment with Tocopheryl Succinate resulted in a marked reduction in UVB-induced cytotoxicity.

ANIMAL TOXICOLOGY

Acute Toxicity

Dermal

Tocopheryl Acetate. The dermal LD_{50} of Tocopheryl Acetate was stated to be >3 g/kg for rats (Hoffmann-LaRoche 1995; Roche 1994).

Tocophersolan. The acute dermal toxicity of 75% Tocophersolan was determined using five male and five female Crl: CD (SD)BR rats (Eastman Kodak Co. 1989). A single dose of 2 g/kg was applied to shaved skin under an occlusive patch for 24 hours. After dosing, the test site was rinsed. The animals were observed for 2 weeks. All animals survived until study termination and no abnormal clinical signs were observed. No treatment-related gross lesions were observed at necropsy. The oral LD_{50} was >2 g/kg for male and female rats.

Oral

Tocopherol. The oral LD_{50} of undiluted Tocopherol was determined using groups of five male ddY mice (CTFA 1972). The animals were given a single dose at a volume of 10 ml/kg and then observed for 14 days. An acute oral LD_{50} of undiluted Tocopherol of >25 ml/kg was calculated by probit analysis—Tocopherol was considered practically nontoxic.

The oral LD_{50} of Tocopherol was stated as >4 g/kg for rats (BASF 1995).

The oral toxicity of a mixture consisting of Tocopherol (<0.1%), Arnica Montana Extract (1% to 5%), and Soybean (Glycine Soja) Oil (>50%) (Chemisches Laboratorium Dr. Kurt Richter GmbH [CLR] 1997) was determined using groups of five male and five female SPF-Wistar rats (International Bio-Research, Inc. (IBR) 1972a). The animals were given one oral dose of 10, 15, or 20 ml/kg of the test mixture, and were observed for 14 days. None of the animals died during the study, and signs of toxicity were not observed. The acute oral LD_{50} of the mixture was >20 ml/kg for SPF-Wistar rats.

Tocopheryl Acetate. The oral LD_{50} of Tocopheryl Acetate was stated as >5 g/kg for rats (BASF 1993b; 1996a) and >4 g/kg for both the rats and mice (Hoffmann-LaRoche 1995). Roche reported the acute oral LD_{50} of Tocopheryl Acetate as >16 g/kg for rats (Roche 1994).

Tocopheryl Nicotinate. The oral LD_{50} of Tocopheryl Nicotinate was stated as >10 g/kg for rats (BASF 1993c, 1994b, 1996b). (Details not provided.)

Tocopheryl Succinate. A group of 10 Charles River COBS, CD rats were dosed with 7 g/kg *d*-α-Tocopheryl Succinate by gavage (Krasavage and Terhaar 1977). The animals were observed for 2 weeks and then killed. With the exception of an initial transient (24-hour) period of lassitude and diarrhea, no clinical signs were noted. No treatment-related deaths occurred. The acute oral LD_{50} of Tocopheryl Succinate was >7 g/kg for male and female rats.

Krasavage and Terhaar (1977) also orally dosed groups of 14 to 20 male and 14 to 20 female 2-day-old neonates from Charles River COBS, CD rats with 0.075, 0.1, or 0.15 ml/neonate *d*-α-tocopheryl polyethylene glycol (PG) 1000 succinate (Tocophersolan), its components, polyethylene glycol 1000 and *d*-α-Tocopheryl Succinate, or corn oil (control). The small body weight and the difficulty in measuring the volume of material delivered made it impossible to calculate a dosage on a mg/kg basis. In addition, the delicacy of the procedure resulted in several deaths by mechanical injury, further complicating the determination of an LD_{50}. The animals were observed daily, and body weights were measured on days 3, 10, and 17 after dosing. No difference was seen in the acute effect of Tocophersolan, PG 1000, or Tocopheryl Succinate, but all three test substances produced a higher mortality than did corn oil. In all groups, females had better survival than males. No significant difference was observed in weight gain between surviving treated and control neonates. The acute oral LD_{50} for Tocopheryl Succinate was stated to be in excess of 7 g/kg body weight.

The acute oral LD_{50} of Tocopheryl Succinate was stated to be approximately 7 g/kg for rats (Spectrum Chemical Manufacturing Corporation 1998b).

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Dioleyl Tocopheryl Methylsilanol. Groups of five male and five female mice were given a single oral dose of a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol (0.05% Tocopheryl Acetate, 0.5% monomethylsilanetriol, remainder oleic alcohol) (Exsymol 1988). The mixture had an LD_{50} >20 ml/kg for mice (Exsymol 1988).

Tocophersolan. A group of 10 Charles River COBS, CD rats were dosed with 7 g/kg *d*-α-Tocophersolan by gavage (Krasavage and Terhaar 1977). The animals were observed for 2 weeks and then killed. With the exception of an initial transient (24-hour) period of lassitude and diarrhea, no gross changes were noted. No treatment-related deaths occurred. The acute oral LD_{50} of Tocophersolan was >7 g/kg for male and female rats.

In the study by Krasavage and Terhaar (1977) described above, no difference was seen in the acute effect of Tocophersolan, PG 1000, or Tocopheryl Succinate, but all three test substances produced a higher mortality than did corn oil; in all groups females had a better survival than males. The acute oral LD_{50} for Tocophersolan was stated to be in excess of 7 g/kg body weight.

The acute oral toxicity of 75% Tocophersolan was determined using five male and five female Crl:CD (SD)BR rats (Eastman Kodak Co. 1989). The animals were given a single oral dose of 5 g/kg and observed for 2 weeks. All animals survived until study termination. The oral LD_{50} was >5 g/kg for male and female rats.

Inhalation

Tocopheryl Nicotinate. No mortality was reported for rats after an 8-hour exposure to Tocopheryl Nicotinate in a highly enriched and/or saturated atmosphere at 20°C (BASF 1993c, 1994b, 1996b).

Parenteral

Tocopherol. The parenteral LD_{50} of Tocopherol was stated as 440 mg/kg for mice (Phelps 1981).

Tocopheryl Acetate. The IP LD_{50} of Tocopheryl Acetate was stated as 840 mg/kg for rats (Hoffmann-LaRoche 1995).

Tocopheryl Nicotinate. The IP LD_{50} of 46.4% and 50% Tocopheryl Nicotinate in olive oil was stated as >10 g/kg for mice (BASF 1993c).

Short-Term Toxicity

Oral

Tocopherol. A group of six adult male Wistar rats was given 400 mg/kg vitamin E (assumed to be Tocopherol) in feed for 60 days, and a control group was fed basal chow (Manimegalai, Geetha, and Rajalakshmi 1993). Tocopherol was not toxic. When compared to animals fed a high-fat diet, Tocopherol prevented the increase in plasma lipid concentrations, decreased the low-density lipoprotein cholesterol to high-density lipoprotein cholesterol (LDLc/HDLc) ratio and lipid peroxide concentrations, and increased reduced glutathione.

Tocopheryl Acetate. Groups of 10 male Holtzman rats were fed basal chow (which contained 60 IU *dl*-α-Tocopheryl Acetate) plus 600 or 6000 IU Tocopheryl Acetate/kg with and without 2.9×10^6 IU vitamin A/kg (as retinyl acetate) for 8 weeks (Jenkins and Mitchell 1975). Body weights were measured weekly and feed consumption was determined twice weekly. All animals were killed at the termination of dosing.

Body weight gain and feed consumption were significantly increased in animals fed Tocopheryl Acetate compared to those given basal chow alone. Body weight gains and feed consumption of animals given Tocopheryl Acetate and vitamin A were similar to control values. Tocopheryl Acetate alone significantly decreased the amount of globulin in the plasma and increased the amount of cholesterol in the blood, did not affect the amount of plasma albumin or blood hemoglobin, and increased plasma and hepatic vitamin A content. Tocopheryl Acetate significantly decreased adrenal gland weight.

Two groups of 18 male Sprague-Dawley rats were fed a basal vitamin E–deficient diet supplemented with 200 ppm *dl*-α-Tocopheryl Acetate or 200 ppm *dl*-α-Tocopheryl Acetate with 1000 ppm $NaNO_2$ for 9 weeks (Chow et al. 1984). A group of 22 rats was given the diet supplemented with 1000 ppm $NaNO_2$ and a control group of 18 rats was given the basal diet only. Nutritional Tocopherol status was monitored by measuring the degree of erythrocyte hemolysis. At study termination, 10 to 13 animals per group were killed; hematological evaluation and a necropsy were performed.

After 5 weeks of dosing, animals fed Tocopheryl Acetate had <5% erythrocyte hemolysis, compared to >85% hemolysis in the animals of the groups not given Tocopheryl Acetate. Nine of the animals given $NaNO_2$ with the basal diet died during the study; no other test or control animals died. Animals fed $NaNO_2$ without Tocopheryl Acetate had massive hepatic necrosis, mild to marked muscular degeneration, tubular nephrosis, and eosinophilic enteritis; only eosinophilic enteritis and mild muscular degeneration were observed in the control group. No lesions were seen in the organs of animals given Tocopheryl Acetate. Tocopheryl Acetate deprivation significantly elevated serum creatine phosphokinase, lactate dehydrogenase, glutamic oxaloacetic transaminase, and pyruvate kinase activities; $NaNO_2$ caused a greater elevation of these enzymes in rats fed the deficient diet. Animals given nitrite, with or without Tocopheryl Acetate, had increased methemoglobin and decreased hematocrit values and erythrocyte counts. Animals given nitrite without Tocopheryl Acetate had a greater number of white blood cells, neutrophils, lymphocytes, monocytes, and eosinophils than animals of any other group.

In a 4-week oral toxicity study using rats and dogs, no adverse effects were reportedly observed with 2500, 5000, 10,000, or 20,000 ppm Tocopheryl Acetate (BASF 1993b).

Parenteral

Tocopherol. Newborn albino rabbit neonates that were delivered by cesarean section were dosed intravenously with

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Tocopherol for 7 days (Rivera et al. 1990). The Tocopherol solution, which was prepared as 25 mg of Tocopherol per milliliter of vehicle solution (90 mg/ml polysorbate-80 and 10 mg/ml polysorbate-20 in sterile water) was administered once daily at a dose of 4 ml/kg. Six and eight animals were given Tocopherol with a low-energy (LE) and a high-energy (HE) diet, respectively. (The LE diet was a standard pediatric solution given to newborn infants and the HE diet was a preparation similar to mature rabbit milk.) Control groups of 10 and 7 animals, which were not dosed, were fed the LE and HE diets, respectively, and 11 and 6 animals were given the polysorbate vehicle with the LE and HE diets, respectively.

Average weight gain for the animals given the LE diet was much less than those given the HE diet. All animals given the LE diet survived until study termination, whereas two animals given Tocopherol and one given the polysorbate vehicle with the HE diet died before day 6. The cause of death was not determined, but it was not thought to be treatment-related. Increased concentrations of Tocopherol were present in the liver and lungs and increased concentrations of γ-Tocopheryl Acetate were present in the tissues of animals given Tocopherol with either the LE or HE diet. Animals fed either the LE or HE diet had no significant treatment-related changes in serum chemistries. However, blood urea nitrogen values were significantly increased and serum alkaline phosphatase activities were significantly decreased in the animals fed the HE diet compared to those fed the LE diet. During microscopic examination of the tissues of animals fed the LE diet, hepatic injury comprised of centrilobular degeneration, necrosis, and pigment accumulation was observed in animals of all groups. A very mild splenic lipidosis was present in animals of the control group, but not in those of the treated groups. In the animals fed the HE diet with Tocopherol, minimal hepatic lipidosis in two animals and cholestasis in one animal, mild to moderate splenic lipidosis in all animals, and minimal adrenal gland lipidosis in one animal were observed. With the exception of mild splenic lipidosis in one animal given the polysorbate vehicle, lesions were not observed in animals of the other groups.

Tocopheryl Acetate. Rivera et al. (1990) (study described previously) also dosed neonatal rabbits intravenously with Tocopheryl Acetate and LE (10 animals) or HE (5 animals) diets. The Tocopheryl Acetate solution was prepared as 25 mg of Tocopheryl Acetate per milliliter of vehicle solution and administered once daily for 7 days at a dose of 4 ml/kg. Observations were similar to those described previously for Tocopherol. All animals given Tocopheryl Acetate with the LE diet survived until study termination, whereas one animal given Tocopheryl Acetate with the HE diet died before day 6. The cause of death was not determined, but it was not thought to be treatment-related. Again, increased concentrations of Tocopherol were present in the liver and lungs and increased concentrations of γ-Tocopheryl Acetate were present in the tissues of animals given Tocopheryl Acetate with both LE and HE diets. The plasma chemistry and microscopic findings for the animals given Tocopheryl Acetate were also similar for those given Tocopherol. In the animals given the HE diet with Tocopheryl Acetate, minimal hepatic lipidosis in one animal, minimal to mild hepatic cholestasis in three animals, minimal splenic lipidosis in two animals, and minimal to mild adrenal gland lipidosis in all animals were observed.

The toxicity of Tocopheryl Acetate in an aqueous polysorbate 80 (90 mg/ml) and polysorbate 20 (10 mg/ml) vehicle was examined using 1 to 2-day-old neonatal pigs (Hale et al. 1995). *dl*-α-Tocopherol, 50 IU/kg/day, was administered to a group of six animals via a bolus IV injection over 90 seconds for 13 days, to a group of four animals via slow IV infusion over a 7-h/day period for 6 days, and to a group of six animals via one daily intramuscular (IM) injection for 13 days. Groups of six animals were dosed with 2 to 4 ml/kg/day polysorbate and groups of five control animals were given saline.

Following bolus administration, microscopic lesions of the spleen, liver, and lungs were observed. The spleen was most affected; marked cytoplasmic vacuolation of cells in the ellipsoids and sinuses were seen. These changes were not observed in animals of the slow IV infusion, IM injection, polysorbate, or control groups. Animals given bolus administration of Tocopheryl Acetate had a massive accumulation of Tocopheryl Acetate in the spleen, and to a lesser amount, the lungs and liver. Slow infusion did not result in a large increase of Tocopheryl Acetate in the tissues; the largest increase with this administration was seen in the lungs. Following IM administration, Tocopheryl Acetate concentrations were slightly but significantly increased in the spleen. Tissue concentrations of Tocopherol were also significantly increased following administration of Tocopheryl Acetate. Following bolus IV administration, a massive accumulation of Tocopherol was seen in the spleen, with lesser increases observed in the liver and lungs. With slow infusion, a statistically significant increase in the Tocopherol concentration in the lungs was observed. After IM administration, small but significant increases in Tocopherol concentration were seen in the spleen and liver. Generally, the increases in the tissue concentrations of Tocopherol were greater than those of Tocopheryl Acetate.

Subchronic Toxicity

Oral

Tocopheryl Acetate. Groups of 30 male and 30 female Fischer 344 rats were given 125, 500, or 2000 mg/kg *d*-α-Tocopheryl Acetate in corn oil by gavage at a dose of 3.5 ml/kg for 90 days, and two control groups were given 3.5 ml/kg vehicle or were untreated (Abdo et al. 1986). Body weights and feed consumption were measured and clinical observations were made weekly. Ten males and 10 females were killed on days 5 and 45, and all surviving animals were killed at study termination. Hematology and clinical chemistry determinations were made for the animals killed at each of these time periods. In the control and high-dose groups, all tissues were examined and in the low- and mid-dose groups, those tissues designated as targets for vitamin E toxicity were examined microscopically.

Seven (of 10) male rats of the high-dose group died or were killed in a moribund state during weeks 9 to 11; these deaths

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were treatment-related and a result of internal hemorrhage. Mean body weights and feed consumption were generally similar to that of the vehicle control group. Relative liver-to-body weights were significantly increased in females of the 500- and 2000-mg/kg groups; this increase was treatment-related and occurred with a significant positive trend. Signs of toxicity, including diarrhea, tachypnea, bleeding through the nose, dark feces, and a red crust around the eyes (often 1 day before death), were observed in males of the high-dose group. After 90 days, statistically significant changes with a dose-related trend were observed in several hematological parameters for males of the mid- and high-dose groups; these changes were attributed to a response to blood loss caused by hemorrhagic diathesis. No treatment-related hematological trends were observed for females. No treatment-related trends were observed for clinical chemistry values; significant increases in thyroid-stimulating hormone were observed in animals of all test groups at day 90. At microscopic examination, treatment-related pulmonary adenomatous hyperplasia and chronic interstitial inflammation, characterized by increased cellularity, vascular congestion, thickened alveolar walls, and the presence of foamy macrophages, occurred in animals of all test groups. The incidence and severity increased in a dose-dependent manner. Increased extramedullary erythropoiesis was observed in four males of the high-dose group.

Tocophersolan. Groups of 30 male and 30 female Charles River COBS, CD albino rats were fed chow containing 0.002%, 0.2%, or 2.0% Tocophersolan supplemented with 5.0% corn oil for 91 days; a control group was fed the basal chow with corn oil (Krasavage and Terhaar 1977). The animals were housed five per cage. Body weights were determined prior to dosing and body weights and feed consumption were measured twice during week 1 and weekly thereafter. Hematological and clinical chemistry evaluations were made for 15 high-dose and control rats per sex on days 42 and 84. These 30 animals of the control and high-dose groups and 15 males and 15 females from the low- and mid-dose groups were killed after 90 days of dosing; the animals of the control and high-dose groups were necropsied. The remaining animals in each group were maintained on their respective diets and used in a reproduction study (described in 'Reproductive and Developmental Toxicity').

Animals of the low-, mid-, and high-dose groups consumed an average of 0.32 to 0.48, 31.5 to 45.6, and 316.8 to 443.1 mg Tocophersolan/day, respectively. No effect on body weight gain or feed consumption was observed for any of the test groups. Behavior and appearance were normal. Hematological and clinical chemistry parameters were normal. No gross lesions were observed and, at necropsy, mean body and organ weights were similar for test and high-dose animals. No treatment-related lesions were observed at microscopic examination.

Chronic Toxicity

Oral

Tocopheryl Acetate. Groups of 60 male and 60 female Charles River CD rats were fed basal chow containing 500, 1000, or 2000 mg/kg/day *dl*-α-Tocopheryl Acetate and a control group was fed basal chow only for 104 weeks (Wheldon et al. 1983). The animals were housed five per cage. Vitamin K_1 was added to the drinking water of all animals during weeks 24, 25, and 26 to "counteract an observed haemostatic failure"; vitamin K was added to the chow for the remainder of the study. Body weights and feed consumption were measured weekly. Hematological and urinalysis parameters were determined using 10 males and 10 females of the control and high-dose groups after 4, 8, 13, 26, 52, 78, and 95 weeks; serum chemistry parameters were evaluated at the same times using 5 males and 5 females from the control and high-dose groups. Ten males and 10 females per group were killed after 52 weeks, and the remaining surviving animals were killed after 104 weeks. A microscopic examination was made of all tissues of animals of the control and high-dose groups and selected tissues of animals of the low- and mid-dose groups.

Body weight gains and feed consumption were similar for animals of the treated and control groups. (Hemorrhagic microscopic lesions and hematological effects that occurred are described in 'Hemorrhagic Activity'.) No other signs of toxicity were observed. At week 8, a slight but statistically significant decrease was observed in hematocrit, hemoglobin concentration, and erythrocyte count in males and females of the high-dose group, and serum alkaline phosphatase activity was significantly increased during several time periods for this group; these changes were not considered treatment-related. However, dose-related increases were observed in activity of alanine aminotransferase during weeks 4 to 26. In females of the high-dose group killed after 52 weeks of dosing, absolute liver weights were increased compared to control values; after 104 weeks, no differences were observed in absolute or relative liver weights for this group. A significant increase was observed in the relative liver weight of females of the mid-dose group when all data for females were pooled. At microscopic examination, no hepatic lesions were observed except for centrolobular aggregates of vacuolated ("foamy") macrophages in the liver of some treated animals.

Dermal Irritation

Tocopherol. Eight male Japanese white rabbits were used to determine the dermal irritation potential of 1.0% Tocopherol in "paraffinum liquidum" (CTFA 1972). The backs of the animals were clipped free of hair 1 day prior to dosing, and the skin on the backs of four of the animals was abraded. The test material, 0.3 ml, was applied to the back of each animal under an occlusive patch (using "patch-test plaster") for 24 hours. The test sites were scored for irritation 24 and 72 hours after application of the test material. The primary irritation index (PII), calculated according to the Draize scoring system, was 0.13/8.0. Tocopherol, 1.0%, was a weak primary skin irritant.

Three male Hartley guinea pigs were used to determine the cumulative skin irritation potential of 1.0% Tocopherol in paraffinum liquidum (CTFA 1972). The test material, 0.05 ml, was applied to a shaved 2 $\times$ 2-cm^2 area on the flank of each animal

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once daily for 3 consecutive days. The test sites were scored 24 hours after each application. The cumulative irritation index was 0.44/4.0. Tocopherol, 1.0%, was a weak cumulative skin irritant.

Three cosmetic formulations, one containing 2% *dl*-Tocopherol, one containing 12% vitamin E (form not specified) in wheat germ (100 IU/g), and one containing 32% mixed Tocopherols in a base of wheat germ and vegetable oils (24,000 IU/2 oz), were evaluated for dermal irritation using New Zealand albino rabbits (Marzulli and Maibach 1975). Each test material, 0.05 ml, was applied to a clipped site on the backs of six rabbits five times per week for a total of 14 open applications. Each site was scored every 24 hour. The mean cumulative irritation score (maximum of 64) for the formulations containing 2%, 12%, and 32% Tocopherol were 31/64, 7/64, and 12/64, respectively. The product containing 2% Tocopherol was also tested on abraded skin; the mean cumulative irritation score was 29/64.

The irritation potential of a mixture consisting of Tocopherol (<0.1%), arnica montana extract (1% to 5%), and soybean (Glycine Soja) oil (>50%) (CLR 1997), applied as a 10% paraffin oil solution, was determined using six New Zealand white rabbits (IBR 1976). The hair was clipped from the back of each animal, and one test site was abraded and one was left intact. A dose of 0.5 ml was applied to each site for 24 hours under an occlusive patch. The test sites were scored for irritation according to the Draize scale 24 and 72 hours after dosing. Irritation was not observed at the intact or abraded sites, and the PII was 0.

Tocopheryl Acetate. The dermal irritation potential of Tocopheryl Acetate was determined using six New Zealand white rabbits (Roche 1999a). Occlusive patches containing 0.5 ml of undiluted Tocopheryl Acetate were applied for 4 hours to intact and abraded skin of each animal. The test sites were washed with ethanol after patch removal. The test sites were scored 4, 24, and 48 hours after application. Tocopheryl Acetate had a primary irritation index of 0.2 and was not a primary irritant.

In a primary skin irritation study using rabbits, details not provided, Tocopheryl Acetate was not irritating (BASF 1993b).

Using Organization for Economic Cooperation and Development (OECD) test methods, Tocopheryl Acetate was not a primary irritant to rabbit skin (BASF 1996a).

Tocopheryl Acetate may be slightly irritating to skin in several species (Hoffmann-LaRoche 1995). In a primary skin irritation study using rabbits, detail not provided, Tocopheryl Acetate produced no to well-defined irritation (Hoffmann-LaRoche 1996).

Tocopheryl Nicotinate. In a primary skin irritation study using rabbits, undiluted Tocopheryl Nicotinate was not irritating (BASF 1993c, 1994b, 1996b). (Details not provided.)

Dioleyl Tocopheryl Methylsilanol. A mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol (0.05% Tocopheryl Acetate, 0.5% monomethylsilanetriol, remainder oleic alcohol), 0.5 g, was applied under a semiocclusive patch to the clipped skin of six New Zealand white rabbits for 4 hours (Exsymol 1988). The test sites were scored for irritation 1, 24, 48, 72, and 96 hours after patch removal. The animals had a PII of 0, and the mixture was not irritating to rabbit skin.

The dermal irritation potential of a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol (0.05% Tocopheryl Acetate, 0.5% monomethylsilanetriol, remainder oleic alcohol) was also determined by applying 2 g of the mixture to the clipped dorsal area of three male and three female albino rabbits daily for 6 weeks (Exsymol 1988). Whether the sites were occluded was not stated; it was stated that the animals were prevented from grooming themselves. From day 2 of the study, slight erythema was observed in all animals. No erythema was observed for any animals at the end of week 7 of the study.

Tocophersolan. Dermal irritation potential following a single application of 75% Tocophersolan was determined using five female Hartley guinea pigs (Eastman Kodak Co. 1989). One-half milliliter was applied under an occlusive patch for 24 hours to the depilated abdomen of each animal. The animals were observed for 2 weeks. No irritation was observed.

The dermal irritation potential of 75% Tocophersolan was also determined after multiple applications to a group of five Hartley guinea pigs (sex not specified) (Eastman Kodak Co. 1989). One-half milliliter was applied to the shaved backs of each animal for a total of nine doses over 11 days. It was not stated whether occlusive patches were used. Slight erythema was observed after a single dose. Moderate erythema was observed for all animals after four doses given (5 days), and moderate erythema was observed after the ninth application.

Sensitization

Tocopherol. The sensitization potential of a mixture consisting of Tocopherol (<0.1%), arnica montana extract (1% to 5%), and soybean (Glycine Soja) oil (>50%) (Chemisches Laboratorium Dr. Kurt Richter GmbH 1997) was determined in an open epicutaneous test using 10 Pirbright white guinea pigs; a group of five guinea pigs was used as a control (IBR 1977). The hair on the left side of the back was clipped, and 0.5 ml of the mixture was applied to the test site daily for 10 days. Following a 14-day nontreatment period, the test material was applied to a previously untreated site of both test and control animals. The test sites were scored according to the method of Draize 24 and 48 hours after application of the challenge dose. Signs of irritation were not observed following challenge, and the mixture was not a sensitizer.

Tocopheryl Acetate. Testing with "higher concentrations (≥30%)" of Tocopheryl Acetate can cause sensitization in the open epicutaneous test for sensitization (Hoffmann-LaRoche 1996). However, Tocopheryl Acetate was not sensitizing in the guinea pig maximization test.

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Tocophersolan. The sensitization potential of 75% Tocophersolan was determined in a Buehler test using five male and five female Hartley guinea pigs (Eastman Kodak Co. 1989). A dose of 0.5 ml was used during induction and challenge. No erythema or edema was observed during induction or at challenge. Tocophersolan was not a sensitizer.

Ocular Irritation

Tocopherol. Three male Japanese white rabbits were used to determine the ocular irritation potential of Tocopherol (CTFA 1972). The test substance, 0.1 ml, was instilled undiluted into the conjunctival sac of one eye of each animal, and the eye was not rinsed. The eyes were evaluated at 1 and 4 hours and 1, 2, 3, 6, and 7 days after instillation according to the Draize scoring system. The maximum score was observed 1 hour after dosing; the average total score at this time was 6.0/110. Tocopherol was a minimal eye irritant.

A volume of 0.1 ml *dl*-α-Tocopherol was instilled into the conjunctival sac of one eye of each of nine New Zealand white rabbits; the eyes of three of the animals were rinsed 2 minutes after instillation, while the eyes of the remaining animals were not rinsed (Roche 1999b). The eyes were evaluated 1, 2, 3, and 7 days after instillation. For 7 days, "very slight conjunctival redness and chemosis was observed" in both rinsed and nonrinsed eyes. The eyes of two animals which were not rinsed had "well-defined conjunctival redness" 2 days after instillation. Also, there was "positive retention of 2% sodium fluorescein stain" 1 day after instillation; retention was observed "occasionally" at 7 days. It was stated that Tocopherol "produced very slight irritation when instilled into rabbit eyes."

Tocopherol was stated to be nonirritating to the eyes of rabbits (BASF 1995).

The ocular irritation potential of a mixture consisting of Tocopherol (<0.1%), arnica montana extract (1–5%), and soybean (Glycine Soja) oil (>50%) (Chemisches Laboratorium Dr. Kurt Richter GmbH 1997) was determined using three New Zealand white rabbits (IBR 1972b). The undiluted mixture, 0.5 ml, was placed in the conjunctival sac of the left eye of each rabbit. (It was not stated whether the eye was rinsed.) The right eye was untreated and used as a control. The eyes were scored for irritation after 1, 2, 8, 24, 48, and 72 hours, and 4, 5, 6, and 7 days. Reddening of the conjunctiva was observed for 24 hours and slight chemosis was observed after 2 hours for all animals; other changes were not observed. The researchers concluded that the mixture "caused no concern in regard to application in the vicinity of the eyes."

Tocopheryl Acetate. In ocular irritation studies, Tocopheryl Acetate was stated to be not irritating to rabbit eyes (BASF 1993b; Hoffmann-LaRoche 1995, 1996).

Using OECD test methods, Tocopheryl Acetate was nonirritating to rabbit eyes (BASF 1996a).

Tocopheryl Nicotinate. In an ocular irritation study, undiluted Tocopheryl Nicotinate was stated to be not irritating to rabbit eyes (BASF 1993c, 1994b, 1996b).

Dioleyl Tocopheryl Methylsilanol. The ocular irritation of a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol (0.05% Tocopheryl Acetate, 0.5% monomethylsilanetriol, remainder oleic alcohol) was determined in a Draize test using four New Zealand white rabbits (Exsymol 1988). The undiluted test material was applied to the conjunctival sac of each animal, and the eyes were not rinsed. A maximum irritation score of 6.75/110 was observed at 1 hour. The score was 0 at 96 hours. The mixture was a nonirritant.

Tocophersolan. The ocular irritation potential of 75% Tocophersolan was determined using six New Zealand white rabbits (sex not specified) (Eastman Kodak Co. 1989). One-tenth milliliter was placed in the conjunctival sac of each rabbit. The eyes of three of the animals were rinsed. In the animals with unrinsed eyes, two had slight and one had moderate erythema of the conjunctiva and the nictitating membrane after 1 hour, and one animal had slight erythema of the eyelids. In the animals with rinsed eyes, slight erythema of the conjunctiva and the nictitating membrane was observed after 1 hour. Tocophersolan was a "slight eye irritant."

Hemorrhagic Activity

Tocopherol. Groups of six male Jcl:SD rats were given *d*-α-Tocopherol orally at doses of 0.63% and 1.0% in basal chow or intraperitoneally at doses of 1.14, 1.82 or 2.91 mmol/kg for 7 days, and the hemorrhagic effect was determined (Takahashi, Ichikawa, and Sasaki 1990). Control animals for oral administration were fed basal chow and for IP administration were given olive oil. Body weights were measured daily. All animals were killed at study termination, and coagulation activities were measured. In the oral study, the mean intake of Tocopherol from feed was 618 and 994 mg/kg/day. Body weights were not affected by oral or IP administration. External hemorrhages were observed on day 6 in rats fed 0.63% and on days 4 and 5 in rats fed 1.0% Tocopherol. At necropsy, one, one, and five animals fed 0.63% Tocopherol had hemorrhages in the cranial cavity, penis, and both epididymes, respectively. One, one, and five animals fed 1.0% Tocopherol had perivascular hemorrhages around the inferior vena cava, blood pooling in the stomach, and hemorrhages in both epididymes, respectively, and one animal given 2.91 mmol/kg Tocopherol intraperitoneally had a hemorrhage in the abdominal cavity. Prothrombin indices were significantly reduced for all test animals fed Tocopherol and in animals given 2.91 mmol/kg Tocopherol intraperitoneally; kaolin-activated partial thromboplastin times (APTT) were significantly reduced for all test animals given Tocopherol by either route. The Tocopherol concentration in the plasma was increased almost 6-fold after oral dosing and 2.4-fold after IP administration.

Takahashi (1995) fed groups of six male Jcl:SD rats 0.5% *d*-α-, *d*-γ-, or *d*-δ-Tocopherol in one experiment and 0.5% *d*-α-and *d*-β-Tocopherol in another. A control group was fed a basal diet. All animals were weighed and feed consumption

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was measured daily. All animals were killed on day 7. In the first experiment, hemorrhages in the right eyeball and exophthalmos were observed on days 5 to 7 in two rats and blood was found in the cages on days 6 and 7 for the animals given α-Tocopherol and a spot of blood was found on day 5 on a feeder box of animals given γ-Tocopherol. In the second experiment, exophthalmos was observed in one animal on day 4, epistaxis was found in one, two, and two animals on days 4, 5, and 6, respectively, and blood was found in the cages on day 5 for the animals given α-Tocopherol and epistaxis was induced on day 5 in one animal given β-Tocopherol. Mild diarrhea was observed on days 4 to 6 in one animal given α-Tocopherol.

Tocopheryl Acetate. Groups of 60 male and 60 female Charles River CD rats were given chow supplemented with 500, 1000, or 2000 mg/kg *dl*-α-Tocopheryl Acetate for 104 weeks, and a control group was fed basal chow (Wheldon et al. 1983). Blood samples were taken from 10 males and 10 females of the control and high-dose groups and 5 males and 5 females of the low- and mid-dose groups after weeks 4, 8, 13, 26, 52, 78, and 95. Hemorrhages in the gut, urinary tract, orbits, and meninges, and after minor trauma of the claws and vibrissal pits, were observed in males of the low-, mid-, and high-dose groups at weeks 18, 16, and 15, respectively. The animals were normal following vitamin K supplementation at week 24. Prothrombin times were increased for males of all dose groups at weeks 4 and 16; these increases were not seen following vitamin K administration.

Groups of 30 male and 30 female Wistar rats were given 125, 500, or 2000 mg/kg *d*-α-Tocopheryl Acetate in corn oil by gavage at a dose of 3.5 ml/kg for 90 days (Abdo et al. 1986). Ten males and 10 females were killed on days 5, 45, and 90, and hematological determinations were made. Compared to animals of the vehicle-control group, males dosed with 2000 mg/kg Tocopheryl Acetate for 90 days had a statistically significant increase in thromboplastin time, APTT, and fibrinogen and males of the 500-mg/kg group had a statistically significant increase in APTT. At 90 days, a dose-related positive trend in APTT was observed for females, but the difference was only statistically significantly increased compared to controls at 2000 mg/kg. Treatment-related hemorrhagic diathesis, as indicated by hemorrhage or hemorrhagic inflammation in the nose, esophagus, salivary gland, trachea, mediastinum, epididymis, or meninges of the brain, was observed in seven and two of the males and females, respectively, killed at 90 days.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Tocopherol. Groups of 12 and 11 gravid Walter Reed-Carworth Farms rats were dosed orally with 0.07 and 0.2 g *d*-γ-Tocopherol, respectively (duration of dosing not specified) (Telford, Woodruff, and Linford 1962). The animals were killed on day 22 of gestation. In the group given 0.07 g Tocopherol, 50% of the animals had one or more resorptions, 5.7% of all implantations were resorbed, and 94% of the implantations resulted in normal fetuses. In the group given 0.2 g Tocopherol, 45.5% of the animals had one or more resorptions, 4.7% of all implantations were resorbed, and 95% of the implantations resulted in normal fetuses. Control data specified that of 126 untreated normal gravid rats, 40.8% of the animals had one or more resorptions, 10.6% of all implantations were resorbed, and 89% of the implantations resulted in normal fetuses.

Groups of 10 male and female Wistar rats were fed a diet containing 0.75, 7.5, or 75 mg/day α-Tocopherol 20 days prior to mating and during gestation; the groups fed 0.75 mg/day served as the control group (Sato 1973). Gravid animals were killed on day 20 of gestation. All of the animals given control feed were gravid, but the pregnancy rate for the animals of the 7.5- and 75-mg/day groups was 90% and 50%, respectively. The number of viable fetuses was slightly less in the 75-mg/day group compared to controls; a few resorptions were observed for both test groups. Fetal weight was similar for all groups. Very mild cases of ossification retardation were observed. No abnormal fetuses were reported. The researchers concluded that Tocopherol had "little effect on the rat fetus."

Three groups of 10 gravid Sprague-Dawley rats were made diabetic on day 6 of gestation; one group was fed a diet providing 400 mg/day vitamin E (assumed to be Tocopherol) on days 0 to 12 of gestation, one was fed basal chow, and one was treated with insulin (Sivan et al. 1996). Two control groups were used, one of which was fed a Tocopherol-supplemented diet. All animals were killed on day 12 of gestation. Unsupplemented diabetic animals had a statistically significant increase in the rate of neural tube defects and resorptions as compared to control animals. Tocopherol supplementation in diabetic rats reduced these rates to values similar to that of control animals. Tocopherol supplementation in nondiabetic animals did not affect these rates.

Tocopheryl Acetate. The Food and Drug Research Labs, Inc. (FDRL) conducted a study in which groups of 12, 13, 10, and 14 gravid belted rabbits were dosed orally with 16, 74.3, 345, or 1600 mg/kg *dl*-α-Tocopheryl Acetate, respectively, in corn oil by gavage on days 6 to 18 of gestation (FDRL 1973). Negative- and positive-control groups, both consisting of 12 gravid animals, were used. Body weights were measured on days 0, 6, 12, 18, and 29 of gestation, and the animals were observed daily. All animals were killed on day 29 of gestation, and the fetuses were examined. "The administration of up to 1600 mg/kg (body weight) of [Tocopheryl Acetate] to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

A study following a similar protocol and same dosages of *dl*-α-Tocopheryl Acetate was performed using groups of 23, 20, 23, and 24 gravid outbred golden hamsters (FDRL 1973). The animals were dosed by gavage on days 6 to 10 of gestation. Body weights were recorded on days 0, 8, 10, and 14 of gestation. All

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animals were killed on day 14 of gestation, and the fetuses examined. "The administration of up to 1600 mg/kg (body weight) of [Tocopheryl Acetate] to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Groups of 14 and 12 gravid Walter Reed-Carworth Farms rats were dosed orally with 0.1 and 0.2 g *dl*-α-Tocopheryl Acetate, respectively (duration of dosing not specified) (Telford, Woodruff, and Linford 1962). The animals were killed on day 22 of gestation. In the group given 0.1 g Tocopheryl Acetate, 71.4% of the animals had one or more resorptions, 14% of all implantations were resorbed, and 86% of the implantations resulted in normal fetuses. In the group given 0.2 g Tocopheryl Acetate, 41.7% of the animals had one or more resorptions, 4.1% of all implantations were resorbed, and 96% of the implantations resulted in normal fetuses. Control data were summarized previously.

Groups of 21, 23, 21, and 22 gravid albino Wistar rats were dosed orally with 16, 74.3, 345, or 1600 mg/kg, respectively, *dl*-α-Tocopheryl Acetate in corn oil by gavage (FDRL 1973). The animals were dosed on days 6 to 15 of gestation. Body weights were recorded on days 0, 6, 11, 15, and 20 of gestation. The animals were killed on day 20 of gestation and the fetuses examined. "The administration of up to 1600 mg/kg (body weight) of [Tocopheryl Acetate] to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

The teratogenic effect of *dl*-α-Tocopheryl Acetate was determined using groups of Sprague-Dawley rats (Martin and Hurley 1977). Gravid animals were fed stock chow supplemented with the crystalline form of vitamin E (three times per week). The following doses, dosage protocols, and matings were used: experiment I: 22.5, 45, 90, 450, and 900 mg/kg/day given during gestation; experiment II: 0, 450, 900, and 2252 mg/kg/day given during gestation and lactation; experiment III: 0, 450, 900, and 2252 mg/kg/day given during gestation and lactation; experiment IV: 0 and 2252 mg/kg/day given during gestation; fetuses taken at term; experiment V: progeny of rats from experiment I mated; experiment VI: progeny of rats from experiment III mated. Results are shown in Table 10.

Groups of 20, 22, 22, and 22 gravid albino CD-1 outbred mice were dosed orally with 16, 74.3, 345, and 1600 mg/kg, respectively, *dl*-α-Tocopheryl Acetate in corn oil (FDRL 1973). The animals were dosed on days 6 to 15 of gestation. Body weights were measured on days 0, 6, 11, 15, and 17 of gestation. The animals were killed on day 17 of gestation and the fetuses examined. "The administration of up to 1600 mg/kg (body weight) of [Tocopheryl Acetate] to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Groups of seven and six gravid ICR mice were dosed orally with 0.4 ml of *d*-α-Tocopheryl Acetate (591 IU) either on days 7 to 11 or day 10 of gestation, respectively (Hook et al. 1974). Groups of 13 and 8 gravid mice were used as untreated or saline control groups (dosed on days 7 to 11 of gestation), respectively. In the Tocopheryl Acetate–treated groups dosed on days 7 to 11 or day 10 of gestation, 3.3% and 4.3% of the fetuses were resorbed, respectively, compared to resorption rates of 5.1% and 3.4% in the untreated and saline control groups, respectively. One fetus of the multiple-dose Tocopheryl Acetate group had exencephaly, open eye, and micrognathia.

Tocopheryl Acetate was stated to be not teratogenic (Hoffmann-LaRoche 1995).

Tocophersolan. Groups of 15 gravid Charles River COBS, CD albino rats were fed chow containing 0.002%, 0.2%, or 2.0% Tocophersolan on days 6 to 16 of gestation (Krasavage and Terhaar 1977). Negative- and positive-controls were used. Body weights were measured daily and feed consumption was determined from days 0 to 6, 6 to 16, and 16 to 20 of gestation. The dams were killed on day 20.

Dams of the low-, mid-, and high-dose groups consumed an average of 4.6, 475, and 4613 mg Tocophersolan per rat, respectively. Mean body weight and feed consumption were similar for treated and negative-control animals. No differences in reproductive indices or the incidence of skeletal anomalies were observed between treated and negative-control animals.

Groups of 15 male and 15 female Charles River COBS, CD albino rats (F_0) that had been fed chow containing 0.002%, 0.2%, or 2% Tocophersolan or basal diet for 112 days (in a subchronic toxicity study described earlier) were mated to produce the F_{1a} offspring while continuing to eat their respective diets (Krasavage and Terhaar 1977). After weaning of the last F_{1a} litter, on day 175 of the study, the males were mated with different females in their respective groups to produce F_{1b} offspring. All offspring, who were given the same feed as their parents, were killed 5 weeks after weaning and necropsied. Tissues from four animals per litter, preferably two males and two females, were examined microscopically. Animals of the F_0 generation were killed after 265 to 268 days of dosing. Hematological and clinical chemistry determinations were made for the control and high dose animals 2 weeks prior to study termination.

Body weight gains were similar for pups of the treated and control groups. Hematological and clinical chemistry parameters of the F_0 control and high-dose animals were normal. No differences in reproductive indices were observed between test and control animals, and organ weights were similar in treated and control F_0 animals. No differences were observed in tissues of high dose and control F_0 and F_{1b} animals at microscopic examination.

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TABLE 10
Reproductive and developmental toxicity of Vitamin E (Martin and Hurley 1977)

Parameter	Experiment	Dose (mg/kg/day)	Observation
Maternal body weight	I	450, 900	Statistically significant increase during lactation
Neonate body weight	II	450	Increased on day 35 of lactation
		900	Increased on days 35, 47 of lactation
Neonate survival rate	I	90	Statistically significant decrease
	V	900	Statistically significant decrease
Maternal liver weight			
Absolute	I	90, 900	Statistically significant increase
	II, III	900	Statistically significant increase
	IV	2252	Statistically significant decrease at the end of gestation
Relative	I	45, 90, 450, 900	Statistically significant increase
	IV	2252	Statistically significant decrease at the end of gestation
Plasma lipids			
Maternal	III	2252	Statistically significantly increased at the end of lactation
	IV	2252	Statistically significantly increased at the end of gestation
Plasma vitamin E			
Maternal	IV	2252	Statistically significantly increased at the end of gestation
Neonate	III	2252	Statistically significantly increased at day 21
Hepatic vitamin E			
Maternal	III	2252	Statistically significantly increased at the end of lactation
	IV		Statistically significantly increased at the end of gestation
Neonate	III		Statistically significantly increased at day 21
Neonate anomalies	I	900	Pups of three litters had missing small toes on their hind feet
	III	2252	Three neonates had closed, infected eyes; one litter had half-open, dry eyes
	V	2252	Eight neonates had closed, infected right eyes or eyes that were half-open
	VI	2252	Three litters had closed eyes on day 14; eyes were open at weaning
General observations			The livers of dams given Tocopheryl Acetate were generally darker red than those of controls and had white spots
			Neonates of all test groups had "scaly skin" during week 1 of lactation or until appearance of the hairy coat
			Delayed delivery in some test groups

Effect on Teratogenic Agents

Tocopherol. Sprague-Dawley rats were used to determine the effect of vitamin E (form not specified; assumed to be Tocopherol) on zinc deficiency–induced teratogenicity (Hurley et al. 1983). Groups of 10 gravid animals were fed control diet (100 μg Zn/g diet) or zinc-deficient diet (<0.4 μg Zn/g diet) supplemented with Tocopherol (22312.5 IU/kg diet; 200 × control). Control groups of 10 or 11 gravid animals were fed control or zinc-deficient diet, respectively, without supplementation. All animals were killed on day 21 of gestation.

The maternal and fetal weight gains were statistically significantly decreased in both groups fed the zinc-deficient diet as compared to those fed the control diet only. The incidence of resorbed and malformed fetuses was statistically significantly increased for these groups. Feeding Tocopherol with control diet did not result in any significant increases in the incidence of resorption or malformation.

Eight groups of gravid Sprague-Dawley rats were used to determine the ability of a dietary cocktail that contained 400 mg/day α-Tocopherol, 1.0 ml/day safflower oil, and 0.08 mg/day *myo*-inositol to reduce the incidence of diabetic embryopathy (Reece and Wu 1997). Diabetes was induced by IV injection of streptozotocin in six of the groups on day 6 of gestation. Of the diabetic groups, four groups of 4, 8, 9, and 10 animals received quarter-, half-, full-, and double-strength cocktail, respectively, on days 0 to 12 of gestation. Two diabetic groups of 10 animals each were not given the cocktail; one was given daily insulin therapy and the other was given normal chow only. Animals of two groups were not made diabetic; one group of 10 animals served as a control and was fed basal diet and the

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other group of 6 animals was given double-strength cocktail on days 0 to 12 of gestation. All animals were killed on day 12 of gestation, and the fetuses were examined for overall growth and differentiation. α-Tocopherol, superoxide dismutase, and *myo*-inositol were determined.

No significant difference in weight gain was observed among any of the groups. Diabetic animals given half-, full-, and double-strength cocktail had neural tube defect rates of 6.4%, 5.4%, and 4.1%, respectively; these rates were statistically significantly decreased compared to the rates of 19.9% seen in the diabetic group given quarter-strength cocktail and 23.7% in the diabetic group given basal chow. In the two nondiabetic groups, rates of 4.0% and 4.4% were seen in the animals given basal chow and the double-strength cocktail, respectively. No significant difference in somite number was found between supplemented diabetic animals and nondiabetic controls. Maternal serum α-Tocopherol concentrations were significantly increased for diabetic animals given half-strength cocktail as compared to nondiabetic and diabetic animals that were not supplemented and diabetic animals given quarter-strength cocktail. Serum dismutase activity was statistically significantly increased in diabetic animals given quarter-, half-, and full-strength cocktail as compared to nondiabetic and diabetic animals that were not given the cocktail; the increase observed in diabetic animals given double-strength cocktail was not statistically significant. Maternal serum *myo*-inositol concentrations were not statistically significantly different between any of the groups. The cocktail containing Tocopherol reduced the malformation rate in diabetic rats.

Tocopheryl Acetate. Diabetes was induced in female Wistar rats by a single IV dose of streptozotocin, and the effect of Tocopheryl Acetate on diabetes-induced teratogenesis was examined (Viana, Herrera, and Bonet 1996). Five days after injection, the animals were mated and any dosing (done by gavage) was started on day 1 of gestation. Groups of 9, 11, and 7 gravid diabetic rats were given 150 mg α-Tocopheryl Acetate in safflower oil, no supplement, or safflower oil only, respectively. Groups of 9, 14, and 11 gravid diabetic animals followed the same regimen just described, respectively, and were given insulin. Three groups of normal rats were also used, again following the same regimen. All animals were killed on day 11.5 of gestation.

Plasma glucose, β-hydroxybutyrate, and fructosamine were measured, and the plasma concentration of all three was greater in all three groups of non–insulin-treated diabetic animals compared to insulin-treated diabetic animals and normal animals. The crown-rump length and number of somites of embryos from diabetic animals given Tocopheryl Acetate without insulin were statistically significantly greater than those of fetuses of untreated diabetic rats without insulin; these values were less than control values. No effect was observed in the protein and DNA content of embryos from diabetic animals given Tocopheryl Acetate compared to those from unsupplemented diabetic animals. Tocopheryl Acetate did not have an effect on the incidence of reabsorption in diabetic animals, 30% in diabetic rats given Tocopheryl Acetate compared to 24% in diabetic animals not supplemented, but it did significantly decrease the number of malformations, from 24.3% to 4.6%.

The effect of Tocopheryl Acetate administration on cortisone-induced embryotoxicity was investigated using random bred ICR mice (Dostál and Bláhová 1985). In the first study, 60 mg α-Tocopheryl Acetate was administered intraperitoneally to two groups of eight gravid mice on days 12 and 13 of gestation, and a third group was given olive oil on these days (route of administration not specified); all animals were mated on the same day. On day 14 of gestation, the first and second groups were dosed intraperitoneally with 60 mg Tocopheryl Acetate and either intramuscularly with 4.0 mg cortisone acetate or with the cortisone vehicle, respectively, and the third group was dosed with olive oil and 4.0 mg cortisone acetate intramuscularly. The animals were killed on day 18 of gestation, and the fetuses were examined. Of a total of 114, 127, and 113 fetuses, 63 (55%), 13 (10%), and 9 (8%) were dead in groups 1, 2, and 3, respectively. The increase in the proportion of dead fetuses was statistically significant. Of the live fetuses, 9 (18%), 1 (1%), and 13 (13%) in groups 1, 2, and 3, respectively, had a cleft palate. The mean body weights of surviving fetuses were similar for all groups.

In a second experiment using groups of seven gravid mice mated on the same day, the effects of IP administration of 0.6 or 6.0 mg Tocopheryl Acetate on day 12 of gestation and Tocopheryl Acetate given in combination with IM administration of 4.0 mg cortisone acetate on day 13 of gestation were examined. A third group of eight gravid mice that were also mated on the same day was given olive oil (route of administration not specified) on day 12 of gestation and olive oil and cortisone acetate on day 13 of gestation. The animals were killed on day 18 of gestation. Of a total of 104, 96, and 131 fetuses, 20 (19%), 42 (44%), and 9 (7%) were dead in groups 1, 2, and 3, respectively. The differences in the proportion of dead fetuses in each group was statistically significant, but only mortality after the administration of 6.0 mg Tocopheryl Acetate and olive oil was significantly different. Of the live fetuses, 30 (36%), 6 (11%), and 23 (19%) in groups 1, 2, and 3, respectively, had a cleft palate. The mean body weights of surviving fetuses were similar for all groups.

Dostál and Bláhová (1985) also examined the effect of a single combined dose of 60 mg Tocopheryl Acetate given intraperitoneally and 4.0 mg cortisone acetate given intramuscularly on day 11, 12, 13, or 14 of gestation using groups of six, seven, eight, or nine gravid mice, respectively; animals from the same mating were used only for groups dosed on day 12 or 13. The animals were killed on day 18 of gestation. Of a total of 88, 101, 110, and 115 fetuses, 22 (25%), 65 (64%) 19 (17%), and 71 (62%) were dead in groups 1, 2, 3, and 4, respectively. Of the live fetuses, 0, 0, 10 (11%), and 10 (23%) in groups 1, 2, 3, and 4, respectively, had a cleft palate. The researchers stated that they could not fully analyze the significance of the difference in the proportion of dead fetuses because females from the same matings were not used on the same day. For the groups of

TABLE 11
Genotoxicity

Assay	Form	Dose	Protocol	Results	Reference
			Tocopherol		
Ames	*dl*-α	≤1000 μg/plate	*S. typhimurium* TA98 and TA100 without and with metabolic activation	Negative	Wood 1998
Ames	*d*-α	≤10,000 μg/plate	*S. typhimurium* TA1535, TA1537, TA1538, TA98, and TA100 without and with metabolic activation; *Escherichia coli* WP2 (uvrA) without and with metabolic activation	Negative	SRI International, Inc. 1979
Ames	*dl*-α	Not stated	*S. typhimurium* TA100 and TA98	Negative	Kawachi et al. 1980
Ames	*dl*-α	≤5 mg/plate	*S. typhimurium* TA92, TA1535, TA100, TA1537, TA94, and TA98	Negative	Ishidate et al. 1984
Ames	*d*-α	0.033–10 mg/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 without and with metabolic activation; *E. coli* WP2 (uvrA) without and with metabolic activation	Negative	Prival, Simmon, and Mortelmans 1991
Ames	*dl*-α	1 mg/plate	*S. typhimurium* TA102	Negative	Anderson et al. 1995
rec	*dl*-α	Not stated	*Bacillus subtilis* without and with metabolic activation	Negative	Kawachi et al. 1980
Micronucleus	*dl*-α	75 mg/kg	Male CD-1 mice were dosed with a single dose of Tocopherol in corn oil	Negative	Anderson et al. 1995
SCE	*dl*-α	Not stated	Hamster lung fibroblast cells without metabolic activation	Negative	Kawachi et al. 1980
SCE	*dl*-α	Not stated	Human embryo fibroblasts without metabolic activation	Negative	Kawachi et al. 1980
Chromosome aberrations	*dl*-α	≤0.5 mg/ml	Chinese hamster fibroblast cell line without metabolic activation	Negative	Ishidate et al. 1984
Chromosome aberrations	*dl*-α	Not stated	Rat bone marrow cells for chromosomal aberration in vivo	Negative	Kawachi et al. 1980
Chromosome aberrations	α	1000 mg/kg twice weekly	Groups of 5 mice were dosed IP with Tocopherol twice weekly for 1, 2, 3, or 4 weeks	No significant difference from control values	Kodýtková, Madar, and Šrám 1980
Chromosome aberrations	α	100, 300-mg/kg	6–7 male albino rats were dosed orally with Tocopherol in olive oil for 6 months, and then examined for chromosomal aberrations; controls were given water or olive oil	Did not produce chromosomal aberrations; animals of the 300-mg/kg group had a decrease in aberrant cells and the number of pulverized cells	El-Nahas, Mattar, and Mohamed 1993
Chromosome aberrations	*dl*-α	400 μg/plate	Human lymphocytes were exposed	Negative	Anderson et al. 1995

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DNA strand breaks	*dl*-α	0.625–5 mg/kg	Sprague–Dawley rats were injected with Tocopherol in olive oil and killed after 4 hours; some animals dosed with 2.5 mg/kg were killed after 0.15–72 hours	A dose-dependent increased elution rate of DNA in alkali was observed	Russo et al. 1984
Thymidine incorporation	α	≤0.1 mM	Hepatocytes from male Fischer 344 rats were used	Incorporation of [^{3}H]-thymidine was inhibited 50%	Novicki, Rosenberg, and Michalopoulos 1985
SOS chromatest	α	Not stated	Details not provided	Negative	von der Hude et al. 1987
Forward mutations	*dl*-α	Not stated	Mutagenic potential using silk worms	Negative	Kawachi et al. 1980
			Tocopheryl Acetate		
Ames	α	Not stated	Details not provided	Negative	BASF 1993b, 1996a
Micronucleus	*dl*-α	0, 30, or 1000 mg/kg diet	Groups of 8 male ICR mice were fed chow containing 0, 30, or 1000 mg Tocopheryl Acetate/kg diet for 50 weeks; blood samples were taken at various intervals for micronucleus analysis	No effect was observed	Umegaki, Uramoto, and Esashi 1997
SCE			SCE in bone marrow was determined at study termination	No effect was observed	
DNA strand breaks	*dl*-α	0.625–5 mg/kg	Sprague-Dawley rats were injected with Tocopherol in olive oil and killed after 4 hours; some animals dosed with 2.5 mg/kg were killed after 0.15–72 hours	Maximum effect observed between 4 and 24 hours; almost no effect observed after 48 hours	Russo et al. 1984
			Tocopheryl Succinate		
Ames	D-α	100–10,000 μg/plate	*S. typhimurium* strains TA100, TA1535, TA97, and TA98 without and with metabolic activation	Negative	Zeiger et al. 1988
Chromosomal aberrations	α	25 μM	Chinese hamster V79 cells were treated for 24 hours	Negative	Sugiyama, Lin, and Costa 1991
			Dioleyl Tocopheryl Methylsilanol		
SOS chromatest	Mix*	Not stated	Details not provided	Negative	Exsymol 1998

*A mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol (0.05% Tocopheryl Acetate, 0.5% monomethylsilanetriol, remainder oleic alcohol).

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animals that were from the same matings (groups 2 and 3), the difference in embryolethality was statistically significant.

In a fourth experiment, Dostál and Bláhová (1985) administered 6.0 mg Tocopheryl Acetate either intraperitoneally or intramuscularly to groups of 11 gravid mice on days 12 and 13 of gestation; 4.0 mg cortisone acetate given intramuscularly was also administered on day 13 of gestation. The animals were killed on day 18 of gestation. Of a total of 153 and 154 fetuses, 93 (61%) and 16 (10%) were dead in groups 1 and 2, respectively. The difference in the proportion of dead fetuses was statistically significant. Of the live fetuses, 30 (50%) and 33 (24%) in groups 1 and 2, respectively, had a cleft palate. The mean body weights of surviving fetuses were similar for all groups. The embryolethal effect of cortisone acetate was potentiated by Tocopheryl Acetate when Tocopheryl Acetate was administered intraperitoneally on days 11 to 14 of gestation.

Tocopheryl Succinate. The effect of Tocopheryl Succinate on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced developmental toxicity was determined using gravid C57BL/6J mice (Hassoun et al. 1997). Groups of 9 or 10 animals were given by gavage 100 mg/kg Tocopheryl Succinate in corn oil on days 10, 11, and 12 of gestation, and 40 mg/kg on day 13 of gestation. The group of 10 animals was also dosed by gavage with 30 μg/kg TCDD in corn oil on day 12 of gestation 2 hours prior to dosing with Tocopheryl Succinate. A group of nine animals was dosed with TCDD only and a negative-control group of nine animals was dosed with corn oil. Four animals of each group were killed on day 14 of gestation, and determinations of reactive oxygen species, lipid peroxidation, and DNA single-strand breaks were made. The remainder of the animals were killed on day 18 of gestation.

Tocopheryl Succinate alone did not induce teratogenic effects. Tocopheryl Succinate decreased TCDD-induced embryo/fetolethality and fetal and placental growth retardation 65%, 86%, and 67%, respectively. However, Tocopheryl Succinate did not prevent TCDD-induced cleft palate or hydronephrosis. Tocopheryl Succinate administration reduced TCDD-induced production of superoxide anion, lipid peroxidation, and DNA single strand breaks by 77% to 88%, 70% to 87%, and 21% to 47%, respectively.

GENOTOXICITY

Assays on the mutagenic potential of Tocopherol, Tocopherol Acetate, Tocopheryl Succinate, and Dioleyl Tocopheryl Methylsilanol were almost uniformly negative. These data are summarized in Table 11.

Modulation of Genotoxicity

Tocopherol. Human leukocytes from a male subject were incubated for 3 days; 15 hours prior to chromosome harvest, 10 μM *dl*-α-Tocopherol and DMBA were added separately (Shamberger 1974). Tocopherol reduced chromosome breakage 63.2% as compared to DMBA alone. Malonaldehyde and β-propiolactone were mutagenic toward *Salmonella typhimurium* strains hisG46, TA1975, hisC207, his3076, TA1977, hisD3052, and TA1978 (Shamberger et al. 1979). *dl*-α-Tocopherol, at doses of 0.33 to 33 μM, reduced the mutagenesis in most runs.

The antimutagenic activity of *dl*-α-Tocopherol against solvent extracts of coal dust, diesel emission particles, airborne particles, fried beef, and tobacco snuff was determined (Ong et al. 1989). Tocopherol inhibited <16% of the activity of coal dust and approximately 20% of the activity of tobacco snuff, but did not inhibit the mutagenic activity of diesel emission particles, airborne particles, or fried beef.

Vitamin E (assumed to be Tocopherol) was evaluated for an antimutagenic effect against 2-nitrofluorene, 4-nitroquinoline 1-oxide, 2-aminoanthracene, and benzo(a)pyrene (BaP) (Houk et al. 1992). Tocopherol inhibited the mutagenic effects of 2-nitrofluorene, 2-aminoanthracene, and BaP by 70% to 90%.

The ability of Tocopherol in DMSO to modulate the spontaneous revertants in *S. typhimurium* strains carrying the *hisG428* mutation was examined (De Flora et al. 1994). α-Tocopherol was tested in triplicate at doses of $\leq$10 μmol/plate with *S. typhimurium* TA104 without metabolic activation. Antimutagenic effects of Tocopherol toward 4-nitroquinoline 1-oxide in *S. typhimurium* TA100 without metabolic activation and cigarette smoke in *S. typhimurium* TA98 with metabolic activation were also determined. Tocopherol did not affect the number of spontaneous revertants in *S. typhimurium* strain TA104 or the mutagenicity induced by 4-nitroquinoline in *S. typhimurium* TA100 without metabolic activation or cigarette smoke in *S. typhimurium* TA98 with metabolic activation.

The protective effect of 3 to 12.5 μg/ml *dl*-α-Tocopherol on the frequency and repair of oxidant-mediated DNA-strand breaks in mononuclear leukocytes exposed to activated autologous neutrophils was determined (van Staden, van Rensburg, and Anderson 1993). Tocopherol completely protected cells against initial DNA damage caused by activated neutrophils. In phagocyte-free systems, Tocopherol had no effect on DNA damage in the mononuclear leukocytes. Tocopherol produced a statistically significant inhibition of superoxide generation.

The effect of vitamin E (assumed to be Tocopherol) on bacterial growth inhibition, the number of spontaneous revertants, and aflatoxin B_1 (AFB_1)-induced mutagenesis was determined in *S. typhimurium* TA98 and TA100 (Raina and Gurtoo 1985). Tocopherol, 2×10^{-5} M, produced <5% bacterial growth inhibition, and it did not affect the number of spontaneous revertants. Tocopherol inhibited AFB_1 metabolism to mutagenic metabolites and the expression of AFB_1-induced mutations.

Male SD rats were fed basal chow and then injected (route not specified) with 750 mg/kg *dl*-α-Tocopherol in peanut oil or fed Tocopherol-deficient chow, and the effect of Tocopherol on the mutagenicity of BaP or AFB_1 was determined (Narbonne et al. 1990). The animals were killed 48 hours after dosing. Tocopherol decreased the ability of S9 fractions to activate BaP, whereas Tocopherol deficiency increased the mutagenic activity of BaP towards *S. typhimurium* TA98. Tocopherol did not have an effect on mutagenicity of AFB_1.

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The effect of Tocopherol on the incidence of chromosomal aberrations in bone marrow cells induced by cyclophosphamide was examined (Kodýtková, Madar, and Šrám 1980). Groups of five female ICR mice were given 0.01% or 0.02% cyclophosphamide in the drinking water for 1, 2, 3, or 4 weeks (approximately 100 or 200 mg/kg weekly). The animals were dosed intraperitoneally with 1000 mg/kg α-Tocopherol twice weekly, starting on the second day of dosing with cyclophosphamide. Dosing with cyclophosphamide alone and with cyclophosphamide plus Tocopherol significantly increased the incidence of aberrant cells as compared to the incidence found in control groups or those given only Tocopherol. The average incidence of aberrant cells was greater in the animals given Tocopherol with 0.01% cyclophosphamide as compared to those given 0.01% cyclophosphamide only.

Ovariectomized and sham-ovariectomized female Swiss mice were given 25 mg α-Tocopherol by gavage for 15 days, and its effect on clastogenicity was determined (Lacava and Luna 1994). Tocopherol inhibited chromosome damage in ovariectomized mice by approximately 80%. Tocopherol also statistically significantly reduced the frequency of chromosomal aberrations in ovariectomized mice dosed with *N*-benzyl-2-nitroimidazoleacetamide.

An Ames assay using *S. typhimurium* strain TA102, a chromosomal aberration assay using human peripheral lymphocytes, and a mouse micronucleus assay using peripheral lymphocytes and bone marrow cells were performed to determine the protective effect of *dl*-α-Tocopherol against bleomycin-induced mutagenicity (Anderson et al. 1995). In the Ames and chromosomal aberration assays, Tocopherol did not affect the mutagenic response to bleomycin. In the micronucleus test, Tocopherol decreased the mutagenic response in peripheral blood cells, but the decrease was not statistically significant, and it did not affect the response in bone marrow.

Assays were performed to evaluate the antimutagenic effect of and a possible interaction between α-Tocopherol and methylazoxymethanol (MAM) and to estimate the protective effect of Tocopherol towards the genotoxic action of MAM using *S. typhimurium* strain TA100 (Tavan et al. 1997). Tocopherol significantly reduced the mutagenicity of MAM, and a significant negative linear correlation was found between the decrease of MAM mutagenicity and the increase in Tocopherol. In determining the protective effect of Tocopherol, a nonsignificant, non–dose-dependent inhibition of MAM mutagenicity was observed, and the number of viable bacteria was the same with or without Tocopherol.

The protective effect of 30 μM α-Tocopherol on Raji lymphoblastoid cells exposed to x-rays or hydrogen peroxide was examined using the comet assay and colony-forming assay (Sweetman, Strain, and McKelvey-Martin 1997). Tocopherol, alone and in combination with 60 μM ascorbic acid, significantly decreased endogenous DNA damage in Raji cells. In cells treated with hydrogen peroxide, a nonsignificant decrease in damage with Tocopherol pretreatment was found at time 0 with 50 μM hydrogen peroxide, and a significant decrease in tail moment was found over the 0- to 90-minute post-treatment period with 5 and 20 μM hydrogen peroxide. Tocopherol increased the survival of cells exposed to 1-Gy x-ray treatment, but supplementation of Tocopherol in combination with ascorbic acid did not affect treatment. Tocopherol, alone or in combination with ascorbic acid, did not have an effect on survival after treatment with 3- and 5-Gy x-ray treatment. Tocopherol also increased survival of cells treated with 5 and 20 μM hydrogen peroxide.

Tocopheryl Acetate. The effect of 0.1 mM *dl*-α-Tocopheryl Acetate in conjunction with 0.1 or 0.5 mM ascorbic acid on hyperoxia-induced mutagenicity toward CHO-K1-BH4 Chinese hamster ovary cells was examined at 20% and 90% oxygen (Gille et al. 1991). With 90% oxygen, 0.1 mM ascorbic acid increased the incidence of aberrant cells and 0.5 mM had an antimitotic effect; Tocopheryl Acetate did not alter either effect. At 20% oxygen, neither mutagenic nor antimutagenic effects were observed.

The effect of α-Tocopheryl Acetate on the yield of sex-linked recessive lethal mutants induced by irradiation in mature *Drosophila* sperm was examined (Beckman, Roy, and Sproule 1982). No significant difference was observed between Oregon-R males raised on Tocopherol- or nonsupplemented medium that were irradiated (30 Gy) and mated with females raised on nonsupplemented medium. A significant reduction in the frequency of sex-linked recessive lethal mutations was observed in the progeny of F_1 females derived from FM7a females raised on a Tocopherol-supplemented medium and mated with irradiated Oregon-R males.

Tocopheryl Succinate. The effect of *dl*-α-Tocopheryl Succinate on oxygen radical–induced sister-chromatid exchanges (SCEs) were determined (Weitberg 1987). Preincubation with Tocopheryl Succinate decreased the number of oxygen radical–induced SCEs; however, control values were not obtained. In target cells that were preincubated with Tocopheryl Succinate and treated with superoxide dismutase or catalase, the number of SCEs was reduced to control values and was significantly less than the number observed with Tocopheryl Succinate alone.

The ability of Tocopheryl Succinate to decrease sodium chromate-induced chromosomal aberrations was examined using Chinese hamster V79 cells (Sugiyama, Lin, and Costa 1991). Pretreatment with 25 μM α-Tocopheryl Succinate prior to exposure to Na_2CrO_4 resulted in a decrease in metal-induced chromosomal aberrations.

Effect on UV-Induced Genotoxicity

Tocopherol. In order to evaluate the radioprotective effect of Tocopherol, groups of four male albino rats were dosed orally with 100 or 300 mg/kg α-Tocopherol in olive oil for 6 months and then subjected to whole-body irradiation of 400 rad for 13 seconds (El-Nahas, Mattar, and Mohamed 1993). Control

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groups were given water or olive oil. The animals were killed 6 hours after irradiation. No difference in the percentage of cells with chromosomal aberrations was found for animals dosed with 100 mg/kg Tocopherol and those given olive oil. In the animals dosed with 300 mg/kg Tocopherol, a statistically significantly increase in the number of aberrant cells was observed when compared to the control animals given olive oil. No difference was observed between cells from any of the treated animals and the control animals given water.

The effect of Tocopherol on DNA repair was examined by measuring unscheduled DNA synthesis (UDS) in UVB-irradiated human skin fibroblasts (Kondo et al. 1990). Fibroblasts, which were obtained from the elbow, back, inguinal area, or upper arm of five healthy subjects, were grown with or without 100 μg/ml Tocopherol. UDS was measured following irradiation with UVB from a polychromatic light from a two-tube sun lamp (Toshiba FL 20SE), wavelength range of 280 to 320 nm with a maximum at 305 nm, at a dose of 500 J/m^2. Tocopherol did not affect UDS.

The protective effect of α-Tocopherol toward the photomutagenicity induced by 8-MOP was determined at various partial pressures of oxygen (pO_2) using *S. typhimurium* strain TA102 and *Saccharomyces cerevisiae* D7 (Bianchi et al. 1996). A dose of 1.86 or 18.6 μM Tocopherol (alone and in combination with β-carotene) and a dose of 4.6×10^{-6} M 8-MOP was used. Both the bacteria and the yeast were irradiated for 10 minutes with UV light from a Philips high-pressure mercury vapor lamp that emitted radiation in a range of 300 to 400 nm with the maximum emission band at 365 nm at various pO_2's.

With *S. typhimurium*, Tocopherol had a protective effect at the higher dose; a 1.86-μM dosage of Tocopherol and carotene had a significant inhibition of the photomutagenic effect induced by 8-MOP. In partial pressure, Tocopherol had a significant antimutagenic effect at 190 and 380 mm Hg pO_2. With *S. cerevisiae*, Tocopherol alone did not have a protective effect at any dose or pressure, and a Tocopherol/carotene mixture had a protective effect only at 380 mm Hg pO_2.

Tocopheryl Succinate. The effect of Tocopheryl Succinate on UVB-induced mutagenicity was examined using Chinese hamster V-79 cells (Sugiyama et al. 1992). The cells were pretreated for 24 hours with 25 μM α-Tocopheryl Succinate and then irradiated with UVB light from four Toshiba FL 20SE-30 lamps, wavelength range of 280 to 360 nm with maximum at 305 nm; UVB doses of 200, 400, and 600 J/m^2 were tested. Control cells were cultured with DMSO only. Pretreatment with Tocopheryl Succinate did not reduce the number of UVB-induced DNA single-strand breaks, chromosomal aberrations, or mutations at the *HGPRT* locus.

CARCINOGENICITY

Oral

Tocopherol. A group of 15 male A/J mice were fed chow containing 550.0 mg/kg Tocopherol and groups of 15 A/J and ddY male mice were fed control diet (which contained 25.5 mg/kg Tocopherol) for 40 weeks (Yano et al. 1994). A/J mice are more susceptible to the induction of spontaneous pulmonary tumorigenesis than ddY strain mice, and this study was designed to determine whether Tocopherol would reduce the incidence of pulmonary tumorigenesis in the A/J mice to an incidence similar to that of ddY mice. Five animals per group were killed after 28 weeks to determined the effect of Tocopherol on oxidative stress on the pulmonary nuclei. The remaining 10 animals per group were killed at the termination of dosing.

No significant difference was observed in body weights between A/J mice of the test and control groups. At 28 weeks, the reduced nicotinamide adenine dinucleotide diphosphate (NADPH)-driven active oxygen generation, the nuclear thiobarbituric acid reactive substances (TBARS), and DNA single-strand breaks were statistically significantly greater in A/J control animals than in ddY control animals; dosing of A/J mice with Tocopherol decreased the amount of TBARS and DNA single-strand breaks compared to A/J controls. Nuclear α-Tocopherol concentrations in A/J controls were significantly decreased compared to ddY controls; Tocopherol increased the concentrations. A/J test animals had a 53% lower incidence of pulmonary neoplasms and 60% less pulmonary neoplasm multiplicity as compared to A/J controls; the incidence of pulmonary neoplasms in A/J test animals was similar to that of ddY controls. The plasma α-Tocopherol concentration in A/J controls was 45% lower than that of ddY controls, and the plasma α-Tocopherol concentration in tumor-bearing A/J controls was significantly decreased compared to that in non–tumor-bearing A/J controls. Plasma α-Tocopherol concentrations of test A/J animals was increased 140% compared to A/J controls. The researchers concluded that Tocopherol reduced spontaneous pulmonary tumorigenesis.

A group of 35 male Wistar rats was fed a diet supplemented with α-Tocopherol (50.0 units/100 g) and a group of 45 male Wistar rats was fed a Tocopherol-"sufficient" diet (2.0 units/100 g), and the effect on iron-mediated, free radical–induced apoptosis, 8-hydroxydeoxyguanosine (8-OHdG), and the incidence of renal cancer was determined (Zhang et al. 1997). After 1 month, five animals per group were killed and the α-Tocopherol concentrations in the sera, liver, and kidneys were determined. Also after 1 month, 10 rats per group were dosed intraperitoneally with ferric nitrilotriacetate (Fe-NTA) and were killed after 0, 1, 6, or 24 hours. The remaining animals in each group were injected with 7.5 mg/kg Fe-NTA once or twice weekly for 3 months and then not treated for 9 months. All surviving animals were killed at study termination.

Iron-induced lipid peroxidation, 8-OHdG formation, and apoptosis were observed in the Fe-NTA–treated animals. Five animals of the Tocopherol-sufficient group died during the first 3 months. The incidence of cancer was statistically significantly reduced in the animals of the Tocopherol-supplemented group (5%) as compared to the surviving animals of the Tocopherol-sufficient group (44%). Severe renal cystic lesions were observed in animals of the Tocopherol-sufficient group.

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Tocopheryl Acetate. In a chronic feeding study performed by Wheldon et al. (1983) in which groups of 60 male and 60 female rats were fed chow supplemented with 500, 1000, or 2000 mg/kg *dl*-α-Tocopheryl Acetate, the researchers examined the incidence of neoplasms. "The group distribution of tumours did not suggest a neoplastic response to treatment with [Tocopheryl Acetate]." The number of neoplasms was similar for all groups after 52 and 104 weeks of treatment. "In both sexes, there were indications of an inverse relation between dosage and the incidence of mammary fibro-adenomas, but in males the intergroup differences were not statistically significant at the 5% level.... The distribution of pituitary adenomas displayed no related trend. Small incidences of liver cell tumours and of tumours of the biliary epithelium were scattered across the groups without relation to dosage or to treatment."

Hoffmann-LaRoche (1995) stated that Tocopheryl Acetate was not carcinogenic, but no basis was provided.

Parenteral

Tocopherol. Groups of 22 male BALB mice were dosed subcutaneously with 16 mg α-Tocopherol in 0.1 ml soya oil, 16 mg α-Tocopherol, 0.1 ml soya oil, or 0.1 ml physiological saline for 10 months (Constantinides and Harkey 1985). With the exception of the Tocopherol + soya oil group, in which the animals were killed 3 to 4 weeks after the first detection of a neoplasm, the animals were killed 7 months after the termination of dosing. In the Tocopherol + soya oil group, 17 of the 22 animals developed tumors in the subcutaneous (SC) space of the dorsum; these tumors were transplantable. No tumors developed in the other three groups. No significant response was observed at the site of Tocopherol (only) injection; however, most soya oil (only) injection sites were massively infiltrated by polymorphonuclear (neutrophil) leukocytes.

Groups of seven female NSF/N and 10 6HF1 mice were dosed subcutaneously with 20 mg of natural vitamin E (8.9% α-Tocopherol, 0.9% β-Tocopherol, 41% γ-Tocopherol, and 49.2% δ-Tocopherol), natural vitamin E + 0.1 ml soya oil, or soya oil (Nitta et al. 1991). Starting at 8 weeks of age, the animals were dosed once a week at four independent sites on the dorsum in rotation for a total of 52 treatments. The animals were killed either when a tumor was 10 mm in diameter or when the animals were 60 weeks of age. In both NFS/N and 6HF1 mice, natural vitamin E + soya oil induced neoplasms (incidence of 85.6% and 60%, respectively). Some of the neoplasms were transplantable. Natural vitamin E or soya oil alone did not induce neoplasms.

Tocopheryl Acetate. Groups of 10 male and 10 female NFS/N mice were dosed subcutaneously with 20 mg *dl*-α-Tocopheryl Acetate + 0.1 ml soya oil and groups of 5 male and 5 female NFS/N mice were dosed subcutaneously with *dl*-α-Tocopheryl Acetate + 0.1 ml palm oil, *dl*-α-Tocopheryl Acetate, soya oil, or palm oil (Nitta et al. 1991). At 8 weeks of age, the animals were injected once a week at four independent sites on the dorsum in rotation; treatment continued until the animals were 60 weeks in age. The animals were killed either when a neoplasm was 10 mm in diameter or when the animals were 68 weeks of age. Tocopheryl Acetate + soya oil induced tumors in 20% and 40% of the male and females, respectively. Both Tocopheryl Acetate + palm oil and Tocopheryl Acetate alone induced tumors in 20% of the males. The neoplasms were transplantable. No neoplasms were induced in females of any of the other groups.

Nitta et al. (1991) also dosed male F344 rats with Tocopheryl Acetate. A group of 17 animals was dosed subcutaneously with 40 mg *dl*-α-Tocopheryl Acetate, 15 or 18 animals were dosed with *dl*-α-Tocopheryl Acetate + soya or palm oil, respectively, and groups of 12 animals were dosed with soya or palm oil. Dosing was initiated at 9 to 11 weeks of age following the same dosing rotation described previously for a total of 52 treatments. The animals were killed either when a neoplasm was 20 mm in diameter or 8 weeks after the last injection. The incidence of neoplasms was 82.4%, 66.7%, and 22.2% for the animals dosed with Tocopheryl Acetate, Tocopheryl Acetate + soya oil, and Tocopheryl Acetate + palm oil, respectively. The neoplasms were transplantable. No neoplasms were induced in the animals dosed with soya or palm oil only.

A group of 15 male Fischer rats were dosed subcutaneously in the dorsum with 40 mg Tocopheryl Acetate in 0.2 ml soybean oil once a week for 10 to 12 months (Ishinaga et al. 1991). Fibrosarcomas developed in 73% of the animals. The neoplasms were transplantable. The phospholipid composition in the primary and transplanted neoplasms were similar, with phosphatidylcholine and phosphatidylethanolamine representing 54% to 56% and 25% to 26% of the total phospholipids.

Modulation of Carcinogenicity

Many studies have investigated the modulation of carcinogenicity by Tocopherol and its derivatives. These studies are included in Table 12. In most cases, Tocopherol appeared protective. One study, however, concluded that Tocopherol acted as a complete tumor promoter in DMBA-initiated mouse skin.

Mitchel and McCann (1993) studied the tumor promotion effects of Tocopherol. Female SENCAR mice, 6 to 8 weeks of age, were initiated with DMBA (10 nmol in 0.2 ml acetone) on the shaved dorsal skin.

Promotion was begun 1 week after initiation. Groups of 25 mice were treated twice weekly with Tocopherol (*d,l* racemic mixture) from two sources (80 μmol in 0.2 ml acetone per application) or a known tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (2 μg in 0.1 ml acetone per application). After 13 weeks of treatment, one tocopherol group was further treated twice weekly with mezerein, a stage II promoter (4 μg in 0.1 ml acetone). Throughout the procedures all animals were given anesthesia in a carrier of pure oxygen. Treatments continued until the number of papillaomas was judged to have stabilized. Mice were followed for their natural life span.

The time to first tumor (papilloma) appearance was 32, 39, and 50 days for the TPA and the two Tocopherol treatment

TABLE 12
Modulation of carcinogenicity

Form	Exposure	Cancer type	Protocol*	Results	Reference
			Tocopherol		
α	Dermal	Skin	Groups of 30 female Ha/ICR mice were shaved 4 days prior to treatment; only those without hair regrowth were used; 43 μg Tocopherol in 80 μl acetone was dropped on the back of each animal; after 5 minutes, 25 μg DMBA in 80 μl acetone was dropped on the same site; after 1 week, 80 μl 1% croton oil in acetone was applied topically once per week for 4 months	Tocopherol did not affect the number of animals with tumors or the number of tumors/animal compared to animals dosed with acetone prior to DMBA	Wattenberg 1972
Vitamin E	Dermal	Skin	30 female Charles River CD-1 mice/group were shaved 2 days prior to treatment; only animals in the resting phase of the hair cycle were used; Initiation was with 2.56 μg DMBA; 10 μg of the promoter TPA was applied twice per week 1 week later; 1000 μg Tocopherol was applied 5 minutes before the initiator	29 animals survived until week 28; 68% of the surviving animals had papillomas, and there were 2.1 papillomas/surviving animal; this is compared to 82% and 3.4 papillomas/animal in the animals that were dosed with DMBA and TPA, but no modifier; Tocopherol had little effect on aryl hydrocarbon hydroxylase activity	Slaga and Bracken 1977
D-α	Dermal	Skin	Groups of 36 female CF-1 mice were used (the dorsal skin was shaved). In an initiation/promotion study, initiation was with 0.1 μmol DMBA; 2 weeks after initiation, 8.5 nmol TPA was applied 2$\times$ weekly; 40 μmol Tocopherol was applied dermally 15 minutes prior to, and at the same site of, TPA	Initiator/promotion study: compared to the animals dosed with DMBA and TPA but not a modifier, Tocopherol reduced the % of animals with papillomas and number of papillomas/animal from 100% to 79% and from 12.6% to 5.8%, respectively; the inhibition was enhanced by combined treatment with GSH and/or Na_2SeO_3	Perchellet et al. 1987
D-α	Dermal	Skin	Groups of 36 female CF-1 mice were used (the dorsal skin was shaved). In a 2-stage promotion study, 8.5 nmol TPA was applied 4$\times$ to DMBA initiated mice, and the animals were then promoted 2$\times$ weekly with 8.5 nmol mezerein; Tocopherol + IP Na_2SeO_3 or IP GSH were applied 15 minutes before TPA or before mezerein was used (the dorsal skin was shaved)	2-stage promotion: mezerein-induced stage 2 promotion was inhibited with both Tocopherol + Na_2SeO_3 or GSH; Tocopherol + GSH did not inhibit and Tocopherol + Na_2SeO_3 enhanced stage 1–promoting activity of TPA	

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D-α	Dermal	Skin	Groups of 36 female CF-1 mice. Using the complete carcinogen DMBA, tumors were induced by a single dermal application of 3.6 μmol or by 2× weekly applications of 0.1 μmol DMBA; Tocopherol + IP Na_2SeO_3 or IP GSH was applied 15 minutes prior to each dose	Complete carcinogen: Tocopherol + GSH or Na_2SeO_3 did not inhibit the carcinogenicity of 3.6 μmol DMBA; Tocopherol + GSH or Na_2SeO_3 enhanced the formation of skin papillomas and carcinomas with 0.1 μmol DMBA from 7% of animals with carcinomas and 2.5 papillomas/animal to 25% and 29%, respectively, and 9.7% and 7.4%, respectively	
DL-α	Oral	Oral	Four groups of 10 male and 10 female noninbred Syrian golden hamsters/group; housed 5/cage. Group 1: the left buccal pouch of each animal was painted 3× per week with 0.5% DMBA in heavy mineral oil; given peanut oil on days opposite DMBA. Group 2: treated with DMBA and dosed orally with 10 mg Tocopherol in peanut oil 2× per week on days opposite DMBA. Group 3: dosed with Tocopherol. Group 4: control group given peanut oil); 2 males and 2 females were killed at 8, 10, 12, 14, 16 weeks	There was a statistically significant decrease in the number of tumors in group 2 compared to group 1; both the appearance of leukoplakia and the development of visible tumors was delayed, and the tumors were smaller at all stages of development; no abnormalities were observed in groups 3 or 4	Shklar 1982
α	Oral	Oral	Four groups of 8 male and 8 female golden hamsters/group. Group 1: the left buccal pouch of each animal was painted 3× per week with 0.25% DMBA in heavy mineral oil. Group 2: treated with DMBA and dosed orally with 7 IU Tocopherol 2× per week on days opposite DMBA. Group 3: treated with DMBA and given vehicle. Group 4: untreated control group. 2 males and 2 females were killed at 8, 10, 12, 14 weeks	There was a delay in the appearance of leukoplakia and the development of visible tumors, and the tumors were smaller and fewer in the group 2 animals compared to groups 1 and 3; microscopically, the development of lesions in group 2, although similar to groups 1 and 3, was delayed	Weerapradist and Shklar 1982
DL-α	Oral	Oral	Four groups of 6 male and 6 female golden hamsters/group. Groups 1: the left buccal pouch of each animal was painted with 0.5% DMBA in heavy mineral oil 3× per week for 7 weeks. Group 2: treated with DMBA followed by painting with 47.5 mg Tocopherol 3× per week for 4 weeks. Group 3: untreated for 7 weeks and then treated with Tocopherol for 4 weeks. Group 4: untreated control group. 6 animals/group were killed after 11 weeks, and the remaining 6 were killed at week 12	Group 2 had statistically significantly less tumors than group 1 (30 vs. 54), and the tumors were smaller in diameter (2–2.5 mm vs. 3–4 mm); groups 3 and 4 had no tumors	Odukoya, Hawach, and Shklar 1984

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TABLE 12
Modulation of carcinogenicity *(Continued)*

Form	Exposure	Cancer type	Protocol*	Results	Reference
DL-α	Oral	Oral	Four groups of 10 male and 10 female noninbred Syrian golden hamsters/group. Group 1: the left buccal pouch of each animal was painted 3× per week for 28 weeks with 0.1% DMBA in heavy mineral oil. Group 2: treated with DMBA and dosed orally with 10 mg Tocopherol in peanut oil 3× per week on days opposite DMBA. Group 3: dosed with Tocopherol. Group 4: untreated control group. All animals were killed after 28 weeks	All group 1 and no group 2, 3, or 4 animals had visible tumors at week 28; the number of tumors/animal was 2.45. Microscopically, the left buccal pouches of group 1 animals had areas of hyperkeratosis, dysplasia, carcinoma in situ, and frank papillary and invasive epidermoid carcinomas; the pouches of group 2 animals had areas of hyperkeratosis and occasional mild dysplasia, but no areas of carcinoma in situ or frank epidermoid carcinoma	Trickler and Shklar 1987
α	Oral	Esophageal	10–17 female C57BL/6 mice/group; housed 4/cage. All animals were dosed by gavage with 0.2 mg/kg NMBzA or corn oil 3× per week for 3 weeks; some animals were fed 142 mg/kg Tocopherol or fed ethanol; other groups were given Tocopherol + NMBzA, ethanol + NMBzA. Ethanol + Tocopherol, or Tocopherol + NMBzA + ethanol; dosing ended 20 weeks after the last NMBzA treatment	Treatment with Tocopherol produced a statistically significant decrease in the number of animals with tumors, tumors per animal, and the size of tumors in NMBzA + ethanol–treated animals; and the number of animals with tumors and tumors per animal in NMBzA-treated animals. Animals given Tocopherol only did not develop tumors. Tocopherol did not affect body weight gain, nor did it cause a statistically significant reduction in indexes of peroxidation in NMBzA- or ethanol-treated animals; Tocopherol did not prevent ethanol- or NMBzA-induced hepatotoxicity	Odeleye et al. 1992
α	Oral	Esophageal	10–17 female C57BL/6 mice/group; housed 4/cage. All animals were dosed by gavage with 0.2 mg/kg NMBzA or corn oil 3× per week for 3 weeks; some animals were fed 142 mg/kg Tocopherol or fed ethanol; other groups were given Tocopherol + NMBzA, ethanol + NMBzA, ethanol + Tocopherol, or Tocopherol + NMBzA + ethanol; dosing ended 22 weeks after the last NMBzA treatment	Compared to nonsupplemented animals, Tocopherol reduced the number of animals with tumors and the number of tumors per animal in the NMBzA and NMBzA + ethanol–treated animals. Animals given Tocopherol only did not develop tumors	Eskelson et al. 1993

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α	Oral	Mammary	18 female Sprague-Dawley rats were given 200 mg/ml Tocopherol in olive oil by gavage; after 1 hour, the animals were dosed with 12 mg/ml DMBA in olive oil; tumors were counted 12 weeks after dosing. The animals were killed 18 weeks after dosing	Compared to animals given vehicle prior to DMBA, Tocopherol slightly but insignificantly reduced the number of animals with tumors	Wattenberg 1972
α	Oral	Lung	20 (initiated) or 10 (noninitiated) male F344/DuCrj mice per group. Initiation consisted of 0.1% DHPN in the drinking water for 2 weeks. An initiated and a noninitiated group were fed 1% Tocopherol for 30 weeks.	Tocopherol significantly decreased the incidence of pulmonary carcinomas, but not adenomas, in DHPN-initiated animals. Tocopherol did not affect the incidence of lesions in the thyroid gland, urinary bladder, or kidneys. Animals given Tocopherol only did not develop tumors. Lung weights of initiated animals given Tocopherol were statistically significantly increased compared to noninitiated animals and decreased compared to animals given DHPN only	Hasegawa et al. 1990
α	Oral	Forestomach	16–19 female Ha/ICR mice/group; housed 5/cage. Animals were fed chow containing 10 mg Tocopherol/g diet and 0.05 mg DMBA/g diet for 28 days (diet intake 4.0 g/mouse/day); the animals then ate a basal diet for 14 weeks	Compared to animals fed only DMBA, Tocopherol slightly but insignificantly reduced the number of animals with tumors and the number of tumors/animal	Wattenberg 1972
DL-α	Oral	Gastroduodenal	20 male Wistar rats/group. Initation consisted of 100 mg/l MNNG in the drinking water and a sodium chloride diet for 8 weeks; the animals were then given tap water and chow containing 1% Tocopherol for 36 weeks. A noninitiated group of 10 animals was fed Tocopherol for 36 weeks	Compared to animals given only MNNG, Tocopherol did not significantly reduce the incidence of gastric lesions. Animals given Tocopherol only did not develop tumors	Takahashi et al. 1986
α	Oral	Stomach, liver, pancreas	30 (initiated) or 20 (noninitiated) Syrian golden hamsters/group. Initiation consisted of a single SC injection of 20 mg/kg DOPN. 1 week after DOPN administration, initiated and noninitiated animals were fed chow containing 1% Tocopherol for 39 weeks	Compared to animals given DOPN and then basal chow, a significant decrease in the number of ductular proliferations in the pancreas and the number of foci in the liver was observed in animals whose chow contained Tocopherol. No other significant changes in lesions of the forestomach, liver, or pancreas were observed. In noninitiated animals, the number of forestomach papillary hyperplasias was significantly increased in the animals whose chow contained Tocopherol compared to controls	Moore et al. 1987

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TABLE 12
Modulation of carcinogenicity *(Continued)*

Form	Exposure	Cancer type	Protocol*	Results	Reference
α		Liver	Groups of 15–17 male Fischer rats were fed basal chow containing 1.5% Tocopherol for 8 days; on day 7, the animals were dosed with 100 mg/kg IQ by gavage after two-thirds partial hepatectomy; after 2 weeks on basal diet, they were fed basal diet containing 0.05% phenobarbitol for 8 weeks; 1 week after the start of this diet, a single IP injection of 300 mg/kg D-galactosamine was given. Surviving animals were killed at week 11. 5 rats were fed Tocopherol without IQ	Compared to animals given IQ then basal chow, Tocopherol resulted in a decrease in the number of GST-P$^+$ foci; the researchers used this as an indicator of the ability of Tocopherol to inhibit the induction of preneoplastic hepatic lesions	Tsuda et al. 1994
α	Oral	Bladder	20 male inbred F344/DuCrj rats/group; housed 5/cage. Initiation consisted of 0.05% BBN in water for 4 weeks. Initiated animals were fed chow containing 0.38, 0.75, or 1.5% and noninitiated animals were fed chow with 1.5% (food additive grade) Tocopherol during weeks 5–36	Compared to animals given BBN then basal chow, there was no statistically significant difference in the incidence of urinary bladder lesions. Animals given Tocopherol only did not develop tumors	Tamano et al. 1987
α	Oral	Prostate	19–20 (initiated) or 15 (noninitiated) male F344/DuCrj rats/group. Animals were fed chow with 0.75 ppm EE for 1 week cycled with non-EE chow for 1 week until week 20; initation consisted of an SC injection with 50 mg/kg DMAB 3 days after each diet cycle transfer of non-EE chow (total of 10 injections). Animals were fed chow containing 1% Tocopherol from weeks 21–60	Compared to animals given DMAB then basal chow, there was no statistically significant difference in the incidence of prostate lesions or lesions in the small or large intestine, pancreas, skin, subcutis, preputial gland, or zymbal gland. Animals given Tocopherol only did not develop prostate tumors	Nakamura et al. 1991
α	Oral	Multiple	10 (noninitiated) or 20 (initiatied) male F344 rats/group; housed 5/cage. Initiation consisted of a single intragastric administration of 100 mg/kg MNNG and of 750 mg/kg EHEN, 2 SC injections of 0.5 mg/kg MBN once/6 days, 4 SC injections of 40 mg/kg DMH once/	Compared to animals initiated and given basal diet only, Tocopherol (1) significantly increased the incidence of glandular stomach atypical foci; (2) significantly decreased the incidence and multiplicity of renal atypical tubules, but not renal cell tumors; and (3) did not	Hirose et al. 1993

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			3–4 days, and drinking water with 0.05% DBN for 4 weeks and 0.1% DHPN for 2 weeks. Initiated and noninitiated animals were fed chow containing 1% Tocopherol from weeks 6–36	significantly affect thyroid gland, tongue, esophageal, forestomach, duodenum, small intestine, liver, pulmonary, or bladder lesions	
α	Parenteral	Lung	20–30 male A/J mice/group. Initiation consisted of an IP injection of 500 mg/kg urethane. 1 week after initiation, the animals were dosed IP with 1000 mg/kg Tocopherol 1× per week for 8 weeks. The animals were killed 4 months after initiation	Tocopherol did not affect tumor formation	Witschi, Hakkinen, and Kehrer 1981
			Tocopheryl Acetate		
α	Oral	Mammary	30 female Sprague-Dawley rats/group. Initiation consisted of a single dose of 10 mg DMBA given by gavage. Animals were given 0.2% Tocopheryl Acetate in a high (20%) polyunsaturated fat, high saturated fat, and low (2%) fat diet	Tocopheryl Acetate did not affect the rate of tumor development in any of the dietary groups	McCay, King, and Pitha 1981
DL-α	Oral	Intestinal	50 male and 50 female Swiss albino mice/group; housed 5/cage. Animals were fed chow containing 4% Tocopheryl Acetate from 43 days of age until the end of their life span; one group was dosed SC with 20 μg/g 1,2-DMH 1× per week for 10 weeks starting 4 days after feeding with Tocopheryl Acetate	Compared to the animals given 1,2-DMH then basal feed, Tocopheryl Acetate enhanced the induction of duodenal, cecal, colon, rectal, and anal tumors; it did not affect the induction of renal or blood vessel tumors. Animals given Tocopheryl Acetate only did not develop renal, duodenal, cecal, colon, rectal, or anal tumors. Animals given 1,2-DMH had a statistically significant decrease in survival	Toth and Patil 1983
α	Oral	Liver	30 female Sprague-Dawley rats/groups; housed 3/cage. Animals were fed diets containing 10, 50, or 500 ppm Tocopheryl Acetate and 0.025% ciprofibrate for 6 or 21 months; 15/group were killed at each time point. A control group was fed 50 ppm Tocopheryl Acetate without ciprofibrate	No hepatic tumors or altered foci were observed in animals killed after 6 months. Animals killled after 21 months had a statistically significant increase in hepatocellular carcinomas and total tumors, but not tumors/animal compared to controls. Animals given 500 ppm Tocopheryl Acetate had a significant increase in foci/cm^3, foci/liver, and % of liver occupied by foci when compared to animals given 10 ppm, and a decrease in body weight from weeks 30+	Glauert et al. 1990

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TABLE 12
Modulation of carcinogenicity *(Continued)*

Form	Exposure	Cancer type	Protocol*	Results	Reference
DL-α	Oral	Liver	10 male B6C3F1 mice/group; housed 5/cage. Initiation consisted of 2 IP injections of 35 mg/kg DEN 1× per week for 8 weeks. When focal lesions were apparent (3 months), animals were then fed chow containing 50, 250, or 450 mg/kg Tocopheryl Acetate for 30 or 60 days	(1) Compared to animals given DEN + 50 mg/kg Tocopheryl Acetate, animals given no Tocopheryl Acetate had a significant increase in the hepatic labeling index after 30 and 60 days (2) The incidence of hepatic apoptosis after 30 and 60 days was significantly greater in animals not given Tocopheryl Acetate and significantly decreased after 60 days in animals given 250 mg/kg and after 30 and 60 days in animals given 450 mg/kg (3) The number and volume of basophilic lesions was increased in animals given 450 mg/kg Tocopheryl Acetate for 60 days (4) An increase in total relative hepatic volume and in total relative focal volume was seen after 60 days in animals given no Tocopheryl Acetate or 450 mg/kg Tocopheryl Acetate, respectively (5) An increase in the labeling index of basophilic lesions was increased after 30 days in animals given no Tocopheryl Acetate and after 60 days in animals given 450 mg/kg Tocopheryl Acetate	Kolaja and Klaunig 1997
			Tocopheryl Succinate		
d-α	Oral	Skin	45 female Shk-1 hairless mice/group; housed 5/cage until initiation. Animals were fed chow containing 0.12% *w/w* Tocopheryl Succinate, Tocopheryl Succinate + 0.5% *w/w* β-carotene. Initiation consisted of a single dermal application of 150 μg DMBA at week 11; 5 μg of the promoter PMA was	Compared to animals given DMBA, a statistically significant decrease in tumors was seen in the Tocopheryl Succinate + carotene animals during weeks 8 and 9 after initiation; the week of appearance of tumors 1–8 for the Tocopheryl Succinate + carotene group was	Lambert et al. 1994

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			applied 2× per week during weeks 12–23, then 1× per week for 9 weeks. The animals were killed 27 week after initiation	significantly delayed compared to tumors 1–10; at the last observation, the cumulative number of tumors was significantly less for all supplemented groups. Compared to the animals given Tocopheryl Succinate, a statistically significant increase in regressed tumors was observed in the Tocopheryl Succinate + carotene and in the basal chow group	
dl-α	Oral	Oral	20 Syrian golden hamsters/group. For induction, the right buccal pouch of each animal was painted 3× per week for 13 weeks with 0.5% DMBA in heavy mineral oil, then group 1: injections of 250 μg Tocopheryl Succinate in Eagles' minimum essential medium were given 2× per week for 4 weeks; group 2: dosed with vehicle; and group 3: untreated	Administration of Tocopheryl Succinate resulted in significant tumor regression	Shklar et al. 1987
α	Oral	Oral	10 male Syrian golden hamsters/group; housed 5/cage. group 1: the right buccal pouch of each animal was painted 3× per week with 0.5% DMBA in heavy mineral oil. Group 2: treated with DMBA and dosed orally with 10 mg Tocopherol in 0.5 ml mineral oil 3× per week days opposite DMBA. Group 3: untreated control group. Group 4: dosed with Tocopheryl Succinate. All animals were killed after 14 weeks	The number of tumors in group 2 was statistically significantly decreased compared to group 1. Angiogenesis and TNFα expression were inhibited in group 2 compared to group 1	Shklar and Schwartz 1996

*Abbreviations: AOM = azoxymethane; BBN = *N*-butyl-*N*-(4-hydroxybutyl)nitrosoamine; BrdU = 5′-bromo-2′-deoxyuridine; DBN = *N*-dibutylnitrosamine; DEN = diethylnitrosamine; DHPN = *N*-bis(2-hydroxypropyl)nitrosoamine; DMAB = 3,2′-dimethyl-4-aminobiphenyl; DMBA = dimethylbenz[a]anthracene; DMH = 1,2-dimethylhydrazine; DOPN = 2,2′-dioxo-*N*-nitrosodipropylamine; EE = ethinyl estradiol; EHEN = *N*-ethyl-*N*-hydroxyethylnitrosamine; GSH = reduced glutathione; IQ = 2-amino-3-methylimidazo[4,5-*f*]quinoline; MBN = *N*-methylbenzylnitrosamine; MNNG = *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NMBzA = *N*-nitrosomethylbenzylamine; PMA = phorbol 12-myristate 13-acetate; TPA = 12-*O*-tetradecanoylphorbol-13-acetate.

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groups, respectively. The maximum percentage of animals that developed tumors was 100%, 92%, and 96% for the groups in the order described above. The Tocopherol group with the longest time to appearance of the first papilloma (7 weeks) was selected to also receive mezerein at 13 weeks. With this subsequent treatment, the number of animals with tumors increased to 100%.

The TPA-treated group developed 19 skin carcinomas in 15 animals, with the first appearing at 167 days. The tocopherol-treated animals developed 20 carcinomas in 15 animals, the first appearing at 190 days. The tocopherol/mezerein-treated group developed 14 carcinomas on 12 animals, with the first carcinoma appearing at 223 days. The control group receiving no promotion followed by mezerein treatment developed 18 carcinomas on 12 animals, with the first appearing at 254 days. The authors concluded that these data indicate that Tocopherol can act as a complete tumor promoter.

Effect on Photocarcinogenicity

Dermal

Tocopherol. Groups of 35 specific pathogen–free female C3H/HeNTac (H-2^k; MTV^-) mice, housed five per cage, were dosed dermally with 25 mg *dl*-α-Tocopherol in 200 μl acetone three times per week; dosing was initiated 3 weeks prior to the start of irradiation and continued throughout the experiment (Gensler and Magdaleno 1991). (To avoid oxidation, Tocopherol was applied 30 minutes after irradiation.) The animals were irradiated with 6.0 J/m^2/sec UVB from a bank of six FS40 Westinghouse fluorescent sunlamps (80% of the output was in the range of 280 to 340 nm) at a distance of 20 cm for 30 minutes per day, 5 days per week, for 27 weeks. Control mice were treated with solvent. Irradiated and nonirradiated animals were fed either basal chow or chow supplemented with 1% β-carotene starting 3 weeks prior to irradiation.

The probability of tumor formation 33 weeks after the first UV exposure in control mice fed basal diet and irradiated was 81%. This probability at 33 weeks for animals dosed with Tocopherol was statistically significantly decreased; this value was 42% and 24% for animals dosed with Tocopherol or dosed with Tocopherol and fed β-carotene, respectively. (A statistically significant decrease was not observed for those animals only fed β-carotene and irradiated.) Tumor burden in animals dosed with Tocopherol was also statistically significantly decreased compared to irradiated control animals. The researchers concluded that Tocopherol reduced photocarcinogenesis.

Tocopheryl Acetate. Groups of 30 specific pathogen–free female BALB/cAnNTacfBR (H-2^d) mice were dosed dermally with 12.5, 25, or 50 mg *dl*-α-Tocopheryl Acetate in 0.2 ml acetone three times per week; dosing was initiated 3 weeks prior to the start of irradiation and continued throughout the experiment (Gensler et al. 1996). (During dosing, Tocopheryl Acetate was applied 30 minutes after irradiation.) The animals were irradiated with 6.44 J/m^2/sec UVB from a bank of six unfiltered FS40 Westinghouse fluorescent sunlamps that emit a continuous spectrum from 270 to 390 nm, with a peak emission of 313 nm (75% of the output was in the range of 280–320 nm), at a distance of 20 cm for 30 minutes per day, 5 days per week, for 18 weeks. Control mice were treated with solvent.

Compared to UV alone, photocarcinogenesis appeared to be enhanced by 12.5 mg Tocopheryl Acetate. A dose-response was not observed. Because no significant difference was found between groups, tumor incidence data were pooled for the different dose groups; pooled data indicated a "significant enhancement of photocarcinogenesis" by Tocopheryl Acetate. The researchers stated the "mechanism underlying the difference in photoprotective capacity by α-Tocopherol and its acetate . . . ester is not known." The researchers postulated that "the limited capacity of skin to cleave the ester forms to the antioxidant form of α-tocopherol may explain the inability of α-tocopheryl acetate . . . to prevent photocarcinogenesis." The researchers noted that α-Tocopheryl Acetate "may have less photoabsorptive capacity than does α-tocopherol . . . Thus it is possible that the α-tocopherol form is a better sunscreen . . ." Also, "the immunoprotection by α-tocopherol and lack of it by α-tocopheryl acetate . . . may be related to the role of antioxidants in regulating transcription factors."

Tocopheryl Succinate. Groups of 28 specific pathogen–free female BALB/cAnNTacfBR (H-2^d) mice were dosed dermally with 2.5, 12.5, or 25 mg *dl*-α-Tocopheryl Succinate in 0.2 ml acetone and irradiated following the same protocol described previously (Gensler et al. 1996). Photocarcinogenesis appeared to be enhanced by 25 mg Tocopheryl Succinate. A dose-response was not observed. Because no significant difference was observed between groups, tumor incidence data were pooled for the different dose groups; pooled data indicated a "significant enhancement of photocarcinogenesis" by Tocopheryl Succinate. The researchers made the same postulations as those described for Tocopheryl Acetate as to the difference in photoprotection between Tocopherol and Tocopheryl Succinate.

Oral

Tocopherol. Female albino hairless mice were used to determine the effect of dietary Tocopherol on UV-induced tumor induction (Black 1974). The animals were exposed to suberythemic doses of UV light from a mercury arc lamp 5 days per week for 18 weeks. Daily exposure was 1.09 J/cm^2 for the first 2 weeks; the dose was increased by 0.27 J/cm^2 every 2 weeks until a dose of 1.91 J/cm^2 was reached. One group of animals was fed a diet containing 2% antioxidants, 0.2% of which was *dl*-α-Tocopherol, and the other group was fed basal chow. At 24 weeks, none of the animals of the test group had carcinomas, and 4 had actinic lesions, as compared to 5 animals of the control group having carcinomas and 33 with actinic lesions.

In a study to determine the effect of Tocopherol on UV-light induced tumor formation, two groups of 100 female albino hairless mice (hrhr) were irradiated 5 days per week with

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suberythemic doses of UV light from a GE-UA3 mercury arc lamp for 16 weeks (Black and Chan 1975). The animals were exposed to 1.13 J/cm^2/day for 2 weeks, and the dose was then increased 0.28 J/cm^2 every 2 weeks until reaching a daily dose of 1.97 J/cm^2. One group of animals was fed a diet containing 0.2% DL-α-Tocopherol (and an additional 1.8% *w/w* of other antioxidants), and the other was fed basal chow, for the duration of the study. Animals were evaluated for actinic effects during weeks 14 to 22; actinic lesions 1 mm in diameter was considered the biological end point in evaluation. After 22 weeks, 30% of animals fed basal chow had frank squamous cell carcinomas as compared to 7% of animals fed chow containing Tocopherol.

Three groups of 30 to 51 female Skh-HR-1 hairless mice were fed basal chow containing a 2% *w/w* antioxidant supplement, 0.2% of which was *dl*-α-Tocopherol, and three groups of control mice were fed the basal chow without the supplement (Black et al. 1983). Two weeks after dose initiation, the animals were irradiated 5 days per week with 0.46 Sunburn Units (SBUs) (0.92 MED) (1 SBU = 4 J/cm^2) in a 24-minute period; the irradiation source was Westinghouse BZS-WLG lamps at a distance of 14 cm from the dorsal surface of the animals. Irradiation of test and control animals was discontinued after 21.6, 31.6, or 44 SBUs had been delivered.

Control animals had a "notably greater tumor latency period" at the low dose; median tumor times were not significantly different at the two highest doses. The median time to tumor development was significantly increased in a nearly linear fashion at all three doses when compared to control values. Tumor multiplicity for all three UV doses had a dose-dependent relationship. The anticarcinogenic effect of the antioxidant supplementation diminished with increasing UV dose.

Tocopheryl Acetate. A study was conducted to determine the effect of Tocopheryl Acetate on photocarcinogenicity (Gerrish and Gensler 1993). Female C3H/HeN mice, housed five per cage, were fed basal chow or chow containing 100 or 200 IU/kg *d*-α-Tocopheryl Acetate for a total 37 weeks. (The number of animals per group was not specified.) After 3 weeks of dosing, the dorsal hair of 20 animals of each group was clipped and these animals were irradiated with UVB light at a distance of 20 cm from the light source for 30 minutes per day, 5 days per week. The light source, a bank of six Westinghouse FS40 sun lamps, from which approximately 80% of the radiation emitted was in the UVB range (280 to 340 nm), delivered 6.4 J/m^2/s. The animals were subjected to irradiation for 13 weeks, with a total UVB dose of 7.49×10^5 J/m^2. Irradiation was discontinued for 3 weeks because of toxicity in the Tocopheryl Acetate dose groups, and then resumed for 2 weeks, resulting in a total UVB dose of approximately 8.6×10^5 J/m^2. The animals were killed at study termination.

Feed consumption was similar for all irradiated animals. The amount of Tocopheryl Acetate consumed per g of mouse averaged 0.0026, 0.0149, and 0.0262 IU for the control, 100, and 200 IU/kg groups, respectively. Thirty-one weeks after the start of UV irradiation, 46% and 19% of the mice fed 100 and 200 IU/kg Tocopheryl Acetate, respectively, had developed neoplasms, as compared to 67% of the mice fed basal chow. The probability of an animal being a tumor-bearer was statistically significantly decreased for animals fed 200 IU/kg compared to controls. After 15 weeks on study, the survival rate of animals fed 100 or 200 IU/kg Tocopheryl Acetate and irradiated was only 75%, as compared to a rate of 100% for those fed basal chow with irradiation and for animals fed basal and test diets and not irradiated. After approximately 34 weeks on study, the survival rate for animals fed 200 IU/kg Tocopheryl Acetate and irradiated was 60%. Relative spleen weights were statistically significantly decreased in irradiated animals of both test groups compared to respective nonirradiated animals fed Tocopheryl Acetate, whereas relative spleen weights were statistically significantly increased in mice fed basal chow and irradiated compared to those fed basal chow and not irradiated. At microscopic examination, an increase in hemosiderin was observed in the spleens of irradiated animals fed Tocopheryl Acetate. Dietary administration of Tocopheryl Acetate reduced the incidence of skin cancer in a dose-dependent manner, but toxicity was observed.

Effects on Other Skin Damage/Photoprotective Effects

Tocopherol. Female albino hairless Skh:HR-1 mice were used to examine the photoprotective effect of 5% (*w/v*) Tocopherol in ethanol (Bissett, Hillebrand, and Hannon 1989). The animals were irradiated individually with a dose of 30 mJ/cm^2 UVB from a bank of four Westinghouse FS-40 sunlamps (peak near 315 nm) at a distance of 10 cm, three times per week, 2 hours after application of the test material. Delay in wrinkle onset and decrease in wrinkle development were used to assess efficacy. In a group of 10 animals, Tocopherol provided protection equal to approximately half that provided by a sun protection factor (SPF) 4 sunscreen. Tocopherol reduced tumor yield at 22 weeks of UVB irradiation from approximately four neoplasms per animal to approximately two neoplasms per animal.

Groups of 10 female hairless albino Skh:HR-1 mice, housed five per cage, were used to determine the photoprotective effect of α-Tocopherol (Bissett et al. 1990). Vehicle (ethanol/propylene glycol/water; 2:1:1 *v/v/v*) controls were used. Animals were irradiated individually with a dose of 15 J/cm^2 UVA from a bank of four General Electric F-40 black lights (peak near 365 nm, no emission <340 nm) at a distance of 45 cm five times weekly or with a dose of 30 mJ/cm^2 UVB (same protocol as described above) three times weekly 2 hours after application of 0.1 ml of test material. Skin wrinkling (UVB-induced) and skin sagging (UVA-induced) were assessed.

Application of 5% (*w/v*) Tocopherol prior to each UVB dose significantly reduced the severity of UVB-induced skin wrinkling compared to controls. In a dose-response study, it was determined that the near-maximum effects were at 5%. Tocopherol

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significantly delayed the average time of tumor onset compared to the controls, from 19.4 to 21.6 weeks, and Tocopherol significantly reduced lesions observed microscopically. Oral administration of 1% α-Tocopherol did not affect UVB damage. Dermal application of 5% Tocopherol did not protect against damage from UVA irradiation.

Groups of 10 female hairless albino Skh:HR-1 mice, housed five per cage, were used to determine the photoprotective effect of α-Tocopherol in conjunction with an anti-inflammatory agent (Bissett, Chatterjee, and Hannon 1992). Vehicle (ethanol/propylene glycol/water; 2:1:1 *v/v/v*) controls were used. Animals were irradiated individually with UVB (same protocol as described previously) 2 hours after application of 0.1 ml of test material for 20 weeks. Skin wrinkling (UVB-induced) was assessed.

In examining the percent wrinkle reduction (compared to controls), 5% Tocopherol significantly protected the skin. Tocopherol (5%) + 0.5% hydrocortisone or 1% ibuprofen significantly reduced wrinkling as compared to Tocopherol alone at wrinkle grades 0.5/3, 1.0/3, and 1.5/3, and Tocopherol +1% naproxen significantly reduced wrinkling compared to Tocopherol alone at wrinkle grades 1.0 and 1.5. At microscopic examination, it was observed that 5% Tocopherol + 0.5% hydrocortisone significantly prevented UVB-induced cutaneous lesions compared to those seen with Tocopherol alone. However, Tocopherol also had some preventive effects when compared to the vehicle.

The effect of Tocopherol on UVB-induced sunburn cell (SBC) production was examined using domestic Yorkshire swine skin (Darr et al. 1996). Tocopherol, 3% (eight animals), reduced the number of UVB-induced SBCs (4-mm biopsy) to a value that was approximately 31% of vehicle control values; this was a statistically significant decrease. Tocopherol + ascorbic acid (seven animals) reduced it to a value that was 15% of control values. Treatment with 2% Tocopherol +10% ascorbic acid once a day for 3 days reduced the number of psoralen-UVA (PUVA)-induced SBCs (4-mm biopsy) from >100 to 59.5. Treatment with Tocopherol + ascorbic acid + oxybenzone reduced this number to 4.3, a statistically significant decrease compared to Tocopherol + ascorbic acid alone.

The photoprotective effect of Tocopherol was determined by examining its ability to inhibit thymidine dimer production (McVean and Liebler 1997). Groups of four female C3H/HeNTac mice received a dermal application of 50 mg of a neutral cream containing 1% to 15% (*w/w*) *d*-α-Tocopherol; controls were dosed with vehicle only. The applications were made 15 minutes prior to irradiation to a 3 cm × 4 cm shaved area on the back of each animal. The animals were irradiated for 60 minutes at a distance of 19.5 cm using a bank of six Westinghouse FS20 lamps (80% of the output in the UVB range) with 2.5 J/m^2/s. After irradiation, the mice were killed, the dorsal skin was removed, and the epidermal cells were isolated for thymine dimer analysis. Duplicate experiments were performed. α-Tocopherol reduced thymidine dimer formation in a dose-dependent manner; 1% and 10% reduced thymidine dimer formation to 43% and 84% of control values, respectively. Reductions were statistically significant at doses of 3%, 5%, and 10% α-Tocopherol.

In order to determine whether α-Tocopherol could protect against thymidine dimer formation induced by wavelengths only in the UVB range or greater, a 285-nm cutoff filter was used to filter UVC. Thymidine dimer formation was reduced to values that were 60% and 17% of control values by 1% and 10% α-Tocopherol dispersions, respectively.

McVean and Liebler (1997) determined that γ- and δ-Tocopherol were less potent than α-Tocopherol in reducing thymidine dimer formation. Dispersions containing 5% (*w/w*) γ- or δ-Tocopherol inhibited dimer formation to 45% of the control value.

Tocopheryl Acetate. Bissett, Chatterjee, and Hannon (1990) used groups of 10 female hairless albino Skh:HR-1 mice to determine the photoprotective effect of Tocopheryl Acetate using the protocol described previously (with Tocopherol) for exposure to UVB radiation. Application of 5% (*w/v*) Tocopheryl Acetate prior to each UVB dose significantly increased the percentage of wrinkle protection compared to application of vehicle only for wrinkle grades 0.5/3 and 1.0/3; significant protection was not observed at wrinkle grade 1.5/3.

McVean and Liebler (1997) used the procedure described previously to determine whether *dl*-α-Tocopheryl Acetate can inhibit thymidine dimer formation in female C3H/HeNTac mice. A 10% (*w/w*) dispersion of Tocopheryl Acetate inhibited dimer formation 44% of the control value.

Tocopheryl Linoleate. Groups of 10 female hairless albino Skh:HR-1 mice were used to determine the photoprotective effect of Tocopheryl Linoleate using the protocol described previously (for Tocopherol) for exposure to UVB radiation (Bissett, Chatterjee, and Hannon 1990). Application of 5% (*w/v*) Tocopheryl Linoleate prior to each UVB dose significantly increased the percentage of wrinkle protection compared to application of vehicle only for wrinkle grade 0.5/3; significant protection was not observed at wrinkle grades 1.0/3, 1.5/3, or 2.0/3. Tocopheryl Linoleate did not significantly affect the average week of tumor onset when compared to control values.

Tocopheryl Nicotinate. Groups of 10 female hairless albino Skh:HR-1 mice were used to determine the photoprotective effect of Tocopheryl Nicotinate using the protocol described previously (for Tocopherol) for exposure to UVB radiation (Bissett, Chatterjee, and Hannon 1990). Application of 5% (*w/v*) Tocopheryl Nicotinate prior to each UVB dose did not reduce wrinkling when compared to control values.

Tocopheryl Succinate. Groups of 10 female hairless albino Skh:HR-1 mice were used to determine the photoprotective effect of Tocopheryl Succinate using the protocol described previously (for Tocopherol) for exposure to UVB radiation (Bissett, Chatterjee, and Hannon 1990). Application of 5% (*w/v*)

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Tocopheryl Succinate prior to each UVB dose did not reduce wrinkling when compared to control values.

CLINICAL ASSESSMENT OF SAFETY

Irritation/Sensitization

Tocopherol. The irritation potential of 1.0% Tocopherol in paraffinum liquidum was determined using 55 subjects (number per sex not specified) (CTFA 1972). The test material, 0.05 ml, was placed on human patch test plaster and applied to the intact surface of the forearm for 24 hours. The test sites were scored on a scale of 0 (−) to 5 (4+) upon removal of the plaster. The positive rate was defined as the percent rate of the number of subjects with a positive reactive above the score 2 (1+) (distinct erythema). The positive rate was 0/55 (0%). Tocopherol, 1.0%, was not a primary skin irritant.

Three cosmetic formulations, one containing 2% *dl*-Tocopherol, one containing 12% vitamin E (form not specified) in wheat germ (100 IU/g), and one containing 32% mixed Tocopherols in a base of wheat germ and vegetable oils (24,000 IU/2 oz), were evaluated for dermal irritation using six subjects (Marzulli and Maibach 1975). Each test material, 0.05 ml, was applied under an occlusive patch to a site on the back in a 21-day cumulative irritation test. Each test site was scored every 24 hours. The mean cumulative irritation scores for the formulations containing 2%, 12%, and 32% Tocopherol were 3.9/84, 7.0/84, and 0.0/84, respectively.

The primary and cumulative irritation potential and the sensitization potential of a cream containing 5% Tocopherol was determined in a repeat-insult patch test (RIPT) using 113 subjects, 35 males and 78 females (Consumer Product Testing Co. 1997a, 1997b). A semiocclusive patch containing 0.2 ml of the test material was applied for 24 hours to the upper back of each subject three times per week for a total of 10 applications. The test sites were scored prior to each application. After a 2-week nontreatment period, the challenge was performed by applying the test material to the original site and a previously untreated site on the volar forearm. These sites were evaluated 24 and 48 hours after application. A cream containing 5% Tocopherol was not an irritant or a sensitizer.

Between July 1, 1985, and June 30, 1989, the North American Contact Dermatitis Group (NACDG) patch-tested 4887 patients with 5% *dl*-α-Tocopherol in petrolatum (NACDG 1999). Twelve patients (0.2%) "were thought to be allergic," two patients (0.04%) "were thought to be irritated," and two patients (0.04%) "had questionable reactions" to Tocopherol.

Adams and Maibach (1985) reported on a 64-months study (during the years 1977 to 1983) involving 12 dermatologists that researched patient reactions to cosmetics. Of an estimated number of 281,100 patients seen, an estimated number of 13,216 patients had contact dermatitis and in 713 of those patients, it was related to cosmetics. Patch tests were performed according to the methods of the NACDG on 56% of the subjects. There were two cutaneous reactions to Tocopherol.

Ninety-seven patients were patch-tested with 20% *dl*-α-Tocopherol in petrolatum (Roed-Petersen and Hjorth 1975). No reactions were observed at day 2 or 4. One patient had a positive reaction 3 weeks after application of the patch test. Subsequent testing with 20% *dl*-α-Tocopherol in petrolatum was positive at day 2, whereas patch tests with 5% *dl*-α-Tocopherol, 5% and 20% *d*-α-Tocopherol, and 5% and 20% *d*-α-Tocopheryl Succinate were negative.

One-hundred sixteen patients with eczematous dermatitis were patch tested with *dl*-α-Tocopherol (Roed-Petersen and Hjorth 1976). One positive reaction was observed.

Tocopheryl Acetate. An RIPT of a lotion containing 0.1% Tocopheryl Acetate was completed using 110 subjects, 18 males and 92 females (AMA Laboratories, Inc. 1996). A semiocclusive patch containing 0.2 g of the test material was applied for 24 hours to the infrascapular region of the back of each subject three times weekly for a total of nine applications. A challenge patch was applied for 24 hours to a previously unexposed site 10 to 14 days after the last induction patch. The sites were scored 24 and 48 hours after application. No reactions were observed during induction or challenge. A lotion containing 0.1% Tocopheryl Acetate was not a primary irritant or sensitizer.

The primary irritation potential of 100% *dl*-α-Tocopheryl Acetate and 1%, 5%, 20%, and 50% Tocopheryl Acetate in petrolatum was determined using eight subjects (Roche 1999c). Petrolatum was used as the control. Occlusive patches containing 0.5 ml of the test materials were applied for 24 hours to the interscapular area of each subject daily for 21 days. The test sites were scored 10 minutes after patch removal and prior to reapplication. The mean irritation indices, on a scale of 0 to 4, were 0 for 100% Tocopheryl Acetate, 0.875, 0.312. 1.0, and 0.312, for 1%, 5%, 20%, and 50% Tocopheryl Acetate in petrolatum, respectively, and 0.125 for petrolatum.

The irritation and sensitization potential of 100% *dl*-α-Tocopheryl Acetate was determined in a Draize study using 209 subjects that had not been previously exposed to vitamin E (Roche 1999c). Tocopheryl Acetate was applied under an occlusive patch three times per week for a total of 10 applications. After a 2-week nontreatment period, reapplications were made for 3 days. The total irritation index for the 203 subjects was 15.5, and the mean irritation intensity index was 0.076. All the sensitization readings were negative. Tocopheryl Acetate was not a primary irritant and did not produce delayed hypersensitivity.

Tocopheryl Linoleate. With the use of a new line of cosmetics that contained Tocopheryl Linoleate, a large number of outbreaks was observed in Switzerland between April and November 1992 (263 patients consulted dermatologists and 642 cases were reported directly to manufacturers; a rate of at least three incidences per 1000 units); the products originated from the same manufacturer (Perrenoud et al. 1994). No geographical clustering was observed. Patch and use tests were performed. A body lotion, an o/w emulsion containing 1% Tocopheryl Linoleate, was patch-tested using 77 patients, whereas

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other products of the cosmetic line, including six batches of Tocopheryl Linoleate, were tested using 26 patients. Artificially aged Tocopheryl Linoleate was tested using six patients and the lotion with 2% or 10% Tocopheryl Linoleate (instead of 1%) was tested using four patients. *d*-α-Tocopherol, *dl*-α-Tocopherol, *dl*-α-Tocopheryl Acetate, all 10% in petrolatum, and undiluted Tocophersolan were also tested at different concentrations using 6 to 11 patients. Two formulations, one that did not contain Tocopheryl Linoleate and one that did not contain Tocopheryl Linoleate or any ingredients containing Tocopherol derivatives, were tested using 12 and 11 patients, respectively.

Twenty-three subjects (13%) had a past history of dermatitis and 14 subjects (8%) had past or present signs of atopic dermatitis. Most patients had a "very itchy, papular and follicular dermatitis that was symmetrically distributed and located in most cases on the trunk and the extremities. In some cases, the papules were surrounded by a halo of vasoconstriction . . . In a few cases, the papules were located on intensely erythematous, well-defined plaques, suggesting irritation rather than allergy." A secondary extension to the face was seen in one of five cases. Control subjects were included in the study. The test substances were applied to the upper back under an occlusive patch for 48 hours, and the reactions were read at 2 and 4 or 5 days using the scale of the International Contact Dermatitis Research Group.

Positive patch-test results ranged from 21% to 64% for the cosmetic products. Increasing the concentration of Tocopheryl Linoleate in the lotion did not increase the rate of positive reactions. Reactions were seen in all patients tested with aged Tocopheryl Linoleate. Positive or doubtful reactions were seen in 1/7, 1/10, 3/11, and 1/6 patients tested with *d*-α-Tocopherol, *dl*-α-Tocopherol, *dl*-α-Tocopherol Acetate, and Tocophersolan, respectively. Testing of the Tocopheryl Linoleate–free formulation resulted in 3/12 patients with doubtful reactions; testing of the Tocopheryl Linoleate– and Tocopherol derivative–free formulation resulted in 0/11 subjects having doubtful or positive reactions. Control subjects did not have positive reactions. Skin biopsies from lesional skin of four patients that had positive patch-test results with Tocopheryl Linoleate had "spongiosis of the interfollicular epidermis with a perifollicular and perivascular infiltrate containing a predominantly mononuclear cell infiltrate with some neutrophils."

Perrenoud et al. (1994) also performed repeated open application tests (ROATs) using products containing Tocopheryl Linoleate. At least 3 months after the last application of a Tocopheryl Linoleate–containing product or after patch-testing, the lotion containing Tocopheryl Linoleate and a Tocopheryl Linoleate–free lotion were applied to the right and left outer arm and an artificially aged product was applied to the anterior right thigh of 15 patients. The ROAT was performed for 4 weeks. The Tocopheryl Linoleate–containing product was not used on the controls.

Eighty percent (12/15) of the subjects had a papular erythematous eruption at the site of application of the lotion that contained Tocopheryl Linoleate. The reactions appeared after 1 to 13 applications (mean of 4) and lasted 2 to 24 days (mean of 9). Four of the subjects reacted to the Tocopheryl Linoleate–free lotion as well. Control subjects did not react to the lotion without Tocopheryl Linoleate.

Perrenoud et al. (1995) stated that "the differences of the results between patients and control subjects, analyzed with Fisher's exact test, clearly indicated that the positive reactions to the cosmetics and vitamin E linoleate were highly specific, suggesting an allergic mechanism." Initially, Perrenoud et al. (1994) suggested that either an in vivo metabolite of α-Tocopherol or a common contaminant of industrially produced Tocopherols was responsible for the reactions. Perrenoud et al. (1995) stated "the results of our compared investigations on patients and healthy control subjects support the hypothesis that a metabolite or contaminant of vitamin E linoleate could act as a hapten and/or irritant with possible synergistic effects."

Tocopheryl Nicotinate. Tocopheryl Nicotinate, 10% in paraffin oil, was stated to be not irritating or sensitizing in a clinical maximization study (BASF 1993c).

Case Studies

Case studies of dermal reactions from exposures to Tocopherol and Tocopheryl Acetate are described in Table 13.

Photosensitization

Tocopheryl Acetate. Eleven subjects (number per sex not specified) with skin types I to III were used to determine the photosensitization potential of Tocopheryl Acetate (Consumer Product Testing Co. 1992). Approximately 0.2 ml of the test material was applied for 24 hours under an occlusive patch to two sites on the lower back of each subject. Upon patch removal, one of the treated sites and an untreated site were irradiated with UVA for 5 to 8 minutes (10.5 to 16.8 J) until 1 MED was achieved. (The MED for each subject had previously been determined.) The light source was a solar UV simulator with a 150-W xenon arc lamp that produced a continuous emission spectrum in the UVA and UVB regions; a Schott WG 345 filter was used to block UVB. The distance from the light source to the test site was not specified. The test and control sites were scored 15 minutes and 24 and 48 hours after irradiation. Tocopheryl Acetate was not phototoxic, and no responses were observed at the exposed, nonirradiated site.

Skin Effects

Tocopheryl Acetate. Transepidermal water loss (TEWL) was measured after application of 1%, 2.5%, and 5% Tocopheryl Acetate (Djerassi, Machlin, and Nocka 1986). One percent and 2.5% Tocopheryl Acetate did not produce a significant decrease in TEWL, but 5% Tocopheryl Acetate applied for 30 minutes reduced TEWL by 19%. TEWL was reduced by 24% after 4 days of twice-daily application of 5% Tocopheryl Acetate.

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TABLE 13
Clinical case studies reporting adverse reactions to Tocopherol and Tocopheryl Acetate

Case description	Reference
Tocopherol	
3 cases: Three males developed dermal reactions after using a deodorant containing *dl*-α-Tocopherol. The first developed erythema, pruritus, and vesiculobullous lesions within 1 day; the second developed acute dermatitis after 3 weeks; and the third developed erythematous pruritic dermatitis after 2 weeks. All three were patch tested with 4% Tocopherol and/or anhydrous alcohol, perfume, silicone or a production sample containing 1% Tocopherol and had positive (4+) reactions. Two patients were patch tested with Tocopheryl Acetate capsules; no reactions were observed at 48 hours. One patient was patch tested with a mixture of α-, β-, γ-, and δ-Tocopherol; a 2+ reaction was observed at 48 hours.	Aeling, Panagotacos, and Andreozzi 1973
Surgeons have developed contact dermatitis from the use of vitamin E–containing soaps and lotions.	Fisher 1975
1 case: A woman who applied two vitamin E creams and a vitamin E oil had multiple erythematous, edematous patches and plaques, papules, and macules. Patch testing was positive to the creams and the oil, and the only common ingredient was vitamin E (either as Tocopherol or Tocopheryl Acetate).	Saperstein, Rapaport, and Rietschel 1984
1 case: A woman with a history of vitiligo applied two different vitamin E preparations to the chest, neck, and face twice daily for 4 weeks and developed extensive pruritic eruptions. Patch tests were positive to both vitamin E preparations (2+ at 48 hours; 1+ at 72 hours).	Goldman and Rapaport 1986
1 case: Eczematous contact dermatitis was observed in a woman who applied a Tocopherol cream to her ear; a patch test to α-Tocopherol was positive.	Fisher 1991
1 case: Urticarial contact dermatitis was observed in two boys who had burns treated with Tocopherol oil—confluent, erythematous, urticarial eruptions appeared that quickly generalized.	
1 case: Erythema multiforme was observed in three patients who used a deodorant containing Tocopherol—erythema and edema of the axillae were observed, with widespread morbilliform eruption.	
1 case: Eczematous contact dermatitis was observed in two patients who applied Tocopherol to a scar—generalized erythema multiforme was observed, and patch tests were positive.	
1 case: Eczematous contact dermatitis was observed in a woman who applied Tocopherol oil to her face—immediate pruritus and edema and urticaria within 20 minutes were observed.	
1 case: Urticarial contact dermatitis was observed in a woman who applied Tocopherol oil to her skin—a large wheal occurred within 15 minutes.	
1 case: Urticarial contact dermatitis was observed in a woman who applied Tocopherol oil to her face for 4 days—on day 5, a bright red eruption resembling erysipelas was observed.	
1 case: Eczematous contact dermatitis was observed in a woman who applied Tocopherol oil to her eyelids—after the third application, a severe, pruritic, edematous eruption appeared, and a patch test with the oil and 5% α-Tocopherol were positive.	
Eye moisturizers containing vitamin E can cause dermatitis. Patients that patch test positively to the formulation usually patch test negatively to vitamin E in bland vegetable oil.	Draelos 1993
1 case: A woman developed eczematous lesions over the arms and legs after using a cream that contained 5% Tocopherol. She had positive reactions in patch tests to Tocopherol and vitamin A.	Bazzano et al. 1996
Tocopheryl Acetate	
1 case: A woman applied a "pure vitamin E" preparation to burn areas two to three times daily for a few days. After 3 days, a generalized dermatosis with large numbers of erythematous, edematous lesions were observed. Patch testing was positive (4+) to the preparation, which contained 100% pure vitamin E in the form of *dl*-α-Tocopheryl Acetate.	Saperstein, Rapaport, and Rietschel 1984
1 case: A woman used a cream containing Tocopheryl Acetate—erythema and urticarial papules developed on the face and neck after 1 day; she patch-tested positive (++ on day 2 and 3) to the cream and 10% Tocopheryl Acetate in petrolatum.	de Groot et al. 1991

(Continued on next page)

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TABLE 13
Clinical Case Studies Reporting Adverse Reactions to Tocopherol and Tocopheryl Acetate *(Continued)*

Case description	Reference
2 cases: Two women reacted to a cream containing Tocopheryl Acetate; one had dermatitis of the face and hands, she patch-tested positive (?+ at day 2 and + at day 3) to the cream and 10% Tocopheryl Acetate in petrolatum; the second woman developed papulovesicular dermatitis, she patch-tested positive (++ on day 2 and 3) to 10% Tocopheryl Acetate in petrolatum.	de Groot et al. 1991
1 case: A woman used a cream containing Tocopheryl Acetate for 3 weeks—erythematous papular dermatitis developed; she patch-tested positive (+ to ++ after 2 and 3 days) to the cream, 10% Tocopheryl Acetate in petrolatum, and 10% Tocopheryl Nicotinate in petrolatum.	de Groot et al. 1991
1 case: A man had acute generalized eczema after application of "natural" creams. He patch-tested positive to water-soluble Tocopheryl Acetate (1% aqueous), which was present in one of the creams.	Garcia-Bravo and Mozo 1992
1 case: A woman applied Tocopheryl Acetate to her face; after 24 hours, she had erythema and edema. Patch testing with the NACDG standard series, 4% *dl*-αTocopherol in petrolatum, *dl*-α-Tocopheryl Acetate, and moisturizers containing Tocopheryl Acetate were negative. An ROAT with *dl*-α-Tocopheryl Acetate performed after the patch test resulted in a nonpruritic, spreading rash at the site of application after 24 hours; at day 4, a fine papular eruption was observed at the site of the ROAT.	Harris and Taylor 1997

Tocopheryl Nicotinate. Tocopheryl Nicotinate was reported to stimulate epidermal blood circulation (BASF 1994b).

Photoprotection

Tocopherol. A group of six male and four female subjects (nine with skin type II and one with skin type III) was given a vitamin supplement containing 1000 IU/day *d*-α-Tocopherol and 2000 mg/day ascorbic acid for 8 days, while a control group of four male and six female subjects (seven with skin type II and three with skin type III) was given a placebo (Eberlein-König, Placzek, and Przybilla 1998). Prior to supplementation, 12, 1.8-cm^2 areas of the lower back were irradiated with 20 to 226 mJ/cm^2 UVB to determine the MED. After 8 days, the other side of the back was irradiated. Cutaneous blood flow was also determined at each site using a laser doppler flowmeter.

Prior to dosing, no difference was observed in MED between the groups. After dosing, a statistically significant increase in the average MED was observed in the test group; the MED increased from 80 to 96.5 mJ/cm^2. The MED of two test subjects did not change. In the placebo group, the MED was unchanged in six subjects and decreased in four subjects; the average MED significantly decreased from 80 to 68.5 mJ/cm^2. Cutaneous blood flow increased at both irradiated test and control sites.

Clinical Toxicity

Tocopherol. Tocopherol is relatively nontoxic (Tsallas, Molgat, and Jeejeebhoy 1986). However, excess Tocopherol can cause prolongation of clotting time, can depress vitamin K–dependent clotting factors, and may affect platelet aggregation via prostaglandin metabolism. Large doses have caused dizziness, weakness, nausea, abdominal cramps, diarrhea, and other gastrointestinal problems (Pettit and O'Flynn 1990). Headaches, fatigue, blurred vision, inflammation of the mouth, chafing of the lips, low blood sugar, and degenerative changes have been reported by others (Gossel and Wuest 1982).

Ten male and 18 female subjects ingested 100 to 200, 400, or 600 to 800 IU vitamin E daily for an average of 3 years (range of 4 months to 21 years) (Farrell and Bieri 1975). Plasma α-Tocopherol concentrations were increased compared to control values; however, mean plasma Tocopherol concentrations were similar among the dose groups. Abnormal biochemical or hematological indices were generally not observed.

Tocopheryl Acetate. A double-blind study was performed with 800 IU/day *d*-α-Tocopheryl Acetate using eight male subjects (Briggs 1974). Blood and 24-hour urine samples were taken prior to dosing and at 7-day intervals. Two subjects, who were receiving Tocopheryl Acetate, complained of severe fatigue and weakness after 3 weeks. Both subjects had elevated serum creatine kinase accompanied by creatinuria at 7 and 14 days. Serum creatine kinase and urinary creatine were normal 7 days after termination of dosing. No other abnormalities were observed.

In a study (described in 'Immunologic Effects') performed by Meydani et al. (1990), 19 subjects were given 800 mg *dl*-α-Tocopheryl Acetate (7 males and 12 females) or a placebo (9 males and 10 females) daily for 30 days. Meydani et al. (1994) examined the toxicological effects of this dose. Tocopheryl Acetate supplementation did not affect the weight gain or health of the subjects. Plasma α-Tocopherol concentration increased almost three-fold in the test group, whereas no change was observed for the controls. A significant decrease in plasma lipid peroxides and a small but statistically significant increase in plasma zinc concentration was observed in the test subjects. No

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significant changes in hematological status or hepatic and renal function indexes were observed.

Tocopherol Deficiency

Tocopherol deficiency in humans has only been observed in premature infants and in patients with long-term fat malabsorption (Tomassi and Silano 1986). Tocopherol deficiency can cause diminished erythrocyte life span, neurological dysfunction, and myopathies in patients with malabsorption syndromes (Bjørneboe, Bjørneboe, and Drevon 1990), and it can manifest itself as skin collagenosis, red cell hemolysis, xanthomatosis, cirrhosis of the gall bladder, steatorrhea, and creatinuria (Tsallas, Molgat, and Jeejeebhoy 1986). It has also been linked to the development of bronchopulmonary dysplasia, intraventricular hemorrhage in the brain, retrolental fibroplasia, and anemia in premature infants (Bjørneboe, Bjørneboe, and Drevon 1990).

SUMMARY

Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan all function as antioxidants in cosmetic formulations; they also have other functions, such as skin-conditioning agents. Vitamin E is comprised of α-, β-, γ-, and δ-Tocopherol and α-, β-, γ-, and δ-tocotrienol. A summary of uses in cosmetic formulations reported to FDA in 1998 is given in Table 14.

Data submitted to CTFA reported that Tocopherol was used at concentrations of $\leq$5%, Tocopheryl Acetate was used at concentrations of $\leq$36% (and at 100% in vitamin E oil), Tocopheryl Linoleate was used at concentrations of $\leq$2%, Tocopheryl Nicotinate was used at concentrations of $\leq$2% (with recommended concentrations of use of 0.1% to 1.0%), Dioleyl Tocopheryl Methylsilanol and oleyl alcohol was recommended for use at 3% to 6%, Potassium Ascorbyl Tocopheryl Phosphate was used at concentrations of 0.02%, and Tocophersolan was used at concentrations of $\leq$0.2%. Tocopherol, Tocopheryl Acetate, Tocopheryl Succinate, and Tocophersolan have functions as dietary supplements.

TABLE 14
Summary of uses

Ingredient	Frequency of use
Tocopherol	1072
Tocopheryl Acetate	1322
Tocopheryl Linoleate	279
Tocopheryl Nicotinate	3
Tocopheryl Succinate	4
Dioleyl Tocopheryl Methylsilanol	12
Potassium Ascorbyl Tocopheryl Phosphate	15
Tocophersolan	2

The following limitations on impurities are indicative of the limitations that can be found on these ingredients as used in foods:

Tocopherol: <20 mg/kg heavy metals, $\leq$10 mg/kg lead, $\leq$25 mg/kg zinc, $\leq$50 mg/kg zinc + copper, and $\leq$0.1% sulfate ash. Tocopherol is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, residual reactants, or other substances.

Tocopheryl Acetate: $\leq$20 ppm heavy metals, $\leq$10 ppm lead, $\leq$0.1% sulfate ash, and $\leq$3 ppm arsenic. Tocopheryl Acetate is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, or other substances.

Tocopheryl Nicotinate: $\leq$20 ppm heavy metals. Tocopheryl Nicotinate is not expected to contain pesticides, 1,4-dioxane, free ethylene oxide, monochloroacetic acid, sulfite, organic solvents, nitrosamines, or other substances.

Tocopheryl Succinate: heavy metals (as Pb) must be <10 mg/kg.

In a dermal absorption study using human subjects, Tocopheryl Acetate was substantially absorbed in the skin, but systemic availability was not observed. Also, conversion to Tocopherol was not seen. In a study using rats, approximately 6% of the applied dose penetrated into the epidermis after 5 days. Most studies found that some Tocopheryl Acetate was converted to Tocopherol. Irradiation of animals dosed with Tocopheryl Acetate resulted in a significant increase in the amount of Tocopheryl Acetate found in the skin as compared to nonirradiated animals, and irradiation significantly increased the amount of Tocopherol found in the skin of test animals. Tocopherol was "more efficiently" absorbed from w/o than o/w emulsions. The liposomal form increased absorption from o/w emulsions but did not have an effect on w/o emulsions.

In oral ingestion studies with human subjects, administration of Tocopherol and Tocopheryl Acetate resulted in increased serum Tocopherol concentrations; the mean serum concentration peaked at approximately 7 to 8 hours following dosing. In one study, *d*-α-Tocopherol had a greater bioavailability than *dl*-α-Tocopherol. In a study using rats, Tocopheryl Acetate generally had a greater uptake than Tocopheryl Nicotinate. The liver was the principal storage site, but the adrenal glands had the greatest uptake. Most of the radioactivity was recovered as tocopheryl quinone, but in a few tissues, such as the adrenal glands, it was recovered mostly as Tocopherol. In another study using rats that were fed Tocopheryl Acetate for up to 14 weeks, a linear relationship was found between time and tissue concentration of Tocopherol. In mice that were fed Tocopheryl Acetate and irradiated, a dose-dependent increase was seen for Tocopherol in the ventral skin compared to controls, whereas irradiation decreased dorsal skin Tocopherol concentrations.

Tocopherol is the major lipid-soluble chain-breaking antioxidant of membranes and an important cellular protectant against

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oxidative damage. It exerts antioxidant effects by trapping peroxyl radicals. Some researchers found that Tocopherol was depleted from the skin upon exposure to UV light; they postulated that other antioxidants that can recycle Tocopherol can also be depleted.

Tocopherol was generally found to inhibit UVB-induced lipid peroxidation. Dermal application of Tocopheryl Acetate also decreased lipid peroxidation, but oral administration was reported not to have an effect. Both single and multiple applications of Tocopherol inhibited 8-MOP photobinding to DNA/RNA and protein. A single application of Tocopheryl Acetate did not affect photobinding, but multiple applications protected.

Application of Tocopherol both prior to and after irradiation increased the MED. Tocopheryl Acetate application after irradiation resulted in decreased skin thickness.

Tocopherol supplementation enhanced indexes of T cell–mediated function in elderly human subjects, and Tocopheryl Acetate improved elderly immune responsiveness. Tocopherol abrogated the capacity of splenocytes from UV-irradiated mice to inhibit normal rejection of UVM12 cells, whereas Tocopheryl Acetate and Tocopheryl Succinate enhanced the growth of injected UVM12 tumor cells in mice. Tocopherol and Tocopheryl Acetate had a number of immunologic effects.

α-Tocopherol is a good inhibitor of nitrosation because its phenol ring is fully substituted. α-Tocopherol might inhibit formation of skin nitrosating agents from NO_2, but it did not inhibit nitrosamine production from skin nitrosating agents. Tocopheryl Acetate altered the cellular response of rats to nitrite; it prevented nitrite-related mortality and the decrease in glutathione *S*-transferase activity.

Tocopheryl Succinate has some cytotoxic activity. Tocopherol and Tocopheryl Succinate protected against UV-induced cytotoxicity.

The dermal LD_{50} values of Tocopherol and 75% Tocophersolan for rats were >3 and >2 g/kg, respectively. The oral LD_{50} values of Tocopherol, Tocopheryl Acetate, Tocopheryl Nicotinate, Tocopheryl Succinate, Tocophersolan, and 75% Tocophersolan for rats were greater than 4, 16, 10, 7, 7, and 5 g/kg, respectively. The oral LD_{50} values of Tocopherol and Tocopheryl Acetate were >25 ml/kg and >4 g/kg , respectively, for mice. Tocopherol and Tocopheryl Acetate were not toxic in short-term oral studies. In a subchronic study, 7 of 10 male rats dosed orally with 2000 mg/kg *d*-α-Tocopheryl Acetate died in 9 to 11 weeks because of internal hemorrhage; other signs of toxicity were observed in a dose-dependent manner. Rats fed ≤2.0% Tocophersolan did not have any treatment-related effects. In a chronic toxicity study in which rats were fed ≤2000 mg/kg/day *dl*-α-Tocopheryl Acetate and supplemented with vitamin K, no significant treatment-related effects were observed. High doses of Tocopherol and Tocopheryl Acetate have hemorrhagic activity.

In one study, Tocopherol was a weak primary skin irritant in rabbits and in another, it was a weak cumulative skin irritant in guinea pigs. Cosmetic formulations containing 2% *dl*-Tocopherol, 12% vitamin E in wheat germ, and 32% mixed Tocopherols in a wheat germ and vegetable oil base had mean cumulative irritation scores of 31, 7, and 12 (maximum possible of 64) in rabbits, respectively. Tocopheryl Acetate and Tocopheryl Nicotinate were generally not irritating to rabbit skin. A single dose of a mixture of Dioleyl Tocopheryl Methylsilanol and oleic acid was not irritating to rabbits, but slight erythema was observed following multiple applications. The same was observed with 75% Tocophersolan in guinea pigs. A mixture containing <0.1% Tocopherol was not a sensitizer in an open epicutaneous test, whereas "higher concentrations (30%≤)" of Tocopheryl Acetate can cause sensitization in this test. However, Tocopheryl Acetate was not sensitizing in a guinea pig maximization test. Tocophersolan was not a sensitizer in a Buehler test. In ocular irritation studies, Tocopherol was nonirritating in some tests and a minimal or very slight ocular irritant in others. Tocopheryl Acetate, Tocopheryl Nicotinate, and a mixture of Dioleyl Tocopheryl Methylsilanol and oleic alcohol were not irritating to rabbit eyes. Tocophersolan was a slight ocular irritant.

Oral administration of Tocopherol, Tocopheryl Succinate, and Tocophersolan did not have reproductive or developmental effects in rats, and Tocopheryl Acetate generally did not have any reproductive or developmental effects in rabbits, hamsters, rats, or mice. Tocopherol and Tocopheryl Acetate had some effect on reducing the number of malformations observed in neonates from diabetic dams. Tocopherol did not have an effect on zinc deficiency–induced teratogenicity. In some studies, Tocopheryl Acetate potentiated the embryolethal effect of cortisone acetate. Tocopheryl Succinate reduced some reproductive effects, but not all, induced by TCDD.

Tocopherol, Tocopheryl Acetate, Tocopheryl Succinate, and a mixture of Dioleyl Tocopheryl Methylsilanol and oleic acid were generally not mutagenic. The only effects observed were a dose-dependent increased elution rate of DNA in alkali in a DNA strand breakage assay and 50% inhibition in the incorporation of [^{3}H]-thymidine in a thymidine incorporation assay using Tocopherol. Tocopherol has some antimutagenic activity and was able to modulate some mutagenic effects. Tocopheryl Succinate also had some mutagenicity modulatory activity. Tocopherol and Tocopheryl Succinate generally did not affect UV-induced mutagenicity.

In an oral study, Tocopheryl Acetate was not carcinogenic. Neoplasms developed in animals injected subcutaneously with Tocopherol or Tocopheryl Acetate and soya oil; however, neoplasms were not seen in animals dosed with Tocopherol or Tocopheryl Acetate only.

The modulation of the carcinogenic effect of other agents was studied with Tocopherol, Tocopheryl Acetate, and Tocopheryl Succinate was mixed. In most cases, there was inhibition of the effect of the other agent; in some cases, no effect was seen. However, in one study, Tocopherol acted as a complete tumor promoter, with an efficiency approaching a standard promoter, the same promoter, whose activity was inhibited by Tocopherol in other studies. In a modulation study, Tocopherol reduced

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spontaneous pulmonary tumorigenesis in A/J mice. In a dermal study using mice, Tocopherol reduced photocarcinogenesis. However, dermally applied Tocopheryl Acetate and Tocopheryl Succinate were reported to enhance photocarcinogenesis. After oral administration, Tocopherol appeared to reduce UV-induced lesions. Orally administered Tocopheryl Acetate reduced the incidence of skin cancer, but toxicity was observed. Tocopherol had some other protective effects on UV-induced skin damage. Slight protection from wrinkling was observed in hairless mice with Tocopheryl Acetate and Tocopheryl Linoleate; Tocopheryl Nicotinate and Tocopheryl Succinate did not have any protective effects.

In clinical studies, Tocopherol and Tocopheryl Acetate were not irritants or sensitizers. A very small percentage of patients patch-tested by the NACDG reacted to Tocopherol. A cosmetic line containing Tocopheryl Linoleate introduced in Switzerland in 1992 resulted in a large number of outbreaks; positive patch tests with Tocopheryl Linoleate were seen. However, the outbreaks were thought to be due to a metabolite or contamination of the product. Tocopheryl Nicotinate was not an irritant or a sensitizer. Case reports exist for Tocopherol- and Tocopheryl Acetate–containing products. Tocopheryl Acetate was not phototoxic. In a study in which subjects were given a supplement containing Tocopherol and ascorbic acid, a significant increase in MED was observed as compared to the controls.

Clinically, Tocopherol is relatively nontoxic. Excess Tocopherol can, however, prolong clotting time, depress vitamin K–dependent clotting factors, and affect platelet aggregation via prostaglandin metabolism. Large doses can produce a number of other effects.

DISCUSSION

The Expert Panel found the data included in this review adequate to determine that Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan are safe as used in cosmetic formulations. It is understood that the Tocopherol in cosmetic products is of similar grade to that used in foods.

The Expert Panel was initially concerned with possible irritation and sensitization because of a large number of outbreaks reported in Switzerland with the release of a new line of cosmetics that contained Tocopheryl Linoleate. However, the researchers thought that the outbreaks were due either to a contaminant or a metabolite. Safety data summarized in this report indicated that Tocopherol was not an irritant or sensitizer. Also, the limited number of case studies available on the ingredients of the Tocopherol family, which have widespread use, was an indication that dermal irritation and sensitization was not a concern.

Tocopherol and Tocopheryl Acetate were reported to be used in hair sprays. Initially, the Expert Panel was concerned with the lack of inhalation toxicity data. However, this concern was allayed because of the low reported concentrations used in hair sprays, 0.0001% to 0.2%.

The Panel did carefully consider that the tumor promoting ability of Tocopherol, Tocopheryl Acetate, and Tocopheryl Succinate has been extensively studied. In most studies, Tocopherol is reported to inhibit tumor promotion. Of concern to the Panel, however, was one study in mice in which Tocopherol acted as a complete tumor promoter. The procedures used in this study were different from most tumor promotion studies and the results have not been repeated. The general experience of the Cosmetic Ingredient Review (CIR) Expert Panel is that Tocopherol is not a tumor promoter.

Finally, the Expert Panel was concerned that hydroquinone is used in the manufacture of Tocopherol and could be an impurity. The Expert Panel stated that residual levels of hydroquinone were to be limited to those achieved by good manufacturing processes.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Linoleate/Oleate, Tocopheryl Nicotinate, Tocopheryl Succinate, Dioleyl Tocopheryl Methylsilanol, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan are safe as used in cosmetic formulations.

REFERENCES

Abdo, K. M., G. Rao, C. A. Montgomery, M. Dinowitz, and K. Kanagalingam. 1986. Thirteen-week toxicity study of *d*-α-tocopheryl acetate (vitamin E) in Fischer 344 rats. *Food Chem. Toxicol.* 24(10/11):1043–1050.

Adams, R. M., and H. I. Maibach. 1985. A five year study of cosmetic reactions. *J. Am. Acad. Dermatol.* 13:1062–1069.

Aeling, J. L., P. J. Panagotacos, and R. J. Andreozzi. 1973. Letter: Allergic contact dermatits to vitamin E aerosol deodorant. *Arch. Dermatol.* 108:579–580.

Alberts, D. S., R. Goldman, M. J. Xu, et al. 1996. Disposition and metabolism of topically administered alpha-tocopherol acetate: A common ingredient of commercially available sunscreens and cosmetics. *Nutr. Cancer* 26(2):193–201.

AMA Laboratories, Inc. 1996. 100 subject repeat insult patch test skin irritation/sensitization evaluation. Unpublished data submitted by CTFA. (10 pages.)[2]

Anderson, D., N. Basaran, S. D. Blowers, and A. J. Edwards. 1995. The effect of antioxidants on bleomycin treatment in *in vitro* and *in vivo* genotoxicity assays. *Mutat. Res.* 329:37–47.

BASF. 1993a. Technical information for Tocopheryl Acetate. Unpublished data submitted by CTFA. (3 pages.)[2]

BASF. 1993b. Toxicological data summary for Tocopheryl Acetate. Unpublished data submitted by CTFA. (1 page.)[2]

BASF. 1993c. Toxicological data summary for Tocopheryl Nicotinate. Unpublished data submitted by CTFA. (2 pages.)[2]

BASF. 1994a. Technical information for Tocopherol. Unpublished data submitted by CTFA. (4 pages.)[2]

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

Distrbuted for comment only -- do not cite or quote

BASF. 1994b. Technical leaflet for Tocopheryl Nicotinate. Unpublished data submitted by CTFA. (4 pages.)[2]

BASF. 1995. Safety data sheet for Tocopherol. Unpublished data submitted by CTFA. (4 pages.)[2]

BASF. 1996a. Safety data sheet for Tocopherol Acetate. Unpublished data submitted by CTFA. (4 pages.)[2]

BASF. 1996b. Safety data sheet for Tocopheryl Nicotinate. Unpublished data submitted by CTFA. (3 pages.)[2]

BASF. 1996c. Impurities of Tocopheryl Nicotinate. Unpublished data submitted by CTFA. (5 pages.)[2]

BASF. 1998a. Cosmetic Ingredient Chemical Description (CICD) form for Tocopherol. Unpublished data submitted by CTFA. (4 pages.)[2]

BASF. 1998b. CICD form for Tocopherol Acetate. Unpublished data submitted by CTFA. (4 pages.)[2]

BASF. 1998c. Impurities of Tocopherol. Unpublished data submitted by CTFA. (8 pages.)[2]

BASF. 1998d. Impurities of Tocopheryl Acetate. Unpublished data submitted by CTFA. (5 pages.)[2]

Bateman, N. E., and D. A. Uccellini. 1985. Kinetics of D-alpha-tocopherol in a water soluble base in man. *J. Pharm. Pharmacol.* 37:728–729.

Bazzano, C., S. De Angeles, G. Kleist, and N. Macedo. 1996. Allergic contact dermatitis from topical vitamins A and E. *Contact Dermatitis* 35:261–262.

Beckman, C., R. M. Roy, and A. Sproule. 1982. Modification of radiation-induced sex-linked recessive lethal mutation frequency by tocopherol. *Mutat. Res.* 105(1/2):73–77.

Beijersbergen van Henegouwen, G. W., H. E. Junginger, and H. de Vries. 1995. Hydrolysis of *RRR*-α-tocopheryl acetate (vitamin E acetate) in the skin and its UV protecting activity. (An *in vivo* study with the rat.) *J. Photochem. Photobiol. B: Biology* 29:45–51.

Bianchi, L., R. Melli, R. Pizzala, et al. 1996. Effects of β-carotene and α-tocopherol on photogenotoxicity induced by 8-methoxypsoralen: The role of oxygen. *Mutat. Res.* 369:183–194.

Bissett, D. L., R. Chatterjee, and D. P. Hannon. 1990. Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse. *Photodermatol. Photoimmunol. Photomed.* 7:56–62.

Bissett, D., R. Chatterjee, and D. Hannon. 1992. Protective effect of a topically applied antioxidant plus an antiinflammatory agent using ultraviolet radiation-induced chronic skin damage in the hairless mouse. *J. Soc. Cosmet. Chem.* 43:85–92.

Bissett, D. L., G. G. Hillebrand, and D. P. Hannon. 1989. The hairless mouse as a model of skin photoaging: Its use to evaluate photoprotective materials. *Photodermatol. Photoimmunol. Photomed.* 6:228–233.

Bjørneboe, A., G. E. Bjørneboe, and C. A. Drevon. 1990. Absorption, transport and distribution of vitamin E. *J. Nutr.* 120:233–242.

Black, H. S. 1974. Effects of dietary antioxidants on actinic tumor induction. *Res. Commun. Chem. Pathol. Pharmacol.* 7:783–786.

Black, H. S., and J. T. Chan. 1975. Suppression of ultraviolet light-induced tumor formation by dietary antioxidants. *J. Invest. Dermatol.* 65:412–414.

Black, H. S., W. Lenger, V. McCann, and J. L. Thornby. 1983. Relation of UV dose to antioxidant modification of photocarcinogenesis. *J. Am. Coll. Toxicol.* 2:201–207.

Briggs, M. 1974. Vitamin E supplements and fatigue. *N. Engl. J. Med.* 290:579–580.

Budavari, S., ed. 1989. *The Merck index. An encyclopedia of chemicals, drugs, and biologicals*, 11th ed., 1496, 1579–1580. Rahway, NJ: Merck & Co.

Bulger, E. M., W. S. Helton, C. M. Clinton, et al. 1997. Enteral vitamin E supplementation inhibits the cytokine response to endotoxin. *Arch. Surg.* 132:1337–1341.

Chemisches Laboratorium Dr. Kurt Richter GmbH (CLR). 1997. Correspondence regarding the composition of Arnica Oil CLR. Unpublished data submitted by CTFA. (3 pages.)[2]

Chow, C. K., C. B. Hong, M. E. Reese, and C. Gairola. 1984. Effect of dietary vitamin E on nitrite-treated rats. *Toxicol. Lett.* 23:109–117.

Constantinides, P., and M. Harkey. 1985. Initiation of a transplantable fibrosarcoma by the synergism of two non-initiators, alpha-tocopherol and soya oil. *Virchows Arch. [Pathol. Anat.]* 405:285–297.

Consumer Product Testing Co. 1992. Final Report on the phototoxicity of vitamin E Acetate CG, Lot #181032. Experiment ref. no. TS-214-92. Report dated Dec 21. Unpublished data submitted by CTFA. (6 pages.)[2]

Consumer Product Testing Co. 1997a. Final report of a repeated insult patch test, protocol no. 1.01, of vitamin E cream formula #26447-154 (containing 5% Tocopherol). Experiment ref. no. C97-0056. Report dated Mar 26. Unpublished data submitted by CTFA. (12 pages.)[2]

Consumer Product Testing Co. 1997b. Final report of a repeated insult patch test, protocol no. 1.01, of vitamin E (*dl*-alpha Tocopherol) 5% in corn oil, formula #26447-154. Experiment ref. no. C97-0056. Report dated Apr 8. Unpublished data submitted by CTFA. (13 pages.)[2]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1972. Acute oral toxicity, primary skin irritation potential, cumulative skin irritation potential, ocular irritation potential, and clinical primary skin irritation potential of Tocopherol. Unpublished data submitted by CTFA. (6 pages.)[2]

CTFA. 1999a. CTFA Chemical Description of Tocopherol. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 1999b. CTFA Chemical Description of Tocopheryl Acetate. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 1999c. CTFA Chemical Description of Potassium Ascorbyl Tocopheryl Phosphate. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 1999d. CTFA Chemical Description of Tocopheryl Linoleate/Oleate. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 1999e. CTFA Chemical Description of Tocopheryl Nicotinate. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 1999f. CTFA Chemical Description of Tocopheryl Succinate. Unpublished data submitted by CTFA. (1 page.)[2]

CTFA. 1999g. Concentration of use data on Tocopherol, Tocopheryl Acetate, Tocopheryl Linoleate, Tocopheryl Nicotinate, Potassium Ascorbyl Tocopheryl Phosphate, and Tocophersolan. Updated July 15. Unpublished data submitted by CTFA. (6 pages.)[2]

Darr, D., S. Dunston, H. Faust, and S. Pinnell. 1996. Effectiveness of antioxidants (vitamin C and E) with and without sunscreens as topical photoprotectants. *Acta Derm. Venerol.* 76:264–268.

DeFlora, S., C. Bennicelli, A. Rovida, L. Scatolini, and A. Camoirano. 1994. Inhibition of the 'spontaneous' mutagenicity in *Salmonella typhimurium* TA102 and TA104. *Mutat. Res.* 307:157–167.

de Groot, A. C., P. J. Berretty, C. J. W. van Ginkel, et al. 1991. Allergic contact dermatitis from tocopheryl acetate in cosmetic creams. *Contact Dermatitis* 25:302–304.

DeLeenheer, A. P., V. O. De Bevere, and A. E. Claeys. 1979. Measurement of alpha-, beta-, and gamma tocopherol in serum by liquid chromatography. *Clin. Chem.* 25:425–428.

Díez-Marques, M. L., F. J. Lucio-Cazaña, and M. Rodriguez-Puyol. 1986. *In vitro* response of erythrocytes to α-tocopherol exposure. *Int. J. Vitam. Nutr. Res.* 56:311–315.

Djerassi, D., L. J. Machlin, and C. Nocka. 1986. Vitamin E: Biochemical function and its role in cosmetics. *Drug Cosmet. Ind.* 138:46–49.

Dostál, M., and S. Bláhová. 1985. Embryotoxicity of cortisone combined with vitamin E. *Folia Morphol. (Prague)* 34:132–140.

Draelos, Z. D. 1993. Four common problematic cosmetics discussed. *Cosmet. Dermatitis* 6:15–16, 19.

Eastman Chemical Co. 1999. Identification of material tested in Eastman Kodak Co. (1989) and impurity data. Unpublished data submitted by CTFA. (1 page.)[2]

Eastman Kodak Co. 1989. Acute dermal toxicity, oral toxicity dermal irritation, dermal sensitization, and ocular irritation studies. Unpublished data submitted by CTFA. (10 pages.)[2]

Eberlein-König, B., M. Placzek, and B. Przybilla. 1998. Protective effect against sunburn of combined systemic ascorbic acid (vitamin C) and *d*-α-tocopherol (vitamin E). *J. Am. Acad. Dermatol.* 38:45–48.

Distrbuted for comment only -- do not cite or quote

El-Nahas, S. M., F. E. Mattar, and A. A. Mohamed. 1993. Radioprotective effect of vitamins C and E. *Mutat. Res.* 301:143–147.

Ennagram. 1999. Purity of Tocopherol used in the manufacture of Tocopheryl Linoleate demonstrated using HPLC. Unpublished data submitted by CTFA. (2 pages.)[2]

Eskelson, C. D., O. E. Odeleye, R. R. Watson, D. L. Earnest, and S. I. Mufti. 1993. Modulation of cancer growth by vitamin E and alcohol. *Alcohol* 28: 117–125.

European Economic Community (EEC). 1995. *EEC Cosmetics Directive 76/768/EEC, as amended, Annexes I through VII.* Brussels: EEC.

Exsymol. 1988. Structure, specifications, composition, acute toxicity, dermal irritation, and ocular irritation data on a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol. Unpublished data submitted by CTFA. (42 pages.)[2]

Exsymol. 1998. Structure, specifications, composition, recommended concentration of use, toxicity summary information on a mixture of Dioleyl Tocopheryl Methylsilanol and oleyl alcohol. Unpublished data submitted by CTFA. (2 pages.)[2]

Farrell, P. M., and J. G. Bieri. 1975. Megavitamin E supplementation in man. *Am. J. Clin. Nutr.* 28:1381–1386.

Ferslew, K. E., R. V. Acuff, E. A. Daigneault, T. W. Woolley, and P. E. Stanton, Jr. 1993. Pharmacokinetics and bioavailability of the RRR and all racemic stereoisomers of alpha-tocopherol in humans after single oral administration. *J. Clin. Pharmacol.* 33:84–88.

Fisher, A. A. 1975. Contact dermatitis in surgeons. *J. Dermatol. Surg.* 1:63–67.

Fisher, A. A. 1991. Three faces of vitamin E topical allergy. *Cutis* 48:272–274.

Food and Drug Administration (FDA). 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw composition statements. *Federal Register* 57:3128–3130.

FDA. 1998a. Property information on *dl*-alpha-Tocopherol (no date given—anonymous source). Submitted by FDA in response to an FOI request—1998. (1 page.)[2]

FDA. 1998b. Determination of α-Tocopherol content of bacon by high pressure liquid chromatography with fluorescence detection (no date given—anonymous source). Submitted by FDA in response to an FOI request—1998. (2 pages.)[2]

FDA. 1998c. Determination of α-Tocopherol content of bacon by Emmerie-Engel reaction (no date given—anonymous source). Submitted by FDA in response to an FOI request—1998. (3 pages.)[2]

FDA. 1998d. Frequency of use of cosmetic ingredients. *FDA database.* Washington, DC: FDA.

Food and Drug Research Labs, Inc. (FDRL). 1973. *Teratologic evaluation of FDA 71-58 (dl-alpha Tocopherolacetate).* Report dated June 1. NTIS Report No. PB223809.

Gallo-Torres, H. E., O. N. Miller, J. G. Hamilton, and C. Tratnyek. 1971. Distribution and metabolism of two orally administered esters of tocopherol. *Lipids* 6:318–325.

Garcia-Bravo, B., and P. Mozo. 1992. Generalized contact dermatitis from vitamin E. *Contact Dermatitis* 26:280.

Gensler, H. L., M. Aickin, Y. M. Peng, and M. Xu. 1996. Importance of the form of topical vitamin E for prevention of photocarcinogenesis. *Nutr. Cancer* 26:183–191.

Gensler, H. L., and M. Magdaleno. 1991. Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by ultraviolet radiation. *Nutr. Cancer* 15:97–106.

Gerrish, K. E., and H. L. Gensler. 1993. Prevention of photocarcinogenesis by dietary vitamin E. *Nutr. Cancer* 19:125–133.

Gille, J. J. P., P. Pasman, C. G. M. Van Berkel, and H. Joenje. 1991. Effect of antioxidants on hyperoxia-induced chromosomal breakage in Chinese hamster overy cells: Protection by carnosine. *Mutagenesis* 6:313–338.

Glauert, H. P., M. M. Beaty, T. D. Clark, et al. 1990. Effect of dietary vitamin E on the development of altered hepatic foci and hepatic tumors induced by the peroxisome proliferator ciprofibrate. *J. Cancer Res. Clin. Oncol.* 116:351–356.

Goldman, M. P., and M. Rapaport. 1986. Contact dermatitis to vitamin E oil. *J. Am. Acad. Dermatol.* 14:133–134.

Gossel, T. A., and J. R. Wuest. 1982. Vitamin megadoses and toxicity. *U.S. Pharm.* 7:48–52, 54.

Hale, T. W., K. Rais-Bahrami, D. L. Montgomery, C. Harkey, and R. W. Habersang. 1995. Vitamin E toxicity in neonatal piglets. *Clin. Toxicol.* 33:123–130.

Harris, B. D., and J. S. Taylor. 1997. Contact allergy to vitamin E capsules: False-negative patch tests to vitamin E? *Contact Dermatitis* 36:273.

Hasegawa, R., F. Furukawa, K. Toyoda, et al. 1990. Inhibitory effects of antioxidants on *N*-bis(2-hydroxypropyl)nitrosamine-induced lung carcinogenesis in rats. *Jpn. J. Cancer Res.* 81:871–877.

Hassoun, E. A., A. C. Walter, N. Z. Alsharif, and S. J. Stohs. 1997. Modulation of TCDD-induced fetotoxicity and oxidative stress in embryonic and placental tissues of C57BL/6J mice by vitamin E succinate and ellagic acid. *Toxicology* 124:27–37.

Henkel. 1994. Information sheet on Copherol® 1250 [Tocopherol Acetate.] Dated Aug 1994. Unpublished data submitted by CTFA. (1 page.)[2]

Henkel. 1996. Information sheet on Copherol®F-1300 [Tocopherol]. Dated Aug 1996. Unpublished data submitted by CTFA. (1 page.)[2]

Henkel. 1998. CICD form for Tocopherol. Unpublished data submitted by CTFA. (4 pages.)[2]

Hirose, M., H. Yadz, K. Hakoi, S. Takahashi, and N. Ito. 1993. Modification of carcinogenesis by α-tocopherol, *t*-butylhydroquinone, propyl gallate and butylated hydroxytoluene in a rat multi-organ carcinogenesis model. *Carcinogenesis* 14:2359–2364.

Hoffmann-LaRoche. 1995. Information file for cosmetic ingredient: DL-α-Tocopheryl Acetate. Product safety assessment. Report dated Dec. 21. Unpublished data submitted by CTFA. (13 pages.)[2]

Hoffmann-LaRoche. 1996. Chemistry, concentration of use, and skin penetration, dermal irritation, sensitization, and ocular irritation summary data on Tocopheryl Acetate. Dated Jan. 31. Unpublished data submitted by CTFA. (5 pages.)[2]

Hook, E. B., K. M. Healty, A. M. Niles, and R. G. Skalko. 1974. Letter: Vitamin E: Teratogen or anti-teratogen? *Lancet* 1:809.

Horwitt, M. K., W. H. Elliott, P. Kanjananggulpan, and C. D. Fitch. 1984. Serum concentrations of alpha-tocopherol after ingestion of various vitamin E preparations. *Am. J. Clin. Nutr.* 40:240–245.

Houk, V. S., H. G. Brooks, L. D. Claxton, and H. E. Brockman. 1992. Evaluation of antimutagenicity in the spiral *Salmonella* assay. *Environ. Mol. Mutagen* 19:26.

Hurley, L. S., D. D. Dungan, C. L. Keen, and B. Lönnerdal. 1983. The effects of vitamin E on zinc deficiency teratogenesis in rats. *J. Nutr.* 113:1875–1877.

International Bio-Research, Inc (IBR). 1972a. Acute oral toxicity test of Arnica Oil-CLR (a mixture composed of <0.1% Tocopherol, 1–5% arnica montana extract, and >50% soybean (Glycine Soja) oil) on rats. Study dated July 18. Unpublished data submitted by CTFA. (6 pages.)[2]

IBR. 1972b. Examination of Arnica Oil (a mixture composed of <0.1% Tocopherol, 1–5% arnica montana extract, and >50% soybean (Glycine Soja) oil) in the eye irritation test on rabbits. Study dated June. Unpublished data submitted by CTFA. (9 pages.)[2]

IBR. 1976. Patch test for primary skin irritation and corrosivity of the compound: "Arnicaöl CLR" (a mixture composed of <0.1% Tocopherol, 1–5% arnica montana extract, and >50% soybean (Glycine Soja) oil) (10% paraffin oil solution). Study dated December. Unpublished data submitted by CTFA. (7 pages.)[2]

IBR. 1977. Investigation of sensitising effects on guinea pigs of "Arnicaöl CLR" (a mixture composed of <0.1% Tocopherol, 1–5% arnica montana extract, and >50% soybean (Glycine Soja) oil) (undiluted). Study dated May. Unpublished data submitted by CTFA. (6 pages.)[2]

Ishidate, M., Jr., T. Sofuni, K. Yoshikawa, et al. 1984. Primary mutagenicity screening of food additives currently used in Japan. *Food Chem. Toxicol.* 22:623–336.

Distrbuted for comment only -- do not cite or quote

Ishinaga, M., M. Tanimoto, S. Sugiyama, R. Kumamoto, and K. Yokoro. 1991. Molecular species of phospholipid in rats in primary and transplanted fibrosarcomas induced by soybean oil containing tocopherol acetate. *Biochem. Cell Biol.* 69:655–660.

Jeng, K. C., C. S. Yang, W. Y. Siu, et al. 1996. Supplementation with vitamins C and E enhances cytokine production by peripheral blood mononuclear cells in healthy adults. *Am. J. Clin. Nutr.* 64:960–965.

Jenkins, M. Y., and G. V. Mitchell. 1975. Influence of excess vitamin E on vitamin A toxicity in rats. *J. Nutr.* 105:1600–1606.

Jurkiewicz, B. A., D. L. Bissett, and G. R. Buettner. 1995. Effect of topically applied tocopherol on ultraviolet radiation-mediated free radical damage in skin. *J. Invest. Dermatol.* 104:484–488.

Kagan, V., E. Witt, R. Goldman, G. Scita, and L. Packer. 1992. Ultraviolet light-induced generation of vitamin E radicals and their recycling. A possible photosensitizing effect of vitamin E in skin. *Free Radic. Res. Commun.* 16:51–64.

Kamimura, M., and T. Matsuzawa. 1968. Percutaneous absorption of α-Tocopheryl Acetate. *J. Vitaminol.* 14:150–159.

Kamm, J. J., T. Dashman, H. Newmark, and W. J. Mergens. 1977. Inhibition of amine-nitrite hepatotoxicty by α-tocopherol. *Toxicol. Appl. Pharmacol.* 41:575–583.

Kawachi, T., T. Yahagi, T. Kada, et al. 1980. Cooperative programme on short-term assays for carcinogenicity in Japan. *IARC Sci. Publ.* 27:323–330.

Kelleher, J., T. Davies, C. L. Smith, B. E. Walker, and M. S. Losowsky. 1972. The absorption of α-tocopherol in the rat. I. The effect of different carriers and different dose levels. *Int. J. Vitam. Nutr. Res.* 42:394–402.

Khettab, N., M. C. Amory, G. Briand, et al. 1988. Photoprotective effect of vitamins A and E on polyamine and oxygenated free radical metabolism in hairless mouse epidermis. *Biochimie* 70:1709–1713.

Klain, G. J. 1989. Dermal penetration and systemic distribution of ^{14}C-labeled vitamin E in human skin grafted athymic nude mice. *Int. J. Vitam. Nutr. Res.* 59:333–337.

Kock, R., S. Seitz, B. Delvous, and H. Greiling. 1997. Two high performance liquid chromatographic methods for the determination of alpha-tocopherol in serum compared to isotope dilution-gas chromatography-mass spectrometry. *Eur. J. Clin. Chem. Clin. Biochem.* 35:371–378.

Kodýtková, I., J. Madar, and R. J. Šrám. 1980. Chromosomal aberrations in mouse bone marrow cells and antibody production changes induced by long-term exposure to cyclophosphamide and α-tocopherol. *Folia Biol. (Praha)* 26:94–102.

Kolaja, K. L., and J. E. Klaunig. 1997. Vitamin E modulation of hepatic focal lesion growth in mice. *Toxicol. Appl. Pharmacol.* 143:380–387.

Kondo, S., A. Mamada, J. Yamaguchi, and S. Fukuro. 1990. Protective effect of *dl*-α-tocopherol on the cytotoxicity of ultraviolet B against human skin fibroblasts *in vitro*. *Photodermatol. Photoimmunol. Photomed.* 7:173–177.

Kramer, K. A., and D. C. Liebler. 1996. Conversion of α-tocopheryl acetate to α-tocopherol in mouse skin. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 37:267. Abstract 1820.

Krasavage, W. J., and C. J. Terhaar. 1977. *d*-α-Tocopherol poly(ethylene glycol) 1000 succinate. Acute toxicity, subchronic feeding, reproduction, and teratologic studies in the rat. *J. Agric. Food Chem.* 25:273–278.

Lacava, Z. G., and H. Luna. 1994. The anticlastogenic effect of tocopherol in peritoneal macrophages of benznidazole-treated and ovariectomized mice. *Mutat. Res.* 305:145–150.

Lambert, L. A., W. G. Warner, R. R. Wei, et al. 1994. The protective but non-synergistic effect of dietary β-carotene and vitamin E on skin tumorigenesis in Skh mice. *Nutr. Cancer* 21:1–12.

Lewis, R. J., Sr., ed. 1993a. *Hazardous chemicals desk reference*, 3rd ed., 1318. New York, NY: Van Nostrand Reinhold.

Lewis, R. J., Sr., ed. 1993b. *Hawleys's condensed chemical dictionary*, 12th ed., 1156. New York: Van Nostrand Reinhold.

Lide, D. R., ed. 1993. *CRC Handbook of Chemistry and Physics*, 74th ed., 3–487. Boca Raton, FL: CRC Press.

Liebler, D. C., J. A. Burr, L. Philips, and A. J. Ham. 1996. Gas chromatography-mass spectrometry analysis of Vitamin E and its oxidation products. *Anal. Biochem.* 236:27–34.

MacCrehan, W. A., and E. Schönberger. 1987. Determination of retinol, alpha-tocopherol, and beta-carotene in serum by liquid chromatography with absorbance and electrochemical detection. *Clin. Chem.* 33:1585–1592.

Machlin, L. J., and E. Gabriel. 1982. Kinetics of tissue alpha-tocopherol uptake and depletion following administration of high levels of vitamin E. *Ann. N.Y. Acad. Sci.* 393:48–60.

Madhavi, D. L., and D. K. Salunkhe, eds. 1996. Toxicological aspects of food antioxidants. In *Food Antioxidants: Technological, Toxicological and Health Perspectives VIII*. 267–359. New York: Marcel Dekker.

Manimegalai, R., A. Geetha, and K. Rajalakshmi. 1993. Effect of vitamin-E on high fat diet induced hyperlipidemia in rats. *Indian J. Exp. Biol.* 31:704–707.

Martin, M. M., and L. S. Hurley. 1977. Effect of large amounts of vitamin E during pregnancy and lactation. *Am. J. Clin. Nutr.* 30:1629–1637.

Marzulli, F. N., and H. I. Maibach. 1975. The rabbit as a model for evaluating skin irritants: A comparison of results obtained on animals and man using repeated skin exposures. *Food Cosmet. Toxicol.* 13:533–540.

McCay, P. B., M. M. King, and J. V. Pitha. 1981. Evidence that the effectiveness of antioxidants as inhibitors of 7,12-dimethylbenz(a)anthracene-induced mammary tumors is a function of dietary fat composition. *Cancer Res.* 41:3745–3748.

McClure, T. D., and D. C. Liebler. 1995. A rapid method for profiling the products of antioxidant reactions by negative ion chemical ionization mass spectrometry. *Chem. Res. Toxicol.* 8(1):128–135.

McVean, M., and D. C. Liebler. 1997. Inhibition of UVB induced DNA photodamage in mouse epidermis by topically applied α-tocopherol. *Carcinogenesis* 18:1617–1622.

Mergens, W. J., J. J. Kamm, H. L. Newmark, W. Fiddler, and J. Pensabene. 1978. Alpha-tocopherol: Uses in preventing nitrosamine formation. *IARC* 19:199–212.

Meydani, S. N., M. P. Barklund, S. Liu, et al. 1990. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am. J. Clin. Nutr.* 52:557–563.

Meydani, S. N., M. Meydani, J. B. Blumberg, et al. 1997. Vitamin E supplementation and *in vivo* immune response in healthy elderly subjects: A randomized control trial. *JAMA* 277:1380–1386.

Meydani, S. N., M. Meydani, L. C. Rall, F. Morrow, and J. B. Blumberg. 1994. Assessment of the safety of high-dose, short-term supplementation with vitamin E in healthy older adults. *Am. J. Clin. Nutr.* 60:704–709.

Mirvish, S. S. 1986. Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis, and cancer. *Cancer* 58(8 Suppl):1842–1850.

Mitchel, R. E. J., and R. McCann. 1993. Vitamin E is a complete tumor promoter in mouse skin. *Carcinogenesis* 14:659–662.

Mochida, K., M. Goto, and K. Saito. 1985. Human cell cultures as an assay for the toxicity of antioxidants. *Bull. Environ. Contam. Toxicol.* 35:427–429.

Moore, M. A., H. Tsuda, W. Thamavit, T. Masui, and N. Ito. 1987. Differential modification of development of preneoplastic lesions in the Syrian golden hamster initiated with a single dose of 2,2′-dioxo-*N*-nitrosodipropylamine: Influence of subsequent butylated hydroxyanisole, α-tocopherol, or carbazole. *J. Natl. Cancer Inst.* 78:289–293.

Moyano, D., M. A. Vilaseca, M. Pineda, J. Campistol, and A. Vernet. 1997. Tocopherol in inborn errors of intermediary metabolism. *Clin. Chim. Acta* 263:147–155.

Nachbar, F., and H. C. Korting. 1995. The role of vitamin E in normal and damaged skin. *J. Mol. Med.* 73:7–17.

Nakamura, A., T. Shirai, S. Takahashi, et al. 1991. Lack of modification by naturally occuring antioxidants of 3,2′-dimethyl-4-aminophenyl-initiated rat prostate carcinogenesis. *Cancer Lett.* 58:241–246.

Narbonne, J. F., P. Cassand, M. Daubeze, C. Colin, and F. Leveque. 1990. Chemical mutagenicity and cellular status in vitamins A, E, and C. *Food Addit. Contam.* 7(Suppl. 1):S48–S52.

Distrbuted for comment only -- do not cite or quote

National Academy of Sciences (NAS). 1996. *Food chemicals codex*, 4th ed., 417–418, 421–424. Washington, DC: National Academy Press.

Nitta, Y., K. Kamiya, M. Tanimoto, et al. 1991. Induction of transplantable tumors by repeated subcutaneous injections of natural and synthetic vitamin E in mice and rats. *Jpn. J. Cancer Res.* 82:511–517.

North American Contact Dermatitis Group (NACDG). 1999. NACDG patch test data on 5% *d,l*-α-Tocopherol in petrolatum. Unpublished data submitted by Donald V. Belsito, MD, President of NACDG. (1 page.)[2]

Novicki, D. L., M. R. Rosenberg, and G. Michalopoulos. 1985. Inhibition of DNA synthesis by chemical carcinogens in cultures of initiated and normal proliferating rat hepatocytes. *Cancer Res.* 45:337–344.

Odeleye, O. E., C. D. Eskelson, S. I. Mufti, and R. R. Watson. 1992. Vitamin E inhibition of lipid peroxidation and ethanol-mediated promotion of esophageal tumorigenesis. *Nutr. Cancer* 17:223–224.

Odukoya, O., F. Hawach, and G. Shklar. 1984. Retardation of experimental oral cancer by topical vitamin E. *Nutr. Cancer* 6:98–104.

Ong, T., W.-Z. Whong, J. D. Stewart, and H. E. Brockman. 1989. Comparative antimutagenicity of 5 compounds against 5 mutagenic complex mixtures in *Salmonella typhimurium* strain TA98. *Mutat. Res.* 222:19–25.

Papas, A. M. 1993. Oil-soluble antioxidants in foods. *Toxicol. Ind. Health* 9:123–149.

Perchellet, J.-P., N. L. Abney, R. M. Thomas, Y. L. Guislain, and E. M. Perchellet. 1987. Effects of combined treatments with selenium, glutathione, and vitamin E on glutathione peroxidase activity, ornithine decarboxylase induction, and complete and multistage carcinogenesis in mouse skin. *Cancer Res.* 47:477–485.

Perrenoud, D., C. Hauser, P. Piletta, et al. 1995. Papular and follicular contact dermatitis: Irritation and/or allergy? *Curr. Probl. Dermatol.* 23:9–17.

Perrenoud, D., H. P. Homberger, P. C. Auderset, et al. 1994. An epidemic outbreak of papular and follicular contact dermatitis to tocopheryl linoleate in cosmetics. *Dermatology* 189:225–233.

Pettit, J., and N. O'Flynn. 1990. Current status of vitamins. (Part 2.) *IPU Rev.* 15:18–19.

Phelps, D. L. 1981. Local and systemic reactions to the parenteral administration of vitamin E. *Dev. Pharmacol. Ther.* 2:156–171.

Prasad, K. N., and J. Edwards-Prasad. 1982. Effects of tocopherol (vitamin E) acid succinate on morphological alterations and growth inhibition in melanoma cells in culture. *Cancer Res.* 42:550–555.

Prival, M. J., V. F. Simmon, and K. E. Mortelmans. 1991. Bacterial mutagenicity testing of 49 food ingredients gives very few positive results. *Mutat. Res.* 260:321–329.

Raina, V., and H. L. Gurtoo. 1985. Effects of vitamins A, C, and E on aflatoxin B_1-induced mutagenesis in *Salmonella typhimurium* TA-98 and TA-100. *Teratog. Carcinog. Mutagen* 5:29–40.

Rama, B. N., and K. N. Prasad. 1983. Study on the specificity of α-tocopheryl (vitamin E) acid succinate effects on melanoma, glioma and neuroblastoma cells in culture. *Proc. Soc. Exp. Biol. Med.* 174:302–307.

Record, I. R., I. E. Dreosti, M. Konstantinopoulos, and R. A. Buckley. 1991. The influence of topical and systemic vitamin E on ultraviolet light-induced skin damage in hairless mice. *Nutr. Cancer* 16:219–225.

Reece, E. A., and Y. K. Wu. 1997. Prevention of diabetic embryopathy in offspring of diabetic rats with use of a cocktail of deficient substrates and an antioxidant. *Am. J. Obstet. Gynecol.* 176:790–798.

Rempe, J. M., and L. G. Santucci. 1997. *CTFA List of Japanese Cosmetic Ingredients*, 3rd ed., 68, 125. Washington, DC: CTFA.

Rivera, A., Jr., K. M. Abdo, J. R. Bucher, et al. 1990. Toxicity studies of intravenous vitamin E in newborn rabbits. *Dev. Pharmacol. Ther.* 14:231–237.

Roche. 1994. Material safety data sheet on Vitamin E Acetate (cosmetic grade). Approved 10/14. Unpublished data submitted by CTFA. (6 pages.)[2]

Roche. 1999a. Technical data sheet. Rabbit dermal irritation testing of Roche Vitamin E Acetate N. F. (*dl*-alpha-Tocopheryl Acetate). Unpublished data submitted by CTFA. (2 pages.)[2]

Roche. 1999b. Technical data sheet. Draize eye irritation testing of Vitamin E (*dl*-alpha-Tocopherol). Unpublished data submitted by CTFA. (3 pages.)[2]

Roche. 1999c. Vitamin E Acetate: Irritation and sensitization study. Unpublished data submitted by CTFA. (8 pages.)[2]

Roed-Petersen, J., and N. Hjorth. 1975. Patch test sensitization from *d,l*-alpha-tocopherol (vitamin E). *Contact Dermatitis* 1:391.

Roed-Petersen, J., and N. Hjorth. 1976. Contact dermatitis from antioxidants. Hidden sensitizers in topical medications and foods. *Br. J. Dermatol.* 94:233–241.

Roshchupkin, D. I., M. Y. Pistsov, and A. Y. Potapenko. 1979. Inhibition of ultraviolet light-induced erythema by antioxidants. *Arch. Dermatol. Res.* 266:91–94.

Russo, P., M. Pala, S. Parodi, et al. 1984. Effect of vitamin E on liver DNA. *Cancer Lett.* 25:163–170.

Sakai, S., and S. Moriguchi. 1997. Long-term feeding of high vitamin E diet improves the decreased mitogen response of rat splenic lymphocytes with aging. *J. Nutr. Sci. Vitaminol.* 43:113–122.

Saperstein, H., M. Rapaport, and R. L. Rietschel. 1984. Topical vitamin E as a cause of erythema multiforme-like eruption. *Arch. Dermatol.* 120:906–908.

Sarria, A., and K. N. Prasad. 1984. *dl*-α-Tocopheryl succinate enhances the effect of γ-irradiation on neuroblastoma cells in culture. *Proc. Soc. Exp. Biol. Med.* 175:88–92.

Sato, Y. 1973. Developmental pharmacological study of vitamin E. Report 1: Study of the effect of vitamin E on a rat fetus. *Folia Pharmacol. Jpn.* 69:293–298.

Savolainen, K. E., K. M. Pynnönen, S. P. Lapinjoki, and M. T. Vidgren. 1988. Determination of fat-soluble vitamins in a pharmaceutical dosage form by solid-phase extraction and reversed-phase liquid chromatography. *J. Pharm. Sci.* 77:802–803.

Schmidt, K., and D. A. Nikoleit. 1993. Natural vitamin E, synthetic vitamin E: How do they differ? *STP Pharma Pratiques* 3:259–273.

Schoonderwoerd, S. A., G. M. J. Beijersbergen van Henegouwen, and K. C. M. Persons. 1991. Effect of alpha-tocopherol and di-butylhydroxytoluene (BHT) on UV-A-induced photobinding of 8-methoxypsoralen to Wistar rat epidermal biomacromolecules *in vivo*. *Arch. Toxicol.* 65:490–494.

Schwartz, J., O. Odukoya, E. Stoufi, and G. Shklar. 1985. Alpha tocopherol alters the distribution of Langerhans cells in DMBA-treated hamster cheek pouch epithelium. *J. Dent. Res.* 64:117–121.

Schwartz, J., and G. Shklar. 1992. The selective cytotoxic effect of carotenoids and α-tocopherol on human cancer cell lines *in vitro*. *J. Oral Maxillofac. Surg.* 50:367–374.

Schwartz, J. L., J. Tanaka, V. Khandekar, T. S. Herman, and B. A. Teicher. 1992. β-Carotene and/or vitamin E as modulators of alkylating agents in SCC-25 human squamous carcinoma cells. *Cancer Chemother. Pharmacol.* 29:207–213.

Shamberger, R. J. 1974. Antioxidants and cancer. III. Selenium and other antioxidants decrease carcinogen-induced chromosome breakage. *Trace Elem. Metab. Anim. (Proc. Int. Symp. 2nd, 1973)*:593–597.

Shamberger, R. J., C. L. Corlett, K. D. Beaman, and B. L. Kasten. 1979. Antioxidants reduce the mutagenic effect of malonaldehyde and β-propiolactone. Part IX, Antioxidants and cancer. *Mutat. Res.* 66:349–355.

Sheppard, E. P., and M. J. Stutsman. 1977. Determination of Vitamin E in cosmetic products by gas liquid chromatography. *J. Soc. Cosmet. Chem.* 28:115–123.

Shklar, G. 1982. Oral mucosal carcinogenesis in hamsters: Inhibition by vitamin E. *J. Natl. Cancer Inst.* 68:791–797.

Shklar, G., and J. Schwartz. 1988. Tumor necrosis factor in experimental cancer regression with alpha-tocopherol, beta-carotene, canthaxanthin and algae extract. *Eur. J. Cancer Clin. Oncol.* 24:839–850.

Shklar, G., and J. L. Schwartz. 1996. Vitamin E inhibits experimental carcinogenesis and tumour angiogenesis. *Eur. J. Cancer B Oral Oncol.* 32b:114–119.

Shklar, G., J. Schwartz, D. P. Trickler, and K. Niukian. 1987. Regression by vitamin E of experimental oral cancer. *J. Natl. Cancer Inst.* 78:987–992.

Sivan, E., E. A. Reece, Y.-K. Wu, et al. 1996. Dietary vitamin E prophylaxis and diabetic embryopathy: Morphologic and biochemical analysis. *Am. J. Obstet. Gynecol.* 175 (4 Pt 1):793–799.

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Slaga, T. J., and W. M. Bracken. 1977. The effects of antioxidants on skin tumor initiation and aryl hydrocarbon hydroxylase. *Cancer Res.* 37:1631–1635.

Spectrum Chemical Manufacturing Corporation. 1998a. Material safety data sheet for Vitamin E Acetate (Tocopherol Acetate). Unpublished data submitted by CTFA. (6 pages.)[2]

Spectrum Chemical Manufacturing Corporation. 1998b. Material safety data sheet for Vitamin E Succinate (Tocopherol Succinate). Unpublished data submitted by CTFA. (6 pages.)[2]

SRI International, Inc. 1979. *Microbial mutagenesis testing of substances. Compound report: F76-057,* d-*alpha-Tocopherol.* NTIS Report No. PB89-169064.

Stuyvesant, V. W., and W. B. Jolley. 1967. Anti-inflammatory activity of *d*-α-tocopherol (vitamin E) and linoleic acid. *Nature* 216:585–586.

Sugiyama, M., X. Lin, and M. Costa. 1991. Protective effect of vitamin E against chromosomal aberrations and mutation induced by sodium chromate in Chinese hamster V79 cells. *Mutat. Res.* 260:19–23.

Sugiyama, M., K. Tsuzuki, K. Matsumoto, and R. Ogura. 1992. Effect of vitamin E on cytotoxicity, DNA single strand breaks, chromosomal aberrations, and mutation in Chinese hamster V-79 cells exposed to ultraviolet-B light. *Photochem. Photobiol.* 56:31–34.

Sweetman, S. F., J. J. Strain, and V. J. McKelvey-Martin. 1997. Effect of antioxidant vitamin supplementation on DNA damage and repair in human lymphoblastoid cells. *Nutr. Cancer* 27:122–130.

Szulc, J., B. Woyczikowski, S. Janicki, et al. 1995. Effect of liposomes on skin absorption of vitamin E (translated from Polish) *Farm. Pol.* 51:604–607.

Takahashi, M., F. Furukawa, K. Toyoda, et al. 1986. Effects of four antioxidants on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine initiated gastric tumor development in rats. *Cancer Lett.* 30:161–168.

Takahashi, O. 1995. Haemorrhagic toxicity of a large dose of α-, β-, γ- and δ-tocopherols, ubiquinone, β-carotene, retinol acetate and L-ascorbic acid in the rat. *Food Chem. Toxicol.* 33:121–128.

Takahashi, O., H. Ichikawa H, and M. Sasaki. 1990. Hemorrhagic toxicity of *d*-α-tocopherol in the rat. *Toxicology* 63:157–165.

Tamano, S., S. Fukushima, T. Shirai, M. Hirose, and N. Ito. 1987. Modification by α-tocopherol, propyl gallate and tertiary butylhydroquinone of urinary bladder carcinogenesis in Fischer 344 rats pretreated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. *Cancer Lett.* 35:39–46.

Tanaka, J., H. Fujiwara, and M. Torisu. 1979. Vitamin E and immune response. I. Enhancement of helper T cell activity by dietary supplementation of vitamin E in mice. *Immunology* 38:727–734.

Tavan, E., S. Maziere, J. F. Narbonne, and P. Cassand. 1997. Effects of vitamins A and E on methylazoxymethanol-induced mutagenesis in *Salmonella typhimurium* strain TA100. *Mutat. Res.* 377:231–237.

Telford, I. R., C. S. Woodruff, and R. H. Linford. 1962. Fetal resorption in the rat as influenced by certain antioxidants. *Am. J. Anat.* 110:29–36.

Tojo, K., and A. C. Lee. 1989. Skin penetration of vitamins C and E. *J. Soc. Cosmet. Chem.* 40:119–125.

Tomassi, G., and V. Silano. 1986. An assessment of the safety of tocopherols as food additives. *Food Chem. Toxicol.* 24:1051–1061.

Toth, B., and K. Patil. 1983. Enhancing effect of vitamin E on murine intestinal tumorigenesis by 1,2-dimetylhydrazine dihydrochloride. *J. Natl. Cancer Inst.* 70:1107–1111.

Trevithick, J. R., and K. P. Mitton. 1993. Topical application and uptake of vitamin E acetate by the skin and conversion to free vitamin E. *Biochem. Mol. Biol. Int.* 31:869–878.

Trevithick, J. R., D. T. Shum, S. Redae, et al. 1993. Reduction of sunburn damage to skin by topical application of vitamin E acetate following exposure to ultraviolet B radiation: Effect of delaying application or of reducing concentration of vitamin E acetate applied. *Scanning Micros.* 7:1269–1281.

Trevithick, J. R., H. Xiong, S. Lee, et al. 1992. Topical tocopherol acetate reduces post-UVB, sunburn-associated erythema, edema and skin sensitivity in hairless mice. *Arch. Biochem. Biophys.* 296:575–582.

Trickler, D., and G. Shklar. 1987. Prevention by vitamin E of experimental oral carcinogenesis. *J. Natl. Cancer Inst.* 78:165–169.

Tsallas, G., T. Molgat, and K. Jeejeebhoy. 1986. Vitamins. Part 1. *On Contin. Pract.* 13:28–41.

Tsuda, H., N. Uehara, Y. Iwahori, et al. 1994. Chemopreventive effects of β-carotene, α-tocopherol and five naturally occurring antioxidants on initiation of hepatocarcinogenesis by 2-amino-3-methylimidazo[4,5-*f*]quinoline in the rat. *Jpn. J. Cancer Res.* 85:1214–1219.

Umegaki, K., H. Uramoto, and T. Esashi. 1997. Lack of influence of a long-term high or low vitamin E diet on the oxidative DNA damage in the bone marrow of mice. *Int. J. Vitam. Nutr. Res.* 67:149–154.

Vanderveen, E., and J. E. Vanderveen. 1990. Vitamins and other nutrients. In *Remington's pharmaceutical sciences*, 18th ed., ed. A. R. Gennaro, 1002–1034. Easton, PA: Mack Publishing.

van Staden, A. M., C. E. van Rensburg, and R. Anderson. 1993. Vitamin E protects mononuclear leucocyte DNA against damage mediated by phagocyte-derived oxidants. *Mutat. Res.* 288:257–262.

Viana, M., E. Herrera, and B. Bonet. 1996. Teratogenic effects of diabetes mellitus in the rat. Prevention by vitamin E. *Diabetologia* 39:1041–1046.

von der Hude, W. J., J. Potenberg, R. Kahl, C. Behm, and A. Basler. 1987. The influence of antioxidants on the genotoxicity of chemical mutagens detected with the SOS chromotest. *Mutat. Res.* 182:293.

Wattenberg, L. W. 1972. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. *J. Natl. Cancer Inst.* 48:1425–1430.

Weber, P., A. Bendich, and L. J. Machlin. 1997. Vitamin E and human health: Rationale for determining recommended intake levels. *Nutrition* 13:450–460.

Weerapradist, W., and G. Shklar. 1982. Vitamin E inhibition of hamster buccal pouch carcinogenesis. A gross, histologic, and ultrastructural study. *Oral Surg. Oral Med. Oral Pathol.* 54:304–312.

Weitberg, A. B. 1987. Effect of combinations of antioxidants on oxygen radical-induced sister chromatid exchanges. *Clin. Genet.* 32:114–117.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1997. *International cosmetic ingredient dictionary and handbook*, 7th ed., Vols. 1–2, 454, 1101, 1411–1413. Washington, DC: CTFA.

Wheldon, G. H., A. Bhatt, P. Keller, and H. Hummler. 1983. *d,l*-α-Tocopheryl acetate (vitamin E): A long term toxicity and carcinogenicity study in rats. *Int. J. Vitam. Nutr. Res.* 53:287–296.

Witschi, H. P., P. J. Hakkinen, and J. P. Kehrer. 1981. Modification of lung tumor development in A/J mice. *Toxicology* 21:37–45.

Wood, A. W. 1998. Evaluation of the mutagenic potential of *d,l*-α-Tocopherol, *d,l*-α-Tocopherol quinone (tocoquinone) and *d,l*-α-Tocopherol ortho-Quinone (Toco Red) in *Salmonella typhimurium* strains TA98 and TA100. Submitted by FDA in response to an FOI request—1998. (15 pages.)[2]

Yano, T., Y. Obata, Y. Yano, S. Otani, and T. Ichikawa. 1994. Vitamin E acts as a useful chemopreventive agent to reduce spontaneous lung tumorigenesis in mice. *Cancer Lett.* 87:205–210.

Zeiger, E., B. Anderson, S. Haworth, R. Lawlor, and K. Mortelmans. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen* 11(Suppl. 12):1–158.

Zhang, D., S. Okada, Y. Yu, et al. 1997. Vitamin E inhibits apoptosis, DNA modification, and cancer incidence induced by iron-mediated peroxidation in Wistar rat kidney. *Cancer Res.* 57:2410–2414.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: December 11, 2013

SUBJECT: d,l-alpha-Tocopherol

It has been brought to our attention, that recently d,l-alpha-Tocopherol has tested positive in an animal sensitization assay. It is likely that a study summary will be available on the ECHA website sometime in late 2014.

During the discussion of the tocopherol ingredients at the December 2013 meeting, Dr. Belsito and Dr. Marks noted that clinically, they do not see a sensitization issue with tocopherol ingredients.

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Personal Care Products Council
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Memorandum

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

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

DATE: December 5, 2013

SUBJECT: Comments on the Draft Report on Tocopherols Prepared for the December 2013 CIR Expert Panel Meeting

Key Issues

The anti-carcinogenicity section is currently unbalanced. Either the section should be deleted, or epidemiology studies that show a lack of cancer prevention activity among persons taking vitamin E supplements should be added to the report..

As the European Chemicals Agency (ECHA) did not write the study summaries included in the dossier of this ingredient, they should not be listed as the "author" in the reference section. ECHA is the source, rather than the author of this material.

This report has no page numbers. Therefore, comments are provided by section heading.

Additional Comments

Table of Contents - The table of contents is not helpful if the pages of the report are not numbered.

Introduction - As there are ascorbyl containing compounds included in this report, it would also be helpful to mention that CIR has reviewed the safety of Ascorbic Acid and found it safe for use in cosmetic products (report published in 2005).

Absorption, Distribution, Metabolism, Excretion (ADME), from the original report on Tocopherols - What was the route of exposure used in the rat study of Tocopheryl Acetate and Tocopheryl Nicotinate?

ADME - Were there any Tocotrienols in the tocopherol mixture fed to CF-1 mice (reference 35)?

ADME, Tocopherol Depletion by UV in the Skin - It is not clear what is meant by "a 5% cream" (reference 38). Is this a cream containing 5% Tocopheryl Acetate? In what species was the study described in reference 39 completed?

Miscellaneous Effects - Please correct: "A-Tocopherol"

Reproductive and Developmental Toxicity, From the original report on Tocopherols - Please provide some indication of the doses used in the studies summarized in this section.

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Genotoxicity, In Vitro, Tocopheryl Succinate - Units of µg/ml represent concentration and should not be called "dose" (references 48, 49).

Carcinogenicity, From the original report on Tocopherols - Please give some indication of the doses used in the carcinogenicity studies.

Anti-Carcinogenicity - Please correct "was studies"

Dermal Irritation and Sensitization, Non-Human, From the original report on Tocopherols - What concentration of Tocopherol was a primary skin irritant in rabbits? What concentration was a weak cumulative irritant in guinea pigs?

Contact Allergy - Case Studies - What was the "active ingredient" in the lipogel studied in reference 58?

Phototoxicity and Photoallergenicity, From the original report on Tocopherols - On what type of information was the following statement based? "Tocopheryl acetate was not phototoxic."

Summary - It would be helpful to note that this is a summary of new information (when the Summary appears in the Compendium without the rest of the report, it will be useful to know that it is not summarizing all the available information on the tocopherol ingredients). Following which route of exposure does natural vitamin E have about twice the systemic availability of synthetic tocopherol?

Table 4 - Use tables should be prepared using the same protocol under which the information was collected. For the reader that is not familiar with FDA product categories "confirmed" will be confusing. How was the product form "confirmed"? Was FDA contacted to determine that there were no errors in the information reported to the VCRP? With the exception of a few product categories, FDA does not collect use information by product form. Saying "confirmed" suggests that it is really known that the products not included in the spray row are not sprays, and the products not included in the powder row are not powders, when in fact the new as well as the old protocols are still a guesses at which products reported to the FDA might be sprays or powders.

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Personal Care Products Council
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Memorandum

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

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

DATE: January 17, 2014

SUBJECT: Comments on the Tentative Report on Tocopherols and Tocotrienols as Used in Cosmetics

Key Issues

Epidemiology studies of vitamin E supplementation - It is not appropriate to introduce vitamin E supplementation epidemiology studies in the Discussion, and say "After reviewing the mixed results reported in vitamin E supplement studies...." when no references are discussed earlier in the report. Please add at least one study, such as the following examples, to the Carcinogenicity section.

Yang CS, Suh N, Kong AN. 2012. Does vitamin E prevent or promote cancer? Cancer Prev Res 5(5):701-5.

Abstract

The cancer preventive activity of vitamin E has been suggested by many epidemiologic studies. However, several recent large-scale human trials with alpha-tocopherol, the most commonly recognized and used form of vitamin E, failed to show a cancer preventive effect. The recently finished follow-up of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) even showed higher prostate cancer incidence in subjects who took alpha-tocopherol supplementation. The scientific community and the general public are faced with a question: "Does vitamin E prevent or promote cancer?" Our recent results in animal models have shown the cancer preventive activity of gamma- and delta-tocopherols as well as a naturally occurring mixture of tocopherols, and the lack of cancer preventive activity by alpha-tocopherol. On the basis of these results as well as information from the literature, we suggest that vitamin E, as ingested in the diet or in supplements that are rich in gamma- and delta-tocopherols, is cancer preventive; whereas supplementation with high doses of alpha-tocopherol is not.

Nicastro HL, Dunn BK. 2013. Selenium and prostate cancer prevention: insights from the selenium and vitamin E cancer prevention trial (SELECT). Nutrients 5(4):1122-48.

Abstract

The Selenium and Vitamin E Cancer Prevention Trial (SELECT) was conducted to assess the efficacy of selenium and vitamin E alone, and in combination, on the incidence of prostate cancer. This randomized, double-blind, placebo-controlled, 2 × 2 factorial design clinical trial found that neither selenium nor vitamin E reduced the incidence of prostate cancer after seven years and that vitamin E was associated with a 17% increased risk of prostate cancer compared to placebo. The null result was surprising given the strong preclinical and clinical evidence suggesting chemopreventive activity of selenium. Potential explanations for the null findings include the agent formulation and dose, the characteristics of the cohort, and the study design. It is likely that only specific subpopulations may benefit from selenium supplementation; therefore, future studies should consider the baseline selenium status of the participants, age of the cohort, and genotype of specific selenoproteins, among other characteristics, in order to determine the activity of selenium in cancer prevention.

Additional Comments

p.5 - Please correct "9% or γ-tocopherol" to "9% for γ-tocopherol"

p.6 - From the description of the study cited to reference 37, it is not clear if AIN-93M diet was the basal diet, or if two different diets were used.

p.6 - In the study cited to reference 38, were tissues really collected from just 1 neonate? It does not make sense to state that "α-tocopherol content increased by 0.1-fold". A change of "0.1-fold" represents a decrease in α-tocopherol content.

p.6-7 - In the study described in reference 40, α-tocopherol was applied in an inert cream or in DMSO. Did the investigators see any effect based on vehicle?

p.8 - Are the units (cm) correct for the size of the skin punch (reference 47)? Or should it be cm^2?

p.9 - What doses of Tocopherol and Tocopheryl Acetate were used in the 60-day and 8-week feeding studies described in the original CIR report? What species were used in these studies?

p.10 - Please give some indication of the concentrations tested in the ocular irritation studies described in the original report.

p.11 - Please correct: "suspended n 1% carboxymethyl cellulose"

p.12 - Please correct: "Test group sere exposed"

p.13 - What concentration of Dioleyl Tocopheryl Methylsilanol was used in the dermal irritation study included in the original report? What concentration of Tocophersolan was not a sensitizer in a Buehler test?

p.13 - Please provide some indication of the concentrations of tocopherol ingredients tested in the clinical studies that were described in the original report. What did the case reports concerning Tocopherol and Tocopheryl Acetate-containing products show?

p.14 - What concentrations of Tocopherol were used in the studies of photoprotective effects described in the original report?

p.15 - In the Summary, please include the concentration of Tocopheryl Acetate and that was irritating to the eyes, and the concentration of Tocopheryl Phosphate that was not irritating.

p.16 - Please correct: "5 mg d-α-tocopherol for 15 weeks tocopherol"

p.16 - What concentrations of Tocopheryl Acetate and Tocopheryl Phosphate were not irritating to rabbit skin?

p.16 - The Discussion should state the current maximum use concentration of Tocopherol.

p.17 - As there are two ingredients with no uses reported, the footnote needs to be revised from "If this ingredient was..." to "If these ingredients were..."

p.19-24, Table 1 - The following CAS numbers that are included in the Dictionary are missing from Table 1: Tocopheryl Linoleate: 36148-84-2; Tocopheryl Nicotinate: 16676-75-8; 43119-47-7; 51898-34-1; 86362-36-9; Tocopheryl Succinate: 17407-37-3, 4345-03-3; Tocopheryl Phosphate: 425429-22-7

p.25, Table 3 - The initials of the person that named the plant should either be with each genus species name, or initials should not be included with any name. Currently, 7/12 names in this table include initials.

p.27, Table 4 - What is meant by "confirmed"? Based on a survey, the Council reported that some use in spray products. These uses were not "confirmed".

p.29, Table 7 - Please correct "bu wk". The *in vitro* study (reference 41) should be in a separate subsection.