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## Safety Assessment of Polysorbates as Used in Cosmetics

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Status: Re-Review for Panel Review  
Release Date: February 20, 2015  
Panel Meeting Date: March 16-17, 2015

The 2015 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

## MEMORANDUM

To: CIR Expert Panel and Liaisons

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

Date: February 20, 2015

Subject: Re-review of Polysorbates as Used in Cosmetics

Attached is the re-review of polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 as used in cosmetics. [PSorba\_032015\_Rep] In 1984, these ingredients were found to be safe as used. [PSorba\_032015\_Data1] The CIR staff proposes that polysorbates from two other reports also be included in this re-review. These also had a safe as used conclusion [PSorba\_032015\_Data2; PSorba\_032015\_Data3] Additionally, other polysorbate ingredients have been identified and it is proposed that these also be included in this report for a total of 79 ingredients.

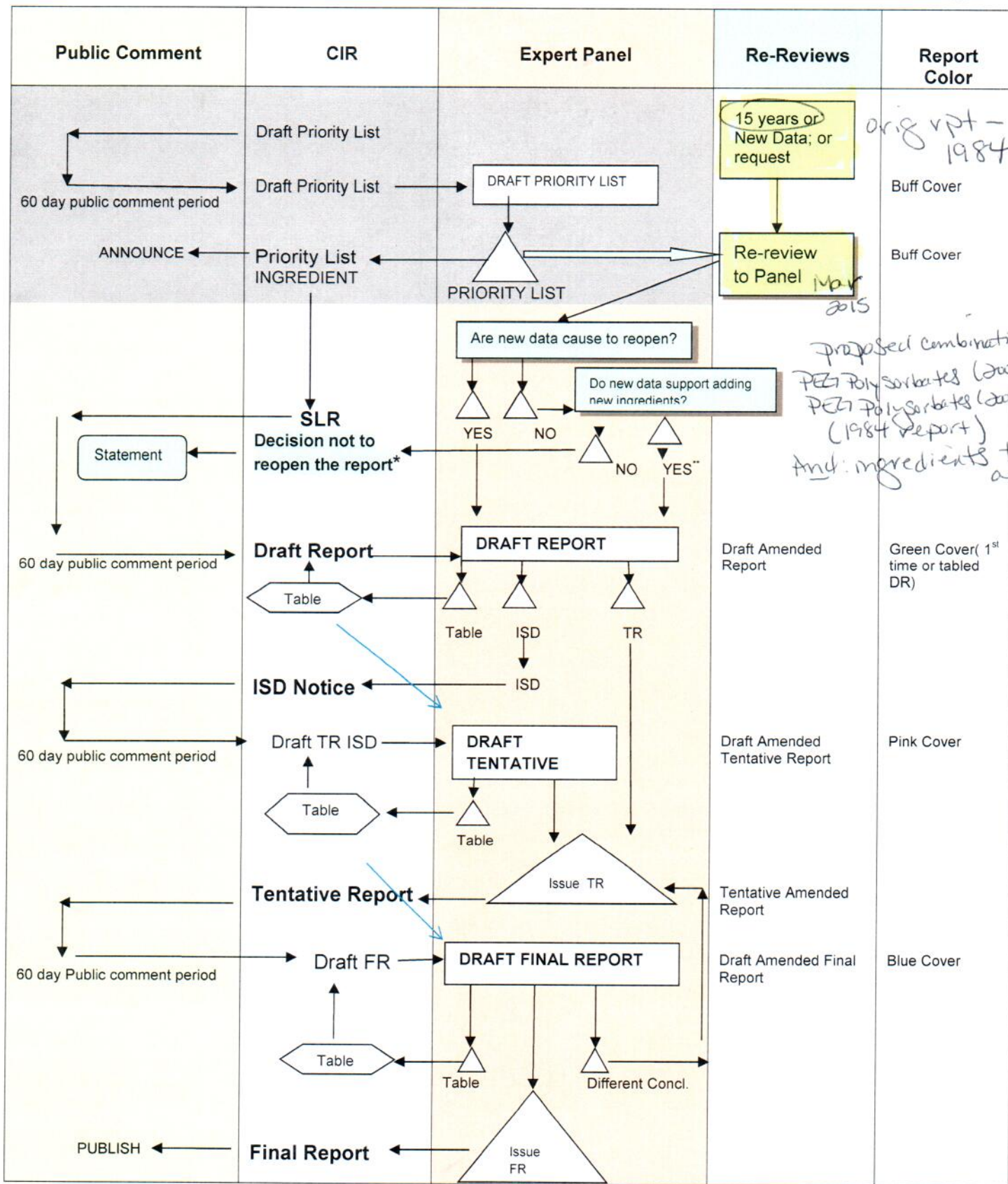
At the time of the safety assessment of sorbeth beeswaxes, the Panel was concerned about the use of PEGs on damaged skin and included a caveat that use on damaged skin should be avoided. In the 2010 re-review of PEGs, the Panel concluded that they were no longer concerned about the use of PEGs on damaged skin and removed the caveat. They also included language in the Discussion that this caveat should be removed from other safety assessments that contained this restriction based on the conclusion of safety of PEGs used on damaged skin.

Concentration of use information was submitted and is included in the report. [PSorba\_032015\_Data4; PSorba\_Data5; PSorba\_032015\_Data7] No other data have been submitted.

The Panel is to determine if the new data provided in this re-review raises safety concerns that would lead to reopening the polysorbate safety assessment to change the conclusion. If so, the Panel should reopen the safety assessment and issue the new conclusion. In addition to deciding if the new data are a reason for reopening the safety assessment, the Panel should decide whether to add the polysorbates from the two additional previous reports and/or any of the un-reviewed polysorbate ingredients. If the Panel re-opens the report, they are to develop the basis for the Discussion.

# SAFETY ASSESSMENT FLOW CHART

Mar 2015



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

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

## **History – Polysorbates**

**1984** – Report on polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 published with a safe as used conclusion.

**2000** – A safety assessment was published of a re-review of the above polysorbates as well as additional polysorbates with a safe as used conclusion.

**2001** – A safety assessment was published of Sorbeth-6 beeswax, Sorbeth-8 beeswax, and Sorbeth-20 beeswax with a safe as used conclusion.

**December, 2014** – The re-review of these ingredients are reviewed by the Panel.



[illegible]

Polysorbate Data Profile for March, 2015. Writer - Lillian Becker																		
	ADME			Acute toxicity			Repeated dose toxicity			Irritation			Sensitization					
	Dermal Penetration	Log K <sub>ow</sub>	Use	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
PEG-4 sorbitan triisostearate																		
PEG-20 sorbitan triisostearate																		
PEG-160 sorbitan triisostearate																		
PEG-3 sorbitan tristearate																		
Sorbeth-3 tristearate																		
Sorbeth-160 tristearate																		
Sorbeth-450 tristearate																		
PEG-2 sorbitan trioleate																		
PEG-18 sorbitan trioleate																		
Polysorbate 85			ON	O			O			O		O		O			O	
Sorbeth-20 tetraisostearate																		
Sorbeth-30 tetraisostearate			N															
Sorbeth-40 tetraisostearate																		
Sorbeth-50 tetraisostearate																		
PEG-60 sorbitan tetrastearate			N															
Sorbeth-60 tetrastearate (PEG-60 sorbitol tetrastearate)																		
Sorbeth-4 tetraoleate			N															
Sorbeth-6 tetraoleate			N															
PEG-20 Sorbitan Tetraoleate																		
PEG-30 sorbitan tetraoleate			N															
Sorbeth-30 tetraoleate			N															
PEG-40 sorbitan tetraoleate			ON															
Sorbeth-40 tetraoleate			N															
PEG-60 sorbitan tetraoleate																		
Sorbeth-60 tetraoleate			N															
Sorbeth-20 pentaisostearate																		
Sorbeth-30 pentaisostearate																		
Sorbeth-40 pentaisostearate																		
Sorbeth-50 pentaisostearate																		
Sorbeth-40 pentaoleate																		
Sorbeth-30 tetraoleate laurate (PEG-30 sorbitol tetraoleate										O	O			O				

Polysorbate Data Profile for March, 2015. Writer - Lillian Becker																		
	ADME			Acute toxicity			Repeated dose toxicity			Irritation			Sensitization					
	Dermal Penetration	Log K <sub>ow</sub>	Use	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
laurate)																		
Sorbeth-2 hexalaurate																		
Sorbeth-2 hexaisostearate																		
PEG-40 sorbitan perisostearate																		
Sorbeth-6 hexastearate																		
Sorbeth-150 hexastearate																		
Sorbeth-2 hexaoleate																		
Sorbeth-40 hexaoleate (PEG-40 sorbitol hexaoleate)																		
Sorbeth-50 hexaoleate (PEG-50 sorbitol hexaoleate)										O	O			O				
PEG-40 sorbitan peroleate			ON							O				O		O		
Sorbeth-2 hexacaprylate/caprate																		
Sorbeth-12 hexacocoate																		
Sorbeth-2/oleate/dimer dilinoleate crosspolymer																		
Sorbitan monooleate, ethoxylated																	N	
Sorbitan monolaurate, ethoxylated						N					N		N				N	
Sorbitan monostearate, ethoxylated				N	N						N						N	
Sorbitan esters	O			O			O			O	O	O		O		O	O	O

X – New data

O – Data from previous reports

## Search Strategy - Polysorbates

### SciFinder

Search Terms: Polysorbate 20, 9005-64-5, Polysorbate 21, Polysorbate 40, 9005-66-7, Polysorbate 60, 9005-67-8, Polysorbate 61, Polysorbate 65, 9005-71-4, Polysorbate 80, 9005-65-6, Polysorbate 81, Polysorbate 85, 9005-70-3

#### HITS:

9005-64-5 – 664; Remove patents – 120; refine by index terms (toxicity, etc.) – 120. 4 selected.

- 9005-66-7 - 981; remove patents – 365; refine by index terms (toxicity, etc.) – 365.
- 4482: remove patents – 1245; refine by index terms (toxicity, etc.) – 1245; English – 922; Toxicity – 66
- 2592; Remove patents – 994;

9005-66-7, 9005-70-3 – 1044; remove patents 404; English 315; toxicity 17 hits, none useful.

9005-71-4 – 689; remove patents 136; English 98; 4 selected

9005-67-8 – 7818; 4779; remove patents 1428; English 1001; toxicity 70 hits; 4 selected

9005-65-6 – 33,579; 24,295; remove patents 9947; English 7337; "toxicity" 576; 1990 – 486; Index term "toxicity" – 64; 4 selected

Search Terms: PEG-2 Sorbitan Isostearate, PEG-5 Sorbitan Isostearate, PEG-20 Sorbitan Isostearate, PEG-40 Sorbitan Lanolate, PEG-75 Sorbitan Lanolate, PEG-10 Sorbitan Laurate, PEG-40 Sorbitan Laurate, PEG-44 Sorbitan Laurate, PEG-75 Sorbitan Laurate, PEG-80 Sorbitan Laurate

#### HITS:

-Substance Identifier "PEG-20 Sorbitan Cocoate; PEG-4... - 12412; remove patents – 4524; refine by index term toxicity - 225

-4524 – remove patents, English 3741; "toxicity" 276; Index term "toxicity" – 276; 8 selected.

Search Terms: PEG-3 Sorbitan Oleate, PEG-6 Sorbitan Oleate, PEG-20 Sorbitan Oleate, PEG-40 Sorbitan Oleate, PEG-80 Sorbitan Palmitate, PEG-40 Sorbitan Perisostearate, PEG-40 Sorbitan Peroleate, PEG-3 Sorbitan Stearate, PEG-6 Sorbitan Stearate, PEG-40 Sorbitan Stearate, PEG-60 Sorbitan Stearate

-4866 hits, remove patents – 1546 hits, English – 1126 hits, Toxicology – 49 hits

- 4866- remove patents – 1546 hits, refine "1980-" 1296 hits; refine "1990-" 990 hits

Search Terms: PEG-30 Sorbitan Tetraoleate, PEG-40 Sorbitan Tetraoleate, PEG-60 Sorbitan Tetraoleate, PEG-60 Sorbitan Tetrastearate, PEG-4 Sorbitan Triisostearate, PEG-20 Sorbitan Triisostearate, PEG-160 Sorbitan Triisostearate, PEG-2 Sorbitan Trioleate, PEG-18 Sorbitan Trioleate, PEG-3 Sorbitan Tristearate

49 hits all patents except CIR report.

Search Terms: Sorbeth-2 Beeswax, Sorbeth-6 Beeswax, Sorbeth-8 Beeswax, Sorbeth-20 Beeswax. Sorbeth-2 Cocoate, Sorbeth-2 Hexacaprylate/Caprates, Sorbeth-12 Hexacocoate, Sorbeth-2 Hexaisostearate, Sorbeth-2 Hexalaurate, Sorbeth-2 Hexaoleate, Sorbeth-40 Hexaoleate (PEG-40 Sorbitol Hexaoleate), Sorbeth-50 Hexaoleate (PEG-50 Sorbitol Hexaoleate), Sorbeth-6 Hexastearate, Sorbeth-150 Hexastearate

3 hits. Not useful

Search Terms: Sorbeth-3 Isostearate, Sorbeth-6 Laurate, Sorbeth-2/Oleate/Dimer Dilinoleate Crosspolymer, Sorbeth-20 Pentaisostearate, Sorbeth-30 Pentaisostearate, Sorbeth-40 Pentaisostearate, Sorbeth-50 Pentaisostearate, Sorbeth-40 Pentaoleate, Sorbeth-20 Tetraisostearate, Sorbeth-30 Tetraisostearate, Sorbeth-40 Tetraisostearate, Sorbeth-50 Tetraisostearate, Sorbeth-4 Tetraoleate, Sorbeth-6 Tetraoleate, Sorbeth-30 Tetraoleate, Sorbeth-40 Tetraoleate, Sorbeth-60 Tetraoleate, Sorbeth-30 Tetraoleate Laurate (PEG-30 Sorbitol Tetraoleate Laurate), Sorbeth-60 Tetrastearate (PEG-60 Sorbitol Tetrastearate), Sorbeth-3 Tristearate, Sorbeth-160 Tristearate, Sorbeth-450 Tristearate

16 hits, none useful.

90 total selections when duplicates removed.

**ECHA** – CAS Nos and INCI names. 3 hits.

## **Historical Minutes of Polysorbates**

### **Polysorbates**

Since these ingredients were incorporated into the PEG Sorbitan/Polysorbate report in 1997, the 1982 and 1983 minutes were not retrieved.

### **PEG Sorbitans and Polysorbates**

**April, 1997**

#### PEG-10, -40, -44, -75, and -80 Sorbitan Laurate and PEG-40 Sorbitan Peroleate

Dr. Belsito noted that his Team had recommended that the Panel consider removing PEG-40 Sorbitan Peroleate from the Draft Report for the following reasons: (1) At some point, the Panel will have to address the safety of the Sorbitan Oleates, and PEG-40 Sorbitan Peroleate should be included in this review. (2) The Panel does not have data on the toxicity of this ingredient; thus, any safety determination would have to be based on data on PEGs Sorbitan Laurate. (3) The structure of PEG-40 Sorbitan Peroleate is a trade secret.

Dr. Belsito's Team also concluded that the available data are insufficient for arriving at a conclusion on the safety of the PEGs Sorbitan Laurate, and determined that the following data are needed: (1) Current concentration of use, (2) Skin irritation and sensitization in animals or humans at concentrations of use, and (3) Photosensitization data. Regarding item (3), Dr. Belsito noted that these data are needed because of the low absorbance of UV light by PEG-20 Sorbitan Laurate.

Dr. Schroeter noted that the UV absorbance observed was extremely low, and that his Team was concerned that this observation may not have been true absorbance and was probably concentration-related. A rather high test concentration (0.1504 mg/ml of aqueous PEG-20 Sorbitan Laurate) was used in the UV spectral analysis. Thus, Dr. Schroeter's Team did not request photosensitization data.

Dr. Schroeter's Team also determined that PEG-40 Sorbitan Peroleate should not be deleted from the present review. Dr. Schroeter acknowledged the lack of data on this ingredient and noted that specific studies could be requested.

Dr. McEwen suggested that the PEGs Sorbitan Oleate be added to the present review.

Ms. Johnson recalled that a number of other PEG Sorbitan fatty acid esters are included in the International Cosmetic Ingredient Dictionary, namely, stearates, isostearates, lanolates, palmitates, and oleates.

Dr. McEwen recommended that all of these ingredients should be included in the present safety assessment.

Dr. Andersen suggested that the fatty acid moiety is not likely to be the area of concern in these molecules; therefore, creating this family of ingredients may be a very useful construct.

Ms. Johnson noted that nine of the ingredients that would be added to this review have already been reviewed in the CIR report on Polysorbates.

Dr. Bergfeld recommended that the present review be tabled until information on other ingredients is incorporated. With this done, the Panel could then reconsider its requests for data.



The Panel unanimously agreed to table the Draft Report on PEGs Sorbitan Laurate and PEG-40 Sorbitan Peroleate, pending the incorporation of information on other PEG Sorbitan fatty acid esters.

The PEGs Sorbitan Fatty Acid Esters that likely will be added are as follows: PEG-20 Sorbitan Cocoate, PEG-40 Sorbitan Diisostearate, PEGs-2, -5, and -20 Sorbitan Isostearate, PEGs-3, and -6 Sorbitan Oleate, PEG-80 Sorbitan Palmitate, PEG-40 Sorbitan Perisostearate, PEGs-3, -6, -40, and -60 Sorbitan Stearate, PEGs-30, -40, and -60 Sorbitan Tetraoleate, PEG-60 Sorbitan Tetrastearate, PEGs-20 and -160 Sorbitan Triisostearate, PEG-40 Sorbitan Lanolate, PEGs-40 and -50 Sorbitol Hexaoleate, and PEG-30 Sorbitol Tetraoleate Laurate.

Dr. Bailey noted that a significant number of *in vitro*, non-animal studies are included in the ocular irritation section of the Draft Report. He wanted to know how much weight will be placed on these studies.

Dr. Bergfeld said that approximately 15 years ago the Panel had an opportunity to review *in vitro* tests for ocular irritation, and, at that time, decided that these tests could not replace the standard animal tests. Since that time, *in vitro* ocular irritation test data have been incorporated into CIR reports; however, the Panel's evaluation of ocular irritation potential has been based on *in vivo* data.

Dr. McEwen noted that companies are now using a combination of methods (alternative methods) to determine the irritation potential of products, to insure that animal testing, when not necessary, is not done. Furthermore, in many instances, companies have validated a particular method for the testing of its ingredients and products against historical information. The methodology chosen may vary from company to company.

Dr. Bergfeld said that in order for *in vitro* ocular irritation test data to be considered by the Panel information on validation of the test method must be provided.

Dr. Bailey noted that, a few years ago, the EU moved to disallow the use of animal testing. Furthermore, this initiative was delayed recently, acknowledging that no validated alternative methods are currently available to replace the traditional methods.

Dr. Bergfeld suggested that someone make a presentation to the Panel on the validation of *in vitro* methods.

## **September, 1997**

PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3 and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitan Triisostearate; PEG-40 and -50 Sorbitol Hexaoleate; and PEG-30 Sorbitol Tetraoleate Laurate

Dr. Schroeter noted that even though the Draft Report now contains data on other Sorbitan/Sorbitol Fatty Acid Esters, the available data are insufficient for evaluating the safety of this group of ingredients.

The Panel voted unanimously in favor of issuing an Insufficient Data Announcement with the following data requests:

- (1) Current concentration of use

(2) Dermal absorption of PEG-2 Sorbitan Isostearate, PEG-3 Sorbitan Oleate, or PEG-3 Sorbitan Stearate; if significantly absorbed, then a 28-day dermal toxicity study and reproductive and developmental toxicity study may be needed

(3) Skin irritation and sensitization data, at the concentration of use, in humans

(4) Because of the UV absorption data included in this report, photosensitization data are needed

### **March, 1998**

PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3 and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitan Triisostearate; PEG-40 and -50 Sorbitol Hexaoleate; and PEG-30 Sorbitol Tetraoleate Laurate, and PEG-60 Sorbitol Tetrastearate

Dr. Belsito noted that after reviewing the data on the above ingredients, and data on the polysorbates, sorbitan fatty acid esters, fatty acids, and PEGs from CIR Final Reports, his Team concluded that the PEG Sorbitan and Sorbitol fatty acid group of compounds is safe as used in cosmetics. Dr. Belsito also said that though there are no current data on ingredient use concentrations, the 1984 FDA data indicated that some of the ingredients were used at concentrations greater than 50%. Thus, it was concluded that it is not likely that current use concentrations would be substantially increased over those reported in 1984.

Dr. Belsito also stated that the only concern expressed by his Team relates to reports that some of the ingredients being reviewed increase the skin penetration of lidocaine. He said that manufacturers should exercise caution when blending these ingredients with chemicals that the Panel has previously found to be safe as used based on poor skin penetration.

Dr. Bergfeld said that Dr. Belsito's concerns should be addressed in the report discussion that will be developed.

Dr. Schroeter noted that there are no skin irritation or sensitization data on the ingredients being reviewed. However, he said that these data are available on the sorbitan fatty acid esters that have been reviewed by CIR and could be used in the evaluation of skin irritation and sensitization potential. Additionally, Dr. Schroeter recalled that data on impurities and dermal absorption and reproductive and developmental toxicity data requested from industry have not been received and are still needed.

Dr. Shank noted that the data needs referred to by Dr. Schroeter are required for the low molecular compounds, PEG-2 and PEG-3 compounds.

Dr. Belsito said that in terms of sensitization, the Panel has data on the polysorbates. He also said that the Panel also has photosensitization data, and, during this type of study, any evidence of sensitization would be noted as well. With this in mind, he said that there really is no need at this point to request skin irritation or sensitization data.

Dr. Shank noted that these data are available on the high molecular weight compounds (which are probably not absorbed), but not on the low molecular weight compounds.

Dr. Carlton wanted to know if it is likely that the ingredients being reviewed are sensitizers.

Dr. Schroeter agreed that it is not likely that these ingredients are sensitizers, but also noted that the Panel does not have data to substantiate this.

Dr. Belsito said that the PEGs and sorbitan laurate, palmitate, and tristearate are known not to be sensitizing, and wanted to know why there would be any reason to suspect that an even larger molecule would be sensitizing, even if it were broken down into its constituents.

Dr. Shank said that the larger molecule is a different molecule. He noted that amino acids are not allergenic, but proteins are. Dr. Shank also said that for sensitization, the larger molecule, if it can be absorbed, may have different sensitizing properties when compared to the individual components.

Dr. Belsito said that in delayed-type hypersensitivity, usually, the allergen is ingested by the antigen presenting cell and broken down into smaller allergenic groups. Thus, if anything, one would expect the larger compounds to be broken down into their constituents, which have already been determined not to be significant sensitizers. Dr. Belsito also recalled that the data on PEG-20 Sorbitan Laurate reviewed by the Panel indicate neither photosensitization nor sensitization. He then restated his position that skin irritation and sensitization data are not needed.

Dr. Schroeter said that the issue of dermal absorption and dermal toxicity in relation to reproductive and developmental toxicity should definitely be addressed, and is a more important issue than sensitization potential in this safety assessment.

Dr. Shank noted that the Panel does not have dermal toxicity and reproductive and developmental toxicity data on the low molecular weight PEG sorbitan fatty acids.

Dr. Bailey said that there is a considerable amount of ocular irritation data in the CIR report that were generated using alternative methods, non-animal test methods for ocular irritation. He wanted to know if the inclusion of these data is indicative of the Panel's formal or informal acceptance of alternative methods as adequate for the assessment of safety.

Dr. Andersen recalled that the CIR final safety assessment on Alpha Hydroxy Acids (AHAs) was likely the first safety assessment in which extensive eye toxicity *in vitro* assay data were included. He said that while there was no formal acknowledgment in that report of the validity of that type of non-animal test, the overall sense of the Panel was that there was no reason for concern about AHA ocular toxicity.

Dr. Bailey said that he had raised the issue of alternative methods because these methods are somewhat controversial. He added that the extrapolation of data from non-animal tests to a human safety evaluation cannot be approached casually, and that, literally, a lot has to happen in order to make those tests reliable for assessing human safety. Dr. Bailey said that this should be taken into consideration whenever the Panel considers the issue of ocular safety.

Dr. Bergfeld noted that from a historical perspective, it was the position of the Panel that alternative tests (non-animal tests) would only be reviewed, until such tests have been validated and accepted. She said that the Panel prefers the standard ocular irritation studies (animal tests).

Dr. McEwen said that the position that has been taken by the Panel in the past is that the Panel encourages the submission of data based on alternative methods and will review the data in light of other information that is available.

Dr. Klaassen wanted to know whether the National Toxicology Program or some other organization is coordinating the process of validating *in vitro* tests.

Dr. Bailey said that the Interagency Coordinating Committee For the Validation of Alternative Methods (ICVAM), which is under NIH, is overseeing the issue of validation and, also, positions for acceptance, focusing specifically on regulatory studies.

Dr. Klaassen wanted to know whether a declaration on any method or test has been made or whether ICVAM is in the process of doing this.

Dr. Bailey said that he thinks that the general framework has been discussed.

Dr. Schroeter reiterated his concern about the potential reproductive and developmental toxicity of the low molecular weight compounds being reviewed.

The Panel voted in favor of issuing a Tentative Report with the following conclusion: The CIR Expert Panel concludes that PEG-20 Sorbitan Cocoate, PEG-40 Sorbitan Diisostearate, PEG-2, -5, and -20 Sorbitan Isostearate, PEG-40 and -75 Sorbitan Lanolate, PEG-10, -40, -44, -75, and -80 Sorbitan Laurate, PEG-3, and -6 Sorbitan Oleate, PEG-80 Sorbitan Palmitate, PEG-40 Sorbitan Perisostearate, PEG-40 Sorbitan Peroleate, PEG-3, -6, -40, and -60 Sorbitan Stearate, PEG-20, -30, -40, and -60 Sorbitan Tetraoleate, PEG-60 Sorbitan Tetrastearate, PEG-20 and -160 Sorbitan Triisostearate, PEG-18 Sorbitan Trioleate, PEG-40 and -50 Sorbitol Hexaoleate,

PEG-30 Sorbitol Tetraoleate Laurate, and PEG-60 Sorbitol Tetrastearate are safe for use as cosmetic ingredients under the present practices of use.

Three Panel members voted in favor of the conclusion, and two voted against it.

Dr. Bergfeld expressed concern over the fact that Dr. Slaga is not present at this meeting and that the vote was rather close (3 to 2). She wanted to establish a position as to what will happen once Dr. Slaga returns. Dr. Bergfeld noted that the Tentative Report that will be issued will contain some of today's discussion, and that the Panel will have an opportunity to revisit any issues that were discussed before a Final Report is issued.

Dr. McEwen added that if Dr. Slaga should determine that the data are insufficient for determining safety and the final vote turns out to be 3 to 3, Dr. Bergfeld will have an opportunity to vote and break the tie.

## **December, 1998**

PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3 and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitan Triisostearate; PEG-40 and -50 Sorbitol Hexaoleate; and PEG-30 Sorbitol Tetraoleate Laurate, and PEG-60 Sorbitol Tetrastearate

Dr. Belsito noted that the Panel had previously elected to table the report on this group of ingredients because of the Panel's concern about the enhancement of chemical carcinogenicity of the skin when these compounds were applied concomitantly. He added that, on the preceding day, his Team had an opportunity to review two studies that address this concern. In these reports, high concentrations of the PEG Sorbitan Fatty Acids were applied frequently to the skin; however, there was no dose-response effect with respect to the ability of these compounds to promote the carcinogenicity of dimethylbenzanthracene. After considering these data along with data from another report on the

inhibition of DNA by these compounds, Dr. Belsito noted that his Team did not find these data very persuasive in terms of the ability of these compounds to promote carcinogenicity or induce DNA inhibition.

Dr. Belsito said that his Team concluded that based on the preceding data as well as the other studies included in the Tentative Report, the PEG Sorbitan Fatty Acids are safe as used, with the caveat (to be included in the report discussion) that these ingredients should not be applied to damaged skin and that the contamination by 1,4 dioxane or ethylene oxide (potential byproducts of the manufacturing process) should not occur in cosmetic grade products. Dr. Belsito added that it should also be stated in the report discussion that the Panel had expressed concern over the tumor promotion potential of the PEG Sorbitan Fatty Acids, but that after reviewing the data, the Panel concluded that this is no longer a concern with respect to cosmetic products containing these ingredients.

Dr. Schroeter noted that his Team also agrees with the statements on impurities, use on damaged skin, and tumor promotion potential that will be included in the report discussion. He said that his Team also recommends a 10% limitation on ingredient use concentrations, based on human skin sensitization data.

Dr. Belsito said that some of the concentrations quoted in the human studies are patch test concentrations for determining whether or not sensitization exists, and not studies to determine whether sensitization can be induced with these compounds.

Dr. Schroeter acknowledged that the human study that he had referred to is not a predictive study in terms of sensitization.

Dr. Belsito noted that the negative human test data on 10% Sorbitan Isostearate (24 h patch test) that Dr. Schroeter was probably referring to is skin irritation data, and that other data indicate that PEG-20 Sorbitan Oleate (100%) was noncorrosive to human skin in an *in vitro* assay. Furthermore, Dr. Belsito noted that the ingredients reviewed in the CIR report are used in well over 500 products and that there is very little case literature suggesting that cosmetic products containing PEG Sorbitan compounds have caused adverse effects.

Dr. Shank said that because current concentration of use data are not available, he could not support a safe as used conclusion for this group of ingredients.

Dr. Schroeter said that if the Panel's conclusion on the PEG Sorbitan Fatty Acid Esters is safe as used, then the basis for this conclusion should be stated in the report discussion.

Dr. Andersen stated that each time a safe as used conclusion is issued, the possibility that there are no reported uses exists. He added that because a number of PEG Sorbitan Fatty Acid Esters are not reported as being used in 1998, the meaning of safe as used for this group of ingredients is unclear.

Dr. Bailey said that it is important to maintain the highest level of technical scientific credibility that is possible, taking into consideration the concentration of use data that are available.

Dr. Shank said that he favors a conclusion of "safe for use" when current ingredient use concentrations are not known.

Ms. Fise said that even if current concentration of use data are not available, 1984 concentration of use data can serve as the basis for a safe as used conclusion; however, a conclusion of safe for use

implies that any concentration is safe. With this in mind, she agreed that a conclusion of safe as used is much more acceptable.

Dr. Belsito noted that 1998 concentration of use data, available only on three of the 17 PEGs Sorbitan Fatty Acid Esters included in this review, indicate use concentrations up to 10%. He favored using the 1984 database (use concentrations up to 25% indicated) to establish any concentration limit because this database is more complete.

Dr. Bergfeld said that the Panel is moving toward approval of a Final Report with a safe conclusion, as stated. The report will also contain a discussion in which information on concentration of use (basis for safe conclusion) is included.

Dr. Slaga added that the issues of cocarcinogenicity and the promotion of DNA repair inhibition will also be included in the report discussion.

Dr. Bergfeld indicated that the issue of potential ingredient contamination with 1,4-dioxane will also be addressed in the report discussion.

The Panel voted unanimously in favor of approving the safe conclusion and discussion that were proposed and issuing a Final Report on the following ingredients: PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3 and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitan Triisostearate; PEG-40 and -50 Sorbitol Hexaoleate; and PEG-30 Sorbitol Tetraoleate Laurate, and PEG-60 Sorbitol Tetrastearate.

## **Sorbeth Beeswax**

### **December, 1997**

#### PEG-6, -8, and -20 Sorbitan Beeswax

Dr. Schroeter noted that his Team concluded that these ingredients are safe as used, because the Expert Panel previously determined that the components of these ingredients are safe as used in cosmetics.

Dr. Belsito said that the Panel does not know how these ingredients are manufactured or the impurities that may be present. Therefore, his Team determined that the available data are insufficient and that the data needs are either the method of manufacture and impurities, or, in the absence of this, a 28-day dermal toxicity study on PGE-6 Sorbitan Beeswax. Dr. Belsito noted that if results of the 28-day dermal toxicity study indicate any evidence suggestive of irritation or sensitization, then dermal irritation and sensitization data on PEG-6 Sorbitan Beeswax (at concentration of use) would be needed.

The Panel voted unanimously in favor of issuing an Insufficient Data Announcement with the following data requests:

- (1) Current concentration of use
- (2) Method of manufacture and impurities, or



(3) Gross pathology and histopathology in skin and other major organ systems associated with repeated exposures to PEG-6 Sorbitan Beeswax; depending on the results, sensitization and irritation data may be needed

### **May, 1998**

#### PEG-6, -8, and -20 Sorbitan Beeswax

Dr. Schroeter stated that his Team concluded that the available data are insufficient for arriving at a conclusion on the safety of these ingredients, and that the following data are needed:

- (1) Method of manufacture and impurities, or
- (2) Gross pathology and histopathology in skin and other major organ systems associated with repeated exposures to PEG-6 Sorbitan Beeswax
- (3) Sensitization and irritation data may be needed, pending the treatment of concentration of use data

Dr. Schroeter clarified item 3 above by saying that if the Panel had access to concentration of use data, then there probably would be no need for this data request. He noted that the test concentrations of PEG-6 and -20 Sorbitan Beeswax were not stated in a human skin irritation and sensitization study that was received from industry.

Dr. Belsito stated that his Team concluded that, based on the available data, PEG-6, -8, and -20 Sorbitan Beeswax are safe for use in cosmetics. He noted that the carcinogenicity study on Sorbitan esters was negative, and that the results for Sorbitan Oleate were negative in a dermal tumor promotion study. Dr. Belsito also said that though data on methods of manufacture are not available, his Team was still able to conclude that PEG-6, -8, and -20 Sorbitan Beeswax are safe as used.

Dr. Bergfeld asked if there had been any discussion of the component parts of the beeswax.

Dr. Belsito stated that the Panel has already ruled on the safety of the component parts.

Dr. Schroeter noted that the Panel does not have subacute dermal toxicity data on PEG-6, -8, and -20 Sorbitan Beeswax. He also said that information on the methods of manufacture and impurities (especially on Sorbitan) is needed, and that possible unscheduled DNA synthesis is reported in the Carcinogenicity section of the Tentative Report.

Dr. Belsito confirmed that Dr. Schroeter was referring to the study on Sorbitan Oleate in which inhibition of DNA repair *in vitro* was noted at a concentration of 0.01%.

Rebecca Lanigan noted that in the published CIR Final Report on Sorbitan Oleate and other Sorbitan esters, Sorbitan Oleate was negative for tumor promotion in a dermal study. Furthermore, both Sorbitan Laurate and Sorbitan Trioleate were possible cocarcinogens when they were undiluted.

Dr. Shank said that the inhibition of DNA repair by Sorbitan fatty acids is questionable.

Dr. Belsito noted that the Panel concluded that Sorbitan Oleate, Sorbitan Laurate, and Sorbitan Trioleate are safe in the Final Report published in 1985. With this in mind, he asked whether or not the safety of these ingredients should be reevaluated.

Dr. Klaassen noted that carcinogenicity studies on Sorbitan Stearate and Sorbitan Laurate were done.

Dr. Shank said that he is concerned about whether or not PEG-6, -8, or -20 Sorbitan Beeswax will cause a problem in terms of sunlight-induced DNA damage, and that this has not been examined.

Dr. Bergfeld asked the Panel to comment on the types of studies that are needed for the safety assessment on PEG-6, -8, and -20 Sorbitan Beeswax.

Dr. Shank said that gross pathology, histopathology, and repeat exposure are needed.

Dr. Belsito did not understand this request, taking into consideration that Dr. Shank's concern seems to be photocarcinogenicity.

Dr. Shank said that he is concerned about cocarcinogenicity.

Dr. Belsito said that there must also be concern about light-induced skin damage. He noted that Dr. Shank is actually requesting a sunburn cell study, which is the same type of study that was requested for the alpha hydroxy acids. He also said that Dr. Shank had expressed concern over what will happen to the skin during exposure to sunlight, after considering that Sorbitan fatty acids may inhibit DNA repair.

Dr. Slaga agreed that a photocarcinogenicity study is needed.

Referring to the Carcinogenicity section of the Tentative Report, Dr. Klaassen wanted to know if the results indicating inhibition of *in vitro* DNA repair are from an unscheduled DNA synthesis test.

Dr. Slaga said that the results are from a transformation assay using Syrian golden hamsters. In this assay, cells have colonized to give rise to tumors when injected into a syngeneic host.

Dr. Shank noted that Sorbitan Oleate inhibited *in vitro* DNA repair at a concentration of 0.01% in an unscheduled DNA synthesis assay. More specifically, he said that there was 50% inhibition of unscheduled DNA synthesis at a test concentration of 0.01%.

Dr. Klaassen wanted to know how these results are being interpreted.

Dr. Shank said that the results are to be interpreted as inhibition of DNA repair in an *in vitro* system.

Dr. Klaassen said that, perhaps, there was less injury, and, thus, there was less that needed to be repaired.

Dr. Shank noted that in this type of test (unscheduled DNA synthesis assay), injury is induced and, in the control, there is a certain amount of incorporation into DNA. Compared to the control, Sorbitan Oleate (0.01%) caused 50% inhibition of unscheduled DNA synthesis.

Dr. Bergfeld asked for comments on the types of studies that are needed for completion of the Panel's safety assessment of PEG-6, -8, and -20 Sorbitan Beeswax.

Dr. Shank said that he would like to see the effect of the cosmetic ingredients on thymine dimer repair in skin.

Dr. Slaga agreed. He said that in order to show that there are no effects on repair in the skin, a UV-induced thymine dimer (or any of the photoproducts) assay would be acceptable.

Dr. Belsito stated that one could evaluate the appearance of thymine dimers in skin irradiated *in vitro* and the capability for thymine dimer repair in saline versus a Sodium Oleate solution.

Dr. McEwen solicited the Panel's help in identifying laboratories that would do this type of study.

Dr. Bergfeld suggested that Dr. McEwen meet with the Panel at the conclusion of the meeting.

Dr. Andersen asked the Panel to identify the compound that would be optimal with respect to looking for thymine dimer production in human skin (i.e., PEG Sorbitan Beeswax or Sorbitan Oleate?).

Dr. Shank selected the lowest molecular weight compound being reviewed (PEG-6 Sorbitan Beeswax).

In summary, the Panel expressed concern over the cocarcinogenesis and tumor promotion data on Sorbitan Trioleate and Sorbitan Laurate, and the DNA repair inhibition data on Sorbitan Oleate. Data showing that the formation of thymine dimers in human skin with UV radiation is not increased in the presence of the PEGs Sorbitan Beeswax would resolve the matter for the Panel.

Dr. Bergfeld called for the vote on a Tentative Report on PEG-6, -8, and -20 Sorbitan Beeswax with an insufficient data conclusion, the data needs being those described during the Panel discussion (See preceding paragraph).

Dr. Bergfeld said that it appears, by majority vote, that the Panel has determined that the Tentative Report on PEG-6, -8, and -20 Sorbitan Beeswax is insufficient. She also stated that the data needs are those that were recorded during the Panel discussion, unless there are other requests that need to be added. [Drs. Belsito and Carton opposed the motion and Dr. Klaassen abstained.]

Concerning the human dermal irritation and sensitization study provided by CTFA, Dr. Schroeter noted that the test concentrations were not specified and that this information is needed. He said that this information might clarify the accuracy of the data.

Dr. Andersen interpreted Dr. Schroeter's comments as follows: If the data already provided are characterized as to the concentration used, then further sensitization/irritation data may not be needed.

Dr. Schroeter said that the laboratory that could do the study for assaying the suppression of DNA repair and thymine dimers would be at Harvard University (Barbara Gilcrest, investigator).

Dr. Belsito said that the National Institute of Arthritis, Muscular, Skeletal, and Skin Diseases is another possibility.

Dr. Klaassen suggested that Sorbitan Laurate should also be tested in the DNA repair assay.

Dr. Shank said that the Sorbitan Fatty Acid Esters (Sorbitan Laurate included) could also be tested.

Dr. McEwen was concerned that even if the results for PEG-6 Sorbitan Beeswax in the DNA repair assay were negative, the Panel may decide to request test data on the Sorbitan Fatty Acid Esters.

Dr. Shank said that if the assay results for PEG-6 Sorbitan Beeswax are negative, these data will be sufficient.

Dr. Andersen stated that these data will resolve the issue for the PEGs Sorbitan Beeswax, but will not resolve the issue relating to the earlier Panel conclusion on the Sorbitan Fatty Acids, which will now

have to be revisited. He said that in order to resolve those questions, it will be necessary to obtain data on Sorbitan Oleate.

Dr. Bergfeld said that it would seem appropriate that each time a safety question is raised concerning a previous CIR report, that the Panel should automatically reopen the review of that ingredient or ingredients. She recommended that this policy be adopted by CIR.

[It is important to note that the Panel's tentative insufficient data conclusion on the PEGs Sorbitan Beeswax was rescinded during its review of the Sorbitan Fatty Acid Esters group report later in the day. The Panel's final decision was that of tabling the review of both group reports. The Panel discussion that led to this decision is included in the section of the minutes on the Sorbitan Fatty Acid Esters ingredient family.]

## **December, 1998**

### PEG-6, -8, and -20 Sorbitan Beeswax

Dr. Belsito recalled that the same issues discussed in the preceding section on Sorbitan Fatty Acid Esters also relate to this ingredient family, and that both reports were tabled at the May 18-19, 1998 Panel meeting. He noted from the preceding discussion that the carcinogenicity issue has been resolved and proposed that PEG-6, -8, and -20 Sorbitan Beeswax are safe as used.

Dr. Belsito also noted that a report discussion with the following components needs to be developed: (1) Caveat on the use of PEGs on damaged skin, (2) 1,4-dioxane and ethylene oxide should not be present in the finished product, (3) the boiler plate on ethylene glycol teratogenicity/reproductive toxicity that was developed, and (4) explanation of the Panel's interpretation of the co-carcinogenicity or tumor promotion study.

Dr. Andersen said that he recalled that the Panel had previously requested the incorporation of data from the CIR Final Report on Beeswax into the present report on

PEG-6, -8, and -20 Sorbitan Beeswax, to indicate the Panel's understanding that the issue of contaminants/impurities associated with Beeswax is not a problem that is associated with PEG-6, -8, and -20 Sorbitan Beeswax.

The Panel agreed that only a statement (with a reference to the Final Report on Beeswax), not actual data, addressing Dr. Andersen's observation should be incorporated into the present report.

Dr. Schroeter recommended that the reason why a concentration limit is not being established for this group of ingredients should be stated in the report discussion.

Dr. Bergfeld said that it is the general consensus of the Panel that issues relating to concentration limits should not be included in the report discussion.

The Panel voted unanimously in favor of issuing a Tentative report on PEG-6, -8, and -20 Sorbitan Beeswax with a "safe as used" conclusion.

The 69th meeting of the CIR Expert Panel was adjourned.

## Safety Assessment of Polysorbates as Used in Cosmetics

Status:	Re-Review for Panel Review
Release Date:	February 20, 2015
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The 2015 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

## INTRODUCTION

This is a re-review of the available scientific literature and unpublished data relevant to assessing the safety of polysorbates as used in cosmetics. In the original safety assessment published in 1984, the Cosmetic Ingredient Review (CIR) Expert Panel (Panel) concluded that 9 polysorbates were safe as used.<sup>1</sup> In this re-review, these ingredients are combined into a group of 79 polyethoxylated sorbitan esters of fatty acids; each ingredient has a common core structure of sorbitan, etherified with polyethoxy (PEG) chains, some of which are also esterified with fatty acids. These ingredients are mostly used as surfactants (Table 1).

The following are the 9 previously reviewed polysorbates that are included in this safety assessment (additional data are found in Table 2):<sup>1</sup>

Polysorbate 20	Polysorbate 65
Polysorbate 21	Polysorbate 80
Polysorbate 40	Polysorbate 81
Polysorbate 60	Polysorbate 85
Polysorbate 61	

Other polysorbates, which are also polyethoxylated sorbitan esters of fatty acids and contain a PEG moiety, have been reviewed by the CIR Panel. In 2000<sup>2</sup>, a safety assessment was published with a safe as used conclusion for the following 33 PEG sorbitan/sorbitol fatty acid esters:

PEG-20 sorbitan cocoate	PEG-40 sorbitan stearate
PEG-40 sorbitan diisostearate	PEG-60 sorbitan stearate
PEG-2 sorbitan isostearate	PEG-20 sorbitan tetraoleate
PEG-5 sorbitan isostearate	PEG-30 sorbitan tetraoleate
PEG-20 sorbitan isostearate	PEG-40 sorbitan tetraoleate
PEG-40 sorbitan lanolate	PEG-60 sorbitan tetraoleate
PEG-75 sorbitan lanolate	PEG-60 sorbitan tetrastearate
PEG-10 sorbitan laurate	PEG-20 sorbitan triisostearate
PEG-40 sorbitan laurate	PEG-160 sorbitan triisostearate
PEG-44 sorbitan laurate	PEG-18 sorbitan trioleate
PEG-75 sorbitan laurate	Sorbeth-40 hexaoleate (previously PEG-40 sorbitol hexaoleate)
PEG-80 sorbitan laurate	Sorbeth-50 hexaoleate (previously PEG-50 sorbitol hexaoleate)
PEG-3 sorbitan oleate	Sorbeth-30 tetraoleate laurate (previously PEG-30 sorbitol tetraoleate laurate)
PEG-6 sorbitan oleate	Sorbeth-60 tetrastearate (previously PEG-60 sorbitol tetrastearate)
PEG-80 sorbitan palmitate	
PEG-40 sorbitan perisostearate	
PEG-40 sorbitan peroleate	
PEG-3 sorbitan stearate	
PEG-6 sorbitan stearate	

There were 2 ingredients that were included in the 2000 report, but were not listed in the *International Cosmetic Ingredient Dictionary and Handbook*<sup>3</sup> (*Dictionary*) at the time of the original review, and are not listed as cosmetic ingredients in the current *Dictionary*.<sup>4</sup> One is PEG-18 sorbitan trioleate, which has 1 use listed in the Food and Drug Administration's (FDA) Voluntary Cosmetic Registration Program (VCRP)<sup>5</sup> and is therefore included in this safety assessment. However, PEG-20 sorbitan tetraoleate has no uses listed in the VCRP, so is not included in this safety assessment.

In 2001<sup>6</sup>, a safety assessment was published with a safe as used conclusion for the following sorbitan beeswaxes:

Sorbeth-6 beeswax (formerly PEG-6 sorbitan beeswax)  
 Sorbeth-8 beeswax (formerly PEG-8 sorbitan beeswax)  
 Sorbeth-20 beeswax (formerly PEG-20 sorbitan beeswax)

At the time, the Panel recommended that cosmetic formulations containing PEG-6, PEG-20, or PEG-75 not be used on damaged skin. Since then, PEGs have been re-reviewed and the Panel has removed the caveat that PEGs should not be used on damaged skin.<sup>7</sup>

A brief summary of pertinent data from each report is provided below. The original polysorbate reports can be found on the CIR website, <http://www.cir-safety.org/ingredients>. Please refer to the original reports for detailed information.

The following 35 ingredients, which are also polyethoxylated sorbitan esters of fatty acids, are proposed as additions to this group:



PEG-20 sorbitan oleate	Sorbeth-20 pentaisostearate
PEG-40 sorbitan oleate	Sorbeth-30 pentaisostearate
PEG-4 sorbitan stearate	Sorbeth-40 pentaisostearate
PEG-4 sorbitan triisostearate	Sorbeth-50 pentaisostearate
PEG-2 sorbitan trioleate	Sorbeth-40 pentaoleate
PEG-3 sorbitan tristearate	Sorbeth-20 tetraisostearate
Sorbeth-2 beeswax	Sorbeth-30 tetraisostearate
Sorbeth-2 cocoate	Sorbeth-40 tetraisostearate
Sorbeth-2 hexacaprylate/caprate	Sorbeth-50 tetraisostearate
Sorbeth-12 hexacocoate	Sorbeth-4 tetraoleate
Sorbeth-2 hexaisostearate	Sorbeth-6 tetraoleate
Sorbeth-2 hexalaurate	Sorbeth-30 tetraoleate
Sorbeth-2 hexaoleate	Sorbeth-40 tetraoleate
Sorbeth-6 hexastearate	Sorbeth-60 tetraoleate
Sorbeth-150 hexastearate	Sorbeth-3 tristearate
Sorbeth-3 isostearate	Sorbeth-160 tristearate
Sorbeth-6 laurate	Sorbeth-450 tristearate
Sorbeth-2/oleate/dimer dilinoleate crosspolymer	

CIR has conducted safety assessments of the acids and related chemical structure moieties of these ingredients (Table 2). The Panel concluded that beeswax, coconut acid, isostearic acid, lanolin acid, oleic acid, lauric acid, myristic acid, stearic acid, and multiple stearates were safe as used.<sup>8-16</sup> Alkyl esters and PEGs are also safe as used.<sup>7,17,18</sup> Sorbitan esters have been reviewed with safe as used conclusions.<sup>19-21</sup>

Much of the new data included in this safety assessment were found on the European Chemicals Agency (ECHA) website.<sup>22-24</sup> The ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited. Some of this data are for generic sorbitan monolaurate, ethoxylated and sorbitan monostearate, ethoxylate; these chemicals fit the general definition of several of these ingredients with the same CAS No. (ie, polysorbate 21, PEG-10 sorbitan laurate, PEG-40 sorbitan laurate, polysorbate 20, PEG-44 sorbitan laurate, PEG-75 sorbitan laurate, and PEG-80 sorbitan laurate all have the CAS No. 9005-64-5). It is expected that data under these chemicals names are for one or some mixture of the ingredients with that CAS No and are useful for read across information.

## **SUMMARIES OF PREVIOUS SAFETY ASSESSMENTS**

### ***Polysorbates, 1984***

*The Polysorbates are a series of polyoxyethylenated sorbitan esters that differ with respect to the number of polymerized oxyethylene subunits and the number and type of fatty acid moieties present.<sup>1</sup> They are used as general purpose, hydrophilic, nonionic surfactants in a variety of cosmetic products. Some of the Polysorbates are also approved by the Food and Drug Administration for use in various pharmaceuticals and food products.*

*Studies employing radioactive tracer techniques show that the Polysorbates are hydrolyzed by pancreatic and blood lipases; the fatty acid moiety is released to be absorbed and metabolized, whereas the polyoxyethylene sorbitan moiety is very poorly absorbed and is excreted unchanged. As expected, the Polysorbates are active at levels of biological structure and function from basic biochemical pathways to the cardiovascular and immune systems. Most or all of these effects can most likely be related to the surface active properties of the intact Polysorbate molecule.*

*Polysorbate 80 was shown to be nonmutagenic in the Ames and micronucleus tests. The polysorbates have been shown in numerous studies to be noncarcinogenic when administered in a variety of ways to laboratory animals, although Polysorbate 80 produced some neoplastic changes in mixed mouse epidermal and dermal in vitro tissue culture. Multiple studies have shown that the Polysorbates enhance the activity of known chemical carcinogens while not actually being carcinogenic themselves. Proposed mechanisms of this tumor enhancement effect include induction of cellular hyperproliferation, inhibition of DNA repair, and others. The Polysorbates also exhibit tumor growth inhibition activity under certain conditions.*

*Extensive testing for acute and long-term oral toxicity in animals has resulted in evidence indicating the low order of toxicity with oral ingestion of the Polysorbates. Most of the reported toxicity can be attributed either directly or indirectly to the osmotic diarrhea caused by the polyoxyethylene sorbitan moiety retained within the intestinal lumen. Polysorbate 20 and product formulations containing 1.0 to 8.4 percent of Polysorbate 20, 40, 80, or 85 produced no evidence of acute or subchronic percutaneous toxicity, the only effects being erythema, edema, and desquamation at the site of application. Acute intravenous and intraperitoneal injection of the Polysorbates into rats or mice resulted in LD<sub>50</sub> values indicative of a low order of parenteral toxicity. Daily intravenous injections of Polysorbates 60 and 80 into rabbits for up to 65 days produced pathology limited mainly to the renal and reticuloendothelial systems.*

The Polysorbates showed little potential for rabbit and mouse skin irritation in acute studies. Those of the Polysorbates that were tested in subchronic skin irritation tests for up to 60 days produced local skin reactions ranging from minimal inflammation to necrosis. These changes were attributable to damage of epidermal cell membranes by the emulsifying action of the Polysorbates. The Polysorbates produced no more than minimal, transient eye irritation in Draize rabbit eye irritation tests. Polysorbate 80 produced superficial, mild damage to the intestinal mucosae of rabbits and rats. Polysorbate 20 produced no inflammation when applied to the hamster cheek pouch, and Polysorbate 40 caused no inflammation when infused into the guinea pig urinary bladder. The Magnusson-Kligman guinea pig maximization test showed moderate to strong skin sensitization to Polysorbate 20 in one study. Another guinea pig skin sensitization assay reported no evidence of skin sensitization to Polysorbates 65 and 80.

The Polysorbates have been ingested by human beings in situations ranging from an accidental administration of 19.2 g of Polysorbate 80 to an infant on 2 consecutive days to daily therapeutic administration of up to 6.0 g of Polysorbate 80 to adults for up to 4 years. These studies consistently showed little or no adverse effects from oral ingestion of the Polysorbates. Extensive clinical skin testing in the Schwartz prophetic patch test showed little potential for human skin irritation and no evidence of skin sensitization in a total of 580 subjects. A total of 1206 patients with eczema were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to a mixture of 5 percent Polysorbate 60 and 5 percent Polysorbate 80 in petrolatum; allergic reactions were shown by only 2 of the patients (< 0.2 percent). Several product formulations containing the Polysorbates have been tested for human skin sensitization on a total of 3481 subjects using a variety of testing methods; there were no reactions indicative of sensitization to any of the Polysorbates in these assays. Investigations with patients known to have skin disease revealed isolated instances of skin sensitization to Polysorbate 40 or 80. Intravenous injection of Polysorbate 80 produced hemodynamic changes in 5 patients. Studies involving exposure to ultraviolet light showed no instance of photocontact sensitization to the Polysorbates, although there were isolated instances of mild irritation following UV exposure when testing product formulations containing the Polysorbates.

#### **PEGs Sorbitan/Sorbitol Fatty Acid Esters 2000**

The PEGs Sorbitan/Sorbitol Fatty Acid Esters are ethoxylated sorbitan and sorbitol esters of fatty acids that function as surfactants in cosmetic formulations.<sup>2</sup> These ingredients were used in a total of 81 cosmetic formulations in 1998. The Polysorbates, which are food additives, were used in 1418 formulations. They are formed by the esterification of sorbitol or sorbitan with a fatty acid, followed by the chemical addition of ethylene oxide. Typical impurities can include the free fatty acids, alcohol, peroxides, isosorbide ethoxylates, and other compounds; 1,4-dioxane and other water-soluble by-products are removed during the manufacturing process.

Few data on the ingredients in this review were available; therefore, relevant data from the previous CIR safety assessments on the Polysorbates (other PEGs Sorbitan Fatty Acid Ester), PEGs, and Sorbitan Esters were included in this report as a further basis for assessing their safety in cosmetics.

During feeding studies, the Polysorbates were absorbed and hydrolyzed by blood and pancreatic lipases. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid, and the PEG Sorbitan moiety was excreted mainly in the urine. The gastrointestinal absorption of PEGs was dependent on the molecular weight; the greater the molecular weight, the lesser the absorption that occurs. In oral and IV studies, the PEGs were not metabolized and were rapidly eliminated in the feces and urine. PEGs were readily absorbed through damaged skin.

A number of cytotoxicity assays has been performed on the Polysorbates; they caused both membrane damage and reduced mitochondrial activity. A concentration of 5% PEG-20 Sorbitan Oleate in rats caused the "destruction" of the mitochondria of the epithelium of the small intestine of Wistar rats. The Polysorbate (concentration = 10%) caused a portion of the microvilli to disappear with flattening of the surfaces of the epithelial cells. PEG-20 Sorbitan Oleate had immunosuppressive effects in Balb/c mice that had been immunized with ovalbumin. PEG-20 Sorbitan Oleate was also a histamine-releasing agent, and increased recruitment of peritoneal macrophages without modifying phagocytic activity. PEG-20 Sorbitan Oleate (100 mg/ml) depressed cardiac potential in dogs and guinea pigs; the Polysorbate reduced mean arterial blood pressure and left ventricular dP/dt.

The Polysorbates had low toxicity in both acute and longterm toxicity studies using animals. In rats, the LD<sub>50</sub> values for these ingredients were >5 to >38.9 g/kg (oral), ~1.4 g/kg (IV), and 0.7 to >5 ml/kg (IP). When administered to rats by IP injection, 16% PEG-20 Sorbitan Laurate and 32% PEG-20 Sorbitan Oleate decreased locomotor activity. During an inhalation toxicity study, PEG-20 Sorbitan Oleate (7%; 0.1 to 0.2 ml) was relatively nontoxic. The Sorbitan Esters and PEGs also were relatively nontoxic to animals.

During a 14-day feeding study of 3000 to 50,000 ppm PEG-20 Sorbitan Oleate, the high dose caused decreased body weight in male rats and mice, but no other clinical findings were reported. A vehicle containing 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate was mildly hepatotoxic to rabbits and, when given intraperitoneally, caused massive peritoneal fibrosis and degeneration of the kidneys in mice and rats. No adverse effects were observed in chicks fed 2% to 5% PEG-20 Sorbitan Stearate for 7 weeks. Rats fed 10% of the Polysorbate for 8 weeks had diarrhea for the first few days of treatment, but no other signs of toxicity. Rats fed 1.5 ml PEG-20 Sorbitan Oleate (1%-4%) for 3 months had congestive and degenerative changes in the heart, liver, and kidneys. In 6-week studies using rats and monkeys, PEG-4 Sorbitan Stearate, PEG-20 Sorbitan Stearate, and PEG-5 Sorbitan Oleate produced no significant adverse effects. In dermal toxicity studies, the PEGs did not cause signs of toxicity other than transient, mild erythema. Evidence of systemic toxicity

was only observed in rabbits that received repeated topical applications of a PEG-based cream to abraded skin. Rats fed 1% to 4% Sorbitan Laurate for 6 weeks had decreased growth rates, and hamsters fed 15% for 68 days had degenerative changes of the gastrointestinal tract, and other lesions. Similar changes were observed in rats fed 25% Sorbitan Laurate for 70 days. Rhesus monkeys fed 2 g/day had no signs of toxicity after 6 weeks of treatment.

Growth retardation and diarrhea were noted in subchronic feeding studies of up to 1% PEG-20 Sorbitan Stearate using mice. Diarrhea in these and other studies was attributed to the high concentrations of the unabsorbed PEG Sorbitan moiety in the intestinal lumen. PEG-20 Sorbitan Oleate (up to 50,000 ppm) was nontoxic to rats and mice during a 13-week feed study. A concentration of 25% PEG-20 Sorbitan Laurate caused microscopic changes of the urinary bladder, spleen, kidneys, and gastrointestinal tract in rats during a 21-week study. The PEGs were nontoxic during a 90-day oral toxicity study using rats. Feeding of 10% to 25% Sorbitan Laurate for 90 days to 23 weeks caused decreased body and organ weights, diarrhea, and hepatic lesions in rats.

During a chronic toxicity study using hamsters, 5% to 15% PEG-20 Sorbitan Laurate caused microscopic lesions of the urinary bladder, kidneys, spleen, and gastrointestinal tract. In monkeys, 1 g/day PEG-20 Sorbitan Laurate did not cause adverse effects after 17 months of treatment. Rats fed up to 2% PEG-20 Sorbitan Laurate for over 2 years had no signs of toxicity. PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Tristearate at concentrations <20% were nontoxic in long-term feeding studies using mice, rats, dogs, and hamsters. At concentrations of 20%, these Polysorbates caused some growth retardation and diarrhea, and had minor effects on longevity and reproduction. Studies using 2% PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Trioleate were also negative. In chronic studies, dogs fed 2% PEG-8, PEG-32, or PEG-75 for 1 year had no adverse effects; rats fed 5% Sorbitan Laurate for 2 years had no signs of toxicity, but only 15% of the treated and control rats survived to the end of the study.

The Polysorbates were nonirritating to mildly irritating in both in vivo and in vitro ocular irritation assays. The concentrations tested ranged from 1% to 100%. PEG-6 and PEG-75 did not cause corneal injuries when instilled into the conjunctival sac of rabbits, but 35% PEG-8 and 0.1 ml PEG-32 (melted in water bath) induced mild ocular irritation. Sorbitan Laurate (30%-100%) was not an ocular irritant in Draize ocular irritation tests using rabbits.

The Polysorbates had little potential for rabbit and mouse skin irritation in acute studies. Moderate to strong sensitization to PEG-20 Sorbitan Laurate was observed in a Magnusson Kligman guinea pig maximization test; PEG-20 Sorbitan Oleate and PEG-20 Tristearate were not sensitizers. PEG-20 Sorbitan Laurate (1%) did not have comedogenic potential in rabbits. The Sorbitan Esters were generally mild skin irritants, but did not cause sensitization in animals. The PEGs were neither irritants nor sensitizers.

In teratology studies of thalidomide, the PEG-20 Sorbitan Laurate vehicle (10 ml/kg) had no effect on the developing mouse embryo. In other studies, reproductive and developmental effects were seen primarily at exposure levels that were maternally toxic. PEG-20 Sorbitan Laurate caused dose-dependent malformations of offspring when administered to Swiss and NMRI mice via IP injections. In the Chernoff-Kavlock assay using Alpk/AP rats, 10 ml/kg/day PEG-20 Sorbitan Laurate reduced offspring litter size, survival, and weight gain when the Polysorbate was administered intraperitoneally, but the parameters did not differ from controls after dermal, oral, or subcutaneous administration. In another study using rats, PEG-20 Sorbitan Laurate had a maternal no-observable-effect level (NOEL) of 500 mg/kg/day, a maternal low effect level of 5000 mg/kg/day, and a developmental NOEL of > 5000 mg/kg/day.

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate caused serious developmental effects in sea urchin embryos when administered at concentrations as low as 0.004% in sea water. Mice fed 10% PEG-20 Sorbitan Stearate or PEG-20 Sorbitan Laurate during a multigeneration study had offspring with decreased weanling weights, significantly smaller litters, and delivered more dead fetuses than mice of the control group. PEG-20 Sorbitan Oleate was not teratogenic in a rat whole-embryo culture study. In in vivo studies using neonatal rats, PEG-20 Sorbitan Oleate (1%-10%, IP injection) accelerated maturation, prolonged the estrous cycle, and induced chronic estrogenic stimulation. The ovaries were without corpora lutea and had degenerative follicles, and the uterus had epithelial squamous cell metaplasia and cytological changes. PEG-20 Sorbitan Oleate (2500 mg/kg/day in one study; 1.25 ml/l drinking water in another) and PEG-20 Sorbitan Stearate (0.1%-10% in one study; 5200 mg/kg/day in another) did not cause developmental effects in rats and mice, but PEG-20 Sorbitan Oleate in drinking water increased locomotor activity and exploratory behavior of offspring of treated rats.

The PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. The CIR Expert Panel concluded that, as the PEGs Sorbitan and Sorbitol Esters are chemically different from the alkyl ethers of ethylene glycol and the alkyl ethers are not present as impurities, these ingredients pose no reproductive or developmental hazard. In subchronic and chronic oral toxicity studies, the PEGs did not cause adverse reproductive effects.

The Polysorbates were nonmutagenic in a number of bacterial and mammalian systems, with the exception of PEG-20 Sorbitan Stearate, which produced both positive and negative results in genotoxicity assays.

In carcinogenicity studies, feeding of PEG-20 Sorbitan Oleate (up to 50,000 ppm) to rats and mice resulted in equivocal evidence of carcinogenicity; the male rats had an increased incidence of pheochromocytomas. The test compound was associated with inflammation and squamous hyperplasia of the nonglandular stomach in mice and with ulcers of the nonglandular stomach in female mice. PEG-20 Sorbitan Stearate did not increase the incidence of neoplasms in the nonglandular stomach and glandular stomach when administered with the carcinogens ENNG and MNNG. In general, the Polysorbates were not oral or dermal carcinogens, and were weak tumor promoters. PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate (0.002%) inhibited metabolic cooperation in V79 Chinese Hamster cells in vitro, which could result in tumor

promotion. PEG-20 Sorbitan Stearate has been reported to have an *in vivo* promoter response, and the Polysorbate induced the cytoplasmic accumulation of proliferin transcripts in mouse fibroblasts; proliferin is an antagonistic regulator of muscle-specific transcription, and can promote morphological transformation. The Polysorbates also had antitumor activity in animal studies. PEG-8 was noncarcinogenic in studies using mice, rats, and guinea pigs. Sorbitan Laurate and Sorbitan Stearate were also noncarcinogenic. At concentrations  $\geq 10\%$ , Sorbitan Laurate was a tumor promoter in mouse skin.

The Polysorbates were nontoxic by the oral route in clinical studies, but a Polysorbate vehicle (9% PEG-20 Sorbitan Oleate, 1% PEG-20 Sorbitan Laurate) for a neonatal parenteral supplement caused the deaths of 38 premature infants. The symptoms and lesions observed included pulmonary deterioration, hepatomegaly, metabolic acidosis, and renal failure. Investigators concluded that human infant membranes were more sensitive to the effects of the Polysorbates and could not efficiently metabolize the compounds. Oleic acid and PEG moieties released during *in vivo* hydrolysis of PEG-20 Sorbitan Oleate could have contributed to the pulmonary deterioration and renal failure, as could ethylene glycol formed from ethylene oxide moieties.

The Polysorbates had little potential for human skin irritation, sensitization, and phototoxicity in extensive clinical studies. PEG-20 Sorbitan Oleate at a concentration of 100% was noncorrosive, and it and PEG-20 Sorbitan Laurate were not irritating to living skin equivalents. The PEGs were nonsensitizers, but cases of systemic toxicity and contact dermatitis were observed in burn patients that were treated with PEG-based topical ointments. The Sorbitan Esters had the potential to cause cutaneous irritation in humans, and could cause sensitization in patients with damaged skin. Sorbitan Stearate and Sorbitan Oleate were not photosensitizing; Sorbitan Laurate, Sorbitan Palmitate, Sorbitan Sesquioleate, and Sorbitan Trioleate did not absorb UVA or UVB light, suggesting that these compounds were not photosensitizers.

In clinical ocular irritation studies, PEG-20 Sorbitan Laurate was nonirritating, but at a concentration of 1%, it markedly increased the permeability of the corneal epithelium to fluorescein in the human eye. PEG-20 Sorbitan Oleate was classified as an ocular irritant, but further details were not available.

### **Sorbitan Beeswax, 2001**

PEG-6, -8, and -20 Sorbitan Beeswax are ethoxylated derivatives of Beeswax that function as surfactants in cosmetic formulations.<sup>6</sup> In 1998, PEG-20 Sorbitan Beeswax was reported used in 16 cosmetic formulations; PEG-6 and -8 Sorbitan Beeswax were not reported used. Data submitted by industry indicated that PEG-20 Sorbitan Beeswax was used at concentrations from 0.2% in make-up fixatives to 11% in blushers. In 1984, it was reported used at concentrations  $\geq 10\%$ .

Few data were available on the PEGs Sorbitan Beeswax. Toxicology data on Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and Polysorbates were reviewed as a further basis for the assessment of safety.

The ester link of the Polysorbate (PEG Sorbitan Fatty Acid Ester) molecule was hydrolyzed by blood and pancreatic lipases after oral administration. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid, and the PEG Sorbitan moiety was poorly absorbed from the GI tract. GI absorption of PEG was inversely related to the molecular weight of the compound. PEGs are readily absorbed through damaged skin. Sorbitan Stearate was hydrolyzed to the stearic acid and anhydrides of sorbitol, and did not accumulate in the fat stores of the rat.

PEG-6 Sorbitan Beeswax was "practically nontoxic" when rats were treated with doses of 10.0 g/kg during acute IP studies. PEGs had low oral, dermal, and inhalation toxicity; greater molecular weight PEGs were less toxic than smaller molecular weight PEGs. The Polysorbates were not toxic during acute and long-term feeding studies, or during acute and short-term IV and IP injection studies. Formulations containing the Polysorbates produced no evidence of acute or subchronic percutaneous toxicity. Formulations containing up to 13% Beeswax (5 to 15 g/kg doses) were not toxic to rats. Undiluted Beeswax killed 2 of 10 rats within 2 days during an acute oral toxicity study. Ten rats fed 5 to 14.4 g/kg Synthetic Beeswax had chromorhinorrhea and chromodacryorrhea; rats fed 5 to 10.4 g/kg had diarrhea, ptosis, bulging eyes, and sniffing. Two rats died after ingestion of the high dose.

The Sorbitan Esters ( $<10\%$ ) were relatively nontoxic via ingestion. The lowest  $LD_{50}$  (rats) reported was 31 g/kg Sorbitan Stearate. No adverse effects were observed when rats, mice, and dogs were fed 5% Sorbitans Laurate, Oleate, and Stearate for up to 2 years. In other studies, the feeding of 0.5%, 4%, and 10% Sorbitan Stearate to mice and rats resulted in depressed growth and renal and/or hepatic abnormalities.

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the eyes of rabbits, and a 30% aqueous solution of PEG-20 Sorbitan Beeswax was minimally irritating (Draize score = 3.5/11 0). Eye makeup formulations containing 1.5% to 2.0% PEG-20 Sorbitan Beeswax were non- to minimally irritating to the eyes of rabbits. PEGs, Polysorbates, Sorbitan Esters, Beeswax, and Synthetic Beeswax were non- to mild ocular irritants. Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the intact and abraded skin of rabbits. Cosmetic formulations containing 1.5% to 2.0% PEG-20 Sorbitan Beeswax were non- to minimal irritants to the skin of rabbits. The PEGs were not irritating to the skin of rabbits or guinea pigs, and PEG-75 was not a sensitizer. The Polysorbates had little potential for rabbit and mouse skin irritation during acute studies. Polysorbate 20 was a moderate to strong sensitizer in one study using guinea pigs, and Polysorbates 65 and 80 were nonsensitizers. Synthetic Beeswax (5 g in 1 ml corn oil) had Draize scores of 0 to 2.08 (out of 8.00) during primary irritation studies using rabbits. At a concentration of 50% in water, Synthetic Beeswax was nonsensitizing to guinea pigs. Sorbitan Esters (3% to 100%) were minimal to mild irritants.

Ethylene glycol and certain of its monoalkyl ethers are reproductive and developmental toxins. As PEGs Sorbitan Beeswax are chemically different from these ethers, reproductive and developmental toxicity due to the ethers was not of concern. PEGs did not cause adverse reproductive effects during subchronic and chronic feeding studies.

PEG-8 and -150 were not mutagenic in several genotoxicity assays. Polysorbate 80 was nonmutagenic in the Ames test. Sorbitan Stearate was not mutagenic in tests using bacteria, with or without metabolic activation, and did not transform hamster embryo cells *in vitro*. Sorbitan Oleate (0.01%) inhibited *in vitro* DNA repair. PEG-8 was not carcinogenic during oral, IP, or

subcutaneous (SC) administration. The Polysorbates were generally noncarcinogenic, but enhanced the activity of some known chemical carcinogens. Sorbitan Stearate was not carcinogenic in mice during a feeding study, but Sorbitan Laurate was a tumor promoter during a mouse skin-painting study. Sorbitans Oleate and Trioleate were inactive as tumor promoters. In another study, undiluted Sorbitans Laurate and Trioleate were not cocarcinogens.

In clinical studies, PEG-6 and -20 Sorbitan Beeswax were nonsensitizers. Formulations containing up to 3.0% PEG-20 Sorbitan Beeswax were mildly irritating and nonsensitizing during in-use, minicumulative, andRIPTs. Systemic toxicity and contact dermatitis were observed in burn patients treated with PEG-containing ointments, but PEGs were not sensitizing to normal skin. The Polysorbates and Sorbitan Esters were nontoxic after oral ingestion. Polysorbates, Beeswax, and Synthetic Beeswax did not cause irritation, sensitization, or photosensitization. The Sorbitan Esters were minimal to mild skin irritants in humans, but were nonsensitizing, nonphototoxic, and nonphotoallergenic.

## **CHEMISTRY**

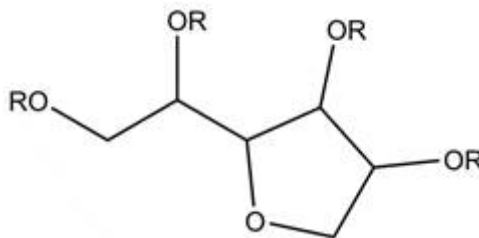
### **Definition and Method of Manufacture**

The polysorbates are a series of general purpose, hydrophilic, nonionic surfactants.<sup>1</sup>

Presented here are 2 possible routes for the synthesis of polysorbates.<sup>25</sup> In the first, sorbitol is esterified with fatty acids or anhydrides, which is typically performed with acid catalysis at 130-180°C. At the temperature required for the esterification, water is eliminated from sorbitol to form 3 possible isomers of sorbitan and (with elimination of another water molecule) isosorbide. These dehydration products react with a fatty acid to form corresponding sorbitan esters. These products, which are known as spans, are ethoxylated to produce polysorbates.

In the other method of manufacture, sorbitol is reacted with ethylene oxide and a basic catalyst at 200-250°C. Under these conditions, sorbitol is isomerized as above. Addition of ethylene oxide yields ethoxylated products, which are called carbowaxes, are subsequently esterified with fatty acids to produce oligomers of polyoxyethylene sorbitan esters (aka polysorbates).

While those ingredients with the nomenclature “polysorbate” form sorbitan by dehydration of sorbitol during the above reactions (which is consequently ethoxylated and esterified), those ingredients herein with the nomenclature “PEG-x sorbitan fatty ester” are the product of the ethoxylation of a preformed sorbitan ester. Regardless of the nomenclature, however, all of these ingredients are related as polyethoxylated sorbitan esters. Each ingredient therefore, has the following core structure:



**Figure 1.** Polysorbates, wherein R is hydrogen or a PEG chain with or without a fatty ester end-cap.

### **Chemical and Physical Properties**

Polysorbates (several of which are often referred to by the commercial name of Tween in the literature) are amphiphilic molecules, which are fatty esters of polyoxyethylated sorbitan.<sup>25</sup> The polysorbates are, for the most part, viscous liquids that range in color from yellow to orange to tan.<sup>1</sup> They possess a faint, characteristic odor and a warm, somewhat bitter taste (Table 3). The reported physical and chemical properties of generic sorbitan monolaurate, ethoxylated and sorbitan monostearate, ethoxylated are provided in Table 4.

Since the fatty acids used in the production of cosmetic ingredients frequently contain fatty acids other than the principal acid named (ie, a mixture), each of the polysorbates may contain a complex mixture of fatty acid moieties.<sup>1,26</sup> Table 5 provides an example of the approximate ester content of polysorbate 20, 21, 40, 60, and 80. Polysorbate 21 is reported to be 30%-80% monoesters, <50% diesters, and <20% triesters.<sup>22</sup> Sorbitan monolaurate is reported to be a mixture of esters of different lengths with the highest percentage being C12 at 40%-60%.

### **Impurities**

During the manufacturing process, the polysorbates are steam stripped to remove unwanted water-soluble byproducts such as 1,4-dioxane.<sup>1</sup> Since PEGs are the condensation products of ethylene oxide and water, with the chain length controlled by the number of moles of ethylene oxide that are polymerized, they may contain trace amounts of 1,4-dioxane, a by-product of ethoxylation. 1,4-Dioxane is a known animal carcinogen.<sup>27</sup> The Food and Drug Administration (FDA) has been periodically monitoring the levels of 1,4-dioxane in cosmetic products, and the cosmetic industry reported that it is aware that 1,4-dioxane may be an impurity in PEGs and, thus, uses additional purification steps to limit it in these ingredients before blending into cosmetic formulation.<sup>28,29</sup>

## USE **Cosmetic**

The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its VCRP.<sup>5</sup> The highest number of uses were reported for polysorbate 21 at 2857 (an increase from 770 in 1998), polysorbates 60 at 1562 (an increase from 332 in 1998), and polysorbates 80 at 907 (an increase from 231 in 1998).<sup>1,2,5</sup> Almost all of the previously reviewed ingredients had increases in the number of reported uses. All of the ingredients not previously reviewed had less than 10 reported uses (Tables 6 and 7).

A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for ingredients in this group.<sup>30,31</sup> The highest concentrations of use were reported for polysorbate 20 at 19.6% in bath soaps and detergents (a decrease from >50% in 1984), polysorbate 80 at 18.1% in paste masks and mud packs (a decrease from up to 25% in 1984), polysorbate 81 at 25.6% in skin cleansing products (an increase from up to 5% in 1984), and polysorbate 85 at 21.9% skin cleansing products (a decrease from >50% in 1984).<sup>1,2,30</sup>

In the report published in 2000, the only concentration of use data that were provided was the following: "...PEG-60 sorbitan tetraoleate, PEG-40 sorbitan tetraoleate, and PEG-160 sorbitan triisostearate are used in cosmetics at concentrations of 0.5% to 10%..."<sup>2</sup> Since the data from the 2000 report are limited, the concentration of use data from the 1984 report were provided in Table 6 to give a better historical perspective.

PEG-18 sorbitan trioleate is no longer listed as a cosmetic ingredient in the *Dictionary*.<sup>4</sup> However, the VCRP reported 1 use in a moisturizer, which is a decrease from 10 uses reported in 1998.<sup>5</sup> The VCRP reported single uses for 3 ingredients that are not listed in the Dictionary, PEG-20 sorbitan laurate (used in 1 other personal cleanliness product), PEG-20 sorbitan stearate (used in 1 night skin product), and PEG-30 sorbitan beeswax (used in 1 mascara). There were no concentrations of use reported for PEG-30 sorbitan beeswax.<sup>31</sup> No further information was found.

The 44 ingredients with no reported uses or concentrations of use are listed in Table 8.

All of the polysorbates named in this report, except Sorbeth-450 tristearate, are listed in the European Union inventory of cosmetic ingredients.<sup>32</sup>

In some cases, reports of uses were received in the VCRP, but no concentration of use data were available. For example, PEG-3 sorbitan stearate was reported to be used in 3 formulations, but no use concentration data were reported. In other cases, no reported uses were received in the VCRP, however a use concentration was provided in the industry survey. For example, PEG-40 sorbitan laurate was not reported in the VCRP to be in use, but the industry survey indicated that it is used in leave-on formulations at up to 2% and rinse-off formulations up to 0.5%. It should be presumed that PEG-40 sorbitan laurate was used in at least 2 cosmetic formulations.

Polysorbates were reported to be used in cosmetic sprays, including aerosol and pump hair sprays, spray deodorants, spray body and hand products, and spray moisturizing products, and could possibly be inhaled. The highest reported to be in spray deodorants up to 4%. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles below 10 µm compared with pump sprays.<sup>33-36</sup> Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (ie, they would not enter the lungs) to any appreciable amount.<sup>33,35</sup> 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.<sup>33</sup> 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.

## **Non-Cosmetic**

The acceptable daily intake (ADI) for humans of polysorbates is 10 mg/kg.<sup>25</sup> The largest food sources of polysorbates are confectionery, ices, desserts, fine bakery wares, milk analogues, emulsified sauces, chewing gums, and fat emulsions for baking.

The polysorbates are used in the drug, food, and animal feed industries; several have been approved by the FDA as direct and indirect food additives for human consumption with certain restrictions (Table 9).

## TOXICOKINETICS

Following oral administration of polysorbate 20 to rats, the ester bond sites of polysorbates are hydrolyzed within the digestive tract by pancreatic lipase.<sup>24</sup> Free fatty acids were absorbed from the digestive tract and oxidized and excreted, mainly as carbon dioxide in exhaled breath. No migration of the polyoxyethylene sorbitan into the thymus lymph nodes has been demonstrated. No sex difference has been detected in the disposition of polysorbates in rats.

Following oral ingestion of polysorbate 20 in humans, 90% or more of the administered substance was excreted in the feces as metabolites, with the polyoxyethylene sorbitan structure maintained, and 2%-3% of these metabolites were excreted in the urine.<sup>24</sup>



### ***Penetration Enhancement***

Polysorbate 20, polysorbate 65, and polysorbate 80 enhanced the dermal penetration of albuterol sulfate through rat skin using Franz cells (Table 10).<sup>37</sup>

## **TOXICOLOGICAL STUDIES**

### **Acute Toxicity**

#### ***Oral – Non-Human***

##### **POLYSORBATE 81**

The oral LD<sub>50</sub> of polysorbate 81 was reported to be > 20 000 mg/kg for rats (n=11).<sup>23</sup>

#### ***Oral - Human***

##### **SORBITAN MONOSTEARATE, ETHOXYLATED**

No toxic effects were observed in human subjects (n=6) orally administered sorbitan monostearate, ethoxylated (20g).<sup>24</sup> The amount of gastric acid was slightly reduced. It was concluded that sorbitan monostearate, ethoxylated was not orally toxic to humans.

#### ***Dermal – Non-Human***

##### **SORBITAN MONOSTEARATE, ETHOXYLATED**

The acute dermal LD<sub>50</sub> of sorbitan monostearate, ethoxylated in Wistar albino rats (n=10/sex) was >2000 mg/kg.<sup>24</sup>

#### ***Inhalation – Non-Human***

##### **SORBITAN MONOLAUATE, ETHOXYLATED**

The inhalation LC<sub>50</sub> was reported to be 5.1 mg/L air for sorbitan monolaurate, ethoxylated administered to Crl:WI(Han) rats (n=5) for 4 h in a nose-only apparatus.<sup>22</sup> No clinical signs of systemic toxicity were observed up to the end of the 14-day observation period. No abnormalities were observed at macroscopic post mortem examination of the animals.

#### ***Intravenous – Non-Human***

##### **POLYSORBATE 20**

The intravenous LD<sub>50</sub> for polysorbate 20 in mice was reported to be 1420 mg/kg.<sup>22</sup>

### **Repeated Dose Toxicity**

In a survey of 4 laboratories of the use of vehicles for in vivo experiments, the highest no-observed-adverse-effect levels (NOAEL) of various routes of administration were assembled.<sup>38</sup> The highest oral NOAELs for polysorbate 20 were 250 and 500 mg/kg/d for 1 month and 90 days in rats, respectively, and 10 mg/kg/d for 1 month in mice (Table 11). For polysorbate 80, the highest oral NOAEL for 90 days in dogs was 5 mL/kg/d, and for 4 weeks in rats was 5 mL/kg/d. The NOAEL for intranasal administration of polysorbates 80 for 3 days to mice was 10 µL/nostril/d at 0.2%.

#### ***Oral – Non-Human***

##### **POLYSORBATE 20**

In a 22-month feeding study, the NOAEL of polysorbate 20 in male C57BL/6 Jax mice was 114285.71 mg/kg/d (10% in feed).<sup>24</sup> Decreased hematologic values were observed but not specified. No characteristic morphologic anemia was observed. The feed contained 5% or 10% polysorbate 20. No further details were provided.

##### **POLYSORBATE 80**

There were no adverse effects or mortalities related to polysorbate 80 (0.005, 0.05, or 0.15 g/kg/d) when administered by gavage to Sprague-Dawley rats (n=5) for 5 days.<sup>39</sup> There were no clinical signs and no significant findings at necropsy. There were decreased serum glucose and increased serum sodium at all dose levels, as well as decreases in uric acid in the mid- and high-dose groups. The high-dose group exhibited a modest reduction in serum calcium levels.

There were no adverse effects or mortalities reported when Sprague-Dawley rats (n=6/sex) were orally administered polysorbate 80 (148, 740, or 3700 mg/kg/d in saline) for 28 days after 28 days of a high fat diet.<sup>40</sup> It was not clear if the rats continued on the high fat diet during treatment.

In the same study, there were no adverse effects or mortalities reported when C57BL/6J mice (n=6/sex) were orally administered polysorbates 80 (400, 1600, or 6400 mg/kg/d in saline) for 28 days after 28 days of a high fat diet. In additional studies, there were no adverse effects or mortalities reported when the same strain of mice (n=5/sex) were orally administered polysorbate 20, polysorbate 40, or polysorbate 60 (1600 mg/kg/d in saline) for 28 days also after 28 days of a high fat diet.<sup>40</sup>

## **REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

### **POLYSORBATE 60**

The teratogenic and reproductive NOAEL was 7693 mg/kg/d when polysorbate 60 (0, 0.1%, 1.0% or 10% in feed; 0, 99 mg/kg, 960 mg/kg, 7693 mg/kg) was administered to pregnant Wistar rats on gestations days 7-14 in feed.<sup>24</sup> There were no effects by polysorbate 60 on the number, sex ratio and body weights of live fetuses. There were no differences between the polysorbate 60-treated and control groups observed in the numbers of resorptions, dead fetuses and live fetuses per litter, the sex ratio of live fetuses, and the fetal body weight of both sexes. External, skeletal, and internal examinations of the fetuses revealed no evidence of teratogenesis. It was concluded that polysorbate 60 had no harmful effects on the prenatal development of the rat offspring.

### **POLYSORBATE 80**

In a reproductive and developmental study where polysorbate 80 (500 and 5000 mg/kg/d in distilled water; 5 mL) was administered by gavage to Crl:CD BR VAF/PlusTM outbred albino rats (n=25) on gestation days 6-15, the maternal and the developmental NOAELs were >5000 mg/kg/d.<sup>23</sup> The control group was administered 5 mL/kg distilled water.

No maternal mortalities or treatment-related clinical signs of toxicity were observed. No effects on weight gain, organ weights (except non-adverse increased relative liver weights), and feed and water consumption. There were no differences in the number of corpora lutea per dam, number of implantations per litter, percent preimplantation loss per litter, percent resorptions per litter, and percent litters with resorptions. No adverse fetal effects were observed, including growth, viability, or development of the fetuses. There were no observed differences in malformations between treatment groups and controls.<sup>23</sup>

## **GENOTOXICITY**

### **In Vitro**

### **POLYSORBATE 20**

After conducting the series of assays of the cyto/genotoxicity of polysorbate 20 below, the authors concluded that this ingredient can induce apoptosis in human umbilical vein endothelial cells (HUVEC) and A549 lung cancer cells.<sup>41</sup> The authors stated that when the following assays are considered together, they show that polysorbate 20 can interact with DNA in treated cells to cause DNA damage and fragmentation. Therefore, they concluded that polysorbate 20 inhibits the growth of both normal and cancer cell lines by inducing apoptosis via chromatin and DNA fragmentation.

In an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, there was a dose- and time-dependent reduction in cell growth for both the HUVEC and A549 cells with IC<sub>50</sub>s of approximately 0.3 and 0.4 µL/mL polysorbate 20, respectively. There was >90% cell death observed after treatment with 2 µL/mL, and the greatest cell death was observed in the highest test group. For the assay, the cells were incubated in the various concentrations of polysorbate 20 (2, 4, 6, 8, or 10 µL/mL) for 24, 48, and 72 h, and then washed.

In a DAPI (4',6-diamidino-2-phenylindole) staining assay, morphological changes and fragmentation in the chromatin and DNA rings within the nucleus were observed in the polysorbate-treated cells of both cell lines, but morphology was unaltered in untreated cells. Polysorbate 20-treated cells showed chromatin and DNA fragmentation as a high positive control of 5% dimethyl sulfoxide (DMSO). For the assay, the cells were treated with polysorbates 20 (4 µL/mL) for various durations (not provided), then fixed and stained with DAPI.

In a DNA fragmentation assay analyzed by agarose gel electrophoresis, polysorbate 20 (concentration not clear) induced apoptosis by DNA fragmentation after incubation for 24 h. The gel showed the formation of DNA ladders of both treated cell lines.

An alkaline comet assay showed that polysorbate 20 (2 µL/mL)-treated A549 cells exhibited increased DNA cleavages compared to untreated cells and similar DNA cleavages to the positive control, hydrogen peroxide (200 mM)-treated cells. Only A549 cells were used in this assay; HUVEC cells were not used.

When polysorbate 20 (2 µL/mL)-treated A549 cells were analyzed with a fluorescein isothiocyanate (FITC)-labeled annexin V apoptosis assay and flow cytometry analysis was used to estimate early and late apoptosis, the results were similar to the results of the DAPI staining assay. Almost all of the treated cells were in early and late stages of apoptosis after 24 h; less than half of DMSO-treated control cells were in early and late stages of apoptosis for the same period of exposure. Only A549 cells were used in this assay; HUVEC cells were not used.<sup>41</sup>

### **POLYSORBATE 80**

Polysorbate 80 was not genotoxic to *Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537) up to 10 000 µg/plate (in distilled water) with and without metabolic activation.<sup>23</sup> The controls had the expected results.

Polysorbate 80 was not genotoxic to *S. typhimurium* (strains TA1535, TA1537, TA98 and TA100) and *Escherichia coli* (strain WP2 uvr A) up to 5000 µg/plate (in ethanol) with and without metabolic activation.<sup>23</sup> The controls had the expected results.

**SORBITAN MONOLAUROATE, ETHOXYLATED**

Sorbitan monolaurate, ethoxylated was not mutagenic, with or without metabolic activation, in an Ames assay using *S. typhimurium* (strains TA1535, TA1537, TA98, and TA100) and *E. coli* (strain WP2 uvr A) in 3 separate experiments.<sup>22</sup> In experiment 1, *S. typhimurium* (strains TA1535, TA1537, TA98) was tested at 10-3330 µg/plate in ethanol; and *S. typhimurium* (strain TA100) and *E. coli* were also tested at 3 and 5000 µg/plate with and without metabolic activation. In experiment 2, *S. typhimurium* (strains TA1535 and TA98) was tested at 33-5000 µg/plate in ethanol with and without metabolic activation. In experiment 3, all strains were tested again at 33-5000 µg/plate in ethanol with and without metabolic activation. Controls had the expected results.

In a chromosomal aberration assay using human lymphocytes, sorbitan monolaurate, ethoxylated was not genotoxic up to 100 µg/mL in ethanol, with and without metabolic activation, but was cytotoxic at 300 µg/mL.<sup>22</sup> Assays were run for 3, 24 and 48h. Controls had the expected results.

In 2 mammalian cell gene mutation assays using mouse lymphoma L5178Y cells, sorbitan monolaurate, ethoxylated was not found to be genotoxic.<sup>22</sup> In the first experiment, the cells were tested for 3 h at 0.3-275 µg/mL without metabolic activation and at 0.3-300 µg/mL with metabolic activation in ethanol. In the second experiment, the cells were tested for 3 h at: 0.3-150 µg/mL without metabolic activation and at 0.3-350 µg/mL with metabolic activation in ethanol. Controls had the expected results.

**SORBITAN MONOOLEATE, ETHOXYLATED**

Sorbitan monooleate, ethoxylated produced ambiguous results in a chromosome aberration assay using Chinese hamster ovary (CHO; CHO-W-B1) cells.<sup>23</sup> The number and percentages of aberrations did not change in a concentration-dependent manner. Sorbitan monooleate, ethoxylated was tested at 300-1600 µg/mL without metabolic activation and 100-1000 µg/mL in DMSO. The positive controls were mitomycin and cyclophosphamide.

Sorbitan monooleate, ethoxylated was not genotoxic in a chromosome aberration assay using CHO (CHO-W-B1) cells.<sup>42</sup> Sorbitan monooleate, ethoxylated was tested at 300-1600 µg/mL without metabolic activation and 16-500 µg/mL in DMSO. The positive controls were mitomycin and cyclophosphamide. The controls had the expected results.

**SORBITAN MONOSTEARATE, ETHOXYLATED**

Sorbitan monostearate, ethoxylated (concentration and vehicle were not specified) was not mutagenic in a bacterial gene mutation assay using *S. typhimurium* (strain TA 98) with metabolic activation.<sup>24</sup>

**CARCINOGENICITY**

No new carcinogenicity data on polysorbates were found in the published literature nor were unpublished data provided.

**IRRITATION AND SENSITIZATION****Irritation*****Dermal – Non-Human*****POLYSORBATE 60**

In a daily skin-painting study of polysorbate 60 (5% aqueous) on rabbits for 30 days, there was moderate irritation observed; skin necrosis occurred when a 10% solution was tested.<sup>24</sup> In a further study on rabbits, there were no dermal effects from a 15% aqueous solution administered for 60 consecutive days; there was mild irritation after administration of an undiluted solution. Local inflammation also occurred after long-term (time not specified) administration of undiluted polysorbate 60 solution to mouse skin (n not specified).

**SORBITAN MONOLAUROATE, ETHOXYLATED**

Sorbitan monolaurate, ethoxylated (100%; 0.5mL) had a Draize score of 0.89 out of 4 when administered to New Zealand White rabbits (n=3) for 4 h under occlusion.<sup>22</sup> Scaliness was observed in all 3 animals at 72 h after exposure and in 1 rabbit at 7 days after exposure. The test sites were observed at 1, 24, 48, and 72 h and 7 days. An untreated site on each rabbit served as the control.

**SORBITAN MONOSTEARATE, ETHOXYLATED**

When sorbitan monostearate, ethoxylated (5% and 10% aqueous) was dermally administered to rabbits (n not specified) for 30 days, the test substance caused necrosis of the skin at 10%.<sup>24</sup> The necrosis was reversible after stopping treatment. Moderate irritation was observed at 5%.

Administration of sorbitan monostearate, ethoxylated (100%) for 60 days did not cause irritation in rabbits.<sup>24</sup> No further information was provided.

Sorbitan monostearate, ethoxylated (100%; 0.5 g) did not produce any skin reaction when administered to the shaved backs (approximately 6 cm<sup>2</sup>) of New Zealand white rabbits (n=3).<sup>24</sup> The irritation score was 0.8 out of 8. The test substance was administered under occlusion for 4 h; the test site was observed for 14 days after removal.

**Dermal – Human**

In human irritation studies, polysorbate 60 (100%), polysorbate 80 (100%), and sorbitan monostearate, ethoxylated (25%) were not dermally irritating (Table 12).<sup>24,43-45</sup>

**Ocular – In Vivo****POLYSORBATE 20**

In vivo tests of polysorbate 20 (up to 10%) and polysorbate 81 (up to 100%) showed that these ingredients were not ocular irritants in rabbits (Table 13).<sup>46-48</sup> Sorbitan monostearate, ethoxylated (0.1 g in water) and sorbitan monolaurate, ethoxylated (100%; 0.1 mL) were not ocular irritant to rabbits.<sup>22,23</sup>

**Ocular – In Vitro****POLYSORBATE 20**

In vitro ocular irritation tests of polysorbate 20 had mixed results. EpiOcular tests, a red blood cell hemolysis assay, and a k562 cell assay predicted polysorbate 20 to be a non- or minimal ocular irritant at 2% and 100% (Table 13).<sup>49,50</sup> Polysorbate 20 was predicted to be an ocular irritant in a short time exposure (STE) assay using SIRC cells, Hen's Egg test-Chorioallantoic Membrane (HET-CAM) assays, and Bovine Corneal Opacity and Permeability (BCOP) assay.<sup>49</sup>

Polysorbate 20 was classified as a non-ocular irritant according to the Globally Harmonized System of Classification and Labeling of Chemicals.<sup>51</sup>

**Sensitization****Non-Human****POLYSORBATE 81**

Polysorbate 81 (2% and 4% in corn oil) was not sensitizing to female Dunkin-Hartley guinea pigs (n=10) when challenged 21 days after last induction at 100% (0.5 mL).<sup>22,23</sup> There were no signs of sensitization up to 72 h after the challenge. The positive control,  $\alpha$ -hexyl cinnamic acid (20%), had the expected results.

**SORBITAN MONOLAUROATES, ETHOXYLATED**

In a local lymph node assay, using female CBA mice (n=5) of sorbitan monolaurates, ethoxylated (25%, 50% and 100% in acetone/olive oil [4:1 v/v]; 25  $\mu$ L), the stimulation indexes (SI) were calculated to be 1.9, 6.0 and 5.0, respectively. The test substance was considered sensitizing.<sup>22</sup> The authors noted that the response of the 100% group did not follow the expected dose-response relationship, which they also noted was common in this kind of study. The response might be less due to differences in skin penetration (no vehicle present) or viscosity. The estimated concentration of polysorbates that would give an SI of 3 was calculated to be 34%. The positive control, hexyl cinnamic aldehyde, had the expected results.

**Human****POLYSORBATE 81**

In a human patch test (n=50), polysorbate 81 (100%) was not sensitizing.<sup>23</sup> There were no signs of irritation or sensitization observed on any subject. The test material was administered under occlusion for 3 days. After 7 days, challenge patches were administered for 72 h.

In a human patch test (n=10), polysorbate 81 (100%) was not sensitizing.<sup>23</sup> There were no signs of irritation or sensitization observed on any subject. The test material was administered under occlusion for 5 days. After 10 days, challenge patches were administered for 48 h.

In a human patch test (n=10), polysorbate 81 (12%; vehicle not specified) was not sensitizing.<sup>23</sup> There were no signs of irritation or sensitization observed on any subject. The test material was administered under occlusion for 5 days. After 10 days, challenge patches were administered for 48 h.

**SUMMARY OF NEW DATA**

This is a re-review of the safety of polysorbates as used in cosmetics. Safety assessments of various polysorbates were published in 1984, 2000, and 2001 with conclusions of safe as used. These safety assessments have been combined, and additional polysorbate ingredients have been identified and included, in this assessment for a total of 79 ingredients. All of these polysorbate ingredients are related in that they have a common core structure of sorbitan etherified with PEG chains.

The highest number of uses were reported for polysorbate 21 at 2857 (an increase from 770 in 1998), polysorbate 60 at 1562 (an increase from 332 in 1998), and polysorbates 80 at 907 (an increase from 231 in 1998). Almost all of the previously reviewed ingredients had increases in the number of reported uses. The highest maximum concentrations of use were reported for polysorbate 20 at 19.6% (a decrease from >50% in 1984), polysorbate 80 at 18.1% (a decrease from up to 25% in 1984), polysorbate 81 at 25.6% (an increase from up to 5% in 1984), and polysorbate 85 at 21.9% (a decrease from >50% in 1984) in rinse-off products. The highest maximum concentration of use for leave-on products was 11.9% polysorbate 80 in perfumes.

Polysorbate 20, polysorbate 65, and polysorbate 80 enhanced the dermal penetration of albuterol sulfate through rat skin.

The oral LD<sub>50</sub> of polysorbate 81 was reported to be > 20 000 mg/kg for rats. The acute dermal LD<sub>50</sub> of sorbitan monostearate, ethoxylated in rats was >2000 mg/kg. The inhalation LC<sub>50</sub> was reported to be 5.1 mg/L air for sorbitan monolaurate, ethoxylated administered to rats for 4 h. The intravenous LD<sub>50</sub> for mice was reported to be 1420 mg/kg.

There were no adverse effects or mortalities related to polysorbate 80 (up to 0.15 g/kg) when administered by gavage to rats for 5 days or in rats orally administered polysorbate 80 (up to 3700 mg/kg/d) for 28 days. There were no adverse effects observed in mice orally administered polysorbate 80 (up to 6400 mg/kg/d), or polysorbate 20, polysorbate 40, or polysorbate 60 (1600 mg/kg/d) for 28 days.

The teratogenic and reproductive NOAEL of polysorbate 60 was 7693 mg/kg/d (ie, the highest dose tested) when administered to pregnant rats on gestations days 7-14 in feed. In a reproductive and developmental study where polysorbate 80 was administered by gavage to rats on gestation days 6-15, the maternal and the developmental NOAELs were >5000 mg/kg/d.

Polysorbate 80 was not genotoxic to *S. typhimurium*, up to 10 000 µg/plate, and *E. coli*, up to 5000 µg/plate, with and without metabolic activation

The combined results of MTT, DAPI, DNA fragmentation, alkaline comet, and FITC-labeled annexin V apoptosis assays led to the conclusion that polysorbate 20 had the capability of interaction with DNA in treated HUVEC and A549 lung cancer cells that resulted in DNA damage and fragmentation. It was concluded that polysorbates 20 inhibits the growth of both normal and cancer cell lines by inducing apoptosis via chromatin and DNA fragmentation.

In a 30-d skin-painting study of polysorbate 60 in rabbits, there was moderate irritation observed at 5% and skin necrosis at 10%. In a clinical test, polysorbate 60 was not a dermal irritant at 1%.

In vivo tests of polysorbate 20 (up to 10%) and polysorbate 81 (up to 100%) showed these ingredients not to be ocular irritants. In vitro predictions tests had mixed results. EpiOcular tests, a red blood cell hemolysis assay, and a k562 cell assay predicted that polysorbate 20 to be a non- or minimal ocular irritant at 2% and 100%. STE at 5%, HET-CAM at 100%, and BCOP (100%) assays predicted that polysorbate 20 would be a mild to severe ocular irritant.

Polysorbate 81 up to 4% was not sensitizing to guinea pigs when challenged 21 days after last induction at 100%. Polysorbate 81 at 100% was not sensitizing in human patch tests.

### **DISCUSSION**

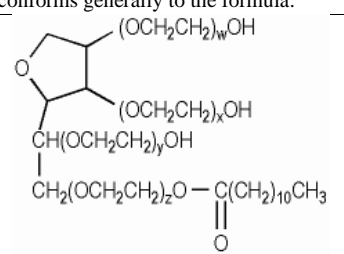
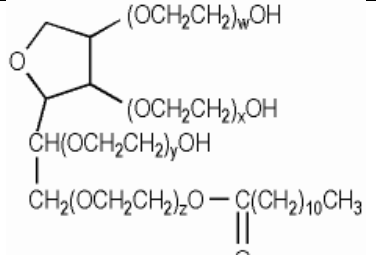
*Discussion may be developed at the March, 2015 Panel meeting.*

### **CONCLUSION**

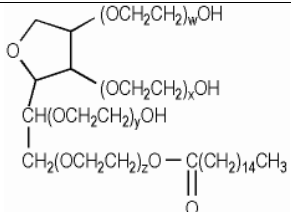
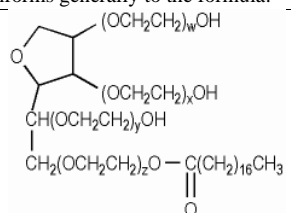
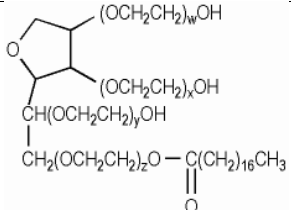
*Conclusion may be developed at the March, 2015 Panel meeting.*

## TABLES AND FIGURES

**Table 1.** The definitions, structure, and functions of the polysorbates in this safety assessment.<sup>4</sup>

Ingredient and CAS No.	Definition	Function
<b>Polysorbate Monoesters</b>		
Polysorbate 21 9005-64-5 (generic)	<p>A mixture of laurate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 4 moles of ethylene oxide. It conforms generally to the formula:</p> <div style="text-align: center;">  </div> <p>where <math>w + x + y + z</math> has an average value of 4.</p>	Fragrance ingredient; surfactant-emulsifying agent
Sorbeth-6 laurate	The ester of lauric acid and a polyethylene glycol ether of sorbitol containing an average of 6 moles of ethylene oxide.	Surfactant-emulsifying agent; surfactant-solubilizing agent
PEG-10 sorbitan laurate 9005-64-5 (generic)	PEG-10 Sorbitan Laurate is an ethoxylated sorbitan ester of lauric acid with an average of 10 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent
PEG-40 sorbitan laurate 9005-64-5 (generic)	An ethoxylated sorbitan ester of lauric acid with an average of 40 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent
Polysorbate 20 9005-64-5 (generic)	<p>A mixture of laurate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 20 moles of ethylene oxide. It conforms generally to the formula:</p> <div style="text-align: center;">  </div> <p>where <math>w + x + y + z</math> has an average value of 20.</p>	Fragrance ingredient; surfactant-emulsifying agent; surfactant-solubilizing agent
PEG-44 sorbitan laurate 9005-64-5 (generic)	An ethoxylated sorbitan ester of lauric acid with an average of 44 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent
PEG-75 sorbitan laurate 9005-64-5 (generic)	An ethoxylated sorbitan ester of lauric acid with an average of 75 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent
PEG-80 sorbitan laurate 68154-33-6 (generic) 9005-64-5 (generic)	An ethoxylated sorbitan ester of lauric acid with an average of 80 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent

**Table 1.** The definitions, structure, and functions of the polysorbates in this safety assessment.<sup>4</sup>

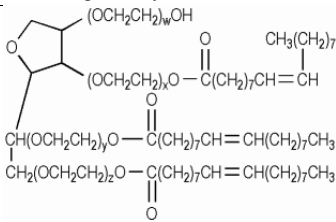
Ingredient and CAS No.	Definition	Function
Polysorbate 40 9005-66-7 (generic)	A mixture of palmitate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 20 moles of ethylene oxide. It conforms generally to the formula: <div></div> where $w + x + y + z$ has an average value of 20.	Surfactant-emulsifying agent; surfactant-solubilizing agent
PEG-80 sorbitan palmitate 9005-66-7 (generic)	An ethoxylated sorbitan monoester of palmitic acid with an average of 80 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-solubilizing agent
Sorbeth-3 isostearate	The ester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 3 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-2 sorbitan isostearate 66794-58-9 (generic)	An ethoxylated sorbitan monoester of isostearic acid with an average of 2 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-5 sorbitan isostearate 66794-58-9 (generic)	An ethoxylated sorbitan monoester of isostearic acid with an average of 5 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-20 sorbitan isostearate 66794-58-9 (generic)	An ethoxylated sorbitan monoester of isostearic acid with an average of 5 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-emulsifying agent; surfactant-solubilizing agent
PEG-3 sorbitan stearate 9005-67-8 (generic)	An ethoxylated sorbitan monoester of stearic acid with an average of 3 moles of ethylene oxide.	Fragrance ingredient; surfactant-emulsifying agent
PEG-4 sorbitan stearate 9005-67-8 (generic)	An ethoxylated sorbitan monoester of stearic acid with an average of 4 moles of ethylene oxide.	Fragrance ingredient; surfactant-emulsifying agent
Polysorbate 61 9005-67-8 (generic)	A mixture of stearate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 4 moles of ethylene oxide. It conforms generally to the formula: <div></div> where $w + x + y + z$ has an average value of 4.	Fragrance ingredient; surfactant-emulsifying agent
PEG-6 sorbitan stearate 9005-67-8 (generic)	An ethoxylated sorbitan monoester of stearic acid with an average of 6 moles of ethylene oxide.	Fragrance ingredient; surfactant-emulsifying agent
Polysorbate 60 9005-67-8 (generic)	A mixture of stearate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 20 moles of ethylene oxide. It conforms generally to the formula: <div></div> where $w + x + y + z$ has an average value of 20.	Fragrance ingredient; surfactant-emulsifying agent; surfactant-solubilizing agent

**Table 1.** The definitions, structure, and functions of the polysorbates in this safety assessment.<sup>4</sup>

Ingredient and CAS No.	Definition	Function
Polysorbate 65 9005-71-4	A mixture of stearate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the triester, condensed with approximately 20 moles of ethylene oxide. It conforms generally to the formula:  <div data-bbox="662 254 954 478" data-label="Chemical-Block"> <math display="block">  \begin{array}{c}  \text{(OCH}_2\text{CH}_2\text{)}_w\text{OH} \\    \\  \text{O} \\    \\  \text{CH}(\text{OCH}_2\text{CH}_2)_x\text{O}-\text{C}(=\text{O})\text{(CH}_2\text{)}_{16}\text{CH}_3 \\    \\  \text{CH}(\text{OCH}_2\text{CH}_2)_y\text{O}-\text{C}(=\text{O})\text{(CH}_2\text{)}_{16}\text{CH}_3 \\    \\  \text{CH}_2(\text{OCH}_2\text{CH}_2)_z\text{O}-\text{C}(=\text{O})\text{(CH}_2\text{)}_{16}\text{CH}_3  \end{array}  </math> </div> where $w + x + y + z$ has an average value of 20.	Surfactant-emulsifying agent
PEG-40 sorbitan stearate 9005-67-8 (generic)	An ethoxylated sorbitan ester of stearic acid with an average of 40 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent
PEG-60 sorbitan stearate 9005-67-8 (generic)	An ethoxylated sorbitan ester of stearic acid with an average of 60 moles of ethylene oxide.	Fragrance ingredient; surfactant-cleansing agent; surfactant-solubilizing agent
PEG-3 sorbitan oleate 9005-65-6 (generic)	An ethoxylated sorbitan ester of oleic acid with an average of 3 moles of ethylene oxide.	Fragrance ingredient; surfactant-emulsifying agent
Polysorbate 81 9005-65-6 (generic)	A mixture of oleate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 5 moles of ethylene oxide. It conforms generally to the formula:  <div data-bbox="630 854 987 1045" data-label="Chemical-Block"> <math display="block">  \begin{array}{c}  \text{(OCH}_2\text{CH}_2\text{)}_w\text{OH} \\    \\  \text{O} \\    \\  \text{CH}(\text{OCH}_2\text{CH}_2)_x\text{OH} \\    \\  \text{CH}(\text{OCH}_2\text{CH}_2)_y\text{OH} \\    \\  \text{CH}_2(\text{OCH}_2\text{CH}_2)_z\text{O}-\text{C}(=\text{O})\text{(CH}_2\text{)}_7\text{CH}=\text{CH}(\text{CH}_2\text{)}_7\text{CH}_3  \end{array}  </math> </div> where $w + x + y + z$ has an average value of 5.	Fragrance ingredient; surfactant-emulsifying agent
PEG-6 sorbitan oleate 9005-65-6 (generic)	An ethoxylated sorbitan ester of oleic acid with an average of 6 moles of ethylene oxide.	Fragrance ingredient; surfactant-emulsifying agent
PEG-20 sorbitan oleate	An ethoxylated sorbitan ester of oleic acid with an average of 20 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-emulsifying agent; surfactant solubilizing agent
Polysorbate 80 9005-65-6 (generic)	A mixture of oleate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the monoester, condensed with approximately 20 moles of ethylene oxide. It conforms generally to the formula:  <div data-bbox="630 1344 987 1535" data-label="Chemical-Block"> <math display="block">  \begin{array}{c}  \text{(OCH}_2\text{CH}_2\text{)}_w\text{OH} \\    \\  \text{O} \\    \\  \text{CH}(\text{OCH}_2\text{CH}_2)_x\text{OH} \\    \\  \text{CH}(\text{OCH}_2\text{CH}_2)_y\text{OH} \\    \\  \text{CH}_2(\text{OCH}_2\text{CH}_2)_z\text{O}-\text{C}(=\text{O})\text{(CH}_2\text{)}_7\text{CH}=\text{CH}(\text{CH}_2\text{)}_7\text{CH}_3  \end{array}  </math> </div> where $w + x + y + z$ has an average value of 20.	Denaturant; fragrance ingredient; surfactant-emulsifying agent; surfactant solubilizing agent
PEG-40 sorbitan oleate	An ethoxylated sorbitan ester of oleic acid with an average of 40 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-emulsifying agent; surfactant solubilizing agent
PEG-20 sorbitan cocoate	An ethoxylated sorbitan ester of coconut acid with an average of 20 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-solubilizing agent
Sorbeth-2 cocoate	The ester of the fatty acids derived from cocos nucifera (coconut) oil and a polyethylene glycol ether of Sorbitol containing an average of 2 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-40 sorbitan lanolate 8036-77-9	An ethoxylated sorbitan derivative of lanolin acid with an average of 40 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant solubilizing agent



**Table 1.** The definitions, structure, and functions of the polysorbates in this safety assessment.<sup>4</sup>

Ingredient and CAS No.	Definition	Function
PEG-75 sorbitan lanolate 8051-13-6	An ethoxylated sorbitan derivative of lanolin acid with an average of 75 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant solubilizing agent
Sorbeth-2 beeswax	An ethoxylated sorbitan derivative of beeswax with an average of 2 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-6 beeswax 8051-15-8	An ethoxylated sorbitan derivative of beeswax with an average of 6 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-8 beeswax	An ethoxylated sorbitan derivative of beeswax with an average of 8 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-20 beeswax	An ethoxylated sorbitan derivative of beeswax with an average of 20 moles of ethylene oxide.	Surfactant-emulsifying agent; surfactant-solubilizing agent
<b>Polysorbate Diester</b>		
PEG-40 sorbitan diisostearate	An ethoxylated sorbitan diester of isostearic acid with an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent; surfactant solubilizing agent
<b>Polysorbate Triesters</b>		
PEG-4 sorbitan triisostearate	The triester of isostearic acid and a polyethylene glycol ether of sorbitol with an average of 4 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-20 sorbitan triisostearate	The triester of isostearic acid and a polyethylene glycol ether of sorbitol with an average of 20 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-160 sorbitan triisostearate	The triester of isostearic acid and a polyethylene glycol ether of sorbitol with an average of 160 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-solubilizing agent
PEG-3 sorbitan tristearate	The triester of stearic acid and a polyethylene glycol ether of sorbitol with an average of 3 moles of ethylene oxide.	Skin-conditioning agent-emollient
Sorbeth-3 tristearate	The triester of stearic acid and a polyethylene glycol ether of sorbitol containing an average of 3 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-160 tristearate	The triester of stearic acid and a polyethylene glycol ether of sorbitol with an average of 160 moles of ethylene oxide.	Surfactant-cleansing agent; surfactant-solubilizing agent
Sorbeth-450 tristearate	The triester of stearic acid and a polyethylene glycol ether of sorbitol with an average of 450 moles of ethylene oxide.	Surfactant-dispersing agent; surfactant - emulsifying agent; surfactant – foam booster; viscosity increasing agent – aqueous
PEG-2 sorbitan trioleate	A triester of oleic acid and a polyethylene glycol ether of sorbitol with an average of 2 moles of ethylene oxide.	Surfactant-emulsifying agent
Polysorbate 85 9005-70-3	A mixture of oleate esters of sorbitol and sorbitol anhydrides, consisting predominantly of the triester, condensed with approximately 20 moles of ethylene oxide. It conforms generally to the formula:	Surfactant-dispersing agent; surfactant-emulsifying agent
 <p>where <math>w + x + y + z</math> has an average value of 20.</p>		
<b>Polysorbate Tetraesters</b>		
Sorbeth-20 tetrastearate	The tetraester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 20 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-30 tetrastearate	The tetraester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 30 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-40 tetrastearate	The tetraester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-50 tetrastearate	The tetraester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 50 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-60 sorbitan tetrastearate	The tetraester of stearic acid and a polyethylene glycol ether of sorbitol, with an average of 60 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-60 tetrastearate	The tetraester of stearic acid and a polyethylene glycol ether of sorbitol containing an average of 60 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-4 tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol containing an average of 4 moles of ethylene oxide.	Surfactant-emulsifying agent

**Table 1.** The definitions, structure, and functions of the polysorbates in this safety assessment.<sup>4</sup>

<b>Ingredient and CAS No.</b>	<b>Definition</b>	<b>Function</b>
Sorbeth-6 tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol containing an average of 6 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-30 sorbitan tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol, with an average of 30 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-30 tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol containing an average of 30 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-40 sorbitan tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol, with an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-40 tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol with an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-60 sorbitan tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol, with an average of 60 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-60 tetraoleate	The tetraester of oleic acid and a polyethylene glycol ether of sorbitol with an average of 60 moles of ethylene oxide.	Surfactant-emulsifying agent
<b>Polysorbate Pentaesters</b>		
Sorbeth-20 pentaistearate	The pentaester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 20 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-30 pentaistearate	The pentaester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 30 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-40 pentaistearate	The pentaester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-50 pentaistearate	The pentaester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 50 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-40 pentaoleate	The pentaester of oleic acid and a polyethylene glycol ether of sorbitol containing an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-30 tetraoleate laurate	The oleic acid tetraester and lauric acid ester of sorbitol ethoxylated with an average of 30 moles of ethylene oxide.	Surfactant-emulsifying agent
<b>Polysorbate Hexaesters</b>		
Sorbeth-2 hexalaurate	The hexaester of lauric acid and a polyethylene glycol ether of sorbitol containing an average 2 moles of ethylene oxide.	Skin-conditioning agent-emollient
Sorbeth-2 hexaisostearate	The hexaester of isostearic acid and a polyethylene glycol ether of sorbitol containing an average of 2 moles of ethylene oxide.	Skin-conditioning agent-emollient
PEG-40 sorbitan perisostearate	A mixture of isostearic acid esters of sorbitol condensed with an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-6 hexastearate	The hexaester of stearic acid and a polyethylene glycol ether of sorbitol containing an average of 6 moles of ethylene oxide.	Surfactant-emulsifying agent
Sorbeth-150 hexastearate	The hexaester of stearic acid and a polyethylene glycol ether of sorbitol containing an average of 150 moles of ethylene oxide.	Viscosity increasing agent-aqueous
Sorbeth-2 hexaoleate	The hexaester of oleic acid and a polyethylene glycol ether of sorbitol containing an average of 2 moles of ethylene oxide.	Skin-conditioning agent-emollient
Sorbeth-40 hexaoleate (formerly PEG-40 sorbitol hexaoleate)	The hexaester of oleic acid and sorbeth-40.	Surfactant-emulsifying agent
Sorbeth-50 hexaoleate (formerly PEG-50 sorbitol hexaoleate)	The hexaester of oleic acid with a polyethylene glycol ether of sorbitol containing an average of 50 moles of ethylene oxide.	Surfactant-emulsifying agent
PEG-40 sorbitan peroleate	A mixture of oleic acid esters of sorbitol condensed with an average of 40 moles of ethylene oxide.	Surfactant-emulsifying agent; surfactant solubilizing agent
Sorbeth-2 hexacaprylate/caprates	The hexaester of a mixture of caprylic and capric acids with a polyethylene glycol ether of sorbitol containing an average of 2 moles of ethylene oxide.	Skin-conditioning agent-emollient
Sorbeth-12 hexacocoate	The hexaester of coconut acid with a polyethylene glycol ether of sorbitol containing an average of 12 moles of ethylene oxide.	Skin-conditioning agent-emollient
<b>Other</b>		
Sorbeth-2/oleate/dimer dilinoleate crosspolymer	The crosslinked polymer of a 2-mole ethoxylate of sorbitol, oleic acid, and dilinoleic acid.	Skin-conditioning agent – emollient

**Table 2.** Previous safety assessment of polysorbates and component moieties of the ingredients in this safety assessment.

Ingredients	Conclusion	Maximum concentration in report	Reference
<b>Previous safety assessment of polysorbates</b>			
<b>Polysorbates</b> – polysorbate 20, 21, 40, 60, 61, 65, 80, 81, 85	Safe as used.	>50%	<sup>1</sup>
<b>Polysorbates</b> – above plus PEG-20 sorbitan cocoate, PEG-40 sorbitan diisostearate, PEG-2 sorbitan isostearate, PEG-5 sorbitan isostearate, PEG-20 sorbitan isostearate, PEG-40 sorbitan lanolate, PEG-75 sorbitan lanolate, PEG-10 sorbitan laurate, PEG-40 sorbitan laurate, PEG-44 sorbitan laurate, PEG-75 sorbitan laurate, PEG-80 sorbitan laurate, PEG-3 sorbitan oleate, PEG-6 sorbitan oleate, PEG-80 sorbitan palmitate, PEG-40 sorbitan perisostearate, PEG-40 sorbitan peroleate, PEG-3 sorbitan stearate, PEG-6 sorbitan stearate, PEG-40 sorbitan stearate, PEG-60 sorbitan stearate, PEG-30 sorbitan tetraoleate, PEG-40 sorbitan tetraoleate, PEG-60 sorbitan tetraoleate, PEG-60 sorbitan tetrastearate, PEG-20 sorbitan triisostearate, PEG-160 sorbitan triisostearate, PEG-40 sorbitol hexaoleate (currently sorbeth-40 hexaoleate), PEG-50 sorbitol hexaoleate (currently sorbeth-50 hexaoleate), PEG-30 sorbitol tetraoleate laurate (currently sorbeth-30 tetraoleate laurate), PEG-60 sorbitol tetrastearate (currently sorbeth-60 tetrastearate)	Safe as used.	10%	<sup>2</sup>
Sorbeth-6 beeswax, Sorbeth-8 beeswax, Sorbeth-20 beeswax	Safe for use as cosmetic ingredients under the present practices of use. The Expert Panel recommends that cosmetic formulations containing PEG-6, PEG-20, or PEG-75 not be used on damaged skin.*	11%	<sup>6</sup>
<b>Safety assessments of components</b>			
Beeswax, candelilla wax, carnauba wax, and Japan wax	Safe as used.	56%	<sup>8,14</sup>
Coconut oil, acid and related ingredients	Safe as used.	100%	<sup>8,10,11,32</sup>
Isostearic acid	Safe as used.	26%	<sup>8,13</sup>
Lanolin acid	Safe as used.	65%	<sup>8,12</sup>
Oleic acid, lauric acid, myristic acid, stearic acid	Safe in the present practices of use and concentration.	> 50%; 43%	<sup>9,16</sup>
Polyethylene glycols (PEG) - triethylene glycol and polyethylene (PEGs) -4, -6, -7, -8, -9, -10, -12, -14, -16, -18, -20, -32, -33, -40, -45, -55, -60, -75, -80, -90, -100, -135, -150, -180, -200, -220, -240, -350, -400, -450, -500, -800, -2M, -5M, -7M, -9M, -14M, -20M, -23M, -25M, -45M, -65M, -90M, -115M, -160M, and -180M and any PEG >= 4	Safe in the present practices of use and concentration.	85%	<sup>7,17</sup>
Sorbitan esters - sorbitan caprylate, sorbitan cocoate, sorbitan diisostearate, sorbitan dioleate, sorbitan distearate, sorbitan isostearate, sorbitan laurate, sorbitan oleate, sorbitan olivate, sorbitan palmitate, sorbitan sesquiosostearate, sorbitan stearate, sorbitan sesquioleate, sorbitan triisostearate, sorbitan trioleate, and sorbitan tristearate	Safe as used.	9.1%	<sup>19-21</sup>
Stearates - butyl stearate, cetyl stearate, isobutyl stearate, isocetyl stearate, isopropyl stearate, myristyl stearate, and octyl stearate	Safe as used.	87%	<sup>8,15</sup>
Alkyl Esters	Safe as used.	78%	<sup>18</sup>

\* In 2010, the Panel concluded that PEGs were safe as used and removed the caveat that PEGs should not be used on damaged skin.<sup>7</sup>

**Table 3.** Chemical and physical properties of some polysorbates.

Property	Value	Reference
<b>Polysorbate 21</b>		
Physical Form	Liquid/Oily liquid	<sup>22</sup>
Molecular Weight g/mol	390.5	<sup>22</sup>
Water Solubility	Dispersible	<sup>53</sup>
Other Solubility		
Ethanol	Soluble	<sup>53</sup>
Corn oil	Soluble	<sup>53</sup>

**Table 3.** Chemical and physical properties of some polysorbates.

Property	Value	Reference
<b>Sorbeth-6 laurate PEG-10 sorbitan laurate</b>		
Physical Form	Liquid	53
Color	Clear yellow	53
Odor	Mild	53
Water Solubility g/L @ °C & pH	Soluble	53
Other Solubility		
Acetone	Soluble	53
Ethyl acetate	Soluble	53
Mineral oil	Insoluble	53
<b>Polysorbate 20</b>		
Physical Form	Liquid	22,54
Color	Lemon-amber	22,54
Odor	Characteristic	22,54
Molecular Weight g/mol	~1228	54
Molecular Volume m <sup>3</sup> /kmol		
Density/Specific Gravity @ 25°C	1.095	22,54
Water Solubility	Soluble	53,54
Other Solubility		
Ethanol	Soluble	53,54
Ethyl acetate	Soluble	53,54
<b>Polysorbate 40</b>		
Physical Form	Oily liquid or Vaseline-like	55-58
Odor	Characteristic	2,53
Density/Specific Gravity @ °C	1.05	58
Water Solubility	Soluble	53
Other Solubility		
Methanol	Soluble	53
Ethanol	Soluble	53
Mineral oil	Insoluble	53
<b>Polysorbate 61</b>		
Physical Form	Waxy solid	53,55,59
Color	Tan	53
Water Solubility	Dispersable	60
Other Solubility		
Ethylene glycol	Insoluble	60
Propylene glycol	Insoluble	60
<b>Polysorbate 60</b>		
Physical Form	Oily liquid	55
	Semigel	61
	Wax	62
Color	Lemon yellow	53
<b>Polysorbate 65</b>		
Physical Form	Waxy solid	55,56
Color	Tan	53
Odor	Faint, characteristic	53
Water Solubility	Dispersible	53
Other Solubility		
Ethanol	Soluble	53
Methanol	Soluble	53
Vegetable and mineral oil	Soluble	53

**Table 3.** Chemical and physical properties of some polysorbates.

Property	Value	Reference
<b>Polysorbate 81</b>		
Physical Form	Liquid	23
	May gel at room temperature	56
Color	Clear	23
Odor	Faint	53
Density/Specific Gravity @ 20°C	1.0356	23
@ 25 °C	1.032	23
@ 20 °C	10299	23
@ 25 °C	1.0264	23
Viscosity kg/(s m) @ 20°C	0.672	23
	0.84	23
@ 25°C	0.328	23
Discuss different Samples	0.383	23
Vapor pressure mmHg@ °C	0.002	23
Vapor Density mmHg		
Melting Point °C	-33.9	23
	-32.7	23
Boiling Point °C		
Water Solubility g/L	~0.100	23
	~0.035	23
@ 20°C & pH 8.29-9.39	>0.500	23
Other Solubility		
Ether	Dispersible	53
Ethylene glycol	Dispersible	53
Ethanol	Soluble	53
<b>PEG-20 sorbitan oleate</b>		
Density/Specific Gravity @ °C	1.1/1.064	2
Other Solubility		
Dimethyl sulfoxide	Soluble	53
Ethanol	Soluble	53
Mineral oil	Soluble	53
Toluene	Soluble	53
<b>Polysorbate 80</b>		
Physical Form	Viscous, oily liquid	55,57,58,61-64
Color	Lemon to orange/amber	53,65
Odor	Characteristic	53
Density/Specific Gravity @ °C	1.08	63
	1.06-1.10	64,65
	1.07-1.09	58
Viscosity kg/(s m)@ °C	0.3-0.5	65
Water Solubility	Soluble	53
<b>PEG-40 sorbitan lanolate</b>		
Physical Form	Soft paste	60
Water Solubility @ 65°C	Soluble	60
Other Solubility @ 65°C		
Dioxane	Soluble	60
Carbon tetrachloride	Soluble	60
<b>Sorbeth-6 beeswax</b>		
Physical Form	Waxy solid	66
Color	Tan	66
Odor	Fatty	66
Water Solubility	Insoluble	66
Other Solubility		
Corn oil	Soluble	66
Ethylene glycol	Insoluble	66
Mineral oil	Insoluble	66
<b>Sorbeth-20 beeswax</b>		
Physical Form	Waxy solid	66
Color	Tan	66
Odor	Mild, fatty	66
Water Solubility g/L @ °C & pH	Insoluble	66
Other Solubility		66
Warm corn oil	Soluble	
Mineral oil	Dispersible	

**Table 3.** Chemical and physical properties of some polysorbates.

Property	Value	Reference
<b>Polysorbate 85</b>		
Physical Form	Liquid	55,57
	May gel at room temperature	53,56
Color	Clear amber	53
Odor	Characteristic	53
Water Solubility	Dispersible	53
Other Solubility		53
Vegetable and mineral oils	Soluble	
<b>PEG-40 sorbitan peroleate</b>		
Physical Form	Viscous, oily liquid	60
Color	Clear yellow	60
Odor	Faint characteristic	60
Water Solubility	Dispersible	60
Other Solubility		60
Mineral oil	Soluble	

**Table 4.** Chemical and physical properties of generic Sorbitan monolaurate, ethoxylated ingredients.

Property	Value	Reference
<b>Sorbitan monolaurate, ethoxylated</b>		
Physical Form	Liquid	22
Water Solubility g/L @ 20 °C & pH 6.3 and 7.9	<2.0	22
<b>Sorbitan monostearate, ethoxylated</b>		
Physical Form	Solid (wax)	24
Color	Colorless	24
Odor	Odorless	24
Density/Specific Gravity @ 23°C	1.007	24
@ 25°C	1.07	24
Vapor pressure mmHg @ 20°C	<0. 0.75	24
@ 20°C	<0.1	24
Melting Point °C	45-50	24
	39.6	24
Boiling Point °C	90.4	24
Water Solubility g/L @ 23°C	0.300	24
Other Solubility g/L		
Petroleum ether @ 23°C	1.800	24
Methanol @ 23°C	0.200	24
log K <sub>ow</sub> @ 23 °C & pH 6.4	0.03	24
Disassociation constants (pKa @ 23°C	0.199 x 10 <sup>-9</sup>	24

**Table 5.** The approximate ester content of some polysorbates.<sup>22,26</sup>

Ingredient	Laurate (%)	Myristate (%)	Palmitate (%)	Stearate (%)	Oleate (%)	Other esters (%)
Polysorbate 20	39±2	26±1	12±1	12±2	ND	11±2
Polysorbate 21	40-60	14-25	6-15	0-7	0-11	0-24
Polysorbate 40	<1	2	87±2	10±1	ND	<1
Polysorbate 60	2±1	4±1	43±1	51±2	ND	<1
Polysorbate 80	<1	2	22±2	11±2	66±1	<1

ND=none detected

**Table 6.** Current and historical frequency and concentration of use of polysorbates according to duration and exposure.<sup>1,2,5,6,14,30</sup>

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2014	1998**	2014	1981***	2014	1998	2014	1981
	<b>Polysorbate 20</b>				<b>Polysorbate 21</b>			
<b>Totals*</b>	<b>2857</b>	<b>770</b>	<b>0.00001-19.6</b>	<b>0.09-&gt;50</b>	<b>54</b>	<b>4</b>	<b>0.33-8</b>	<b>0.1-1</b>
<b>Duration of Use</b>								
Leave-On	1571	446	0.00001-9.1	0.09->50	16	4	0.33-2	0.1-1
Rinse-Off	1197	297	0.0006-19.6	0.09-25	38	NR	0.5-8	NR
Diluted for (Bath) Use	89	27	0.0097-8.9	0.1-50	NR	NR	NR	NR
<b>Exposure Type</b>								
Eye Area	211	39	0.00015-3.5	0.1-10	4	NR	0.5	NR
Incidental Ingestion	32	12	0.01-5.8	0.09-5	NR	NR	NR	NR
Incidental Inhalation-Spray	25; 545 <sup>a</sup> ; 354 <sup>c</sup>	22; 169 <sup>a</sup> ; 50 <sup>c</sup>	0.00001-3 <sup>d</sup>	0.09-1; <0.1->50 <sup>a</sup> ; 0.09-5 <sup>c</sup>	5 <sup>a</sup>	4 <sup>a</sup>	0.33 <sup>g</sup>	0.1-1 <sup>a</sup>
Incidental Inhalation-Powder	51; 5 <sup>b</sup> ; 545 <sup>c</sup>	43; 50 <sup>c</sup>	0.00075- 0.0027; 0.6-2 <sup>b</sup>	0.1-1; 0.09-5 <sup>c</sup>	3 <sup>b</sup>	NR	NR	NR
Dermal Contact	2057	493	0.00001-19.6	0.09-5	13	4	0.38-2	NR
Deodorant (underarm)	11 <sup>a</sup>	3 <sup>a</sup>	0.00018-4 <sup>c</sup> ; 0.00082-3 <sup>f</sup>	0.1-5 <sup>a</sup>	NR	NR	NR	NR
Hair - Non-Coloring	634	205	0.006-12.6	0.09-25	14	NR	0.33-8	NR
Hair-Coloring	99	50	0.4-3.8	0.09-5	24	NR	2.4	NR
Nail	10	6	0.000041-3.3	0.09-5	NR	NR	NR	NR
Mucous Membrane	683	66	0.0006-19.6	0.09->50	3	NR	NR	NR
Baby Products	31	3	0.00078-12.6	0.1-25	NR	NR	NR	NR
	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1981</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1981</b>
	<b>Polysorbate 40</b>				<b>Polysorbate 60</b>			
<b>Totals*</b>	<b>78</b>	<b>32</b>	<b>0.008-5</b>	<b>0.09-10</b>	<b>1562</b>	<b>332</b>	<b>0.0000001-6</b>	<b>0.09-25</b>
<b>Duration of Use</b>								
Leave-On	63	24	0.008-5	0.09-10	1155	255	0.00009-4	0.09-25
Rinse-Off	15	8	1.5-3	0.09-5	404	77	0.0000001-6	0.09-5
Diluted for (Bath) Use	NR	NR	NR	NR	3	NR	0.0015-0.06	0.1-10
<b>Exposure Type</b>								
Eye Area	12	1	0.015-3.75	1-5	71	35	0.0021-3.8	0.09-10
Incidental Ingestion	1	NR	NR	NR	15	NR	0.2-0.4	0.09-5
Incidental Inhalation-Spray	23 <sup>a</sup> ; 21 <sup>c</sup>	13 <sup>a</sup> ; 3 <sup>c</sup>	0.5-2.5 <sup>a</sup>	0.1-10 <sup>a</sup> ; 0.1-5 <sup>c</sup>	1; 604 <sup>a</sup> ; 311 <sup>c</sup>	93 <sup>a</sup> ; 59 <sup>c</sup>	0.083-0.8 <sup>b</sup> ; 0.0005-4 <sup>a</sup> ; 2.4 <sup>c</sup>	0.1-10 <sup>a</sup>
Incidental Inhalation-Powder	21 <sup>c</sup>	3 <sup>c</sup>	NR	0.1-5 <sup>c</sup>	7; 10 <sup>b</sup> ; 311 <sup>c</sup>	59 <sup>c</sup>	0.053; 0.053-2.4 <sup>b</sup> ; 2.4 <sup>c</sup>	0.09-5
Dermal Contact	74	29	0.008-5	0.09-10	1208	297	0.00009-6	0.09-10
Deodorant (underarm)	NR	NR	NR	NR	1 <sup>a</sup>	NR	0.02 <sup>f</sup>	NR
Hair - Non-Coloring	1	2	NR	0.09-5	218	22	0.0000001-5	0.1-25
Hair-Coloring	NR	NR	NR	NR	107	1	0.002-2.5	1-5
Nail	NR	1	NR	0.1-5	2	5	3.5	0.1-5
Mucous Membrane	4	NR	3	NR	52	NR	0.0008-2	0.09-10
Baby Products	NR	NR	NR	NR	11	3	0.00009-1.5	NR

**Table 6.** Current and historical frequency and concentration of use of polysorbates according to duration and exposure.<sup>1,2,5,6,14,30</sup>

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2014	1998	2014	1981	2014	1998	2014	1981
	<b>Polysorbate 61</b>				<b>Polysorbate 65</b>			
<b>Totals*</b>	<b>14</b>	<b>8</b>	<b>1-1.8</b>	<b>0.1-5</b>	<b>24</b>	<b>2</b>	<b>0.0003-3</b>	<b>1-5</b>
<b>Duration of Use</b>								
<i>Leave-On</i>	14	8	1-1.8	0.1-5	21	NR	0.0003-3	NR
<i>Rinse-Off</i>	NR	NR	NR	0.1-5	3	2	0.002-0.15	1-5
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	NR	NR	NR	NR
<b>Exposure Type</b>								
Eye Area	NR	NR	NR	NR	5	NR	0.5	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	4 <sup>c</sup>	NR	1-5 <sup>a</sup> ; 0.1-1 <sup>c</sup>	7 <sup>a</sup> ; 8 <sup>c</sup>	NR	NR	NR
Incidental Inhalation-Powder	NR	3 <sup>b</sup> ; 4 <sup>c</sup>	NR	1-5 <sup>b</sup> ; 0.1-1 <sup>c</sup>	8 <sup>c</sup>	NR	NR	NR
Dermal Contact	14	8	1-1.8	0.1-5	24	1	0.0003-3	1-5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	0.003 <sup>f</sup>	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	1	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	0.002-0.003	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	1	4	NR	1-5	NR	NR	NR	NR
	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1981</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1981</b>
	<b>Polysorbate 80</b>				<b>Polysorbate 81</b>			
<b>Totals*</b>	<b>907</b>	<b>231</b>	<b>0.00031-18.1</b>	<b>0.01-25</b>	<b>NR</b>	<b>4</b>	<b>0.4-25.6</b>	<b>0.1-5</b>
<b>Duration of Use</b>								
<i>Leave-On</i>	734	132	0.00031-11.9	0.01-10	NR	4	0.5-7.5	0.1-5
<i>Rinse-Off</i>	166	89	0.0038-18.1	0.09-10	NR	NR	0.4-25.6	0.1-5
<i>Diluted for (Bath) Use</i>	7	10	NR	0.1-25	NR	NR	5-7.5	NR
<b>Exposure Type</b>								
Eye Area	111	16	0.0024-11	0.09-5	NR	NR	0.5	NR
Incidental Ingestion	46	15	0.00031-1.5	0.09-1	NR	NR	NR	NR
Incidental Inhalation-Spray	6; 319 <sup>a</sup> ; 191 <sup>c</sup>	16; 71 <sup>a</sup> ; 13 <sup>c</sup>	0.02-1.6 <sup>i</sup> ; 0.0038-11.9 <sup>a</sup>	0.1-1; 0.09-10 <sup>a</sup> ; 0.09-10 <sup>c</sup>	NR	1 <sup>a</sup>	5 <sup>a</sup>	0.1-5 <sup>a</sup> ; 0.1-1 <sup>c</sup>
Incidental Inhalation-Powder	18; 2 <sup>b</sup> ; 191 <sup>c</sup>	7; 13 <sup>c</sup>	0.42-2; 0.42-2 <sup>c</sup>	1-10; 0.09-10 <sup>b</sup> ; 0.09-10 <sup>c</sup>	NR	NR	5 <sup>b</sup>	0.1-1 <sup>c</sup>
Dermal Contact	710	122	0.00075-18.1	0.01-25	NR	3	0.5-25.6	0.1-1
Deodorant (underarm)	1 <sup>a</sup>	NR	NR	0.09-1 <sup>a</sup>	NR	NR	NR	NR
Hair - Non-Coloring	115	80	0.02-10	0.09-10	NR	1	0.4	0.1-5
Hair-Coloring	24	10	0.36	NR	NR	NR	NR	NR
Nail	7	2	NR	0.1-1	NR	NR	7.5	NR
Mucous Membrane	76	29	0.00031-11.9	0.09-25	NR	NR	5-7.5	NR
Baby Products	4	4	10	0.1-10	NR	NR	NR	NR



**Table 6.** Current and historical frequency and concentration of use of polysorbates according to duration and exposure.<sup>1,2,5,6,14,30</sup>

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2014	1998	2014	1981	2014	1998	2014	1998
	Polysorbate 85				PEG-20 sorbitan isostearate			
<b>Totals*</b>	<b>51</b>	<b>35</b>	<b>0.03-21.9</b>	<b>0.01-&gt;50</b>	<b>3</b>	<b>2</b>	<b>0.3</b>	<b>NR</b>
<b>Duration of Use</b>								
<i>Leave-On</i>	23	28	0.03-6	0.01-10	3	2	0.3	NR
<i>Rinse-Off</i>	15	7	5.5-21.9	0.09->50	NR	NR	NR	NR
<i>Diluted for (Bath) Use</i>	13	NR	0.03-0.055	NR	NR	NR	NR	NR
<b>Exposure Type</b>								
Eye Area	4	5	NR	0.09-5	1	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	0.01-10 <sup>a</sup> ; 0.1-1 <sup>c</sup>	1 <sup>a</sup> ; 2 <sup>c</sup>	1 <sup>a</sup>	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	0.01-1 <sup>b</sup> ; 0.1-1 <sup>c</sup>	2 <sup>c</sup>	NR	0.3 <sup>b</sup>	NR
Dermal Contact	51	30	0.03-21.9	0.01-10	3	NR	0.3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	4	NR	0.01-5	NR	NR	NR	NR
Hair-Coloring	NR	1	NR	>50	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	1	NR	NR
Mucous Membrane	14	NR	0.03-0.55	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>
	PEG-40 sorbitan lanolate				PEG-10 sorbitan laurate			
<b>Totals*</b>	<b>NR</b>	<b>7</b>	<b>NR</b>	<b>NR</b>	<b>2</b>	<b>2</b>	<b>NR</b>	<b>NR</b>
<b>Duration of Use</b>								
<i>Leave-On</i>	NR	4	NR	NR	1	NR	NR	NR
<i>Rinse-Off</i>	NR	3	NR	NR	1	2	NR	NR
<i>Diluted for (Bath) Use</i>	NR	NR	NR	NR	NR	NR	NR	NR
<b>Exposure Type</b>								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	1 <sup>a</sup>	NR	NR	1 <sup>a</sup>	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	2	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	5	NR	NR	NR	1	NR	NR
Hair-Coloring	NR	2	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	1	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

**Table 6.** Current and historical frequency and concentration of use of polysorbates according to duration and exposure.<sup>1,2,5,6,14,30</sup>

	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2014	1998	2014	1998	2014	1998	2014	1998
	PEG-44 sorbitan laurate				PEG-80 sorbitan laurate			
<b>Totals*</b>	<b>8</b>	<b>8</b>	<b>0.5-2</b>	<b>NR</b>	<b>84</b>	<b>34</b>	<b>0.0002-4.2</b>	<b>NR</b>
<b>Duration of Use</b>								
Leave-On	6	6	2	NR	19	6	0.0002-0.059	NR
Rinse-Off	2	2	0.5	NR	53	28	2-4.2	NR
Diluted for (Bath) Use	NR	NR	NR	NR	12	NR	2.5	NR
<b>Exposure Type</b>								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	2	NR	0.059	NR
Incidental Inhalation-Spray	1 <sup>a</sup>	1 <sup>a</sup>	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	8	8	2	NR	61	14	0.0002-4.2	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	0.5	NR	21	20	4.2	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	34	2	0.059-4.2	NR
Baby Products	NR	NR	NR	NR	30	15	4.2	NR
	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>
	PEG-3 sorbitan oleate				PEG-6 sorbitan oleate			
<b>Totals*</b>	<b>1</b>	<b>4</b>	<b>NR</b>	<b>NR</b>	<b>NR</b>	<b>NR</b>	<b>0.43</b>	<b>NR</b>
<b>Duration of Use</b>								
Leave-On	1	4	NR	NR	NR	NR	0.43	NR
Rinse-Off	NR	NR	NR	NR	NR	NR	NR	NR
Diluted for (Bath) Use	NR	NR	NR	NR	NR	NR	NR	NR
<b>Exposure Type</b>								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	1 <sup>a</sup>	1 <sup>a</sup>	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	1	3	NR	NR	NR	NR	0.43	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	1	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>	<b>2014</b>	<b>1998</b>
	PEG-40 sorbitan peroleate				PEG-40 sorbitan stearate			
<b>Totals*</b>	<b>49</b>	<b>13</b>	<b>0.16-4</b>	<b>NR</b>	<b>1</b>	<b>1</b>	<b>NR</b>	<b>NR</b>
<b>Duration of Use</b>								
Leave-On	47	8	0.16-4	NR	1	NR	NR	NR
Rinse-Off	2	NR	NR	NR	NR	1	NR	NR
Diluted for (Bath) Use	NR	5	NR	NR	NR	NR	NR	NR
<b>Exposure Type</b>								
Eye Area	5	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	13 <sup>a</sup> ; 13 <sup>c</sup>	6 <sup>a</sup> ; 1 <sup>c</sup>	4 <sup>a</sup>	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	13 <sup>c</sup>	1; 1 <sup>c</sup>	0.3-0.9 <sup>b</sup>	NR	1 <sup>a</sup>	NR	NR	NR
Dermal Contact	46	13	0.16-1	NR	1	1	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	3	NR	4	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	5	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

**Table 6.** Current and historical frequency and concentration of use of polysorbates according to duration and exposure.<sup>1,2,5,6,14,30</sup>

	<i># of Uses</i>		<i>Max Conc of Use (%)</i>		<i># of Uses</i>		<i>Max Conc of Use (%)</i>	
	2014	1998	2014	1998	2014	1998	2014	1999
	<b>PEG-40 sorbitan tetraoleate</b>				<b>Sorbeth-20 beeswax</b>			
<b>Totals*</b>	<b>1</b>	<b>1</b>	<b>NR</b>	<b>NR</b>	<b>8</b>	<b>16</b>	<b>0.5-2.8</b>	<b>0.5-2.8</b>
<b><i>Duration of Use</i></b>								
<i>Leave-On</i>	<i>1</i>	<i>1</i>	<i>NR</i>	<i>NR</i>	<i>8</i>	<i>16</i>	<i>0.5-2.8</i>	<i>0.5-2.8</i>
<i>Rinse-Off</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>
<i>Diluted for (Bath) Use</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>	<i>NR</i>
<b><i>Exposure Type</i></b>								
Eye Area	NR	NR	NR	NR	6	11	2.8	2.8
Incidental Ingestion	NR	NR	NR	NR	1	4	2.5	2.5
Incidental Inhalation-Spray	1 <sup>c</sup>	1 <sup>c</sup>	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	1 <sup>c</sup>	1 <sup>c</sup>	NR	NR	NR	NR	NR	NR
Dermal Contact	1	1	NR	NR	1	4	0.5-1	0.5-1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	1	4	2.5	2.5
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

NR – no reported use

\* 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.

\*\* The year that the Council survey was conducted in the previous report. In the report published in 2000, the only concentration of use data that were provided was the following: "...PEG-60 sorbitan tetratoate, PEG-40 sorbitan tetraoleate, and PEG-160 sorbitan Triisostearate are used in cosmetics at concentrations of 0.5% to 10%..." in 1998. Since the data from the 2000 report is limited, the concentration of use data from the 1984 report are provided here to give a better historical perspective.

\*\*\* At the time of the 1984 safety assessment, concentration of use data were not reported by the FDA; 1981 data were presented. These data were presented in ranges so the limits of the ranges are represented here.

<sup>a</sup> It is possible these products are sprays, but it is not specified whether the reported uses are sprays.<sup>b</sup> It is possible these products are powders, but it is not specified whether the reported uses are powders.<sup>c</sup> 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.<sup>d</sup> Aerosol hair spray 0.027%-3%; pump hair spray 0.4%-1%; spray body and hand products 0.00001%-1.2%; spray moisturizing products 0.1%.<sup>e</sup> Spray deodorants.<sup>f</sup> Not spray deodorants.<sup>g</sup> Aerosol hair spray.<sup>h</sup> Spray body and hand products 0.083%-0.8%.<sup>i</sup> Aerosol hair spray 0.078%-1.6%; pump hair spray 0.02%-0.2%; spray face and neck products 0.39%.

**Table 7.** Frequency of use according to duration and exposure of polysorbates that are reviewed for the first time in this safety assessment.<sup>5,30</sup>

Use type	Maximum Concentration (%)		Maximum Concentration (%)		Maximum Concentration (%)		Maximum Concentration (%)	
	Uses		Uses		Uses		Uses	
	PEG-20 sorbitan cocoate		PEG-40 sorbitan diisostearate		PEG-40 sorbitan laurate		PEG-75 sorbitan laurate	
Total/range	9	0.003-0.3	2	1	NR	0.25-2	NR	0.5-2
Duration of use								
Leave-on	9	0.03-0.3	2	1	NR	2	NR	2
Rinse-off	NR	0.06	NR	NR	NR	0.25-0.5	NR	0.5
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	NR	NR
Exposure type*								
Eye area	1	NR	NR	NR	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	5 <sup>a</sup> ; 3 <sup>c</sup>	NR	2 <sup>a</sup>	1 <sup>a</sup>	NR	NR	NR	NR
Incidental inhalation-powders	3 <sup>c</sup>	0.03-0.3 <sup>b</sup>	NR	NR	NR	NR	NR	NR
Dermal contact	9	0.03-0.3	2	NR	NR	2	NR	2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	1	NR	0.5	NR	0.5
Hair-coloring	NR	NR	NR	NR	NR	0.25	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby	NR	NR	NR	NR	NR	NR	NR	NR

	PEG-3 sorbitan stearate		PEG-6 sorbitan stearate		PEG-30 sorbitan tetraoleate		PEG-60 sorbitan tetraoleate	
Total/range	3	NR	2	NR	1	10	NR	0.5-0.9
Duration of use								
Leave-on	2	NR	NR	NR	NR	NR	NR	0.5-0.9
Rinse-off	1	NR	2	NR	1	10	NR	NR
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	NR	NR
Exposure type								
Eye area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	NR	NR	NR	NR	NR	NR	NR	0.8 <sup>a</sup> ; 0.5-0.9 <sup>c</sup>
Incidental inhalation-powders	NR	NR	NR	NR	NR	NR	NR	0.5-0.9 <sup>c</sup>
Dermal contact	3	NR	2	NR	1	10	NR	0.8-0.9
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	NR	NR	NR	NR	0.5
Hair-coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby	NR	NR	NR	NR	NR	NR	NR	NR

**Table 7.** Frequency of use according to duration and exposure of polysorbates that are reviewed for the first time in this safety assessment.<sup>5,30</sup>

Use type	Maximum Concentration (%)		Maximum Concentration (%)		Maximum Concentration (%)		Maximum Concentration (%)	
	Uses		Uses		Uses		Uses	
	Sorbeth-6 beeswax		Sorbeth-30 tetraisostearate		Sorbeth-4 tetraoleate		Sorbeth-6 tetraoleate	
<b>Total/range</b>	<b>7</b>	<b>2</b>	<b>1</b>	<b>NR</b>	<b>4</b>	<b>NR</b>	<b>NR</b>	<b>0.21</b>
<i>Duration of use</i>								
Leave-on	7	2	1	NR	4	NR	NR	0.21
Rinse-off	NR	NR	NR	NR	NR	NR	NR	NR
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	NR	NR
<i>Exposure type</i>								
Eye area	3	NR	NR	NR	NR	NR	NR	NR
Incidental ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	3 <sup>a</sup>	NR	1 <sup>c</sup>	NR	NR	NR	NR	NR
Incidental inhalation-powders	NR	NR	1 <sup>c</sup>	NR	NR	NR	NR	0.21
Dermal contact	7	2	1	NR	4	NR	NR	0.21
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair-noncoloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby	NR	NR	NR	NR	NR	NR	NR	NR

	Sorbeth-30 tetraoleate		Sorbeth-40 tetraoleate		Sorbeth-60 tetraoleate		
<b>Total/range</b>	<b>8</b>	<b>0.11-10.8</b>	<b>NR</b>	<b>0.5</b>	<b>1</b>	<b>NR</b>	
<i>Duration of use</i>							
Leave-on	3	NR	NR	0.5	1	NR	
Rinse-off	5	0.11-10.8	NR	NR	NR	NR	
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	
<i>Exposure type</i>							
Eye area	NR	NR	NR	NR	NR	NR	
Incidental ingestion	NR	NR	NR	NR	NR	NR	
Incidental Inhalation-sprays	NR	NR	NR	NR	1 <sup>a</sup>	NR	
Incidental inhalation-powders	NR	NR	NR	NR	NR	NR	
Dermal contact	8	0.11-10.8	NR	0.5	1	NR	
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	
Hair-noncoloring	NR	NR	NR	NR	NR	NR	
Hair-coloring	NR	NR	NR	NR	NR	NR	
Nail	NR	NR	NR	NR	NR	NR	
Mucous Membrane	NR	NR	NR	NR	NR	NR	
Baby	NR	NR	NR	NR	NR	NR	

NR = Not Reported; Totals = Rinse-off + Leave-on Product Uses.

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

<sup>a</sup> 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.<sup>b</sup> It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.<sup>c</sup> Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.<sup>c</sup> It is possible these products may be powders, but it is not specified whether the reported uses are powders.

**Table 8.** Ingredients for which there were no reported uses from the VCRP or the Council.<sup>5,30</sup>

PEG-5 sorbitan isostearate	Sorbeth-2 Hexaoleate
PEG-20 sorbitan isostearate	Sorbeth-40 Hexaoleate (PEG-40 sorbitol hexaoleate)
PEG -75 sorbitan lanolate	Sorbeth-50 hexaoleate (PEG -50 sorbitol hexaoleate)
PEG -20 sorbitan oleate	Sorbeth-6 hexastearate
PEG-40 sorbitan oleate	Sorbeth-150 hexastearate
PEG -80 sorbitan palmitate	Sorbeth-3 isostearate
PEG-4 sorbitan stearate	Sorbeth-6 laurate
PEG -60 sorbitan stearate	Sorbeth-2/oleate/dimer dilinoleate crosspolymer
PEG -60 sorbitan tetrastearate	Sorbeth-20 pentaisostearate
PEG -4 sorbitan triisostearate	Sorbeth-30 pentaisostearate
PEG -20 sorbitan triisostearate	Sorbeth-40 pentaisostearate
PEG -160 sorbitan triisostearate	Sorbeth-50 pentaisostearate
PEG -2 sorbitan trioleate	Sorbeth-40 pentaoleate
PEG -18 sorbitan trioleate	Sorbeth-20 tetraisostearate
PEG -3 sorbitan tristearate	Sorbeth-30 tetraisostearate
Sorbeth-2 beeswax	Sorbeth-40 tetraisostearate
Sorbeth-8 beeswax	Sorbeth-50 tetraisostearate
Sorbeth-2 cocoate	Sorbeth-30 tetraoleate laurate (PEG -30 sorbitol tetraoleate laurate)
Sorbeth-2 hexacaprylate/caprate	Sorbeth-60 tetrastearate (PEG -60 sorbitol tetrastearate)
Sorbeth-12 hexacocoate	Sorbeth-3 tristearate
Sorbeth-2 hexaisostearate	Sorbeth-160 tristearate
Sorbeth-2 hexalaurate	Sorbeth-450 tristearate

**Table 9.** Regulations controlling the use of polysorbates.

<b>Ingredient</b>	<b>Regulation</b>	<b>Citation</b>
Polysorbate 20, 60, 65, and 80	Approved as dilutents in color additives for drug use.	21CFR73.1; 21CFR73.1001
Polysorbates 20, 60, and 80	Approved for direct use in all food types as synthetic flavorings.	21CFR172.623
Polysorbate 80	Approved to be used with carrageenan to make chewing gum bases and related substances.	21CFR172.623
Polysorbate 60, 65, and 80	Approved as multipurpose additives.	21CFR172.836; 21CFR172.838; 21CFR172.840]
Polysorbate 20	Permitted as a secondary direct food additive for human consumption.	21CFR173.310
Polysorbate 60, 65, and 80	Approved as defoaming agents in food for human consumption.	21CFR173.340
Polysorbate 20, 40, 60, and 80; PEG-3 sorbitan stearate; and PEG-3 sorbitan oleate	Approved for indirect addition to all food types as components of adhesives.	21 CFR 175.105
PEG-40 sorbitan laurate, PEG-6 sorbitan stearate, PEG-40 sorbitan stearate, PEG-6 sorbitan oleate, PEG-40 sorbitan tetraoleate, and PEG-40 sorbitan peroleate	May be used as indirect food additives as a defoaming agent in the manufacture of paper and paperboard.	12CFR176.210
Polysorbate 20, 40, 60, 65, 80, and 85, and PEG-3 sorbitan oleate	Approved for indirect addition to all food types as emulsifiers and/or surfactants.	21 CFR 178.3400
PEG-3 sorbitan oleate	May be used as a component of paper and paperboard in contact with dry food.	21CFR180
Polysorbate 80	Approved as an ophthalmic demulcent.	21CFR349.12
Polysorbate 60 and 80	Approved for use in animal feed and drinking water.	21CFR573.840; 21CFR573.860
Polysorbate 80	May be used to denature spirits.	27CFR21.68; 27CFR21.151

**Table 10.** Penetration enhancement studies of some polysorbates.<sup>37</sup>

Ingredient	Chemical/drug tested	Results; notes
Polysorbate 20 (5%)	Albuterol sulfate	ER compared to control (saline buffer)=3.43±0.52; ER compared to vehicle (ethanol)=1.26±0.32. Thawed rat skin pretreated with test substance using Franz cells.
Polysorbate 65 (5%)	Albuterol sulfate	ER compared to control (saline buffer)=4.74±0.23; ER compared to vehicle (ethanol)=1.75±0.29. Thawed rat skin pretreated with test substance using Franz cells.
Polysorbate 80 (5%)	Albuterol sulfate	ER compared to control (saline buffer)=2.95±0.45; ER compared to vehicle (ethanol)=1.09±0.17. Thawed rat skin pretreated with test substance using Franz cells.

ER=Enhancement ratio

**Table 11.** Highest reported NOAELs for polysorbate 20 and polysorbate 80 reported in a survey of 4 research organizations.<sup>38</sup>

Animal	Route	Duration	Dose	Comments
<b>Polysorbate 20</b>				
Rat	Oral	1 month	250 mg/kg	Well tolerated
	Oral	90 days	500 mg/kg	Diarrhea
Mouse	Oral	1 month	10 mg/kg	Well tolerated
<b>Polysorbate 80</b>				
Dog	Oral	90 days	5 mL/kg	As 1% of formulation; well tolerated
Rat	Oral	Not reported	350 mg/kg	Well tolerated
	Oral	4 weeks	5 mL/kg	1%; well tolerated
	Oral	7 days	10 mL/kg	1%; well tolerated
Mouse	Intravenous	Not reported	100 mg/kg	Well tolerated
	Intraperitoneal	1 month	10 mL/kg	2%; well tolerated
	Intranasal	3 days	10 µL/nostril	0.2%; well tolerated
Primate	Oral	Efficacy	5 mL/kg	1%; well tolerated

**Table 12.** Human irritation studies of some polysorbates.

Ingredient (concentration)	Assay	Results; notes	Reference
<b>Dermal irritation in vivo</b>			
Polysorbate 60 (concentration not specified in a cream or 100%)	Administered to the foreheads. Amount and n not specified.	Urticaria observed at application sites at 20 min caused by both polysorbate 60-based cream and polysorbate 60. There was no effect of either the polysorbate 60 or the cream on the dorsal and arm skin	24
Polysorbate 60 (1% in DMEM)	Human patch test scored according to ICDRG. Patches were in place for 2 days in Haye's chambers. n=30.	Irritation score=0.4 out of 4.	44
Polysorbate 80 (100%)	Test substance administered for increasing time periods: 15 min-4 h and observed at 24, 48, and 72 h. n=29	1 positive reaction. Control of 20% sodium dodecyl sulfate exhibited 24 of 29 reactions.	43
Polysorbate 80 (100%)	Test substance administered for increasing time periods: 15 min-4 h and observed at 24, 48, and 72 h. n=24	1 positive reaction. Control of 20% sodium dodecyl sulfate exhibited 8 of 27 reactions.	45
Sorbitan monostearate, ethoxylated (25% aqueous)	10 drops of the solution administered to the scalp twice/d for 16 weeks. n=68	Irritation score 1 out of 68. Mild redness observed in 1 subject. Not irritating.	24

**Table 13.** Ocular irritation assays of some polysorbates.

<b>Ingredient (concentration)</b>	<b>Assay</b>	<b>Results; notes</b>	<b>Reference</b>
<b>In vivo</b>			
Polysorbate 20 (10%)	Draize test	Maximal average score=0.7; 24-h average score=0.0	47
Polysorbate 20 (2%)	Draize test	Not an ocular irritant	46
Polysorbate 20 (10%)	Draize test	Maximum average total score=0.7; 24-h score=0. Not an ocular irritant.	48
Polysorbate 81 (10% in light mineral oil)	Draize test using New Zealand White Rabbits (n=9)	Irritation score=0 out of 4; not irritating. Eyes were wash 2 sec after administration in 3 rabbits. Eyes were observed at 1, 24, 48, 72 h and 7 days.	23
Polysorbate 81 (100%)	Draize test using New Zealand White Rabbits (n=9)	Irritation score=0 out of 4; not irritating. Eyes were wash 2 sec after administration in 3 rabbits. Eyes were observed at 1, 24, 48, 72 h and 7 days.	23
Sorbitan monostearate, ethoxylated (0.1 g in water)	Draize test using New Zealand White Rabbits (n=3)	Irritation score=0 out of 110; not irritating. Did not produce any eye irritation or any eye discharge throughout the 72-h observation period. No lesions such as pannus, staining were observed.	24
Sorbitan monolaurate, ethoxylated (100%; 0.1 mL)	Draize test using New Zealand White rabbits (n=9)	Irritation score=0 out of 4; not irritating. Eyes were wash 2 sec after administration in 3 rabbits.	22
<b>In vitro</b>			
Polysorbate 20 (not provided)	EpiOcular test over 7 laboratories	Not predicted to be an ocular irritant. Average mean cell viability 97.40±6.49% of distilled water control.	50
Polysorbate 20 (2%)	Red blood cell hemolysis assay	Predicted to be a minimal ocular irritant.	46
Polysorbate 20 (2%)	K562 cell assay	Predicted to be a minimal ocular irritant.	46
Polysorbate 20 (5% in saline; 200 µL)	STE using SIRC cells (CCL-60). Exposure for 5 min.	Predicted to be an irritant.	49
Polysorbate 20 (100%; 50 µL)	EpiOcular assay	Predicted to be a non-irritant.	49
Polysorbate 20 (100%; 200 µL)	HET-CAM assay (Fertilized chicken eggs (white leghorn species) with microscopic evaluation of hemorrhage, lysis, and coagulation at 0.5, 2, and 5 min.	Predicted to be an irritant.	49
Polysorbate 20 (100%)	HET-CAM assay (Same as above but evaluation of time to hemorrhage, lysis, and coagulation)	Predicted to be a severe irritant.	49
Polysorbate 20 (100%)	BCOP assay	Predicted to be a mild irritant.	49

BCOP=Bovine Corneal Opacity and Permeability assay; DMEM= Dulbecco's modified Eagle's medium; HET-CAM=Hen's Egg Test-Chorioallantoic Membrane assay; ICDRG=International Contact Dermatitis Research Group; STE=Short Time Exposure test.



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## 1

# Final Report on the Safety Assessment of Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85

The Polysorbates are a series of polyoxyethylenated sorbitan esters that are used as hydrophilic, nonionic surfactants in a variety of cosmetic products. Polysorbates are hydrolyzed by pancreatic and blood lipases; the fatty acid moiety is released to be absorbed and metabolized, whereas the polyoxyethylene sorbitan moiety is very poorly absorbed and is excreted unchanged. Acute and long-term oral toxicity in animals indicates a low order of toxicity with oral ingestion of the Polysorbates.

Polysorbate 80 was shown to be nonmutagenic in the Ames and micronucleus tests. The Polysorbates were noncarcinogenic in laboratory animals. Multiple studies have shown that the Polysorbates enhance the activity of known chemical carcinogens while not actually being carcinogenic themselves.

Extensive clinical skin testing showed Polysorbates to have little potential for human skin irritation or evidence of skin sensitization or phototoxicity. The available data indicate that these ingredients are used in numerous preparations without clinical reports of significant adverse effects. It is concluded that they are safe for use in cosmetics at present concentrations of use.

## CHEMICAL AND PHYSICAL PROPERTIES COMPOSITION

The Polysorbates are a series of general purpose, hydrophilic, nonionic surfactants. They are obtained by reaction of sorbitol and its anhydrides with ethylene oxide ( $C_2H_4O$ ) under conditions that cause splitting of water from the sorbitol, leaving sorbitan. A specified molar ratio of ethylene oxide to sorbitol and its mono- and dianhydrides is used in the condensation to effect an oxyethylene copolymerization at the free hydroxyl groups of sorbitan. The resulting polyoxyethylene sorbitans are esterified with 1 or 3 moles of a fatty acid (lauric, palmitic, stearic, oleic) to produce the Polysorbates. Therefore, in summary the Polysorbates are polyoxyethylene ( $W + X + Y + Z$ ) sorbitan mono- or triesters, where the sum of  $w + x + y + z$  is the average number of moles of ethylene oxide per mole of sorbitol, and where  $R$  denotes 1 or 3 moles of an esterified fatty acid. Specific data for these variables are listed in Table 1 for each of the Polysorbates.<sup>(1-19)</sup>

**TABLE 1.** Compositions of the Polysorbates.

Ingredient	Chemical Name	Average Moles of Ethylene Oxide (w + x + y + z)	Major Fatty Acid Moieties	
			R	No. of Moles
Polysorbate 21	Polyoxyethylene(21) sorbitan monolaurate	21	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_{11}\text{CH}_3 \end{array}$	1
Polysorbate 21	Polyoxyethylene(4) sorbitan monolaurate	4	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_{11}\text{CH}_3 \end{array}$	1
Polysorbate 41	Polyoxyethylene(21) sorbitan monopalmitate	21	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_{14}\text{CH}_3 \end{array}$	1
Polysorbate 61	Polyoxyethylene(21) sorbitan monostearate	21	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_{16}\text{CH}_3 \end{array}$	1
Polysorbate 61	Polyoxyethylene(4) sorbitan monostearate	4	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_{16}\text{CH}_3 \end{array}$	1
Polysorbate 65	Polyoxyethylene(21) sorbitan tristearate	21	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_{16}\text{CH}_3 \end{array}$	3
Polysorbate 81	Polyoxyethylene(21) sorbitan monooleate	21	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}_3 \end{array}$	1
Polysorbate 81	Polyoxyethylene(5) sorbitan monooleate	5	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}_3 \end{array}$	1
Polysorbate 85	Polyoxyethylene(21) sorbitan trioleate	21	$\begin{array}{c} \text{O} \\    \\ -\text{O}-\text{C}(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}_3 \end{array}$	3

### Properties

The Polysorbates are, for the most part, viscous liquids that range in color from yellow to orange to tan. They possess a faint, characteristic odor and a warm, somewhat bitter taste.<sup>(11,13-16,18,19)</sup>

The solubility characteristics, physical properties, and chemical properties of the Polysorbates are listed in Tables 2, 3, and 4, respectively.

Polyoxyethylene esters are characterized by a very strong infrared absorption peak at 9  $\mu\text{m}$ .<sup>(20)</sup> Detailed discussions of other physical properties, especially regarding the surface active properties of the Polysorbates, are available in the scientific literature. These include studies on oil/water interfacial tension,<sup>(21)</sup> emulsion stability,<sup>(22)</sup> suspension stability,<sup>(23,24)</sup> rheological characteristics of emulsions,<sup>(25,26)</sup> hydrophile:hydrophobe proton ratio,<sup>(27)</sup> and critical micelle concentration.<sup>(28-32)</sup>

### Chemical Reactivity

Aqueous solutions of Polysorbate 20 undergo autoxidation on storage at room temperature, with changes in the peroxide number, pH, surface tension, and cloud point. Autoxidation is accelerated by light, elevated temperature, and copper sulfate. Hydrolysis of Polysorbate 20 also occurs at room temperature, whereas the oxyethylene moieties undergo chain shortening at temperatures above 40°C. Evidence of such degradation has been detected in unopened commercial samples of Polysorbates 20, 40, and 60.<sup>(33)</sup> Degradation of Polysorbates might also occur in cosmetic formulations because of microorganisms. Bacteria

**TABLE 2.** Solubilities of the Polysorbates.<sup>(11,13-15,18,19)</sup>

Solvent	Polysorbate								
	20	21	40	60	61	65	80	81	85
Water	+		+/-	+/-	+	+	+	+	+
Mineral oil	-		-	-		+/-	-	+	+
Fixed oils							+		
Vegetable oils				-		+			
Cottonseed oil							+		
Corn oil							+		
Alcohol	+		+	+/-	+	+	+	+	+
Methanol	+					+	+		
Polyols	-			-			-		
Etheralcohols				+/-					
Acetone				+/-		+			
Toluene				+			+		
Carbon tetrachloride				+		+			
Petroleum solvents				+/-					
Mineral spirits	-					+			
Ethyl acetate	+			+			+		
Dioxane	+			+		+			
Aniline				+					
Aromatic hydrocarbons				+					

+ = Soluble or dispersible.

- = Insoluble.

+/- = Slightly soluble, depending on temperature; or conflicting reports.

Blank spaces indicate no data.

**TABLE 3.** Physical Properties of the Polysorbates.

<i>Ingredient</i>	<i>Density</i>	<i>Physical State</i>	<i>Setting Point</i>	<i>Viscosity</i>	<i>Refr. Index</i>	<i>pH</i> (5 percent aq. sol.)	<i>HLB</i>
Polysorbate 20		Viscous, oily liquid <sup>(11,13,15,16,42)</sup>	14°–16°C <sup>(15)</sup>	4000–6000 centipoise at 25°C <sup>(15)</sup>	1.472 <sup>(15)</sup>		16.7 <sup>(42)</sup>
Polysorbate 21		Liquid <sup>(42)</sup>					13.3 <sup>(42)</sup>
Polysorbate 40	1.05 <sup>(15)</sup>	Oily liquid or Vaseline-like <sup>(11,15,16,42)</sup>					15.6 <sup>(42)</sup>
Polysorbate 60		Oily liquid <sup>(42)</sup> or semigel, <sup>(13)</sup> wax, <sup>(15)</sup> Vaseline-like <sup>(16)</sup>	45°–55°C <sup>(15)</sup>				14.9 <sup>(42)</sup>
Polysorbate 61		Waxy solid <sup>(11,42)</sup>					6.9 <sup>(42)</sup>
Polysorbate 65		Waxy solid <sup>(11,13,42)</sup>	31°C <sup>(11)</sup>				10.5 <sup>(42)</sup>
Polysorbate 80	1.08 <sup>(14)</sup> 1.06–1.10 <sup>(19)</sup> 1.07–1.09 <sup>(18)</sup>	Viscous, oily liquid <sup>(13-16,18,19,42)</sup>	5°–6°C <sup>(15)</sup>	345–445 centistokes at 25°C and 150–210 centistokes at 38°C <sup>(18)</sup> 270–430 centistokes <sup>(19)</sup>		6.0–8.0 <sup>(15,18)</sup> 5–7 <sup>(19)</sup>	15.0 <sup>(42)</sup>
Polysorbate 81		Liquid, <sup>(42)</sup> may gel at room temperature <sup>(11)</sup>		350–550 centipoise at 25°C <sup>(11)</sup>			10.0 <sup>(42)</sup>
Polysorbate 85		Liquid, <sup>(16,42)</sup> may gel at room temperature <sup>(11)</sup>					11.0 <sup>(42)</sup>



**TABLE 4.** Chemical Properties of the Polysorbates.

<i>Ingredient</i>	<i>Fatty Acid Moiety</i>	<i>Oxyethylene Content (%)</i>	<i>Fatty Acid Content (g/100g-sample)</i>	<i>Acid Value</i>	<i>Hydroxyl Value*</i>	<i>Saponification Value</i>
Polysorbate 20	Monolaurate	70.0–74.0 <sup>(13)</sup>	15–17 <sup>(13)</sup>	2.0 max <sup>(11,13)</sup> 7.0 max <sup>(15)</sup> 4.0 max <sup>(16)</sup>	95–115 <sup>(11)</sup> 96–108 <sup>(13)</sup>	50–51 <sup>(11)</sup> 40–50 <sup>(13)</sup> 43–57 <sup>(16)</sup>
Polysorbate 21	Monolaurate			3.0 max <sup>(6)</sup>	225–255 <sup>(6)</sup>	100–115 <sup>(6)</sup>
Polysorbate 40	Monopalmitate			2.0 max <sup>(11)</sup> 4.0 max <sup>(16)</sup>	89–105 <sup>(11)</sup>	43–49 <sup>(11)</sup> 41–55 <sup>(16)</sup>
Polysorbate 60	Monostearate	65.0–69.5 <sup>(13)</sup>	24–26 <sup>(13)</sup>	2.0 max <sup>(7,13)</sup> 5.0 max <sup>(15)</sup> 4.0 max <sup>(16)</sup>	81–96 <sup>(7,13)</sup>	45–55 <sup>(7,13)</sup> 43–55 <sup>(16)</sup>
Polysorbate 61	Monostearate			2.0 max <sup>(8,11)</sup>	170–200 <sup>(11)</sup> 180–190 <sup>(8)</sup>	95–114 <sup>(11)</sup> 45–55 <sup>(8)</sup>
Polysorbate 65	Tristearate	46.0–50.0 <sup>(13)</sup>	42–44 <sup>(13)</sup>	2.0 max <sup>(9,11,13)</sup>	44–60 <sup>(9,11,13)</sup>	88–98 <sup>(9,11,13)</sup>
Polysorbate 80	Monooleate	65.0–69.5 <sup>(13)</sup>	22–24 <sup>(13)</sup>	2.0 max <sup>(13)</sup> 10.0 max <sup>(15)</sup> 4.0 max <sup>(16)</sup>	65–80 <sup>(13)</sup>	45–55 <sup>(13)</sup> 135–140 <sup>(15)</sup> 40–55 <sup>(16)</sup>
Polysorbate 81	Monooleate			2.0 max <sup>(11)</sup>	136–152 <sup>(11)</sup>	95–105 <sup>(11)</sup>
Polysorbate 85	Trioleate			2.0 max <sup>(11)</sup> 4.0 max <sup>(16)</sup>	39–52 <sup>(11)</sup>	82–95 <sup>(11)</sup> 83–105 <sup>(16)</sup>

\*Number of milligrams potassium hydroxide equivalent to one gram of sample.

in the deionized water used to manufacture cosmetic products were found to enzymatically decompose Polysorbate 20.<sup>(34)</sup>

The kinetics of the hydrolysis of Polysorbate 80 in aqueous buffers was studied over the pH range of 1.10 to 10.28. The hydrolysis was specific acid-catalyzed at pH values below 3 and specific base-catalyzed at pH values greater than 7.6.<sup>(35)</sup>

### Analytical Methods

Positive identification of the Polysorbates can be made through close matching to standard infrared spectra<sup>(11)</sup> or through any of several physical or chemical assays.<sup>(13,16,18,20)</sup> Quantitative and/or qualitative determinations of the Polysorbates have been made using gas chromatography,<sup>(36)</sup> thin-layer chromatography,<sup>(37)</sup> paper chromatography,<sup>(38,39)</sup> acidic complex precipitation tests,<sup>(40)</sup> and solubility assays.<sup>(41)</sup> The fatty acid moieties are determined as methyl esters after saponification of the Polysorbates and esterification of the resulting fatty acids.<sup>(43)</sup> Methods adapted for the quantitative and qualitative analysis of Polysorbate 80 in pharmaceutical preparations have been reviewed.<sup>(44)</sup>

The surface active properties of the Polysorbates have been evaluated with such methods as proton magnetic resonance,<sup>(27)</sup> interference refractometry,<sup>(29)</sup> membrane osmometry,<sup>(45)</sup> surface tension determination,<sup>(31,32)</sup> and density determination of aqueous solutions of the Polysorbates.<sup>(30)</sup>

Chemical properties of the Polysorbates for which assay methods have been described include hydroxyl value, saponification value, acid value, oxyethylene content, free fatty acid content, arsenic content, heavy metal content, water content, and residue on ignition.<sup>(13)</sup>

### Impurities

Since the fatty acids used in the production of cosmetic ingredients frequently contain fatty acids other than the principal acid named, each of the Polysorbates may contain a complex fatty acid moiety. In a study on Polysorbates 20, 40, 60, and 80, 15 different fatty acids were detected. The main constituents are listed in Table 5. The fatty acid compositions of the minor Polysorbates (21, 61, 65, 81, and 85) can be expected to reflect those of their major counterparts listed in Table 5.<sup>(46)</sup>

Peroxides, the ethoxylates of isosorbide, and unreacted free fatty acids may be present at unspecified concentrations.<sup>(2-10)</sup> A method for the removal of per-

**TABLE 5.** Fatty Acid Moiety Compositions of the Polysorbates.<sup>(46)</sup>

Ingredient	Fatty Acid (%)					
	Lauric	Palmitic	Stearic	Oleic	Myristic	Palmitoleic
Polysorbate 20 (monolaurate)	36.9	15.3		13.7	22.8	
Polysorbate 40 (monopalmitate)		86.4	10.2			
Polysorbate 60 (monostearate)		44.4	45.0			
Polysorbate 80 (monooleate)		6.4		76.9		6.4

oxides has been reported.<sup>(47)</sup> Birkel et al.<sup>(48)</sup> detected 1,4-dioxane at levels of 5.5 to 378 ppm in samples of Polysorbates 60 and 80. One manufacturer who followed this work has reported that dioxane is no longer detectable in their product.<sup>(49)</sup> During the manufacturing process, the Polysorbates are steam stripped to remove such unwanted water-soluble byproducts as 1,4-dioxane.<sup>(2-10)</sup> Since 1,4-dioxane is reported to induce carcinoma of the nasal turbinates in rats and hepatocellular carcinoma in mice when given in the drinking water at 0.5 to 1.0 percent,<sup>(50)</sup> the presence of traces of 1,4-dioxane is undesirable. More specific data on impurities are listed in Table 6.

## USE

### Purpose in Cosmetics

The Polysorbates are a series of general purpose, hydrophilic, nonionic surfactants supplied by the manufacturers at 100 percent concentration.<sup>(42)</sup> Various terms used to describe their roles in cosmetics include oil/water emulsifier, detergent, dispersing agent, solubilizer, and stabilizer.<sup>(1,13,15,51)</sup>

The Polysorbates exhibit high hydrophile-lipophile balance (HLB) values, indicating they will function to disperse oil in water as opposed to water in oil. HLB values for most emulsifiers fall in the range of 1.8 to 18.6, with the Polysorbates in the range 9.6 to 16.7 (Table 3). Because of their nonionic nature, the Polysorbates are comparatively insensitive to hard water and electrolytes and may be used in both acidic and basic formulations.<sup>(52)</sup>

### Scope and Extent of Use in Cosmetics

The Polysorbates are used in a wide variety of cosmetic products. Table 7 lists product types and the number of product formulations containing the Polysorbates as reported by the Food and Drug Administration (FDA) in 1981. The 1979 totals for all product categories are listed in Table 7 for comparison to the 1981 figures.

**TABLE 6.** Impurities.

<i>Ingredient</i>	<i>Arsenic Content (as As)</i>	<i>Heavy Metal Content (as Pb)</i>	<i>Water Content</i>	<i>Residue on Ignition</i>
Polysorbate 20	< 3 ppm <sup>(13)</sup>	< 10 ppm <sup>(13)</sup>	< 3.0% <sup>(11,13,16)</sup>	< 0.15% <sup>(13)</sup> < 1.0% <sup>(16)</sup>
Polysorbate 21			< 3.0% <sup>(6)</sup>	
Polysorbate 40			< 3.0% <sup>(11,16)</sup>	< 1.0% <sup>(16)</sup>
Polysorbate 60	< 3 ppm <sup>(13)</sup>	< 10 ppm <sup>(13)</sup>	< 3.0% <sup>(7,13,16)</sup>	< 0.25% <sup>(13)</sup> < 1.0% <sup>(16)</sup>
Polysorbate 61			< 3.0% <sup>(11)</sup> < 1.0% <sup>(8)</sup>	
Polysorbate 65	< 3 ppm <sup>(13)</sup>	< 10 ppm <sup>(13)</sup>	< 3.0% <sup>(9,11,13)</sup>	< 0.25% <sup>(13)</sup>
Polysorbate 80	< 3 ppm <sup>(13,18)</sup>	< 10 ppm <sup>(13,18)</sup>	< 3.0% <sup>(13,16)</sup>	< 0.15% <sup>(13,18)</sup> < 1.0% <sup>(16)</sup>
Polysorbate 81			< 3.0% <sup>(11)</sup>	
Polysorbate 85			< 5.0% <sup>(11)</sup> < 3.0% <sup>(16)</sup>	< 1.0% <sup>(16)</sup>

TABLE 7. Product Formulation Data.<sup>(53)</sup>

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25-50	>10-25	>5-10	>1-5	>0.1-1	≤0.1
Polysorbate 20										
Baby shampoos	35	6	—	—	—	2	—	2	2	—
Other baby products	15	1	—	—	—	—	—	—	1	—
Bath oils, tablets, and salts	237	7	—	—	2	2	—	1	2	—
Bubble baths	475	7	—	—	—	—	3	2	2	—
Bath capsules	3	10	—	—	—	1	—	1	8	—
Eyeliners	369	11	—	—	—	—	1	4	6	—
Eye shadow	2582	1	—	—	—	—	—	—	1	—
Eye makeup remover	81	2	—	—	—	—	—	2	—	—
Mascara	397	2	—	—	—	—	—	—	2	—
Other eye makeup preparations	230	3	—	—	—	—	—	1	2	—
Colognes and toilet waters	1120	13	—	3	—	1	—	9	—	—
Perfumes	657	7	—	1	—	5	—	—	—	—
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	28	—	—	—	—	—	—	28	—
Other fragrance preparations	191	7	—	—	—	1	—	3	2	1
Hair conditioners	478	33	—	—	—	—	1	10	21	1
Hair sprays (aerosol fixatives)	265	2	—	—	—	—	—	—	1	1
Permanent waves	474	54	—	—	—	—	2	6	38	8
Hair rinses (noncoloring)	158	14	—	—	—	—	1	2	10	1
Hair shampoos (noncoloring)	909	47	—	—	—	2	2	8	33	2
Tonics, dressings, and other hair grooming aids	290	14	—	—	—	—	—	6	7	1
Wave sets	180	31	—	—	—	—	—	1	19	11
Other hair preparations (noncoloring)	177	7	—	—	—	—	—	1	5	1
Hair dyes and colors (all types requiring caution statement and patch test)	811	4	—	—	—	—	—	4	—	—
Hair tints	15	14	—	—	—	—	—	13	1	—
Hair shampoos (coloring)	16	7	—	—	—	—	—	—	7	—

Other hair coloring preparations	49	3	—	—	—	—	—	2	1	—
Blushers (all types)	819	10	—	—	—	—	—	5	4	1
Makeup foundations	740	20	—	—	—	—	—	1	16	3
Lipstick	3319	1	—	—	—	—	—	1	—	—
Makeup bases	831	60	—	—	—	—	1	6	36	17
Rouges	211	4	—	—	—	—	—	—	3	1
Makeup fixatives	22	1	—	—	—	—	—	—	1	—
Other makeup preparations (not eye)	530	5	—	—	—	—	—	2	3	—
Cuticle softeners	32	4	—	—	—	—	—	4	—	—
Nail creams and lotions	25	3	—	—	—	—	—	1	1	1
Nail polish and enamel remover	41	1	—	—	—	—	—	—	1	—
Other manicuring preparations	50	1	—	—	—	—	—	—	1	—
Mouthwashes and breath fresheners (liquids and sprays)	53	4	—	—	—	—	—	—	3	1
Bath soaps and detergents	148	4	—	—	—	—	—	4	—	—
Deodorants (underarm)	239	5	—	—	—	—	—	2	3	—
Douches	26	5	—	—	—	—	—	3	2	—
Other personal cleanliness products	227	14	—	—	—	—	—	—	14	—
Aftershave lotions	282	6	—	—	—	—	1	3	2	—
Preshave lotions (all types)	29	1	—	—	—	—	—	—	1	—
Shaving cream (aerosol, brushless, and lather)	114	7	—	—	—	—	—	4	3	—
Other shaving preparation products	29	1	—	—	—	—	—	—	—	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	51	—	—	—	—	5	21	18	7
Face, body, and hand skin care preparations (excluding shaving preparations)	823	35	—	—	—	—	—	22	11	2
Moisturizing skin care preparations	747	23	—	—	—	—	—	5	13	5
Night skin care preparations	219	7	—	—	—	—	—	3	2	2
Paste masks (mud packs)	171	12	—	—	—	—	1	5	6	—
Skin lighteners	44	1	—	—	—	—	—	1	—	—
Skin fresheners	260	44	—	—	—	—	—	22	20	2
Wrinkle smoothers (removers)	38	2	—	—	—	—	—	1	1	—

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25-50	>10-25	>5-10	>1-5	>0.1-1	≤0.1
Polysorbate 20—Cont.										
Skin lighteners	44	1	—	—	—	—	—	1	—	—
Skin fresheners	260	44	—	—	—	—	—	22	20	2
Wrinkle smoothers (removers)	38	2	—	—	—	—	—	1	1	—
Other skin care preparations	349	26	—	—	—	—	—	10	14	2
Suntan gels, creams, and liquids	164	5	—	—	—	—	—	3	2	—
Indoor tanning preparations	15	2	—	—	—	—	—	1	1	—
Other suntan preparations	28	2	—	—	—	—	—	—	2	—
1981 TOTALS		702	—	4	2	14	18	209	383	72
1979 TOTALS <sup>(54)</sup>		719	52	6	2	14	17	205	363	60
Polysorbate 21										
Night skin care preparations	219	1	—	—	—	—	—	—	1	—
1981 TOTALS	—	1	—	—	—	—	—	—	1	—
1979 TOTALS <sup>(54)</sup>	—	1	—	—	—	—	—	—	1	—
Polysorbate 40										
Eye lotion	13	2	—	—	—	—	—	2	—	—
Other eye makeup preparations	230	3	—	—	—	—	—	3	—	—
Colognes and toilet waters	1120	3	—	—	—	—	3	—	—	—
Hair conditioners	478	3	—	—	—	—	—	1	1	1
Hair straighteners	64	1	—	—	—	—	—	1	—	—
Permanent waves	474	3	—	—	—	—	—	—	—	3
Hair shampoos (noncoloring)	909	1	—	—	—	—	—	—	1	—
Tonics, dressings, and other hair grooming aids	290	3	—	—	—	—	—	3	—	—
Makeup foundations	740	1	—	—	—	—	—	—	—	1
Nail creams and lotions	25	1	—	—	—	—	—	1	—	—
Other manicuring preparations	50	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	21	—	—	—	—	—	17	4	—

Face, body, and hand skin care preparations (excluding shaving preparations)	823	8	—	—	—	—	—	6	2	—
Moisturizing skin care preparations	747	8	—	—	—	—	—	4	4	—
Night skin care preparations	219	2	—	—	—	—	—	2	—	—
Skin fresheners	260	2	—	—	—	—	—	—	2	—
Other skin care preparations	349	2	—	—	—	—	—	—	2	—
Suntan gels, creams, and liquids	164	3	—	—	—	—	—	3	—	—
Indoor tanning preparations	15	1	—	—	—	—	—	1	—	—
<b>1981 TOTALS</b>		<b>69</b>	<b>—</b>	<b>—</b>	<b>—</b>	<b>—</b>	<b>3</b>	<b>44</b>	<b>17</b>	<b>5</b>
<b>1979 TOTALS<sup>(34)</sup></b>		<b>59</b>	<b>11</b>	<b>—</b>	<b>—</b>	<b>—</b>	<b>2</b>	<b>31</b>	<b>12</b>	<b>3</b>
<i>Polysorbate 60</i>										
Bubble baths	475	1	—	—	—	—	1	—	—	—
Other bath preparations	132	2	—	—	—	—	—	1	1	—
Eyebrow pencil	145	3	—	—	—	—	—	—	3	—
Eyeliners	369	2	—	—	—	—	—	—	2	—
Eye shadow	2582	116	—	—	—	—	—	2	—	114
Mascara	397	10	—	—	—	—	2	1	7	—
Other eye makeup preparations	230	7	—	—	—	—	1	4	2	—
Colognes and toilet waters	1120	1	—	—	—	—	—	1	—	—
Perfumes	657	1	—	—	—	—	—	—	1	—
Sachets	119	7	—	—	—	—	—	7	—	—
Other fragrance preparations	191	5	—	—	—	—	—	—	5	—
Hair conditioners	478	2	—	—	—	—	—	—	2	—
Hair straighteners	64	3	—	—	—	—	—	3	—	—
Hair rinses (noncoloring)	158	1	—	—	—	—	—	1	—	—
Tonics, dressings, and other hair grooming aids	290	3	—	—	—	—	1	1	1	—
Other hair preparations (noncoloring)	177	2	—	—	—	1	—	1	—	—
Other hair coloring preparations	49	1	—	—	—	—	—	1	—	—
Blushers (all types)	819	24	—	—	—	—	1	—	1	22
Face powders	555	26	—	—	—	—	—	1	—	25
Makeup foundations	740	22	—	—	—	—	1	12	8	1
Lipstick	3319	1	—	—	—	—	—	—	—	1
Makeup bases	831	12	—	—	—	—	—	3	4	5
Rouges	211	8	—	—	—	—	—	1	—	7

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				> 50	> 25-50	> 10-25	> 5-10	> 1-5	> 0.1-1	≤ 0.1
<i>Polysorbate 60—Cont.</i>										
Nail creams and lotions	25	2	—	—	—	—	—	1	1	—
Mouthwashes and breath fresheners (liquids and sprays)	53	2	—	—	—	—	—	1	1	—
Other personal cleanliness products	227	1	—	—	—	—	—	—	1	—
Shaving cream (aerosol, brushless, and lather)	114	19	—	—	—	—	—	9	10	—
Other shaving preparation products	29	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	67	—	—	—	—	—	48	19	—
Face, body, and hand skin care preparations (excluding shaving preparations)	823	46	—	—	—	—	—	27	17	2
Moisturizing skin care preparations	747	64	—	—	—	—	1	37	24	2
Night skin care preparations	219	23	—	—	—	—	—	20	3	—
Paste masks (mud packs)	171	7	—	—	—	—	—	4	3	—
Skin lighteners	44	2	—	—	—	—	—	—	2	—
Skin fresheners	260	2	—	—	—	—	—	—	2	—
Other skin care preparations	349	8	—	—	—	—	—	7	1	—
Suntan gels, creams, and liquids	164	12	—	—	—	—	1	5	6	—
Indoor tanning preparations	15	5	—	—	—	—	—	5	—	—
Other suntan preparations	28	5	—	—	—	—	—	3	2	—
1981 TOTALS		526	—	—	—	1	9	207	130	179
1979 TOTALS <sup>(54)</sup>		512	44	—	—	—	4	181	103	180
<i>Polysorbate 61</i>										
Baby lotions, oils, powders, and creams	56	1	—	—	—	—	—	1	—	—



Makeup bases	831	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	—	—	—	—	—	1	1	—
Face, body, and hand skin care preparations (excluding shaving preparations)	823	1	—	—	—	—	—	—	1	—
Moisturizing skin care preparations	747	1	—	—	—	—	—	1	—	—
Night skin care preparations	219	1	—	—	—	—	—	1	—	—
Skin lighteners	44	1	—	—	—	—	—	1	—	—
Other skin care preparations	349	2	—	—	—	—	—	2	—	—
1981 TOTALS		10	—	—	—	—	—	7	3	—
1979 TOTALS <sup>(54)</sup>		7	—	—	—	—	—	4	3	—
<i>Polysorbate 65</i>										
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	2	—	—	—	—	—	2	—	—
Hormone skin care preparations	747	2	—	—	—	—	—	—	2	—
1981 TOTALS		4	—	—	—	—	—	2	2	—
1979 TOTALS <sup>(54)</sup>		2	—	—	—	—	—	2	—	—
<i>Polysorbate 80</i>										
Baby shampoos	35	1	—	—	—	—	1	—	—	—
Baby lotions, oils, powders, and creams	56	1	—	—	—	—	—	—	1	—
Bath oils, tablets, and salts	237	4	—	—	—	2	—	1	1	—
Bubble baths	475	2	—	—	—	—	—	1	1	—
Eyeliners	369	4	—	—	—	—	—	1	—	3
Eye shadow	2582	18	—	—	—	—	—	1	8	9
Other eye makeup preparations	230	1	—	—	—	—	—	—	1	—
Colognes and toilet waters	1120	3	—	—	—	—	—	1	2	—
Other fragrance preparations	191	1	—	—	—	—	—	1	—	—
Hair conditioners	478	8	—	—	—	—	—	—	7	1
Hair sprays (aerosol fixatives)	265	1	—	—	—	—	—	—	1	—

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				> 50	> 25-50	> 10-25	> 5-10	> 1-5	> 0.1-1	≤ 0.1
Polysorbate 80 – Cont.										
Permanent waves	474	3	—	—	—	—	—	—	—	3
Wave sets	180	5	—	—	—	—	—	—	4	1
Other hair preparations										
(noncoloring)	177	1	—	—	—	—	—	—	—	1
Blushers (all types)	819	9	—	—	—	—	—	1	2	6
Face powders	555	2	—	—	—	1	1	1	—	—
Makeup foundations	740	7	—	—	—	1	1	1	4	1
Makeup bases	831	17	—	—	—	—	—	—	2	15
Rouges	211	2	—	—	—	—	—	1	1	—
Other makeup preparations										
(not eye)	530	1	—	—	—	—	—	—	1	—
Cuticle softeners	32	1	—	—	—	—	—	—	1	—
Other manicuring preparations										
Mouthwashes and breath fresheners (liquids and sprays)	53	4	—	—	—	—	—	—	3	1
Bath soaps and detergents	148	1	—	—	—	—	—	—	1	—
Deodorants (underarm)	239	2	—	—	—	—	—	—	1	1
Douches	26	1	—	—	—	—	—	—	1	—
Other personal cleanliness products										
Shaving cream (aerosol, brushless, and lather)	114	3	—	—	—	—	—	3	—	—
Other shaving preparation products										
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	29	1	—	—	—	—	—	1	—	—
Face, body, and hand skin care preparations (excluding shaving preparations)	680	23	—	—	—	—	—	12	9	2
Moisturizing skin care preparations	823	18	—	—	—	—	1	4	11	2
	747	25	—	—	—	—	2	8	11	4

Night skin care preparations	219	2	—	—	—	—	—	2	—	—
Paste masks (mud packs)	171	4	—	—	—	—	—	2	1	1
Skin fresheners	260	16	—	—	—	—	—	8	7	1
Other skin care preparations	349	3	—	—	—	—	1	—	2	—
Suntan gels, creams, and liquids	164	4	—	—	—	—	—	1	3	—
1981 TOTALS		203	—	—	—	2	7	51	91	52
1979 TOTALS <sup>(54)</sup>		166	18	—	—	2	4	42	57	43
<i>Polysorbate 81</i>										
Hair conditioners	478	2	—	—	—	—	—	1	1	—
Tonics, dressings, and other hair grooming aids	290	2	—	—	—	—	—	1	1	—
Blushers (all types)	819	2	—	—	—	—	—	—	2	—
Makeup foundations	740	5	—	—	—	—	—	—	5	—
Other makeup preparations (not eye)	530	1	—	—	—	—	—	—	1	—
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	1	—	—	—	—	—	—	1	—
Face, body, and hand skin care preparations (excluding shaving preparations)	823	1	—	—	—	—	—	—	1	—
Moisturizing skin care preparations	747	1	—	—	—	—	—	—	1	—
1981 TOTALS		15	—	—	—	—	—	2	13	—
1979 TOTALS <sup>(54)</sup>		12	—	—	—	—	—	1	11	—
<i>Polysorbate 85</i>										
Eye shadow	2582	3	—	—	—	—	—	—	—	3
Eye makeup remover	81	1	—	—	—	—	—	1	—	—
Hair conditioners	478	4	—	—	—	—	—	4	—	—
Tonics, dressings, and other hair grooming aids	290	4	—	—	—	—	—	3	1	—
Hair lighteners with color	2	1	—	1	—	—	—	—	—	—
Hair bleaches	111	1	—	1	—	—	—	—	—	—
Blushers (all types)	819	4	—	—	—	—	—	—	—	4

TABLE 7. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	Unreported Concentration	No. Product Formulations Within Each Concentration Range (%)*						
				>50	>25-50	>10-25	>5-10	>1-5	>0.1-1	≤0.1
Polysorbate 85 – Cont.										
Other makeup preparations (not eye)	530	3	—	—	—	—	—	—	2	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	4	—	—	—	—	—	2	1	1
Face, body, and hand skin care preparations (excluding shaving preparations)	823	1	—	—	—	—	—	—	1	—
Moisturizing skin care preparations	747	2	—	—	—	—	1	—	1	—
Night skin care preparations	219	1	—	—	—	—	—	1	—	—
Paste masks (mud packs)	171	1	—	—	—	—	—	—	1	—
1981 TOTALS		30	—	2	—	—	1	11	7	9
1979 TOTALS <sup>(54)</sup>		43	28	2	—	—	1	8	4	—

\*Preset product categories and concentration ranges in accordance with federal filing regulations (21 CFR 720.4); see Scope and Extent of Use in Cosmetics.

The cosmetic product formulation computer printout that is made available by the FDA is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations. Ingredients are listed in prescribed concentration ranges under specific product type categories. Although the Polysorbates are thought to be supplied only in undiluted form, certain cosmetic ingredients are supplied by the manufacturer at less than 100 percent concentration. The value reported by the cosmetic formulator in such a case may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for a two- to tenfold overestimation of the actual concentration of an ingredient in a particular product.

Table 7 lists the total number of reported formulations containing each of the Polysorbates as well as a summary analysis of the concentrations at which they are used; total figures for 1981 and 1979 data were compiled separately for each of the Polysorbates. These figures can be found in the columns labeled 1981 TOTALS and 1979 TOTALS at the end of the product category listings for each ingredient.

### **Surfaces to Which Commonly Applied**

Products containing the Polysorbates are applied to all areas of the skin, hair, nails, and mucous membranes (Table 7).

### **Frequency and Duration of Application**

Formulations containing the Polysorbates are applied as many as several times a day and remain in contact with the skin for variable periods of time following each application. Daily or occasional use may extend over many years.

### **Potential Interactions with Other Ingredients**

As surface active agents, the Polysorbates interact with many of the other ingredients used in cosmetic formulations; these interactions can go beyond the primary action of oil/water emulsification. As shown in biopharmaceutical and drug release studies, the Polysorbates can produce marked effects on suspensions of insoluble ingredients.<sup>(55,56)</sup> Numerous studies on the interactions of the Polysorbates with specific drugs can be found in the scientific literature.

The Polysorbates have been shown to inactivate preservatives in several studies. The micellar preservative concentration increased with surfactant concentration, causing a decrease in the amount of free, biologically active preservative. The preservatives were bound to two distinct loci within the Polysorbate micelle. A high-affinity site was assumed to be located near the junction of the hydrocarbon core, and a very low affinity site was located in the polyoxyethylene region. The cosmetic ingredients found to be influenced in these studies included *p*-hydroxybenzoic acid and its methyl, ethyl, propyl, and butyl esters; benzoic acid; benzyl alcohol; capric acid; chlorbutol; chlorocresol; chloroxyleneol; glycerol monolaurate; *o*-phenylphenol; and sorbic acid.<sup>(57-65)</sup> In another study, the effect of Polysorbates on preservatives was not simple inactivation; decrease or increase in the activity of cationic germicidal agents depended upon the critical micelle concentration.<sup>(66)</sup>

### Noncosmetic Use

The Polysorbates find numerous uses in industry, research, pharmacy, and food production. They are used in the textile industry as antistatic agents, fiber lubricants, and finish emulsifiers.<sup>(42,51)</sup> In biological research, they find uses in membrane protein extraction, virus deactivation, and growth culture preparation.

The Polysorbates are used in pharmaceuticals for various reasons, including the modification of an active ingredient's absorption.<sup>(67)</sup> The FDA has approved Polysorbate 80 at up to 1.0 percent as an active demulcent in ophthalmic preparations; it is "recognized as safe and effective" at the recommended concentrations of 0.2 to 1.0 percent.<sup>(68)</sup> Polysorbates 20 and 80 are listed as wetting or clarifying agents in ophthalmic products and as cleaning, wetting, or solvent agents for contact lenses in concentrations not to exceed 1.0 percent. Polysorbate 20 is classified as an "inactive ingredient or pharmaceutical necessity" in topical analgesic, antirheumatic, otic, burn, and sunburn treatment/prevention products. Polysorbate 80 holds the same status in dentifrices and other dental care agents; it may also be used as an alcohol denaturant in mouthwashes.<sup>(69)</sup>

The Polysorbates find almost ubiquitous use in the food industry and have been approved by the FDA as direct and indirect food additives for human consumption with certain restrictions.<sup>(68,70)</sup> The details of these FDA regulations for the food use of Polysorbates are listed in Table 8. Polysorbates 20, 60, and 80 are approved for direct use in all food types as synthetic flavorings (21 CFR 172.515). Polysorbates 60, 65, and 80 are approved for direct use in a wide variety of specified food types as emulsifiers, solubilizers, dispersing agents, surfactants, wetting agents, opacifiers, defoaming agents, dough conditioners, and/or adjuvants. Usage limits range from 10 ppm to 4.5 percent of the finished product; limits for vitamin-mineral preparations range from 175 to 475 mg/day, based on the recommended daily dose (21 CFR 172.836, 172.383, 172.840 and as amended 9/5/80). Polysorbates 20, 40, 60, and 80 are approved for indirect addition to all food types as components of adhesives (21 CFR 175.105). Polysorbates 20, 40, 60, 65, 80, and 85 are approved for indirect addition to all food types as emulsifiers and/or surfactants (21 CFR 178.340). The FDA has also approved Polysorbates 60 and 80 for various uses in animal feeds (21 CFR 573.840-.860).

## BIOLOGICAL PROPERTIES

### Absorption, Metabolism, Storage, and Excretion

The metabolism of Polysorbates in rats has been studied in detail with <sup>14</sup>C-label tracer techniques. When administered orally, the ester link of the Polysorbate molecule is hydrolyzed by pancreatic lipase, and the fatty acid moiety is released to be absorbed and metabolized as any other dietary fatty acid. The efficiencies with which rats hydrolyzed and absorbed the labeled fatty acid portions of Polysorbates 80, 60, and 65 when fed at a dietary level of 10 percent were 100 percent, 98 percent, and 84 percent, respectively.<sup>(71)</sup> Treon et al.<sup>(72)</sup> found that the labeled lauric acid moiety of Polysorbate 20 was rapidly absorbed and oxidized by rats. After 24 hours, some 75 percent of the lauric acid was oxidized and expired as CO<sub>2</sub>; 4 percent was not absorbed from the alimentary tract. Nelson et al.<sup>(73)</sup> fed Polysorbate 20 to rats and followed the distribution of labeled lauric acid to various tissues. The approximate distribution of radioactivity 24 hours after oral

**TABLE 8.** FDA Regulation Status of Polysorbates Found Safe for Human Consumption.<sup>(68,71)</sup>

<i>Ingredient</i>	<i>Category</i>	<i>Food Type</i>	<i>Purpose</i>	<i>Usage Limit</i>	<i>1979 21 CFR Code</i>
Polysorbate 21	DFA (Direct Food Additive)	All	Synthetic flavoring	Minimum required for intended effect	172.515
	IFA (Indirect Food Additive)	All	Component of adhesives	GMP (Good Manufacturing Practices)	175.115
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3411
Polysorbate 41	IFA	All	Component of adhesives	GMP	175.115
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3411
Polysorbate 61	DFA	All	Synthetic flavoring	Minimum required for intended effect	172.515
	DFA	Whipped edible oil topping	Emulsifier	≤ 1.4% (≤ 1.77% if with sorbitan monostearate)	172.836
	DFA	Cakes and cake mixes	Emulsifier	≤ 1.46%	172.836
	DFA	Nonstandardized confectionery coatings and cacao products	Emulsifier	≤ 1.5%	172.836
	DFA	Cake icings and cake fillings	Emulsifier	≤ 1.46%	172.836
	DFA	Sugar-type confection coating	Opacifier	≤ 1.2%	172.836
	DFA	Nonstandardized dressings	Emulsifier	≤ 1.3%	172.836

TABLE 8. (Continued.)

<i>Ingredient</i>	<i>Category</i>	<i>Food Type</i>	<i>Purpose</i>	<i>Usage Limit</i>	<i>1979 21 CFR Code</i>
Polysorbate 61 continued	DFA	Shortenings and edible oils	Emulsifier	≤ 1.1% (may be exceeded if properly labeled)	172.836
	DFA	Vegetable fat-water coffee creamers	Emulsifier	≤ 1.4%	172.836
	DFA	Alcoholic drink mixes	Foaming agent	≤ 4.5%	172.836
	DFA	Yeast-leavened bakery products	Dough conditioner	≤ 1.5%	172.836
	DFA	White mineral oil and/or petrolatum wax for protective coating on raw fruits and vegetables	Emulsifier	GMP	172.836
	DFA	Gelatin desserts and mixes	Dispersing agent	≤ 0.5%	172.836
	DFA	Chocolate flavored syrups	Emulsifier	≤ 0.5%	172.836
	DFA	Natural and artificial colors in soft drinks, gelatin desserts, and pudding mixes	Surfactant and wetting agent	GMP	172.836 (as amended 9/5/80)
	IFA	All	Component of adhesives	GMP	175.105
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400
Polysorbate 65	DFA	Frozen desserts	Emulsifier	≤ 0.1%	172.838
	DFA	Cakes and cake mixes	Emulsifier	≤ 0.32%	172.838
	DFA	Whipped edible oil topping	Emulsifier	≤ 0.4%	172.838



Polysorbate 80	DFA	Vegetable fat-water coffee creamers	Emulsifier	$\leq 0.4\%$	172.838
	DFA	Cake icings and cake fillings	Emulsifier	$\leq 0.32\%$	172.838
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400
	DFA	All	Synthetic flavoring	Minimum required for intended effect	172.515
	DFA	Frozen desserts	Emulsifier	$\leq 0.1\%$	172.840
	DFA	Yeast-defoamer	Defoaming agent	$\leq 0.4\%$ ( $\leq 4$ ppm in finished food)	172.840
	DFA	Pickles and pickle products	Solubilizing and dispersing agent	500 ppm	172.840
	DFA	Vitamin-mineral preparations containing calcium caseinate but not fat-soluble vitamins	Solubilizing and dispersing agent	$\leq 175$ mg/day (based on recommended daily dose)	172.840
	DFA	Vitamin-mineral preparations containing fat-soluble vitamins but not calcium caseinate	Solubilizing and dispersing agent	$\leq 300$ mg/day (based on recommended daily dose)	172.840
	DFA	Vitamin-mineral preparations containing both calcium caseinate and fat-soluble vitamins	Solubilizing and dispersing agent	$\leq 475$ mg/day (based on recommended daily dose)	172.840
	DFA	Sodium chloride crystals	Surfactant	$\leq 10$ ppm	172.840
	DFA	Special dietary foods	Emulsifier	$\leq 360$ mg/day	172.840
	DFA	Dill oil in canned spiced green beans	Solubilizing and dispersing agent	$\leq 30$ ppm	172.840

**TABLE 8.** (Continued.)

<i>Ingredient</i>	<i>Category</i>	<i>Food Type</i>	<i>Purpose</i>	<i>Usage Limit</i>	<i>1979 21 CFR Code</i>
Polysorbate 80 continued	DFA	Shortenings and edible oils	Emulsifier	≤ 1.0 percent (may be exceeded if properly labeled)	172.840
	DFA	Whipped edible oil topping	Emulsifier	≤ 0.4%	172.840
	DFA	Scald water for poultry defeathering	Wetting agent	≤ 0.0175%	172.840
	DFA	Gelatin desserts and mixes	Dispersing agent	≤ 0.082%	172.840
	DFA	Residues from herbicides and plant growth regulators	Adjuvant	No tolerance requirement	172.840
	DFA	Creaming mixture for cottage cheese	Defoaming agent	≤ 0.008%	172.840
	DFA	Natural and artificial colors used in barbecue sauce	Surfactant and wetting agent	GMP	172.840 (as amended 9/5/80)
	IFA	All	Component of adhesives	GMP	175.105
	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400
Polysorbate 85	IFA	All	Emulsifier and/or surfactant	Minimum required for intended technical effect	178.3400

administration was: expired CO<sub>2</sub>, 80 percent; carcass, 12 percent; unabsorbed from the gastrointestinal tract, 4 percent; urine, 2.5 percent; and liver, 1.2 percent.

The polyoxyethylene sorbitan moiety left after hydrolysis of the ester is poorly absorbed from the rat's gastrointestinal tract. In one study with a radioactive carbon label in the polyoxyethylene portion of Polysorbate 20, 90 percent was excreted in the feces and 8 percent in the urine. No radioactivity was found in the liver, carcass, or expired CO<sub>2</sub>.<sup>(73)</sup> When the sorbitol moiety of Polysorbate 80 was labeled, 91 percent of the radioactivity was recovered in the feces, 2.1 percent in the urine, 1.6 percent in the carcass, and none in expired CO<sub>2</sub>, liver, kidney, spleen, adrenals, brain, gonads, or fat.<sup>(72)</sup>

After intravenous injection into rats, the ester bond is hydrolyzed by blood lipases. When Polysorbate 20 was injected into rats, the labeled lauric acid moiety was metabolized and appeared mostly as expired CO<sub>2</sub>. The polyoxyethylene moiety was not catabolized, since no radioactivity was recovered as CO<sub>2</sub> when this portion of the molecule was labeled. Most of the labeled polyoxyethylene appeared in the urine, but some was present in the feces, indicating biliary excretion.<sup>(72)</sup> After intravenous injection of Polysorbate 20 into rats in another study, the distribution of the labeled lauric acid moiety was: expired CO<sub>2</sub>, 68 percent; carcass, 22 percent; urine, 5 percent; feces and gastrointestinal contents, 2.5 percent; and liver 0.7 percent. The distribution of the labeled polyoxyethylene moiety was: urine, 83 percent; feces, 11 percent; carcass, 2 percent; liver, 0.15 percent; and expired CO<sub>2</sub> nil.<sup>(73)</sup>

Clinical tests have shown that essentially the same pattern of metabolism is followed as in the rat. The ethoxyl values of the urine and stools of four subjects fed 4.5 g of Polysorbate 80 per day were determined to ascertain the amount of the polyoxyethylene portion excreted. The results showed that the polyoxyethylene fraction was excreted quantitatively; approximately 95 percent was excreted in the feces and 5 percent in the urine. Since there were no polyoxyethylenated fatty acids detected in the urine, it was concluded that the polyoxyethylene moiety in the urine represented polyoxyethylene sorbitan and not the parent ester. The Polysorbate 80 was most likely hydrolyzed by pancreatic lipase, with the liberated oleic acid following the normal metabolic pathways of unsaturated fatty acids. The source of the polyoxyethylene in the urine was that portion absorbed from the upper intestinal tract following hydrolysis of the ester bonds. Since there was no carryover of the polyoxyethylene sorbitan in the urine during the post-medication control periods, there was no storage of this moiety in the body.<sup>(74)</sup> The possibility of oxalic acid poisoning from the polyoxyethylene component would seem negligible in light of its quantitative excretion. Urinary studies for oxalate content in patients taking oral Polysorbate 80 indicated no increase in oxaluria.<sup>(75)</sup>

## General Effects

### Biochemistry

The Polysorbates have been shown to activate or inhibit the numerous in vitro biochemical reactions listed in Table 9. The particular effect of Polysorbate 80 on one in vitro reaction listed in Table 9 was most likely due to its surface active properties alone.<sup>(76)</sup> The mechanisms of activation or inhibition include changes in substrate or enzyme dispersal and availability and are sometimes dependent

**TABLE 9.** Specific in Vitro Biochemical Effects of the Polysorbates.

<i>Polysorbate</i>	<i>Effect</i>	<i>Enzyme or Reaction</i>	<i>Determination Made in</i>	<i>Reference</i>
80	Inhibited	Dimethylnitrosamine demethylase, and ethylmorphine demethylase	Rat hepatic microsome	79
80	Inhibited	Dimethylnitrosamine demethylase	Hepatic extract	78
80	Inhibited (weakly)	Dichloro- <i>p</i> -nitro-anisole O-demethylase	Rat hepatic microsome	80
80	Inhibited	Biphenyl 2- and 4-hydroxylation	Hamster hepatic microsome	81
80	Inhibited	Biphenyl 4-hydroxylase	Rabbit hepatic microsome	82
80	Inhibited	Aniline 4-hydroxylase	Rat hepatic microsome	79
80	Activated	Aniline 4-hydroxylase	Hamster hepatic microsome	81
80	Inhibited	Glucose-6-phosphate phosphohydrolase	Rat hepatic microsome	83
80	Inhibited	Esterification of cholesterol by palmitoyl coenzyme A	Rabbit adrenal microsome	84
80	Concentration-dependent activation and inhibition	Palmitoyl coenzyme A carnitine O-palmityltransferase	Purified enzyme	85
80	Inhibited	Ribonuclease and deoxyribonuclease	Rat hepatic lysosome	86
80	Inhibited	Acid phosphatase	Rat hepatic lysosome	86
80	Activated	4-Hydroxybenzoate: polyprenyl transferase	Aged rat and guinea pig liver mitochondria	87
80	Activated	Acetylcholinesterase	Rabbit and ox caudate nuclei homogenates	76
80	Activated at low concentration and inhibited at high concentration of Polysorbate	Na/K ATPase and Mg ATPase	Isolated brush border membrane of rat intestinal epithelium	88
80	Accelerated	Photodegradation of flavin mononucleotide	Kinetic study on purified FMN	89
60	Inhibited	Serine dehydratase	Rat hepatic microsome	90
40	Inhibited	Serine dehydratase	Rat hepatic microsome	90
20	Inhibited	Biphenyl 4-hydroxylase	Rabbit hepatic microsome	82
20	Inhibited	Glucose-6-phosphate phosphohydrolase	Rat hepatic microsome	83

20	Inhibited	Esterification of cholesterol by palmitoyl coenzyme A	Rabbit adrenal microsome	84
20	Concentration-dependent activation and inhibition	Palmitoyl coenzyme A carnitine o-palmitoyl transferase	Purified enzyme	85
20	Activated low concentration and inhibited at high concentration of Polysorbate	Na/K ATPase and Mg ATPase	Isolated brush border of rat intestinal epithelium	88
20	Inhibited	Cholesterol oxidase	Purified enzyme	77
20	Inhibited	Diacylglycerol choline and ethanolamine phosphotransferases	Rat fat cell microsome	91
20	Activated	Alkaline phosphatase	Dissolved calf thymus plasma membranes	92
20	Activated	Alkaline phosphatase	Solubilized bovine milk fat globule membrane	93
20	Inhibited	Acid phosphatase	Solubilized bovine milk fat globule membrane	93
20	Activated	Phosphodiesterase I and gamma-glutamyl transpeptidase	Solubilized bovine milk fat globule membrane	93
20	Inhibited	Lipoxygenase	Human and rabbit washed platelets	94
20	Activated	5' Adenylic acid deaminase	Particulate fraction from rat brain and liver	95
20	Inhibited	Potassium phosphatase and Na/K ATPase	Purified enzyme from dog kidney	96

on Polysorbate concentration.<sup>(77)</sup> However, these *in vitro* actions may not be necessarily indicative of the *in vivo* effects of the Polysorbates. Whereas Polysorbate 80 inhibited rat hepatic dimethylnitrosamine demethylase *in vitro*, it slightly induced the enzyme when tested *in vivo*.<sup>(78)</sup>

### Cellular Metabolism

The Polysorbates have been shown to influence directly or indirectly the processes of DNA replication, transcription, and translation. Polysorbate 80 in the incubation medium of isolated nuclei from hamster kidney cells stimulated the synthesis of DNA to 150 percent of controls.<sup>(97)</sup> A single intraperitoneal injection of Polysorbate 40 at 600 to 800 mg/kg into albino mice increased short-term <sup>32</sup>P labeling of mRNA in the liver and increased the turnover rates of both rRNA and mRNA in this organ over a period of 24 hours. The activity of DNA-dependent RNA polymerases in the nuclei of treated animals was also stimulated up to 60 percent over that of control nuclei.<sup>(98)</sup> When tested *in vitro* on normal embryonic chicken, hamster, or murine or human renal cells, Polysorbate 80 stimulated the incorporation of labeled amino acids into the acid-soluble fraction of the cell suspensions. It was suggested that the Polysorbate might affect a system located in the cell membrane that controls intracellular protein synthesis.<sup>(99)</sup>

The Polysorbates affect the processes of cellular respiration in a dose-dependent manner. Inhibition of oxygen consumption in rat small intestine epithelial cells by the Polysorbates was in the order Polysorbate 20 > Polysorbate 80 > Polysorbate 60. Lactic acid formation was increased by low concentrations and decreased by high concentrations of Polysorbates 20 and 80.<sup>(100)</sup> At low concentrations and in the absence of exogenous adenosine diphosphate, Polysorbate 80 provoked a threefold to fourfold increase of *in vitro* mitochondrial respiration. At higher concentrations, Polysorbate 80 slightly inhibited mitochondrial oxidation; it progressively decreased phosphorylating capacity with increasing concentration.<sup>(101)</sup>

The effects of the Polysorbates on mitochondrial respiration seem to reflect a direct action on the ferrocytochrome C step in the electron transport chain of oxidative phosphorylation. The addition of Polysorbate 80 to suspensions of liver mitochondria from normal and tumor-bearing rats led to an increase in the activity of succinate-cytochrome C reductase in one study<sup>(102)</sup> and an increase in the activity of cytochrome C oxidase in another.<sup>(103)</sup> The activation of cytochrome C oxidase by the Polysorbates is well documented. They have been shown to reversibly convert cytochrome oxidase from an inactive to an active coupling state by providing a suitable environment for the most active conformational state.<sup>(104-106)</sup> This effect was pH independent when demonstrated with purified cytochrome oxidase.<sup>(103)</sup>

### Biological Membranes

Due to the surface active properties of the Polysorbates and the physicochemical nature of cellular membrane bilayers, the Polysorbates can affect the structure and function of biological membranes. Extensive studies have been made on the action of nonionic surfactants using test systems ranging from artificial lipid monolayers to natural multilayer epithelia.

Whether the effect the Polysorbates have on membranes is solely a function of their hydrophile-lipophile balance or whether the specific structure of the

Polysorbate molecule may also determine its biological activity is unclear. In an effort to answer this question, the hemolytic action of Polysorbates 20, 40, 60, and 80 on human erythrocytes was measured and correlated with the physical properties of the surfactants. The hemolytic power depended on the mutual effect of the hydrophobic and hydrophilic fragments of the Polysorbate molecule and did not depend on the hydrophile-lipophile balance as such. It was suggested that the role of the polyoxyethylene moiety in the action of the Polysorbates on membranes lies with its effect on the relative lipophilicity of the compound. The polyoxyethylene fragment may have also interacted with surface components when the molecule was adsorbed onto the membrane. It was concluded that the lysis of erythrocytes by the Polysorbates was caused not by the destruction of the membrane but by some rearrangement of the membrane structure accompanying adsorption of the surfactant.<sup>(107)</sup>

Surfactants are well known to generally increase the permeability of skin; although the degree of permeability to different substances varies greatly, the rate of water desorption could provide an indication of the skin's overall barrier function.<sup>(67,108)</sup> An in vitro method showed that excised rabbit skin treated with petrolatum containing 10 percent Polysorbate 85 had a greater transepidermal water desorption rate than skin treated with petrolatum alone. It was concluded in this study that Polysorbate 85 affected membrane structure, thereby increasing permeability.<sup>(67)</sup> An in vivo method for monitoring water desorption from human forearms confirmed the increase in epidermal permeability caused by Polysorbate 85.<sup>(108)</sup> In another test of epithelial water permeability, it was observed that the injection of Polysorbate 80 in normal saline into the anterior chambers of rabbits' eyes promptly and regularly produced corneal edema, which was accompanied by marked corneal endothelial cytolysis and increased limbal vascular permeability.<sup>(109)</sup> Polysorbate 80 also alternatively increased and decreased the osmotic resistance of erythrocytes, the nature of the effect depending on surfactant concentration. The response to Polysorbate 80 was presumed to be due to its influence on the erythrocyte plasma membrane.<sup>(110)</sup>

Closely related to the maintenance of osmotic equilibria are the transmembrane diffusion and active transport of electrolytes. The normal function of all cell types is dependent on proper ionic permeability characteristics of the plasma membrane, but changes in the ability to control ion exchange are most prominently seen in such cell types as neurons, secretory cells, and others whose primary function depends directly on an electrical potential difference across the membrane. In a study on artificial membranes, Polysorbates 20 and 60 penetrated a lecithin monolayer and produced blockade of charge transfer through the interface.<sup>(111)</sup> When added to a bimolecular oxidized cholesterol membrane, these Polysorbates increased membrane resistance and decreased its stability. It was suggested that the Polysorbates (1) lowered the conductance of the membrane by making it less permeable to charged molecules and (2) decreased membrane stability by becoming incorporated into the membrane structure.<sup>(112)</sup>

The electrophysiologic effect of the Polysorbates on several natural tissues has also been studied. Addition of Polysorbate 80 to diluted rat blood, at concentrations having no hemolytic effect, produced an increase in the transmembrane electrical potential of the erythrocytes.<sup>(113)</sup> In the isolated rat jejunum, Polysorbate 80 increased the transmural potential differences by 20 to 34 percent and short-circuit currents by 66 to 112 percent. It decreased net tissue resistance by 19 to 30 percent.<sup>(114)</sup>

The Polysorbates also influence the transport of larger molecules across membranes and thus can affect drug activity and toxicity. Toxic synergy in golden hamsters was revealed by Polysorbate-type surface active substances when used as emulsifiers and stabilizers in foodstuffs and food colorants.<sup>(115)</sup> In contrast, Polysorbate 80 decreased the acute oral toxicity in mice of tetracycline, norsulfazole, theophylline, tubazid, procainamide, amidopyrine, and pentobarbital.<sup>(116)</sup>

Other studies support the concept that the influence of the Polysorbates on the permeability of some larger molecules is due to a change in the membrane. Polysorbate 80 increased the solubilized concentration of butylparaben, yet it decreased percutaneous penetration of the preservative through in vitro guinea pig skin.<sup>(117)</sup> Although Polysorbate 80 caused a general increase in the membrane permeability of in situ rat intestine, the absorption of *p*-aminobenzoic acid was significantly inhibited. The specific inhibitory effect in this study was attributed to the solubilization and then release of membrane proteins, which are responsible for the absorption of *p*-aminobenzoic acid.<sup>(118)</sup> Support for the theory that the Polysorbates influence the absorption of some drugs by interacting with membrane proteins also comes from the observation that Polysorbate 80 did not affect the permeability of rat small intestine to benzocaine and sulfoxazole, which are thought to be absorbed by a passive diffusion mechanism independent of membrane proteins.<sup>(119)</sup>

The potential also exists for the major site of action for Polysorbate-induced changes in epithelial and tissue permeability to be the intercellular space instead of the cellular membrane. In two studies on the mechanism of the inhibitory effect of Polysorbate 80 on the intramuscular absorption of drugs, the inhibition of absorption could not be attributed to a direct or indirect effect on the capillary wall. It was concluded that the effect was mainly due to its influence on the extracellular space and the permeability of connective tissue.<sup>(120,121)</sup>

### Neuromuscular Systems

The Polysorbates produce various, seemingly disparate effects in neuromuscular systems. Polysorbate 80 stimulated colonic motility in anesthetized rabbits.<sup>(122)</sup> In contrast, it depressed in vitro small intestinal smooth muscle cell contraction when this function was assessed by measuring the contractile activity of electrically stimulated guinea pig ileum.<sup>(123)</sup> Polysorbates 20, 40, 60, 80, 81, and 85 inhibited the spasmogenic effect of acetylcholine, barium chloride, and histamine when tested on isolated guinea pig duodenum. This spasmolytic activity of the Polysorbates was of both musculotropic and neurotropic origin; the musculotropic spasmolytic activity was similar to that of papaverine, an alkaloid with smooth muscle relaxant properties.<sup>(124)</sup> In another study, Polysorbates 20 and 80 caused 30 to 100 percent inhibition of the effect of 11 different spasmogenic compounds on the isolated guinea pig ileum. They also inhibited the spasmogenic effects of some of these compounds when tested on isolated rabbit jejunum, uteri of estrus-induced rats, and isolated guinea pig seminal vesicles. They did not reduce the inhibitory effect of epinephrine and isoproterenol on the rabbit jejunum.<sup>(125)</sup>

### Lipid Metabolism

Polysorbates 20 and 80 markedly stimulated secretion of bile when injected intraduodenally at 1 ml/kg into rats.<sup>(126)</sup> Polysorbate 80 produced a weak stimulation of pancreatic enzyme secretion under the same conditions.<sup>(127)</sup> Surface ac-



tive agents are also thought to produce micellar solutions in the intestinal lumen in much the same way as bile salts, thus enhancing the uptake of fatty acids.<sup>(128)</sup> Polysorbate 80 acted synergistically with low concentrations of bile salts in the anesthetized rat, increasing the absorption and esterification of oleic acid.<sup>(129)</sup> When fed to rats for 1 week at 0.1 percent and 1 percent of the diet, Polysorbate 80 augmented the absorption of fats when they were present at 10 to 33 percent of the diet; this effect was not seen when fats comprised less than 7 percent of the diet.<sup>(130)</sup>

Polysorbate 80 and other surface active agents have been reported to reduce the severity of atherosclerosis in rabbits. It inhibited and reversed the in vitro insoluble complex formation between low-density lipoproteins from cholesterol-fed rabbits and such sulfated polysaccharides as heparitin sulfate and dextran sulfate. The binding of low-density lipoproteins to collagen and elastin was also inhibited, as were platelet adhesiveness and the production of aortic atheromas.<sup>(131,132)</sup> These effects were not observed in similar experiments with pigeons.<sup>(133)</sup>

### Hemodynamics

There have been several studies on the hemodynamic effects of the Polysorbates. The effects of the Polysorbates vary from species to species, with a general trend toward a depression of cardiac output. When a 5 percent aqueous solution of Polysorbate 80 was injected intravenously in doses of 1 ml/kg into cats, rabbits, and rhesus monkeys, there was a slight and transient fall in blood pressure; dogs exhibited a prolonged depressor response. This effect was never elicited by oral administration of the Polysorbates, and the depressor reaction has not been obtained in man.<sup>(134)</sup>

Polysorbate 80 showed a coronary vasodilatory effect and increased the cardiac output in isolated guinea pig and rabbit hearts when present at 2.4 mg/L in the perfusion fluid. If the concentration of Polysorbate 80 was increased stepwise from 0.7 to 4.0 mg/L, there was a dose-related increase in the coronary output. At higher doses, a slight decrease in the amplitude of contraction and an increase in the heart rate were seen.<sup>(135)</sup> In another study, the cardiovascular effects of two concentrations of Polysorbate 80 were examined in dogs. A 10 percent dextran solution with 0.05 percent Polysorbate 80 injected into the left atrium caused systemic and/or cardiac alterations in all four dogs studied. Reactions consisted of a reduction in cardiac dimensions with or without hypotension and tachycardia. Administration of a lower concentration of Polysorbate 80 (0.01 percent) induced reactions in 6 of 14 dogs. Subsequent administration of this concentration on the same day rarely induced adverse reactions.<sup>(136)</sup> When injected intravenously into dogs, Polysorbate 20 initially increased cardiac output, coronary sinus flow, and heart rate, with maintenance of systemic pressure. After 3 to 4 minutes, this was followed by greatly decreased cardiac output, vascular pressure, and coronary flow, with a continuance of tachycardia.<sup>(137)</sup>

The intravenous infusion of 5 ml of Polysorbate 20 at a rate of 0.2 ml/15 sec into 14 intact and 8 splenectomized dogs evoked anaphylactic-like symptoms that may have been mediated by endogenous histamine release. Histamine release was indicated by skin changes, tachyphylaxis, and protection by antihistamines. Other changes included decreased arterial pressure, heart rate, and plasma volume and increased respiratory rate, lymph flow, and hematocrit.<sup>(138)</sup> The Polysorbates have been shown to be nonspecific histamine releasers,<sup>(139,140)</sup> and their

hemodynamic effects are entirely compatible with histamine release. The later response of decreased output, hypotension, and tachycardia is almost indistinguishable from that found in endotoxic or hemorrhagic shock.<sup>(137)</sup>

When 10 percent aqueous Polysorbate 85 was applied repeatedly to the skin of guinea pigs for 5 hours, it caused an increase in cutaneous bloodflow. Polysorbate 20 had no effect, regardless of the duration of its application.<sup>(141)</sup>

### Immune System

Mice given 0.3 ml intraperitoneal injections of 25 percent Polysorbate 80 in saline solution prior to immunization with ovalbumin absorbed to  $\text{Al}(\text{OH})_3$  demonstrated no primary IgE response, indicating that Polysorbate 80 inhibited this response.<sup>(142)</sup> Prior intraperitoneal injection of 0.3 ml of 25 percent Polysorbate 80 in saline also caused a total suppression of the primary IgG response and a partial suppression of the passive hemagglutination response to ovalbumin in mice. Jerne plaque assays showed significant suppression of the primary antibody response. Contact sensitivity to oxazolone was not suppressed.<sup>(143)</sup>

Polysorbate 80 inactivated the rabbit serum antibody to crystalline bovine serum albumin in vitro when added to bentonite flocculation suspensions at concentrations used in immunologic studies (0.01 to 5.0 percent). It was thought either to inactivate irreversibly the antibody bound to the bentonite or to remain adsorbed to the bentonite in some manner so as to cause continued inhibition.<sup>(144)</sup>

### Mutagenesis

Polysorbate 80 was tested in the micronucleus and Ames tests as part of an evaluation of the micronucleus test as a short-term mutagenicity assay for the identification of potential carcinogens. Polysorbate 80 was negative in both assays for mutagenicity. Metabolic activation was not specified in the Ames test; the micronucleus test is an in vivo method, which implies some degree of metabolism.<sup>(145)</sup>

### Carcinogenesis

Table 10 summarizes several bioassays for carcinogenesis that have been performed on the Polysorbates. Oral studies showed no evidence for carcinogenicity by this route. Upon topical application to the skin, the Polysorbates did produce skin tumors in some studies, mostly benign dermal tumors with a tendency to regression. After reviewing many of these studies and conducting multiple experiments, Setala<sup>(146)</sup> concluded that the Polysorbates are not carcinogenic when applied to the skin. Several studies have also investigated the production of tumors after subcutaneous injection, with variable results. In one such study by Grasso et al.,<sup>(147)</sup> repeated subcutaneous injections of 2 ml of a 6 percent aqueous solution of Polysorbate 80 three times weekly for 40 weeks induced local sarcomas in 11/17 rats. As shown by concurrent tests with the food additives Blue VRS, Patent Blue V, calcium cyclamate, and sodium cyclamate, the induction of sarcomas and the tissue reaction at the site of repeated subcutaneous injections were predictable and dependent on the surface activity and calcium ion concentration of the injected solution. The authors concluded that such local sarcomas in the rat, produced by long-continued repeated injections into the same subcutaneous site, do not constitute a valid index of chemical carcinogenicity for purposes of safety evaluation.

**TABLE 10.** Bioassays for Carcinogenesis.

<i>Ingredient</i>	<i>Reference</i>	<i>Animal</i>	<i>Preparation and Dose</i>	<i>Route or Site</i>	<i>Tumors</i>	<i>Survival</i>	<i>Duration of Experiment</i>
Polysorbate 20	150	14 rats	25% of diet	PO	0	6/14	59 days
	151	36 hamsters	5% of diet	PO	0	22/36	68 days
	152	10 hamsters	5% in bread diet	PO	0	9/10	39 weeks
		10 hamsters	10% in bread diet	PO	0	2/10	39 weeks
		10 hamsters	15% in bread diet	PO	0	1/10	39 weeks
		10 rats	25% in synthetic diet	PO	0	9/10	21 weeks
	153	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor at 36 weeks	30/50 at 36 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks
	155	mice	0.18 mole. conc. twice daily, 6 days/week	Skin	0	100%	30 days
		mice	0.18 mole. conc. once daily, 6 days/week	Skin	0	100%	30 days
Polysorbate 40	153	50 mice	100% once daily, 6 days/week	Skin	2 with benign dermal tumors, first at 24 weeks	43/50 at 24 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor	43/50	24 weeks
Polysorbate 60	156	3 beagles	10% of diet	PO	0	3/3	1 year
		36 hamsters	5% of diet	PO	0	33/36	13 months
		36 hamsters	1% of diet	PO	0	36/36	13 months
		24 mice	2.5, 5, or 10% of diet	PO	0		4 months
	157	6 hamsters	0.05 ml of 1% aq. sol. once weekly	Intra-tracheal	0	3/6 at 9 months	15 months
		50 mice	100% once daily, 6 days/week	Skin	5 with 6 benign dermal tumors, first at 16 weeks. Liquid paraffin control gave no tumors	45/50 at 16 weeks	52 weeks
	158	3 rabbits	2.5% in olive oil once daily	Skin	0		116 days
	154	50 mice	100% once daily, 6 days/week	Skin	2 with benign dermal tumor	45/50	24 weeks
	159	30 mice	100% once daily, 6 days/week	Skin	1 papilloma		16 months

TABLE 10. (Continued.)

<i>Ingredient</i>	<i>Reference</i>	<i>Animal</i>	<i>Preparation and Dose</i>	<i>Route or Site</i>	<i>Tumors</i>	<i>Survival</i>	<i>Duration of Experiment</i>
Polysorbate 60 (cont'd.)	160	60 rats	60 mg undiluted 6 times/week	Skin	10 with 23 epithelial tumors	42/60	40 weeks
		60 mice	60 mg undiluted 6 times/week	Skin	12 with 67 epithelial tumors and 2 carcinomas	29/60	55 weeks
	155	Mice	0.18 mole. conc. in Carbowax 400, 6 times/week	Skin	0		30 days
		Mice	As above, 6 times/week	Skin	0	100%	30 days
		Mice	As above, 12 times/week	Skin	0	100%	30 days
		Mice	As above, 12 times/week	Skin	0	100%	30 days
		Mice	As above, 6 times/week	Skin	0	100%	30 days
		Mice	As above, 6 times/week	Skin	0	100%	30 days
	161	48 mice	100%, 60 mg once daily, 6 days/week	Skin	14 with 30 benign dermal tumors (20 regressed)	41/48 at 35 weeks, 14/48 at 60 weeks	70 weeks
		54 mice	As above	Skin	12 with 17 benign dermal tumors (11 regressed)	25/54 at 25 weeks, 9/54 at 60 weeks	70 weeks,
		60 mice	100%, 2 drops daily	Skin	22 with 202 benign dermal tumors, 5 squamous cell carcinomas, and 1 basal cell carcinoma	10/60	60-82 weeks
		60 mice (M + F)	100%, 60 mg twice weekly	Skin	19 with 91 benign dermal tumors, 1 squamous cell carcinoma, and 1 dermal fibrosarcoma	20/60 at 60 weeks	75-80 weeks
		30 mice (F)	100%, 30 mg twice weekly	Skin	2 with 2 benign dermal tumors	22/30 at 50 weeks	50 weeks
		30 mice (F)	100%, 30 mg once daily, 6 days/week	Skin	1 benign dermal tumor, 1 squamous cell carcinoma	15/30 at 50 weeks	50 weeks
		50 mice (tumor-susceptible strain)	0.18 mole. aq. sol.	Skin	Local skin tumors beginning at 7 weeks	1	7 weeks

163	30 mice	100% once daily	Skin	16 with 150 epidermal tumors (114 regressions) and 3 squamous cell carcinomas	10/30	60 weeks
	30 mice	As above	Skin	6 with 52 epidermal tumors (19 regressions) and 1 squamous cell carcinoma	7/30 at 40 weeks	60 weeks
	30 mice	100% twice weekly	Skin	9 with 46 epidermal tumors (32 regressions) and 1 squamous cell carcinoma	11/30	60 weeks
	30 mice	As above	Skin	10 with 44 epidermal tumors (32 regressions)	8/30	60 weeks
164	50 mice (F)	100% once daily, 6 days/week	Skin	1 skin tumor in neck region at 22 weeks	50/50	32 weeks
	40 mice (M)	As above	Skin	1 skin tumor in handling region at 16 weeks	40/40	32 weeks
	50 mice (F; tumor-susceptible CFW strain)	As above	Skin	4 with tumors in neck region and 9 animals with 13 tumors in handling region	49/50	32 weeks
	40 mice (M; tumor-susceptible CFW strain)	As above	Skin	11 with tumors in neck region and 15 animals with 25 tumors in handling region	40/40	32 weeks
	50 mice (F; tumor-susceptible CF-1 strain)	As above	Skin	2 with tumors in neck region and 14 animals with 24 tumors in handling region	50/50	32 weeks
	40 mice (M; tumor-susceptible CF-1 strain)	As above	Skin	1 skin tumor in neck region and 15 animals with 22 tumors in handling region	39/40	32 weeks
165	50 mice (F)	0.06 ml of 25% aq. sol. twice weekly	Skin	1 skin tumor with regression, 4 malignant lymphomas, 2 mammary adenocarcinomas, 8 lung adenomas	31/50	60 weeks

**TABLE 10.** (Continued.)

<i>Ingredient</i>	<i>Reference</i>	<i>Animal</i>	<i>Preparation and Dose</i>	<i>Route or Site</i>	<i>Tumors</i>	<i>Survival</i>	<i>Duration of Experiment</i>
Polysorbate 60 (cont'd)		19 mice (M)	As above	Skin	3 skin tumors in 1 mouse with 1 regression and 6 lung adenomas	8/19	60 weeks
	166	20 mice	100%, 0.1 ml twice weekly	Skin	1 skin tumor with regression		19 weeks
	167	30 mice	100% twice weekly	Skin	0	25/30	20 weeks
	168	30 rats	1 ml of 6% aq. sol. weekly	SC	5 with injection site fibrosarcomas and 3 introabdominal lymphosarcomas (probably spontaneous)		73 weeks
	169	24 rats	5 ml/kg body wt of a 6% aq. sol. once weekly x 28	SC	No subcutaneous tumors	All died or sacrificed	2 years
		24 rats	As above but x 16 weeks		No subcutaneous tumors	All died or sacrificed	2 years
	170	Mice	100% once weekly x 4	SC	1% with subcutaneous sarcoma, 5% with hepatoma	Sacrificed at 1 year	1 year
	171	49 mice	1.1 mg in 0.1 ml saline on days 1 and 7; 2.2 mg in 0.2 ml on days 14 and 21; total dose 6.6 mg	SC	2 malignant lymphomas and 6 pulmonary tumors at 49-53 weeks; 1 thyroid adenoma at 50 weeks. 5/73 untreated controls with pulmonary tumors and 2/73 with single hepatomas	48/49 at 49 weeks	53 weeks
	172	49 mice	1% on days 1, 7, 14, and 21 of life	SC	No significant increase in tumor incidence as compared to controls		1 year
		22 mice	As above but 10% concentration	SC	As above		1 year
		10 mice	As above but 100% concentration	SC	As above		1 year

Polysorbate 65	155	Mice	0.18 mole. conc. in Carbowax 400 6 times weekly	Skin	0		30 days
Polysorbate 80	173	28 mice	100 mg daily in diet for 10 weeks	PO	0	25/28 at 30 weeks, 20/28 at 50 weeks	51 weeks
	174	25 ducks	25 ml of 1% saline sol. x 1	Intra-tracheal	0		400 days
	175	1 dog	30 ml of 1% saline sol x 1	Intrabronchial	0	Died at 177 days	177 days
	153	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor at 43 weeks	34/50 at 43 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks
	176	10 mice	0.1 ml of 0.5% in saline, once weekly x 15	SC	1 pulmonary adenoma		270 days
	147	20 rats	2 ml of 6% aq. sol., 3 weeks	SC	11 with injection site fibrosarcomas (with varying degrees of differentiation), first at 27 weeks; some invasive but 0 metastasis; 5 successful transplants	17/20 at 27 weeks	40 weeks
Polysorbate 81	153	50 mice	100% once daily, 6 days/week	Skin	0	18/50	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks
Polysorbate 85	153	50 mice	100% once daily, 6 days/week	Skin	1 benign dermal tumor at 27 weeks	40/50 at 27 weeks	52 weeks
	154	50 mice	100% once daily, 6 days/week	Skin	0		24 weeks

The National Toxicology Program has begun a long-term oral carcinogenesis bioassay on Polysorbate 80. About 64 months are required from inception of the study to publication of the final report; prechronic testing began in February, 1981.<sup>(148)</sup>

Mixed cultures of epidermal and dermal cells from term fetuses of Balb/cAn mice were exposed to a medium containing Polysorbate 80 as a control for a study on the neoplastic changes caused by 50 µg/ml of 7,12-dimethylbenz[a]anthracene (DMBA) in the Polysorbate 80 medium. The cultures began to exhibit accelerated growth in vitro and an epithelioid morphology at 15 weeks in culture; similar changes were shown by DMBA plus Polysorbate 80 at 5 weeks. Injection of cells beginning approximately 21 weeks after treatment into syngeneic hosts gave rise to undifferentiated, apparently malignant epidermoid tumors. Polysorbate 80 did not produce the degree of in vivo malignancy nor the same types of changes in morphology and cell differentiation in culture as DMBA.<sup>(149)</sup>

### **Tumor Enhancement\***

Numerous reports are available on tumor promotion and cocarcinogenesis by the Polysorbates. Tumor promotion and cocarcinogenesis have been demonstrated with a number of known carcinogenic agents and are such that succedent or concurrent administration of a Polysorbate produces increased yields of tumors. These data are summarized in Table 11.

The terms "tumor promotion" and "cocarcinogenesis" are used because the Polysorbates are neither genotoxic nor carcinogenic. Tumor promoters, by definition, enhance the effects of complete carcinogens when given subsequently; examples of tumor promoters include phorbol esters, phenol, anthralin, bile acids, tryptophan metabolites, and saccharin. Cocarcinogens enhance the effects of complete carcinogens when given at the same time; examples include phorbol esters, pyrene, catechol, ethanol, *n*-dodecane, and SO<sub>2</sub>.<sup>(185)</sup>

A comprehensive review and discussion of tumor promotion and cocarcinogenesis by the Polysorbates was published by Setala in 1960.<sup>(146)</sup> As in that report, the term "tumor enhancement" is used in this review to encompass both concepts. Setala concluded that "tumor enhancement in mouse skin is a fully benign, slow, and at least partly reversible process. It merely provides the conditions for the manifestations of the actual carcinogenic process started by pretreatment with carcinogen, or, in susceptible mouse strains, even without it. Accordingly, the tumor-enhancing process as brought about by dipole-type agents is not part of the carcinogenic process itself." The reader is referred to this paper by Setala for a detailed review of the data and knowledge available in 1960. Following is a summary of the literature on the subject published after 1960.

When applied to the skin of mice, Polysorbates 40 and 60 have been shown to increase the mitotic index, shorten the mitotic cycle, and accelerate cell differentiation in the basal layer of the epidermis. This action seems to involve the selection of cells with a shortened G1 phase, which may be associated with a weakened response to the factors regulating cell proliferation. These changes in the kinetics of epidermal growth are also found when carcinogens alone are applied to the skin.<sup>(186-189)</sup> It has been hypothesized that the transformation of normal cells into cancer cells is determined by two factors: a genotoxic factor and a cell

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\* The term "tumor enhancement" encompasses both tumor promotion and cocarcinogenesis.<sup>(146)</sup>



**TABLE 11.** Tumor Promotion and Cocarcinogenesis.

<i>Ingredient</i>	<i>Reference</i>	<i>Animal</i>	<i>Route or Site</i>	<i>Carcinogenic Agent</i>	<i>Preparation and Dose</i>	<i>Results</i>	<i>Duration of Experiment</i>
Polysorbate 20	177	ICR Swiss mice	Skin	7, 12-dimethyl- benz[a]anthracene (DMBA)	0.125 mg DMBA followed by repeated appl. of 0.2 ml 0.3-3% Poly- sorbate 20	Weak tumor promotion	
	178	Wistar rats	PO	N-methyl-N'-nitro- N-nitrosoquandine (MNNG)	50 mg/L MNNG in drink- ing water containing 0.4% Polysorbate 20	Increased incidence of glandular stomach tumors as compared to MNNG controls	26-30 weeks
Polysorbate 40	179	Wistar rats	PO	MNNG	50 mg/L MNNG in drink- ing water containing 0.4% Polysorbate 40	Increased incidence of glandular stomach tumors as compared to MNNG controls	26-30 weeks
Polysorbate 60	164	50 CF-1 mice (skin tumor-susceptible strain)	Skin	DMBA	100% Polysorbate 60 containing 0.001% DMBA, 3/week	Tumors in handling region; cocarcinogenesis not demonstrated	13 weeks
		50 CF-1 mice (skin tumor-susceptible strain)	Skin	DMBA	As above, except 25% aqueous Polysorbate 60	As above	13 weeks
	166	Swiss mice	Skin	DMBA	0.1 ml 100% Polysorbate 60, 2 weeks, preceded 1 week by single application of 0.1 ml 1.5% DMBA in Nujol	Tumor promotion as compared to DMBA controls	19 weeks
	167	Swiss mice	Skin	DMBA	As above, except DMBA in mineral oil	As above	20 weeks
	180	Golden Syrian hamsters	Cheek pouch	DMBA	0.5% DMBA in undiluted Polysorbate 60, 3/week until tumor appearance	Shorter latency of cheek pouch tumors than with DMBA control	58 days
		Golden Syrian hamsters	Cheek pouch	DMBA	As above, except 3/week x 5	As above	139 days
		Golden Syrian hamsters	Cheek pouch	DMBA	As above, except 0.2% DMBA and 3/week until appearance of first tumor	As above	139 days

TABLE 11. (Continued.)

<i>Ingredient</i>	<i>Reference</i>	<i>Animal</i>	<i>Route or Site</i>	<i>Carcinogenic Agent</i>	<i>Preparation and Dose</i>	<i>Results</i>	<i>Duration of Experiment</i>
Polysorbate 60 (cont'd.)	181	SWR mice	Gastric intub- ation	3-methyl-chloanthrene (MCA)	0.1 ml undiluted Poly- sorbate 60 with 0.25% MCA, 2/week for life	Skin carcinomas in 14–34 weeks; MCA controls had no skin tumors	Life
		SWR mice	Gastric intub- ation	MCA	As above, except 1.0% MCA	As above	Life
		C57BL/6 mice	Gastric intub- ation	DMBA	0.15 ml undiluted Poly- sorbate 60 with 2% DMBA; 2/week for life	Benign skin tumors in 8–30 weeks; DMBA controls had no skin tumors	Life
		SWR mice	Gastric intub- ation	3,4-benz[a]-pyrene (BP)	0.1 ml undiluted Poly- sorbate 60 with 0.25% BP, 2/week for life	No skin tumors	Life
		SWR mice	Gastric intub- ation	BP	As above, except 1.0% BP	No skin tumors	Life
	181	C57BL/6 mice	Gastric intub- ation	BP	0.15 ml undiluted Poly- sorbate 60 with 2.0% BP, 2 weeks × 1 week	No skin tumors	Life

	182	Wistar rats	PO	MNNG	100 mg/L MNNG in drinking water containing 0.4% Polysorbate 60	Insignificant increase in incidence of glandular stomach tumors as compared to MNNG controls	18 weeks
	178	Wistar rats	PO	MNNG	50 mg/L MNNG in drinking water containing 0.4% Polysorbate 60	Increased incidence of glandular stomach tumors as compared to MNNG controls	26-30 weeks
	183	Wistar rats	PO	MNNG	100 mg/L MNNG in drinking water containing 0.4% Polysorbate 60	Insignificant increase in incidence of glandular stomach tumors as compared to MNNG controls	
	184	Rats, hamsters and dogs	PO	MNNG	50-83 mg/L MNNG in drinking water containing Polysorbate 60	Increased incidence of anaplastic stomach tumors as compared to MNNG controls	7-12 months
Polysorbate 80	177	ICR Swiss mice	Skin	DMBA	0.25 mg DMBA followed by repeated appl. of 0.2 ml 0.3-3% Polysorbate 80	Tumor promotion	-
	178	Wistar rats	PO	MNNG	50 mg/L MNNG in drinking water containing 0.4% Polysorbate 80	No increase in tumor incidences as compared to MNNG controls	26-30 weeks

proliferation/promotion factor. Both carcinogens and cocarcinogens produce epidermal hyperplasia, but only the carcinogens are genotoxic.<sup>(190)</sup> Tumor enhancement by the Polysorbates has been linked to epidermal hyperplasia in studies involving either skin painting with both the carcinogen and Polysorbate or gastric administration of the carcinogen followed by application of the Polysorbate to the skin. Other tumor promoters have also been tested under the same conditions in some studies, and it was found that the promoting activity of the particular agent is directly related to its ability to induce epidermal hyperplasia.<sup>(167,190-193)</sup> One study showed that Polysorbate 20 does not produce such epidermal hyperplasia.<sup>(194)</sup>

A second possible mechanism for tumor enhancement by the Polysorbates is inhibition of DNA repair. A variety of tumor enhancers has been examined for their effects on DNA excision repair by following the incorporation of tritiated thymidine into the DNA of cell suspensions damaged by ultraviolet, neutron, or gamma-ray irradiation. Hydroxyurea was included in these studies to produce concomitant suppression of ordinary semiconservative DNA replication. These *in vitro* assay systems used suspensions of mouse spleen cells, rat blood cells, or normal human lymphocytes. Polysorbate 80 produced a concentration-dependent suppression of DNA excision repair in every study, with 50 percent inhibition evident at concentrations as low as 0.002 percent.<sup>(195-200)</sup> Another study also demonstrated an inhibition of semiconservative DNA synthesis.<sup>(201)</sup>

A third possible mechanism of tumor enhancement by the Polysorbates is that of an effect on biological membranes. The important factor may be the effect the Polysorbates have on lipid integrity<sup>(177)</sup> and, more specifically, lysosomal membranes.<sup>(202)</sup> Alternatively, the increase in plasma membrane permeability caused by the Polysorbates may initiate RNA and protein synthesis in the G1 phase of the tumor cells and, in this way, cause the initiation of cell division.<sup>(203)</sup> This mechanism would coordinate well with that of cellular hyperplasia.

Other effects of the Polysorbates that have been linked to tumor enhancement include (1) facilitation of direct contact of a carcinogen with mucosal cell surfaces,<sup>(179)</sup> (2) induction of ornithine decarboxylase, a polyamine biosynthetic enzyme in mouse epidermis,<sup>(204)</sup> (3) inhibition of epidermal histidase activity, an enzyme found in normal epidermis but not in mouse squamous cell carcinoma,<sup>(205)</sup> and (4) enhancement of *in vitro* cell transformation and plaque formation by viruses.<sup>(206,207)</sup>

In addition, another effect of the Polysorbates on cell membranes manifested by a change in cellular adhesiveness and volume may have important consequences for tumor cell metastases. The incidence of experimental metastases after intravenous injection of Walker tumor cell suspensions into rats was increased by prior incubation with Polysorbate 80. This effect was attributed to an increase in cellular adhesiveness and volume.<sup>(208,209)</sup> In contrast, however, another study showed a decrease in human amniotic cell adhesion when incubated with Polysorbate 80.<sup>(210)</sup>

Sivak and Goyer<sup>(211)</sup> prepared an evaluation of the skin tumor enhancement potential of Polysorbates as used in cosmetic products for consideration in the safety review.

### **Tumor Growth Inhibition**

Several studies have shown that the Polysorbates at higher concentrations also have tumor growth inhibition activity. *In vitro* tests with Polysorbates 20, 21,

40, 60, and/or 80 on mouse Ehrlich ascites carcinoma cells produced reversible alterations in cellular membranes,<sup>(212)</sup> inhibition of respiration,<sup>(213)</sup> increased sensitivity to hyperthermia,<sup>(214)</sup> and an unspecified cytotoxicity.<sup>(215)</sup> In vivo tests produced different results for different Polysorbates. Intraperitoneal injection of Polysorbate 80 into mice inoculated with Ehrlich ascites carcinoma cells or of Polysorbate 60 into rats inoculated with Morris hepatoma cells significantly reduced the formation and size of tumors and increased survival time of the animals.<sup>(216-219)</sup> One author concluded that the cytotoxicity of Polysorbate 80 for the tumor cells was related to the oleic acid component, since substitution of the polyoxyethylene sorbitan residue by diethanolamine did not eliminate the cytotoxic action.<sup>(219)</sup> On the other hand, Polysorbates 20 and 40 did not exhibit in vivo tumor growth inhibition activity when assayed in mice with Ehrlich ascites carcinomas.<sup>(215,220)</sup>

### Other Physiologic Effects

The Polysorbates have been shown to produce other physiologic effects in biological assay systems. Polysorbate 80 reduced the size of litters when administered orally to rats at doses of approximately 0.8 to 3.0 g/kg.<sup>(221)</sup> At 0.01 percent in human serum, it decreased the binding of atropine sulfate to serum albumin.<sup>(222)</sup> Polysorbates 60 and 80 increased the incorporation of inorganic phosphate into rat pituitary glands and several other tissues by inducing the release of pituitary glands and several other tissues by inducing the release of pituitary corticotropin.<sup>(223)</sup> Polysorbate 20 decreased the plateletlike thromboplastic activity of bovine brain cephalin due to a change in the physical properties of the dispersion.<sup>(224)</sup> It also induced reticuloendothelial system (RES) effects in a pattern typical of many RES-activating materials; small doses stimulated and high doses depressed RES function. The low doses were thought to stimulate the RES by way of a surface active effect on RES cell membranes.<sup>(225)</sup>

## Animal Toxicology

### Oral Toxicity

#### *Acute Studies*

The acute oral toxicity of the Polysorbates has been reported in primary studies<sup>(134,226-232)</sup> and in reviews of published and unpublished literature.<sup>(233-236)</sup> The acute oral LD<sub>50</sub> values for the Polysorbates are listed in Table 12. The doses tolerated by rodents in these studies show each of the Polysorbates to be relatively harmless by acute oral administration. Product formulations, each containing one of the Polysorbates at concentrations of 1.0 to 8.4 percent, have also been tested with similarly high LD<sub>50</sub> values.<sup>(237-243)</sup>

#### *Long-term Studies*

Numerous long-term feeding studies have been carried out using a variety of animal species. Animals were fed Polysorbates at dietary levels of up to 25 percent, for periods of up to 2 years, and, in some cases, over multiple generations. Most of these studies included detailed clinical, gross pathologic, and histopathologic observations. One such study is reported below, and others are summarized in Table 13. After reviewing many of these studies, the FAO/WHO Committee on Food Additives<sup>(244)</sup> concluded that the Polysorbates cause no toxicological ef-

**TABLE 12.** Acute Oral Toxicity.

<i>Ingredient</i>	<i>Species</i>	<i>LD<sub>50</sub></i>	<i>Reference</i>
Polysorbate 20	Rat	> 38.9 g/kg	233,234
	Rat	36.7 ml/kg	231
	Rat	> 34.7 g/kg	233
	Rat	> 30 ml/kg	226
	Rat	> 5 g/kg	229
	Rat	> 4.6 g/kg	228
	Mouse	> 30 ml/kg	226
	Mouse	> 25 g/kg	232
	Hamster	18.0 ml/kg	231
Polysorbate 21	Rat	> 38.0 g/kg	233
	Rat	> 33.8 g/kg	233,236
	Rat	> 10 ml/kg	235
Polysorbate 40	Rat	> 38.4 g/kg	233,234
	Rat	> 34.2 g/kg	233,236
	Rat	> 20 ml/kg	235
	Rat	> 5 g/kg	230
Polysorbate 60	Rat	> 38.0 g/kg	233,234
	Rat	> 33.8 g/kg	233,236
	Rat	> 20 g/kg	235
	Rat	> 5 g/kg	227
Polysorbate 61	Rat	> 39.8 g/kg	233,236
	Rat	> 35.5 g/kg	233
	Rat	> 8 g/kg	235
Polysorbate 65	Rat	> 39.8 g/kg	233,234
	Rat	> 35.5 g/kg	233
	Rat	> 10 g/kg	235
Polysorbate 80	Rat	54.5 ml/kg	231
	Rat	> 38.0 g/kg	233,234
	Rat	> 33.8 g/kg	233
	Rat	> 20 ml/kg	235
	Mouse	> 25 g/kg	232
Polysorbate 81	Mouse	> 20 ml/kg	134
	Rat	> 36.6 g/kg	233,236
	Rat	> 32.6 g/kg	233
	Rat	> 20 ml/kg	235
Polysorbate 85	Rat	> 36.4 g/kg	233,236
	Rat	> 32.4 g/kg	233
	Rat	> 20 ml/kg	235

fects at a level of 5 percent in the daily diet of test animals. Indeed, many species tolerated much greater quantities for extended periods of time.

A definitive long-term study was conducted by Oser and Oser<sup>(71,245-247)</sup> in which the effects of Polysorbates 60, 65, and 80 at dosage levels of 5, 10, and 20 percent in the diet of rats were observed for 2 years and over four successive generations. The rats were evaluated by various criteria, which can be summarized under the headings of growth, feeding efficiency, clinical observations, reproductive efficiency, hematology, urology, and histopathology. The 20 percent dosage level was chosen as one that "was expected to induce an adverse response." The most notable effect at this level was diarrhea; there were also some effects on postnatal survival, lactation efficiency, breeding activity, growth rate, and longevity. The 10 percent dosage level produced only diarrhea. The problems with diar-

**TABLE 13.** Subchronic and Chronic Oral Toxicity.

<i>Ingredient</i>	<i>Length of Study</i>	<i>Species</i>	<i>Number of Animals</i>	<i>Level of Polysorbate in Diet</i>	<i>Results</i>	<i>Reference</i>
Polysorbate 20	7 weeks	Chick	12	0.1%	No adverse effects	256
	7 weeks	Chick	12	1.0%	No adverse effects	
	7 weeks	Chick	12	2.0%	No adverse effects	
	8 weeks	Rat		3%	Slow weight gain attributed	257
	8 weeks	Rat		5%	to mild diarrhea; no gross abnormalities nor significant histopathological findings	
	10 weeks	Hamster	36	5%	High mortality, perhaps due to diarrhea	150,151
	10 weeks	Hamster	36	15%		
	21 weeks	Rat	10	25%	1 fatality; significant gross and histopathological findings in bladder, kidney, spleen, and GI tract	231
	28-39 weeks	Hamster	10	5%	18/30 fatalities, 14 before week 12; significant gross and histopathologic findings in bladder, kidney, spleen, and GI tract	231
	28-39 weeks	Hamster	10	10%		
	28-39 weeks	Hamster	10	15%		
	17 months	Monkey	4	1 g/day	No significant gross or histopathologic changes	257
	Lifespan	Rat		0.5-2.0%	No significant gross, hematologic, or histopathologic changes	235,258
		Hamster	10	5% 10% 15%	Diarrhea and retarded growth, high mortality, perhaps due to diarrhea	152
Polysorbate 21	2 years	Rat		2%	No significant gross or histopathologic changes	235
Polysorbate 40	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235
		Rat		2%	No gross or histologic abnormalities	259

**TABLE 13.** (Continued.)

<i>Ingredient</i>	<i>Length of Study</i>	<i>Species</i>	<i>Number of Animals</i>	<i>Level of Polysorbate in Diet</i>	<i>Results</i>	<i>Reference</i>
Polysorbate 60	7 weeks	Chick	12	0.1%	No adverse effects	256
	7 weeks	Chick	12	1.0%	No adverse effects	
	7 weeks	Chick	12	2.0%	No adverse effects	
	8 weeks	Rat		2%	No adverse effects	235
	8 weeks	Rat		5%	No adverse effects	
	8 weeks	Rat		10%	Diarrhea after first few days with recovery after continued feeding	
	12-16 weeks	Mouse	10-12	2.5%	No adverse effects	156
	12-16 weeks	Mouse	10-12	5%	No adverse effects	
	12-16 weeks	Mouse	10-12	10%	No adverse effects	
	12-16 weeks	Mouse	10-12	15%	Some GI disturbance with reduced food intake and growth retardation	
	14 weeks	Weanling rat	12	5% in purified casein	Diarrhea and growth retardation	249
	14 weeks	Weanling rat	12	5% in soybean meal	No adverse effects	249
	14 weeks	Weanling rat	12	10% in soybean meal	No adverse effects	
	14 weeks	Adult rat	12	5% in soybean meal	No adverse effects	
	14 weeks	Monkey		2 g/day	No adverse effects	235
	15 weeks	Rat		25%	Growth retardation and transient diarrhea; normal hematology and no other gross or histopathologic findings	257
	12 months	Mouse		2.5%	No adverse effects	156
	12 months	Mouse		5%	No adverse effects	
	12 months	Mouse		10%	No adverse effects	



	12 months	Mouse		15%	Reduced food intake and growth retardation; no other adverse effects	
	12 months	Hamster	12	1%	Normal levels of mortality;	156
	12 months	Hamster	12	5%	diarrhea at 5%; kidney changes at autopsy due to water imbalance from chronic diarrhea	
	14 months + 3 generations	Rat		0.25%	No differences from controls	261
	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235
	2 years	Rat	24	2%	All groups showed normal patterns of mortality; no	260
	2 years	Rat	24	5%	adverse effects at 2 and 5%;	
	2 years	Rat	24	10%	marked diarrhea and enlargement of cecum at 10 and 25%; questionable fatty change in liver at 25%;	
	2 years	Rat	24	25%	no other microscopic changes at any feeding level	
	2 years	Beagle puppies		5%	No adverse effects	156
				10%	No adverse effects	
	2 years	Basenji puppies		5%	No adverse effects	156
	2 years + 4 generations	Rat	22	5%	No adverse effects	71,245-247
	2 years + 4 generations	Rat	22	10%	Diarrhea	
	2 years + 4 generations	Rat	22	20%	Diarrhea and some minor effects on growth, longevity, and reproduction	
		Rat		2%	No gross or histologic abnormalities	262
Polysorbate 61	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235

**TABLE 13.** (Continued.)

<i>Ingredient</i>	<i>Length of Study</i>	<i>Species</i>	<i>Number of Animals</i>	<i>Level of Polysorbate in Diet</i>	<i>Results</i>	<i>Reference</i>
Polysorbate 65	12 months	Dog	2	13.5%	Periods of diarrhea and dehydration; dog fed 34% showed phosphate kidney stones on autopsy as result of dehydration; no other gross, hematologic, urologic, or histopathologic findings	156
	12 months	Dog	1	34%		
	2 years	Rat		2%	No significant gross, hematologic, or histopathologic changes	235
	2 years + 4 generations	Rat	22	5%	No adverse effects	71,245–247
	2 years + 4 generations	Rat	22	10%	Diarrhea	
	2 years + 4 generations	Rat	22	20%	Diarrhea and some minor effects on growth, longevity, and reproduction	

Polysorbate 80	3 months	Rat	12	1.5 ml at 1%	1.5 ml of solution given daily;	263
	3 months	Rat	12	1.5 ml at 2%	congestion and degenerative	
	3 months	Rat	12	1.5 ml at 4%	changes in heart, liver, and	
					kidney, thought to result	
					from capillary wall damage	
	17 months	Monkey	2	1 g/day	No significant gross or histo-	257
					pathologic findings	
	2 years	Rat	30	2%	No adverse effects	134
	2 years	Rat	22	5%	No adverse effects	71,245-247
	2 years	Rat	22	10%	Diarrhea	
Polysorbate 81	+ 4 generations					
	2 years	Rat	22	20%	Diarrhea and some minor	
	+ 4 generations				effects on growth, longevity,	
					and reproduction	
	3 generations	Rat	30	2%	No alteration in fecundity or	264
					growth pattern; no histo-	
					pathologic findings in liver	
					and kidney	
	3 generations	Rat		2%	No adverse effects	265
	6 weeks	Rat		1%	No adverse effects	235
Polysorbate 85	6 weeks	Rat		4%	No adverse effects	
	6 weeks	Monkey	2	2 ml/day	No adverse effects	235
	2 years	Rat		2%	No adverse effects	235
	6 weeks	Rat	12	1%	No adverse effects	235
	6 weeks	Rat	12	4%	No adverse effects	
	2 years	Rat		2%	No adverse effects	235

rhea and reproduction at high dosage levels were alleviated by the addition of fat to the diet. The 5 percent level was chosen as a "substantial multiple of the maximum conceivable human level"; there were no adverse effects noted at this level. Even the highest levels of the Polysorbates gave no evidence of cumulative toxicity or of progressively changing physiologic response through the four consecutive generations.

It is likely that the diarrhea noted in many feeding studies with the Polysorbates resulted from having high concentrations of the unabsorbed polyoxyethylene sorbitan moiety within the intestinal lumen.<sup>(248)</sup> This diarrhea may either directly or indirectly cause many of the other observed adverse effects. The symptom of diarrhea by itself is of questionable significance, for it was found by Chow et al.<sup>(249)</sup> to depend directly upon the type of basal diet fed to the test animals. A purified casein diet that contained 5 percent Polysorbate 60 caused diarrhea and growth retardation in rats, whereas a soybean meal diet with up to 15 percent Polysorbate 60 caused neither diarrhea nor any other adverse reactions. More recent studies have confirmed this protective effect against toxicity by certain diets and have attributed it to dietary fiber.<sup>(250-254)</sup>

### Dermal Toxicity

#### *Acute Studies*

Undiluted Polysorbate 20 was tested for acute dermal toxicity following a single percutaneous exposure.<sup>(255)</sup> Each of six albino guinea pigs received 3 g/kg on the clipped intact or abraded skin of the back and flank, and the material was allowed to remain in contact with the skin for 24 hours under occlusion. No deaths resulted from the exposure, all animals appeared normal throughout the study, and there was no observable gross pathology at necropsy on the seventh day.

Six albino guinea pigs were clipped free of abdominal hair and immersed up to their axillae in a 0.5 percent aqueous solution of a product formulation containing 8.4 percent Polysorbate 20; the effective ingredient concentration was 0.042 percent.<sup>(266)</sup> The animals were immersed 4 hours per day for 3 consecutive days. There were no signs of systemic toxicity, and all skin appeared normal 48 hours after the last exposure.

#### *Subchronic Studies*

A body lotion containing 4 percent Polysorbate 40 was tested for percutaneous toxicity in a 28-day study.<sup>(267)</sup> Doses of 0.3 or 0.9 ml/kg/cm<sup>2</sup> body surface area were applied to the backs of albino rabbits 5 days a week for a total of 20 applications. The two test groups and one control group each contained eight animals, four of which received epidermal abrasions twice a week. No deaths occurred in test or control animals during the study. Slight peripheral leukocytosis was observed in several male and female rabbits after 2 weeks of treatment but was not observed after the last application; the cause of the leukocytosis is unknown. All other hematologic, urologic, and histopathologic findings were within normal ranges. A dose-related dermatitis was seen in treated rabbits as evidenced by mild to moderate erythema and edema and scaly desquamation.

A cream product formulation containing 2.5 percent Polysorbate 80 was tested for percutaneous toxicity in a 90-day study.<sup>(268)</sup> Doses of 6 mg/cm<sup>2</sup> of the product were applied to the backs of 10 rabbits for 90 consecutive days; 10 untreated ani-

mals served as a control. Three of the 20 animals (2 control and 1 treated) died during the study from intestinal and respiratory problems not considered to be treatment-related. Hematology, clinical chemistry, urinalyses, and histopathology revealed no unusual findings, and no signs of systemic toxicity were observed. Dermal lesions were characterized clinically by moderate edema and erythema, with slight to moderate desquamation, and histologically by mild dermatitis.

A cream product formulation containing 1 percent Polysorbate 85 was tested

Single 1 ml injections of 5 percent Polysorbate 80 in water were made into the central artery of the rabbit ear, intravenously into the margin ear vein of the rabbit, and intradermally at two different sites on the abdomen of the rabbit. The resultant mild intra-arterial irritation was entirely clear within 24 hours, there was no abnormal reaction intravenously, and the intradermal wheals were completely absorbed within 1 hour.<sup>(270)</sup>

The minimum lethal dose with intravenous administration of Polysorbate 80 was 1.0 g/kg for rats and 0.5 g/kg for cats and dogs. After reviewing this and other parenteral toxicity data, Badden et al.<sup>(271)</sup> recommended that the maximum concentration of Polysorbate 80 for use in pharmacologic testing without any solvent toxicity should be 6.8 percent.

#### *Subchronic Studies*

Grossman et al.<sup>(272)</sup> investigated the acute and subchronic intravenous toxicity in rabbits of Polysorbate 60 alone and of fat emulsions made with Polysorbate 60. Daily injection of 10 ml/kg of a fat emulsion containing 0.5 percent Polysorbate 60 for 3 weeks produced no biochemical or histologic abnormalities. Daily intravenous Polysorbate 60 at unspecified doses for 9 weeks led to a marked elevation of serum cholesterol and phospholipid, a slight enlargement of the adrenals, and a marked enlargement of the spleen.

Payne and Duff<sup>(273)</sup> injected intravenously a 20 percent solution of Polysorbate 80 in saline into each of 10 rabbits daily for 40 to 65 days; the daily dose of 25 ml was split into two separate injections of 10 and 15 ml. Six of the animals died between 40 and 61 days of treatment. Histologic examination showed greatly enlarged spleens with tremendous foam cell accumulation in the reticuloendothelial system and marked lipid infiltration of the renal tubular epithelium.

#### *Chronic Studies*

In a study by Huy et al.<sup>(274)</sup> on tetrahydrocannabinol toxicity, a 4 percent solution of an unidentified Polysorbate in saline was administered daily by intraperitoneal injection (0.3 ml) for 6 months to a group of 10 guinea pigs as a solvent control. Physical and biochemical parameters did not differ significantly from absolute controls receiving saline solution alone.

A reported lethal subcutaneous dose of Polysorbate 80 administered to rats over 40 weeks was a cumulative dose of 49 g/kg.<sup>(275)</sup>

### **Skin Irritation**

#### *Acute Studies*

The potential for primary skin irritation caused by Polysorbates 20, 40, 60, and 80 was evaluated using the Draize rabbit skin patch test technique in several studies.<sup>(233,279-285)</sup> In each study, 0.5 ml samples were applied and occluded for 24 hours, after which the patch sites were graded for erythema and edema on the Draize scale or another, similar scale. The results and other details of these studies are summarized in Table 15. The undiluted Polysorbates produced no or only mild skin irritation. When Polysorbate 60 was diluted to 15 percent in water, the test showed no signs of irritation.

In another test system used by Lansdown and Grasso,<sup>(286)</sup> a single 0.5 ml application of 10 percent Polysorbate 80 in distilled water to the backs of 15 mice produced no gross or histologic changes in the skin.

**TABLE 15.** Primary Rabbit Skin Irritation.

<i>Ingredient</i>	<i>Conc. (%)</i>	<i>No. of Rabbits</i>	<i>Primary Irritation Index*</i>	<i>Comments</i>	<i>Reference</i>
Polysorbate 20	100	6	0.0	No signs of irritation	233
	100	9	0.17 (max = 4.0)	Minimal irritation; modified Draize scoring	281
	100	1	0.0	No signs of irritation; three intact and three abraded patch sites	283
Polysorbate 40	100	1	0.0	No signs of irritation; three intact and three abraded patch sites	284
Polysorbate 60	100	6	0.0	No signs of irritation	233
	100	6	0.29	Minimal irritation	285
	100	9	0.50 (max = 4.0)	Mild irritation; modified Draize scoring	280
	100	1	0.0	No signs of irritation	282
	15 in water	6	0.0	No signs of irritation	285
Polysorbate 80	100	6	0.0	No signs of irritation	233
	100	9	0.17 (max = 4.0)	Minimal irritation; modified Draize scoring	279

\*Maximum = 80 unless otherwise noted; those results reported on a scale with maximum = 4.0 were calculated as the mean of individual Primary Skin Irritation (PSI) scores.

Cosmetic formulations containing the Polysorbates have also been tested for rabbit primary skin irritation. Formulations containing Polysorbates 20 and 60 at concentrations of 2.0 and 2.5 percent, respectively, produced minimal to mild irritation in a Draize 24-hour test.<sup>(287,288)</sup> Formulations containing Polysorbates 40, 60, 80, and 85 at concentrations of 1.0 to 4.0 percent produced mild to moderate irritation when applied for 4 successive days.<sup>(237,240-242)</sup>

### *Subchronic Studies*

Repeated application of 1 percent or 2.5 percent Polysorbate 80 in distilled water to the backs of 15 mice for up to 9 days produced no macroscopic or histologic changes.<sup>(286)</sup>

When 100 percent Polysorbate 60 was applied daily to the backs of three rabbits for 60 days, it was "relatively well tolerated" with only slight dermal congestion. A 15 percent solution of Polysorbate 60 in water was "very well tolerated" under the same test conditions, resulting in a normal histologic picture.<sup>(285)</sup>

Polysorbates 20, 60, 80 and 85 were applied daily to the backs of rabbits for 30 days to determine their irritative potentials and physiologic properties. They were applied either undiluted or diluted to 10, 5, or 1 percent; the solvents used were water, petrolatum, and a hydrophilic ointment. Each of the Polysorbates at 100 percent produced erythema by the third day and thickening of the skin by Day 10. Microscopic examination after 10 days revealed minimal to mild inflammation but neither acanthosis nor necrosis. After 30 days application, microscopic examination showed mild to moderate inflammation with acanthosis and, for Polysorbate 80, necrosis. At dilutions of 10, 5, and 1 percent, the Polysorbates produced only slight erythema after 10 days of application. Microscopic evaluation at 10 days showed minimal inflammation. After 30 days of application, the degree of inflammation ranged from minimal to marked for the various Polysorbates, with Polysorbate 60 producing some necrosis at 10 percent in water.<sup>(289)</sup>

A series of further studies was conducted by the same investigators in an attempt to determine the mechanism by which 10 percent Polysorbate 85 in petrolatum damages the skin of rabbits upon daily application.<sup>(290-293)</sup> Polysorbate 85 increased the phospholipid content of the rabbit epidermis<sup>(290)</sup> and increased the in vitro incorporation of labeled inorganic phosphorus into phospholipids.<sup>(291)</sup> The composition of epidermal phospholipids remained unchanged, but the biosynthetic and turnover rates of all identified phospholipids were greatly increased.<sup>(292)</sup> There were also increases in the biosynthetic rates of nucleic acids and acid-soluble materials.<sup>(293)</sup> It was concluded that the damage to rabbit skin caused by the Polysorbates reflected a direct effect on the cell membranes of the epidermis.

### **Eye Irritation**

The Draize rabbit eye irritation procedure or a modification of the test was used to evaluate the Polysorbates in a number of studies.<sup>(233,282-284,294-296)</sup> In each study, a 0.1 ml sample was instilled into one eye of each rabbit with no subsequent washing; some rabbits received a water wash, as noted in Table 16. Treated eyes were examined and graded on the Draize eye irritation scale at 1, 2, 3, 4, and 7 days. The results and other details of these studies are summarized in Table 16. The undiluted Polysorbates produced, at most, only minimal eye irritation that cleared by Day 3. Some of the Polysorbates diluted to 75 or 30 percent in water



**TABLE 16.** Draize Rabbit Eye Irritation.

Ingredient	Conc. (%)	No. of Rabbits	Ocular Irritation Index (max = 110)					Comments	Reference
			Day 1	Day 2	Day 3	Day 4	Day 7		
Polysorbate 20	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	100	6	1.0	0	0	0	0	Minimal irritation	296
	100	1	0	0	0	0	0	No irritation	283
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 21	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 40	100	1	0	0	0	0	0	No irritation	284
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 60	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	100	6	2.0	1.0	0	0	0	Minimal irritation	295
	100	1	0	0	0	0	0	No irritation	282
	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 61	60 w/v in distilled water	6 unwashed	0.33	0	0	0	0	Minimal irritation	233
		3 washed	0.67	0	0	0	0	Minimal irritation; eyes washed with water after 2 seconds	

TABLE 16. (Continued.)

Ingredient	Conc. (%)	No. of Rabbits	Ocular Irritation Index (max = 110)					Comments	Reference
			Day 1	Day 2	Day 3	Day 4	Day 7		
Polysorbate 65	30 w/v in distilled water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	10 w/v in light mineral oil	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 80	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	100 30 w/v in distilled water	6	2.0	1.0	0	0	0	Minimal irritation	294
		6 unwashed 3 washed	0 0	0 0	0 0	0 0	0 0	No irritation No irritation; eyes washed with water after 2 seconds	233
Polysorbate 81	100	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	10 w/v in light mineral oil	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
Polysorbate 85	100	6 unwashed	0.33	0	0	0	0	Minimal irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	75 in water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	30 in water	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	
	10 in light mineral oil	6 unwashed	0	0	0	0	0	No irritation	233
		3 washed	0	0	0	0	0	No irritation; eyes washed with water after 2 seconds	

or 10 percent in mineral oil produced no signs of irritation; Polysorbate 61 at 60 percent in water produced a minimal transient irritation at 1 hour after instillation, which cleared before subsequent readings.

Hazleton<sup>(297)</sup> found undiluted Polysorbates 20, 40, 60, 65, and 80 to be "tolerated" by the rabbit eye. Guillot et al.<sup>(285)</sup> tested undiluted Polysorbate 60 in a Draize eye irritation test on six rabbits with scores indicative of minimal transient irritation. Draize and Kelley<sup>(298)</sup> reported the "maximum tolerated concentration" of Polysorbate 80 to be 100 percent.

Polysorbate 60 was also tested for rabbit eye irritation by a method that involved the determination of dry tissue weight as a measure of corneal and conjunctival edema and the estimation of dye diffusion as a measure of vascular leakage in the conjunctiva and aqueous humor. The results indicate minimal to mild irritation with instillation of 100 percent Polysorbate 60 for up to 3 days and "only very minor" effects on the eye with instillation of 25 percent Polysorbate 60 in water for up to 13 days.<sup>(299)</sup>

Several product formulations containing Polysorbate 20, 40, 60, 80, or 85 at concentrations of 1.0 to 8.5 percent have also been tested in the Draize rabbit eye irritation test with scores indicative of minimal to mild irritation.<sup>(237,240-242,300-302)</sup> A bubble bath formulation produced severe irritation when instilled into rabbit eyes as evidenced by damage to the conjunctiva, iris, and cornea.<sup>(303)</sup> A subsequent test with the same formulation diluted to 0.5 percent in water showed minimal transient eye irritation.<sup>(304)</sup>

### Mucosal Irritation

In an attempt to produce a pathophysiologic model of an intestinal malabsorption syndrome, a series of studies was conducted in which detergents were used to damage the intestinal mucosa. Yonezawa<sup>(305,306)</sup> applied aqueous solutions of Polysorbate 80 to the intestinal mucosa of adult male albino rabbits. Exposure to 10 percent Polysorbate 80 for up to 4 hours produced no morphologic changes in the intestinal villi. There was a hypersecretion of mucus from goblet cells, but there was no desquamation or necrosis. Exposure to 20 percent Polysorbate 80 facilitated glucose absorption and stimulated goblet cell mucus secretion, but this concentration too caused no histopathologic changes. Oshumi<sup>(307)</sup> used transmission electron microscopy and other methods to determine the histologic and biochemical effects of a 30-minute treatment with 10 percent aqueous Polysorbate 80 on the rat intestinal mucosa. Marked deformation and desquamation of the microvilli occurred after treatment, without removal of the enteric surface coat. Histologically, the destruction was strongly localized within the level of the brush border, while the terminal web and level beneath remained intact. Glucose absorption was accelerated as a result of this mucosal damage. Further tests were conducted by Oshumi et al.,<sup>(308)</sup> with the duration of treatment lasting 3 months. Polysorbate 80 was given at 10 percent in the drinking water of rats for the duration of the test. As compared to controls, Polysorbate 80 produced body weight gain and increased glucose absorption, which declined after long-term treatment, hypersecretion of goblet cells, deformation of villi, destruction of microvilli, and changes in mitochondria. Nadai et al.<sup>(309)</sup> studied the effect of Polysorbate 80 on the intestinal mucosa of the rat by measuring the exsorption rate of intravenously administered sulfaguanidine. Polysorbate 80 increased the transfer of sulfaguanidine from the blood vessel to the intestinal lumen, indicating damage to the mucosa.

A study on the effects of Gastrografin, a water-soluble radiographic contrast material, on the colonic mucosa of rats suggested that Polysorbate 80 played a major role in the production of inflammation.<sup>(310)</sup> A later study in which rats were given enemas of 10 percent aqueous Polysorbate 80 showed no deleterious effects with volumes sufficient to fill the colon. Severe changes did result, however, from volumes that produced overdilatation.<sup>(311)</sup>

Studies have been carried out to determine the effect of Polysorbates on mucosa other than that of the intestine. Polysorbate 20 produced no inflammation when applied to the cheek pouch mucosa of hamsters at an unspecified volume and concentration,<sup>(312)</sup> and 10 percent aqueous Polysorbate 40 produced neither inflammation nor toxic effects when infused into the urinary bladder of guinea pigs.<sup>(313)</sup>

Mucosal irritation tests have been conducted on product formulations containing the Polysorbates. A lotion containing 4 percent Polysorbate 40 and a cream formulation containing 1 percent Polysorbate 85 produced no signs of irritation after 0.1 ml doses were applied to the penile and vaginal mucosae of rabbits.<sup>(241,314)</sup> A bubble bath containing 6 percent Polysorbate 20 produced severe irritation when instilled into the vaginal vaults of three beagle dogs once daily, 5 days a week, for 3 weeks. Mucosal lesions were characterized by redness, blistering, mucosal sloughing, and, in one case, fibrous adhesions. Histologic examination revealed marked diffuse erosion of the vaginal mucosa with necrotic and fibrinous exudate on the mucosal surface and a marked infiltration of polymorphonuclear leukocytes in the submucosal tissue.<sup>(315)</sup> A subsequent, similar test with the same bubble bath diluted to 0.5 percent in water produced no visible findings that were attributable to treatment. Gross pathologic and histopathologic observations revealed no differences from saline-treated controls.<sup>(316)</sup>

### Skin Sensitization

The Magnusson-Kligman guinea pig maximization test<sup>(317)</sup> was used to determine the sensitization potential of Polysorbate 20; five assays were completed on three different batches of the material.<sup>(318)</sup> The procedure consisted of an induction phase of intradermal injection and topical application followed by a series of two or three topical challenges. Animals were injected intradermally with 0.1 ml of 50 percent complete Freund's adjuvant in saline, a 0.1 ml of an irritant concentration of Polysorbate 20 in paraffin oil (5.0 to 7.5 percent), and 0.1 ml of the test material in 50 percent complete Freund's adjuvant in saline. One week following the injections, undiluted Polysorbate 20 was applied topically under occlusion for 24 hours to a site pretreated with 10 percent sodium lauryl sulfate. After a 2-week rest period, undiluted Polysorbate 20 was again applied topically for 24 hours under occlusion. Second and, in one case, third challenge applications were made at 1 week intervals. Four of the five assays evoked responses at challenge indicative of moderate sensitization; one batch of Polysorbate 20 produced strong sensitization under the conditions of the test.

The sensitization potentials of Polysorbates 65 and 80 in guinea pigs were tested by repeated intradermal injections of a 0.1 percent suspension in saline followed by a challenge dose after 2 weeks.<sup>(235)</sup> There was no indication of sensitization reported.

## Clinical Assessment of Safety

### Ingestion Studies

#### *Acute Oral Toxicity*

Polysorbates 60 and 80 have been given a toxicity rating of practically non-toxic (1/6), with a probable oral lethal dose in humans greater than 15 g/kg.<sup>(319)</sup>

Chusid and Diamond<sup>(320)</sup> reported an accidental overdose of Polysorbate 80 fed to a 4 month old male infant weighing less than 8 pounds, wherein 19.2 g of Polysorbate 80 was ingested daily for 2 consecutive days with no other food. The infant passed six loose stools but showed no other evidence of intoxication.

Johnson et al.<sup>(321)</sup> administered four daily doses of 200 mg Polysorbate 20 to 13 premature and 2 fullterm infants with steatorrhea. No increase in fat absorption was observed, but it was noted that no adverse effects resulted with respect to anorexia, vomiting, defecation, or growth.

Snyderman et al.<sup>(322)</sup> studied the administration of Polysorbate 80 as a dietary supplement to nine premature infants ranging in body weight from 1.41 to 2.01 kg. Daily doses of 0.179 to 0.335 g/kg for 4 consecutive days were well tolerated with no reported adverse effects.

In an attempt to determine the effect of large doses of Polysorbate 60 on the alimentary tract of man, Steigmann et al.<sup>(323)</sup> fed a single 20 g-dose to each of 11 subjects of both sexes and various ages. There were no significant changes in gastric motility or gastric acidity and no subjective reports of adverse symptoms.

A single oral dose of 5 ml of Polysorbate 80 was given by Fisherman and Cohen<sup>(324)</sup> to 86 non-aspirin-sensitive patients with intrinsic chronic rhinitis, nasal polyps, and asthma. A positive reaction was shown by 21 of the patients (only 7 of whom were atopic), as evidenced by exacerbation of their respiratory disease. Comparisons between these patients and those of other groups tested suggest that the incidence of Polysorbate 80 intolerance in patients with intrinsic respiratory disease is about one-half the incidence of aspirin intolerance and twice the incidence of iodide intolerance.<sup>(324)</sup>

#### *Long-term Feeding*

The FAO/WHO Expert Committee on Food Additives<sup>(244)</sup> has established a maximum acceptable daily intake of Polysorbates of 25 mg/kg/day.

There have been a number of human feeding studies published primarily because of interest in using Polysorbates for therapy in lipid malabsorption syndromes. Krantz and Carr<sup>(264)</sup> described Polysorbate 80 as useful in promoting fat absorption from the alimentary tract and reported that long-term use for such purposes is "apparently without harmful effects."

Krantz et al.<sup>(134)</sup> reported that Polysorbate 80 was prescribed to more than 100 patients of both sexes and various ages for its beneficial effect on the intestinal absorption of fats. This group of patients ranged in age from 5 to 72 years and was of approximately equal sex distribution. Doses of 4.5 to 6.0 g were taken daily by 10 patients for 3 to 4 years, 17 for 2 to 3 years, 19 for 1 to 2 years, and more than 54 for less than 1 year. The large body of clinical and laboratory data collected during the course of the study indicated no adverse effects after long-term consumption of Polysorbate 80. It was judged to be harmless for human consumption in amounts of at least 6.0 g per day.

King et al.<sup>(325)</sup> studied the effects of Polysorbates 20 and 80 in 13 patients with

bile salt-deficient steatorrhea, 8 of whom received Polysorbate 80 and 5 Polysorbate 20. When 2 g were administered three times a day, the Polysorbates increased micellization of ingested fat and improved fat absorption. Therapy reduced steatorrhea in 10 of the 13 patients.

Jones et al.<sup>(75)</sup> fed as much as 15.0 g of Polysorbate 80 daily for a period of several months to patients with steatorrhea for modification of fat absorption. There were no untoward symptoms and no evidence of toxicity as measured by changes in peripheral blood erythrocytes, leukocytes, liver function, or renal function. The only symptom attributable to the use of Polysorbate 80 has been the rare manifestation of increased bowel activity.

Boyd and Helfrick<sup>(326)</sup> reported that a 2-year old child with severe celiac disease remained free of any symptoms when treated for over 1 year with 40 mg of Polysorbate 80 for each gram of ingested fat.

Mindrum<sup>(327)</sup> used Polysorbate 80 at a level of 2 percent in a high-calorie emulsion diet for nine critically ill patients. Ingestion of this emulsion for 30 to 120 days caused no apparent adverse effects.

Waldstein et al.<sup>(328)</sup> evaluated the pharmacologic effect of Polysorbate 60 per os. A group of 34 patients of an elderly and chronic disease infirmary and a group of 10 normal hospital personnel were fed 6 g of Polysorbate 60 daily for 28 days. Clinical and laboratory tests produced no evidence of adverse effects.

Steigmann et al.<sup>(323)</sup> fed 6 g of Polysorbate 60 per day for 28 days to each of 10 subjects. No significant effects were found on the physiologic activity of the gastrointestinal tract in any of the subjects.

Preston et al.<sup>(329)</sup> fed daily 1-g doses of Polysorbate 60 to three normal children; one child received treatment for 13 days, another for 31 days, and the third for 34 days. No harmful effects were observed in any of the patients as reflected by careful clinical examinations, including tests for duodenal enzymes and fecal fat and nitrogen.

Page<sup>(330)</sup> studied 20 normal adults who were fed 4 g/day for 28 days of an emulsifier mixture containing 20 percent Polysorbate 60 as a supplement to their regular diet. An additional 20 subjects were each fed 8 g/day for 28 days of a mixture consisting of 80 percent Polysorbate 61 and 20 percent Polysorbate 60. A third group of 20 subjects was fed 4 g/day for 28 days of an emulsifier mixture containing 6 percent Polysorbate 60. The test doses were administered in three equal portions daily in conjunction with a chocolate syrup. No significant variations were observed in any of the subjects as evidenced by physical examination, hematology, and urinalysis.

Jeans and Stearns<sup>(331)</sup> studied the effects of adding emulsifier mixtures containing Polysorbates 60 and 80 to the daily diets of nine infants ranging in age from 1 week to 7 months. Daily administration of approximately 0.2 g Polysorbate 60 with 0.04 g Polysorbate 80 was continued for periods of 1.5 to 5 months, with three of the infants receiving approximately 0.4 g Polysorbate 60 with 0.04 g Polysorbate 80 per day for an additional 1 to 2 months. Careful observation of the patients, including comparative growth curves and nutritional balance studies, indicated no adverse effects as a result of feeding the emulsifiers.

A review of animal studies indicates that the Polysorbates may induce diarrhea when given in the diet at high doses (see Oral Toxicity in Animal Toxicology section). To test the effects of Polysorbates 65 and 80 on gastrointestinal motility in man, Janowitz et al.<sup>(332)</sup> fed normal individuals of both sexes and various ages 9 g/day for 13 consecutive days; each of the Polysorbates was administered to 12

adult subjects. No subjective reactions or changes in bowel habit, character of stools, or body weight were induced. No evidence was obtained to indicate any effect of the Polysorbates on intestinal transit time or gastrointestinal mucosal patterns.

Polysorbate 80 has been used by McCorriston<sup>(333)</sup> as an oral therapeutic agent for the treatment of atopic dermatitis, psoriasis, and other dermatoses. Daily doses of 6 g were administered to a total of 85 patients with chronic dermatoses for up to 3 months in an attempt to alter lipid metabolism. There was a significant increase in serum lipid levels but no evidence of toxic effects.

### Skin Irritation

Undiluted Polysorbates 20, 21, 40, 60, 80, 81, and 85, and Polysorbates 61 and 65 diluted to 60 percent in water were tested in 50 subject panels by Schwartz.<sup>(334)</sup> A 72-hour occlusive patch was applied to the skin, followed after 7 days with a second 72-hour patch. No evidence of irritation was observed in any of the patients. Other investigators using a similar technique with 48-hour patches on 10 subject panels found no irritation to undiluted Polysorbates 20, 21, 65, 80, and 81 or to Polysorbates 20, 40, 60, 61, 80, 81, and 85 diluted to 30 percent in water.<sup>(335,336)</sup> Polysorbates 20 and 80 were also tested in 24-hour single insult patch tests with no resultant irritation in 19 to 20 subject panels.<sup>(337,338)</sup> These studies are summarized in Table 17; they show no potential for primary skin irritation caused by the Polysorbates.

A number of product formulations containing various Polysorbates at concentrations of 1.0 to 8.4 percent have also been tested for human skin irritation. The results and other details of these studies are summarized in Table 18. Single insult occlusive patch tests on three formulations produced no or only minimal to mild irritation.<sup>(339-341)</sup> Daily skin patching of eight product formulations for 21 days produced ratings of "essentially nonirritating" to "highly irritating."<sup>(342-348)</sup> Results indicative of irritation cannot be interpreted without knowledge of the other ingredients in a formulation.

Mezei<sup>(349)</sup> applied, under occlusion, 10 percent Polysorbate 85 in white petrolatum daily for 4 days to the upper arms of 15 normal individuals (9 male, 6 female; 20 to 32 years old). The other arm was similarly treated with petrolatum to provide the control area. At the end of the treatment, macroscopic observations indicated only minor erythema in 11 cases; no visible changes were noted in the treated areas of 4 persons or in any of the control areas. No definite histologic changes were observed by microscopic evaluation. The results of biochemical assays, however, were more definitive. The content of epidermal phospholipids was elevated 5 to 65 percent as a result of the treatment with the Polysorbate 85 preparation. Radioactive tracer studies indicated higher rates of phosphorus incorporation into epidermal phospholipids, DNA, RNA, and trichloroacetate-soluble fractions of the treated skin. Results resembled those that were documented in earlier studies by the same researcher with rabbit skin (see Skin Irritation in Animal Toxicology section).

### Eye Irritation

The effect of Polysorbate 20 on corneal permeability to fluorescein was investigated in human subjects in an effort to find safe and effective agents that increase permeability to drugs. Subjects were normal volunteers of both sexes,

**TABLE 17.** Clinical Skin Patch Tests with the Polysorbates.

<i>Ingredient</i>	<i>Test Method</i>	<i>No. of Applications</i>	<i>Duration of Each Exposure (hours)</i>	<i>Conc. (%)</i>	<i>No. of Subjects</i>	<i>Results</i>	<i>Reference</i>
Polysorbate 20	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	335
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
	Single insult	1	24	40 in water	19	No irritation	338
Polysorbate 21	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	336
Polysorbate 40	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
Polysorbate 60	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
Polysorbate 61	Prophetic patch	2	72	60 in water	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
Polysorbate 65	Prophetic patch	2	48	100	10	One subject had questionable reaction on first application only	336
Polysorbate 80	Prophetic patch	2	72	60 in water	50	No irritation; no sensitization	334
	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	335
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
	Single insult	1	24	100	20	No irritation	337
Polysorbate 81	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	100	10	No irritation; no sensitization	335
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335
	Prophetic patch	2	48	12 in water	10	No irritation; no sensitization	335
Polysorbate 85	Prophetic patch	2	72	100	50	No irritation; no sensitization	334
	Prophetic patch	2	48	30 in water	10	No irritation; no sensitization	335



**TABLE 18.** Clinical Skin Irritation Tests with Product Formulations Containing a Polysorbate.

<i>Test Method</i>	<i>Material Tested</i>	<i>Conc. of Polysorbate (%)</i>	<i>No. of Subjects</i>	<i>Results</i>	<i>Reference</i>
24-hour single insult occlusive patch	Unspecified product formulation	8.4—Polysorbate 20	19	Mean PSI = 0.47 (max = 4.0); minimal to mild irritation in 11 subjects	341
	Unspecified product formulation	2.0—Polysorbate 20	18	Mean PSI = 0.08 (max = 4.0); minimal irritation in 2 subjects	339
Cumulative irritancy test (daily 23-hour occlusive patch for 21 days)	Unspecified product	2.5—Polysorbate 60	20	No signs of irritation	340
	Bubble bath	6.0—Polysorbate 20	12	Highly irritating; total composite score was 533/630 max	342
	Bubble bath	0.03—Polysorbate 20 (0.5% aqueous dilution of product containing 6%)	10	Moderately irritating; total composite score was 433/630 max	343
	Lotion	4.0—Polysorbate 40	15	Slightly irritating; total composite score was 71/630 max	344
	Cream	4.0—Polysorbate 40	11	Slightly irritating; total composite score was 80/630 max	345
	Moisturizer cream	4.0—Polysorbate 40	9	Slightly irritating; total composite score was 48/630 max	346
	Moisturizer cream	4.0—Polysorbate 40	9	Slightly irritating; total composite score was 127/630 max	346
	Cream	6.0—Polysorbate 60	11	Essentially nonirritating; total composite score was 16/630 max	347
	Cream	1.0—Polysorbate 85	11	Slightly irritating; total composite score was 72/630 max	348

aged 20 to 46 years. Single drops of Polysorbate 20 at various concentrations in saline were administered to one eye; the other eye served as a control. Polysorbate 20 caused no adverse effects on the eye at concentrations up to 40 percent. During the initial screening for effective agents in this study, Polysorbates 40 and 81 were also administered at 1 percent in saline with no harmful effects.<sup>(350)</sup>

### Skin Sensitization

In 1975 and 1976, a total of 1206 patients with eczema (505 male, 701 female) were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to several common emulsifiers. One of the materials tested consisted of a mixture of 5 percent Polysorbate 60 and 5 percent Polysorbate 80 in petrolatum. Allergic reactions were shown by only 2 of the patients (< 0.2 percent of the test population). Only one of the other emulsifiers tested produced fewer reactions.<sup>(351)</sup>

Each of the Polysorbates was tested undiluted and/or diluted in water according to the Schwartz prophetic patch test technique.<sup>(334-336)</sup> The results and other details of these studies are summarized in Table 17. There were no reactions indicative of skin sensitization in a total of 580 subjects.

Several product formulations containing the Polysorbates have been tested for human skin sensitization on a total of 3481 subjects using a variety of testing methods. These studies included: four Schwartz-Peck prophetic patch tests on product formulations containing 0.3 to 2.4 percent Polysorbates 20, 60, or 80; four controlled-use tests on product formulations containing Polysorbate 85 at 1.0 percent or Polysorbates 60 and 80 at 2.5 percent each; 15 Draize-Shelanski repeated insult patch tests on product formulations containing 0.084 to 6.0 percent Polysorbates 20, 40, 60, or 80; and one Kligman maximization test on a product formulation containing 6.0 percent Polysorbate 20. The results and other details of these studies are summarized in Table 19. Of the 3481 subjects reported in Table 19, there were no reactions indicative of sensitization to any of the Polysorbates.

A group of 100 patients who had suffered eczematous eruptions as complications following application of topical preparations for other conditions were tested for allergic contact dermatitis caused by ingredients in the base or vehicle of the topical preparations. The patients were patch tested with 15 such substances, including one of the Polysorbates, and returned to the laboratory 48 hours after the patches were applied. The Polysorbate produced no positive reactions.<sup>(352)</sup>

Of 130 patients suffering from eczematous complications of lower leg chronic venous insufficiency, 3 were sensitive to topical administration of 2.5 percent Polysorbate 40 in water.<sup>(353)</sup>

Investigations into an isolated case of contact urticaria caused by a therapeutic cream showed Polysorbate 60 to be the responsible ingredient. The reaction was manifested on the forehead but not on the arm or back.<sup>(354)</sup>

### Intravenous Injection

The intravenous injection of 10 mg/kg of Polysorbate 80 produced hemodynamic changes in five patients. The effects included an increase in cardiac output and a decrease in peripheral resistance.<sup>(355)</sup>

**TABLE 19.** Clinical Skin Sensitization Tests with Product Formulations Containing a Polysorbate.

<i>Test Method</i>	<i>Material Tested</i>	<i>Conc. of Polysorbate (%)</i>	<i>No. of Subjects</i>	<i>Results</i>	<i>Reference</i>
Schwartz-Peck prophetic patch test (open and closed 48-hour patches, repeated after 2 weeks)	Shaving preparation	2.4—Polysorbate 20	197	No irritation; no sensitization	358
	Bubble bath	0.3—Polysorbate 20 (5% aqueous dilution of product containing 6%)	103	Minimal irritation in 3 subjects; no sensitization; supplemental UV exposure after second insult showed no photosensitization	359
	Shaving preparation Makeup	0.6—Polysorbate 60	197	No irritation; no sensitization	360
		0.6—Polysorbate 80	303	Mild irritation with closed patch in 25 subjects at first exposure and in 14 subjects at second; no evidence of sensitization	361
Prophetic patch and in-use testing (48-hour occlusive patch followed by 4 weeks of daily use and final 48-hour challenge patch)	Shaving foam	2.5—Polysorbate 60 and 2.5—Polysorbate 80	100	No reactions after either patch; 2 subjects discontinued use after 2 weeks because of minor pruritis, but no clinical signs were observed; no evidence of sensitization	362
	Shaving foam	2.5—Polysorbate 60 and 2.5—Polysorbate 80	82	Minor irritation after first patch in 6 subjects; no reactions with use of challenge patch; no evidence of sensitization	363
	Shaving foam	2.5—Polysorbate 60 and 2.5—Polysorbate 80	110	Minimal irritation in 5 subjects and moderate irritation in 1 after first patch; no reactions with use; minimal irritation in 3 subjects after challenge; one subject previously sensitized to fragrance component, no other evidence of sensitization	364
	Moisturizer cream	1.0—Polysorbate 85	204	Minimal to mild irritation in 2 subjects; no other evidence of irritation or sensitization	365

TABLE 19. (Continued.)

<i>Test Method</i>	<i>Material Tested</i>	<i>Conc. of Polysorbate (%)</i>	<i>No. of Subjects</i>	<i>Results</i>	<i>Reference</i>
Draize-Shelanski repeated insult patch test (24- or 48-hour patches 3 days/week for 10 induction patches; challenge patch after 2 weeks rest)	Shaving preparation	2.4—Polysorbate 20	101	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	358
	Skin care product	2.0—Polysorbate 20	99	Minimal irritation; no sensitization	366
	Skin care product	1.0—Polysorbate 20	98	Minimal irritation; no sensitization	367
	Bubble bath	0.3—Polysorbate 20 (5% aqueous dilution of product containing 6%)	55	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	359
	Shampoo	0.084—Polysorbate 20 (1% aqueous dilution of product containing 84%)	97	Essentially no irritation; no sensitization	368
	Lotion	4.0—Polysorbate 40	206	Isolated transient irritation; no sensitization	369
	Lotion	4.0—Polysorbate 40	206	Isolated transient irritation; no sensitization	370

	Lotion	4.0—Polysorbate 40	205	Isolated transient irritation; equivocal sensitization in 5 subjects	371
	Cream	6.0—Polysorbate 60	107	No irritation; no sensitization	372
	Facial makeup	2.5—Polysorbate 60	116	Minimal irritation; no confirmed sensitization to Polysorbate 60	373
	Baby lotion	1.0—Polysorbate 60	200	Minimal irritation; skin fatigue in 5/200; no sensitization	374
	Shaving preparation	0.6—Polysorbate 60	101	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	360
	Cream	2.5—Polysorbate 80	210	Minimal irritation; no sensitization	375
	Makeup	0.6—Polysorbate 80	149	Minimal irritation; no sensitization; supplemental UV exposure after induction patches 1, 4, 7, and 10 and after challenge showed no photosensitization	361
	Cream	1.0—Polysorbate 85	210	Essentially no irritation; no sensitization	376
Kligman maximization test (5 successive 48-hour patches with challenge after 10-day rest; sodium lauryl sulfate pretreatment before induction and challenge)	Bubble bath	6.0—Polysorbate 20	25	No sensitization	377

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### Photocontact Sensitization

Two products containing 2.5 percent Polysorbate 60 were tested by a photomaximization procedure for evidence of photo-induced contact sensitization.<sup>(356,357)</sup> On Monday of the first week in the test protocol, each patient was patched with a 5 percent aqueous sodium lauryl sulfate solution for 30 minutes. After 6 to 8 hours, a 48-hour open patch and a 48-hour ultraviolet-exposed photopatch of the product were applied at pretreated sites. This was followed on Wednesday by a second 48-hour open patch. This procedure was repeated for a total of 3 weeks, and single 48-hour open and photopatches were applied on Wednesday of the fourth week. In one study,<sup>(357)</sup> 2 of the 49 subjects showed weak, nonvesicular reactions to the first photopatch. One of these subjects also showed a weak reaction to the challenge photopatch. In the other study,<sup>(356)</sup> none of the 50 subjects showed positive reactions.

Some of the studies summarized in Table 19 include exposure to ultraviolet (UV) light as a supplement to the Schwartz-Peck prophetic patch test<sup>(359)</sup> or Draize-Shelanski repeated insult patch test.<sup>(358-361)</sup> The UV light exposure was to a Hanovia Tanette Mark I quartz lamp at a distance of 12 inches for 1 minute. This lamp has a wavelength coverage of 240 to 370 nm with a peak at 265 nm. One of the 103 subjects in the Schwartz-Peck test on a product containing Polysorbate 20 showed a weak, nonvesicular reaction after a single UV exposure; the significance of this reaction was not interpreted.<sup>(359)</sup> The Draize-Shelanski tests included UV exposure after induction patches 1, 4, 7, and 10 and after the challenge patch. With products containing Polysorbates 20 and 60, there were few instances of irritation and no reactions indicative of photosensitization.<sup>(358-360)</sup> The product containing 0.6 percent Polysorbate 80 produced several instances of irritation after UV exposure but no photosensitization.<sup>(361)</sup> When testing such whole product formulations, positive reactions are of questionable significance with respect to any one ingredient.

### Industry Complaint Experience

Complaint experience data are available on three product formulations containing Polysorbate 20. A shampoo containing 8.4 percent Polysorbate 20 had two safety-related complaints in 3 years with an estimated 5.88 million uses.<sup>(378)</sup> A cuticle softener containing 2.0 percent Polysorbate 20 had 24 complaints in 4 years with 131 million uses.<sup>(379)</sup> A paste mask containing 2.0 percent Polysorbate 20 had 11 complaints in 4 years with 12.7 million uses.<sup>(380)</sup> These complaints were listed in the category "allergy/irritation."

Complaint experience data on a moisturizing cream containing 4 percent Polysorbate 40 show five safety-related complaints in 3.5 years with 10.1 million uses; three of these were listed as "rash" and two as "redness or swelling."<sup>(381)</sup>

There were no safety-related complaints over a 2-year period for a moisturizing product containing 2.5 percent Polysorbate 60 used an estimated 26.7 million times.<sup>(382)</sup> A shaving preparation containing 2 percent Polysorbate 60 had one complaint over a 2-year period from 3.5 million units sold.<sup>(383)</sup>

### SUMMARY

The Polysorbates are a series of polyoxyethylenated sorbitan esters that differ with respect to the number of polymerized oxyethylene subunits and the num-

ber and type of fatty acid moieties present. They are used as general purpose, hydrophilic, nonionic surfactants in a variety of cosmetic products. Some of the Polysorbates are also approved by the Food and Drug Administration for use in various pharmaceuticals and food products.

Studies employing radioactive tracer techniques show that the Polysorbates are hydrolyzed by pancreatic and blood lipases; the fatty acid moiety is released to be absorbed and metabolized, whereas the polyoxyethylene sorbitan moiety is very poorly absorbed and is excreted unchanged. As expected, the Polysorbates are active at levels of biological structure and function from basic biochemical pathways to the cardiovascular and immune systems. Most or all of these effects can most likely be related to the surface active properties of the intact Polysorbate molecule.

Polysorbate 80 was shown to be nonmutagenic in the Ames and micronucleus tests. The polysorbates have been shown in numerous studies to be noncarcinogenic when administered in a variety of ways to laboratory animals, although Polysorbate 80 produced some neoplastic changes in mixed mouse epidermal and dermal in vitro tissue culture. Multiple studies have shown that the Polysorbates enhance the activity of known chemical carcinogens while not actually being carcinogenic themselves. Proposed mechanisms of this tumor enhancement\* effect include induction of cellular hyperproliferation, inhibition of DNA repair, and others. The Polysorbates also exhibit tumor growth inhibition activity under certain conditions.

Extensive testing for acute and long-term oral toxicity in animals has resulted in evidence indicating the low order of toxicity with oral ingestion of the Polysorbates. Most of the reported toxicity can be attributed either directly or indirectly to the osmotic diarrhea caused by the polyoxyethylene sorbitan moiety retained within the intestinal lumen. Polysorbate 20 and product formulations containing 1.0 to 8.4 percent of Polysorbate 20, 40, 80, or 85 produced no evidence of acute or subchronic percutaneous toxicity, the only effects being erythema, edema, and desquamation at the site of application. Acute intravenous and intraperitoneal injection of the Polysorbates into rats or mice resulted in LD<sub>50</sub> values indicative of a low order of parenteral toxicity. Daily intravenous injections of Polysorbates 60 and 80 into rabbits for up to 65 days produced pathology limited mainly to the renal and reticuloendothelial systems.

The Polysorbates showed little potential for rabbit and mouse skin irritation in acute studies. Those of the Polysorbates that were tested in subchronic skin irritation tests for up to 60 days produced local skin reactions ranging from minimal inflammation to necrosis. These changes were attributable to damage of epidermal cell membranes by the emulsifying action of the Polysorbates. The Polysorbates produced no more than minimal, transient eye irritation in Draize rabbit eye irritation tests. Polysorbate 80 produced superficial, mild damage to the intestinal mucosae of rabbits and rats. Polysorbate 20 produced no inflammation when applied to the hamster cheek pouch, and Polysorbate 40 caused no inflammation when infused into the guinea pig urinary bladder. The Magnusson-Kligman guinea pig maximization test showed moderate to strong skin sensitization to Polysorbate 20 in one study. Another guinea pig skin sensitization assay reported no evidence of skin sensitization to Polysorbates 65 and 80.

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\* The term "tumor enhancement" encompasses both tumor promotion and cocarcinogenesis.<sup>(146)</sup>

The Polysorbates have been ingested by human beings in situations ranging from an accidental administration of 19.2 g of Polysorbate 80 to an infant on 2 consecutive days to daily therapeutic administration of up to 6.0 g of Polysorbate 80 to adults for up to 4 years. These studies consistently showed little or no adverse effects from oral ingestion of the Polysorbates. Extensive clinical skin testing in the Schwartz prophetic patch test showed little potential for human skin irritation and no evidence of skin sensitization in a total of 580 subjects. A total of 1206 patients with eczema were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to a mixture of 5 percent Polysorbate 60 and 5 percent Polysorbate 80 in petrolatum; allergic reactions were shown by only 2 of the patients (< 0.2 percent). Several product formulations containing the Polysorbates have been tested for human skin sensitization on a total of 3481 subjects using a variety of testing methods; there were no reactions indicative of sensitization to any of the Polysorbates in these assays. Investigations with patients known to have skin disease revealed isolated instances of skin sensitization to Polysorbate 40 or 80. Intravenous injection of Polysorbate 80 produced hemodynamic changes in 5 patients. Studies involving exposure to ultraviolet light showed no instance of photocontact sensitization to the Polysorbates, although there were isolated instances of mild irritation following UV exposure when testing product formulations containing the Polysorbates.

## DISCUSSION

Polysorbates are not mutagens or complete carcinogens. However, some are known tumor enhancers\* in certain laboratory animals. Data are not available on the possible tumor-enhancement activity of Polysorbates in man. The Panel considered the published studies on Polysorbates as a tumor enhancer as well as those comments submitted during the public comment period of the Tentative Report. The FDA has approved Polysorbates 20 and 80 at up to 1.0 percent in ophthalmic preparations and Polysorbate 60 at up to 4.5 percent in foods. It has also approved, without limit to concentration, the use of Polysorbate 80 in vitamin-mineral preparations to a maximum recommended daily consumption of 475 mg/day.

In cosmetic preparations, the preponderance of uses does not exceed the 5 to 10 percent range. Presently available data indicate that these ingredients are used in numerous preparations at these concentrations without clinical reports of significant adverse effects. It is recognized that rinse-off preparations and those that are diluted with use carry a lower potential for adverse effects than might be indicated by the ingredient concentration.

## CONCLUSION

On the basis of the available data, the Panel concludes that Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 are safe as cosmetic ingredients in the concentration of present use.

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\* The term "tumor enhancement" encompasses both tumor promotion and cocarcinogenesis.<sup>(146)</sup>



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# Final Report on the Safety Assessment of PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 and -75 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3, and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-20, -30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitantriisostearate; PEG-18 Sorbitan Trioleate; PEG-40 and -50 Sorbitol Hexaoleate; PEG-30 Sorbitol Tetraoleate Laurate; and PEG-60 Sorbitol Tetrastearate—Addendum to the Final Report on the Safety Assessment of Polysorbates<sup>1</sup>

The PEGs Sorbitan/Sorbitol Fatty Acid Esters are ethoxylated sorbitan and sorbitol esters of fatty acids that function as surfactants in cosmetic formulations. PEG is the terminology used in the cosmetics industry for polyethylene glycol. Ingredients in a subset of this group are referred to by the cosmetics industry as Polysorbates and were previously reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. These ingredients are formed by the esterification of sorbitol or sorbitan with a fatty acid, followed by the chemical addition of ethylene oxide. 1,4-Dioxane and other water-soluble by-products may be formed. Most of the available safety test data relate to the Polysorbates or their components, Sorbitan Fatty Acids, PEGs, and Fatty Acids, which also have completed safety assessments. These ingredients are readily hydrolyzed by blood and pancreatic lipases, with the fatty acid moiety absorbed and metabolized as any dietary fatty acid and the PEG Sorbitan moiety excreted mainly in the urine. It is well recognized that PEGs are readily absorbed through damaged skin. Polysorbates have low toxicity in both acute and long-term toxicity studies using animals. Sorbitan Esters and PEGs also were relatively nontoxic to animals.

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<sup>1</sup>Reviewed by the Cosmetic Ingredient Review Expert Panel. Rebecca S. Lanigan, former Scientific Analyst and Writer, prepared this report. The original safety assessment of Polysorbates was published in 1984. *J. Am. Col. Toxicol.* 3:1–82. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

Growth retardation and diarrhea in mice, microscopic changes of the urinary bladder, spleen, kidneys, and gastrointestinal tract in rats, and decreased body and organ weights, diarrhea, and hepatic lesions in rats were noted in subchronic feeding studies, whereas other studies found no effects. One chronic toxicity study using hamsters noted microscopic lesions of the urinary bladder, kidneys, spleen, and gastrointestinal tract, whereas other studies in monkeys, mice, rats, dogs, and hamsters were negative. The Polysorbates were nonirritating to mildly irritating in both in vivo and in vitro ocular irritation assays at concentrations ranging from 1% to 100%. In teratology studies of thalidomide, the PEG-20 Sorbitan Laurate vehicle (10 ml/kg) had no effect on the developing mouse embryo. In other studies, reproductive and developmental effects were seen primarily at exposure levels that were maternally toxic. It is recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. The CIR Expert Panel concluded that, as the PEGs Sorbitan and Sorbitol Esters are chemically different from the alkyl ethers of ethylene glycol and the alkyl ethers are not present as impurities, these ingredients pose no reproductive or developmental hazard. In subchronic and chronic oral toxicity studies, the PEGs did not cause adverse reproductive effects. The Polysorbates were nonmutagenic in a number of bacterial and mammalian systems. Data were available showing that treatment of cells in culture with Sorbitan Oleate reduces DNA repair following UV irradiation, but these data were not considered significant in view of the available carcinogenesis data. In general, the Polysorbates were not oral or dermal carcinogens. Data on the cocarcinogenesis of certain Sorbitan Esters were positive, but only with high exposure levels and a high frequency of exposure, and the results lacked a dose response.

The Polysorbates also had antitumor activity in animal studies. The Polysorbates were nontoxic by the oral route in clinical studies, but a Polysorbate vehicle for a neonatal parenteral supplement caused the deaths of 38 premature infants. The Polysorbates had little potential for human skin irritation, sensitization, and phototoxicity in extensive clinical studies. Likewise, PEGs were nonsensitizers, but cases of systemic toxicity and contact dermatitis were observed in burn patients that were treated with PEG-based topical ointments. The Sorbitan Esters had the potential to cause cutaneous irritation in humans, and could cause sensitization in patients with damaged skin. Several of the Polysorbates enhanced skin penetration of other chemicals. Overall, these data were considered an adequate basis for assessing the safety of the entire group. The CIR Expert Panel concluded that these ingredients were safe for use in cosmetics at the levels in current use (not more than a 25% concentration) with the caveat that they should not be used on damaged skin.

## INTRODUCTION

The PEGs Sorbitan Fatty Acid Esters are ethoxylated sorbitol and sorbitan esters of fatty acids that function as surfactants in cosmetic formulations. This assessment is an addendum to the review of the Polysorbates, which is the name given by the cosmetics industry to a series of specific chain length PEGs Sorbitan Fatty Acid Esters (Elder 1984). Table 1 presents the ingredients included in this safety assessment along with a list of those Polysorbates previously reviewed.

The Polysorbates are generally recognized as safe (GRAS) food additives and are used as emulsifiers in pharmaceutical products. Data on the Polysorbates, sorbitan esters, fatty acids, and Polyethylene glycols (PEGs), have been updated as a further

basis for the assessment of safety of the additional PEGs Sorbitan and Sorbitol Fatty Acid Esters. The Cosmetic Ingredient Review (CIR) Expert Panel has concluded previously that:

Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 are safe as cosmetic ingredients in the concentration of present use (Elder 1984).

Sorbitan Stearate, Sorbitan Laurate, Sorbitan Sesquioleate, Sorbitan Oleate, Sorbitan Tristearate, Sorbitan Palmitate, and Sorbitan Trioleate are considered safe as cosmetic ingredients under present conditions of concentration and use (Elder 1985).

Oleic, Lauric, Palmitic, Myristic, and Stearic Acids are safe in present practices of use and concentration in cosmetics (Elder 1987).

Isostearic Acid is safe as a cosmetic ingredient in the present practices of use (Elder 1983).

Coconut Oil, Coconut Acid, Hydrogenated Coconut Oil, and Hydrogenated Coconut Acid are safe for use as cosmetic ingredients (Elder 1986).

Lanolin and related Lanolin materials described herein are safe for topical application to humans in the present practices of use and concentration (Elder 1980).

PEG-6, -8, -32, -75, -150, -14M, and -20M are safe for use at the concentrations reflected in the Cosmetic Use section and in the product formulation safety test data included in the Final Report. The Expert Panel recommends that cosmetic formulations containing these PEGs not be used on damaged skin (Andersen 1993).

## CHEMISTRY

### Definition and Structure

The PEGs Sorbitan and Sorbitol Fatty Acid Esters (Table 2) are ethoxylated fatty acid esters of the hexahydric alcohol,

**TABLE 1**  
Ingredient list

New ingredients (this report)	Polysorbate ingredients previously reviewed (Elder 1984)
PEG-20 Sorbitan Cocoate	
PEG-40 Sorbitan Diisostearate	PEG-4, -20 Sorbitan Laurate ( <i>Polysorbates 21 and 20</i> )
PEG-2, -5, -20 Sorbitan Isostearate	
PEG-40, -75 Sorbitan Lanolate	
PEG-10, -40, -44, -75, -80 Sorbitan Laurate	
PEG-3, -6 Sorbitan Oleate	PEG-5, -20 Sorbitan Oleate ( <i>Polysorbates 81 and 80</i> )
PEG-80 Sorbitan Palmitate	PEG-20 Sorbitan Palmitate ( <i>Polysorbate 40</i> )
PEG-40 Sorbitan Perisostearate	
PEG-40 Sorbitan Peroleate	
PEG-3, -6, -40, -60 Sorbitan Stearate	PEG-4, -20 Sorbitan Stearate ( <i>Polysorbates 61 and 60</i> )
PEG-20, -30, -40, -60 Sorbitan Tetraoleate	
PEG-60 Sorbitan Tetrastearate	
PEG-20, -160 Sorbitan Triisostearate	
PEG-18 Sorbitan Trioleate	PEG-20 Sorbitan Trioleate ( <i>Polysorbate 85</i> )
	PEG-20 Sorbitan Tristerate ( <i>Polysorbate 65</i> )
PEG-40, -50 Sorbitol Hexaoleate	
PEG-30 Sorbitol Tetraoleate Laurate	
PEG-60 Sorbitol Tetrastearate	



sorbitol, and its mono- and dianhydrides. These ingredients conform generally to the formulas in Figure 1 (Chi, Scocca, and Huang 1978; Nikitakis and McEwen 1990a; Radian Corporation 1991; Chemline 1996; Food and Drug Administration [FDA] 1996; Wenninger et al. 2000). Structures were not available for PEG-20 Sorbitan Cocoate, PEG-40 and -75 Sorbitan Lanolate, PEG-40 Sorbitan Perisostearate, and PEG-40 Sorbitan Peroleate.

A subset of this group of ingredients is the Polysorbate family. Even though a different name is used, these are also PEGs Sorbitan Fatty Acid mono- or triesters. This family includes PEG-4 and -20 Sorbitan Laurate, PEG-4 and -20 Sorbitan Stearate, PEG-5 and -20 Sorbitan Oleate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Tristearate, and PEG-20 Sorbitan Trioleate (Elder 1984).

### Chemical and Physical Properties

PEG-40 Sorbitan Lanolate is an amber-colored, soft paste that is soluble in dioxane, carbon tetrachloride, and water at 65°C. The hydroxyl value is 130 to 155, the maximum acid value is 3.0, the saponification value is 20 to 30, and the maximum amount of moisture is 1.0% (Nikitakis and McEwen 1990b).

PEG-4 Sorbitan Laurate (Polysorbate 21) is dispersible in water and soluble in ethanol and corn oil. Its hydroxyl value is 220 to 255, the saponification value is 100 to 115, Sorbitan Laurate (Polysorbate 20) is soluble in water, ethanol, ethyl acetate, and the maximum acid value is 4.0. The maximum amount of water is 3.0%. PEG-10 and -20 Sorbitan Laurate are clear, yellow, unctuous liquids with mild odors. PEG-20 Sorbitan Laurate (Polysorbate 20) is soluble in water, ethanol, ethyl acetate and methanol. PEG-10 Sorbitan Laurate is soluble in water, acetone, ethyl acetate, and the lower alcohols. Both are insoluble in mineral oil. For PEG-10 Sorbitan Laurate, the saponification value is 66 to 76, and the hydroxyl value is 150 to 168. The maximum values for acid, sulfated ash, and water are 2.0%, 0.15%, and 3.0%, respectively. PEG-20 Sorbitan Laurate has a saponification value of 40 to 50, a hydroxyl value of 96 to 108, and a maximum acid value of 2.0. The maximum amounts of sulfated ash and water are 0.15% and 3.0%, respectively (Nikitakis and McEwen 1990a). When the PEGs Sorbitan Laurate were heated to decomposition, acrid, irritating fumes were released (Lewis 1993b). The hydrophile lipophile balance value (HLB) value of PEG-4 Sorbitan Laurate is 13.3 (Cosmetic Science & Technology On-line 1997).

PEG-5 Sorbitan Oleate (Polysorbate 81) is an amber, unctuous liquid which can gel. It has a faint odor and is dispersible in water, ether, and ethylene glycol. It is soluble in ethanol, methanol, ethyl acetate, mineral oil, and corn oil. The saponification value is 96 to 104, the hydroxy value is 135 to 145, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are 0.15% and 3.0%, respectively. PEG-20 Sorbitan Oleate (Polysorbate 80) is a lemon to orange-colored, oily liquid with a faint, characteristic odor. It is very soluble in water, and produces an odorless and nearly colorless aqueous solution

(Nikitakis and McEwen 1990a). PEG-20 Sorbitan Oleate is soluble in dimethyl sulfoxide, ethanol, methanol, cottonseed and corn oils, mineral oil, ethyl acetate, and toluene. It is insoluble in mineral oil (Nikitakis and McEwen 1990a; Radian Corporation 1991). The saponification value is 45 to 55, the hydroxyl value is 65 to 80, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are, respectively, 0.15% and 3.0% (Nikitakis and McEwen 1990a). The molecular weight of PEG-20 Sorbitan Oleate is 1309.68 Da, the specific gravity is 1.1, and the density is 1.064 g/ml. PEG-20 Sorbitan Oleate is "incompatible" with strong alkalis and oxidizers. The flash point of this compound is >110°C, and it is considered "probably combustible." The viscosity is 270 to 430 centistokes, the refractive index is 1.4756 at 20°C, and the pH of a 5% aqueous solution is 5 to 7 (Radian Corporation 1991).

PEG-20 Sorbitan Palmitate (Polysorbate 40) is a clear yellow, unctuous liquid with a faint, characteristic odor. It is soluble in water, methanol, and ethanol, and is insoluble in mineral oil. The saponification value is 43 to 49, the hydroxyl value is 89 to 105, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water found in PEG-20 Sorbitan Palmitate, respectively, are 0.15% and 3.0% (Nikitakis and McEwen 1990a).

PEG-40 Sorbitan Peroleate is a viscous, oily, clear yellow liquid which has a faint characteristic odor. It is soluble in mineral or vegetable oil and is dispersible in water. The hydroxyl value of PEG-40 Sorbitan Peroleate is 20 to 38, the saponification value is 100 to 115, and the acid value is 8.0 to 12.0 (Nikitakis and McEwen 1990b).

PEG-4 Sorbitan Stearate (Polysorbate 61) is a tan, waxy solid at room temperature, and has a mild odor. It is soluble in methanol and ethanol, dispersible in distilled water, and insoluble in ethylene glycol and propylene glycol. The saponification value is 95 to 115, the hydroxy value is 170 to 200, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are 0.25% and 3.0%, respectively. PEG-20 Stearate (Polysorbate 60) is a lemon yellow, oily liquid with a tendency to gel at room temperature. It is soluble in water, ethanol, and ethyl acetate, but is insoluble in mineral and vegetable oils. The saponification value is 45 to 55, the hydroxyl value is 81 to 96, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and water are 0.25% and 3.0%, respectively (Nikitakis and McEwen 1990a).

PEG-20 Sorbitan Trioleate (Polysorbate 85) is a clear, amber, unctuous liquid that can gel, and has a characteristic odor. It is dispersible in water, and is soluble in most vegetable and mineral oils as well as in a variety of organic solvents. The saponification value is 82 to 95, the hydroxyl value is 39 to 52, and the maximum acid value is 2.0. The maximum amount of sulfated ash is 0.15% (Nikitakis and McEwen 1990a).

PEG-20 Sorbitan Tristearate (Polysorbate 65) is a tan, waxy solid with a faint, characteristic odor. It is soluble in ethanol, methanol, mineral oil, vegetable oil, acetone, and ether, and is dispersible in water. The saponification value is 88 to 98, the hydroxyl value is 44 to 60, and the maximum acid value is 2.0. The maximum amounts of sulfated ash and

**TABLE 2**  
Information on PEGs Sorbitan/Sorbitol Fatty Acid Esters\*

Ingredient	CAS number, definition and generic names	Calculated molecular weight <sup>a</sup>	Synonym(s)
PEG-20 Sorbitan Cocoate	Ethoxylated sorbitan ester of coconut acid with average of 20 moles ethylene oxide	>1043 <sup>b</sup>	Polyethylene glycol 1000 sorbitan cocoate Polyoxyethylene (20) sorbitan cocoate
PEG-40 Sorbitan Diisostearate	Ethoxylated sorbitan diester of isostearic acid with average of 40 moles ethylene oxide	2456	Polyethylene glycol 1000 sorbitan diisostearate Polyoxyethylene (40) sorbitan diisostearate
PEG-2 Sorbitan Isostearate		518	Polyethylene glycol 100 sorbitan isostearate Polyoxyethylene (2) sorbitan isostearate
PEG-5 Sorbitan Isostearate	CAS No. 66794-58-9; ethoxylated sorbitan monoesters of isostearic acid with average of <i>n</i> moles ethylene oxide	650	Polyethylene glycol (5) sorbitan isostearate Polyoxyethylene (5) sorbitan isostearate
PEG-20 Sorbitan Isostearate		1310	Polyethylene glycol 1000 sorbitan monoisostearate Polyoxyethylene sorbitan isostearate (20 E.O.) Polyoxyethylene (20) sorbitan monoisostearate
PEG-40 Sorbitan Lanolate	CAS No. 8036-77-9; ethoxylated sorbitan derivatives of lanolin acid (q.v.) with average of <i>n</i> moles ethylene oxide	>2807 <sup>b</sup>	Polyethylene glycol 2000 sorbitan lanolate Polyoxyethylene (40) sorbitol lanolate Polyoxyethylene sorbitol lanolate (40 E.O.)
PEG-75 Sorbitan Lanolate	CAS No. 8051-13-6; ethoxylated sorbitan derivatives of lanolin acid (q.v.) with average of <i>n</i> moles ethylene oxide	>2807 <sup>b</sup>	Polyethylene glycol 4000 sorbitan lanolate Polyoxyethylene (75) sorbitol lanolate
PEG-10 Sorbitan Laurate	CAS No. 9005-64-5; mixtures of laurate partial esters of the hexahydric alcohol, sorbitol and its anhydrides, condensed with average of <i>n</i> moles ethylene oxide for each mole of sorbitol and sorbitol mono- and dianhydrides	788	Polyethylene glycol 500 sorbitan monolaurate
PEG-40 Sorbitan Laurate		2108	Polyethylene glycol 2000 sorbitan monolaurate
PEG-44 Sorbitan Laurate		2284	
PEG-75 Sorbitan Laurate	Generic names include sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivative	3648	Polyethylene glycol 4000 sorbitan monolaurate
PEG-80 Sorbitan Laurate		3868	
PEG-3 Sorbitan Oleate	CAS No. 9005-65-6; ethoxylated sorbitan esters of oleic acid with average of <i>n</i> moles ethylene oxide	560	Polyethylene glycol (3) sorbitan monooleate Polyoxyethylene (3) sorbitan monooleate
PEG-6 Sorbitan Oleate	Generic names include: sorbitan mono-9-octadecanoate poly(oxy-1,2-ethanediyl) derivative; polyoxyethylene oxide sorbitan mono-oleate; sorbitan mono-oleate polyoxyethylene; sorbitan, monooleate polyoxyethylene derivative; and ethosorbitan monooleate	692	Polyethylene glycol 300 sorbitan monooleate Polyoxyethylene sorbitan monooleate (6 E.O.)

PEG-80 Sorbitan Palmitate	CAS No. 9005-66-7; ethoxylated sorbitan monoester of palmitic acid with average 80 moles ethylene oxide	3922	Polyethylene glycol (80)sorbitan monopalmitate Polyoxyethylene (80) sorbitan monopalmitate
PEG-40 Sorbitan Perisostearate	Mixture of isostearic acid esters of sorbitol condensed with average of 40 moles ethylene oxide	>2807 <sup>b</sup>	Polyethylene glycol 2000 sorbitan perisostearate Polyoxyethylene (40) sorbitan perisostearate
PEG-40 Sorbitan Peroleate	Mixture of oleic acid esters of sorbitol condensed with average of 40 moles ethylene oxide	>2807 <sup>b</sup>	Polyethylene glycol 2000 sorbitan peroleate Polyoxyethylene (40) sorbitan peroleate
PEG-3 Sorbitan Stearate	CAS No. 9005-67-8; ethoxylated sorbitan monoesters of stearic acid with average of <i>n</i> moles ethylene oxide	562	Polyethylene glycol (3) sorbitan monostearate Polyoxyethylene (3) sorbitan monostearate
PEG-6 Sorbitan Stearate		685	Polyethylene glycol 300 sorbitan monostearate Polyoxyethylene (6) sorbitan monostearate
PEG-40 Sorbitan Stearate		2190	Polyoxyethylene sorbitan monostearate (6 E.O.) Polyethylene glycol 2000 sorbitan stearate Polyoxyethylene (40) sorbitan stearate
PEG-60 Sorbitan Stearate		3070	Polyethylene glycol 3000 sorbitan monostearate Polyoxyethylene (60) sorbitan monostearate
PEG-20 Sorbitan Tetraoleate	Tetraesters of oleic acid and polyethylene glycol ether of sorbitol, with average of <i>n</i> moles ethylene oxide	2100	
PEG-30 Sorbitan Tetraoleate		2540	Polyethylene glycol (30) sorbitan tetraoleate Polyoxyethylene (30) sorbitan tetraoleate
PEG-40 Sorbitan Tetraoleate		2980	Polyethylene glycol 2000 sorbitan tetraoleate Polyoxyethylene (40) sorbitan tetraoleate
PEG-60 Sorbitan Tetraoleate		4772	Polyethylene glycol 3000 sorbitan tetraoleate Polyoxyethylene (60) sorbitan tetraoleate
PEG-60 Sorbitan Tetrastearate	Tetraester of stearic acid and polyethylene glycol ether of sorbitol, with average of 60 moles ethylene oxide	4780	Polyethylene glycol (60) sorbitan tetrastearate Polyoxyethylene (60) sorbitan tetrastearate
PEG-20 Sorbitan Triisostearate	Triesters of isostearic acid and polyethylene glycol of sorbitol, with average of <i>n</i> moles ethylene oxide	1842	Polyethylene glycol 1000 sorbitan triisostearate Polyoxyethylene (20) sorbitan triisostearate
PEG-160 Sorbitan Triisostearate		64622	
PEG-18 Sorbitan Trioleate	Ethoxylated sorbitan triester of oleic acid with average of 18 moles ethylene oxide	1748	
PEG-40 Sorbitol Hexaoleate	Oleic acid hexaesters of ethoxylated sorbitol with average of <i>n</i> moles ethylene oxide	3525	Polyethylene glycol 2000 sorbitol hexaoleate Polyoxyethylene (40) sorbitol hexaoleate
PEG-50 Sorbitol Hexaoleate		3965	Polyethylene glycol (50) sorbitol hexaoleate Polyoxyethylene (50) sorbitol hexaoleate
PEG-30 Sorbitol Tetraoleate Laurate	Oleic acid tetraester and lauric acid ester of ethoxylated sorbitol with average of 30 moles ethylene oxide	2505	Polyethylene glycol (30) sorbitol tetraoleate laurate Polyoxyethylene (30) sorbitol tetraoleate laurate
PEG-60 Sorbitol Tetrastearate	Stearic acid tetraester of ethoxylated sorbitol with average of 60 moles ethylene oxide	3428	Polyethylene glycol (60) sorbitol tetrastearate Polyoxyethylene (60) sorbitol tetrastearate Polyoxyethylene sorbitol tetrastearate (60 E.O.)

\*Chi, Scocca, and Huang 1978; Nikitakis and McEwen, 1990a; Radian Corporation 1991; Chemline 1996; FDA, 1996; Wenninger et al. 2000.

<sup>a</sup> Molecular weight calculated using  $(W + X + Y + Z) = n$  or  $(U + V + W + X + Y + Z) = n$ , where  $n$  equals the average moles of ethylene oxide (the number in the name).

<sup>b</sup>If structures were unavailable, the value is the sum of the molecular weights of the sorbitan and PEG moieties only.

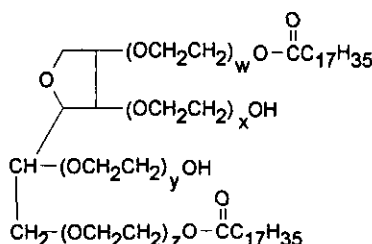
water are 0.25% and 3.0%, respectively (Nikitakis and McEwen 1990a).

### Method of Manufacture

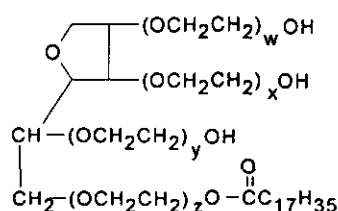
The Polysorbates, including PEG-4 and -20 Sorbitan Laurate, are prepared from sorbitol and sorbitol anhydrides (Figure 2) by the elimination of sorbitan, which is then partially esterified with a fatty acid. The resulting hexitan ester is polymerized with ethylene oxide (Gennaro 1990) and steam-stripped to remove water-soluble impurities such as 1,4-dioxane (Elder 1984; Smolinske 1992).

PEG-20 Sorbitan Palmitate (Polysorbate 40) is prepared by the reaction of ethylene oxide with sorbitan monopalmitate, by the addition of polyoxyethylene chains to the nonesterified hydroxyls, and by the esterification of sorbitol with one or three molecules of fatty acid. PEG-20 Sorbitan Oleate (Polysorbate 80) is manufactured by the partial esterification of sorbitan with fatty acid to yield a hexitan ester or by the chemical addition of ethylene oxide to yield the polyoxyethylene derivative. In addition, it can be formed by the elimination of water from sorbitol to form sorbitan, the cyclic sorbitol anhydride (Hazardous Substances Database [HSDB] 1996).

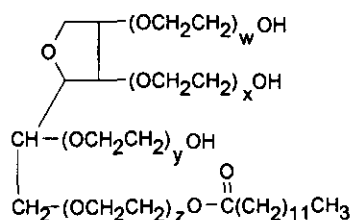
PEG-40 Sorbitan Diisostearate



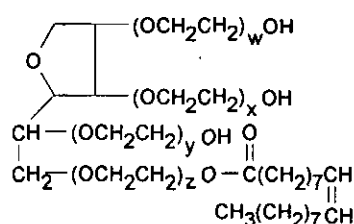
PEG-n Sorbitan Isostearate



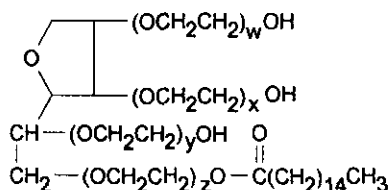
PEG-n Sorbitan Laurate



PEG-n Sorbitan Oleate



PEG-n Sorbitan Palmitate



PEG-n Sorbitan Stearate

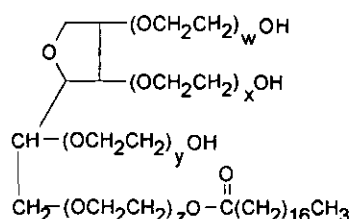
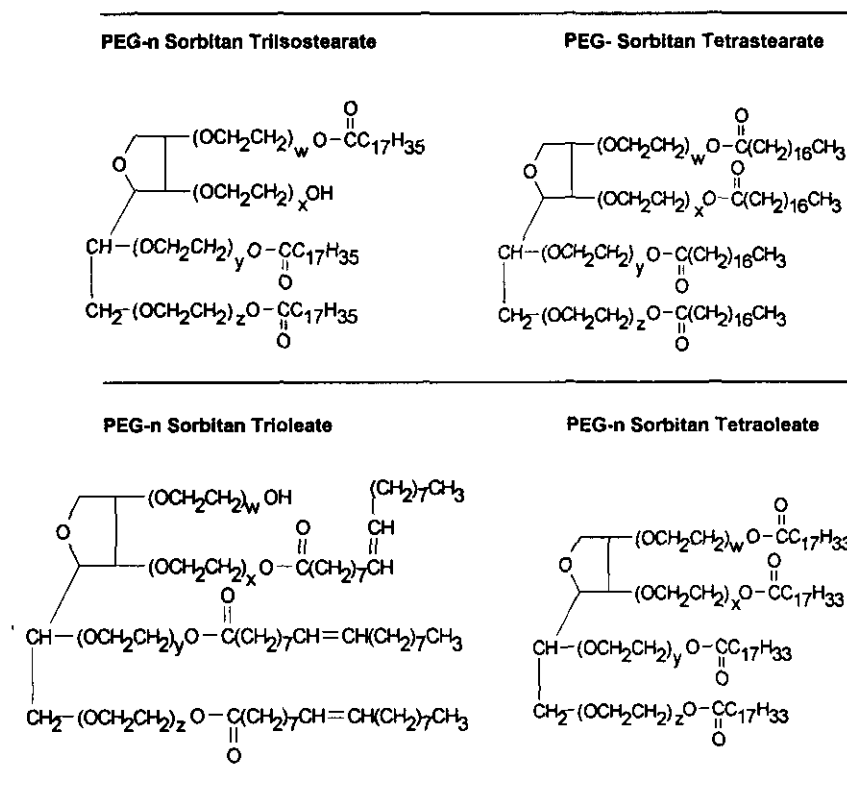


FIGURE 1

Formulas for selected PEGs Sorbitan/Sorbitol Fatty Acid Esters. The naming convention has the value of "n" in the ingredient name as sum of U + V + W + X + Y + Z. Chi, Scocca, and Huang 1978; Nikitakis and McEwen 1990a; Radian Corporation 1991; Chemline 1996; FDA 1996; Wenninger et al. 2000. (Continued)



**FIGURE 1**  
(Continued)

### Impurities

Impurities in the Polysorbates include peroxides, isosorbide ethoxylates, free fatty acids, lead, and arsenic (Elder 1984; Smolinske 1992).

Each of the Polysorbates can have a complex fatty acid moiety, as the fatty acids used in the production of cosmetic ingredients frequently contain fatty acids other than the principal acid named. When the fatty acid moiety compositions of the Polysorbates were determined, Polysorbate 20 (PEG-20 Sorbitan Laurate) was comprised of 36.9% lauric acid, 15.3% palmitic acid, 13.7% oleic acid, and 22.8% myristic acid. Polysorbate 40 (PEG-20 Sorbitan Palmitate) consisted of 86.4% palmitic acid and 10.2% stearic acid. Polysorbate 60 (PEG-20 Sorbitan Stearate) was comprised of 44.4% palmitic acid and 45.0% stearic acid. Polysorbate 80 (PEG-20 Sorbitan Oleate) consisted of 6.4% palmitic acid, 76.9% oleic acid, and 6.4% palmitoleic acid (Elder 1984).

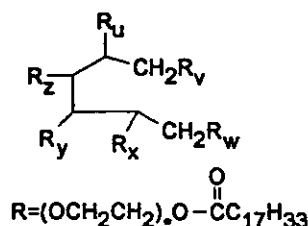
1,4-Dioxane was detected at concentrations of 5.5 to 378 ppm in samples of PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate; however, the Polysorbates are steam-stripped during the manufacturing process to remove water-soluble by-products such as 1,4-dioxane (Elder 1984).

The sorbitan esters could contain some residual free acid and alcohol. Minor impurities include arsenic (not more than 3 ppm), lead (not more than 10 ppm), and water (Elder 1985).

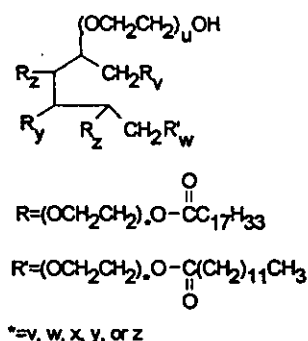
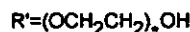
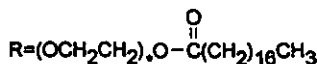
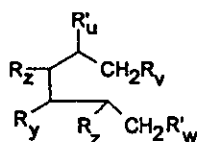
PEG-6 could contain small amounts of monomer and dimers. The amounts were not quantified. Peroxides, formed as a result of autoxidation, were found in PEG-32 and PEG-75. The amount of peroxide in PEG was dependent upon the molecular weight of the PEG and its age. The older the compound, the greater the concentration of peroxides. In a colorimetric assay used to determine the peroxide concentrations in several production lots of PEG, PEG-6 and PEG-8 were each added to acidified potassium iodide solution, and the iodine liberated was titrated against a standard thiosulfate solution. PEG-6 had peroxide concentrations ranging from 1.4 to 9.3  $\mu$ Eq thiosulfate/ml glycol. PEG-8 had concentrations ranging from 3.24 to 5.7  $\mu$ Eq thiosulfate/ml glycol. The specific peroxides present in the PEGs were not determined, but they were considered organic peroxides rather than hydrogen peroxide (Andersen 1993).

### Reactivity

The Polysorbates interacted with specific drugs in biopharmaceutical and drug release studies. They inactivated preservatives and altered the activities of cationic germicidal agents in numerous cosmetic ingredients, including *p*-hydroxybenzoic acid and its methyl, ethyl, propyl, and butyl esters; benzoic acid; benzyl alcohol; capric acid; chlorbutol; chlorocresol; sorbic acid; and glyceryl monolaurate (Elder 1984).

**PEG-n Sorbitol Hexaoleate**

The \* in the R group is either u, v, w, x, y, or z

**PEG-30 Sorbitol Tetraoleate Laurate****PEG-60 Sorbitol**

\* = u, v, w, x, y, or z

**Tetrastearate****FIGURE 1**

(Continued)

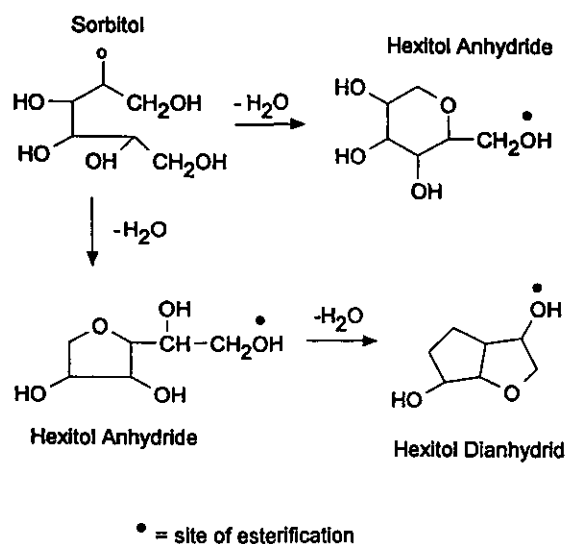
Aqueous solutions of PEG-20 Sorbitan Laurate undergo autoxidation during storage. During autoxidation, the peroxide number increases and subsequently decreases, the acidity increases continuously, pH and surface tension fall and plateau, and the cloud point drops such that turbidity begins at room temperature. Autoxidation is accelerated by light, increased temperature, and a copper sulfate catalyst. Hydrolysis also occurs, releasing lauric acid. Hydrolysis has major effects at room temperature, and the oxyethylene units undergo chain shortening at temperatures above 40°C (Donbrow, Azaz, and Pillersdorf 1978).

Degradation of PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Stearate has been detected in

unopened commercial samples of the Polysorbates, and can occur in cosmetic formulations due to the action of microorganisms. Bacteria in deionized water used to manufacture cosmetics were found to enzymatically decompose PEG-20 Sorbitan Laurate (Elder 1984).

**Analytical Methods**

PEG-10 Sorbitan Laurate has been determined using infrared spectrometry and nuclear magnetic resonance (Nikitakis and McEwen 1990a). PEG-40 Sorbitan Peroleate has been analyzed by infrared spectrometry (Nikitakis and McEwen 1990b). Poly-oxyethylene esters are characterized by a very strong infrared absorption peak at 9  $\mu$ m (Elder 1984).

**FIGURE 2**

Mechanisms of Hexitol Anhydride Derivation (Canterbury 1997).

### Ultraviolet Absorption

The maximum absorbances of 0.1504 mg/ml of aqueous PEG-20 Sorbitan Laurate were 0.100 at 245 nm and approximately 0.140 at 320 nm ( $\lambda_{\max}$ ). PEG-20 Sorbitan Laurate did not absorb ultraviolet (UV) radiation above 365 nm (National Toxicology Program [NTP], 1992a).

Sorbitans Laurate, Sesquioleate, Palmitate, and Trioleate did not absorb UVA or UVB radiation in a UV spectral analysis (Elder 1985).

### USE

#### Cosmetic

The PEGs Sorbitan Fatty Acid Esters are surfactants and function as emulsifying agents, cleansing agents, and solubilizing agents in cosmetic formulations (Wenninger et al. 2000). Current uses of ingredients in this report are presented in Table 3. In 1998, PEG-20 Sorbitan Isostearate, PEG-40 Sorbitan Lanolate, PEG-10, -44, and -80 Sorbitan Laurate, PEG-40 Sorbitan Peroleate, PEG-20 and -40 Sorbitan Tetraoleate, and PEG-18 Sorbitan Trioleate were used in a total of 81 formulations. The Polysorbates were used in 1418 formulations. No uses were reported for the remaining PEGs Sorbitan Fatty Acid Esters (Food and Drug Administration [FDA] 1998).

Data submitted by industry indicated that PEG-60 Sorbitan Tetratoate, PEG-40 Sorbitan Tetraoleate, and PEG-160 Sorbitan Triisostearate are used in cosmetics at concentrations of 0.5% to 10% (Cosmetic, Toiletry, and Fragrance Association [CTFA] 1998a). In 1984, PEG-10 Sorbitan Laurate was used at concentrations  $\leq 10\%$ . Concentrations of 1% to 5% PEG-44 and -75 Sorbitan Laurate were used in cosmetic formulations. The concentration of use of PEG-40 Sorbitan Peroleate was typically

0.1% to 1%, but concentrations of 1% to 5% and 10% to 25% were reported in some categories. PEG-20 Sorbitan Isostearate was used at concentrations of 1% to 10%, PEG-40 Sorbitan Lanolate was used at 0.1% to 1%, and PEG-75 Sorbitan Lanolate was used at concentrations up to 10%. The Polysorbates were generally used at concentrations up to 5%, but a small number of formulations contained these ingredients at concentrations greater than 50% (FDA 1984).

#### Noncosmetic

PEG-20 Sorbitan Oleate (Polysorbate 80; USP grade) is used as an emulsifier and dispersing agent for medicinal products designed for internal use, and is a pharmaceutical aid (surfactant). It is a defoamer and emulsifier in foods, and a neutralizer for quaternary ammonium compounds in disinfectants. PEG-20 Sorbitan Palmitate is used as an emulsifier and dispersing agent, flavoring agent, textile finish, surfactant, and foaming and defoaming agent, and is used in pharmaceuticals, shortenings, and baked goods. In veterinary medicine, PEG-20 Sorbitan Palmitate is used in feeds (as emulsifiers), milk replacers, pesticides, vitamins, parenterals, vaccines, oral pharmaceuticals, and intramammary and assorted topical treatments (Radian Corporation 1991; HSDB 1996).

### GENERAL BIOLOGY

#### Absorption, Metabolism, Distribution, and Excretion

##### *Polysorbates*

The ester link of the Polysorbate molecule was hydrolyzed by blood and pancreatic lipases following oral administration in labeling studies using rats. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid. When fed to rats at a dietary concentration of 10%, the efficiencies at which the radiolabelled fatty acid portions of PEG-20 Sorbitan Oleate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Tristearate were hydrolyzed and absorbed were 100%, 98%, and 84%, respectively. The lauric acid moiety of PEG-20 Sorbitan Laurate was rapidly absorbed and oxidized by rats. After 24 hours, 75% to 80% of the lauric acid was expired as  $\text{CO}_2$  and 4% was not absorbed from the alimentary tract. Twelve percent was found in the carcass, 2.5% in urine, and 1.2% in the liver. The polyoxyethylene sorbitan moiety was poorly absorbed from the gastrointestinal tract. Of the administered PEG group, 90% was excreted in the feces and 8% in the urine. In the case of the sorbitan moiety, 91% of the radioactivity was recovered in the feces, 2.1% in the urine, and 1.6% in the carcass after administration of PEG-20 Sorbitan Oleate. None was detected in expired  $\text{CO}_2$ , liver, kidneys, spleen, adrenal glands, brain, gonads, or fat. Similar results were observed following intravenous injection of PEG-20 Sorbitan Laurate. In a study in which 4.5 g/day of PEG-20 Sorbitan Oleate was fed to rats, approximately 95% of the polyoxyethylene fraction was excreted in the feces and 5% was excreted in the urine. No polyoxyethylated fatty acids

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

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Isostearate		
Cuticle softeners	19	1
Moisturizing preparations	769	1
<b>1998 total for PEG-20 Sorbitan Isostearate</b>		<b>2</b>
PEG-40 Sorbitan Lanolate		
Hair conditioners	636	1
Tonics, dressings, and other hair grooming aids	549	1
Other hair preparations	276	3
Hair lighteners with color	6	1
Other hair coloring preparations	59	1
<b>1998 total for PEG-40 Sorbitan Lanolate</b>		<b>7</b>
PEG-4 Sorbitan Laurate (Polysorbate 21)		
Other fragrance preparations	148	1
Moisturizing preparations	769	1
Suntan gels, creams, and liquids	136	2
<b>1998 total for PEG-4 Sorbitan Laurate</b>		<b>4</b>
PEG-10 Sorbitan Laurate		
Shampoos (noncoloring)	860	1
Nail polish and enamel removers	34	1
<b>1998 total for PEG-10 Sorbitan Laurate</b>		<b>2</b>
PEG-20 Sorbitan Laurate (Polysorbate 20)		
Baby shampoos	21	1
Other baby products	29	2
Bath oils, tablets, and salts	124	2
Bubble baths	200	11
Other bath preparations	159	14
Eyeliner	514	10
Eye lotion	18	4
Eye makeup remover	84	11
Mascara	167	3
Other eye makeup preparations	120	11
Colognes and toilet waters	656	5
Perfumes	195	8
Powders	247	43
Other fragrance preparations	148	4
Hair conditioners	636	40
Hair sprays (aerosol fixatives)	261	14
Permanent waves	192	6
Rinses (noncoloring)	40	2
Shampoos (noncoloring)	860	48
Tonics, dressings and other hair grooming aids	549	61
Wave sets	55	8
Other hair preparations	276	25
Hair dyes and colors	1572	44
Hair rinses (coloring)	33	1
Hair color sprays (aerosols)	4	1
Hair bleaches	113	2



## POLYSORBATES

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

Product category	Total no. of formulations in category	Total no. containing ingredient
<b>PEG-20 Sorbitan Laurate (Polysorbate 20)-continued</b>		
Other hair coloring preparations	59	2
Blushers (all types)	238	4
Foundations	287	21
Lipstick	790	2
Makeup bases	132	29
Makeup fixatives	11	3
Other makeup preparations	135	3
Cuticle softeners	19	1
Nail creams and lotions	17	2
Nail polish and enamel	80	1
Other manicuring preparations	61	2
Dentifrices	38	3
Mouthwashes and breath fresheners	49	7
Bath soaps and detergents	385	16
Deodorants (underarm)	250	3
Douches	5	1
Other personal cleanliness products	291	10
Aftershave lotion	216	9
Shaving cream	139	13
Other shaving preparation products	60	3
Cleansing preparations	653	58
Face and neck preparations (excluding shaving)	263	10
Body and hand preparations (excluding shaving)	796	39
Foot powders and sprays	35	1
Moisturizing preparations	769	33
Night preparations	188	5
Paste masks (mud packs)	255	11
Skin fresheners	184	38
Other skin care preparations	692	44
Suntan gels, creams, and liquids	136	6
Indoor tanning preparations	62	5
Other suntan preparations	38	4
<b>1998 total for PEG-20 Sorbitan Laurate (Polysorbate 20)</b>		<b>770</b>
<b>PEG-44 Sorbitan Laurate</b>		
Cleansing preparations	653	2
Skin fresheners	184	1
Other skin care preparations	692	5
<b>1998 total for PEG-44 Sorbitan Laurate</b>		<b>8</b>
<b>PEG-80 Sorbitan Laurate</b>		
Baby shampoos	21	10
Other baby products	29	5
Hair conditioners	636	1
Rinses (noncoloring)	40	1
Shampoos (noncoloring)	860	7
Other hair preparations	276	1
Bath soaps and detergents	385	2
Cleansing preparations	653	7
<b>1998 total for PEG-80 Sorbitan Laurate</b>		<b>34</b>

(*Continued on next page*)

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

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-5 Sorbitan Oleate (Polysorbate 81)		
Other hair preparations	276	1
Blushers (all types)	238	2
Moisturizing preparations	769	1
<b>1998 total for PEG-5 Sorbitan Oleate (Polysorbate 81)</b>		<b>4</b>
PEG-20 Sorbitan Oleate (Polysorbate 80)		
Baby shampoos	21	2
Baby lotions, oils, powders, and creams	53	2
Bath oils, tablets, and salts	124	1
Bubble baths	200	5
Other bath preparations	159	4
Eyeliners	514	10
Eye makeup remover	84	1
Mascara	167	2
Other eye makeup preparations	120	3
Colognes and toilet waters	656	5
Powders	247	6
Other fragrance preparations	148	3
Hair conditioners	636	32
Hair sprays (aerosol fixatives)	261	16
Hair straighteners	63	1
Permanent waves	192	1
Shampoos (noncoloring)	860	9
Tonics, dressings, and other hair-grooming aids	549	12
Wave sets	55	2
Other hair preparations	276	5
Hair dyes and colors	1572	9
Hair shampoos (coloring)	24	1
Blushers (all types)	238	2
Face powders	250	1
Lipstick	790	1
Makeup bases	132	1
Rouges	12	1
Other makeup preparations	135	3
Nail creams and lotions	17	1
Nail polish and enamel removers	34	1
Dentifrices	38	1
Mouthwashes and breath fresheners	49	13
Bath soaps and detergents	385	2
Other personal cleanliness products	291	2
Aftershave lotion	216	3
Shaving cream	139	3
Other shaving preparation products	60	2
Cleansing preparations	653	4
Face and neck preparations (excluding shaving)	263	3
Body and hand preparations (excluding shaving)	796	10
Moisturizing preparations	769	27
Night preparations	188	4
Paste masks (mud packs)	255	4
Skin fresheners	184	6

## POLYSORBATES

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

Product category	Total no. of formulations in category	Total no. containing ingredient
PEG-20 Sorbitan Oleate (Polysorbate 80)-continued		
Other skin care preparations	692	3
Indoor tanning preparations	62	1
<b>1998 total for PEG-20 Sorbitan Oleate (Polysorbate 80)</b>		<b>231</b>
PEG-20 Sorbitan Palmitate (Polysorbate 40)		
Other eye makeup preparations	120	1
Other fragrance preparations	148	4
Hair straighteners	63	1
Tonics, dressings, and other hair-grooming aids	549	1
Other manicuring preparations	61	1
Cleansing preparations	653	7
Body and hand preparations (excluding shaving)	796	3
Moisturizing preparations	769	6
Night preparations	188	1
Other skin care preparations	692	6
Indoor tanning preparations	62	1
<b>1998 total for PEG-20 Sorbitan Palmitate (Polysorbate 40)</b>		<b>32</b>
PEG-40 Sorbitan Peroleate		
Bath oils, tablets, and salts	124	5
Powders	247	1
Other fragrance preparations	148	1
Face and neck preparations (excluding shaving)	263	1
Moisturizing preparations	769	5
<b>1998 total for PEG-40 Sorbitan Peroleate</b>		<b>13</b>
PEG-4 Sorbitan Stearate (Polysorbate 61)		
Baby lotions, oils, powders, and creams	53	3
Other baby products	29	1
Body and hand preparations (excluding shaving)	796	4
<b>1998 total for PEG-4 Sorbitan Stearate (Polysorbate 61)</b>		<b>8</b>
PEG-20 Sorbitan Stearate (Polysorbate 60)		
Baby lotions, oils, powders, and creams	53	3
Eyebrow pencil	91	14
Eyeliners	514	4
Eye shadow	506	2
Eye lotion	18	2
Mascara	167	7
Other eye makeup preparations	120	6
Other fragrance preparations	148	4
Hair conditioners	636	6
Hair straighteners	63	4
Tonics, dressings, and other hair-grooming aids	549	7
Other hair preparations	276	2
Other hair-coloring preparations	59	1
Blushers (all types)	238	1

(*Continued on next page*)

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

Product category	Total no. of formulations in category	Total no. containing ingredient
<b>PEG-20 Sorbitan Stearate (Polysorbate 60)-continued</b>		
Foundations	287	30
Makeup bases	132	1
Other makeup preparations	135	4
Cuticle softeners	19	3
Nail creams and lotions	17	1
Other manicuring preparations	61	1
Aftershave lotion	216	1
Shaving cream	139	21
Cleansing preparations	653	29
Face and neck preparations (excluding shaving)	263	15
Body and hand preparations (excluding shaving)	796	41
Foot powders and sprays	35	3
Moisturizing preparations	769	58
Night preparations	188	9
Paste masks (mud packs)	255	13
Skin fresheners	184	2
Other skin care preparations	692	24
Suntan gels, creams, and liquids	136	2
Indoor tanning preparations	62	9
Other suntan preparations	38	2
<b>1998 total for PEG-20 Sorbitan Stearate (Polysorbate 60)</b>		<b>332</b>
<b>PEG-40 Sorbitan Stearate</b>		
Eye makeup remover	84	1
<b>1998 total for PEG-40 Sorbitan Stearate</b>		<b>1</b>
<b>PEG-20 Sorbitan Tetraoleate</b>		
Moisturizing preparations	769	3
<b>1998 total for PEG-20 Sorbitan Tetraoleate</b>		<b>3</b>
<b>PEG-40 Sorbitan Tetraoleate</b>		
Body and hand preparations (excluding shaving)	796	1
<b>1998 total for PEG-40 Sorbitan Tetraoleate</b>		<b>1</b>
<b>PEG-18 Sorbitan Trioleate</b>		
Other shaving preparations products	60	1
Cleansing preparations	653	3
Moisturizing preparations	769	1
Other skin care preparations	692	5
<b>1998 total for PEG-18 Sorbitan Trioleate</b>		<b>10</b>
<b>PEG-20 Sorbitan Trioleate (Polysorbate 85)</b>		
Eyeliners	514	2
Eye shadow	506	2
Eye makeup remover	84	1
Hair conditioners	636	1
Tonics, dressings, and other hair-grooming aids	549	3
Hair lighteners with color	6	1
Foundations	287	1
Makeup bases	132	1
Makeup fixatives	11	2

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

Product category	Total no. of formulations in category	Total no. containing ingredient
<b>PEG-20 Sorbitan Trioleate (Polysorbate 85)-continued</b>		
Other makeup preparations	135	3
Cleansing preparations	653	4
Face and neck preparations (excluding shaving)	263	1
Body and hand preparations (excluding shaving)	796	3
Moisturizing preparations	769	3
Other skin care preparations	692	1
Suntan gels, creams, and liquids	136	1
Indoor tanning preparations	62	5
<b>1998 total for PEG-20 Sorbitan Trioleate (Polysorbate 85)</b>		<b>35</b>
<b>PEG-20 Sorbitan Tristearate (Polysorbate 65)</b>		
Hair conditioners	636	1
Cleansing preparations	653	1
<b>1998 total for PEG-20 Sorbitan Tristearate (Polysorbate 65)</b>		<b>2</b>

were detected in the urine, hence the polyoxyethylene moiety in the urine represented PEG Sorbitan, and not the parent ester. PEG-20 Sorbitan Oleate was most likely hydrolyzed by pancreatic lipase, with the liberated oleic acid following the normal metabolic pathways of unsaturated fatty acids (Elder 1984).

In an in vitro study on surfactant-induced alterations of permeability of rabbit oral mucosa, PEG-20 Sorbitan Oleate caused a lesser increase in permeability than other surfactants, including sodium lauryl sulfate (Siegel and Gordon 1986). The lingual frenula was removed from anesthetized adult male New Zealand white rabbits. Mucosal "pieces" were mounted in modified Ussing chambers, which were filled with Krebs-Ringer phosphate solution bubbled with 100% oxygen. The half-chamber facing outside (oral side) of the tissue was filled with oxygenated phosphate solution, one of eight solutes, and the test substance. PEG-20 Sorbitan Oleate only caused significant increases in permeability at the greatest concentration tested (1.0%), and only to three of the eight solutes used (heptanediol, sucrose, inulin). Similar results were reported in an earlier study using canine oral mucosa (Siegel and Gordon 1985).

#### *Sorbitan Esters*

When ingested, Sorbitan Stearate was hydrolyzed to stearic acid and anhydrides of sorbitol. Approximately 90% of Sorbitan Stearate was absorbed and hydrolyzed when fed to rats in oil solution, and ~50% was absorbed and hydrolyzed when fed as a water emulsion. The ingredient did not accumulate in the fat stores of the rat body (Elder 1985).

#### *Polyethylene Glycol*

Gastrointestinal absorption of PEG is dependent on the molecular weight of the compound. In general, the greater the molec-

ular weight of the PEG compound, the lesser the absorption that occurs. In both oral and intravenous studies, no metabolism was observed and the PEGs were rapidly eliminated unchanged in the urine and feces. In a study with human burn patients, monomeric ethylene glycol was isolated in the serum following topical exposure to a PEG-based antimicrobial cream, indicating that PEGs are readily absorbed through damaged skin (Andersen 1993).

#### **Cytotoxic Effects**

Two in vitro cutaneous toxicity assays used skin of male New Zealand white rabbits (2 cm<sup>2</sup> discs), from which the subcutaneous fat had been removed, to determine the cytotoxicity of various chemicals, including PEG-20 Sorbitan Laurate (van de Sandt, Rutten, and Koëter 1993). Concentrations tested ranged from 30% to 100% (w/v), and the test compounds were applied for 4 hours. At 24 and 48 hours mitochondrial activity was assessed by measuring the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a MTT-formazan precipitate by mitochondrial dehydrogenases. Membrane damage was assessed by measuring the uptake of neutral red (NR), a vital dye that normally accumulates in the lysosomes of viable cells. Cytotoxicity was measured and expressed as the concentration that resulted in a 70% reduction in either MTT conversion (MTT-70) or NR uptake (NR-70), compared to the vehicle control, deionized water.

Topical treatment with PEG-20 Sorbitan Laurate inhibited MTT reduction in the cultured skin cells. PEG-20 Sorbitan Laurate reduced the amount of the NR that accumulated in the treated cell lysosomes. PEG-20 Sorbitan Laurate inhibited the accumulation of the dye, indicating that membrane damage had occurred. The MTT-70 was 18.0% and the NR-70 (extrapolated)

TABLE 4

Cytotoxic end points of PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Palmitate (Borenfreund and Shopsis 1985)

Cytotoxicity assay Cell line	NR-90 <sup>a</sup> Murine Balb/c 3T3 fibroblasts	Highest tolerated dose			UI-50 <sup>b</sup> Murine Balb/c 3T3 fibroblasts
		Murine Balb/c 3T3 fibroblasts	HepG2 hepatoma cells	RAW 264-7 macrophages	
PEG-20 Sorbitan Oleate	—	280 µg/ml	300 µg/ml	300 µg/ml	400 µg/ml
PEG-20 Sorbitan Palmitate	—	150 µg/ml	200 µg/ml	200 µg/ml	190 µg/ml
PEG-20 Sorbitan Stearate	0.160 µg/ml	220 µg/ml	250 µg/ml	220 µg/ml	230 µg/ml

<sup>a</sup>Concentration that resulted in 90% reduction of neutral red uptake.

<sup>b</sup>Concentration that induced 50% inhibition in [<sup>3</sup>H] uridine uptake.

was 2.2%. The reductions in MTT conversion and NR uptake were dose-dependent.

In another NR assay (using human lymphocytes) for cytotoxicity, the LC<sub>50</sub> for PEG-20 Sorbitan Stearate was 210 µg/ml (Arechabala et al. 1995). The cytotoxic endpoints were also determined for PEG-20 Sorbitan Stearate (Borenfreund and Shopsis 1985), PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Oleate (Table 4).

The greatest tolerated doses of three Polysorbates to Balb/c murine fibroblasts (3T3 cells), human hepatoma cells (HepG<sub>2</sub>), and murine macrophage cells (RAW 264.7) were 150 to 200 µg/ml for PEG-20 Sorbitan Palmitate, 220 to 250 µg/ml for PEG-20 Sorbitan Stearate, and 280 to 300 µg/ml for PEG-20 Sorbitan Oleate (Borenfreund and Borrero 1984).

A number of other cytotoxic assays to determine ocular or dermal irritation or antitumor activity has been performed on the Polysorbates. These studies are summarized in their respective sections in this report.

### Immunologic Effects

Barnett and Bryant (1980) investigated the immunosuppressive effects of adjuvants—retinol and PEG-20 Sorbitan Oleate—after exposure to the antigen ovalbumin. To determine immunosuppressive effects, Balb/c mice were treated with adjuvant by intraperitoneal (IP) injection on day -1 and immunized with the antigen intravenously on days 1 and 30. The ovalbumin was injected alone, diluted with saline (0.1 µg/0.1 ml), or adsorbed onto 0.1 mg Al(OH)<sub>3</sub> (0.1-ml dose). The effects of the adjuvants on the humoral response were then quantified using the passive hemagglutination assay (PHA) and passive cutaneous anaphylaxis (PCA) assay. The PHA determined titers of specific IgM and IgG antibodies to ovalbumin, and used human type O<sup>+</sup> erythrocytes which were sensitized with chromic chloride and diluted to 0.5% in bovine serum albumin and saline. The 2-hour PCA assay was used to determine IgG<sub>1</sub> titers after immunization with ovalbumin alone. The other assay involved the intradermal injection of 0.05 ml of immune serum at the dorsal surface, followed by an intravenous injection of 500 µg antigen in 0.4% Evans blue dye. A positive reaction was indicated by a blue skin reaction of 5 mm or greater.

Control mice retreated with saline alone produced "substantial amounts" of IgG and IgM after immunization with the adsorbed antigen (Table 5). A similar response was observed after injection of ovalbumin:saline. In the other control group, mice retreated with saline:PEG-20 Sorbitan Oleate (3:1) initially had no detectable antibody response (IgG and IgM) after injection of ovalbumin:saline or ovalbumin:Al(OH)<sub>3</sub>. Mice reimmunized with the adsorbed antigen had significant increases in IgG and IgM antibody titers. Mice retreated with saline:PEG-20 Sorbitan Oleate and immunized with ovalbumin alone had no IgG<sub>1</sub> response throughout the study. After immunization with ovalbumin:Al(OH)<sub>3</sub> no IgG<sub>1</sub> response was detected until after reimmunization. The primary response (IgG and IgM) was not detected until reimmunization with any antigen after the mice were retreated with retinol:PEG-20 Sorbitan Oleate. The IgG<sub>1</sub> titer for mice treated with 1000 IU retinol:PEG-20 Sorbitan Oleate was initially less than that produced by saline:PEG-20 Sorbitan Oleate, but was the same by the end of the study. Pretreatment with 3000 to 9000 IU of retinol:PEG-20 Sorbitan Oleate caused suppression of the primary response, but reimmunization produced high antibody titers.

In general, mice treated with RP on day -1 and immunized with ovalbumin:saline on days 0 and 30 had "excellent" IgE responses, but only after the second injection of the antigen. Retinol apparently promoted priming of IgE-specific components, whereas PEG-20 Sorbitan Oleate inhibited the other components of the immune response. Pretreatment with saline alone resulted in the production of "substantial amounts of antibodies." The investigators concluded that when retinol:PEG-20 Sorbitan Oleate was the sole source of adjuvant, a dose-dependent increase in the secondary response occurred; suppression of the primary adjuvant-independent response was not total. Some suppression of the primary immunoresponse occurred when the mice were immunized with ovalbumin:Al(OH)<sub>3</sub> after pretreatment with retinol:PEG-20 Sorbitan Oleate. The retinol portion of the adjuvant combination induced a secondary response that equaled the secondary response of the antigen alone. The primary IgG<sub>1</sub> response was totally inhibited by PEG-20 Sorbitan Oleate, and the secondary response was dependent on retinol. Retinol presumably acted upon the B-cell component.

**TABLE 5**  
Immunosuppressive effects of adjuvants (Barnett and Bryant 1980)

Adjuvant	Antigen	Results
Saline (control)	OA:Al(OH) <sub>3</sub>	10 <sup>3</sup> →10 <sup>7</sup> throughout study
Saline (control)	OA:saline	Similar results
SP (control)	OA:saline	No detectable IgG or IgM response at first immunization
SP (control)	OA	No IgG <sub>1</sub> response
SP (control)	OA:Al(OH) <sub>3</sub>	No antibody responses until after reimmunization; IgG and IgM titer = 10 <sup>2</sup> –10 <sup>5</sup> at weeks 4–9; peak IgG <sub>1</sub> titer = 80 at week 9
RP (1000 IU)	OA	No IgG <sub>1</sub> response
RP (3000 IU)	OA	No IgG <sub>1</sub> response
RP (5000 IU)	OA	No IgG <sub>1</sub> response until after reimmunization; peak titer = 80
RP (9000 IU)	OA	No IgG <sub>1</sub> response
RP (1000 IU)	OA:saline	Peak IgG and IgM titer = 160 at weeks 2–3; no response at weeks 4–6; second peak = 50 at week 9
RP (3000 IU)	OA:saline	Peak IgG and IgM titer = 10 <sup>5</sup> at weeks 5–6
RP (5000 IU)	OA:saline	Peak IgG and IgM titer = 10 <sup>5</sup> at weeks 5–6
RP (9000 IU)	OA:saline	Peak IgG and IgM titer = 10 <sup>3</sup> at week 8
RP (1000 IU)	OA:Al(OH) <sub>3</sub>	"Lagged behind" other groups in terms of IgG and IgM titer; titer = 100 at week 4, 10 <sup>3</sup> –10 <sup>8</sup> for remainder of study
RP (3000 IU)	OA:Al(OH) <sub>3</sub>	IgG and IgM titer = ~170 at week 4; 10 <sup>8</sup> at week 9; peak IgG <sub>1</sub> titer = 640 at week 8
RP (5000 IU)	OA:Al(OH) <sub>3</sub>	IgG and IgM titer = ~140 at week 3; ~1.7 × 10 <sup>7</sup> at week 9; IgG <sub>1</sub> titer = 80 at week 5; 320 at week 7
RP (9000 IU)	OA:Al(OH) <sub>3</sub>	IgG and IgM titer = 10–100 at weeks 3–4; ~1.7 × 10 <sup>3</sup> at week 5; 10 <sup>4</sup> at week 8; no IgG <sub>1</sub> response until week 5; peak titer = ~200 at weeks 8–9

IU = international units; 0.3 μg.

RP = retinol:PEG-20 Sorbitan Oleate.

OA = ovalbumin.

SP = saline:PEG-20 Sorbitan Oleate.

The majority of the observed immunosuppressive effects could be attributed to the Polysorbate, but retinol could also have been toxic. The investigators concluded that either (a) T-helper cell function was affected or (b) the surfactant properties of PEG-20 Sorbitan Oleate prevented activation of macrophages and/or their interaction with T-helper and B cells (Barnett and Bryant 1980).

In a follow-up study by Barnett (1981), Balb/c mice treated with PEG-20 Sorbitan Oleate (emulsified in saline, 3:1) had significantly decreased numbers of primary (IgM) plaques without a significant change in the number of secondary (IgG) plaques during a Jerne plaque assay. For this assay, the mice (number not available) were immunized on day 0 with 2 × 10<sup>7</sup> sheep erythrocytes administered by the IP route. On day 5, half of the mice per group was killed and assayed for the number of IgM antibody-producing cells in their spleens using a direct hemolytic plaque assay. On day 10, the secondary immune response was boosted in the remaining mice with a second IP administration of sheep erythrocytes. On day 14, the mice were killed, and the secondary IgG response was quantified via the indirect hemolytic plaque assay (using rabbit anti-mouse IgG).

The effect of PEG-20 Sorbitan Oleate on cell-mediated immunity was investigated for this study using the delayed hypersensitivity response to a contact allergen. After treatment, as above, with PEG-20 Sorbitan Oleate, mice were sensitized on day 0 by topical application of 25 μl of 8% (w/v) oxazolone in acetone. One day 5, the mice were challenged with 10 μl of 0.1% oxazolone by topical application to the dorsal surface of the right external ear. Immunologic responses to the allergen were determined for three days using micrometer measurements of the increase in external ear thickness. PEG-20 Sorbitan Oleate had no significant effect on the contact hypersensitivity response; the Polysorbate had no effect on the priming and triggering of T-effector cells. The investigator concluded that the inhibition of the primary, and not the secondary, hemolytic plaque response involved inhibition of the primary IgE antibody response as well as a decrease of the concentration of circulating IgG and IgM antibodies.

PEG-20 Sorbitan Oleate was a histamine-releasing agent (Eschaliere et al. 1988). In a study on the Polysorbate's effects on macrophage activation (Bonhomme et al. 1993), 1% PEG-20 Sorbitan Oleate increased peritoneal macrophage recruitment

without modifying phagocytic activity. This study used female Swiss mice that were injected IP with 2 ml volumes of the sterilized test chemicals. Four days after dosing, the peritoneal macrophages were harvested and viable cells were counted. Macrophage phagocytic activity was measured in terms of chemiluminescence following engulfment of opsonized zymosan.

### Miscellaneous Effects

During a malabsorption study (Ohsumi et al. 1979), Wistar rats were fed 5% to 10% PEG-20 Sorbitan Oleate for 3 months. The body weight was determined weekly, and the effects of the detergents on the small intestine were observed at 1 week, 2 months, and 3 months after the treatment. In addition, glucose absorption was evaluated after the infusion of glucose, sucrose, and dextrin solutions into the rat intestine. The goblet cells, intestinal epithelial cell, and villi were evaluated using light microscopy, and the brush border and mitochondria were examined using electron microscopy. The villi were also examined using scanning electron microscopy. One week after treatment with PEG-20 Sorbitan Oleate, glucose absorption was greater than after treatment with the control vehicle. At 3 months, the blood glucose concentrations were lower in rats given the Polysorbate. Intestinal epithelial cells of rats treated with 5% PEG-20 Sorbitan Oleate were intact, whereas the mitochondria were "destroyed" and the cristae were distributed irregularly. For 10% PEG-20 Sorbitan Oleate, a portion of the microvilli disappeared, and the surface of the epithelial cells appeared flat. Changes of the mitochondria occurred (giant mitochondria, destruction of cristae), and vacuolated cells were observed in the cells.

Using a cascade superfusion bioassay system, Uluoglu et al. (1996) investigated the functional reduction of endothelial function in rabbit thoracic aorta by PEG-20 Sorbitan Oleate. The bioassay tissue was deendothelialized precontracted aorta ring from male albino rats. Segments of rabbit thoracic aortas (from which the fat and connective tissue were removed) were obtained from male New Zealand white rabbits. The segments were either incubated with 1% to 10% PEG-20 Sorbitan Oleate for 30 minutes or 3 hours (or Krebs's solution as a control) before being placed in a donor chamber, or were placed in the chamber without previous incubation. Half of the segments that was not incubated were perfused with Krebs's solution containing  $10^{-1}$  to  $10^{-3}$  ml/l PEG-20 Sorbitan Oleate (perfusion rate = 3 ml/min); the remaining half served as a control and was perfused with Krebs solution alone. The bioassay rings were superfused with the effluents of the rabbit aortas, and removal of endothelium was determined by brief exposure of the bioassay rings to acetylcholine. Incubation with 10% PEG-20 Sorbitan Oleate and perfusion with  $10^{-1}$  ml/l PEG-20 Sorbitan Oleate caused total inhibition of the release of acetylcholine-induced endothelium derived relaxing factor (EDRF). At microscopic examination, significant desquamation of vascular endothelium was observed after treatment with PEG-20 Sorbitan Oleate, but no damage to the underlying smooth muscle was observed. Low to moderate concentrations of the Polysorbate had no significant

effect on the release of EDRF. Perfusion with PEG-20 Sorbitan Oleate also inhibited EDRF release from donor aorta in a dose-dependent manner. The investigators suggested that the endothelial lining could have been denuded by PEG-20 Sorbitan Oleate, resulting in the reduction in the release of EDRF.

PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Stearate released lysosomal enzymes from the intestinal mucosal cells of the female Sprague-Dawley rat. The intestinal permeability was slightly increased to sodium fluorescein in the absence and presence of the Polysorbates at concentrations of 10 mg/ml (instilled into a section of ligated, cannulated gut). The investigators concluded that surface-active agents had the potential to impair the function of the mucosal barrier and to increase the permeability of the gut to potentially toxic and pathogenic compounds (Tagesson and Edling 1984).

Oberle, Moore, and Krummel (1995) reported that surfactants, including PEG-20 Sorbitan Oleate, increased the rates of lactate dehydrogenase and mucus release in the jejunum and colon of male Sprague-Dawley rats (four to nine per group). The lactate dehydrogenase release rate in the jejunum increased approximately twofold after perfusion of 1% PEG-20 Sorbitan Oleate, compared to saline controls. Minimal morphological damage was observed using light and scanning electron microscopy. The enzyme release rate was approximately threefold less in the colon than jejunum in saline and 1% PEG-20 Sorbitan Oleate, but the rate was twofold greater in rats treated with the Polysorbate than in rats of the control group. Total tissue lactose dehydrogenase decreased along the length of the intestine and was approximately sevenfold less in the colon than in the jejunum, indicating that the relative effects of the surfactants were similar between intestinal regions. Release rates were linear for 6 hours with both saline and PEG-20 Sorbitan Oleate. Mucus release in the jejunum was greater after exposure to PEG-20 Sorbitan Oleate.

In a study by Chi, Scocca, and Huang (1978), PEG-20 Sorbitan Oleate did not increase or diminish the effects of UV and gamma irradiation in *Escherichia coli*.

PEG-20 Sorbitan Oleate caused the proliferation and aggregation of *E. coli* K-12. Normally, *E. coli* grows in culture in a dispersed state (Levinson, Allen, and Sung 1978). In this study, maximal aggregation and proliferation occurred when a concentration of 0.005% PEG-20 Sorbitan Oleate was added to the minimal medium.

PEG-20 Sorbitan Oleate had an immediate effect on the permeability barrier of three strains of *Pseudomonas aeruginosa*, but did not affect that of *E. coli* (Brown and Winsley 1969). In this study, effects on cell permeability were assessed by measuring the effects on the leakage of 260 nm-absorbing substances (on entry of a fluorescent dye) and by the viability of bacteria "under stress." Leakage was enhanced when the cells were treated with concentrations up to 1.25% of PEG-20 Sorbitan Oleate. Cells of *P. aeruginosa* treated with the Polysorbate leaked more readily and had greater percentage viability losses when the pH, temperature, or sodium chloride concentration (suspending fluid) was changed rapidly.



PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Laurate caused 30% to 100% inhibition of the spasmogenic effects of histamine, acetylcholine, carbachol, angiotensin, and other compounds in isolated guinea pig ileum and isolated rabbit jejunum (Sabir, Singh, and Bhide 1972).

Gough et al. (1982) reported that PEG-20 Sorbitan Oleate was a potent cardiac depressant in dogs. The Polysorbate was dissolved in deionized water to give a concentration of 100 mg/ml, and was injected intravenously over a 5-minute period. In addition, commercial IV amiodarone (containing 50 mg/ml amiodarone and 100 mg/ml PEG-20 Sorbitan Oleate) was similarly injected. Within 5 minutes of injection of PEG-20 Sorbitan Oleate, blood pressure, left ventricular maximum  $dP/dt$ , and cardiac output were significantly decreased in all four treated dogs. Within 5 minutes of injection of the commercial solution, the three dogs tested had "severely reduced" mean arterial blood pressure and left ventricular maximum  $dP/dt$ . These parameters remained reduced for >1 hour. Cardiac output also decreased, but due to the variation in control values, the significance could not be determined. For both test compounds, >75% reduction remained in left ventricular  $dP/dt$  after 30 minutes; blood pressure was reduced by at least 68%.

PEG-20 Sorbitan Oleate also caused hemodynamic changes in approximately 20% of 33 guinea pigs treated with 6 ml of 0.02% PEG-20 Sorbitan Oleate in isotonic saline via injection into the left atrium. Within 30 seconds of injection, coronary flow was reduced to an average of 30% below control. This reduction lasted for 0.5 to 3 minutes, and was followed by a subsequent hyperemic response in which peak flow averaged 53% above control. Normal flow resumed by 9 minutes after injection. Left ventricular pressure, aortic blood pressure, and left ventricular stroke volume only declined slightly, and heart rate was unaffected. Upon a second injection, only seven guinea pigs had recurrent adverse reactions (Grund et al. 1995).

The Polysorbates have been reported to activate or inhibit various *in vitro* biochemical reactions. Enzyme activities affected by these ingredients included those of biophenyl 4-hydroxylase, glucose-6-phosphate dehydrogenase, cholesterol oxidase, Na/K ATPase  $Mg^{2+}$ , ATPase, dimethylnitrosamine demethylase, ethylmorphine demethylase, dichloro-*p*-nitro-anisole *O*-demethylase, aniline 4-hydroxylase, biphenyl 2- and 4-hydroxylase, palmityl coenzyme A carnitine *O*-palmityltransferase, and acetylcholinesterase, as well as other enzymes. The Polysorbates affected cellular respiration in rat small intestine epithelial cells by inhibiting oxygen consumption. Lactic acid formation was increased by low concentrations and decreased by high concentrations of PEG-20 Sorbitan Laurate or PEG-20 Sorbitan Oleate. The latter ingredient slightly inhibited mitochondrial oxidation by progressively decreasing phosphorylation capacity with increasing concentration of the Polysorbate. PEG-20 Sorbitan Laurate inhibited the spasmogenic effects of acetylcholine, barium chloride, and histamine during *in vitro* studies using isolated guinea pig duodenum and ileum. PEG-20 Sorbitan Laurate also stimulated secretion of bile when injected intraduodenally (1 ml/kg) into rats. The Polysorbates are non-

specific histamine releasers. Intravenous infusion of PEG-20 Sorbitan Laurate (5 ml, 0.2 ml/15 s) into splenectomized dogs produced anaphylactic-like clinical signs that could have been mediated by endogenous histamine release. The Polysorbates affected the structure and function of cellular membranes. PEG-20 Sorbitan Laurate caused the lysis of erythrocytes and, in a study using artificial membranes, penetrated a lecithin monolayer to block charge transfer through the interface. It also increased membrane resistance and decreased membrane stability of a bimolecular oxidized cholesterol membrane. Investigators suggested that Polysorbates lower conductance of the membrane by making it less permeable to charged molecules and decrease membrane stability when incorporated into the membrane structure (Elder 1984).

Skin penetration of lidocaine increased generally in the presence of an aqueous propylene glycol vehicle containing 1% PEG-20 Sorbitan Laurate or PEG-20 Sorbitan Stearate (Sarpotdar and Zatz 1986). The addition of either Polysorbate to a 40% aqueous solution of propylene glycol caused a decrease in lidocaine penetration rate, however. In solutions containing 60% propylene glycol, flux was greater in the solution containing Polysorbate than the control solution with no surfactant. An 80% solution of propylene glycol with the surfactant had three times the flux of the control solution. In the 40% solution, the micellar solubilization of lidocaine lowered its activity in the vehicle, hence the decrease in its penetration rate.

PEG-20 Sorbitan Oleate (9%; 5 ml/kg IV) affected the blood-brain barrier in an *in situ* brain perfusion assay such that the Polysorbate enhanced the brain uptake and analgesic activity of D-kyotorphin in mice and rats (Sakane et al. 1989).

The Polysorbates influenced the transport of larger molecules across membranes and thus affected drug activity and toxicity (Elder 1984). When Polysorbate-type surfactants were used as emulsifiers and stabilizers in foodstuffs and food colorants, toxic synergy occurred in golden hamsters. PEG-20 Sorbitan Oleate, however, decreased the acute oral toxicity in mice of tetracycline, norsulfazole, theophylline, tubazid, procaineamide, amidopyrine, and pentobarbital. In other studies, PEG-20 Sorbitan Oleate decreased percutaneous penetration of butylparaben *in vitro* through guinea pig skin and inhibited absorption of *p*-aminobenzoic acid from the *in situ* small intestine of the rat, despite the increase in solubilized concentration and membrane permeability of the compounds, respectively, caused by PEG-20 Sorbitan Oleate.

Sorbitan Laurate at concentrations of 1% to 60% in petrolatum or water was applied daily to the clipped backs of New Zealand white rabbits for 81 days. Treated skin sites had increased numbers of inflammatory cells in the dermis. Oxygen consumption of skin treated with Sorbitan Laurate for 3 to 13 days increased twofold; at 30 to 81 days of treatment the increase was two- to threefold (Mezei et al. 1966).

## ANIMAL TOXICOLOGY

The available studies on the PEGs Sorbitan/Sorbitol Fatty Acid Esters (including the Polysorbates), PEGs, and Sorbitan Esters are summarized in Table 6.

**TABLE 6**  
Toxicology data summary

Test chemical	In vitro	Animal	Clinical
	New or updated data		
PEG-20 Sorbitan Laurate	Cytotoxicity Primary skin irritation Ocular irritation Genotoxicity	Behavioral effects Short-term toxicity (IV, IP) Primary skin irritation Ocular irritation Comedogenicity Reproductive and developmental toxicity Tumor inhibition	Parenteral toxicity
PEG-20 Sorbitan Stearate	Cytotoxicity Primary skin irritation  Ocular irritation Metabolic cooperation Genotoxicity	Ocular irritation Reproductive and developmental toxicity Cocarcinogenicity Carcinogenicity Tumor inhibition	Sensitization
PEG-20 Sorbitan Oleate	Mucosa permeability effects Cytotoxicity Primary skin irritation Ocular irritation Genotoxicity Metabolic cooperation	Immunosuppressive effects GI and cardiac effects Penetration enhancement Acute toxicity (ICV,* IP) Behavioral effects Short-term toxicity (IV, IP, oral) Subchronic toxicity (oral) Skin sensitization Ocular irritation Pulmonary toxicity Reproductive and developmental toxicity Carcinogenicity Tumor inhibition	Parental toxicity Sensitization
PEG-20 Sorbitan Palmitate	Cytotoxicity Primary skin irritation Ocular irritation Genotoxicity	Ocular irritation Tumor inhibition	Sensitization
PEG-80 Sorbitan Palmitate		Primary skin irritation Ocular irritation	
PEG-40 Sorbitan Lanolate		Acute toxicity (IP) Primary skin irritation Ocular irritation	Sensitization
PEG-50 Sorbitol Hexaoleate		Acute toxicity (IP) Primary skin irritation Ocular irritation	Sensitization
PEG-30 Sorbitol Tetraoleate Laurate		Acute toxicity (IP) Primary skin irritation Ocular irritation	Sensitization
PEG-40 Sorbitan Peroleate	Genotoxicity	Acute toxicity (IP) Ocular irritation	Sensitization
PEG-20 Sorbitan Trioleate	Primary skin irritation Ocular irritation	Acute toxicity (intramuscular)	
Sorbitan Esters		Acute toxicity (intramuscular, oral) Primary skin irritation	Sensitization Primary and cumulative irritation

**TABLE 6**  
Toxicology data summary (*Continued*)

Test chemical	In vitro	Animal	Clinical
		Data from previous safety assessments	
PEG-4 Sorbitan Laurate		Acute toxicity (oral, IP, IV) Chronic toxicity (oral) Primary skin irritation Ocular irritation Tumor inhibition	
PEG-20 Sorbitan Laurate	Membrane permeability Effects on biochemical reactions	Metabolism, distribution, excretion Acute toxicity (oral, IP, percutaneous, IV) Short-term toxicity (oral) Subchronic toxicity (oral) Chronic toxicity (oral) Primary skin irritation Sensitization Ocular irritation Carcinogenicity	Primary skin irritation Sensitization Photosensitization Ocular irritation
PEG-4 Sorbitan Stearate		Acute toxicity (oral) Short-term toxicity (oral) Subchronic toxicity (oral) Chronic toxicity (oral) Ocular irritation	
PEG-20 Sorbitan Stearate	Effects on biochemical reactions	Absorption, metabolism Acute toxicity (oral) Short-term toxicity (oral) Chronic toxicity (oral) Primary skin irritation Ocular irritation Carcinogenicity Cocarcinogenicity Tumor inhibition	Oral toxicity Primary skin irritation Cumulative skin irritation Sensitization
PEG-5 Sorbitan Oleate		Acute toxicity (oral) Short-term toxicity (oral) Primary skin irritation Ocular irritation Carcinogenicity	
PEG-20 Sorbitan Oleate	Effects on biochemical reactions Genotoxicity	Absorption, metabolism, excretion Penetration enhancement Acute toxicity (oral) Short-term toxicity (oral) Subchronic toxicity (percutaneous) Chronic toxicity (oral) Sensitization Reproductive and developmental toxicity Carcinogenicity Cocarcinogenicity Tumor inhibition	Oral toxicity Primary skin irritation Sensitization
PEG-20 Sorbitan Palmitate	Effects on biochemical reactions	Acute toxicity (oral) Short-term toxicity (percutaneous) Chronic toxicity (oral) Primary skin irritation	Cumulative skin irritation

(Continued on next page)

**TABLE 6**  
Toxicology data summary (*Continued*)

Test chemical	In vitro	Animal	Clinical
PEG-20 Sorbitan Trioleate		Ocular irritation	Primary skin irritation Cumulative skin irritation Sensitization
		Carcinogenicity	
		Cocarcinogenicity	
		Tumor inhibition	
		Acute toxicity (oral)	
		Chronic toxicity (oral)	
PEG-20 Sorbitan Tristearate		Ocular irritation	
		Carcinogenicity	
		Absorption, metabolism	
		Acute toxicity (oral)	
		Chronic toxicity (oral)	
		Sensitization	
Sorbitan Esters	Genotoxicity	Ocular irritation	Oral toxicity Primary skin irritation Cumulative skin irritation Sensitization Phototoxicity Photosensitization
		Carcinogenicity	
		Absorption, metabolism, distribution	
		Effects on skin structure and O <sub>2</sub> consumption	
		Acute toxicity (oral, intramuscular)	
		Short-term toxicity (oral)	
		Subchronic toxicity (oral)	
		Chronic toxicity (oral)	
		Ocular irritation	
		Carcinogenicity	
PEGs	Genotoxicity	Primary skin irritation	Primary skin irritation Sensitization
		Absorption, metabolism, excretion	
		Acute toxicity (oral, dermal)	
		Short-term toxicity (dermal)	
		Subchronic toxicity (oral)	
		Chronic toxicity (oral)	
		Primary skin irritation	
		Sensitization	
		Ocular irritation	
		Reproductive and developmental toxicity	
		Carcinogenicity	

\*ICV = intracerebroventricular.

### Acute Toxicity

#### PEGs Sorbitan/Sorbitol Fatty Acid Esters

Fifteen female rats were given 39.8 g/kg PEG-40 Sorbitan Lanolate and five were given 25.1 g/kg of the compound via gastric intubation. The investigators observed signs of intoxication including depression, diarrhea, and stained, wet perineal areas, but none of the rats died before their scheduled necropsy. Gross lesions included hydronephrosis, focal congestion of the lungs and thymus, and congestion of the mesenteric lymph nodes. PEG-40 Sorbitan Lanolate was classified as "relatively harmless" (CTFA 1998b).

When PEG-50 Sorbitol Hexaoleate and PEG-30 Sorbitol Tetraoleate Laurate were tested similarly using rats and mice, the LD<sub>50</sub> values were >31.6 g/kg. The LD<sub>50</sub> of PEG-40 Sorbi-

tan Peroleate in five male and five female rats was greater than 28.2 g/kg (CTFA 1998b).

#### Polysorbates

The acute oral LD<sub>50</sub> values of PEG-20 Sorbitan Laurate were >38.9 g/kg in rats and >25 g/kg in mice. In dermal studies, adverse effects were not observed after undiluted PEG-20 Sorbitan Laurate was injected percutaneously into the intact or abraded skin of albino guinea pigs. No signs of toxicity were observed after guinea pigs were immersed for 4 h/day in a 0.5% aqueous solution of a product formulation containing 8.4% PEG-20 Sorbitan Laurate for 3 consecutive days. The parenteral LD<sub>50</sub> of PEG-20 Sorbitan Laurate in rats was 0.7 ml/kg or 1.45 g/kg (IV). In mice, these values ranged from 1.42 to 3.75 g/kg. When

administered IP, the LD<sub>50</sub> in rats was 3.85 g/kg; in mice, the LD<sub>50</sub> was 2.64 g/kg. The acute oral LD<sub>50</sub> of PEG-4 Sorbitan Laurate in the rat was >38 g/kg. The LD<sub>50</sub> values of PEG-4 Sorbitan Laurate in the rat were 1.38 g/kg (IV) and >5 ml/kg (IP). The acute oral LD<sub>50</sub> values of PEG-20 Sorbitan Palmitate and PEG-4 and -20 Sorbitan Stearate in the rat varied from >5 g/kg to >38.4 g/kg. For PEG-20 Sorbitan Tristearate, the LD<sub>50</sub> was >10 g/kg to >39.8 g/kg. In rats the LD<sub>50</sub>s for PEG-5 Sorbitan Oleate ranged from 20 ml/kg to >36.6 g/kg, and the LD<sub>50</sub> for PEG-20 Sorbitan Oleate ranged from 20 ml/kg to 54.5 ml/kg. In mice, the LD<sub>50</sub> for PEG-20 Sorbitan Oleate was >25 g/kg (Elder 1984).

The acute IV LD<sub>50</sub> of PEG-20 Sorbitan Oleate was 1790 mg/kg in the rat. The IP LD<sub>50</sub> values were 8210 mg/kg in the mouse and 6804 mg/kg in the rat. The acute oral LD<sub>50</sub> in the mouse was 25 g/kg. The IV LD<sub>50</sub> values of PEG-20 Sorbitan Oleate in the mouse, dog, and cat were 1 g/kg, 500 mg/kg, and 500 mg/kg, respectively (Radian Corporation 1991).

Dib and Falchi (1996) gave Ofa albino rats intracerebroventricular injections of 5% PEG-20 Sorbitan Oleate in saline, with or without an ethanol emulsion. Thirty-six rats of group 1 were injected with 10  $\mu$ l PEG-20 Sorbitan Oleate and 5% ethanol. Within 2 to 3 minutes after injection, the rats had strong convulsions and died; blood mixed with mucus was observed around the nasal area. Seven rats of group 2 were treated with the Polysorbate and 0.5% ethanol. Two rats had convulsions and died within 5 minutes of treatment, and the others recovered locomotory activity within an hour. Two rats of group 3 had an injection of the Polysorbate without ethanol. The rats "lay quietly" in their cages during the initial five minute period, then died after convulsions. The six rats injected with 10 to 20  $\mu$ l of 5% ethanol alone were not affected. When capsaicin was dissolved in PEG-20 Sorbitan Oleate, no adverse effects were noted.

The behavioral effects of PEG-20 Sorbitan Laurate and PEG-20 Sorbitan Oleate were evaluated using 8 to 12 male CD2F1 mice per group (Castro et al. 1995). The Polysorbates were administered at various concentrations by IP injection; the dose volume was 10 ml/kg. The mice were observed for 12 hours after treatment. PEG-20 Sorbitan Laurate significantly decreased locomotor activity (by 50% of controls) at a concentration of 16% in saline (v/v). PEG-20 Sorbitan Oleate decreased locomotor activity at a concentration of 32% in saline (v/v).

A volume of 0.5 ml PEG-20 Sorbitan Trioleate was injected  $\frac{1}{2}$  inch deep in the right and left pectoral muscles of six 7 to 8-week-old male Hubbard Crossbred chickens (Hem et al. 1974, 1975). One chicken had inflammation of tissue involving >8.1 cm<sup>2</sup>, one had inflammation involving <2.0 cm<sup>2</sup>, one had necrosis, and three had inflammation involving 2.1 to 8.0 cm<sup>2</sup>. The same results were observed at the left and right injection sites for each chicken.

#### *Sorbitan Esters*

Five female ddY mice were treated with a single oral dose of Sorbitan Sesquiossearate at a volume of 10 ml/kg body weight. The acute oral LD<sub>50</sub> was 25 ml/kg, which was consid-

ered "practically non-toxic" under the conditions of the study (CTFA 1998c).

No toxic effects were observed in 10 male rats that were given 20 g/kg Sorbitan Laurate as a single oral dose. The LD<sub>50</sub> values of Sorbitan Laurate determined by acute oral toxicity studies using rats ranged from 33.6 to 41.5 g/kg. Sorbitan fatty acid esters in low concentrations were relatively nontoxic after ingestion. The lowest reported rat LD<sub>50</sub> in twenty studies was 31 g/kg for Sorbitan Stearate (Elder 1985).

Male Hubbard Crossbred chickens (6/group) which had Sorbitan Trioleate injected  $\frac{1}{2}$  inch deep into the right and left pectoral muscles had inflammation and necrosis of the tissue at the injection site within seven days of treatment (Hem et al. 1974, 1975). One chicken had inflammation involving <2.0 cm<sup>2</sup> at both sites, one chicken had inflammation involving <2.0 cm<sup>2</sup> at the left site and inflammation involving 2.1 to 8.0 cm<sup>2</sup> at the right injection site. One chicken had inflammation involving 2.1 to 8.0 cm<sup>2</sup> at both sites, one had >8.1 cm<sup>2</sup> inflammation at the left site and necrosis at the right site, and one chicken had necrosis at both sites. Four chickens injected with Sorbitan Isostearate had inflammation of the injection site: three had 2.1 to 8.0 cm<sup>2</sup> inflammation at both sites and one had >8.1 cm<sup>2</sup> at both sites. One chicken had no visible signs of tissue damage at either site, and one had 2.1 to 8.0 cm<sup>2</sup> inflammation at one site, but no signs of tissue damage at the other.

#### *Polyethylene Glycol*

The acute oral LD<sub>50</sub> in rabbits of 100% PEG-6 was 17.3 g/kg; that of 100% PEG-75 was 76 g/kg. Acute dermal toxicity studies did not result in mortality after rabbits were given 20 ml/kg doses of undiluted PEG-6 or 40% PEG-20M (Andersen 1993).

#### **Short-Term Toxicity**

##### *Polysorbates*

A parenteral vitamin E supplement containing Polysorbate emulsifiers was implicated in a number of deaths in premature infants (see Clinical Assessment of Safety—Parenteral Toxicity). To study the toxicity of this supplement, newborn (1-week-old) albino rabbits were given IV doses of 4 ml/kg/day (100 mg/kg/day) of one of two vitamin E preparations containing 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate in water, or the Polysorbate vehicle alone. Parenteral nutrition was provided constantly. To determine the influence of diet, rabbits in both the treatment and control groups received either a low-energy (LE) diet or high-energy (HE) diet. The LE diet was the standard pediatric IV nutrient solution given to premature infants. The HE diet was similar in composition to rabbit milk. Rabbits given the LE diet gained weight at an average of 0.6  $\pm$  1.0 g/day, and rabbits given the HE diet gained an average of 3.9  $\pm$  0.5 g/day. After 6 to 7 days of treatment, the rabbits were killed and the blood and tissues were analyzed. Rabbits of all treatment and control groups given the LE diet had centrilobular degeneration and necrosis and pigment accumulation in the liver. No other lesions were attributed to the administration of the vitamin E preparations or the Polysorbate vehicle.

Rabbits given the HE diet had no nutrition-related centrilobular degeneration, but they had other treatment-related changes. These changes included microscopic evidence of mild bile stasis, elevated serum bilirubin, and minimal lipidosis of the liver, spleen, and adrenal cortex. The study suggested that vitamin E ( $\alpha$ -tocopherol or  $\alpha$ -tocopherol acetate) in a Polysorbate vehicle was mildly hepatotoxic and altered fat metabolism (Rivera et al. 1990).

Farkas et al. (1991) treated neonatal Sprague-Dawley rats and 566 mice with the same Polysorbate vehicle by IP injection. The strain of mouse and number of rats were not available. The vehicle was diluted to 10% in saline. In an acute toxicity study using rats, essentially all mortality was in the first 7 days. Multiple injections during a 90-day study had little effect on increasing mortality if the treated rats lived 7 days; the injections, however, produced massive peritoneal fibrosis and adhesions between organs in rats that lived for 2 to 3 weeks. Rats treated with 3.5 to 4 g/kg had swollen, inflamed tails within several days of the injection, and annular constrictions developed. In newborn mice injected daily, gross or microscopic evidence of hepatic damage was not observed. The mortality pattern paralleled that observed in rats. Animals injected with  $\geq 2.5$  g/kg had chylous ascites and hydropic degeneration of the renal tubules. Renal tubular regeneration was observed in rats and mice that survived multiple injections of the Polysorbates. Large areas of Zenker's necrosis of the muscle of the diaphragm were observed at microscopic examination at the same time of the appearance of chylous ascites.

A body lotion containing 4% PEG-20 Sorbitan Palmitate was tested for percutaneous toxicity during a 28-day study. Doses of 0.3 or 0.9 ml/kg/cm<sup>2</sup> were applied daily (5 days/week) to the backs of albino rabbits, half of which received epidermal abrasions twice a week. After 2 weeks of treatment, several rabbits had slight peripheral leukocytosis, and dose-related dermatitis (mild to moderate erythema and edema and scaly desquamation) was observed. No adverse effects were reported when chicks were given 0.1 to 2% PEG-20 Sorbitan Laurate in feed for 7 weeks. In feeding studies using rats, 3% to 5% PEG-20 Sorbitan Laurate caused slow weight gain attributed to mild diarrhea, but no other signs were noted after eight weeks of treatment. In a 10-week study using hamsters, 5% to 15% PEG-20 Sorbitan Laurate caused high mortality that could have been due to diarrhea. No adverse effects were noted when PEG-20 Sorbitan Stearate was fed to chicks at up to 2% for 7 weeks or to rats fed up to 5% for 8 weeks. Rats fed 10% PEG-20 Sorbitan Stearate for 8 weeks had diarrhea after the first few days, with recovery after continued feeding. During a 3-month study, Charles Foster rats fed 1.5 ml PEG-20 Sorbitan Oleate at 1% to 4% had congestion and degenerative changes in the heart, liver, and kidneys, which were attributed to capillary wall damage. In 6-week studies by the same investigators, rats given 1% to 4% PEG-20 Sorbitan Stearate and PEG-5 Sorbitan Oleate and rhesus monkeys given 2 ml/day PEG-4 Sorbitan Stearate had no adverse effects. In these studies, the observed diarrhea was considered likely due to the high concentrations of the unabsorbed polyoxyethylene sorbitan

moiety within the intestinal lumen. It could have been directly or indirectly the cause of the other adverse effects (retarded growth, etc.) observed in the feeding studies (Elder 1984).

During a 14-day feeding study performed by the National Toxicology Program (NTP 1992b), five rats and five mice per sex per group were fed 3000, 6000, 12,500, 25,000, or 50,000 ppm/day PEG-20 Sorbitan Oleate. No deaths occurred prior to study termination. The mean body weight change of males fed 50,000 ppm was significantly lower than that of controls. No clinical findings related to administration of the test compound were observed.

#### *Sorbitan Esters*

The feeding of Sorbitan Stearate to rats for 8 weeks did not affect growth; other studies indicated that the ingredient had nutritive value for rats and dogs. A slight but inconsistent (non-significant) increase in growth occurred in chicks fed 0.1% to 2.0% Sorbitan Laurate for 10 weeks, with or without penicillin supplementation. Mortality, body weight, and necropsy findings were not affected by treatment. Rats (three groups of 12) fed 1% to 4% Sorbitan Laurate for 6 weeks had slightly decreased growth rates; all other parameters were normal. Rhesus monkeys given 2 g/day Sorbitan Laurate for 6 weeks had no signs of toxicity. Hamsters fed 5% Sorbitan Laurate for 68 days had reduced growth rates and slightly greater mortality than controls. Hamsters fed 15% had diarrhea, higher mortality, and gastrointestinal mucosal hyperemia, edema, and renal tubule epithelial degeneration. Increased weights of the brain, kidneys, heart, spleen, lungs, and liver were observed in Sprague-Dawley rats fed 25% Sorbitan Laurate for 59 days. Prior to necropsy, the rats had reduced body weights, diarrhea, nasal hemorrhage, and gangrenous tails. In a 70-day feeding study, the rats had decreased activity and appetite, reduced weight gain, nasal bleeding, gangrene of the tail and hind legs, increased organ weights, and degenerative changes of the gastrointestinal tract, kidneys, and liver (Elder 1985).

#### *Polyethylene Glycol*

No evidence of toxicity was observed in rabbits that had received daily dermal applications of PEG-20M (0.8 g/kg/day) for 30 days; however, transient, mild erythema was observed. The only evidence of systemic toxicity that resulted from dermal exposure was in rabbits that received repeated applications of an antimicrobial cream containing 63% PEG-6, 5% PEG-20, and 32% PEG-75 to excised skin for 7 days (Andersen 1993).

#### **Subchronic Toxicity**

##### *Polysorbates*

When 25% PEG-20 Sorbitan Laurate was fed to 10 rats over 21 weeks, there was only 1 fatality; however, significant gross and microscopic changes were observed in the urinary bladder, spleen, kidneys, and gastrointestinal tract. No adverse effects were reported when mice were fed 2.5% to 10% PEG-20

Sorbitan Stearate for 12 to 16 weeks. Mice given the compound at a concentration of 20% had some gastrointestinal "disturbance" with reduced feed intake and growth retardation. When rats were fed 5% to 10% (in soybean meal) PEG-20 Sorbitan Stearate for 14 weeks, no adverse effects were observed. When administered in purified casein, 5% caused diarrhea and growth retardation. Monkeys fed 2 g/day for 14 weeks had no adverse effects, and rats given 25% had growth retardation and transient diarrhea after 15 weeks of treatment. As noted above (see Short-Term Toxicity), the observed diarrhea was considered likely due to the high concentrations of the unabsorbed PEG sorbitan moiety within the intestinal lumen. A cream formulation containing 2.5% PEG-20 Sorbitan Oleate was tested for percutaneous toxicity. Doses of 6 mg/cm<sup>2</sup> were applied to the backs of rabbits for 90 consecutive days. No signs of systemic toxicity were observed, but moderate edema and erythema, slight to moderate desquamation, and mild dermatitis were noted by investigators. Slight erythema and scaly desquamation were reported when a cream formulation was tested at doses of 0.36 ml/260 cm<sup>2</sup>/3 kg rabbit for 93 consecutive days (Elder 1984).

When diets containing 3100 to 50,000 ppm PEG-20 Sorbitan Oleate were fed to groups of 10 F344/N rats and 10 B6C3F<sub>1</sub> mice of each sex, all animals survived to study termination. No clinical findings, changes in absolute or relative organ weights, changes in mean body weight, or gross or microscopic lesions were observed during the 13-week study (NTP 1992b).

#### *Sorbitan Esters*

In subchronic studies, no toxic effects were noted when chickens, rats, monkeys, and hamsters were fed Sorbitan Laurate at concentrations <10%. At greater concentrations, growth depression, decreased organ weights, diarrhea, unkempt appearance, hepatic and renal abnormalities, and gastrointestinal tract irritation were generally observed. Rats fed <10% Sorbitan Oleate had no abnormalities. At greater concentrations, the same types of abnormalities were observed as were noted in animals fed Sorbitan Laurate. No deaths occurred in Wistar rats fed 2.5% to 10.0% Sorbitan Laurate for 90 days. Treated rats had decreased body weights, hemoglobin concentrations, and packed cell volume values. The average weights of the brain, liver, and kidneys increased, but the average weights of the heart and gastrointestinal tract decreased. Periportal vacuolization of hepatocytes and tubular nephrosis were also observed. In a 13-week feeding study using rats, increased liver and kidney weights, and decreased body weights were observed following treatment with 10% Sorbitan Laurate. In a 23-week study, rats fed 15% to 25% Sorbitan Laurate had diarrhea, unkempt appearance, and severely retarded growth. Eight of ten rats of the high dose group died prematurely. A pale and enlarged liver, enlarged common bile duct, and gangrene of the tail were observed. Hepatic lesions included fatty changes, fibrosis, chronic inflammation, and necrosis. Other lesions observed were focal nephritis, increased numbers of foamy alveolar macrophages, and hyperplasia of cells of the bone marrow and spleen. Rats fed 10% Sorbitan Laurate for 17 weeks had decreased body weights, packed cell

volume, and hemoglobin values. Kidney and liver weights were significantly increased (Elder 1985).

#### *Polyethylene Glycol*

In 90-day oral toxicity studies involving groups of albino rats, the largest and smallest molecular weight PEGs tested (PEG-20M and PEG-6, respectively) did not induce toxicity or death when administered daily in the diet (PEG-20M) or in drinking water (PEG-6) at concentrations of 4% or less (Andersen 1993).

### **Chronic Toxicity**

#### *Polysorbates*

Of hamsters fed 5% to 15% PEG-20 Sorbitan Laurate for 28 to 39 weeks, 18/30 (10 per group) died and significant gross and microscopic lesions were observed in the urinary bladder, kidneys, spleen, and gastrointestinal tract. In a 17-month study using monkeys, dietary administration of 1 g/day PEG-20 Sorbitan Laurate produced no signs of toxicity. Rats fed 0.5% to 2% PEG-20 Sorbitan Laurate over the course of a lifetime had no adverse effects. Rats fed 2% PEG-4 Sorbitan Laurate for 2 years had neither gross nor microscopic changes. Numerous chronic feeding studies were performed on PEG-4 and -20 Sorbitan Stearate. No adverse effects were reported after PEG-20 Sorbitan Stearate was administered for up to 2 years at concentrations up to 10% in the mouse, 5% in the rat, 10% in the dog, and 1% in the hamster. When fed at greater concentrations, the only effects observed were diarrhea and some growth retardation; in multigeneration studies, 20% PEG-20 Sorbitan Stearate caused minor effects on growth, longevity, and reproduction. When dogs were fed PEG-20 Sorbitan Tristearate for 12 months at concentrations of 13.5% and 34%, the high dose caused phosphate kidney stones as a result of dehydration, and both doses caused periods of dehydration and diarrhea. In 2-year studies using rats (four generation studies), concentrations of 10% to 20% caused diarrhea, 20% caused minor effects on growth, longevity, and reproduction, and 2% to 5% caused no adverse effects. No gross or microscopic anomalies were observed after rats were fed 2% PEG-20 Sorbitan Palmitate for 2 years. No adverse effects were noted when two monkeys were treated with 1 g/day PEG-20 Sorbitan Oleate for 17 months. Rats fed 2% to 5% for 2 years had no adverse effects. Doses of 10% and 20% caused diarrhea, and 20% resulted in some minor effects on growth, longevity, and reproduction during a multigeneration study. Rats fed 2% PEG-5 Sorbitan Oleate or PEG-20 Sorbitan Trioleate for 2 years also had no adverse effects (Elder 1984).

#### *Sorbitan Esters*

Chronic feed studies have been conducted with Sorbitan Stearate, Sorbitan Laurate, and Sorbitan Oleate. At 5%, Sorbitan Laurate and Sorbitan Oleate had no adverse effects on rats over a 2-year period. Dogs fed 5% Sorbitan Stearate for 20 months had no changes related to the test compound;  $\geq 10\%$  was required to produce depressed growth and hepatic and renal abnormalities. Mice were more sensitive to Sorbitan Stearate than rats. Growth

depression was observed in rats fed 0.5% Sorbitan Stearate, and 4% caused renal abnormalities as well (Elder 1985).

#### *Polyethylene Glycol*

Toxic effects were not observed in dogs that received 2% PEG-8, PEG-32, or PEG-75 in the diet for 1 year (Andersen 1993).

### **Skin Irritation and Sensitization**

#### *PEGs Sorbitan/Sorbitol Fatty Acid Esters*

PEG-40 Sorbitan Lanolate, PEG-50 Sorbitol Hexaoleate, and PEG-80 Sorbitan Palmitate did not produce erythema or edema when applied undiluted to the intact and abraded skin of New Zealand white rabbits for 24 to 72 hours. The primary irritation index (PII) was 0. Undiluted PEG-30 Sorbitol Tetraoleate Laurate caused very slight to well-defined erythema within 24 hours of treatment; very slight erythema was still evident 72 hours after treatment. The PII was 0.83/8, and the compound was classified as slightly irritating to the skin of rabbits (CTFA 1998b).

#### *Polysorbates*

In primary dermal irritation studies (by Draize methods) using rabbits, minimal to no irritation was observed after administration of undiluted PEGs-4 and -20 Sorbitan Laurate for up to 72 hours. In a 30-day dermal irritation study, PEG-20 Sorbitan Laurate caused erythema by the third day and skin thickening by day 10. At that time, minimal to mild inflammation was observed, but not acanthosis or necrosis. Slight erythema and minimal inflammation were reported when 1%, 5%, and 10% concentrations of PEG-20 Sorbitan Laurate (in water, petrolatum, or a hydrophilic ointment) were tested. Topical application of 100% PEG-20 Sorbitan Palmitate did not cause signs of irritation to the skin of rabbits during a primary skin irritation assay. PEG-20 Sorbitan Stearate (4%–100%) caused no irritation to mild irritation (PII = 0.50/4.0). PEG-20 Sorbitan Oleate (100%) was not an irritant in one study, and was a minimal irritant (PII = 0.17/4.0) in another. During a 28-day percutaneous toxicity study using rabbits, a body lotion containing 4% PEG-20 Sorbitan Palmitate (doses = 0.3 or 0.9 ml/kg/cm<sup>2</sup>) caused mild to moderate erythema and edema, as well as scaly desquamation. A cream formulation containing 2.5% PEG-20 Sorbitan Oleate (doses = 6 mg/cm<sup>2</sup>) caused moderate edema and erythema, slight to moderate desquamation, and mild dermatitis after 90 consecutive days of treatment during a second percutaneous toxicity study using rabbits. In a 93-day study, the cream formulation (doses = 0.36 ml/260 cm<sup>2</sup>/3 kg rabbit) produced slight erythema and scaly desquamation. In Magnusson-Kligman guinea pig maximization tests, PEG-20 Sorbitan Laurate caused moderate to strong sensitization. In this study, the induction concentrations were 5% to 7.5%, and the challenge concentration was 100%. In studies using guinea pigs PEG-20 Sorbitan Tristearate and PEG-20 Sorbitan Oleate were not sensitizers (Elder 1984).

DeLeo et al. (1989) evaluated the cutaneous primary irritancy of several surfactants, including PEG-20 Sorbitan Laurate, using

the guinea pig dermal irritation assay, and the in vitro choline release assay. The latter assay determined the effect of the surfactants on membrane choline phospholipid metabolism in human epidermal keratinocytes. The investigators hypothesized that surfactants stimulated phospholipase activation of human keratinocytes during surfactant-skin interaction, leading to the production of membrane-derived mediators, resulting in skin irritation.

The guinea pig primary dermal irritation test was a modification of the rabbit primary irritation procedure and used adult Hartley guinea pigs of both sexes (500–700 g) instead of rabbits. Feed and water were available ad libitum. The test solution (undiluted; 1 ml) was applied to the shaved abdominal region using a Webril pad that was affixed with surgical tape. Adhesive tape served as an overwrap. After 4 hours, the occlusive patch was removed. This procedure was repeated for 2 more consecutive days. On the sixth day, the guinea pigs were depilated, rinsed, towel-dried, and recaged. The test sites were evaluated 4 hours later. PEG-20 Sorbitan Laurate had a score of <1 (no irritation to very slight erythema).

Human keratinocytes were incubated with [<sup>3</sup>H]choline for 24 hours in the choline release assay. The keratinocytes incorporated 40% to 60% of the radioactivity of the media. The concentrations of PEG-20 Sorbitan Laurate tested were 1–4 × 10<sup>-4</sup> M. Cellular extracts were examined by high performance liquid chromatography (HPLC). To determine the origin of the [<sup>3</sup>H]choline released into the media by surfactant treatment, the acid-soluble and acid-precipitable pools of radioactivity were determined in surfactant-treated and control (distilled water) cultures, and the treated and control membrane phospholipids were extracted and separated by HPLC. PEG-20 Sorbitan Laurate induced choline release only at 4 × 10<sup>-4</sup> M, and was classified as a minimal dermal irritant (DeLeo et al. 1989).

Gajjar and Benford (1987) used a differentiating keratinocyte cell line developed from explant cultures of rat sublingual epithelium as a model for topical skin irritancy. The investigators tested a number of surfactants, including PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Trioleate. The end points used to assess toxicity were acid phosphatase (AP) activity after 4 hours of dosing, NR uptake (see General Biology—Cytotoxic Effects), and kenacid blue (KB) staining after 3 days to assess cell viability and number. No peak in AP activity was observed after treatment with the Polysorbates, with the exception of a slight peak observed after treatment with the highest concentration (1.0 mg/ml) of PEG-20 Sorbitan Laurate; the peak was 158% that of the control. The NR-50 values for the Polysorbates were 0.59, 0.21, 0.34, 1.0, and >1.0 mg/ml for PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Trioleate, respectively. The KB-50 for PEG-20 Sorbitan Laurate was 0.44 mg/ml, the KB-50 for PEG-20 Sorbitan Palmitate was 0.23 mg/ml, the value for PEG-20 Sorbitan Stearate was 0.32 mg/ml, the value for PEG-20 Sorbitan Oleate was 0.89,



and the KB-50 for PEG-20 Sorbitan Trioleate was  $> 1.0$  mg/ml. Of the compounds evaluated in this study, the Polysorbates were the least toxic.

PEG-20 Sorbitan Oleate at a concentration of 50% in ethanol was tested for sensitization potential in a mouse ear sensitization assay (Descotes 1988). The test compound was applied twice to the right external ear of Swiss mice using a scapular subcutaneous injection of complete Freund's adjuvant. The degree of contact hypersensitivity was determined from left external ear swelling, calculated as the difference in external ear thickness measured immediately before challenge and 24 hours later. The external ear thickness measurements taken before and after application of PEG-20 Sorbitan Oleate did not differ significantly, indicating that the Polysorbate was not a skin sensitizer under the conditions of this study.

PEG-20 Sorbitan Oleate was not a sensitizer and did not cause swelling of the external ear in a similar study using mice (Gad et al. 1986).

#### *Sorbitan Esters*

Sorbitan Isostearate was classified as a moderate irritant (primary irritation index, PII = 2.8/8.0) to the skin of rabbits. Sorbitan Isostearate also had very low sensitization potential when tested in four Magnusson-Kligman guinea pig maximization studies. The induction concentrations were 1% to 2% (intra-dermal injection) and 50% to 100% (topical application), and the challenge concentrations were 10% to 25%. In addition, a Landsteiner guinea pig test showed that intradermal injections of 0.2% Sorbitan Isostearate in propylene glycol caused mild to severe irritation in all animals, but did not cause sensitization reactions (Unichema International 1996).

Sorbitan Isostearate was described as "non-irritating, non-sensitizing, non-comedogenic in studies according to industry standard protocols (Repeated Insult Patch Test (RIPT); comedogenicity)" and in the chorioallantoic membrane vascular assay, of which additional details were unavailable (CTFA 1998d).

Sorbitan Isostearate (2.5%) was tested in an RIPT using 201 subjects. During the induction period, 48- to 72-hour occlusive patches containing 0.2 g of the test material were applied to the upper arm or back. Patches were applied three times per week for 3 weeks. After a 2-week nontreatment period, a 72-hour challenge patch was applied to a previously unexposed site. Reactions were scored at 96 hours post-application. Sorbitan isostearate did not induce a sensitization response (CTFA 1998d).

The primary skin irritation potentials of Sorbitan Isostearate and Sorbitan Sesquiosostearate (both 10.0% in squalene) were evaluated using eight male Japanese White rabbits. The test materials were added to abraded and intact skin sites of the clipped back, and the sites were covered for 24 hours using patch test plaster. The test sites were evaluated at 24 and 72 hours after administration of the test material. The PIIs were 0.3/8.0 and 0.5/8.0, respectively, which corresponded to a grade of non- to weak irritant.

Sorbitan Isostearate and Sorbitan Sesquiosostearate were weak cumulative irritants using three male Hartley guinea pigs. A 0.05-ml volume of each test substance (10.0% in squalene) was applied to the clipped and shaved skin of the flank, once daily for 3 consecutive days. The treatment sites were examined for signs of irritancy 24 hours after each application. The cumulative scores were 1.1/4.0 and 1.7/4.0, respectively (CTFA 1998c).

Numerous skin irritation studies in animals indicate that the Sorbitan Esters are minimal to mild irritants. In acute skin irritation tests using rabbits, Sorbitan Stearate was mildly irritating and caused dose-dependent erythema and edema. The rabbit dermal toxicity and irritation potential of Sorbitan Sesquioleate was minimal. Sorbitan Oleate was minimally irritating to rabbit skin, and caused erythema and edema. Sorbitan Palmitate was nonirritating and did not cause systemic toxicity during a short-term dermal toxicity study. Sorbitan Tristearate was nonirritating when applied to the skin of rabbits. In rabbits, Sorbitan Trioleate was generally found to be a skin irritant; it caused erythema, edema, and thickening, but not systemic toxicity. After 3 days of treatment, Sorbitan Laurate at a concentration of 100% caused intense erythema and edema to the clipped skin of New Zealand rabbits; 10% and 60% concentrations resulted in erythema and edema. No visible change was observed after treatment with 1% Sorbitan Laurate. After 10 days of application, thickening of the skin sites was observed at 60% and 100%, and erythema and edema were observed at 1% and 10% (Elder 1985).

#### *Polyethylene Glycol*

In skin irritation tests, undiluted PEG-6 was applied to the skin of rabbits for 4 hours and 50% PEG-75 was applied to guinea pigs for 4 days and to rabbits over a 13-week period. In the guinea pig skin sensitization test, PEG-75 was tested at a concentration of 0.1%. The PEGs were not irritating to the skin of rabbits or guinea pigs, and PEG-75 was not a sensitizer (Andersen 1993).

#### *Ocular Irritation*

##### *PEGs Sorbitan/Sorbitol Fatty Acid Esters*

A series of Draize ocular irritation assays was performed on the PEGs Sorbitan/Sorbitol Fatty Acid Esters. Conjunctival irritation was observed at 1 hour, but not 96 hours after instillation of PEG-40 Sorbitan Lanolate at a concentration of 100%. PEG-30 Sorbitol Tetraoleate Laurate caused slight redness in one eye at 1 hour, but not 24 hours after instillation. The Draize scores for both compounds were 0.33/100, and both were classified as nonirritating to the eyes of rabbits. PEG-40 Sorbitan Peroleate (100% and 10% in water) and PEG-50 Sorbitol Hexaoleate (100%) were nonirritating to the eyes of rabbits. PEG-80 Sorbitan Palmitate (100%) was minimally irritating in unrinse eyes (CTFA 1998b).

#### *Polysorbates*

Undiluted PEGs-4 and -20 Sorbitan Laurate were nonirritating to the eyes of rabbits. PEG-20 Sorbitan Laurate at a

concentration of 30% (in distilled water) was not an ocular irritant. PEG-20 Sorbitan Palmitate (30–100%), PEG-20 Sorbitan Tristearate (30%), PEG-5 Sorbitan Oleate (100%), PEG-20 Sorbitan Stearate (30%), and PEG-20 Sorbitan Trioleate (10%–75%) were not irritating to the eyes of rabbits. At a concentration of 100%, PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate caused either no or minimal irritation. PEG-4 Sorbitan Stearate at a concentration of 60% was minimally irritating to the eyes of rabbits (Elder 1984).

The concentrations of PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Oleate which caused a 50% reduction in cell viability using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay were 219, 227, and 270  $\mu\text{g/ml}$ , respectively. The maximum concentrations (*w/v*) of these compounds that did not cause irritation to the eyes of rabbits were 16%, 41%, and 62%, respectively (Nagami and Maki 1993).

Undiluted PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Oleate were evaluated for ocular irritation using the *in vitro* Skin<sup>2</sup> tissue system model ZK1200. The end point of this assay was MTT reduction, which determined cell viability after exposure to the test chemicals. The times of exposure that resulted in the death of 50% of the treated cells were 9.4, > 10, and > 10 minutes, respectively. On the basis of this test, PEG-20 Sorbitan Laurate was classified as a mild-moderate irritant, and PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Oleate were classified as innocuous chemicals (Rachui et al. 1994). PEG-20 Sorbitan Oleate at a dose of 150 mg was a mild ocular irritant to the eyes of rabbits (Radian Corporation 1991).

North-Root et al. (1992) reported that the concentrations of PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Stearate that resulted in 50% relative survival of SIRC rabbit corneal cells were 11,482 and 36,000 ppm, respectively. A Draize assay was also performed using New Zealand white rabbits (test concentrations did not exceed 30%). The surfactant concentrations predicted to cause Draize scores of 20 were >90% and >>90% for PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Stearate, respectively.

The maximum average scores for PEG-20 Sorbitan Oleate and PEG-20 Sorbitan Laurate in the Draize ocular irritation assay were 3.8 and 5.7, respectively (Roguet et al. 1994). PEG-20 Sorbitan Oleate at a concentration of 10% in water was also nonirritating in a Draize test using six New Zealand white rabbits (Guido 1987). The maximum Draize score for 98% PEG-20 Sorbitan Oleate in another study was 4.0/110 (Bagley et al. 1992). Jacobs and Martens (1989) reported that the mean erythema score of PEG-20 Sorbitan Oleate was 0.55, and no signs of chemosis, corneal opacity, or corneal swelling were observed in the three rabbits tested.

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Trioleate, and PEG-20 Sorbitan Oleate were classified as nontoxic to the eye in a study using the permeability of the mouse cornea as a test for acute ocular toxicity (Maurice and Brooks 1995). In this study, a drop of the test compound

was placed on the cornea for 1 minute and was washed off and replaced with a drop of sulforhodamine B, a fluorophore dye. Permeability was determined by the total fluorescence of the cornea. The investigators noted the potential of PEG-20 Sorbitan Laurate to increase the penetration of the dye.

In a similar study, 1% PEG-20 Sorbitan Oleate did not significantly increase the permeability of the mouse cornea to fluorescein (Etter and Wildhaber 1985). PEG-20 Sorbitan Oleate at a concentration of 100% did not significantly increase the opacity or permeability of the bovine cornea (six per test compound) to sodium fluorescein (Vanparys et al. 1993).

The red blood cell (RBC) assay estimates the irritation potentials of surfactants and detergents by measuring the photometrical absorbance of oxyhemoglobin, an indicator for cell membrane lysis and cell protein denaturation. When tested using this assay, PEG-20 Sorbitan Laurate did not produce hemolysis and the denaturation index (equal to 100% at a concentration of 30 moles sodium dodecyl sulfate per mole oxyhemoglobin as an internal standard) was <1 (Pape, Pfannenbecker, and Hoppe 1987).

Tachon et al. (1989) compared the *in vitro* cellular toxicity and the *in vivo* ocular irritation potency of 16 surfactants, including PEG-20 Sorbitan Laurate. Cellular toxicity was estimated in V79 Chinese hamster lung fibroblasts using a cell mortality test and a cell growth inhibition assay. In the cell mortality test, V79 cells were suspended in Eagle's modified minimal essential medium (EMEM) for 1 hour at 37°C, and various concentrations of the surfactants were added. The treatment solution was discarded and the cells were resuspended in fresh medium to which 25  $\mu\text{l}$  of trypan blue had been added. Cell mortality was assessed in terms of membrane integrity; trypan blue was excluded by intact cells, but penetrated membrane-damaged cells. The lethal concentration 50% (LC<sub>50</sub>) of PEG-20 Sorbitan Laurate was 98.34  $\mu\text{g/ml}$ . When 10% fetal calf serum was added to the medium, the LC<sub>50</sub> increased to 595.30  $\mu\text{g/ml}$ , indicating that the surfactant interacted with serum proteins. In the cell growth inhibition test, V79 cells were incubated in well plates with EMEM and 10% fetal calf serum to which the surfactants had been added. After 72 hours, phase contrast inverted microscopy was used to evaluate cellular morphology changes. Cell growth was assessed by measuring the total protein content per well, with bovine serum albumin as a standard. The PEG-20 Sorbitan Laurate concentration required to reduce growth by 50% was 287.63  $\mu\text{g/ml}$ , as determined using the cell growth inhibition test. This assay did not discriminate between cell death and inhibition of growth, however.

PEG-20 Sorbitan Laurate at a concentration of 10% in water was a very weak ocular irritant in the Draize test using six albino rabbits. The maximum ocular irritation score was 5.67 after 1 or 24 hours, and the irritation score was zero after 7 days. The investigators concluded that the results of the Draize test correlated with the results of the two previous *in vitro* assays (Tachon et al. 1989).

Isolated rabbit eyes were used in an ocular toxicity assay as a model for human corneal damage after exposure to various

chemicals, including PEG-20 Sorbitan Laurate. The eyes were enucleated within 30 minutes of death and were stored at 4°C overnight. The enucleated globes were kept in a temperature-control chamber (32–36°C), held vertically in clamps, and irrigated with Hanks' balanced salt solution at the upper limbus. The globes were examined using a Haag-Streit Slit Lamp before and after treatment, and the corneal thickness was measured. After 90 minutes, 20- and 100- $\mu$ l volumes of PEG-20 Sorbitan Laurate were applied at the superior limbus for 10 seconds and 1 minute, respectively. The larger volume was applied in 20- $\mu$ l aliquots at 10-second intervals. The globes were rinsed for 10 seconds with the salt solution. The corneal thickness was measured every 30 minutes; the total time of the experiment was 7 to 8 hours. Exposure to PEG-20 Sorbitan Laurate had little effect on corneal deturgescence in enucleated rabbit globes, compared to controls. Increased granularity of the epithelium and fluorescein staining in the contact area were observed after treatment with PEG-20 Sorbitan Laurate (Berry and Easty 1993).

Surfactant cytotoxicity was evaluated in primary cultures of rabbit corneal epithelial cells by Grant et al. (1992) and Yao and Acosta (1992) using as end points lactate dehydrogenase (LDH) enzyme leakage from the cytosol to the medium, mitochondrial MTT dye reduction, and lysosomal NR uptake, followed by morphological observations at 1 and 24 hours after treatment. The LDH leakage assay evaluated cell membrane integrity, and the MTT reduction and NR uptake assays determined cell viability (Grant et al. 1992; Yao and Acosta 1992). In the study by Grant et al. (1992), cells treated with PEG-20 Sorbitan Laurate had marked vacuolization with little formation of pseudopodia. Most LDH leakage occurred during the 1-hour treatment time, and was <20% after 24 hours. In the Yao and Acosta study (1992), treated cells had either a pleomorphic epithelial appearance or a keratinocyte-like appearance. Of the surfactants tested (including benzalkonium chloride and sodium dodecyl sulfate [SDS]), PEG-20 Sorbitan Laurate was the least cytotoxic in both studies. The in vitro results were correlated with Draize rabbit ocular irritation data in which PEG-20 Sorbitan Laurate was practically nonirritating to the eyes of rabbits (Grant et al. 1992; Yao and Acosta 1992).

Yang and Acosta (1994) determined cytotoxicity of surfactant mixtures by LDH leakage and MTT reduction in a primary culture system of rabbit corneal epithelial cells. The investigators then correlated the in vitro cytotoxicity with reported Draize ocular irritation data. Binary and tertiary groups of surfactant mixtures were tested. The total active surfactant concentration of both groups was 7%. The binary group contained lauramphocarboxyglycinate (HMS) and SDS in varying proportions, and the ternary group contained HMS, SDS, and 8% PEG-20 Sorbitan Laurate. At low and high surfactant concentrations, LDH leakage increased by <20% compared to controls. A 20% to 50% increase occurred at the middle concentrations. A similar pattern occurred in the MTT reduction assay. The ternary surfactant mixture containing PEG-20 Sorbitan Laurate was slightly more cytotoxic than the binary mixture, although undiluted PEG-20 Sorbitan Laurate was nonirritating to the rabbit eye. The investigators speculated that PEG-20 Sorbitan Lau-

rate affected the toxicity of other surfactants by facilitating the uptake of SDS and HMS (by decreasing the surface tension of the plasma membrane) or by changing the micellar organization of hydrophilic/lipophilic balance ratios of surfactants.

PEG-20 Sorbitan Palmitate was minimally irritating to the eyes of guinea pigs in the Draize ocular irritation assay when tested at a concentration of 5% (*w/v*); the Draize score was 4.4. In vitro assays using mouse embryo fibroblasts (BALB/c 3T3 cells; NR assay only) and BHK 21/C13 cells were also performed; PEG-20 Sorbitan Palmitate was minimally irritating (Bracher et al. 1988). The NR-50 score was 269.3  $\mu$ g/ml, the concentration that caused 25% cell detachment was 750  $\mu$ g/ml, the concentration that caused 50% growth inhibition was 115  $\mu$ g/ml, the concentration that caused fluorescein retention (fluorescence shift, FS-25) was 5.6  $\mu$ g/ml, and the concentration that resulted in a 25% viability ratio based upon ethidium bromide exclusion was 21 g/ml.

In other in vitro assays, PEG-20 Sorbitan Laurate and PEG-20 Sorbitan Oleate at concentrations of 5% to 10% (*w/v*) increased opacity and thickness of isolated bovine cornea after 30 minutes to 4.5 hours of incubation (Igarashi and Northover 1987).

#### *Polyethylene Glycol*

PEG-6 and -75 did not cause corneal injuries when instilled (undiluted, 0.5 ml) into the conjunctival sac of rabbits. PEG-8 (35% solution, 0.1 ml) and PEG-32 (melted in water bath, 0.1 ml) induced mild ocular irritation in rabbits (Andersen 1993).

#### *Sorbitan Esters*

Sorbitan Isostearate was nonirritating to the eyes of rabbits in two studies (Unichema International 1996). When 0.1 ml (10.0% in squalene) was tested using three male Japanese white rabbits, the average total score was 4.0/110.0, which corresponded to a grade of minimal irritant. Using the same procedure, Sorbitan Sesquiisostearate (10.0% in squalene) was a minimal irritant to the eyes of rabbits, with an average total score of 6.7/110.0 (CTFA 1998c).

Sorbitan Stearate was not an ocular irritant in a study using rabbits when the ester was tested at high concentrations. Low concentrations in formulation caused slight conjunctival irritation. High concentrations of Sorbitan Sesquioleate were nonirritating. One study with Sorbitan Laurate and two each with Sorbitan Oleate, Sorbitan Tristearate, and Sorbitan Palmitate were negative for ocular irritation in the rabbit. Concentrations of 30% to 100% Sorbitan Laurate were nonirritating to the eyes of rabbits in Draize ocular irritation tests (Elder 1985).

#### *Comedogenicity*

When applied once daily, five times weekly to the external ear of the New Zealand white rabbit for 3 weeks, a 1% aqueous solution of PEG-20 Sorbitan Laurate had no comedogenic activity (Morris and Kwan 1983).

A product containing 5% Sorbitan Isostearate was tested to determine its comedogenicity potential in 20 human subjects. Reactions that scored a value of one or greater, and were

statistically different from the negative control, were considered positive for comedogenicity. Data from the global assessment of the test and the control values were compared statistically to determine biological significance ( $p \leq 0.05$ ). No significant clinical irritation was observed during the study period. Reactions ranging from +0.5 to +1.0 were observed occasionally in 9 of the 20 subjects. Comparison of the test sites and untreated control sites through statistical analysis for the formation of microcomedones yielded a  $p$  value of greater than 0.05. It was concluded that this product did not produce lesions of comedogenicity (CTFA 1998d).

### Inhalation Toxicity

Martinez and Brown (1991) evaluated the pulmonary toxicity in male Sprague-Dawley rats of 7% PEG-20 Sorbitan Oleate in comparison with the toxicities of a herbicide and 7% polyoxyethyleneamine (POEA). The rats were anesthetized prior to administration of 0.1, 0.2, and 0.4 ml doses of the test compounds directly into the trachea. The negative control was saline. The five rats per group were observed for 1 hour for signs of respiratory distress or early death. After 24 hours, the rats were killed for necropsy. The lungs were removed immediately after death, dissected free from other structures, blotted, and evaluated. Lung weight measurements and a subjective scaling system were used to evaluate the severity of damage. Each lung was given a value of 0 to 5, with 5 being hemorrhages involving the whole lung.

In this study, treatment with 0.1 to 0.2 ml of PEG-20 Sorbitan Oleate did not result in deaths. Of the rats of the high dose group, 30% died. The negative control, saline, killed 20% of rats given the 0.2 ml dose, but none of the rats given the high or low doses. PEG-20 Sorbitan Oleate had no effect on lung weight at 0.1 and 0.2 ml, but the weight was increased from 1.4 to 1.8 g at the high dose. It did not increase lung damage at 0.1 to 0.2 ml, but increased the score to 1.3 ("little obvious dysfunction") at the high dose. The negative control scores were 0.25, 0.1, and 0.4 for 0.1 to 0.4 ml, respectively. It was concluded that PEG-20 Sorbitan Oleate had few significant pulmonary effects except at the highest dose.

The plasticizer di(2-ethylhexyl)phthalate (DEHP; 125–300 mg/kg) caused acute lung injury in adult male rats after it was injected intravenously with 13.3% PEG-20 Sorbitan Oleate and 0.9% saline (Schulz 1974). Labored respiration and cyanosis occurred within minutes of the injection of the high dose, and 80% of the rats died within 3 hours of treatment, but none thereafter. The lungs were grossly enlarged and darkened. After injection of 200 mg/kg DEHP, the wet lung to body weight ratio increased compared to that of controls, but the wet lung to dry lung weight ratio did not differ, suggesting the presence of excess proteinaceous material in the lung. Rats given 125 mg/kg had significant polymorphonuclear leukocytic infiltration of the interalveolar septa. The vehicle itself had no effect on lung weight when compared with non-injected controls. Similarly solubilized preparations of corn oil or di(2-ethylhexyl)sebacate, and solutions consisting of up to 500 mg/kg DEHP in bovine serum albumin or gum arabic did not produce acute lung effects. The in-

vestigator concluded that a specific interaction between DEHP and PEG-20 Sorbitan Oleate had occurred to produce the observed lesions.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### Polysorbates

PEG-20 Sorbitan Laurate was used in teratologic studies as a solubilizer for poorly soluble substances such as thalidomide and its metabolites. In these studies using Swiss mice, the PEG-20 Sorbitan Laurate vehicle had no effect on developing embryos when given doses of 10 ml/kg in saline (Meise, Ockenfeis, and Köhler 1973; Köhler and Koch 1974; Fickentscher et al. 1977). During studies using CBA and C57BL mice, more than 50% of the mice died after being given 2.5 ml/kg IP doses of the PEG-20 Sorbitan Laurate vehicle in saline (Kocher-Becker and Kocher 1981). Scott, Fradkin, and Wilson (1977) reported similar results in IP studies using thalidomide in PEG-20 Sorbitan Laurate. When 40 ml/kg of the vehicle alone was administered, five of five nonpregnant Harlan (ICR-derived) mice died. A test volume of 20 ml/kg killed three of five nonpregnant mice and five of five nonpregnant Royalhart (Wistar-derived) rats. No deaths occurred after administration of 10 ml/kg, but hepatic swelling was observed at necropsy. In mice, 200 mg/kg of the vehicle produced 10.1% dead or resorbed embryos, and 0.8% malformations in survivors out of 139 total implants. Without treatment, 2.5% of the 120 total implants was dead or resorbed and 0.9% of the survivors was malformed. No difference in the mean fetal weight was observed. Pregnant rats given 400 mg/kg of the vehicle had 160 total implants; 7.5% was dead or resorbed, and 0.7% of the survivors was malformed. Untreated rats had 666 total implants, of which 5% was dead or resorbed, and 1% of the survivors was malformed. No difference in fetal weight was observed (Scott, Fradkin, and Wilson 1977).

Six pregnant NMRI mice and 11 pregnant Swiss mice received a single IP injection of PEG-20 Sorbitan Laurate on the ninth day of gestation (Kocher-Becker and Kocher 1981). The PEG-20 Sorbitan Laurate doses were 1.0, 1.7, 2.5, 3.3, and 5.0 ml/kg. The doses were diluted to 10 ml/kg or 20 ml/kg (high-dose only) with physiological saline. For the NMRI mice, five of six dams died after receiving 5.0 ml/kg PEG-20 Sorbitan Laurate. Three of 12 NMRI dams died, and a further 3 NMRI mice aborted after receiving 3.3 ml/kg PEG-20 Sorbitan Laurate. In Swiss mice, 9 of 11 dams died after receiving the high dose. All pregnancies were terminated on day 16 or 18 of gestation. The fetuses were inspected for gross malformations and stained for examination of the skeleton (Table 7). All doses of PEG-20 Sorbitan Laurate caused malformations in offspring of both treated strains. In Swiss mice, the number of malformed fetuses was dose-dependent, but the number of litters with malformed fetuses was not. The same applied to the results using NMRI mice, but only when the 2.5 ml/kg dose was compared with the lower two doses. The percentage of malformed fetuses was greater in the Swiss strain than in the NMRI strain. Malformations observed included wedge-shaped and incomplete vertebrae,

TABLE 7

Reproductive and developmental toxicity of PEG-20 Sorbitan Laurate (Kocher-Becker and Kocher 1981)

	Mouse strain							
	NMRI dose (ml/kg)					Swiss dose (ml/kg)		
	1.0	1.7	2.5	3.3	Control	1.0	2.5	Control
Maternal/litter information								
Dams	7	10	35	12	25	15	21	8
Maternal deaths	0	0	2	3	0	1	4	0
Litters aborted	0	0	2	3	0	0	1	0
Litters with at least one malformed fetus	2	2	17	2	2*	7	9	0
Implantations	83	118	361	79	278	192	237	141
Individual fetus information								
Resorbed	6	11	71	23	18	23	55	8
Percent resorbed	7.2	9.3	19.7	29.1	6.5	12.0	23.2	5.7
Dead	0	0	3	0	1	0	6	3
Living	77	107	287	56	259	169	176	130
Malformed (living)	2	4	41	6	2*	20	57	0
Percent malformed (living)	2.6	3.7	14.3	10.7	0.8	11.8	32.4	0
Types of malformations								
Limbs alone or combined with others	0	0	6	0	0	3	4	0
Vertebrae and ribs without limb involvement	1	4	33	6	0	17	53	0
Others	1	0	2	0	2*	0	0	0

\*Atypical: exencephaly

fused vertebral arches in the thoracic and lumbar region, and/or rib fusions of varying severity in length of fusion and numbers of ribs involved. Limb malformations, including phocomelia, occurred sporadically at all doses, and were mainly of the thalidomide type.

Wickramaratne (1987) evaluated the teratogenicity of PEG-20 Sorbitan Laurate and other chemicals in Alpk:AP (Wistar-derived) rats using the Chernoff-Kavlock assay. Dams (15 per group) were treated with 10 ml/kg/day PEG-20 Sorbitan Laurate at varying concentrations on gestational days (GD) 7 to 17. Maternal body weights were determined on GD 1, 7 to 17, and 22. Offspring were weighted on days 1 and 5 postpartum and the numbers of live and dead pups were counted. No specific examination for malformations was performed. The control was physiological saline. PEG-20 Sorbitan Laurate reduced offspring litter size, survival, and weight gain when the dams were given the chemical intraperitoneally, but the parameters did not differ significantly from controls when the Polysorbate was administered orally, dermally, or subcutaneously (Table 8).

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate caused serious developmental effects in sea urchin embryos when administered at concentrations as low as 0.004% in artificial sea water. At concentrations >0.1%, the Polysorbates blocked cell cleavage and lysed two-cell stage embryos. The investigators noted, however, that the sea urchin embryo model was more sensitive than those using mammalian cells in culture (Bresch and Ockenfels 1977).

The NTP (1992a, 1992c) studied time-mated Sprague-Dawley female rats (8–10-week-old) given 5 ml/kg/day of aqueous PEG-20 Sorbitan Laurate by gavage on GD 6 to 15. The treatment doses were 500 (25 rats) and 5000 mg/kg/day (24 rats). The body weights were recorded at GD 0, 3, 6 to 15, 18, and 20. Feed and water consumption were monitored throughout the study. The dams were killed on GD 20. Pregnancy status was determined by uterine examination. The body, liver, right kidney, heart, and uterus of each rat were weighed and fixed for microscopic examination. Live fetuses were dissected from the uterus, weighed, and examined for external abnormalities and visceral malformations.

All treated female rats survived to scheduled necropsy, and 22 to 24 pregnancies per group were confirmed. Treated dams had transient weight loss (>5 g/dam) between GD 6 and 7; 3/24 dams of the low-dosing group and 7/22 of the high-dosing group experienced weight loss, compared to 1 of the 22 untreated control rats. By GD 20, alopecia (regional hair loss) occurred in 5/24 and 5/22 rats of the low- and high-dosing groups, respectively. No untreated rats had hair loss. No other signs of toxicity were observed, and average maternal body weights did not differ between groups. No treatment-related change in maternal weight gain occurred during gestation. Maternal weight gain during treatment was decreased by 14% at 5000 mg/kg/day relative to that of the vehicle control, but no effect was observed at 500 mg/kg/day. Maternal organ weights and feed intake did not differ between groups. Maternal water intake was increased by 14%

**TABLE 8**  
Reproductive toxicity of PEG-20 Sorbitan Laurate using rats (Wickramaratne 1987)

Concentration, dose, route	Group mean weight change (GD 1-22)	Group mean weight change (GD 7-16)	No. pregnant rats	No. viable litters		Mean litter size (live + dead)
				Day 1	Day 5	
10%, 10 ml/kg, oral	121.3 ± 15.3	105.0 ± 12.6	15	15	14	12.1 ± 2.51
Oral control <sup>a</sup>	115.6 ± 15.5	95.1 ± 29.0	15	15	15	10.7 ± 3.94
10%, 10 ml/kg, IP	68.5 ± 24.5	41.0 ± 29.0	8	6	4	6.2 ± 3.76
IP control	111.9 ± 15.6	85.9 ± 16.6	12	11	11	7.6 ± 3.57
100%, 2 ml/kg, dermal <sup>b</sup>	89.6 ± 14.3	61.9 ± 11.3	15	15	15	11.0 ± 3.49
Dermal control	96.1 ± 11.3	67.1 ± 11.4	15	15	15	13.2 ± 2.24
50%, subcutaneous	95.6 ± 14.7	76.7 ± 9.7	15	15	15	10.6 ± 3.02
100%, subcutaneous	108.7 ± 13.2	69.6 ± 8.2	14	14	13	12.2 ± 2.64
Subcutaneous control	115.9 ± 21.0	77.2 ± 15.1	15	15	15	10.8 ± 2.78

<sup>a</sup>Control and vehicle was physiological saline.

<sup>b</sup>6 h occlusive dressing on GD 7-17.

GD = gestational day.

from GD 6 to 15 at 5000 mg/kg/day compared to the control. The maternal low effect adverse effect level was 5000 mg/kg/day (based on the decrease in weight gain), and the maternal no-observed-adverse-effect level (NOAEL) was 500 mg/kg/day.

No differences between groups occurred in the number of corpora lutea per dam, the number of implantation sites per dam, or the percent preimplantation loss per litter. The incidence of resorptions per litter was significantly lower ( $p < 0.05$ ) in the 5000 mg/kg/day dosing group compared to the both the vehicle control group and historical controls. Resorption incidences were 4.1% (control), 4.2% (500 mg/kg/day), and 0.9% (5000 mg/kg/day), respectively. No significant effects were observed for the percent litters with at least one resorption. No late fetal deaths occurred, and no litters with 100% prenatal mortality were observed. PEG-20 Sorbitan Laurate exposure had no effect on live litter size, percent males per litter, fetal body weight, percent adversely affected fetuses per litter, or percent litters with one or more resorbed or adversely affected implants. The percent fetuses per litter with external or visceral malformations and the overall incidence of malformations did not differ among the groups. Only two fetuses (of 308) of the 5000 mg/kg/day-treated dams had skeletal malformations; these malformations (bipartite vertebra in the thoracic region) were, however, common in Sprague-Dawley rats. PEG-20 Sorbitan Laurate had no adverse effects on the growth, viability, or morphological developments of fetuses of treated dams. The developmental NOAEL was >5000 mg/kg/day (NTP 1992a; 1992c).

PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Laurate were fed to C57BL/6 mice at a concentration of 10% during a three-generation study. The first generation consisted of seven breeding groups: a control group, groups in which both sexes were fed a Polysorbate diet, and groups in which one sex was fed a Polysorbate diet and the other was fed a control diet. Only groups in which both sexes were fed the same diet were continued for the

second and third generations. Four litters of mice were delivered from "brother-sister" matings by the first and second generation mice and three litters by the third generation. Offspring of dams that were fed one of the Polysorbate diets had significantly decreased weaning weights when compared to offspring of dams fed the control diet. The decrease was due to a poorer weight gain in the experimental offspring during the third week of life. Mice fed PEG-20 Sorbitan Stearate had significantly smaller litters than mice fed the control diet, and dams fed PEG-20 Sorbitan Stearate delivered significantly more offspring dead at birth than mice fed the control PEG-20 Sorbitan Laurate diets. Females fed the experimental diets produced their largest litter at a later age than females fed the control diet. Feeding of the Polysorbates did not have any significant effect upon the weight of adult mice, the weight of gonads of adult mice, or the rate of sperm movement. Other details were unavailable (Paschall 1964).

Negative results were reported when the teratogenicity of PEG-20 Sorbitan Oleate was evaluated using the whole-embryo culture. Schmid, Trippmacher, and Bianchi (1998) cultured Han Wistar rat embryos in 5 ml of homologous, undiluted rat serum and incubated them for 48 hours. The test chemicals were added to the medium after dilution with distilled water or suspension in 2% gelatin. The system also contained S9 mix for the entire culture period. When heart beat and flexion were present, and at least 50% of the embryos had established functional yolk sac-blood circulation, the embryos were evaluated for signs of teratogenicity. Embryos classified as abnormal had deviations from the controls and had crown-rump lengths that were at least 80% of the mean control value.

In a study using neonatal female rats (Gadjová, Jakuborshy, and Váľky 1993), PEG-20 Sorbitan Oleate accelerated maturation, prolonged the estrous cycle, and induced persistent vaginal estrous when injected IP on days 4 to 7 after birth. Six rats were

injected with 1% PEG-20 Sorbitan Oleate, five were injected with 5%, and six were injected with 10%; the injection volume was 0.1 ml/rat. The untreated control group included 8 rats, the negative-control group (aqua pro injection) included 10 rats, and the positive-control group (diethylstilboestrolum in oil *helianthus*) contained 5 rats. The estrous cycle was evaluated at weeks 10, 14, and 18, and vaginal smears were obtained daily for 14 days. The rats were killed at 5 months of age, and the uterus, ovaries, adrenal glands, and pituitary gland were removed, weighed, and examined. PEG-20 Sorbitan Oleate did not affect growth or viability of the treated rats. A significant decrease in body weight was observed in rats of the 1% dose group as compared to the control group. All rats given the Polysorbate had significantly advanced vaginal opening in comparison with controls. The average length of the estrous cycle was 9.3 to 14 days in rats of the treatment groups. For rats of the untreated and positive control groups, the lengths were 4.3 and 9.4 days, respectively. The ovaries of high-dose rats had multiple cavities (3 mm diameter) in three rats; two rats of the positive-control group had similar lesions. An enlarged uterus was observed in two rats of the low-dose group. The relative weight of the adrenal glands was increased in all treatment groups compared to the untreated control group, but the increase was significant only in the low-dose group ( $p \leq 0.05$ ). The relative weight of the ovaries was decreased significantly in all PEG-20 Sorbitan Oleate-treated groups when compared to the untreated control. The uterine weight was decreased significantly in rats treated with 5% PEG-20 Sorbitan Oleate and in rats of the positive-control group. Microscopic findings in rats of the treatment groups were similar to those of the positive-control group. In the uterus, squamous cell metaplasia of the epithelium and other cytological changes that were indicative of chronic estrogenic stimulation were observed. The ovaries were without corpora lutea, and had degenerative follicles.

PEG-20 Sorbitan Oleate (2500 mg/kg/day) did not cause developmental toxicity in the CD-1 mice during an in vivo teratology screening study in which pregnant mice were treated by gavage on GD 8 to 12 (Kavlock, Short, and Chernoff 1987).

The teratogenicity of PEG-20 Sorbitan Stearate was evaluated by Ema et al. (1988) using pregnant Wistar rats. The rats were fed 0.1%, 1.0%, or 10% of the Polysorbate from GD 7 to 14. Rats of the low dose group consumed 99 mg/kg/day, rats of the medium dose group consumed 960 mg/kg/day, and rats of the high dose group consumed 7693 mg/kg/day. Under the conditions of this study, PEG-20 Sorbitan Stearate had no harmful effects on the prenatal development of the rat. Changes were not observed in the number, sex ratio, or body weight of live fetuses, and external, visceral, and skeletal malformations of the offspring were not detected.

Hardin et al. (1987) fed pregnant CD-1 mice 5200 mg/kg/day PEG-20 Sorbitan Stearate on GD 6 to 13. None of the 50 treated dams died as a result of treatment with the control diet (corn oil) or the Polysorbate. Values for the control group are stated in parentheses. The maternal weight gain was  $5.0 \pm 3.4$  g (4.6

$\pm 3.4$  g) and the number of viable litters was 34 of 34 (34 of 37). The number of liveborn pups per litter was  $10.5 \pm 2.7$  ( $9.1 \pm 3.9$ ), the percentage survival was  $98.7 \pm 4.3$  ( $98.4 \pm 16.0$ ), and the birth weight per pup was  $1.5 \pm 0.1$  g ( $1.5 \pm 0.3$  g). The only significant finding ( $p < 0.05$ ) was reduced birth weight or weight gain; the weight gain per pup was  $0.3 \pm 0.2$  g ( $0.5 \pm 0.2$ ).

Brubaker, Tayler, and Bull (1982) evaluated the behavioral effects of PEG-20 Sorbitan Oleate in dams milk after dosing female Sprague-Dawley rats with 1.25 ml/l PEG-20 Sorbitan Oleate in drinking water. The 65-day-old rats were fed ad libitum the basal feed for 5 days. The rats were given PEG-20 Sorbitan Oleate for 14 days prior to mating. At parturition, each litter was culled to eight male pups. From 10 days after birth until weaning, each dam and her litter were placed in a home cage apparatus designed to measure the activity of the pups. Mean daily activity was measured by photocell counts. PEG-20 Sorbitan Oleate appeared to enhance locomotor activity and exploratory behavior at ages 16, 17, 18, and 20 days. Mean daily and hourly activities were significantly greater in pups exposed to PEG-20 Sorbitan Oleate than pups of the control group, but only at nighttime.

PEG-20 Sorbitan Oleate decreased the size of litters when fed to rats at doses of approximately 0.8 to 3.0 g/kg (Elder 1984).

#### *Ethylene Glycol And Its Ethers*

It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers (e.g., methoxyethanol, also known as ethylene glycol monomethyl ether) are reproductive and developmental toxins. The CIR Expert Panel undertook a separate, limited scope review of these compounds in order to assess the possibility that PEG-derived cosmetic ingredients could present similar concerns (CIR 1996). In summary, this report concluded that the ethylene glycol monoalkyl ethers are not themselves toxic, but rather that one or more alcohol or aldehyde dehydrogenase metabolites are toxic. From the available data, the report also concluded that the toxicity of the monoalkyl ethers is inversely proportional to the length of the alkyl chain (methyl is more toxic than ethyl than propyl than butyl, etc.).

Given the methods of manufacture of the PEGs Sorbitan Fatty Acid Ester, that there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity, and that the esters are chemically different from the alkyl ethers, the Panel concluded no reproductive or developmental hazard is posed by these compounds.

#### *Polyethylene Glycol*

No adverse reproductive effects occurred during subchronic (90 days) and chronic (2 years) oral toxicity studies of PEG-6-32 and PEG-75. In the subchronic study, PEG-75 was tested at a dose of 0.23 g/kg/day. In the chronic study, PEG-75 was tested at doses up to 0.062 g/kg/day and, PEG-6-32, at doses up to 1.69 g/kg/day (Andersen 1993).

### Sorbitol

MacKensie et al. (1986) performed a multigeneration feeding study to determine the reproductive and developmental effects of Sorbitol. Twelve male and 24 female Charles River CD (SD) BR rats per group were fed a diet containing 2.5%, 5.0%, or 10% Sorbitol (replacing the sucrose content of the basal feed) during a 96-week multigeneration study. The two high concentrations were "built up in 2.5% steps at weekly intervals." The  $F_0$  rats were mated to produce the  $F_{1a}$  and  $F_{1b}$  litters. The  $F_{1b}$  rats were treated and mated to produce the  $F_{2a}$  and  $F_{2b}$  litters. The  $F_{2b}$  rats were treated and mated to produce the  $F_{3a}$  litters. Twelve rats/sex/group were fed the test diets for 4 weeks, then were killed. Gross examinations were performed on all mated animals and two rats/sex of the  $F_{1a}$  and  $F_{2a}$ . Gross and microscopic examinations and biochemical analyses were performed on the  $F_{3a}$  rats. In this study, the feeding of up to 10% Sorbitol to rats had no significant adverse clinical, behavioral, or reproductive effects, and no significant gross or microscopic changes were observed.

The safety of hydrogenated starch hydrolysates (HSH), which are mixtures of polyhydric alcohols such as ~7.0% Sorbitol, was investigated using a 2 year ingestion study (50 Sprague-Dawley rats/sex/group), a multigeneration reproduction study (20 rats/sex/group), and a teratology study (30 dams/group). At a concentration of 18% in drinking water (3000–7000 mg/kg/day), HSH did not produce reproductive or developmental effects (Modderman 1993).

## GENOTOXICITY

### PEG-40 Sorbitan Peroleate

PEG-40 Sorbitan Peroleate was nonmutagenic in the Ames test; details of this study were not available (CTFA 1998b).

### Polysorbates

PEG-20 Sorbitan Laurate was nonmutagenic in the L5178Y TK<sup>+</sup>/− mouse lymphoma assay with and without S9 metabolic activation (Coppinger, Brennan, and Thompson 1981).

PEG-20 Sorbitan Stearate gave positive results in the Rec-assay using *Bacillus subtilis*, but gave negative results in the Ames Test (Kada, Hirano, and Shirasu 1980).

Odashima (1976) reported results of genotoxicity assays of a number of chemicals, including PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate. The investigators performed the newborn test, transplacental carcinogenesis assay, chromosomal aberration assays, and mutagenicity assays in microbial systems, mammalian cells, and insects. For the chromosomal aberration studies, Chinese hamster cell line KC-1 and mouse bone marrow cells were used. During the microbial mutagenicity assays, *Salmonella* strains TA1535, TA1536, TA1537, TA1538, WP2, TA100, and TA98 were used for evaluating mutagenic activity, whereas strains H-17, M-45, W3110, and TA1978 were used for repair testing. In the other assays, mutagenicity was determined using XP cells transformed by SV40 and the silkworm oocyte

system. In the transplacental study, experimental animals (number and species not available) were treated three times on GD 15, 17, and 19 or on GD 14, 16, or 18. The dose administered was approximately the maximum dose that did not cause abortion and early death of the sucklings. The observation period for tumor development was limited to 1 year after birth. For the newborn study, the neonates were given subcutaneous injections on days 1, 8, 15, and 22 after birth. The dose administered was the approximate maximum dose that did not cause early death of greater than 20% of the animals, and the observation period was limited to 1 year after birth. PEG-20 Sorbitan Stearate produced false-negatives in the chromosomal aberration, microbial mutagenicity, and mammalian or insect mutagenicity assays, and produced positive parallelism in the transplacental and newborn tests. PEG-20 Sorbitan Stearate was classified as a carcinogen. PEG-20 Sorbitan Oleate produced false-positive results in the transplacental and newborn assays, and positive parallelism was reported in tests for mammalian, insect, or microbial mutagenicity and chromosomal aberrations; therefore, PEG-20 Sorbitan Oleate was classified as a noncarcinogen. The determination of "false-negative" was based on previous studies using rodents, for which the data are unavailable. The investigators concluded in some cases that the procedures used were not practical for screening suspected carcinogens, as frequencies up to 53% and 56% were reported for false-positives and false-negatives, respectively.

In a later study, however, PEG-20 Sorbitan Stearate was considered noncarcinogenic (Kawachi et al. 1980a). Without metabolic activation, it produced negative results in the silk-worm chromosomal aberration test, hamster sister chromatid exchange assay, and mutagenicity assays using *S. typhimurium* TA100 and TA98. It was positive in the rec assay. Results were not provided for studies performed in the presence of S9. Inoue, Sunakawa, and Takayama (1980) reported that PEG-20 Sorbitan Stearate was not mutagenic in *S. typhimurium* strains TA100 and TA98; the Polysorbate also did not induce in vitro transformation of hamster embryo cells.

PEG-20 Sorbitan Palmitate did not cause chromosome damage in Chinese Hamster ovary cells, but the Polysorbate was cytotoxic and caused marked reductions in cell number compared with detergent-free controls (Flower, Phillips, and Andersen 1988).

PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate (in physiological saline) were evaluated for genotoxic potential using the Chinese hamster cell chromosomal aberration assay (Ishidate and Odashima 1977). The maximum doses tested were 0.2 and 0.1 mg/ml, respectively. Of the treated cells with each Polysorbate, 1% had chromosomal aberrations (chromatid gaps). The chemicals were classified as nonmutagenic and noncarcinogenic, with the exception of PEG-20 Sorbitan Stearate, which produced significant genotoxic effects in metabolic activation systems.

PEG-20 Sorbitan Oleate caused no evidence of mutagenicity when tested using *S. typhimurium* strains TA98, TA100,



TA1535, and TA1537, with and without metabolic activation (NTP, 1992b). It was not genotoxic in microbial systems or in mammalian systems (Kawachi et al. 1980a; 1980b); the systems used were *S. typhimurium* TA100 and TA98 (mutation), *B. subtilis* (rec assay), hamster lung fibroblasts and human embryo fibroblasts (chromosomal aberrations, sister chromatid exchanges), rat bone marrow (in vivo chromosome aberrations), and the silk worm (mutation). PEG-20 Sorbitan Oleate reduced the frequency of acridine orange-induced gene conversions in *Saccharomyces cerevisiae* D7, but did not affect the frequency of UV-induced recombinations (Arni 1985). In one study (Scott and Alderson 1973), *Aspergillus conida* grown in PEG-20 Sorbitan Oleate suspension was less susceptible (by approximately a factor of two) to lethal and mutagenic damage caused by ionizing radiation.

PEG-20 Sorbitan Oleate was nonmutagenic in the micronucleus and Ames tests (Elder 1984).

In a study examining the role of inhibition of DNA repair as a mechanism in cocarcinogenesis, Sorbitan Oleate, at a concentration of 0.01%, was found to inhibit the repair of UV-irradiated DNA extracted from normal human lymphocytes (Gaudin et al. 1971).

#### *Sorbitan Esters*

Sorbitan Stearate was not mutagenic in bacteria with or without metabolic activation, and did not transform primary Syrian golden hamster embryo cells in vitro. Sorbitan Oleate at a concentration of 0.01% inhibited in vitro DNA repair (Elder 1985).

#### *Sorbitol*

After being fed to adult *Drosophila*, Sorbitol was negative for whole chromosome loss and did not cause clastogenic effects or nondisjunction. In these studies, Sorbitol did not appear to cause sex-linked recessive lethals; however, it could not be classified as either positive or negative for mutagenic activity due to an inadequate sample size (Abbott and Bowman 1976).

Chinese hamster ovary cells in medium made hyperosmotic with Sorbitol had significant increases in the incidence of chromosomal aberrations. The test concentrations were 300 to 450 mM. The cells were harvested for aberration analysis 24 to 26 hours after the beginning of the 4-hour treatment period. Cells treated with 300 to 350 mM Sorbitan had 100% survival, and cells treated with 400 and 450 mM had 40% and 15% survival, respectively. Survival was measured after 6 days of colony formation, as a percentage of the untreated control value. The numbers of aberrations per 100 cells were 2 (control), 26 (300 mM; 1 cells was excluded), 11 (350 mM), 29 (400 mM), and 27 (450 mM; only 30 scoreable cells). The incidences of cells with aberrations were 2% (control), 8% (300 mM), 7% (350 mM), and 17% (400 and 450 mM). The investigators concluded that the increase in aberrations represented an indirect effect on the cells (Galloway et al. 1987).

An unspecified Sorbitan Fatty Acid Ester (maximum dose = 5.0 mg/plate, in DMSO) was tested for mutagenicity in the Ames

test using *S. typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537. In the chromosomal aberration test using Chinese hamster fibroblasts, a maximum dose of 0.3 mg/ml of the test compound (in DMSO) resulted in 5.0% polyploid cells and 8.0% structural aberrations 48 h after treatment. The results were considered equivocal, and polyploidization effects were observed (Ishidate et al. 1984).

The addition of sugars such as Sorbitol reduced the mutagenicity of smoke condensates of high- and low-tar cigarettes, as tested using *S. typhimurium* strains TA98 and TA100, with metabolic activation. Cigarettes treated with Sorbitol yielded more tar than untreated cigarettes. When 0.51 g Sorbitol was added to each high-tar cigarette, the percent mutagenicity per mg smoke condensate was 66% (TA100) and 37% (TA98), relative to cigarettes without added sugars. The percent mutagenicity per cigarette was 77% (TA100) and 46% (TA98). When 0.70 g Sorbitol was added to low-tar cigarettes, the percentages were 65% (TA100) and 23% (TA98) per mg smoke condensate and 184% (TA100) and 66% (TA98) per cigarette. The addition of sugars without metabolic activation had no effect on mutagenicity of the cigarette smoke condensates (Sato et al. 1979).

#### *Polyethylene Glycol*

PEG-8 was negative in the Chinese hamster ovary cell mutation test and the sister chromatid exchange test; the maximum test concentration in both studies was 1%. In the unscheduled DNA synthesis assay, a statistically significant increase in radioactive thymidine incorporation into rat hepatocyte nuclei was noted only at the highest concentration tested (0.1% PEG-8). PEG-150 was not mutagenic in the mouse lymphoma forward mutation assay when tested at concentrations up to 150 g/l (Andersen 1993).

## CARCINOGENICITY

#### *Polysorbates*

PEG-20 Sorbitan Oleate was evaluated for carcinogenicity in a 2-year (103-week) feed study (NTP 1992b) using F344/N rats and B6C3F<sub>1</sub> mice. Sixty animals per sex per group were given 25,000 or 50,000 ppm of PEG-20 Sorbitan Oleate in feed daily. Feed and water were available ad libitum. Clinical observations were made twice daily, and findings were recorded weekly for 13 weeks, then monthly or as necessary afterwards. After the first 15 weeks of treatment, an interim evaluation was performed in which 7 to 10 mice and rats given 0 or 50,000 ppm underwent a complete histopathological examination. At this time, no changes in relative or absolute organ weights were observed in any group as compared to controls. Female mice fed 50,000 ppm PEG-20 Sorbitan Oleate had an increased incidence of hyperplasia and inflammation of the nonglandular stomach. Neoplasms observed during this examination were not considered related to the administration of the test compound. At the end of the study, the final mean body weight of female mice fed 50,000 ppm was decreased by 11%, as compared to controls. Male rats fed the high dose had decreased survival (0 ppm,

29/50; 25,000 ppm, 18/50; 50,000 ppm, 18/50) due to neoplasms commonly observed in aging F344/N rats, including mononuclear cell leukemia, pituitary gland adenoma, preputial gland carcinoma, mammary gland fibroadenoma, Zymbal's gland carcinoma, and mesothelioma. Survival in the other groups did not differ from controls (female rats: 23/50, 25/50, 25/50; male mice: 33/49, 34/50, 32/50; female mice: 30/50, 28/50, 26/50, respectively, for 0–50,000 ppm dose groups). No clinical findings were noted. In male rats, the incidences of benign and malignant pheochromocytoma were 21/50, 19/50, and 29/50; the incidence was significantly increased in the high-dose group, compared to the control group. For benign neoplasms, the incidences were 21/50, 16/50, and 28/50. For malignant neoplasms, the incidences were 1/50, 4/50, and 1/50. Male rats of the low-dose group had a decreased incidence of hyperplasia of the adrenal medulla, whereas the incidence in males of the high-dose group was increased compared to the control group. The incidences of hyperplasia of the adrenal medulla for male rats were 11/50, 22/50, and 12/50. For mice treated with PEG-20 Sorbitan Oleate, no increase in the incidence of neoplasms were observed. Investigators, however, observed increased incidences of squamous hyperplasia and inflammation of the nonglandular stomach in mice of the high-dose group. Female mice of the high-dose group also had an increased incidence of ulcers of the nonglandular stomach.

The investigators concluded that, based upon the increased incidence of pheochromocytomas of the adrenal medulla, "equivocal evidence of carcinogenicity" existed for the male F344/N rat (equivocal evidence = marginal increase of neoplasms that may be chemical related). No evidence of carcinogenic activity was observed in female rats or mice of either sex fed up to 50,000 ppm PEG-20 Sorbitan Oleate. The test compound was associated with inflammation and squamous hyperplasia of the nonglandular stomach in male and female mice, and with ulcers of the nonglandular stomach in female mice (NTP 1992b).

Epstein et al. (1970) treated infant Swiss albino mice with 0.11 to 110 mg PEG-20 Sorbitan Stearate in 0.9% saline at test volumes of 0.1 to 0.2 ml on days 1, 7, 14, and 21. The mice were injected subcutaneously (SC) in the nape of the neck, and were examined for signs of carcinogenicity daily during the first month, then monthly thereafter for the duration of the 49- to 53-week study. Mortality at weaning was 100% for the two highest doses, but only 2% in the low-dose group, compared to 14% to 19% in the vehicle and uninjected control groups. The only significant findings upon necropsy were solitary adenomas in 3 of 16 males and a lymphoma in 1 male (total dose = 6.6 mg PEG-20 Sorbitan Stearate) that survived to 49 weeks.

Shirai et al. (1982) investigated the effects of sodium chloride, PEG-20 Sorbitan Stearate, and N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) on gastric carcinogenesis in the male Wistar rat. The Polysorbate did not increase the incidence of tumors of the nonglandular stomach and glandular stomach when given subsequent to treatment with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), which was also the positive control chemical.

A concentration of 0.002% PEG-20 Sorbitan Stearate or PEG-20 Sorbitan Oleate inhibited metabolic cooperation (cell-cell communication) in Chinese hamster V79 cells in vitro (Trosko et al. 1981; 1982). This inhibition can result in tumor promotion via the deregulation of various complex cellular functions, such as proliferation, differentiation, immune response, and gene modulation. The tumor promoters appeared to inhibit contact inhibition or metabolic cooperation between cells, alter growth factor receptor sites, affect the immune system, and/or block chalones. Of the two Polysorbates, PEG-20 Sorbitan Stearate had an in vivo promoter response (Trosko et al. 1981).

PEG-20 Sorbitan Stearate at a dose of 6.6 mg did not promote the formation of tumors in neonatal ICR mice (Fujii 1991). The Polysorbate was injected into the necks of 49 neonatal mice within 24 hours of birth, and the mice were observed for 1 year. Four males (incidence = 16%) and four females (17%) had tumors: the males had lung tumors, two females (9%) had lung tumors, one female (4%) had lymphoma or leukemia, and one female (4%) had thyroid adenoma.

PEG-20 Sorbitan Stearate induced the cytoplasmic accumulation of transcripts of the proliferin gene family in mouse fibroblast C3H/10T1/2 cells (Parfett 1992). Proliferin protein is an antagonistic regulator of muscle-specific transcription, and can promote morphological transformation. The accumulation of proliferin transcripts occurred at or near the effective concentration for promotion of transformation.

The Polysorbates are not considered oral or dermal carcinogens, and were weak tumor promoters (cited in detail in Elder 1984). Skin tumors were produced by topical application of these compounds, but the observed tumors were mostly benign dermal tumors with a tendency toward regression. Various results were reported during studies in which the Polysorbates were injected subcutaneously. Investigators concluded that local sarcomas in the rat produced by long-continued repeated injections into the same subcutaneous site was not a valid index of chemical carcinogenicity. In a 21-week study, mixed cultures of epidermal and dermal cells from term fetuses of Balb/c mice were exposed to medium containing PEG-20 Sorbitan Oleate as a control. The Polysorbate did not produce the degree of in vivo malignancy or the same types of changes in morphology and cell differentiation as the test compound, 50 µg/ml of 7, 12-dimethylbenz[a]anthracene (DMBA) (Elder 1984).

#### *Sorbitan Esters*

Mice fed low concentrations of Sorbitan Stearate for 80 weeks had no difference in tumor type and incidence of tumors as compared to control animals. No carcinogenic effects were observed after undiluted Sorbitan Laurate was applied twice weekly to the clipped skin of the interscapular region of male Swiss mice for 73 weeks. Sorbitan Laurate was, however, a tumor promoter following induction with the tumor initiator DMBA. In a 75-week study, 5 of 50 mice developed a total of eight tumors (including one carcinoma), two of which regressed. Of the 100 control

mice, 1 had five tumors. In a 52-week study, daily application of 80 mg of Sorbitan Laurate (150  $\mu$ g DMBA) resulted in 10 tumors in nine mice (treated once daily) and 33 tumors in 21 animals (twice daily). Undiluted Sorbitan Laurate was also active on mouse skin as a cocarcinogen with 0.003% to 0.3% DMBA. In the same study, Sorbitan Oleate and Sorbitan Trioleate were active as cocarcinogens on mouse skin when applied with 0.003% DMBA (Elder 1985).

Sorbitan Stearate was fed to 48 male and 48 female TO strain mice at dose levels of 0%, 0.5%, 20% or 40% of the diet for 80 weeks. Tumor type and incidence were two of the parameters studied. A majority of the tumors found in this study occurred either with comparable frequency in the test and control groups or more frequently in the control groups (Hendy et al. 1978).

#### *Sorbitol*

At a concentration of 18% in drinking water (3000–7000 mg/kg/day), hydrogenated starch hydrolysates (mixtures of polyhydric alcohols such as ~7.0% Sorbitol) were not carcinogens after 2 years of treatment. This study used 50 Sprague-Dawley rats/sex/group. No significant clinical signs of toxicity were observed (Modderman 1993).

In studies using rats, high dietary concentrations of Sorbitol caused enlargement of the cecum, increased absorption of calcium from the gut, increased urinary excretion of calcium, pelvic and corticomedullary nephrocalcinosis, acute tubular nephropathy, urinary calculus formation, and hyperplasia and neoplasia of the adrenal medulla. The investigator concluded that adrenal neoplasms observed in mice fed 20% Sorbitol were laboratory artifacts, and not indicative of any risk to humans exposed to normal concentrations of Sorbitol in the diet (Roe 1984).

#### *Polyethylene Glycol*

PEG-8 was not carcinogenic when administered orally to mice (30 weeks of dosing), intraperitoneally to rats (6 months of dosing), subcutaneously (20 weeks of dosing for rats; 1 year of dosing for mice), or when injected into the gastric antrum of guinea pigs over a period of 6 months (Andersen 1993).

### **COCARCINOGENICITY**

Sorbitan Laurate was tested for both tumor promoting activity and carcinogenicity in the skin using 50 male Swiss mice. Sorbitan Laurate was applied to a 2  $\times$  2-cm area of the interscapular region kept free of hair by periodic clipping. During the carcinogenicity experiment, Sorbitan Laurate was applied twice weekly to the skin for 73 weeks. All animals were checked twice weekly for skin lesions. No carcinogenic effect was detected, with 1 animal out of 50 developing one papilloma. Control groups of 240 male and female mice from the same colony were kept untreated and observed over their lifespan. One papilloma appeared and regressed in one control female and one skin papilloma and a carcinoma of skin appendages were each found in a control male. Additional control groups of 100 males and 100 females were observed for over 100 weeks and showed no signs of skin tumors. In the test of Sorbitan Laurate as a promoting agent, a

single application of DMBA as a 1% solution in mineral oil was applied 1 week after the single application of the ester (dose not given), and, thereafter the ester was applied twice weekly for 75 weeks. Five of the 50 animals developed eight tumors, one of which regressed. One of the eight tumors was a carcinoma. Two nonconcomitant control groups received the DMBA and no further treatment. One of the 100 control mice developed five tumors (Saffiotti and Shubik 1963).

A study published by Setälä (1956) on the promoting and cocarcinogenic activity of a variety of nonionic-lipophilic-hydrophilic agents that included Sorbitan Laurate, Oleate and Trioleate. An initial single dose of 150  $\mu$ g of DMBA (0.3% in paraffin) was painted on the backs of male mice (50 mice per group). The hair was cut from the treatment site twice weekly. The promoting agents were applied to the test site in doses that ranged between 51 to 87 mg once or twice daily, 6 days per week for 52 weeks. Animals receiving Sorbitan Laurate once or twice daily after initiation had 10 tumors in 9 animals and 33 tumors in 21 animals, respectively. The Sorbitan Oleate group had five tumors in four animals. No tumors were observed in animals that received Sorbitan Trioleate after initiation. Additional details are available in Table 9. Sorbitan Oleate and Trioleate were inactive as tumor promoters. Sorbitan Laurate was considered an active tumor promoter on mouse skin, apparently based on the finding that doubling the frequency of application increased significantly the mean incidence of tumor-bearing mice without increasing the dose of carcinogen.

Setälä (1956) also investigated the cocarcinogenic activity of Sorbitans Laurate, Oleate and Trioleate. DMBA of either 0.3% (150  $\mu$ g), 0.03% (15  $\mu$ g), or 0.003% (1.5  $\mu$ g) was dissolved into the various Sorbitans and applied to the backs of mice (50 per group) three times per week. The hair was cut from the treatment site twice weekly. At the 0.3% DMBA dose the results were: Sorbitan Laurate, 240 tumors in 46 animals after 30 weeks; Sorbitan Oleate, 1 tumor in 1 animal after 10 weeks; Sorbitan Trioleate, 17 tumors in 8 animals after 17 weeks; and controls (DMBA in liquid paraffin), 200 tumors in 46 animals after 26 weeks. The

**TABLE 9**

Mean incidence of tumor-bearing mice during a 10-week period (Setälä 1956)

Compound tested for tumor-promoting capacity	Mean incidence of tumor-bearing mice (%)
PEG Sorbitan Stearate	63
PEG Sorbitan Palmitate	48
PEG Sorbitan Trioleate	37
PEG Sorbitan Oleate (Tween 80)	27
Sorbitan Laurate	2.9
Sorbitan Oleate	1.5
PEG Sorbitan Laurate	1.1
PEG Sorbitan Oleate (Tween 81)	0
Sorbitan Trioleate	0
PEG Sorbitol Tetraoleate	0

results for the 0.03% dose were: Sorbitan Laurate, 155 tumors in 31 animals after 30 weeks; Sorbitan Oleate, 168 tumors in 30 animals after 36 weeks; Sorbitan Trioleate, 130 tumors in 41 animals after 41 weeks; and controls (DMBA in liquid paraffin), 215 tumors in 39 animals after 34 weeks. At the 0.003% carcinogen dose, the results were: Sorbitan Laurate, 155 tumors in 35 animals after 52 weeks; Sorbitan Oleate, 25 tumors in 16 animals after 52 weeks; Sorbitan Trioleate, 57 tumors in 27 animals after 52 weeks; and controls (DMBA in liquid paraffin), 18 tumors in 13 animals after 52 weeks. Sorbitan Laurate and Sorbitan Trioleate were active on mouse skin as cocarcinogens when used as the solvent for 0.003% DMBA. Carcinomas did not develop on mouse skin when Sorbitan Oleate was used as a solvent for 0.003% DMBA.

### TUMOR INHIBITION

Crispens and Sorenson (1990) tested combinations of  $\text{Cu(II)}_2$  (3,5-diisopropylsalicylate)<sub>4</sub> (CuDIPS) a binuclear complex with immunostimulant and superoxide dismutase mimetic activities), PEG-20 Sorbitan Oleate, and cyclophosphamide for their effects on mortality and incidence of reticulum cell sarcoma in female SJL/J mice. Mice treated with CuDIPS and PEG-20 Sorbitan Oleate had a reduced survival rate and accelerated rate of tumorigenesis during the 52-week treatment period. PEG-20 Sorbitan Oleate plus cyclophosphamide caused a reduction in tumor incidence, as did the Polysorbate plus polyvinyl alcohol and other compounds. In general, high concentrations of PEG-4 and -20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate were cytotoxic to mouse Ehrlich ascites carcinoma cells, and produced reversible alterations in cellular membranes, inhibited respiration, and increased sensitivity to hyperthermia during *in vitro* studies. PEG-20 Sorbitan Laurate and PEG-20 Sorbitan Palmitate did not have *in vivo* tumor growth inhibition activities in mice with Ehrlich ascites carcinomas (Elder 1984).

Crispens and Sorenson (1991) reported that PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, and PEG-20 Sorbitan Stearate had little activity against reticulum cell sarcoma in SJL/J mice during a 52-week study, although PEG-20 Sorbitan Oleate had anticancer activity in the SJL/J tumor system. The apparent difference was attributed to the fatty acid components (saturated vs. unsaturated) of the Polysorbates.

When 50 Syrian hamsters were treated weekly for 25 weeks with benzo(a)pyrene via tracheal instillation, simultaneous administration of 5% PEG-20 Sorbitan Oleate reduced the number of benign and malignant neoplasms (Farrell 1974). These neoplasms included papillomas in the larynx and trachea, adenomas and adenocarcinoma in the bronchi, bronchioles, and lung periphery, and other neoplasms outside the respiratory tract. The vehicle was administered for 40 weeks, and from the 41st week to death, and the hamsters received PEG-20 Sorbitan Oleate, vehicle alone, or both. The total number of neoplasms observed after treatment with 3.4 mg benzo(a)pyrene in 0.2 ml of the 0.5% gelatin-in-saline vehicle was 24. Twelve neoplasms were

observed after treatment with benzo(a)pyrene plus 5% PEG-20 Sorbitan Oleate. No malignant neoplasms were observed in the respiratory tract of hamsters given the gelatin-in-saline vehicle, PEG-20 Sorbitan Oleate, or both; hamsters given the Polysorbate plus carcinogen only in the first instillation also had no malignant neoplasms. Hyperplasia of type II alveolar epithelial cells was observed via electronmicroscopy in animals without neoplasms.

In a study using 10 male CDF<sub>1</sub> mice per group, the simultaneous IP administration of 5000 mg/kg PEG-20 Sorbitan Oleate increased the chemotherapeutic activity of Adriamycin against murine leukemia P388 (Harrison, Cusic, and McAfee 1981). This action was due to an apparent reduction of plasma volume. In another study (Stavrovskaya et al. 1975), PEG-20 Sorbitan Oleate at concentrations greater than 0.1% increased the sensitivity of colcemid-resistant transformed mouse cells *in vitro*.

In an earlier study, Casazza et al. (1978) reported that Adriamycin in a 10% aqueous solution of PEG-20 Sorbitan Oleate produced significant increases of antitumor activity in mice against ascites tumors (L 1210 leukemia), disseminated leukemias (transplanted leukemias originally induced by Gross and Moloney leukemia viruses), and solid tumors (sarcoma 180 and MS-2 sarcoma) when the solution was administered intravenously. Toxicity of the antitumor drug, however, was enhanced. When the solution was administered intraperitoneally, no increase in antitumor activity was observed and toxicity was increased significantly.

Menon et al. (1984) investigated the effects of a PEG-20 Sorbitan Oleate vehicle on the natural resistance of sarcoma-180 tumor to the protein synthesis inhibitor, bouvardin. In this study, 10% PEG-20 Sorbitan Oleate enhanced cytotoxicity and increased the life span of Swiss mice bearing the sarcoma. *In vitro*, sarcoma cells exposed to bouvardin alone had inhibition of uridine incorporation by 46%, whereas bouvardin plus the Polysorbate inhibited uridine incorporation by 66%.

In other studies, PEG-20 Sorbitan Oleate at a concentration of 10% enhanced antitumor activity of adriamycin against murine P388 leukemia (Chitnis, Menon, and Gude 1984). At concentrations of 5% to 10% (0.1–0.2 ml/mouse, IP), the Polysorbate had anticancer activity against reticulum cell sarcoma in female SJL/J mice (Crispens, Porter, and Sorenson 1986; Crispens and Sorenson 1988). Kubis et al. (1979) reported that 5% (500 mg/kg, IP) PEG-20 Sorbitan Oleate had a cytotoxic effect on Ehrlich ascites tumors in Balb/c mice.

### CLINICAL ASSESSMENT OF SAFETY

#### Oral Toxicity

Polysorbates are commonly used emulsifiers in foods, and are approved by the FDA as direct and indirect food additives for human consumption (see Noncosmetic Use). The typical concentration of use of Polysorbates as food additives is 0.4% (HSDB 1996).

PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate have a toxicity rating of practically nontoxic (1/6), with a probable oral lethal dose in humans  $>15$  g/kg.

Polysorbates have been used in the treatment of lipid malabsorption syndromes. PEG-20 Sorbitan Oleate was described as useful for promoting fat absorption from the alimentary tract and no harmful effects were reported. It was judged harmless for human consumption in amounts of at least 6.0 g/day. A 4-month-old male infant ( $<8$  lbs) consumed 19.2 g PEG-20 Sorbitan Oleate daily for 2 consecutive days with no other food; the infant had loose stools, but no evidence of toxicity. When 13 premature and two full-term infants with steatorrhea were given four 200-mg daily doses of PEG-20 Sorbitan Laurate, the infants had no increase in fat absorption, but had no adverse effects with respect to anorexia, vomiting, defecation, or growth. In another study, nine premature infants were given 0.179 to 0.335 g/kg PEG-20 Sorbitan Oleate as a dietary supplement for a period of 4 consecutive days. The investigators did not report any adverse effects. Adults fed 20 g PEG-20 Sorbitan Stearate as a single dose had no changes in gastric motility or acidity, and no adverse effects were observed (Elder 1984).

#### *Sorbitan Esters*

Three clinical assessments have evaluated the oral toxicity of Sorbitan Stearate. One dose of 20 g was administered to five subjects, two of whom had increased gastric motility. One subject had an increase in free gastric acidity, and all subjects had normal gastric juices. In another study, nine patients were given 3 g Sorbitan Stearate twice daily for 28 days. Seven patients had normal gas patterns, one had more, and one had less at the end of the observation period. Seven patients had no change in gall bladder function, the eighth had increased emptying time, and the ninth patient had fainter visualization. Normal radiographic intestinal patterns were observed for all nine patients. In the third study, 42 subjects ingested 6 g Sorbitan Stearate daily for 28 days. Eleven subjects had albumin in their urine at the end of the study, and four had glycosuria; one of the four, however, was diabetic, and another had an abnormal glucose tolerance test. No significant changes were found in hemoglobin content, hematocrit, red cell count, or red cell fragility, and blood chemistry values were normal except in one patient who had slightly elevated total serum bilirubin (Elder 1985).

#### **Parenteral Toxicity**

From 1983 to 1984, low-birth-weight infants in neonatal intensive care units were given 25 U/ml supplements of vitamin E (DL- $\alpha$ -tocopheryl acetate) solubilized in 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate. The supplements were administered intravenously as 1 to 2-ml vials, usually admixed with a parenteral nutrition solution. Before the vitamin solution was withdrawn from the market, 38 premature infants died and 43 more became seriously ill. In one study, 14 of 17 affected infants that had been treated with the vitamin supplement died. Affected infants had dose-related, progressive deterioration characterized by unexplained thrombocytopenia, renal dys-

function, azotemia, hepatomegaly, cholestasis, ascites, hypotension, and metabolic acidosis. Oxalic acid-type crystals were observed in the distal renal tubules and collecting ducts. These effects were attributed to the high concentration of Polysorbate emulsifiers in the vitamin E supplement (Bove et al. 1985; Lorch et al. 1985; McKean, Peske, and Koo 1987; Pesce and McKean 1989; McKean 1992; Smolinske 1992). At microscopic examination of autopsy samples, lesions indicative of progressive injury were observed, including Kupffer cell exfoliation, hepatocytolysis, sinusoidal dilatation with accumulation of cellular debris and "free-floating" cells ( $<1$  week after infusion), attenuation of liver cell plates with extreme panlobular-congestion (1–2 weeks), cholestasis, early intralobular fibrosis (2–3 weeks), and ultimately, marked fibrosis with sinusoidal "obliteration." Regression was not apparent after discontinuation of the parenteral solution (Balistreri et al. 1985).

Tissue extracts from the neonates had unmetabolized Polysorbates at concentrations of up to 200  $\mu$ g/ml (McKean 1992). An infant that died had 100  $\mu$ g/ml of Polysorbates in ascites fluid (McKean and Pesce 1985). Compared to the membranes of human adults, human infant membranes were more sensitive to the effects of the surfactants and did not efficiently metabolize Polysorbates (McKean 1992). In adults, 90% of administered Polysorbates was eliminated via the urine in 24 hours. In infants, only the polyoxyethylated metabolite (not identified) was excreted (Pesce and McKean 1989).

Alade et al. (1986) reported that the mean in vitro response of human lymphocytes to phytohemagglutinin (PHA) was stimulated by vitamin E ( $\alpha$ -tocopherol acetate), whereas the commercial supplement and PEG-20 Sorbitan Oleate inhibited the PHA response by 37% and 44%, respectively. The percentage of T11 cells was also decreased by addition of the supplement and either or both of the Polysorbates. Based upon animal toxicology data on PEG-20 Sorbitan Oleate, the investigators suggested that the supplement's toxicity was due to the catabolism of the Polysorbate vehicle. Oleic acid and polyoxyethylene moieties released during in vivo hydrolysis of PEG-20 Sorbitan Oleate could have contributed to the pulmonary deterioration and renal failure of the affected infants. In addition, the renal failure and azotemia could have been due to the conversion of the other constituent, ethylene oxide, to ethylene glycol, a known nephrotoxic agent.

#### **Skin Irritation and Sensitization**

##### *PEGs Sorbitan/Sorbitol Fatty Acid Esters*

One case report described contact sensitivity (on the forehead) to PEG-40 Sorbitan Lanolate in a 28-year-old male using a styling gel (Pazzaglia et al. 1995). Volunteers were patch tested with PEG-40 Sorbitan Lanolate (50 subjects), PEG-50 Sorbitol Hexaoleate (200 subjects), PEG-40 Sorbitan Peroleate (208 subjects), and PEG-30 Sorbitol Tetraoleate Laurate (50 subjects). The test materials (concentration not specified) were each applied to a 1  $\times$  1-inch square of absorbent cotton twill, which was sealed onto the skin with a 2  $\times$  2-inch elastic adhesive patch. The patches were removed after 72 hours, and the skin sites were observed for irritation. This procedure was repeated at the

same site 7 days after the removal of the patch. Under the conditions of this study, PEG-40 Sorbitan Lanolate, PEG-50 Sorbitol Hexaoate, PEG-40 Sorbitan Peroleate, and PEG-30 Sorbitol Tetraoleate Laurate were nonsensitizing (CTFA 1998b).

#### *Polysorbates*

When a number of emulsifiers, including a mixture of 5% PEG-20 Sorbitan Stearate and 5% PEG-20 Sorbitan Oleate in petrolatum, was tested for contact sensitization (Hannuksela, Kousa, and Pirila 1976), two patients had allergic reactions (0.2%) and two had "toxic reactions" (0.2%). For this study, epicutaneous tests were performed with the Finn chamber method using 1206 patients with eczema. The occlusive patches were in place for 24 hours, and the sites were evaluated 20 minutes after removal, and after 2, 4, and 5 days after application. Irritant reactions were marked on the first or second day, and disappeared or became more faint within 4 to 5 days. One patient who reacted to the Polysorbates also reacted to polyoxyethylene oxypropylene stearate and PEG-n Sorbitol Lanolin. The second patient had reactions to a polyoxyethylene sorbitol lanolin derivative, balsam of Peru, neomycin, bacitracin, and tetramethylthioram disulphide (TMTD).

In a similar study, 10% PEG-20 Sorbitan Palmitate in petrolatum did not produce sensitization in 47 patients with chronic or recurrent (> 1 year) inflammatory skin diseases (Pasche-Koo et al. 1994). The same concentration of PEG-20 Sorbitan Oleate caused sensitization in one patient with eczema, but no sensitization was observed in other patients. The control group for this study consisted of 10 healthy volunteers.

A group of 737 patients with suspected cosmetic-related contact dermatitis were patch-tested with six emulsifiers (Tosti et al. 1990). PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Oleate were tested at 10% in petrolatum. Thirty-nine patients (5.3%) had one or more positive patch sites to the emulsifiers. Of that number, two patients reacted to PEG-20 Sorbitan Palmitate, one reacted to PEG-20 Sorbitan Oleate, and three reacted to both Polysorbates. The typical reaction sites were the hands and face. The sensitization was classified as clinically relevant in four patients; three had been sensitized by leave-on cosmetics and one by an antimycotic cream containing 0.1% PEG-20 Sorbitan Oleate, 1.5% PEG-20 Sorbitan Stearate, 2% sorbitan stearate, and other ingredients.

During a 4-hour patch test using the Hill Top chamber, undiluted PEG-20 Sorbitan Oleate caused positive reactions in only 1 of 27 patients (incidence = 4%). The comparable reactivity of 20% sodium dodecyl sulfate was eight of 27 patients (30%). As a result, PEG-20 Sorbitan Oleate was not classified as a human skin irritant (York et al. 1996).

PEG-20 Sorbitan Oleate at a concentration of 100% was non-corrosive to the skin of humans during an in vitro assay (Perkins, Osborne, and Johnson 1996). The test substance was applied topically to the stratum corneum of human skin cultures and cytotoxicity was measured as decreased MTT metabolism (see General Biology—Cytotoxic Effects). In a study by Gay et al.

(1992), PEG-20 Sorbitan Oleate was not irritating to a living skin equivalent. This in vitro system was comprised of an organotypic coculture of human dermal fibroblasts in a collagen-containing matrix overlaid with human keratinocytes that have formed a stratified epidermis. Irritation was determined by the cytotoxicity values of the MTT assay. The results correlated with those reported in in vitro studies using rabbit and human skin.

Roguet et al. (1994) reported that PEG-20 Sorbitan Laurate was nontoxic to Episkin reconstituted human epidermis. The LC<sub>50</sub> of PEG-20 Sorbitan Laurate in human keratinocyte monolayers (MTT assay) was 1.22 mg/ml.

The Polysorbates did not cause dermal irritation or sensitization in patch tests that used up to 50 subjects exposed to concentrations as high as 100%. When product formulations containing 2% to 8.4% PEG-20 Sorbitan Laurate were tested for 24-hour primary irritation, minimal to mild irritation was observed. A bubble bath (0.03%–6% PEG-20 Sorbitan Laurate) was moderately to severely irritating to the skin during cumulative irritancy tests. Lotions and creams containing 4.0% PEG-20 Sorbitan Palmitate were slightly irritating in a cumulative irritancy test. In similar tests, a cream containing 6.0% PEG-20 Sorbitan Stearate was essentially nonirritating, and a cream containing 1% PEG-20 Sorbitan Trioleate was slightly irritating to the skin. PEG-20 Sorbitan Laurate in formulation at concentrations of 0.3%–2.4% was nonsensitizing and nonphotosensitizing in Schwartz-Peck prophetic patch tests using up to 197 subjects. Irritation and sensitization were not observed when a shaving preparation containing 0.6% PEG-20 Sorbitan Stearate was evaluated in a similar study using 197 subjects. Mild irritation was observed when makeup containing 0.6% PEG-20 Sorbitan Oleate was tested using 303 subjects. No signs of sensitization, but minimal to mild irritation were observed when shaving foams and a moisturizer containing 2.5% PEG-20 Sorbitan Stearate, 2.5% PEG-20 Sorbitan Oleate, or 1.0% PEG-20 Sorbitan Trioleate were evaluated during a 48-hour prophetic patch and in-use test using up to 204 subjects. In other studies, formulations containing up to 6.0% of the Polysorbates were minimally irritating but not sensitizing (Elder 1984).

The cutaneous toxicities of PEG-20 Sorbitan Laurate and two other surfactants (sodium dodecyl sulfate [SDS] and Triton X-100) were determined using cultured human keratinocytes (Shivji et al. 1994). Three end points were evaluated: cytotoxicity as determined by crystal violet staining (CVS), the release of [<sup>3</sup>H]arachidonic acid, and the regulation of the proinflammatory cytokine interleukin-1 $\alpha$  (IL-1 $\alpha$ ) message in keratinocytes. Arachidonic acid was released from membrane phospholipids after induction by dermal irritants. IL-1 $\alpha$  was constitutively produced and stored by keratinocytes and was released upon injury to the cells, thereby stimulating the release of other cytokines. The IL-1 $\alpha$  message was semiquantitated using the reverse transcription polymerase chain reaction. PEG-20 Sorbitan Laurate was the least toxic of the surfactants tested. PEG-20 Sorbitan Laurate at concentrations of 0.55% and 0.04% reduced cell viability by 50% (CVS<sub>50</sub>) after 1 and 24 hours

of treatment, respectively. These values were obtained from dose-response curves. PEG-20 Sorbitan Laurate had a time-dependent toxic effect, but not as prominent as that observed after treatment with SDS or Triton X-100. PEG-20 Sorbitan Laurate required greater exposure time to exert an effect on the treated keratinocytes.

The concentration of PEG-20 Sorbitan Laurate needed to release 50% of the arachidonic acid (AAR<sub>50</sub>) in normal keratinocytes was 0.20% after 2 hours of treatment. The highest concentration of PEG-20 Sorbitan Laurate tested, 0.01%, caused an induction of IL-1 $\alpha$  messages. PEG-20 Sorbitan Laurate up-regulated the expression of IL-1 $\alpha$  mRNA compared to the vehicle control (Shivji et al. 1994).

#### *Sorbitan Esters*

A 24-hour occlusive patch test was performed using 56 subjects. A 0.05 ml volume of Sorbitan Isostearate (10.0% in squalene) was applied to the intact skin of the forearm for 24 hours, when the treatment site was examined for signs of primary irritation. None of the subjects reacted to Sorbitan Isostearate under the conditions of this study. Sorbitan Sesquiosate (10.0% in squalene) was evaluated similarly using 10 subjects, none of whom reacted to the test material (CTFA 1998c).

The Sorbitan Esters were minimal to mild skin irritants in humans. Products containing low concentrations of Sorbitan Stearate were mild irritants in 21-day cumulative irritation studies. A Schwartz Prophetic Patch test using 30% Sorbitan Laurate in water or undiluted Sorbitan Laurate did not produce signs of irritation in 10 or 50 subjects, respectively. In two Schwartz Prophetic Patch tests (60 subjects total), high concentrations of Sorbitan Sesquiosate produced no reactions. Several products containing 1.75% to 2% Sorbitan Oleate have been tested on human subjects. In four 21-day cumulative irritation studies, the products were mildly irritating; the specific ingredient(s) causing irritation was not determined. No irritation was observed in maximization tests using Sorbitan Oleate. Two of 53 subjects had mild irritation during a product usage study of Sorbitan Oleate. Sorbitan Tristearate, in a Schwartz Prophetic Patch test, produced no irritation in 211 panelists. Sorbitan Palmitate-containing skin formulations were slightly irritating to humans in a 21-day cumulative irritancy test using 34 subjects. Products containing 5% Sorbitan Trioleate were slightly irritating in 21-day cumulative irritancy tests, a Shelanski-Jordan repeat-insult patch test (RIPT), modified Schwartz-Peck predictive patch tests, and in a 4-week usage test. Results from three RIPTs (involving a total of 420 subjects) indicated that Sorbitan Stearate was not a sensitizer. Four RIPTs involving 339 panelists classified Sorbitan Oleate-containing products as nonsensitizers. In a Shelanski-Jordan RIPT (206 subjects), a skin care product containing Sorbitan Palmitate was neither an irritant nor a sensitizer. Human tests for sensitivity to Sorbitan Sesquiosate indicated that the compound was a nonsensitizer. In five RIPTs involving 352 subjects, results indicated that none of the five products containing 1% to 3% Sorbitan Sesquiosate was a sensitizer; some subjects, however, experienced mild irritation. Products containing

up to 2% of either Sorbitan Stearate or Sorbitan Oleate were nonphototoxic and nonphotoallergenic (Elder 1985).

#### *Polyethylene Glycol*

In clinical studies, PEG-6 and PEG-8 induced mild sensitization in 9% and 4% of 23 male subjects tested, respectively. However, later production lots of PEG-6, as well as PEG-75, did not cause reactions in any of the 100 male and 100 female subjects tested. A product formulation containing 3% PEG-8 induced minimal to mild irritation (induction phase) in over 75% of 90 volunteers participating in a skin irritation and sensitization study. Responses (not classified) were noted in 22 subjects at the 24-hour challenge reading. Cases of systemic toxicity and contact dermatitis in burn patients were attributed to PEG-based topical ointments. The ointment that induced systemic toxicity contained 63% PEG-6, 5% PEG-20, and 32% PEG-75 (Andersen 1993).

#### **Ocular Irritation**

In clinical ocular irritation tests, PEG-20 Sorbitan Laurate was nonirritating (Elder 1984).

PEG-20 Sorbitan Laurate markedly increased the permeability of the corneal epithelium in the human eye to fluorescein. The test compound was instilled at a concentration of 1% in saline (0.9% mixed with 5% Tris buffer, pH = 7.4; fluorescein added for final concentration of 0.75%). Concentrations of PEG-20 Sorbitan Laurate up to 40% did not produce adverse ocular effects in the volunteers tested (Marsh and Maurice 1971).

Enucleated human eyeballs was used in an ocular toxicity assay as a model for human corneal damage after exposure to various chemicals, including PEG-20 Sorbitan Laurate. The majority of human eyeballs were enucleated 3 to 24 hours after death. All were stored at 4°C for at least 12 hours before the experiment, which took place 24 to 72 hours after death. The isolated eyeballs were kept in a temperature-control chamber (32–36°C), held vertically in clamps, and irrigated with Hanks' balanced salt solution at the upper limbus. The eyeballs were examined using a Haag-Streit Slit Lamp before and after treatment, and the corneal thickness was measured. After 90 minutes, 20- and 100- $\mu$ l volumes of PEG-20 Sorbitan Laurate were applied at the superior limbus for 10 seconds and 1 minute, respectively. The larger volume was applied in 20- $\mu$ l aliquots at 10-second intervals. The eyeballs were rinsed for 10 seconds with saline. Cornea thickness was measured every 30 minutes; the total time of the experiment was 7 to 8 hours. Exposure to PEG-20 Sorbitan Laurate had little effect on corneal deturgescence in isolated human eyeballs, compared to controls. Increased granularity of the epithelium and fluorescein staining in the contact area were observed after treatment with PEG-20 Sorbitan Laurate (Berry and Easty 1993).

#### **SUMMARY**

The PEGs Sorbitan/Sorbitol Fatty Acid Esters are ethoxylated sorbitan and sorbitol esters of fatty acids that function as surfactants in cosmetic formulations. These ingredients were



used in a total of 81 cosmetic formulations in 1998. The Polysorbates, which are food additives, were used in 1418 formulations. They are formed by the esterification of sorbitol or sorbitan with a fatty acid, followed by the chemical addition of ethylene oxide. Typical impurities can include the free fatty acids, alcohol, peroxides, isosorbide ethoxylates, and other compounds; 1,4-dioxane and other water-soluble by-products are removed during the manufacturing process.

Few data on the ingredients in this review were available; therefore, relevant data from the previous CIR safety assessments on the Polysorbates (other PEGs Sorbitan Fatty Acid Ester), PEGs, and Sorbitan Esters were included in this report as a further basis for assessing their safety in cosmetics.

During feeding studies, the Polysorbates were absorbed and hydrolyzed by blood and pancreatic lipases. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid, and the PEG Sorbitan moiety was excreted mainly in the urine. The gastrointestinal absorption of PEGs was dependent on the molecular weight; the greater the molecular weight, the lesser the absorption that occurs. In oral and IV studies, the PEGs were not metabolized and were rapidly eliminated in the feces and urine. PEGs were readily absorbed through damaged skin.

A number of cytotoxicity assays has been performed on the Polysorbates; they caused both membrane damage and reduced mitochondrial activity. A concentration of 5% PEG-20 Sorbitan Oleate in rats caused the "destruction" of the mitochondria of the epithelium of the small intestine of Wistar rats. The Polysorbate (concentration = 10%) caused a portion of the microvilli to disappear with flattening of the surfaces of the epithelial cells. PEG-20 Sorbitan Oleate had immunosuppressive effects in Balb/c mice that had been immunized with ovalbumin. PEG-20 Sorbitan Oleate was also a histamine-releasing agent, and increased recruitment of peritoneal macrophages without modifying phagocytic activity. PEG-20 Sorbitan Oleate (100 mg/ml) depressed cardiac potential in dogs and guinea pigs; the Polysorbate reduced mean arterial blood pressure and left ventricular  $dP/dt$ .

The Polysorbates had low toxicity in both acute and long-term toxicity studies using animals. In rats, the  $LD_{50}$  values for these ingredients were  $>5$  to  $>38.9$  g/kg (oral),  $\sim 1.4$  g/kg (IV), and  $0.7$  to  $>5$  ml/kg (IP). When administered to rats by IP injection, 16% PEG-20 Sorbitan Laurate and 32% PEG-20 Sorbitan Oleate decreased locomotor activity. During an inhalation toxicity study, PEG-20 Sorbitan Oleate (7%;  $0.1$  to  $0.2$  ml) was relatively nontoxic. The Sorbitan Esters and PEGs also were relatively nontoxic to animals.

During a 14-day feeding study of 3000 to 50,000 ppm PEG-20 Sorbitan Oleate, the high dose caused decreased body weight in male rats and mice, but no other clinical findings were reported. A vehicle containing 9% PEG-20 Sorbitan Oleate and 1% PEG-20 Sorbitan Laurate was mildly hepatotoxic to rabbits and, when given intraperitoneally, caused massive peritoneal fibrosis and degeneration of the kidneys in mice and rats. No adverse effects

were observed in chicks fed 2% to 5% PEG-20 Sorbitan Stearate for 7 weeks. Rats fed 10% of the Polysorbate for 8 weeks had diarrhea for the first few days of treatment, but no other signs of toxicity. Rats fed 1.5 ml PEG-20 Sorbitan Oleate (1%–4%) for 3 months had congestive and degenerative changes in the heart, liver, and kidneys. In 6-week studies using rats and monkeys, PEG-4 Sorbitan Stearate, PEG-20 Sorbitan Stearate, and PEG-5 Sorbitan Oleate produced no significant adverse effects. In dermal toxicity studies, the PEGs did not cause signs of toxicity other than transient, mild erythema. Evidence of systemic toxicity was only observed in rabbits that received repeated topical applications of a PEG-based cream to abraded skin. Rats fed 1% to 4% Sorbitan Laurate for 6 weeks had decreased growth rates, and hamsters fed 15% for 68 days had degenerative changes of the gastrointestinal tract, and other lesions. Similar changes were observed in rats fed 25% Sorbitan Laurate for 70 days. Rhesus monkeys fed 2 g/day had no signs of toxicity after 6 weeks of treatment.

Growth retardation and diarrhea were noted in subchronic feeding studies of up to 10% PEG-20 Sorbitan Stearate using mice. Diarrhea in these and other studies was attributed to the high concentrations of the unabsorbed PEG Sorbitan moiety in the intestinal lumen. PEG-20 Sorbitan Oleate (up to 50,000 ppm) was nontoxic to rats and mice during a 13-week feed study. A concentration of 25% PEG-20 Sorbitan Laurate caused microscopic changes of the urinary bladder, spleen, kidneys, and gastrointestinal tract in rats during a 21-week study. The PEGs were nontoxic during a 90-day oral toxicity study using rats. Feeding of 10% to 25% Sorbitan Laurate for 90 days to 23 weeks caused decreased body and organ weights, diarrhea, and hepatic lesions in rats.

During a chronic toxicity study using hamsters, 5% to 15% PEG-20 Sorbitan Laurate caused microscopic lesions of the urinary bladder, kidneys, spleen, and gastrointestinal tract. In monkeys, 1 g/day PEG-20 Sorbitan Laurate did not cause adverse effects after 17 months of treatment. Rats fed up to 2% PEG-20 Sorbitan Laurate for over 2 years had no signs of toxicity. PEG-20 Sorbitan Stearate, PEG-20 Sorbitan Oleate, and PEG-20 Sorbitan Tristearate at concentrations  $<20\%$  were nontoxic in long-term feeding studies using mice, rats, dogs, and hamsters. At concentrations of 20%, these Polysorbates caused some growth retardation and diarrhea, and had minor effects on longevity and reproduction. Studies using 2% PEG-20 Sorbitan Palmitate and PEG-20 Sorbitan Trioleate were also negative. In chronic studies, dogs fed 2% PEG-8, PEG-32, or PEG-75 for 1 year had no adverse effects; rats fed 5% Sorbitan Laurate for 2 years had no signs of toxicity, but only 15% of the treated and control rats survived to the end of the study.

The Polysorbates were nonirritating to mildly irritating in both in vivo and in vitro ocular irritation assays. The concentrations tested ranged from 1% to 100%. PEG-6 and PEG-75 did not cause corneal injuries when instilled into the conjunctival sac of rabbits, but 35% PEG-8 and 0.1 ml PEG-32 (melted in water bath) induced mild ocular irritation. Sorbitan



Laurate (30%–100%) was not an ocular irritant in Draize ocular irritation tests using rabbits.

The Polysorbates had little potential for rabbit and mouse skin irritation in acute studies. Moderate to strong sensitization to PEG-20 Sorbitan Laurate was observed in a Magnusson-Kligman guinea pig maximization test; PEG-20 Sorbitan Oleate and PEG-20 Tristearate were not sensitizers. PEG-20 Sorbitan Laurate (1%) did not have comedogenic potential in rabbits. The Sorbitan Esters were generally mild skin irritants, but did not cause sensitization in animals. The PEGs were neither irritants nor sensitizers.

In teratology studies of thalidomide, the PEG-20 Sorbitan Laurate vehicle (10 ml/kg) had no effect on the developing mouse embryo. In other studies, reproductive and developmental effects were seen primarily at exposure levels that were maternally toxic. PEG-20 Sorbitan Laurate caused dose-dependent malformations of offspring when administered to Swiss and NMRI mice via IP injections. In the Chernoff-Kavlock assay using Alpk/AP rats, 10 ml/kg/day PEG-20 Sorbitan Laurate reduced offspring litter size, survival, and weight gain when the Polysorbate was administered intraperitoneally, but the parameters did not differ from controls after dermal, oral, or subcutaneous administration. In another study using rats, PEG-20 Sorbitan Laurate had a maternal no-observable-effect level (NOEL) of 500 mg/kg/day, a maternal low effect level of 5000 mg/kg/day, and a developmental NOEL of > 5000 mg/kg/day.

PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Stearate, and PEG-20 Sorbitan Oleate caused serious developmental effects in sea urchin embryos when administered at concentrations as low as 0.004% in sea water. Mice fed 10% PEG-20 Sorbitan Stearate or PEG-20 Sorbitan Laurate during a multigeneration study had offspring with decreased weanling weights, significantly smaller litters, and delivered more dead fetuses than mice of the control group. PEG-20 Sorbitan Oleate was not teratogenic in a rat whole-embryo culture study. In *in vivo* studies using neonatal rats, PEG-20 Sorbitan Oleate (1%–10%, IP injection) accelerated maturation, prolonged the estrous cycle, and induced chronic estrogenic stimulation. The ovaries were without corpora lutea and had degenerative follicles, and the uterus had epithelial squamous cell metaplasia and cytological changes. PEG-20 Sorbitan Oleate (2500 mg/kg/day in one study; 1.25 ml/l drinking water in another) and PEG-20 Sorbitan Stearate (0.1%–10% in one study; 5200 mg/kg/day in another) did not cause developmental effects in rats and mice, but PEG-20 Sorbitan Oleate in drinking water increased locomotor activity and exploratory behavior of offspring of treated rats.

The PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. The CIR Expert Panel concluded that, as the PEGs Sorbitan and Sorbitol Esters are chemically different from the alkyl ethers of ethylene glycol and the alkyl ethers are not present as impurities, these ingredients pose no reproductive or developmental hazard. In subchronic and chronic oral toxicity studies, the PEGs did not cause adverse reproductive effects.

The Polysorbates were nonmutagenic in a number of bacterial and mammalian systems, with the exception of PEG-20 Sorbitan Stearate, which produced both positive and negative results in genotoxicity assays.

In carcinogenicity studies, feeding of PEG-20 Sorbitan Oleate (up to 50,000 ppm) to rats and mice resulted in equivocal evidence of carcinogenicity; the male rats had an increased incidence of pheochromocytomas. The test compound was associated with inflammation and squamous hyperplasia of the nonglandular stomach in mice and with ulcers of the nonglandular stomach in female mice. PEG-20 Sorbitan Stearate did not increase the incidence of neoplasms in the nonglandular stomach and glandular stomach when administered with the carcinogens ENNG and MNNG. In general, the Polysorbates were not oral or dermal carcinogens, and were weak tumor promoters. PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Oleate (0.002%) inhibited metabolic cooperation in V79 Chinese Hamster cells *in vitro*, which could result in tumor promotion. PEG-20 Sorbitan Stearate has been reported to have an *in vivo* promoter response, and the Polysorbate induced the cytoplasmic accumulation of proliferin transcripts in mouse fibroblasts; proliferin is an antagonistic regulator of muscle-specific transcription, and can promote morphological transformation. The Polysorbates also had antitumor activity in animal studies. PEG-8 was noncarcinogenic in studies using mice, rats, and guinea pigs. Sorbitan Laurate and Sorbitan Stearate were also noncarcinogenic. At concentrations  $\geq 10\%$ , Sorbitan Laurate was a tumor promoter in mouse skin.

The Polysorbates were nontoxic by the oral route in clinical studies, but a Polysorbate vehicle (9% PEG-20 Sorbitan Oleate, 1% PEG-20 Sorbitan Laurate) for a neonatal parenteral supplement caused the deaths of 38 premature infants. The symptoms and lesions observed included pulmonary deterioration, hepatomegaly, metabolic acidosis, and renal failure. Investigators concluded that human infant membranes were more sensitive to the effects of the Polysorbates and could not efficiently metabolize the compounds. Oleic acid and PEG moieties released during *in vivo* hydrolysis of PEG-20 Sorbitan Oleate could have contributed to the pulmonary deterioration and renal failure, as could ethylene glycol formed from ethylene oxide moieties.

The Polysorbates had little potential for human skin irritation, sensitization, and phototoxicity in extensive clinical studies. PEG-20 Sorbitan Oleate at a concentration of 100% was noncorrosive, and it and PEG-20 Sorbitan Laurate were not irritating to living skin equivalents. The PEGs were nonsensitizers, but cases of systemic toxicity and contact dermatitis were observed in burn patients that were treated with PEG-based topical ointments. The Sorbitan Esters had the potential to cause cutaneous irritation in humans, and could cause sensitization in patients with damaged skin. Sorbitan Stearate and Sorbitan Oleate were not photosensitizing; Sorbitan Laurate, Sorbitan Palmitate, Sorbitan Sesquioleate, and Sorbitan Trioleate did not absorb UVA or UVB light, suggesting that these compounds were not photosensitizers.

In clinical ocular irritation studies, PEG-20 Sorbitan Laurate was nonirritating, but at a concentration of 1%, it markedly increased the permeability of the corneal epithelium to fluorescein in the human eye. PEG-20 Sorbitan Oleate was classified as an ocular irritant, but further details were not available.

## DISCUSSION

The CIR Expert Panel has reviewed previously the safety of the Polysorbates, which are specific PEGs Sorbitan/Sorbitol Fatty Acid Esters, as well as that of their components (sorbitan esters, fatty acids, and PEGs). The larger-molecular-weight PEGs Sorbitan Fatty Acid Esters and their components are known not to be toxic. It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers are reproductive and developmental toxins. Given the methods of manufacture of the PEGs Sorbitan/Sorbitol Fatty Acid Esters, there is no likelihood of ethylene glycol or its alkyl ethers being present, and the ingredients are chemically different from the ethylene glycol alkyl ethers of concern.

The Expert Panel was concerned about the lack of data on dermal absorption and/or reproductive and developmental toxicity of the smaller-molecular-weight ingredients. For example, the molecular weights of PEG-2 Sorbitan Isostearate, PEG-3 Sorbitan Stearate, and PEG-4 Sorbitan Laurate are approximately 518 to 562 Da. In contrast, the molecular weights of PEG-20 Sorbitan Stearate and PEG-20 Sorbitan Laurate are approximately 1228 to 1310 Da. Given that the smallest ingredients of this family have relatively large molecular weights, the Panel expressed the view that the penetration into the skin of the smaller-molecular-weight polymers would not be great. This, coupled with the available data on the components of the PEGs Sorbitan/Sorbitol Fatty Acid Esters, led to a basic conclusion of safety.

The Expert Panel's "safe for use" conclusion is based on historical data on the concentration of use of certain of these ingredients. Accordingly, the "present practices of use" in the conclusion means that the Expert Panel does not expect uses of PEGs Sorbitan Fatty Acid Esters, including those not currently used, to exceed 25%. Although there were single-insult patch test data showing these ingredients were not sensitizers at certain concentrations, the Expert Panel did not establish a maximum use concentration on that basis because such patch testing was considered inappropriate to establish a level above which the ingredient would be considered a sensitizer.

The CIR Expert Panel, however, was concerned about the sensitization and toxicity potential of the PEGs Sorbitan/Sorbitol Fatty Acid Esters when applied to damaged skin. This concern arose because of positive patch tests and incidences of nephrotoxicity in burn patients treated with an antimicrobial cream that contained PEG-6, PEG-20, and PEG-75. PEG was the causative agent in both animal and human studies; no evidence of systemic toxicity or sensitization was found in studies with intact skin. The cosmetics industry should consider this information when formulating products with PEGs Sorbitan/Sorbitol Fatty Acid Esters. The Expert Panel recommends that cosmetic

formulations containing these PEGs not be used on damaged skin.

Also of concern to the Expert Panel was the possible presence of 1,4-dioxane and ethylene oxide impurities. The Panel stressed that the cosmetics industry should continue to use the necessary procedures to remove these impurities from the PEGs Sorbitan/Sorbitol Fatty Acid Ester ingredients before blending them into cosmetic formulations.

The Expert Panel recognized that several of the Polysorbates enhanced skin penetration of other chemicals, and recommended that care should be exercised in using these ingredients in cosmetic products where the penetration of other ingredients is a concern.

The Expert Panel considered the finding that treatment of normal, human lymphocytes with 0.01% Sorbitan Oleate reduces DNA repair following UV irradiation, and the authors' hypothesis that this effect may be a mechanism in cocarcinogenesis, but concluded that this was only an hypothesis and does not demonstrate a link between DNA repair inhibition and cocarcinogenesis. The Panel carefully considered the data on the cocarcinogenesis of the Sorbitan Esters, noting the high exposure levels used, the high frequency of exposure, and the lack of a dose response and concluded that the positive response is not likely to be relevant to the use of these PEG Sorbitan/Sorbitol fatty acid esters in cosmetic formulations.

## CONCLUSION

The CIR Expert Panel concludes that PEG-20 Sorbitan Cocoate; PEG-40 Sorbitan Diisostearate; PEG-2, -5, and -20 Sorbitan Isostearate; PEG-40 and -75 Sorbitan Lanolate; PEG-10, -40, -44, -75, and -80 Sorbitan Laurate; PEG-3 and -6 Sorbitan Oleate; PEG-80 Sorbitan Palmitate; PEG-40 Sorbitan Perisostearate; PEG-40 Sorbitan Peroleate; PEG-3, -6, -40, and -60 Sorbitan Stearate; PEG-20, -30, -40, and -60 Sorbitan Tetraoleate; PEG-60 Sorbitan Tetrastearate; PEG-20 and -160 Sorbitan Triisostearate; PEG-18 Sorbitan Trioleate; PEG-40 and -50 Sorbitol Hexaoleate; PEG-30 Sorbitol Tetraoleate Laurate; and PEG-60 Sorbitol Tetrastearate are safe for use as cosmetic ingredients under the present practices of use.

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# Final Report on the Safety Assessment of PEG-6, -8, and -20 Sorbitan Beeswax<sup>1</sup>

Polyethylene Glycol (PEG)-6, -8, and -20 Sorbitan Beeswax are ethoxylated derivatives of Beeswax that function as surfactants in cosmetic formulations. Only PEG-20 Sorbitan Beeswax is currently reported to be used, at concentrations up to 11%. Few data on the PEGs Sorbitan Beeswax ingredients were available. This safety assessment relied upon the available data from previous safety assessments of Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and PEG Sorbitan fatty acid esters, also known as Polysorbates. The ester linkage of PEG Sorbitan fatty acid esters was hydrolyzed after oral administration, and the PEG Sorbitan moiety was poorly absorbed from the gastrointestinal tract. Sorbitan Stearate was hydrolyzed to stearic acid and anhydrides of sorbitol in the rat. PEGs are readily absorbed through damaged skin and are associated with contact dermatitis and systemic toxicity in burn patients. PEGs were not sensitizing to normal skin. PEGs did not cause reproductive toxicity, nor were tested PEGs mutagenic or carcinogenic. Sorbitol was not a reproductive or developmental toxin in multigenerational studies in rats. Neither Beeswax nor Synthetic Beeswax produced significant acute animal toxicity, ocular irritation, skin irritation, or skin sensitization. Polysorbates produced no acute or long-term effects, were generally not irritating or sensitizing, and were noncarcinogenic, although studies did demonstrate enhancement of the activity of chemical carcinogens. Sorbitan fatty acid esters were relatively nontoxic via ingestion, generally were not skin irritants or sensitizers, and were not mutagenic or carcinogenic. Sorbitan Laurate was a cocarcinogen in a mouse skin-painting study. PEG-6 Sorbitan Beeswax delivered via a stomach tube was nontoxic in rats in acute studies. Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the eyes of rabbits and was nonirritating to intact and abraded skin of rabbits. PEG-20 Sorbitan Beeswax was only minimally irritating to rabbit eyes at concentrations as high as 30%, and was not a significant skin irritant in rabbits exposed to a product with PEG-20 Sorbitan Beeswax at 2%. In clinical tests, PEG-6 and -20 Sorbitan Beeswax at concentrations up to 3% were only minimally irritating and were nonsensitizers. Careful consideration was made of the data on the cocarcinogenesis, but the high exposure levels, high frequency of exposure, and absence of a dose-response led to the conclusion that there was not a cocarcinogenesis risk with the use of these ingredients in cosmetic formulations. Accordingly, these ingredients were considered safe for use in cosmetic formulations under the present practices of use.

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## INTRODUCTION

Polyethylene Glycol (PEG)-6, -8, and -20 Sorbitan Beeswax are the ethoxylated derivatives of Beeswax that function as surfactant—emulsifying agents and surfactant—solubilizing agents in cosmetic formulations.

The Cosmetic Ingredient Review (CIR) Expert Panel has previously reviewed the safety in cosmetics of PEGs, Polysorbates (PEGs Sorbitan Fatty Acid Ester), Beeswax, Synthetic Beeswax, and Sorbitan Fatty Acid Esters. The conclusions reached in those reviews are described below:

- PEG-6, -8, -32, -75, -150, -14M, and -20M are safe for use at the concentrations reflected in the Cosmetic Use section and in the product formulation safety test data included in this report. The Expert Panel recommends that cosmetic formulations containing these PEGs not be used on damaged skin (Andersen 1993).
- Polysorbates 20, 21, 40, 60, 61, 65, 80, 81, and 85 (PEGs Sorbitan Fatty Acid Esters) are safe as cosmetic ingredients in the concentration of present use (Elder 1984a).
- Candelilla Wax, Carnauba Wax, Japan Wax, and Beeswax are safe as used in cosmetics under present practices of concentration and use (Elder 1984b).
- Ozokerite, Ceresin, Montan Wax, Paraffin, Microcrystalline Wax, Emulsifying Wax N.F., Synthetic Wax, and Synthetic Beeswax are safe as cosmetic ingredients in present practices of concentration and use (Elder 1984b).
- Sorbitan Stearate, Sorbitan Laurate, Sorbitan Sesquileate, Sorbitan Oleate, Sorbitan Tristearate, Sorbitan Palmitate, and Sorbitan Trioleate (Sorbitan Fatty Acid Esters) are considered safe as cosmetic ingredients under present conditions of concentration and use (Elder 1985).
- Sorbitan Caprylate, Sorbitan Cocoate, Sorbitan Diisostearate, Sorbitan Dioleate, Sorbitan Distearate, Sorbitan Isostearate, Sorbitan Olivinate, Sorbitan Sesquiisostearate, Sorbitan Sesquisteate, and Sorbitan Triisostearate (Sorbitan Fatty Acid Esters) are considered safe for use in cosmetic formulations under the present practices of use (CIR 1999).

Because few data on the PEGs Sorbitan Beeswax were available, selected data on the PEGs, Polysorbates, the Beeswaxes, and the Sorbitan Fatty Acid Esters from these safety assessments, as well as new safety test data available since the reports were written, have been added to this report as a further basis to the assessment of safety in cosmetics of the PEGs Sorbitan Beeswax ingredients.

## CHEMISTRY

### Definition and Structure

#### *PEGs Sorbitan Beeswax*

The PEGs Sorbitan Beeswax (CAS Nos. 8051-15-8 and 8051-73-8) are ethoxylated sorbitan derivatives of Beeswax with an average of  $n$  moles of ethylene oxide, where  $n$  equals the number in the name. Synonyms for PEG-6, -8, and -20 Sorbitan Beeswax are Polyethylene Glycol  $n$  Sorbitan Beeswax, where  $n$  equals 300, 400, and 1000, respectively. These ingredients are also known as Polyoxyethylene ( $n$ ) Sorbitan Beeswax (Wenninger and McEwen 1997).

The definition and structure of most of the related ingredients are presented in the safety assessments described above and will not be repeated here. Because Beeswax is the unusual component in these ingredients, information from previous reports is summarized below.

#### *Beeswax*

Beeswax is a complex mixture of several chemical entities, each with its own chemical and physical properties. Beeswax is synthesized from even-numbered alcohols ranging from C14 to C32. The alcohols are oxidized and combined with higher alcohols to form esters. Mixed dimers can be formed by the combination of certain acids and hydrocarbons by decarboxylation of esters. Beeswax contains 14% hydrocarbons, 73% esters (35% monoesters, 14% diesters, 3% triesters, 4% hydroxymonoesters, 8% hydroxypolyesters, 2% acid monoesters, and 7% acid polyesters), 12% free myristic acid, and unreported amounts of hydroxy acids and diols (Elder 1984b). Synthetic Beeswax is a blend of fatty esters (C32 to C62), fatty acids (C16 to C36), fatty alcohols (C16 to C36), and high-molecular-weight hydrocarbons (C21 to C34). Esters are the most abundant, the hydrocarbons next, the acids, and then the alcohols (Elder 1984c).

### Chemical and Physical Properties

#### *PEGs Sorbitan Beeswax*

PEG-6 Sorbitan Beeswax is a tan, waxy solid with a "fatty odor." It is soluble in corn oil, but is insoluble in ethylene glycol, mineral oil, or water. The pour point is approximately 62°C. The hydroxyl number is 105 to 135, the saponification number is 65 to 90, and the maximum acid number is 4.0. PEG-6 Sorbitan Beeswax contains up to 1.5% moisture.

PEG-20 Sorbitan Beeswax is a tan, waxy solid with a mild fatty odor. It is soluble in warm corn oil, insoluble in water or

ethanol, and dispersible in mineral oil. The pour point is approximately 63°C, the maximum acid number is 3.0, the maximum amount of moisture is 3.0%, and the saponification number is 70 to 105 (Nikitakis and McEwen 1990).

### Impurities

#### *PEGs Sorbitan Beeswax*

Impurities data were not available on the PEGs Sorbitan Beeswax ingredients.

#### *PEGs*

PEG-6 could contain small amounts of monomer and dimers. The amounts have not been quantified. Peroxides, formed as a result of autoxidation, were found in PEG-32 and PEG-75. The amount of peroxide in PEG was dependent upon the molecular weight of the PEG and its age. The older the compound, the greater the concentration of peroxides. In a colorimetric assay used to determine the peroxide concentrations in several production lots of PEG, PEG-6 and PEG-8 were each added to acidified potassium iodide solution, and the iodine liberated was titrated against a standard thiosulfate solution. PEG-6 had peroxide concentrations ranging from 1.4 to 9.3  $\mu$ Eq thiosulfate/ml glycol. PEG-8 had concentrations ranging from 3.24 to 5.7  $\mu$ Eq thiosulfate/ml glycol. The specific peroxides present in the PEGs were not determined, but they were considered organic peroxides rather than hydrogen peroxide (Andersen 1993).

#### *Beeswax*

Natural impurities found in Beeswax include resins, pollens, and insect and plant matter, all of which are removed in the refining process. Refined Beeswax can contain additives such as tallow, paraffin, ceresin, and vegetable waxes (Elder 1984b).

#### *Sorbitan Fatty Acid Esters*

Sorbitan Fatty Acid Esters can contain impurities such as free acid and alcohol, arsenic (<3 ppm), lead (<10 ppm), and water (Elder 1985).

## COSMETIC USE

The PEGs Sorbitan Beeswax are surfactant—emulsifying agents and surfactant—solubilizing agents in cosmetic formulations. Data submitted to the Food and Drug Administration (FDA) in 1998 indicated that PEG-20 Sorbitan Beeswax was used in 16 formulations in five product categories (Table 1), and the remaining PEGs Sorbitan Beeswax were not used (FDA 1998). Data provided by industry in 1998 and 1999 expanded the product categories in which PEG-20 Sorbitan Beeswax is reportedly used and provided current concentrations of use as shown in Table 2 (CTFA 1998a, 1998f, 1999a, 1999b). The highest currently reported concentration of use is 11% in blushers. These concentrations may be compared to data reported to FDA in 1984 indicating that PEG-6 and -20 Sorbitan Beeswax were used at concentrations  $\leq 10\%$  (FDA 1984).

**TABLE 1**  
Frequency of use of PEG-20 Sorbitan Beeswax (FDA 1998)

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Eyeliner	514	2
Mascara	167	8
Other Eye makeup preparations	120	1
Lipstick	790	4
Other Skin care preparations	692	1
<b>1998 PEG-20 Sorbitan Beeswax total</b>		<b>16</b>

## GENERAL BIOLOGY

### Absorption, Distribution, Metabolism, and Excretion

#### *PEGs Sorbitan Beeswax*

No information was available on the absorption, distribution, metabolism, or excretion of the PEGs Sorbitan Beeswax ingredients.

#### *Polysorbates (PEG Sorbitan Fatty Acid Esters)*

The ester link of the Polysorbate molecule was hydrolyzed by blood and pancreatic lipases following oral administration in labeling studies using rats. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid. The lauric acid moiety of PEG-20 Sorbitan Laurate was rapidly absorbed and oxidized by rats. After 24 hours, 75% to 80% of the lauric acid was expired as CO<sub>2</sub> and 4% was not absorbed from the alimentary tract. Twelve percent was found in the carcass, 2.5% in urine, and 1.2% in the liver. The polyoxyethylene sorbitan moiety was poorly absorbed from the gastrointestinal (GI) tract. Of the administered PEG group, 90% was excreted in the feces and 8% in the urine. In the case of the sorbitan moiety, 91% of the radioactivity was recovered in the feces, 2.1% in the urine, and 1.6% in the carcass. Similar results were observed

following intravenous (IV) injection of PEG-20 Sorbitan Laurate (Elder 1984a).

#### *PEGs*

GI absorption of PEG is dependent on the molecular weight of the compound. In general, the greater the molecular weight of the PEG compound, the lesser the absorption that occurs. In both oral and IV studies, no metabolism was observed and the PEGs were rapidly eliminated unchanged in the urine and feces. In a study with human burn patients, monomeric ethylene glycol was isolated in the serum following topical exposure to a PEG-based antimicrobial cream, indicating that PEGs are readily absorbed through damaged skin (Andersen 1993).

#### *Sorbitan Fatty Acid Esters*

Sorbitan Stearate is hydrolyzed to stearic acid and anhydrides of sorbitol when ingested. Approximately 90% of the Sorbitan Stearate is absorbed and hydrolyzed when fed to rats in oil solution, and 50% is absorbed and hydrolyzed when fed as a water emulsion. Sorbitan Stearate does not accumulate to any appreciable amount (<0.5%) in the fat stores of the rat body (Elder 1985).

**TABLE 2**  
Concentration of use of PEG-20 Sorbitan Beeswax

Product type	Reported concentration (%)	Reference(s)
Blushers	11	CTFA 1999a
Eyebrow pencil	3	CTFA 1999b
Eyelash primer	3	CTFA 1998a
Eyeliner	1.4–1.5	CTFA 1998a, 1999b
Eye makeup base	0.5	CTFA 1998a
Foot powders and sprays	1	CTFA 1999b
Lipstick	3–8	CTFA 1999a, 1999b
Makeup fixatives	0.2	CTFA 1999a, 1999b
Mascara	2–8	CTFA 1998a, 1999a, 1999b
Moisturizing creams, lotions, powders, and sprays	1	CTFA 1998f
Other makeup preparations	3	CTFA 1998f, 1999b



## ANIMAL TOXICOLOGY

### Acute Toxicity

#### *PEGs Sorbitan Beeswax*

PEG-6 Sorbitan Beeswax was administered via stomach intubation to two female rats (strain not stated) at a single dose of 10.0 g/kg. No signs of toxicity were noted, and none of the animals died prior to scheduled necropsy. Hydronephrosis and granular spleens were observed at necropsy. In a similar study, rats given 10.0 g/kg PEG-20 Sorbitan Beeswax had granular spleens and focal hemorrhages of the lungs. One rat had a scarlike lesion of the liver. Both compounds were classified as "practically nontoxic" (CTFA 1998b).

#### *Sorbitan Fatty Acid Esters*

Five female ddY mice were treated with a single oral dose of Sorbitan Sesquiossearate at a volume of 10 ml/kg body weight. The acute oral LD<sub>50</sub> was 25 ml/kg, which was considered "practically nontoxic" under the conditions of the study (CTFA 1998c).

#### *PEGs*

In general, PEGs have low oral and dermal toxicity. The greater molecular weight PEGs appear to be less toxic than the smaller molecular weight PEGs in oral studies. Inhalation of aerosolized PEG-75 (20% w:w in water) at concentrations up to 1008 mg/m<sup>3</sup> caused little or no toxicity in rats (Andersen 1993).

#### *Polysorbates (PEGs Sorbitan Fatty Acid Esters)*

Extensive acute and long-term oral toxicity testing in animals has produced evidence indicating the low order of toxicity after oral ingestion of the Polysorbates. Most of the reported toxicity can be attributed either directly or indirectly to the osmotic diarrhea caused by the polyoxyethylene sorbitan moiety retained within the intestinal lumen. Polysorbate 20 and product formulations containing 1.0% to 8.4% of Polysorbate 20, 40, 80, or 85 (PEG-20 Sorbitan Laurate, PEG-20 Sorbitan Palmitate, PEG-20 Sorbitan Oleate, or PEG-20 Sorbitan Trioleate, respectively) produced no evidence of acute or subchronic percutaneous toxicity, the only effects being erythema, edema, and desquamation at the site of application. Acute IV and intraperitoneal (IP) injections of the Polysorbates into rats or mice resulted in LD<sub>50</sub> values indicative of a low order of parenteral toxicity. Injections of Polysorbates 60 (10 ml of 0.5% solution IV daily) and 80 (one 10-ml injection and one 15-ml injection daily IV of a 20% solution) into rabbits for up to 65 days produced lesions limited principally to the kidneys and monocyte-macrophage system (Elder 1984a).

#### *Beeswax*

Four of 10 rats died on day 2 of the 14-day observation period and the survivors had depression and ataxia after being dosed orally with undiluted Beeswax. In other studies, cosmetic formulations containing 0.3% to 13.0% Beeswax (100% or 33.3% in corn oil) were orally administered as 5 to 15 g/kg doses. No

signs of toxicity were observed, and the LD<sub>50</sub> values could not be computed (Elder 1984b).

Ten male Wistar rats fed 5 to 14.43 g/kg Synthetic Beeswax had chromorhinorrhea and chromodacryorrhea. Rats given 5 to 10.4 g/kg had diarrhea, ptosis, bulging eyes, and sniffing. One rat of the high dose group died on day 1, and another died on day 6 (Elder 1984c).

#### *Sorbitan Fatty Acid Esters*

The results of oral toxicity studies of Sorbitan Fatty Acid Esters indicated that these Sorbitans were relatively nontoxic via ingestion when administered at low concentrations. The lowest rat LD<sub>50</sub> in the 20 sorbitan ester studies reported was 31 g/kg for Sorbitan Stearate (Elder 1985).

### Subchronic Toxicity

#### *Sorbitan Fatty Acid Esters*

In subchronic feeding studies of Sorbitan Laurate in a variety of species (chickens, rats, monkeys, and hamsters), no toxic effects were noticed when the ester concentration in the feed was less than 10%. When the feed concentration was  $\geq 10\%$ , growth depression, decreased organ weights, diarrhea, unkempt appearance, hepatic and renal abnormalities, and GI tract irritation were generally observed. Subchronic feeding of Sorbitan Oleate to rats produced no abnormalities until the concentration of the ester was at least 10%. At this concentration, the same types of abnormalities occurred as those observed in the Sorbitan Laurate-fed animals (Elder 1985).

### Chronic Toxicity

#### *Sorbitan Fatty Acid Esters*

Chronic feeding studies have been conducted with Sorbitans Stearate, Laurate, and Oleate. At a 5% dietary concentration, Sorbitan Laurate or Sorbitan Oleate produced no adverse effects when rats were fed the compounds for a 2-year period. Dogs fed 5% Sorbitan Stearate for 20 months had no compound-related changes. A feed concentration of  $\geq 10\%$  Sorbitan Stearate produced depressed growth and hepatic and renal abnormalities. Mice appeared more sensitive to toxic effects of Sorbitan Stearate than rats. A 0.5% dietary concentration produced growth depression in male rats, and a 4% dietary concentration produced renal abnormalities as well (Elder 1985).

### Ocular Irritation

#### *PEGs Sorbitan Beeswax*

Undiluted PEG-6 Sorbitan Beeswax was nonirritating and 30% (in water) PEG-20 Sorbitan Beeswax was minimally irritating (score = 3.5/110) to the eyes of rabbits (CTFA 1998b).

An undiluted eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was instilled three times into the conjunctival sac of three rabbits. Two days after instillation, one rabbit had redness, swelling, and/or discharge of the conjunctiva (Draize score = 2),

but no other reactions were observed. The eyeliner, therefore, was classified as minimally irritating to the eyes of rabbits. In studies using the same procedure, a mascara and lash conditioner containing 2% PEG-20 Sorbitan Beeswax were nonirritating and minimally irritating, respectively, to the eyes of three rabbits (CTFA 1998d).

A liquid eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was tested for irritancy potential in the Eytex assay. The eyeliner was classified as a minimal irritant, and the equivalent Draize score was 1.2/110 (National Testing Corporation 1988).

#### *PEGs*

PEGs caused mild, transient ocular irritation in rabbits (Andersen 1993). The Polysorbates produced no more than minimal, transient ocular irritation in Draize rabbit eye irritation tests (Elder 1984a).

#### *Beeswax*

A cream formulation containing 6% Beeswax and 6% ceresin was evaluated for ocular irritancy using nine New Zealand white rabbits. The eyes of six rabbits were rinsed after instillation of the test material; at 24 hours, four had minimal chemosis and two had minimal conjunctival redness. No signs of irritation were observed in rabbits with unrinsed eyes (Elder 1984b).

A 0.1-ml volume of 3.0% Synthetic Beeswax was instilled into the conjunctival sac of three albino rabbits. No irritation was observed. In another study, six rabbits were treated with 0.1 ml of the compound. On days 1 to 3, the Draize scores were 6.3/110, 3/110, and 2/110, respectively. Synthetic Beeswax was deemed minimally irritating on days 1 and 2, and practically nonirritating on day 3 (Elder 1984c).

#### *Sorbitan Fatty Acid Esters*

Sorbitan Isostearate was nonirritating to the eyes of rabbits during two studies (Unichema International 1996). When 0.1 ml (10.0% in squalene) was tested using three male Japanese white rabbits, the average total score was 4.0/110.0, which corresponded to a grade of minimal irritant. Using the same procedure, Sorbitan Sesquiosostearate (10.0% in squalene) was a minimal irritant to the eyes of rabbits, with an average total score of 6.7/110.0 (CTFA 1998c).

Draize and Modified Draize ocular irritation studies using rabbits were performed. One study using a concentration of 30% Sorbitan Stearate was negative for ocular irritation, and low concentrations (4%) in products caused slight conjunctival irritation. High concentrations of Sorbitan Sesquioleate (3.0% to 100%) produced no ocular irritation. One study with Sorbitan Laurate (30%), and two studies each on Sorbitans Oleate (5% to 100%), Tristearate (30% to 40%), and Palmitate (4.0% to 30%) were negative for ocular irritation in the rabbit (Elder 1985).

### **Dermal Irritation and Sensitization**

#### *PEGs Sorbitan Beeswax*

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the intact and abraded skin of New Zealand white rabbits when

applied for 24 to 72 hours (CTFA 1998b). An eyeliner containing 1.5% PEG-20 Sorbitan Beeswax caused erythema and was minimally irritating in a single insult patch test using nine rabbits. The total primary irritation index (PII) score was 1.44/8.0. A mascara and lash conditioner containing 2.0% PEG-20 Sorbitan Beeswax each had PIIs of 1.33/8.0, and the formulations were classified as minimally irritating to the skin of six rabbits (CTFA 1998d).

#### *Sorbitan Fatty Acid Esters*

Sorbitan Isostearate was classified as a moderate irritant (primary irritation index, PII = 2.8/8.0) to the skin of rabbits. Sorbitan Isostearate also had very low sensitization potential when tested in four Magnusson-Kligman guinea pig maximization studies. The induction concentrations were 1% to 2% (intradermal injection) and 50% to 100% (topical application), and the challenge concentrations were 10% to 25%. In addition, a Landsteiner guinea pig test showed that intradermal injections of 0.2% Sorbitan Isostearate in propylene glycol caused mild to severe irritation in all animals, but did not cause sensitization reactions (Unichema International 1996).

Sorbitan Isostearate was described as "non-irritating, non-sensitizing, non-comedogenic in studies according to industry standard protocols (repeat-insult patch test [RIPT], comedogenicity)" and in the chorioallantoic membrane vascular assay, additional details were unavailable (CTFA 1998e).

The primary skin irritation potentials of Sorbitan Isostearate and Sorbitan Sesquiosostearate (both 10.0% in squalene) were evaluated using eight male Japanese white rabbits. The test materials were added to abraded and intact skin sites of the clipped back, and the sites were covered for 24 hours using patch-test plaster. The test sites were evaluated at 24 and 72 hours after administration of the test material. The PII's were 0.3/8.0 and 0.5/8.0, respectively, which corresponded to a grade of non- to weak irritant.

Sorbitan Isostearate and Sorbitan Sesquiosostearate were weak cumulative irritants using three male Hartley guinea pigs. A 0.05-ml volume of a 10% solution (in squalene) of each test substance was applied to the clipped and shaved skin of the flank, once daily for 3 consecutive days. The treatment sites were examined for signs of irritancy 24 hours after each application. The cumulative scores were 1.1/4.0 and 1.7/4.0, respectively (CTFA 1998c).

Numerous skin irritation studies in animals indicate that the Sorbitan Fatty Acid Esters are minimal to mild irritants. In acute skin irritation tests using rabbits, Sorbitan Stearate (1% to 60%) produced mild irritation. Sorbitan Laurate (1% to 100%) was mildly irritating to rabbit skin, causing dose-dependent erythema and edema. The rabbit dermal toxicity and irritation potential of Sorbitan Sesquioleate (3%) was minimal. Sorbitan Oleate (5% to 100%) was minimally irritating when applied to rabbit skin. When solutions of Sorbitan Oleate were applied to rabbit skin, erythema and edema developed. Sorbitan Palmitate (4% to 50%) when tested for acute dermal irritation in the rabbit

produced no irritation. A subchronic dermal study was negative for any systemic toxicity. Sorbitan Tristearate (30%) was non-irritating when applied to the skin of rabbits. Sorbitan Trioleate (1% to 100%) was generally found to be a skin irritant in rabbits. Sorbitan Trioleate when applied to rabbit skin produced erythema, edema, and thickening. No systemic toxicity was observed (Elder 1985).

#### PEGs

The PEGs were not irritating to the skin of rabbits or guinea pigs. PEG-75 was not a sensitizer (Andersen 1993).

#### Polysorbates (PEG Sorbitan Fatty Acid Esters)

The Polysorbates had little potential for rabbit and mouse skin irritation in acute studies. The Polysorbates that were tested in subchronic skin irritation tests for up to 60 days produced local skin reactions ranging from minimal inflammation to necrosis. These changes were attributable to damage of epidermal cell membranes by the emulsifying action of the Polysorbates. Moderate to strong skin sensitization to Polysorbate 20 was observed in one Magnusson-Kligman guinea pig maximization test. In another guinea pig skin sensitization assay, no skin sensitization to Polysorbates 65 (PEG-20 Sorbitan Tristearate) and 80 was observed (Elder 1984a).

#### Beeswax

When 5 g Synthetic Beeswax (in 1 ml corn oil) was applied to intact and abraded skin sites of six New Zealand white rabbits for 24 hours, the Draize score (at 72 hours) was 2.08/8.0. In a second primary irritation assay, 0.5 ml of the test compound was applied under occlusive patches to abraded and intact skin sites of three albino rabbits. The Draize primary irritation index was 0.0/8.0.

Fifty percent Synthetic Beeswax (in distilled water) with 1% carboxymethyl cellulose and 0.2% Polysorbate 80 was applied to the clipped backs of guinea pigs (number not available) for 3 consecutive days/week for 3 weeks. One application was made in the fourth week. The volume of the first application was 0.5 ml, and the volume of the remaining nine applications was 0.1 ml. Challenge occurred at 14 days after the last application. The scores were 0.16/4.0 (erythema) and 0.05/4.0 (edema). The investigators concluded that the test material had no potential for irritation or sensitization (Elder 1984c).

### Other Safety Tests

#### PEGs Sorbitan Beeswax

A chorioallantoic membrane vascular assay was performed on 50% (aqueous) and 100% concentrations of a cosmetic formulation containing 1.5% PEG-20 Sorbitan Beeswax. Ten eggs per group were treated with the test materials, incubated for 30 minutes, and were examined for signs of vascular hemorrhage, capillary injection, or ghost vessels. The  $RC_{50}$  was >100% (MB Research Labs 1991).

#### Polysorbates (PEG Sorbitan Fatty Acid Esters)

Polysorbate 80 (PEG-20 Sorbitan Oleate) produced superficial, mild damage to the intestinal mucosae of rabbits and rats. Polysorbate 20 (PEG-20 Sorbitan Laurate) produced no inflammation when infused into the guinea pig urinary bladder (Elder 1984a).

### REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

#### PEGs Sorbitan Beeswax

Data on the reproductive or developmental toxicity of the PEGs Sorbitan Beeswax ingredients were not found. Data on PEGs and Sorbitol are provided. Because of concerns about the reproductive and developmental toxicity of the PEG monomer, ethylene glycol, a separate section is included to address that issue.

#### PEGs

No adverse reproductive effects occurred during subchronic (90 days) and chronic (2 years) oral toxicity studies of PEG-6-32 and PEG-75. In the subchronic study, PEG-75 was tested at a dose of 0.23 g/kg/day. In the chronic study, PEG-75 was tested at doses up to 0.062 g/kg/day and, PEG-6-32, at doses up to 1.69 g/kg/day (Andersen 1993).

#### Sorbitol

MacKenzie et al. (1986) performed a multigeneration feeding study to determine the reproductive and developmental effects of Sorbitol. Twelve male and 24 female Charles River CD (SD) BR rats per group were fed diets having 2.5%, 5.0%, or 10% Sorbitol (replacing the sucrose content of the basal feed) during a 96-week multigeneration study. The two high concentrations were "built up in 2.5% steps at weekly intervals." The  $F_0$  rats were mated to produce the  $F_{1a}$  and  $F_{1b}$  litters. The  $F_{1b}$  rats were treated and mated to produce the  $F_{2a}$  and  $F_{2b}$  litters. The  $F_{2b}$  rats were treated and mated to produce the  $F_{3a}$  litters. Twelve rats/sex/group were fed the test diets for 4 weeks, then were killed. Gross examinations were performed on all mated animals and two rats/sex of the  $F_{1a}$  and  $F_{2a}$ . Gross and microscopic examinations and biochemical analyses were performed on the  $F_{3a}$  rats. In this study, the feeding of up to 10% Sorbitol to rats had no significant adverse clinical, behavioral, or reproductive effects, and no significant gross or microscopic changes were observed.

The safety of hydrogenated starch hydrolysates (HSH), which are mixtures of polyhydric alcohols such as ~7.0% Sorbitol, was investigated using a 2-year ingestion study (50 Sprague-Dawley rats/sex/group), a multigeneration reproduction study (20 rats/sex/group), and a teratology study (30 dams/group). At a concentration of 18% in drinking water (3000 to 7000 mg/kg/day), HSH did not produce reproductive or developmental effects (Modderman 1993).

#### Ethylene Glycol and Its Ethers

It is generally recognized that the PEG monomer, ethylene glycol, and certain of its monoalkyl ethers (e.g., methoxyethanol,

a.k.a. ethylene glycol monomethyl ether) are reproductive and developmental toxins. The CIR Expert Panel undertook a separate, limited scope review of these compounds in order to assess the possibility that PEG-derived cosmetic ingredients could present similar concerns (Andersen 1999). In summary, this report concluded that the ethylene glycol monoalkyl ethers are not themselves toxic, but rather that one or more alcohol or aldehyde dehydrogenase metabolites are toxic. From the available data, the report also concluded that the toxicity of the monoalkyl ethers is inversely proportional to the length of the alkyl chain (methyl is more toxic than ethyl than propyl than butyl, etc.).

The PEGs Sorbitan Beeswax are chemically different from the alkyl ethers; therefore, the Panel concluded that no reproductive or developmental hazard is posed by those compounds.

## GENOTOXICITY

### PEGs Sorbitan Beeswax

No data were available on the mutagenicity of the PEGs Sorbitan Beeswax ingredients.

### PEGs

PEG-8 was negative in a Chinese hamster ovary cell mutation test, sister-chromatid exchange test, and unscheduled DNA synthesis assay. PEG-150 was not mutagenic in a mouse TK<sup>+</sup>/→ TK<sup>-</sup>/ forward mutation assay. The mutation index ranged from 0.8 to 2.3 (Andersen 1993).

### Polysorbates (PEG Sorbitan Fatty Acid Esters)

Sorbitan Stearate was not mutagenic in bacteria with or without metabolic activation. Sorbitan Stearate did not transform primary Syrian golden hamster embryo cells in vitro. Sorbitan Oleate at a concentration of 0.01% inhibited in vitro DNA repair (Elder 1985).

Polysorbate 80 (PEG-20 Sorbitan Oleate) was nonmutagenic in the Ames and micronucleus tests (Elder 1984a).

An unspecified Sorbitan Fatty Acid Ester (maximum dose = 5.0 mg/plate, in dimethyl sulfoxide (DMSO)) was negative for mutagenicity in the Ames test using *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1535, and TA1537. In the chromosomal aberrations test using Chinese hamster fibroblasts, a maximum dose of 0.3 mg/ml of the test compound (in DMSO) resulted in 5.0% polyploid cells and 8.0% structural aberrations 48 hours after treatment. The results were considered equivocal, and polyploidization effects were observed (Ishidate et al. 1984).

PEG-20 Sorbitan Stearate was not mutagenic in *S. typhimurium* strains TA100 and TA98; the Polysorbate also did not induce in vitro transformation of hamster embryo cells. But in a study examining the role of inhibition of DNA repair as a mechanism in cocarcinogenesis, Sorbitan (0.01%) was found to inhibit the repair of UV-irradiated DNA extracted from normal human lymphocytes (Elder 1985).

### Sorbitol

After being fed to adult *Drosophila*, Sorbitol was negative for whole-chromosome loss and did not cause clastogenic effects or nondisjunction. In these studies, Sorbitol did not appear to cause sex-linked recessive lethals; however, it could not be classified as either positive or negative for mutagenic activity due to an inadequate sample size (Abbott and Bowman 1976).

Chinese hamster ovary cells in medium made hyperosmotic with Sorbitol had significant increases in the incidence of chromosomal aberrations. The test concentrations were 300 to 450 mM. The cells were harvested for aberration analysis 24 to 26 hours after the beginning of the 4-hour treatment period. Cells treated with 300 to 350 mM Sorbitol had 100% survival, and cells treated with 400 and 450 mM had 40% and 15% survival, respectively. Survival was measured after 6 days of colony formation, as a percentage of the untreated control value. The numbers of aberrations per 100 cells were 2 (control), 26 (300 mM; one cell was excluded), 11 (350 mM), 29 (400 mM), and 27 (450 mM; only 30 scoreable cells). The incidences of cells with aberrations were 2% (control), 8% (300 mM), 7% (350 mM), and 17% (400 and 450 mM). The investigators concluded that the increase in aberrations represented an indirect effect on the cells (Galloway et al. 1987).

The addition of sugars such as Sorbitol reduced the mutagenicity of smoke condensates of high- and low-tar cigarettes, as tested using *S. typhimurium* strains TA98 and TA100, with metabolic activation. Cigarettes treated with Sorbitol yielded more tar than untreated cigarettes. When 0.51 g Sorbitol was added to each high-tar cigarette, the percent mutagenicity per mg smoke condensate was 66% (TA100) and 37% (TA98) relative to cigarettes without added sugars. The percent mutagenicity per cigarette was 77% (TA100) and 46% (TA98). When 0.70 g Sorbitol was added to low-tar cigarettes, the percentages were 65% (TA100) and 23% (TA98) per milligram smoke condensate and 184% (TA100) and 66% (TA98) per cigarette. The addition of sugars without metabolic activation had no effect on mutagenicity of the cigarette smoke condensates (Sato et al. 1979).

## CARCINOGENICITY

### PEGs Sorbitan Beeswax

No data were available on the carcinogenicity of the PEGs Sorbitan Beeswax ingredients.

### PEGs

PEG-8 was not carcinogenic when administered orally, intraperitoneally, or subcutaneously to various test animals (Andersen 1993).

### Polysorbates (PEG Sorbitan Fatty Acid Esters)

The Polysorbates have been found in numerous studies to be noncarcinogenic when administered to laboratory animals, although Polysorbate 80 produced some neoplastic changes in

mixed mouse epidermal and dermal in vitro tissue cultures (Elder 1984a). In multiple studies, the Polysorbates have enhanced the activity of known chemical carcinogens while not actually being carcinogenic themselves. Proposed mechanisms of this tumor enhancement included induction of cellular hyperproliferation or inhibition of DNA repair. The Polysorbates also have tumor growth inhibition activity under certain conditions (Elder 1984a).

Mice fed low concentrations of Sorbitan Stearate for 80 weeks had no difference in tumor type and incidence as compared to control animals (Elder 1985). Sorbitan Laurate was inactive as a carcinogen when painted on mouse skin for 73 weeks (Elder 1985).

#### *Sorbitol*

At a concentration of 18% in drinking water (3000 to 7000 mg/kg/day), hydrogenated starch hydrolysates (mixtures of polyhydric alcohols such as ~7.0% Sorbitol) did not produce evidence of carcinogenicity after 2 years of treatment. This study used 50 Sprague-Dawley rats/sex/group. No significant clinical signs of toxicity were observed (Modderman 1993).

In studies using rats, high dietary concentrations of Sorbitol caused enlargement of the cecum, increased absorption of calcium from the gut, increased urinary excretion of calcium, pelvic and corticomedullary nephrocalcinosis, acute tubular nephropathy, urinary calculus formation, and hyperplasia and neoplasia of the adrenal medulla. The investigator concluded that adrenal neoplasms observed in mice fed 20% Sorbitol were laboratory artifacts, and not indicative of human risk exposed to normal concentrations of Sorbitol in the diet (Roe 1984).

### **Cocarcinogenicity**

#### *PEGs Sorbitan Beeswax*

No data were available on the cocarcinogenicity of the PEGs Sorbitan Beeswax ingredients.

#### *Polysorbates (PEG Sorbitan Fatty Acid Esters)*

Sorbitan Laurate applied twice weekly for 75 weeks to the skin of mice after an initiating exposure to 7,12-dimethylbenz(a)anthracene (DMBA) produced more tumors than did DMBA treatment alone. A comparison of Sorbitan Laurate, Sorbitan Oleate, and Sorbitan Trioleate was done in a similar mouse study. DMBA at 0.3%, 0.03%, and 0.003% was used as the tumor initiator, followed by skin treatment with one of the sorbitan fatty acid esters, once or twice daily, 6 days a week, for 52 weeks. With 0.3% DMBA, Sorbitan Laurate produced more tumors than did DMBA treatment alone, but neither Sorbitan Oleate nor Sorbitan Trioleate promoted DMBA carcinogenesis. With 0.03% DMBA, none of the sorbitan fatty acid esters were tumor promoters. With 0.003% DMBA, Sorbitan Laurate and Sorbitan Trioleate produced more tumors than did DMBA treatment alone, but Sorbitan Oleate was not a tumor promoter (Elder 1985).

## **CLINICAL ASSESSMENT OF SAFETY**

### **Oral Toxicity**

The Polysorbates have been ingested by human beings in situations ranging from an accidental administration of 19.2 g of Polysorbate 80 (PEG-20 Sorbitan Oleate) to an infant on 2 consecutive days to daily therapeutic administration of up to 6.0 g of Polysorbate 80 to adults for up to 4 years. In these studies, oral ingestion of the Polysorbates produced little or no adverse effects (Elder 1984a).

### **Dermal Irritation and Sensitization**

PEG-6 and -20 Sorbitan Beeswax (concentrations not specified) were nonsensitizing when patch-tested in 50 subjects. The test materials were applied to 1 × 1-inch cotton twill pads that were affixed to the skin for 72 hours using 2 × 2-inch elastic adhesive patch. Seven days after patch removal, the test compounds were reapplied in the same fashion; no reactions were observed (CTFA 1998b).

An undiluted liquid eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was tested for primary irritancy using 17 subjects. The PII was 0.00, and the formulation was considered nonirritating in the single insult patch test.

In a cumulative irritation study using 12 subjects, a volume of 0.3 ml of the same eyeliner was applied to the skin of the back under a closed patch with Webril pad. Applications were made daily for 21 consecutive days. The total scores were 299/756 (base n = 10) and 357/630 (base n = 12), and the irritating potential for the formulation was classified as "possibly mild in normal use" (CTFA 1998d).

A mascara containing 2.0% PEG-20 Sorbitan Beeswax was tested for irritancy in a 4-day minicumulative patch test. The PII was 0.24/8.0, and the formulation was classified as mild. The number of subjects was not available. In similar studies, a lash primer/conditioner containing 3.0% PEG-20 Sorbitan Beeswax was "adequately mild when used in a conventional manner," and had a PII of 0.25/8.0 to 0.34/8.0 (CTFA 1998d).

The eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was also tested for sensitization using 94 subjects, 4 of whom withdrew from the study for reasons unrelated to the test material. The test sample was applied to the skin of the upper back under a Webril pad affixed to an adhesive bandage and secured with Scanpor tape. Induction applications were made for 24 hours, three times weekly for 3 consecutive weeks. Challenge applications were made to untreated skin sites in weeks 6 to 7. The patches were removed 24 hours later and reactions were scored 24 and 48 hours after patch removal. Two subjects had possible sensitization reactions and were repatched for 24 hours with the test formulation: one subject received the eyeliner as is, and one was repatched with a 50% aqueous dilution of the formulation. The investigators concluded that the eyeliner was nonsensitizing under the conditions of this study (CTFA 1998d).

In a similar sensitization study, a mascara containing 2.0% PEG-20 Sorbitan Beeswax was tested using 89 subjects, 2 of

whom withdrew for reasons unrelated to the test procedure. Under the conditions of the study, the mascara was not a sensitizer (CTFA 1998d).

A lash conditioner containing 2.0% PEG-20 Sorbitan Beeswax was nonirritating and nonsensitizing when tested in an RIPT using 86 subjects (Hill Top Research, Inc. 1988).

A liquid eyeliner containing 1.5% PEG-20 Sorbitan Beeswax was tested for irritancy of the eye area during normal use by 56 subjects. One half of the panelists used the test formulation and one half used a control formulation. After 3 weeks of use, the two groups switched formulations for another three weeks. Dermatologic examinations of the ocular area (browline, sub-orbital area, lids and lid margins, and outer aspects of the eye) were performed at weeks 3 and 6. One panelist had slight scaling on the lid margins after initially using the test eyeliner, but had no subjective discomfort. The eyeliner "[did] not have the potential to evoke adverse effects on the eye area when used under consumer use conditions" and was "very to moderately gentle to the eyes." Similar results were reported for a cream mascara containing 2.0% PEG-20 Sorbitan Beeswax (CTFA 1998d).

The potential of a lash primer/conditioner containing 2.0% PEG-20 Sorbitan Beeswax to cause irritation and subjective discomfort was determined using 19 panelists who used the product prior to application of mascara for 5 consecutive days. No clinical irritation was observed for the eye area exposed to the conditioner, with or without the mascara, and minimal discomfort was reported (CTFA 1998d).

### PEGs

Cases of systemic toxicity and contact dermatitis in burn patients were attributed to a PEG-based topical ointment. In clinical studies, PEG-6 and PEG-8 caused mild immediate hypersensitivity. However, PEG-6, -8, -32, and -75 were not sensitizers (Andersen 1993).

### Polysorbates (PEG Sorbitan Fatty Acid Esters)

In extensive clinical skin testing using the Schwartz prophetic patch test, the Polysorbates had little potential for human skin irritation and produced no evidence of skin sensitization in 580 subjects. When 1206 patients with eczema were tested in a chamber method 24-hour occlusive patch test for allergic contact dermatitis to a mixture of 5% Polysorbate 60 (PEG-20 Sorbitan Stearate) and 5% Polysorbate 80 (PEG-20 Sorbitan Oleate) in petrolatum, reactions were observed in only 2 of the patients (<0.2%). Several product formulations containing Polysorbates have been tested for human skin sensitization using 3481 subjects and a variety of testing methods; no reactions indicative of sensitization were found to any of the Polysorbates in these assays. Investigations using patients known to have skin disease have produced isolated instances of skin sensitization to Polysorbate 40 (PEG-20 Sorbitan Palmitate) or Polysorbate 80. Polysorbate 80 (IV) produced hemodynamic changes in five patients (Elder 1984a).

Results from three RIPTs (involving a total of 420 subjects) indicated that Sorbitan Stearate was not a sensitizer. Products containing low concentrations of Sorbitan Stearate were mild irritants in 21-day cumulative irritation studies (Elder 1985).

In a Schwartz prophetic patch test, Sorbitan Laurate produced no irritation. Results of human skin tests for sensitivity to Sorbitan Sesquioleate indicated that the compound was a nonsensitizer. In two Schwartz prophetic patch tests (60 subjects total) utilizing 30% and 100% concentrations, Sorbitan Sesquioleate produced no reactions. The results of five RIPTs involving 352 subjects indicated that none of the five products containing 1% to 3% Sorbitan Sesquioleate produced sensitization; however, some subjects experienced mild irritation (Elder 1985).

Several products containing 1.75% to 2.0% Sorbitan Oleate have been tested using human subjects. In four 21-day cumulative irritation studies, the products tested were mildly irritating. In the tests using entire product formulations, the specific ingredient(s) causing irritation was not determined. Four RIPTs involving 339 subjects classified the Sorbitan Oleate-containing products as nonsensitizers. No irritation was observed in maximization tests. A product usage test on 53 subjects produced mild irritation in two individuals (Elder 1985).

In a Schwartz prophetic patch test using Sorbitan Tristearate, 211 panelists had no signs of irritation. Sorbitan Palmitate-containing skin products were slightly irritating to the skin of humans in 21-day cumulative irritation tests (34 subjects total). In a Shelanski/Jordan RIPT (206 subjects), a skin care product containing Sorbitan Palmitate was nonirritating and nonsensitizing. Several products containing 5% Sorbitan Trioleate were tested on human subjects. Sorbitan Trioleate-containing products were slightly irritating in 21-day cumulative irritation tests, Shelanski/Jordan RIPT, Modified Schwartz-Peck predictive patch tests, and in a 4-week usage test (Elder 1985).

Sorbitan Isostearate (2.5%) was tested in a RIPT using 201 subjects. During the induction period 48- to 72-hour occlusive patches containing 0.2 g of the test material were applied to the upper arm or back. Patches were applied three times per week for 3 weeks. After a 2-week nontreatment period, a 72-hour challenge patch was applied to a previously unexposed site. Reactions were scored at 96 hours post application. Sorbitan Isostearate did not induce a sensitization response (CTFA 1998e).

A 24-hour occlusive patch test was performed using 56 subjects. A 0.05-ml volume of Sorbitan Isostearate (10.0% in squalene) was applied to the intact skin of the forearm for 24 hours and then the treatment site was examined for signs of primary irritation. None of the subjects reacted to Sorbitan Isostearate under the conditions of this study. Sorbitan Sesquiosostearate (10.0% in squalene) was evaluated similarly using 10 subjects, none of whom reacted to the test material (CTFA 1998c).

A 24-hour occlusive patch test using 56 subjects exposed to 10% Sorbitan Isostearate produced no signs of irritation. In a similar study in 10 subjects, 10% Sorbitan Sesquiosostearate

produced no irritation. Sorbitan Isostearate at a concentration of 2.5% was negative in an RIPT using 201 subjects (CIR 1999).

#### *Beeswax*

The total irritation score of a cream formulation containing 6% Beeswax and 6% Synthetic Beeswax was 6.4/630 for a 21-day cumulative irritation study using 14 subjects. No irritation was observed when 100 women used the formulation daily for 14 days. A Schwartz-Peck prophetic patch test of the above formulation was performed using 98 subjects. Two applications (at 48-hour intervals) were made using open and closed patches. After the second application, photosensitization potential was evaluated using irradiation from a solar simulator at a distance of 12 inches for 1 minute. No irritation, sensitization, or photosensitization reactions were observed. No irritation, sensitization, or photosensitization was observed in 49 subjects exposed to the above formulation during a Draize-Shelanski RIPT, followed by UV irradiation (360 nm). No evidence of contact sensitization was observed when 22 subjects were treated (volar forearm skin sites) with 5% sodium lauryl sulfate 24 hours prior to treatment with the above formulation under occlusive conditions at 48-hour intervals. Challenge took place 10 to 14 days after the last application (Elder 1984b).

No irritation or sensitization was observed after a lipstick (7.2% to 9.4% Synthetic Beeswax) was tested in 896 subjects using an RIPT.

#### **Comedogenicity**

A product containing 5% Sorbitan Isostearate was tested using 20 human subjects to determine its comedogenicity. Reactions that scored a value of 1 or greater, and were statistically different from the negative control, were considered positive for comedogenicity. Data from the global assessment of the test and the control values were compared statistically to determine biological significance ( $p \leq .05$ ). No significant clinical irritation was observed during the study period. Reactions ranging from +0.5 to +1.0 were observed occasionally in 9 of the 20 subjects. Comparison of the test sites and untreated control sites through statistical analysis for the formation of microcomedones yielded a  $p$  value of greater than .05. It was concluded that this product did not produce evidence of comedogenicity (CTFA 1998e).

#### **Photosensitization**

In studies involving exposure to UV light, no evidence of photocontact sensitization to the Polysorbates was observed, although isolated instances of mild irritation occurred following UV exposure after application of formulations containing the Polysorbates (Elder 1984a).

A formulation containing 6% Beeswax and 6% ceresin produced no evidence of photosensitization potential during a Schwartz-Peck prophetic patch test or an RIPT (see "Dermal Irritation and Sensitization") (Elder 1984b).

No photosensitization reactions were observed when 7.2% to 9.4% Synthetic Beeswax in a lipstick was applied to the skin of 83 subjects. The treated skin sites were irradiated with a 150-W solar simulator set at continuous emission of 290 to 400 nm. The treatment and UV exposure were repeated six times, and challenge application was made at 10 days (Elder 1984c).

Photosensitization assessments of products containing Sorbitan Stearate or Sorbitan Oleate classified both products as nonphototoxic and nonphotoallergenic. Sorbitans Laurate, Sesquioleate, Palmitate, and Trioleate did not absorb radiation in the UVA and UVB range in ultraviolet spectral analysis (Elder 1985).

#### **Ocular Irritation**

Seventy-five subjects used a lash primer/conditioner containing 2.0% PEG-20 Sorbitan Beeswax for 6 weeks prior to mascara application. Ophthalmologic examinations were performed initially and after completion of the study. No irritation was observed and the potential to cause subjective discomfort was low (CTFA 1998d).

#### **SUMMARY**

PEG-6, -8, and -20 Sorbitan Beeswax are ethoxylated derivatives of Beeswax that function as surfactants in cosmetic formulations. In 1998, PEG-20 Sorbitan Beeswax was reported used in 16 cosmetic formulations; PEG-6 and -8 Sorbitan Beeswax were not reported used. Data submitted by industry indicated that PEG-20 Sorbitan Beeswax was used at concentrations from 0.2% in make-up fixatives to 11% in blushers. In 1984, it was reported used at concentrations  $\leq 10\%$ .

Few data were available on the PEGs Sorbitan Beeswax. Toxicology data on Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and Polysorbates were reviewed as a further basis for the assessment of safety.

The ester link of the Polysorbate (PEG Sorbitan Fatty Acid Ester) molecule was hydrolyzed by blood and pancreatic lipases after oral administration. The fatty acid moiety was absorbed and metabolized as any other dietary fatty acid, and the PEG Sorbitan moiety was poorly absorbed from the GI tract. GI absorption of PEG was inversely related to the molecular weight of the compound. PEGs are readily absorbed through damaged skin. Sorbitan Stearate was hydrolyzed to the stearic acid and anhydrides of sorbitol, and did not accumulate in the fat stores of the rat.

PEG-6 Sorbitan Beeswax was "practically nontoxic" when rats were treated with doses of 10.0 g/kg during acute IP studies. PEGs had low oral, dermal, and inhalation toxicity; greater molecular weight PEGs were less toxic than smaller molecular weight PEGs. The Polysorbates were not toxic during acute and long-term feeding studies, or during acute and short-term IV and IP injection studies. Formulations containing the Polysorbates produced no evidence of acute or subchronic percutaneous toxicity. Formulations containing up to 13% Beeswax (5 to 15 g/kg

doses) were not toxic to rats. Undiluted Beeswax killed 2 of 10 rats within 2 days during an acute oral toxicity study. Ten rats fed 5 to 14.4 g/kg Synthetic Beeswax had chromorhinorrhea and chromodacryorrhea; rats fed 5 to 10.4 g/kg had diarrhea, ptosis, bulging eyes, and sniffing. Two rats died after ingestion of the high dose.

The Sorbitan Esters (<10%) were relatively nontoxic via ingestion. The lowest LD<sub>50</sub> (rats) reported was 31 g/kg Sorbitan Stearate. No adverse effects were observed when rats, mice, and dogs were fed 5% Sorbitans Laurate, Oleate, and Stearate for up to 2 years. In other studies, the feeding of 0.5%, 4%, and 10% Sorbitan Stearate to mice and rats resulted in depressed growth and renal and/or hepatic abnormalities.

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the eyes of rabbits, and a 30% aqueous solution of PEG-20 Sorbitan Beeswax was minimally irritating (Draize score = 3.5/110). Eye makeup formulations containing 1.5% to 2.0% PEG-20 Sorbitan Beeswax were non- to minimally irritating to the eyes of rabbits. PEGs, Polysorbates, Sorbitan Esters, Beeswax, and Synthetic Beeswax were non- to mild ocular irritants.

Undiluted PEG-6 Sorbitan Beeswax was nonirritating to the intact and abraded skin of rabbits. Cosmetic formulations containing 1.5% to 2.0% PEG-20 Sorbitan Beeswax were non- to minimal irritants to the skin of rabbits. The PEGs were not irritating to the skin of rabbits or guinea pigs, and PEG-75 was not a sensitizer. The Polysorbates had little potential for rabbit and mouse skin irritation during acute studies. Polysorbate 20 was a moderate to strong sensitizer in one study using guinea pigs, and Polysorbates 65 and 80 were nonsensitizers. Synthetic Beeswax (5 g in 1 ml corn oil) had Draize scores of 0 to 2.08 (out of 8.00) during primary irritation studies using rabbits. At a concentration of 50% in water, Synthetic Beeswax was non-sensitizing to guinea pigs. Sorbitan Esters (3% to 100%) were minimal to mild irritants.

Ethylene glycol and certain of its monoalkyl ethers are reproductive and developmental toxins. As PEGs Sorbitan Beeswax are chemically different from these ethers, reproductive and developmental toxicity due to the ethers was not of concern. PEGs did not cause adverse reproductive effects during subchronic and chronic feeding studies.

PEG-8 and -150 were not mutagenic in several genotoxicity assays. Polysorbate 80 was nonmutagenic in the Ames test. sorbitan Stearate was not mutagenic in tests using bacteria, with or without metabolic activation, and did not transform hamster embryo cells in vitro. Sorbitan Oleate (0.01%) inhibited in vitro DNA repair. PEG-8 was not carcinogenic during oral, IP, or subcutaneous (SC) administration. The Polysorbates were generally noncarcinogenic, but enhanced the activity of some known chemical carcinogens. Sorbitan Stearate was not carcinogenic in mice during a feeding study, but Sorbitan Laurate was a tumor promoter during a mouse skin-painting study. Sorbitans Oleate and Trioleate were inactive as tumor promoters. In another study, undiluted Sorbitans Laurate and Trioleate were not cocarcinogens.

In clinical studies, PEG-6 and -20 Sorbitan Beeswax were nonsensitizers. Formulations containing up to 3.0% PEG-20 Sorbitan Beeswax were mildly irritating and nonsensitizing during in-use, minicumulative, and RIPTs. Systemic toxicity and contact dermatitis were observed in burn patients treated with PEG-containing ointments, but PEGs were not sensitizing to normal skin. The Polysorbates and Sorbitan Esters were nontoxic after oral ingestion. Polysorbates, Beeswax, and Synthetic Beeswax did not cause irritation, sensitization, or photosensitization. The Sorbitan Esters were minimal to mild skin irritants in humans, but were nonsensitizing, nonphototoxic, and nonphotoallergenic.

## DISCUSSION

Because there were few data available on the PEGs Sorbitan Beeswax ingredients, the available data from previous safety assessments of Beeswax, Synthetic Beeswax, Sorbitan Esters, PEGs, and PEG Sorbitan fatty acid esters, also known as Polysorbates, was discussed primarily.

Data summarized in this report indicate that Beeswax did not produce any mutagenicity or toxicity in rats and in skin and eye irritation tests it produced minimal to no irritation in rabbits. Beeswax also did not cause any phototoxic reactions in hairless mice, swine, and humans. Subchronic dermal toxicity tests in rabbits and rats produced no topical or systemic effects. In clinical studies, a 21-day cumulative patch test and an RIPT containing 6% Beeswax and 6% Synthetic Beeswax caused no irritation. Based on this data, the Expert Panel determined Beeswax to be safe as used in cosmetics under present practices of concentration and use. The Expert Panel believes the information from the Beeswax report supports a safe as used conclusion for PEG -6, -8, and -20 Sorbitan Beeswax.

The CIR Expert Panel, however, was concerned about the sensitization and toxicity potential of the PEGs Sorbitan/Sorbitol Fatty Acid Esters when applied to damaged skin. This concern arose because of positive patch tests and incidences of nephrotoxicity in burn patients treated with an antimicrobial cream that contained PEG-6, PEG-20, and PEG-75. PEG was the causative agent in both animal and human studies; no evidence of systemic toxicity or sensitization was found in studies with intact skin. The cosmetics industry should consider this information when formulating products with PEGs Sorbitan/Sorbitol Fatty Acid Esters.

Also of concern to the Expert Panel was the possible presence of 1,4-dioxane and ethylene oxide impurities. The Panel stressed that the cosmetics industry should continue to use the necessary procedures to remove these impurities from the PEGs Sorbitan/Sorbitol Fatty Acid Ester ingredients before blending them into cosmetic formulations.

The Expert Panel considered the finding that treatment of normal, human lymphocytes with 0.01% Sorbitan Oleate reduces DNA repair following UV irradiation, and the researchers' hypothesis that this effect could be a mechanism in



cocarcinogenesis. The Panel carefully considered the data on the cocarcinogenesis of the Sorbitan Esters, noting the high exposure levels used, the high frequency of exposure, and the lack of a dose-response, and concluded that the positive response in these studies does not constitute a risk in cosmetic formulations.

## CONCLUSION

Based on the available data on the ingredients themselves and on data on the components, the Expert Panel concludes that PEG -6, -8, and -20 Sorbitan Beeswax are safe for use as cosmetic ingredients under the present practices of use. The Expert Panel recommends that cosmetic formulations containing PEG-6, PEG-20, or PEG-75 not be used on damaged skin.

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<sup>2</sup>Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington DC, 20036, USA.



**Memorandum**

**TO:** Lillian Gill, D.P.A.  
Director - COSMETIC INGREDIENT REVIEW (CIR)

**FROM:** Beth Lange, Ph.D.  
Industry Liaison to the CIR Expert Panel

**DATE:** July 16, 2014

**SUBJECT:** Concentration of Use by FDA Product Category: Polysorbate and Related Ingredients

**Concentration of Use by FDA Product Category\***

Polysorbate 20	PEG-6 Sorbitan Stearate	Sorbeth-50 Hexaoleate (PEG-50 Sorbitol Hexaoleate)
Polysorbate 21	PEG-40 Sorbitan Stearate	Sorbeth-6 Hexastearate
Polysorbate 40	PEG-60 Sorbitan Stearate	Sorbeth-150 Hexastearate
Polysorbate 60	PEG-20 Sorbitan Tetraoleate	Sorbeth-3 Isostearate
Polysorbate 61	PEG-30 Sorbitan Tetraoleate	Sorbeth-6 Laurate
Polysorbate 65	PEG-40 Sorbitan Tetraoleate	Sorbeth-2/Oleate/Dimer
Polysorbate 80	PEG-60 Sorbitan Tetraoleate	Dilinoleate Crosspolymer
Polysorbate 81	PEG-60 Sorbitan	Sorbeth-20 Pentaistearate
Polysorbate 85	Tetrastearate	Sorbeth-30 Pentaistearate
PEG-20 Sorbitan Cocoate	PEG-4 Sorbitan Triisostearate	Sorbeth-40 Pentaistearate
PEG-40 Sorbitan	PEG-20 Sorbitan	Sorbeth-50 Pentaistearate
Diisostearate	Triisostearate	Sorbeth-40 Pentaoleate
PEG-2 Sorbitan Isostearate	PEG-160 Sorbitan	Sorbeth-20 Tetraistearate
PEG-5 Sorbitan Isostearate	Triisostearate	Sorbeth-30 Tetraistearate
PEG-20 Sorbitan Isostearate	PEG-2 Sorbitan Trioleate	Sorbeth-40 Tetraistearate
PEG-40 Sorbitan Lanolate	PEG-18 Sorbitan Trioleate	Sorbeth-50 Tetraistearate
PEG-75 Sorbitan Lanolate	PEG-3 Sorbitan Tristearate	Sorbeth-4 Tetraoleate
PEG-10 Sorbitan Laurate	Sorbeth-2 Beeswax	Sorbeth-6 Tetraoleate
PEG-40 Sorbitan Laurate	Sorbeth-6 Beeswax	Sorbeth-30 Tetraoleate
PEG-44 Sorbitan Laurate	Sorbeth-8 Beeswax	Sorbeth-40 Tetraoleate
PEG-75 Sorbitan Laurate	Sorbeth-20 Beeswax	Sorbeth-60 Tetraoleate
PEG-80 Sorbitan Laurate	Sorbeth-2 Cocoate	Sorbeth-30 Tetraoleate
PEG-3 Sorbitan Oleate	Sorbeth-2	Laurate (PEG-30 Sorbitol Tetraoleate Laurate)
PEG-6 Sorbitan Oleate	Hexacaprylate/Caprata	Sorbeth-60 Tetrastearate
PEG-20 Sorbitan Oleate	Sorbeth-12 Hexacocoate	(PEG-60 Sorbitol Tetrastearate)
PEG-40 Sorbitan Oleate	Sorbeth-2 Hexaistearate	Sorbeth-3 Tristearate
PEG-80 Sorbitan Palmitate	Sorbeth-2 Hexalaurate	Sorbeth-160 Tristearate
PEG-40 Sorbitan	Sorbeth-2 Hexaoleate	Sorbeth-450 Tristearate
Perisostearate	Sorbeth-40 Hexaoleate (PEG-40 Sorbitol Hexaoleate)	
PEG-40 Sorbitan Peroleate		
PEG-3 Sorbitan Stearate		

<b>Ingredient Name</b>	<b>Product Category</b>	<b>Maximum Concentration of Use</b>
Polysorbate 20	Baby shampoo	12.6%
Polysorbate 20	Baby lotions, oils and creams not powder	2%
Polysorbate 20	Other baby products	0.00078%
Polysorbate 20	Bath oils, tablets and salts	0.0097%
Polysorbate 20	Bubble baths	2-8.9%
Polysorbate 20	Other bath preparations	0.4-5%

Polysorbate 20	Eyebrow pencil	0.01-2%
Polysorbate 20	Eye liner	0.049-3.1%
Polysorbate 20	Eye shadow	0.035-3.5%
Polysorbate 20	Eye lotion	0.00015-3%
Polysorbate 20	Eye makeup remover	0.5-3.5%
Polysorbate 20	Mascara	0.025-2%
Polysorbate 20	Other eye makeup preparations	0.5-0.59%
Polysorbate 20	Colognes and toilet waters	0.97%
Polysorbate 20	Perfumes	0.00021-0.02%
Polysorbate 20	Powders (dusting and talcum)	0.00075-1.1%
Polysorbate 20	Hair conditioners	0.006-2.5%
Polysorbate 20	Hair sprays aerosol pump spray	0.027-3% 0.4-1%
Polysorbate 20	Shampoos (noncoloring)	0.17-6.1%
Polysorbate 20	Tonics, dressings and other hair grooming aids	0.24-6%
Polysorbate 20	Other hair preparations (noncoloring)	0.5%
Polysorbate 20	Hair dyes and colors	1-1.5%
Polysorbate 20	Hair bleaches	0.4%
Polysorbate 20	Other hair coloring preparations	3.8%
Polysorbate 20	Blushers	1.4%
Polysorbate 20	Face powders	0.0027-3%
Polysorbate 20	Foundations	0.007-2.3%
Polysorbate 20	Lipstick	1%
Polysorbate 20	Makeup bases	0.01-0.5%
Polysorbate 20	Makeup fixatives	0.0003-1%
Polysorbate 20	Other makeup preparations	0.3-0.5%
Polysorbate 20	Cuticle softeners	0.23-2%
Polysorbate 20	Nail creams and lotions	0.000041%
Polysorbate 20	Nail polish and enamel	0.03%
Polysorbate 20	Nail polish and enamel removers	0.9%
Polysorbate 20	Other manicuring preparations	3.3%
Polysorbate 20	Mouthwash and breath fresheners	0.34-2.9%
Polysorbate 20	Other oral hygiene products	0.01-5.8%
Polysorbate 20	Bath soaps and detergents	0.0006-19.6%
Polysorbate 20	Deodorants not spray aerosol pump spray	0.00082-3% 0.00018% 4%
Polysorbate 20	Feminine hygiene deodorants	0.76%
Polysorbate 20	Other personal cleanliness products	0.3%
Polysorbate 20	Aftershave lotions	0.00088-0.98%
Polysorbate 20	Shaving cream	0.97-2.4%
Polysorbate 20	Other shaving preparations	1%
Polysorbate 20	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.014-2.5%

Polysorbate 20	Depilatories	1%
Polysorbate 20	Face and neck products not spray	0.002-9.1%
Polysorbate 20	Body and hand products not spray spray	0.0006-2% 0.00001-1.2%
Polysorbate 20	Foot products	0.97-2%
Polysorbate 20	Moisturizing products not spray spray	0.5-2% 0.1%
Polysorbate 20	Night products not spray	0.0013-2%
Polysorbate 20	Paste masks and mud packs	0.05-3%
Polysorbate 20	Skin fresheners	0.95-2.8%
Polysorbate 20	Other skin care preparations	0.015-2.5%
Polysorbate 20	Suntan products not spray	0.03%
Polysorbate 20	Indoor tanning products	0.0019-3%
Polysorbate 20	Other suntan preparations	0.5-1%
Polysorbate 21	Mascara	0.5%
Polysorbate 21	Other eye makeup preparations	0.5%
Polysorbate 21	Hair conditioners	0.5%
Polysorbate 21	Hair sprays aerosol	0.33%
Polysorbate 21	Shampoos (noncoloring)	0.5-8%
Polysorbate 21	Hair dyes and colors	2.4%
Polysorbate 21	Other shaving preparations	0.5%
Polysorbate 21	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.6%
Polysorbate 21	Face and neck products not spray	0.38%
Polysorbate 21	Other skin care preparations	2%
Polysorbate 40	Eyeline	0.015%
Polysorbate 40	Eye lotion	3.75%
Polysorbate 40	Mascara	0.025%
Polysorbate 40	Tonics, dressings and other hair grooming aids	0.8-2.5%
Polysorbate 40	Makeup fixative	0.01%
Polysorbate 40	Bath soaps and detergents	3%
Polysorbate 40	Aftershave lotions	0.008%
Polysorbate 40	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	1.8-2.5%
Polysorbate 40	Depilatories	2.4%
Polysorbate 40	Face and neck products not spray	0.019-5%
Polysorbate 40	Body and hand products not spray	1.8%

Polysorbate 40	Paste masks and mud packs	1.5%
Polysorbate 40	Skin fresheners	0.5%
Polysorbate 40	Suntan products not spray	3%
Polysorbate 60	Baby lotions, oils and creams not powder	1-1.5%
Polysorbate 60	Other baby products	0.00009%
Polysorbate 60	Bath oils, tablets and salts	0.0015-0.06%
Polysorbate 60	Eyebrow pencil	0.21-1%
Polysorbate 60	Eyeline	1-3.8%
Polysorbate 60	Eye shadow	2.9%
Polysorbate 60	Eye lotion	0.0021-0.7%
Polysorbate 60	Eye makeup removers	0.3%
Polysorbate 60	Mascara	0.1-1.4%
Polysorbate 60	Colognes and toilet water	0.0025%
Polysorbate 60	Hair conditioners	0.00001-1%
Polysorbate 60	Hair straighteners	1.5-4%
Polysorbate 60	Permanent waves	5%
Polysorbate 60	Shampoos (noncoloring)	0.0000001-1%
Polysorbate 60	Tonics, dressings and other hair grooming aids	0.29-4%
Polysorbate 60	Wave sets	1%
Polysorbate 60	Other hair preparations (noncoloring)	0.16%
Polysorbate 60	Hair dyes and colors	0.002-2.5%
Polysorbate 60	Hair tints	1%
Polysorbate 60	Blushers	3%
Polysorbate 60	Face powders	0.053%
Polysorbate 60	Foundations	0.016-3.5%
Polysorbate 60	Lipstick	0.2-0.4%
Polysorbate 60	Makeup bases	1-2.5%
Polysorbate 60	Cuticle softeners	3.5%
Polysorbate 60	Bath soaps and detergents	0.0008-2%
Polysorbate 60	Deodorants not spray	0.2%
Polysorbate 60	Aftershave lotions	0.0068-1.8%
Polysorbate 60	Shaving cream	0.75-3%
Polysorbate 60	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.4-6%
Polysorbate 60	Face and neck products not spray	0.018-3.7%
Polysorbate 60	Body and hand products not spray spray	0.025-2% 0.083-0.8%
Polysorbate 60	Foot products	2.4%
Polysorbate 60	Moisturizing products not spray	0.099-2.2%
Polysorbate 60	Night products	

	not spray	0.027-1.5%
Polysorbate 60	Paste masks and mud packs	0.3-2%
Polysorbate 60	Skin fresheners	0.6%
Polysorbate 60	Other skin care preparations	0.3-3.4%
Polysorbate 60	Suntan products not spray	0.083-2.4%
Polysorbate 60	Indoor tanning preparations	0.0005-0.54%
Polysorbate 60	Other suntan preparations	0.0089-0.5%
Polysorbate 61	Foundations	1%
Polysorbate 61	Other makeup preparations	1%
Polysorbate 61	Face and neck products not spray	1.8%
Polysorbate 65	Eye lotion	0.5%
Polysorbate 65	Foundation	0.5%
Polysorbate 65	Basecoats and undercoats (manicuring preparations)	0.002%
Polysorbate 65	Cuticle softeners	0.003%
Polysorbate 65	Other manicuring preparations	0.003% (rinse-off)
Polysorbate 65	Deodorants not spray	0.0003%
Polysorbate 65	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.002%
Polysorbate 65	Face and neck products not spray	0.003%
Polysorbate 65	Body and hand products not spray	0.003-3%
Polysorbate 65	Moisturizing products not spray	0.5-3%
Polysorbate 65	Paste masks and mud packs	0.003-0.15%
Polysorbate 65	Other skin care preparations	0.003%
Polysorbate 80	Other baby products	10%
Polysorbate 80	Eyebrow pencil	0.05%
Polysorbate 80	Eyeliners	0.005-11%
Polysorbate 80	Eye shadow	0.0024-1%
Polysorbate 80	Eye lotion	0.19-0.5%
Polysorbate 80	Eye makeup removers	1%
Polysorbate 80	Mascara	0.5-1%
Polysorbate 80	Perfume	11.9%
Polysorbate 80	Hair conditioner	0.63-1.3%
Polysorbate 80	Hair sprays aerosol pump spray	0.078-1.6% 0.02-0.2%
Polysorbate 80	Shampoo (noncoloring)	0.021-10%
Polysorbate 80	Tonics, dressings and other hair grooming aids	0.35-2%
Polysorbate 80	Hair dyes and colors	0.36%
Polysorbate 80	Blushers	1.2-1.8%

Polysorbate 80	Face powder	0.42-2%
Polysorbate 80	Foundation	0.00075-1%
Polysorbate 80	Lipstick	0.00031-1.5%
Polysorbate 80	Makeup base	0.13%
Polysorbate 80	Dentifrices	0.012%
Polysorbate 80	Mouthwash and breath fresheners	0.0038%
Polysorbate 80	Bath soaps and detergents	0.04%
Polysorbate 80	Aftershave lotion	0.068-3%
Polysorbate 80	Beard softener	0.7%
Polysorbate 80	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.038-8%
Polysorbate 80	Depilatories	1.7%
Polysorbate 80	Face and neck products not spray spray	0.005-2% 0.39%
Polysorbate 80	Body and hand products not spray	0.0075-1.2%
Polysorbate 80	Moisturizing products not spray	0.15-1%
Polysorbate 80	Night products not spray	0.006-0.5%
Polysorbate 80	Paste masks and mud packs	2-18.1%
Polysorbate 80	Skin fresheners	1%
Polysorbate 80	Other skin care preparations	0.23-0.8%
Polysorbate 80	Suntan products not spray	4.9%
Polysorbate 80	Indoor tanning preparations	0.3%
Polysorbate 81	Bath oils, tablets and salts	5-7.5%
Polysorbate 81	Other bath preparations	7.5%
Polysorbate 81	Eye shadow	0.5%
Polysorbate 81	Perfume	5%
Polysorbate 81	Hair conditioner	0.4%
Polysorbate 81	Foundation	0.5%
Polysorbate 81	Nail creams and lotions	7.5%
Polysorbate 81	Other manicuring preparations	7.5% (rinse-off)
Polysorbate 81	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.8-25.6%
Polysorbate 81	Body and hand products not spray	5%
Polysorbate 85	Other bath preparations	0.03-0.055%
Polysorbate 85	Other makeup preparations	6%
Polysorbate 85	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	5.5-21.9%
Polysorbate 85	Face and neck products not spray	0.54%
Polysorbate 85	Body and hand products	



	not spray	0.06-0.11%
Polysorbate 85	Night products not spray	0.03-0.055%
PEG-20 Sorbitan Cocoate	Face and neck products not spray	0.03-0.3%
PEG-20 Sorbitan Cocoate	Body and hand products not spray	0.18%
PEG-20 Sorbitan Cocoate	Moisturizing products not spray	0.03%
PEG-20 Sorbitan Cocoate	Paste masks and mud packs	0.06%
PEG-40 Sorbitan Diisostearate	Tonics, dressings and other hair grooming aids	1%
PEG-20 Sorbitan Isostearate	Body and hand products not spray	0.3%
PEG-40 Sorbitan Laurate	Shampoos (noncoloring)	0.5%
PEG-40 Sorbitan Laurate	Hair dyes and colors	0.25%
PEG-40 Sorbitan Laurate	Other skin care preparations	2%
PEG-44 Sorbitan Laurate	Shampoos (noncoloring)	0.5%
PEG-44 Sorbitan Laurate	Other skin care preparations	2%
PEG-75 Sorbitan Laurate	Shampoos (noncoloring)	0.5%
PEG-75 Sorbitan Laurate	Other skin care preparations	2%
PEG-80 Sorbitan Laurate	Baby shampoo	4.2%
PEG-80 Sorbitan Laurate	Bubble bath	2.5%
PEG-80 Sorbitan Laurate	Shampoos (noncoloring)	4.2%
PEG-80 Sorbitan Laurate	Lipstick	0.059%
PEG-80 Sorbitan Laurate	Bath soaps and detergents	3-4.2%
PEG-80 Sorbitan Laurate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	2%
PEG-80 Sorbitan Laurate	Face and neck products not spray	0.0098%
PEG-80 Sorbitan Laurate	Body and hand products not spray	0.0002%
PEG-6 Sorbitan Oleate	Blushers	0.43%
PEG-40 Sorbitan Peroleate	Tonics, dressings and other hair grooming aids	4%
PEG-40 Sorbitan Peroleate	Aftershave lotion	0.16%
PEG-40 Sorbitan Peroleate	Face and neck products not spray	0.9%
PEG-40 Sorbitan Peroleate	Body and hand products not spray	0.3%
PEG-40 Sorbitan Peroleate	Moisturizing products not spray	1%
PEG-6 Sorbitan Stearate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	3.4%
PEG-30 Sorbitan Tetraoleate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	10%
PEG-60 Sorbitan Tetraoleate	Tonics, dressings and other hair grooming aids	0.5%

PEG-60 Sorbitan Tetraoleate	Body and hand creams, lotions and powders not spray	0.9%
PEG-60 Sorbitan Tetraoleate	Skin fresheners	0.8%
PEG-60 Sorbitan Tetraoleate	Other skin care preparations	0.8%
Sorbeth-6 Beeswax	Moisturizing products not spray	2%
Sorbeth-20 Beeswax	Mascara	2.8%
Sorbeth-20 Beeswax	Lipstick	2.5%
Sorbeth-20 Beeswax	Face and neck products not spray	0.5-1%
Sorbeth-6 Tetraoleate	Face powder	0.21%
Sorbeth-30 Tetraoleate	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.11-10.8%
Sorbeth-40 Tetraoleate	Face and neck products not spray	0.5%
Sorbeth-40 Tetraoleate	Other skin care preparations	0.5%

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

Information collected in 2014  
Table prepared July 15, 2014

**2014 VCRP Use Data - Polysorbates**

01A - Baby Shampoos	POLYSORBATE 20	7
01B - Baby Lotions, Oils, Powders, and Creams	POLYSORBATE 20	5
01C - Other Baby Products	POLYSORBATE 20	19
02A - Bath Oils, Tablets, and Salts	POLYSORBATE 20	17
02B - Bubble Baths	POLYSORBATE 20	56
02D - Other Bath Preparations	POLYSORBATE 20	16
03A - Eyebrow Pencil	POLYSORBATE 20	1
03B - Eyeliner	POLYSORBATE 20	20
03C - Eye Shadow	POLYSORBATE 20	58
03D - Eye Lotion	POLYSORBATE 20	51
03E - Eye Makeup Remover	POLYSORBATE 20	13
03F - Mascara	POLYSORBATE 20	22
03G - Other Eye Makeup Preparations	POLYSORBATE 20	46
04B - Perfumes	POLYSORBATE 20	8
04C - Powders (dusting and talcum, excluding aftershave talc)	POLYSORBATE 20	7
04E - Other Fragrance Preparation	POLYSORBATE 20	13
05A - Hair Conditioner	POLYSORBATE 20	117
05B - Hair Spray (aerosol fixatives)	POLYSORBATE 20	24
05C - Hair Straighteners	POLYSORBATE 20	7
05D - Permanent Waves	POLYSORBATE 20	11
05F - Shampoos (non-coloring)	POLYSORBATE 20	152
05G - Tonics, Dressings, and Other Hair Grooming Aids	POLYSORBATE 20	194
05H - Wave Sets	POLYSORBATE 20	15
05I - Other Hair Preparations	POLYSORBATE 20	107
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	POLYSORBATE 20	95
06E - Hair Color Sprays (aerosol)	POLYSORBATE 20	1
06G - Hair Bleaches	POLYSORBATE 20	2
06H - Other Hair Coloring Preparation	POLYSORBATE 20	1
07A - Blushers (all types)	POLYSORBATE 20	16
07B - Face Powders	POLYSORBATE 20	44
07C - Foundations	POLYSORBATE 20	37
07D - Leg and Body Paints	POLYSORBATE 20	1
07E - Lipstick	POLYSORBATE 20	12
07F - Makeup Bases	POLYSORBATE 20	8
07G - Rouges	POLYSORBATE 20	1
07I - Other Makeup Preparations	POLYSORBATE 20	25
08A - Basecoats and Undercoats	POLYSORBATE 20	2
08B - Cuticle Softeners	POLYSORBATE 20	1
08C - Nail Creams and Lotions	POLYSORBATE 20	1
08E - Nail Polish and Enamel	POLYSORBATE 20	3
08F - Nail Polish and Enamel Removers	POLYSORBATE 20	1
08G - Other Manicuring Preparations	POLYSORBATE 20	2
09A - Dentifrices	POLYSORBATE 20	1
09B - Mouthwashes and Breath Fresheners	POLYSORBATE 20	8
09C - Other Oral Hygiene Products	POLYSORBATE 20	11

10A - Bath Soaps and Detergents	POLYSORBATE 20	405
10B - Deodorants (underarm)	POLYSORBATE 20	11
10C - Douches	POLYSORBATE 20	3
10E - Other Personal Cleanliness Products	POLYSORBATE 20	154
11A - Aftershave Lotion	POLYSORBATE 20	22
11D - Preshave Lotions (all types)	POLYSORBATE 20	2
11E - Shaving Cream	POLYSORBATE 20	8
11F - Shaving Soap	POLYSORBATE 20	2
11G - Other Shaving Preparation Products	POLYSORBATE 20	6
12A - Cleansing	POLYSORBATE 20	155
12C - Face and Neck (exc shave)	POLYSORBATE 20	254
12D - Body and Hand (exc shave)	POLYSORBATE 20	95
12E - Foot Powders and Sprays	POLYSORBATE 20	5
12F - Moisturizing	POLYSORBATE 20	192
12G - Night	POLYSORBATE 20	53
12H - Paste Masks (mud packs)	POLYSORBATE 20	21
12I - Skin Fresheners	POLYSORBATE 20	46
12J - Other Skin Care Preps	POLYSORBATE 20	133
13A - Suntan Gels, Creams, and Liquids	POLYSORBATE 20	3
13B - Indoor Tanning Preparations	POLYSORBATE 20	23
13C - Other Suntan Preparations	POLYSORBATE 20	5
		2857

03F - Mascara	POLYSORBATE 21	3
03G - Other Eye Makeup Preparations	POLYSORBATE 21	1
05A - Hair Conditioner	POLYSORBATE 21	1
05F - Shampoos (non-coloring)	POLYSORBATE 21	7
05G - Tonics, Dressings, and Other Hair Grooming Aids	POLYSORBATE 21	1
05I - Other Hair Preparations	POLYSORBATE 21	5
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	POLYSORBATE 21	24
10A - Bath Soaps and Detergents	POLYSORBATE 21	1
10E - Other Personal Cleanliness Products	POLYSORBATE 21	2
11E - Shaving Cream	POLYSORBATE 21	1
11G - Other Shaving Preparation Products	POLYSORBATE 21	2
12F - Moisturizing	POLYSORBATE 21	3
12J - Other Skin Care Preps	POLYSORBATE 21	2
13A - Suntan Gels, Creams, and Liquids	POLYSORBATE 21	1
		54

03D - Eye Lotion	POLYSORBATE 40	9
03F - Mascara	POLYSORBATE 40	2
03G - Other Eye Makeup Preparations	POLYSORBATE 40	1
05G - Tonics, Dressings, and Other Hair Grooming Aids	POLYSORBATE 40	1
07C - Foundations	POLYSORBATE 40	2
07I - Other Makeup Preparations	POLYSORBATE 40	1
09B - Mouthwashes and Breath Fresheners	POLYSORBATE 40	1
10A - Bath Soaps and Detergents	POLYSORBATE 40	2
10E - Other Personal Cleanliness Products	POLYSORBATE 40	1
12A - Cleansing	POLYSORBATE 40	10
12C - Face and Neck (exc shave)	POLYSORBATE 40	18
12D - Body and Hand (exc shave)	POLYSORBATE 40	2
12E - Foot Powders and Sprays	POLYSORBATE 40	1
12F - Moisturizing	POLYSORBATE 40	16
12G - Night	POLYSORBATE 40	5
12H - Paste Masks (mud packs)	POLYSORBATE 40	1
12J - Other Skin Care Preps	POLYSORBATE 40	5
		78

01B - Baby Lotions, Oils, Powders, and Creams	POLYSORBATE 60	10
01C - Other Baby Products	POLYSORBATE 60	1
02A - Bath Oils, Tablets, and Salts	POLYSORBATE 60	2
02D - Other Bath Preparations	POLYSORBATE 60	1
03A - Eyebrow Pencil	POLYSORBATE 60	9
03B - Eyeliner	POLYSORBATE 60	6
03D - Eye Lotion	POLYSORBATE 60	26
03F - Mascara	POLYSORBATE 60	12
03G - Other Eye Makeup Preparations	POLYSORBATE 60	18
04C - Powders (dusting and talcum, excluding aftershave talc)	POLYSORBATE 60	1
04E - Other Fragrance Preparation	POLYSORBATE 60	2
05A - Hair Conditioner	POLYSORBATE 60	77
05B - Hair Spray (aerosol fixatives)	POLYSORBATE 60	1
05C - Hair Straighteners	POLYSORBATE 60	50
05D - Permanent Waves	POLYSORBATE 60	4
05F - Shampoos (non-coloring)	POLYSORBATE 60	9
05G - Tonics, Dressings, and Other Hair Grooming Aids	POLYSORBATE 60	58
05I - Other Hair Preparations	POLYSORBATE 60	19
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	POLYSORBATE 60	91
06B - Hair Tints	POLYSORBATE 60	1
06C - Hair Rinses (coloring)	POLYSORBATE 60	1
06F - Hair Lighteners with Color	POLYSORBATE 60	1

06H - Other Hair Coloring Preparation	POLYSORBATE 60	13
07A - Blushers (all types)	POLYSORBATE 60	5
07B - Face Powders	POLYSORBATE 60	6
07C - Foundations	POLYSORBATE 60	16
07D - Leg and Body Paints	POLYSORBATE 60	5
07E - Lipstick	POLYSORBATE 60	13
07F - Makeup Bases	POLYSORBATE 60	2
07G - Rouges	POLYSORBATE 60	2
07I - Other Makeup Preparations	POLYSORBATE 60	18
08B - Cuticle Softeners	POLYSORBATE 60	1
08C - Nail Creams and Lotions	POLYSORBATE 60	1
09B - Mouthwashes and Breath Fresheners	POLYSORBATE 60	2
10A - Bath Soaps and Detergents	POLYSORBATE 60	8
10B - Deodorants (underarm)	POLYSORBATE 60	1
10E - Other Personal Cleanliness Products	POLYSORBATE 60	26
11A - Aftershave Lotion	POLYSORBATE 60	11
11E - Shaving Cream	POLYSORBATE 60	12
11G - Other Shaving Preparation Products	POLYSORBATE 60	2
12A - Cleansing	POLYSORBATE 60	78
12C - Face and Neck (exc shave)	POLYSORBATE 60	110
12D - Body and Hand (exc shave)	POLYSORBATE 60	199
12E - Foot Powders and Sprays	POLYSORBATE 60	2
12F - Moisturizing	POLYSORBATE 60	429
12G - Night	POLYSORBATE 60	42
12H - Paste Masks (mud packs)	POLYSORBATE 60	29
12I - Skin Fresheners	POLYSORBATE 60	5
12J - Other Skin Care Preps	POLYSORBATE 60	58
13A - Suntan Gels, Creams, and Liquids	POLYSORBATE 60	10
13B - Indoor Tanning Preparations	POLYSORBATE 60	51
13C - Other Suntan Preparations	POLYSORBATE 60	5
		1562

01B - Baby Lotions, Oils, Powders, and Creams	POLYSORBATE 61	1
12C - Face and Neck (exc shave)	POLYSORBATE 61	2
12D - Body and Hand (exc shave)	POLYSORBATE 61	3
12F - Moisturizing	POLYSORBATE 61	8
		14

03D - Eye Lotion	POLYSORBATE 65	3
03G - Other Eye Makeup Preparations	POLYSORBATE 65	2
12A - Cleansing	POLYSORBATE 65	2
12D - Body and Hand (exc shave)	POLYSORBATE 65	8
12F - Moisturizing	POLYSORBATE 65	6
12G - Night	POLYSORBATE 65	1
12H - Paste Masks (mud packs)	POLYSORBATE 65	1
12J - Other Skin Care Preps	POLYSORBATE 65	1
		24

01A - Baby Shampoos	POLYSORBATE 80	2
01B - Baby Lotions, Oils, Powders, and Creams	POLYSORBATE 80	2
02A - Bath Oils, Tablets, and Salts	POLYSORBATE 80	1
02B - Bubble Baths	POLYSORBATE 80	2
02D - Other Bath Preparations	POLYSORBATE 80	4
03B - Eyeliner	POLYSORBATE 80	23
03C - Eye Shadow	POLYSORBATE 80	26
03D - Eye Lotion	POLYSORBATE 80	35
03E - Eye Makeup Remover	POLYSORBATE 80	2
03F - Mascara	POLYSORBATE 80	3
03G - Other Eye Makeup Preparations	POLYSORBATE 80	22
04A - Cologne and Toilet waters	POLYSORBATE 80	1
04B - Perfumes	POLYSORBATE 80	4
04C - Powders (dusting and talcum, excluding aftershave talc)	POLYSORBATE 80	5
04E - Other Fragrance Preparation	POLYSORBATE 80	4
05A - Hair Conditioner	POLYSORBATE 80	14
05B - Hair Spray (aerosol fixatives)	POLYSORBATE 80	6
05D - Permanent Waves	POLYSORBATE 80	1
05F - Shampoos (non-coloring)	POLYSORBATE 80	17
05G - Tonics, Dressings, and Other Hair Grooming Aids	POLYSORBATE 80	45
05H - Wave Sets	POLYSORBATE 80	3
05I - Other Hair Preparations	POLYSORBATE 80	27
06B - Hair Tints	POLYSORBATE 80	22
06D - Hair Shampoos (coloring)	POLYSORBATE 80	1
06H - Other Hair Coloring Preparation	POLYSORBATE 80	1
07A - Blushers (all types)	POLYSORBATE 80	11
07B - Face Powders	POLYSORBATE 80	13
07C - Foundations	POLYSORBATE 80	19
07E - Lipstick	POLYSORBATE 80	5
07F - Makeup Bases	POLYSORBATE 80	2
07G - Rouges	POLYSORBATE 80	1
07I - Other Makeup Preparations	POLYSORBATE 80	9
08B - Cuticle Softeners	POLYSORBATE 80	1
08C - Nail Creams and Lotions	POLYSORBATE 80	2

08G - Other Manicuring Preparations	POLYSORBATE 80	4
09A - Dentifrices	POLYSORBATE 80	2
09B - Mouthwashes and Breath Fresheners	POLYSORBATE 80	39
10A - Bath Soaps and Detergents	POLYSORBATE 80	8
10B - Deodorants (underarm)	POLYSORBATE 80	1
10C - Douches	POLYSORBATE 80	2
10E - Other Personal Cleanliness Products	POLYSORBATE 80	13
11A - Aftershave Lotion	POLYSORBATE 80	4
11E - Shaving Cream	POLYSORBATE 80	1
11F - Shaving Soap	POLYSORBATE 80	1
11G - Other Shaving Preparation Products	POLYSORBATE 80	4
12A - Cleansing	POLYSORBATE 80	20
12C - Face and Neck (exc shave)	POLYSORBATE 80	103
12D - Body and Hand (exc shave)	POLYSORBATE 80	87
12E - Foot Powders and Sprays	POLYSORBATE 80	1
12F - Moisturizing	POLYSORBATE 80	181
12G - Night	POLYSORBATE 80	22
12H - Paste Masks (mud packs)	POLYSORBATE 80	13
12I - Skin Fresheners	POLYSORBATE 80	9
12J - Other Skin Care Preps	POLYSORBATE 80	42
13A - Suntan Gels, Creams, and Liquids	POLYSORBATE 80	3
13B - Indoor Tanning Preparations	POLYSORBATE 80	10
13C - Other Suntan Preparations	POLYSORBATE 80	1
		907

02A - Bath Oils, Tablets, and Salts	POLYSORBATE 85	13
03B - Eyeliner	POLYSORBATE 85	1
03C - Eye Shadow	POLYSORBATE 85	1
03E - Eye Makeup Remover	POLYSORBATE 85	2
04E - Other Fragrance Preparation	POLYSORBATE 85	1
07B - Face Powders	POLYSORBATE 85	1
07C - Foundations	POLYSORBATE 85	5
07I - Other Makeup Preparations	POLYSORBATE 85	1
10E - Other Personal Cleanliness Products	POLYSORBATE 85	1
11G - Other Shaving Preparation Products	POLYSORBATE 85	2
12A - Cleansing	POLYSORBATE 85	10
12D - Body and Hand (exc shave)	POLYSORBATE 85	5
12F - Moisturizing	POLYSORBATE 85	5
12J - Other Skin Care Preps	POLYSORBATE 85	2
13A - Suntan Gels, Creams, and Liquids	POLYSORBATE 85	1
		51



12F - Moisturizing	PEG-3 SORBITAN OLEATE	1
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12H - Paste Masks (mud packs)	PEG-3 SORBITAN STEARATE	1
12J - Other Skin Care Preps	PEG-3 SORBITAN STEARATE	2
		3

12A - Cleansing	PEG-6 SORBITAN STEARATE	2
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12A - Cleansing	PEG-10 SORBITAN LAURATE	1
12I - Skin Fresheners	PEG-10 SORBITAN LAURATE	1
		2

03D - Eye Lotion	PEG-20 SORBITAN COCOATE	1
12C - Face and Neck (exc shave)	PEG-20 SORBITAN COCOATE	3
12F - Moisturizing	PEG-20 SORBITAN COCOATE	3
12G - Night	PEG-20 SORBITAN COCOATE	2
		9

12D - Body and Hand (exc shave)	PEG-20 SORBITAN ISOSTEARATE	2
12G - Night	PEG-20 SORBITAN ISOSTEARATE	1
		3

12A - Cleansing	PEG-30 SORBITAN TETRAOLEATE	1
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12F - Moisturizing	PEG-40 SORBITAN DIISOSTEARATE	2
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03D - Eye Lotion	PEG-40 SORBITAN PEROLEATE	1
03G - Other Eye Makeup Preparations	PEG-40 SORBITAN PEROLEATE	4
05G - Tonics, Dressings, and Other Hair Grooming Aids	PEG-40 SORBITAN PEROLEATE	3
12C - Face and Neck (exc shave)	PEG-40 SORBITAN PEROLEATE	8
12D - Body and Hand (exc shave)	PEG-40 SORBITAN PEROLEATE	5
12F - Moisturizing	PEG-40 SORBITAN PEROLEATE	10
12H - Paste Masks (mud packs)	PEG-40 SORBITAN PEROLEATE	2
12J - Other Skin Care Preps	PEG-40 SORBITAN PEROLEATE	16
		49

01B - Baby Lotions, Oils, Powders, and Creams	PEG-40 SORBITAN STEARATE	1
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12D - Body and Hand (exc shave)	PEG-40 SORBITAN TETRAOLEATE	1
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12A - Cleansing	PEG-44 SORBITAN LAURATE	2
12I - Skin Fresheners	PEG-44 SORBITAN LAURATE	1
12J - Other Skin Care Preps	PEG-44 SORBITAN LAURATE	5
		8

01A - Baby Shampoos	PEG-80 SORBITAN LAURATE	13
01C - Other Baby Products	PEG-80 SORBITAN LAURATE	17
02B - Bubble Baths	PEG-80 SORBITAN LAURATE	8
02D - Other Bath Preparations	PEG-80 SORBITAN LAURATE	4
05F - Shampoos (non-coloring)	PEG-80 SORBITAN LAURATE	8
07E - Lipstick	PEG-80 SORBITAN LAURATE	2
10A - Bath Soaps and Detergents	PEG-80 SORBITAN LAURATE	11
10E - Other Personal Cleanliness Products	PEG-80 SORBITAN LAURATE	9
12A - Cleansing	PEG-80 SORBITAN LAURATE	11
12H - Paste Masks (mud packs)	PEG-80 SORBITAN LAURATE	1
		84

05F - Shampoos (non-coloring)	PEG-160 SORBITAN TRIISOSTEARATE	3
10A - Bath Soaps and Detergents	PEG-160 SORBITAN TRIISOSTEARATE	1
		4

07A - Blushers (all types)	SORBETH-4 TETRAOLEATE	4
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03D - Eye Lotion	SORBETH-6 BEESWAX	3
12F - Moisturizing	SORBETH-6 BEESWAX	3
12J - Other Skin Care Preps	SORBETH-6 BEESWAX	1
		7

03F - Mascara	SORBETH-20 BEESWAX	6
07E - Lipstick	SORBETH-20 BEESWAX	1
07I - Other Makeup Preparations	SORBETH-20 BEESWAX	1
		8

12C - Face and Neck (exc shave)	SORBETH-30 TETRAISOSTEARATE	1
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07A - Blushers (all types)	SORBETH-30 TETRAOLEATE	1
07C - Foundations	SORBETH-30 TETRAOLEATE	1
07I - Other Makeup Preparations	SORBETH-30 TETRAOLEATE	1
12A - Cleansing	SORBETH-30 TETRAOLEATE	5
		8

12F - Moisturizing	SORBETH-60 TETRAOLEATE	1
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No longer a cosmetic ingredient.

12F - Moisturizing	PEG-18 SORBITAN TRIOLEATE	1
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10E - Other Personal Cleanliness Products	PEG-20 SORBITAN LAURATE	1
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In VCRP, not in On-Line

12G - Night	PEG-20 SORBITAN STEARATE	1
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In VCRP, not in On-Line

03F - Mascara	977144083 PEG-30 SORBITAN BEESWAX	1
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In VCRP, not in On-Line



**Memorandum**

**TO:** Lillian Gill, D.P.A.  
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

**DATE:** December 23, 2014

**SUBJECT:** Concentration of Use by FDA Product Category: PEG-30 Sorbitan Beeswax

PEG-30 Sorbitan Beeswax was included in the October 2014 concentration of use survey. No uses were reported.