Amended Safety Assessment of Octoxynols as Used in Cosmetics

Status:	Draft Final Amended Report for Panel Review
Release Date:	August 15, 2025
Panel Meeting Date:	September 8 – 9, 2025

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Preethi Raj, M.S., former Senior Scientific Analyst/Writer, and Priya Ferguson, M.S., Senior Scientific Analyst/Writer, CIR.

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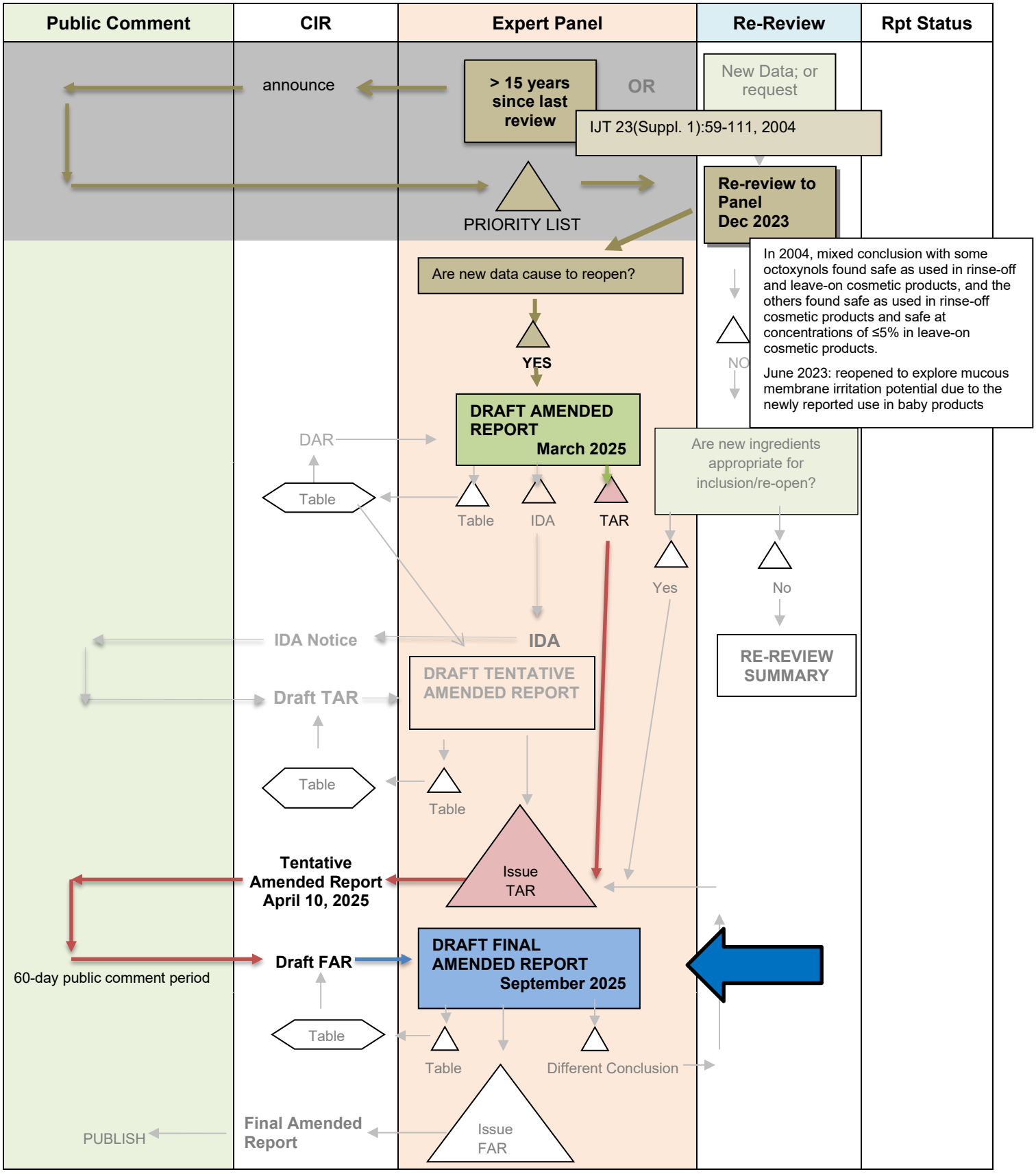
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Octoxynols

MEETING September 2025



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Ferguson, M.S., Senior Scientific Analyst/Writer, CIR
Date: August 15, 2025
Subject: Amended Safety Assessment of Octoxynols as Used in Cosmetics

Enclosed is the Draft Final Amended Report on the Safety Assessment of Octoxynols as Used in Cosmetics. (It is identified as *report_Octoxynols_092025* in the pdf document). At the March 2025 meeting, the Panel concluded that the 25 octoxynol ingredients reviewed in this report are safe in the present practices of use and concentration when formulated to be non-irritating, and a Tentative Amended Report was issued.

Comments received from the Council on the Tentative Amended Report have been addressed (*PCPCcomments_Octoxynols_092025* and *response-PCPCcomments_Octoxynols_092025*). Some of these comments require the Panel's response (as indicated in the response document).

It should be noted that these ingredients were previously reviewed in a report published in 2004 (*originalreport_Octoxynols_092025*). In that report, the Panel issued a conclusion stating that Octoxynol-9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, and -70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products, and Octoxynol-1, -3, -5, -6, -7, and -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave on cosmetic products

Included in this report package are 2025 concentration of use data (*data_Octoxynols_092025*), which have been incorporated into the report (this information is additive to the 2022 concentration of use data). According to these data, Octoxynol-9 may result in mucous membrane exposure as it is reported to be used in disposable wipes at 0.36%.

Additional supporting documents for this report package include a flow chart (*flow_Octoxynols_092025*), report history (*history_Octoxynols_092025*), a search strategy (*search_Octoxynols_092025*), meeting transcripts (*transcripts_Octoxynols_092025*), and a data profile (*dataprofile_Octoxynols_092025*).

The Panel should carefully review the Abstract, Discussion, and Conclusion, and issue a Final Amended Report.

Octoxynols History

2004

- The Expert Panel for Cosmetic Ingredient Safety (Panel) published a Final Report with the conclusion that that Octoxynol-9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, -70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. Additionally, the Panel concluded that Octoxynol-1, -3, -5, -6, -7, and -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave on cosmetic products.

June 2023

- An extensive search of the available published literature since 1999 was conducted in accordance with CIR Procedures regarding re-review of these ingredients after ~ 15 years. The Panel determined that this safety assessment should be reopened due to the previously unreported use of Octoxynol-9 at 0.1% in baby products and to explore the irritation potential of these ingredients in products which come in contact with mucous membranes (e.g. Octoxynol-9 in spermicides and vaginally applied products).

December 2023

- Panel reviews Draft Amended Report and determines to table for receipt of Cosmetics Direct data as mandated by MoCRA

March 2025

- Panel reviews Draft Amended Report with inclusion of 2024 RLD data
- Panel issues TR with safe as used conclusion when formulated to be non-irritating for all ingredients
- TR posted

April 2025

- Comments on Draft Tentative Amended Report received

September 2025

- Panel reviews Draft Final Amended Report

Octoxynols Data Profile* –September 2025 – Priya Ferguson

				Toxicokinetics				Acute Tox			Repeated Dose Tox			DART			Genotox		Carci			Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	ADME	Intravaginal	Percutaneous Absorption	Dermal	Oral	Inhalation	Dermal	Oral	Intravaginal	Dermal	Oral	Intravaginal	In Vitro	In Vivo	Dermal	Oral	Intravaginal	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Octoxynol-1	OX		O						O		O						O							O			O			O		
Octoxynol-3	OX								O		O													O			O			O		
Octoxynol-5	OX		O						O															O			O			O		
Octoxynol-6	OX																															
Octoxynol-7																																
Octoxynol-8																																
Octoxynol-9	OX	O	O		O	O	O	O	O	O	O			O	O	O	O					O	O	O			O		O	O		O
Octoxynol-10	OX																															
Octoxynol-11	OX	O	O																				O							O		
Octoxynol-12	X																															
Octoxynol-13	OX		O						O		O												O	O			O			O		
Octoxynol-16									O																							
Octoxynol-20									O																							
Octoxynol-25																																
Octoxynol-30	OX								O																							
Octoxynol-33																																
Octoxynol-40	OX				O				O			O			O																	
Octoxynol-70																																
Octoxynol-9 Carboxylic Acid																																
Octoxynol-20 Carboxylic Acid																																
Potassium Octoxynol-12 Phosphate	OX																															
Sodium Octoxynol-2 Ethane Sulfonate	OX																															
Sodium Octoxynol-2 Sulfate																																
Sodium Octoxynol-6 Sulfate																																
Sodium Octoxynol-9 Sulfate																																
The safety of nonoxynols is not being reviewed in this assessment but has been included as supporting data.																																
nonoxynols							O						O							O	O					O		O				O
an octoxynol* **								X									X					X	X			X			X			

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

**data were found on this ingredient

***data were found on the trade name, Triton X-100, as this substance can refer to more than one octoxynol, it is listed in the report as "an octoxynol"

Octoxynols

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Octoxynol-1	9002-93-1 9036-19-5 9004-87-9 **2315-67-5**	✓*	✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-3	9002-93-1 9036-19-5 9004-87-9 **27176-94-9** **2315-62-0**	NR	✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-5	9002-93-1 9036-19-5 9004-87-9 **2315-64-2** **27176-99-4**	✓*	✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-6	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-7	9002-93-1 9036-19-5 9004-87-9 **27177-02-2**		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-8	9002-93-1 9036-19-5 9004-87-9 **3520-90-9**		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-9	9002-93-1 9036-19-5 9004-87-9 **9010-43-9** **42173-90-0** **59935-87-4** **2315-65-3**		✓	✓	NR	✓*	✓*	NR	✓	✓*	NR	NR	✓*	✓*	NR	NR	
Octoxynol-10	9002-93-1 9036-19-5 9004-87-9 **2315-66-4** **27177-07-7**		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-11	9002-93-1 9036-19-5 9004-87-9 **108437-62-3**		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR	✓*	NR	NR	
Octoxynol-12	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-13	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Octoxynol-16	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-20	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-25	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-30	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-33	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-40	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-70	9002-93-1 9036-19-5 9004-87-9		✓	✓	NR	NR	✓*	NR	✓	✓*	NR	NR	NR		NR	NR	
Octoxynol-9 Carboxylic Acid	**25338-58-3**		✓	✓	NR		✓*	NR	NR	✓*	NR	NR	NR		NR	NR	
Octoxynol-20 Carboxylic Acid			NR	NR	NR		✓*	NR	NR	NR	NR	NR	NR		NR	NR	
Potassium Octoxynol-12 Phosphate			✓	NR	NR		✓*	NR	NR	NR	NR	NR	NR		NR	NR	
Sodium Octoxynol-2 Ethane Sulfonate	**2917-94-4** **55837-16-6** **67923-87-9**		✓	✓			✓*	NR	NR	✓*	NR	NR	NR		NR	NR	
Sodium Octoxynol-2 Sulfate			NR	NR	NR		✓*	NR	NR	NR	NR	NR	NR		NR	NR	
Sodium Octoxynol-6 Sulfate			NR	NR	NR		✓*	NR	NR	NR	NR	NR	NR		NR	NR	
Sodium Octoxynol-9 Sulfate			NR	NR	NR		✓*	NR	NR	NR	NR	NR	NR		NR	NR	

Bolded CAS number -number most recognized by
NR – not reported or available
✓ - data is available
✓*- in database, but data is not available or relevant

Search Strategy

Search terms used in PubMed and links listed below:

- INCI names
- CAS numbers

- Triton X-100

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (2nd Edition; 2013) - http://abc.herbalgram.org/site/DocServer/AHPABotanicalSafety_FMexcerpt.pdf?docID=4601
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0 http://fragrancematerialsafetyresource.elsevier.com/

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: April 24, 2025

SUBJECT: Tentative Amended Report: Amended Safety Assessment of Octoxynols as Used in Cosmetics (release date: April 10, 2025)

The Personal Care Products Council respectfully submits the following comments on the tentative amended report, Safety Assessment of Octoxynols as Used in Cosmetics.

Key Issues

Since the trade name Triton X-100 has been used in other CIR reports when it is used as a positive control, is it necessary to use "an octoxynol (number of ethoxy repeat units unknown)" in the subheadings of this report. It would be clearer if Triton X-100 was used in these subheadings.

Chemical Properties, Table 2 – Although the original CIR report and the references cited in that report said that Octoxynol-1, -5, and -11 are insoluble in water and Octoxynol-9 is soluble in water, this does not make sense. Rather than repeating the information from the original CIR report, citing new references would be more useful. For example, this Chemical book reference https://www.chemicalbook.com/ProductChemicalPropertiesCB0282743_EN.htm says the water solubility of Octoxynol-9 is 4.55 mg/L at 20ºC. The Sigma reference on Triton X-100 says: "Triton X-100 is soluble in all proportions at 25°C in water."

Cosmetic Use – The results from the PCPC concentration of use survey completed in 2025 still need to be added to this report. Only 2 use concentrations for Octoxynol-9 were reported: 0.36% in disposable wipes and 0.22% in leave-on face and neck products. This information should be added to the report without removing the information from 2022 PCPC concentration of use survey. The concentration for disposable wipes should be added to the text in place of "(concentrations not stated)".

Acute and other sections of the report where reference 32 is described – Please consider deleting reference 32 (studies on the toxicity of a leather cream containing an unstated concentration of Triton X-100) as without information on the concentration of the ingredient in the product this

reference is not helpful in assessing the safety of Octoxynols in cosmetic products.

Discussion – Does the Expert Panel also want to mention the potential for sensory irritation following inhalation exposure in the second paragraph of the Discussion?

All the current inhalation boilerplate language does not seem to be appropriate for the Octoxynols. Inhalation studies showed irritation in the upper respiratory tract. Does the Expert Panel still consider it appropriate to state: "Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients."

Additional Considerations

Introduction – Since all these ingredients are mixtures, the word "specific" should be deleted from the paragraph on Triton X-100.

Percutaneous Absorption, old report summary – The first paragraph concerns the effect of nonoxynols on the skin barrier (measurement of water permeation rates). It does not belong in the Percutaneous Absorption section.

ADME, Dermal, old report summary – The information (guinea pig study) in this section is also presented in the Percutaneous Absorption section. Please delete this study in one of these sections so it is only presented once.

Acute, old report summary – In the following sentence: "The LD_{50} values for ~~the 7 g/kg~~ Octoxynol-20 (70%) and ~~28 g/kg~~ Octoxynol-30 (70%) ~~groups~~ were 3.64 and 21.2 g/kg, respectively." please delete the text as indicated by the strikethroughs. The doses stated in this sentence were the highest doses tested. It generally requires more than one dose group to determine an LD_{50}.

Subchronic, Oral, old report summary – The first paragraph and the first sentence of the second paragraph appear to be describing the same dietary study of 5% Octoxynol-40 in rats. The first sentence of the second paragraph should be deleted.

Developmental and Reproductive Toxicity, Dermal, old report summary – It is not clear what the numbers mean in the following sentence. If they are incidences, why are there no numbers for the rest of the variations? "The following statistically significant skeletal variations were observed only in pups from the highest dose group: poorly ossified lumbar arches, unossified sternebra 6, poorly ossified sternebra, unossified cervical centrum 5, unossified cervical centrum 6, rudimentary bone island, poorly ossified hyoid, poorly ossified zygomatic arch, and poorly ossified supraoccipital."

Developmental and Reproductive Toxicity, Intravaginal, old report summary – The incidence of skeletal malformations is stated for the untreated and sham treated controls. What were the incidences of skeletal malformations in the offspring of Octoxynol-9 treated rats?

Carcinogenicity, Intravaginal, Nonoxynols, old report summary – The study was done in rats,

"species not specified" should be corrected to "strain not specified".

Ocular Irritation, Animal, old report summary – A reference is in the wrong format "(Johnson, 2004 #9)"

Mucous Membrane Irritation, Human, nonoxynols, old report study – It is not clear why the studies are in this section, while similar studies are in the Clinical Studies section. In addition, it should be noted that subjects in the 7-month study of 5 spermicidal formulations containing nonoxynol-9 are a subset of the subjects studied in the trial in 1536 women described in the Clinical Studies section.

Exposure Assessment – It is not clear what the aerodynamic diameter of 4.25 ± 1.5 μm represents. What type of product does this represent?

Summary – It should be made clear that the exposure estimate was for a face cleansing (or makeup remover) product.

Table 1 – In many of the definitions it states: "Error! Reference source not found" for Figure 1.

Octoxynols – September 2025 – Priya Ferguson	
Comment Submitter: Personal Care Products Council **Date of Submission: April 24, 2025**	
Comment	**Response/Action**
Since the trade name Triton X-100 has been used in other CIR reports when it is used as a positive control, is it necessary to use "an octoxynol (number of ethoxy repeat units unknown)" in the subheadings of this report. It would be clearer if Triton X-100 was used in these subheadings.	trade name mixtures are not used in current CIR reports
Chemical Properties, Table 2 – Although the original CIR report and the references cited in that report said that Octoxynol-1, -5, and -11 are insoluble in water and Octoxynol-9 is soluble in water, this does not make sense. Rather than repeating the information from the original CIR report, citing new references would be more useful. For example, this Chemical book reference https://www.chemicalbook.com/ProductChemicalPropertiesCB0282743_EN.htm says the water solubility of Octoxynol-9 is 4.55 mg/L at 20oC. The Sigma reference on Triton X-100 says: "Triton X-100 is soluble in all proportions at 25°C in water."	solubility value for octoxynol-9 updated in report
Cosmetic Use – The results from the PCPC concentration of use survey completed in 2025 still need to be added to this report. Only 2 use concentrations for Octoxynol-9 were reported: 0.36% in disposable wipes and 0.22% in leave-on face and neck products. This information should be added to the report without removing the information from 2022 PCPC concentration of use survey. The concentration for disposable wipes should be added to the text in place of "(concentrations not stated)".	Addressed
Acute and other sections of the report where reference 32 is described – Please consider deleting reference 32 (studies on the toxicity of a leather cream containing an unstated concentration of Triton X-100) as without information on the concentration of the ingredient in the product this 2 reference is not helpful in assessing the safety of Octoxynols in cosmetic products.	Panel response needed.
Discussion – Does the Expert Panel also want to mention the potential for sensory irritation following inhalation exposure in the second paragraph of the Discussion?	Panel response needed.
All the current inhalation boilerplate language does not seem to be appropriate for the Octoxynols. Inhalation studies showed irritation in the upper respiratory tract. Does the Expert Panel still consider it appropriate to state: "Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients."	Panel response needed.
Introduction – Since all these ingredients are mixtures, the word "specific" should be deleted from the paragraph on Triton X-100.	Addressed.
Percutaneous Absorption, old report summary – The first paragraph concerns the effect of nonoxynols on the skin barrier (measurement of water permeation rates). It does not belong in the Percutaneous Absorption section.	Addressed.
ADME, Dermal, old report summary – The information (guinea pig study) in this section is also presented in the Percutaneous Absorption section. Please delete this study in one of these sections so it is only presented once.	Addressed.
Acute, old report summary – In the following sentence: "The LD50 values for the 7 g/kg Octoxynol-20 (70%) and 28 g/kg Octoxynol-30 (70%) groups were 3.64 and 21.2 g/kg, respectively." please delete the text as indicated by the strikethroughs. The doses stated in this sentence were the highest doses tested. It generally requires more than one dose group to determine an LD50.	Addressed.
Subchronic, Oral, old report summary – The first paragraph and the first sentence of the second paragraph appear to be describing the same dietary study of 5% Octoxynol-40 in rats. The first sentence of the second paragraph should be deleted.	Addressed.
Developmental and Reproductive Toxicity, Dermal, old report summary – It is not clear what the numbers mean in the following sentence. If they are	Addressed.

incidences, why are there no numbers for the rest of the variations? "The following statistically significant skeletal variations were observed only in pups from the highest dose group: poorly ossified lumbar arches, unossified sternebra 6, poorly ossified sternebra, unossified cervical centrum 5, unossified cervical centrum 6, rudimentary bone island, poorly ossified hyoid, poorly ossified zygomatic arch, and poorly ossified supraoccipital."	
Developmental and Reproductive Toxicity, Intravaginal, old report summary – The incidence of skeletal malformations is stated for the untreated and sham treated controls. What were the incidences of skeletal malformations in the offspring of Octoxynol-9 treated rats?	Details not provided.
Carcinogenicity, Intravaginal, Nonoxynols, old report summary – The study was done in rats, 3 "species not specified" should be corrected to "strain not specified".	Addressed
Ocular Irritation, Animal, old report summary – A reference is in the wrong format "(Johnson, 2004 #9)"	Addressed.
Mucous Membrane Irritation, Human, nonoxynols, old report study – It is not clear why the studies are in this section, while similar studies are in the Clinical Studies section. In addition, it should be noted that subjects in the 7-month study of 5 spermicidal formulations containing nonoxynol-9 are a subset of the subjects studied in the trial in 1536 women described in the Clinical Studies section.	Addressed
Exposure Assessment – It is not clear what the aerodynamic diameter of 4.25 ± 1.5 µm represents. What type of product does this represent?	hair spray
Summary – It should be made clear that the exposure estimate was for a face cleansing (or makeup remover) product.	Addressed.
Table 1 – In many of the definitions it states: "Error! Reference source not found" for Figure 1.	Addressed

MARCH 2025 MEETING – FIRST REVIEW/DRAFT AMENDED REPORT

Belsito Team – March 13, 2025

The next ingredients is the Octoxynols. Let me go to that, Page 7. Octoxynols, I'm not a chemist. I will never proclaim to be a chemist. Issues with people the same thing, with pathology, diagnosing. So, anyway, it's funny. Okay. so this is a draft amended report. Priya is here with us. Good morning.

MS. CHERIAN: Good morning.

DR. SNYDER: Good to see you. Final report was issued in 2004. In June of 2023, we reopened due to mucus membrane irritation and new uses of Octoxynol-9 at 0.1 percent in other baby products. So, we have new updated data from the RLD. There are still mucus membrane exposures possible through those uses. There are no reported uses in baby products. The highest leave-on concentration is 1.5 percent for Octoxynol-12. There's been published reviews in 1983, 1999, and 2005 of nonoxynols, which are one carbon longer.

So, we received a Wave 2 from the Women's Voices of the Earth comments. So, if no additional data are needed, then we need to draft a discussion and issue a tentative amended report. If additional data are needed, we need to indicate what those are and issue an insufficient data announcement. That's what I have. Anybody have anything different?

DR. BELSITO: No. I thought the summary responses and editorial changes to Women Voices of the Earth comments were good. I had a question for Allan about the add-ons. This is PDF Page 29, that third column, ingredients. Allan, you were okay with all of those as add-ons?

DR. RETTIE: Third column, ingredients, where you start with carboxylic acid, phosphate, ethane sulfonate, et cetera?

DR. BELSITO: Yes.

DR. RETTIE: I mean, the salts I was fine with. I wanted to get a bit more info on the structure of the two carboxylic acids, but I was fine with the other five. I had a brief discussion with Dave Ross about read-across, more from the perspective of Nonoxynol data we had, but this didn't come up. So, that's a decent point. The carboxylic acids I kind of reserve judgment on until I look into that a little bit more.

DR. BELSITO: So, is this something you feel we need to discuss with the other team tomorrow?

DR. RETTIE: Sure. I should know more about them.

MS. FIUME: So, I just want to clarify. These aren't add-ons.

DR. SNYDER: Add-ons?

MS. FIUME: They were in the last.

DR. SNYDER: They were in the last report. Yeah. These weren't add-ons. They were in the last report.

DR. SNYDER: They were in the last report. Yeah. These weren't add-ons.

DR. BELSITO: I see.

DR. SNYDER: Yeah. So, then I guess the question is, should they still be contained in this report?

DR. SNYDER: Yeah. We'll have Allan and David discuss that tomorrow, or we can bring that up for a point of discussion.

DR. BELSITO: Yeah. Because I didn't really see a lot of data on these specific ingredients in the report. Right? I mean, basically, we were relying on read across from Octoxynols for this.

DR. RETTIE: So, it looks like the Octoxynol-9 Carboxylic Acid at least is as you would expect. It's CO2H group at the end of a long chain. That would change. I would change polarity a little bit. But the chain is so long to begin with, it's probably minor in terms of physical chemical characteristics and distribution and all those things we care about for read-across. So, I'm guessing it would be fine. That's a tentative conclusion for me.

DR. SNYDER: Okay.

DR. BELSITO: Yeah. Okay. And then, just on PDF Page 31 where we said that Octoxynol-9, this is the top of the page, the starting fourth point down, is the highest maximum reported concentration of use, two percent, in a cleansing preparation. But we typically also give the highest concentration in the leave-on, which we didn't put in this paragraph. And I think that was 0.1 percent in leave-ons. And it was for, I think, Octoxynol-12.

DR. SNYDER: Nine.

DR. BELSITO: Nine?

DR. SNYDER: I believe it was nine. Yeah.

DR. BELSITO: Was 9 0.1 percent, or was it 12?

MS. FIUME: 1.5 percent Octoxynol-12 in face and neck is what I have written down in my notes.

DR. SNYDER: Oh, I'm sorry. One point what?

MS. FIUME: 1.5.

DR. SNYDER: Okay.

MS. FIUME: Let me double check the table to make sure my notes are correct.

MS. CHERIAN: It says 1.5 in face and neck, Octoxynol-12.

MS. FIUME: Yeah.

DR. SNYDER: 12? Okay. So, we need to add that.

DR. BELSITO: The 1.5 in leave-ons. Yeah. And, just as we're preparing this, I think we need a heavy metal boilerplate for this. Would people agree?

DR. SNYDER: Yes.

DR. BELSITO: I guess this is a philosophical question, again, on PDF Page 31, where it says that this is the second paragraph. VCRP data indicated that some of the ingredients may be used around the eyes and in products that may be incidentally ingested. These uses, however, were not reported in RLD submitted to CIR in 2024. My hypothetical question is, we know that small companies don't have to report.

So, what do we do when we have data like this where we have VCRP data suggesting those uses but then no RLD data, but we know that there are exemptions to those companies that have to report to RLD. Do we assume that it's used in eye and lip, or do we not assume it?

MS. FIUME: You know, Don, I think that's a difficult question to answer. And, if FDA or industry could jump in if they have more information. The VCRP, sometimes those data land there and never get cleaned out. So, if formulations change, they may not have been deleted from the VCRP. So, it may be old information, but I'm not sure.

So, right now we provide both to the Panel. If you feel more comfortable looking at it for both uses, we can make sure that's clear in the discussion. But, the RLD, they're still working through and not cleaning that up, but combing through all that. So, FDA, do you have any input on that?

DR. ZANG: I hear you, Don. You're right because the reality is there are some small business exemptions. And, also, I think it's a case-by-case situation. For example, in VCRP, what is the (inaudible)? Is it just single digit, or is this considered very large? That could contribute to your decision, whether you want to take a look or not.

Secondly, your resource. Right? If given with the uncertainty, even though we don't know if this ingredient is currently used or not, but if there is a conclusion there that might give useful information to small business having that information, say the information is there. I don't know, guidance, reference, some kind of information that they might feel useful no matter what the conclusion is. That's what I think.

MS. FIUME: I'm just thinking out loud. As we go further down, just to avoid issues as we go down the road. So our conclusions are safe as used so, Don, I think your question is actually more wide reaching because when you're saying as used, is it --

DR. SNYDER: And we have a known reported use that's not -- yeah. Right.

MS. FIUME: -- under VCRP that's old or is it only RLD as we get a little further into using the RLD information?

DR. SNYDER: That almost seems like we should almost take on based on current safe as used. You know what I mean? Somehow we need something to say that the use data is based on the current data, not the old data, right, in this case?

MS. FIUME: Yes, But then you also have concentration of use data being reported where you may not have RLD numbers or VCRP numbers.

DR. SNYDER: Right. Yeah.

MS. FIUME: So, we've always looked at that as a combination. If it's the concentration of use or frequency of use, then it's part of the as used.

DR. SNYDER: Okay.

MS. FIUME: So, I think for right now, with both bits of information being reported, if you're erring on the side of caution, if it's in VCRP or -- or RLD -- then that category can be considered if you err on the side of caution.

DR. SNYDER: Okay. That'd be my preference. What about you, Don or Allan?

DR. BELSITO: Yeah. I mean, I think, at this point, since everything is so new, if 2023 VCRP data suggested it was used in lip and eye products, I think we almost need to assume it's used. I don't know. I mean, that was just my thought.

MS. FIUME: That would definitely be erring on the side of caution. But it would be great to have that conversation tomorrow in case the other teams looked at it differently and just wanted to go with the more current information.

DR. BELSITO: Yeah. I mean, I don't have a problem with that. I think we would just need to clarify in the Discussion that, when we're saying as used, it's based upon RLD data and not prior VCRP data.

MS. FIUME: I know. Yeah. If that discussion could be had tomorrow, just so that we're all on the same page.

DR. SNYDER: Yeah. Yeah. I'll bring it up.

MS. FIUME: That would be great. Thank you.

DR. SNYDER: Okay.

DR. RETTIE: Go ahead, Don.

DR. BELSITO: Just another -- go ahead, Allan. I'm sorry.

DR. RETTIE: This is just a minor point. The ethoxylation boilerplate again should be added, I guess. It's an appropriate point. We have limits for dioxin and ethylene oxide for Octoxynol-9.

MS. FIUME: We'll make sure to enter that in the Discussion.

DR. RETTIE: Okay.

DR. SNYDER: Go ahead, Don.

DR. BELSITO: On PDF Page 40, under sensitization, it says that skin sensitization of 0.1 percent Octoxynol-9 is evaluated in a human repeat insult patch test. That description is not an HRIPT. It was applied to a cotton twill and secured with adhesive tape for six days to the arms of men and women. That's not an HRIPT. HRIPT is applied every other day for three weeks, rested for two, and then challenged. So, I mean it is a sensitization test, but to call it an HIRPT is a misnomer.

DR. SNYDER: Where are you at on that page, Don? Under what now?

DR. BELSITO: It's PDF Page 40.

DR. SNYDER: I got it. Page 40. Yeah.

DR. BELSITO: The sensitization, the first one, where it's called an HIRPT.

DR. SNYDER: I got you. Yep, yep, yep, yep, yep. Yeah.

DR. BELSITO: That's not an HRIPT.

DR. SNYDER: Okay. So just remove the reference to an HRIPT?

DR. BELSITO: Yeah. I mean if you just take HRIPT out, it's fine. I mean, they are looking at sensitization, but it's not an HRIPT test.

DR. SNYDER: Got you. Okay. Anything else?

DR. BELSITO: No.

DR. SNYDER: Curt?

DR. KLAASSEN: No.

DR. SNYDER: Allan? Okay.

MS. FIUME: So, what is the faction on this?

DR. SNYDER: So, we're going to discuss about using the read-across with the other team as a basis for safety. It's basically editorial to add the highest concentration used for the leave-on. I think it's editorial just taking out the HRIPT. We're going to add the heavy metal boilerplate.

We're going to have a discussion with the other team regarding VCRP data and RLD data in small companies and whether or not they have to report. And we're going to talk about we prefer to err on the side of caution and utilize the VCRP data still in our reports, and add the ethylene boiler. So, it's all editorial, I think. Right?

MS. FIUME: So, we'll go --

DR. BELSITO: Formulate to be non-irritant?

DR. SNYDER: Yeah.

MS. FIUME: So, TAR, a Tentative Amended Report?

DR. SNYDER: Yeah.

DR. BELSITO: The only other question that I had was on photo. Is everyone okay with that for UV absorption or for need of additional photo testing?

DR. RETTIE: So, Don, when we've discussed this in the last few meetings, the action when we decided we were concerned about phototoxicity is to simply ask for a UV absorbance. That's fine. I was just curious if we needed to formulate an action plan for following up when that absorbance is positive of any significant magnitude.

DR. BELSITO: Right.

DR. RETTIE: Because I had a chat with Dan Liebler about this. In RIFM, of course, they have a very well, nicely laid-out plan for what happens beyond the fact that you observe UV absorbance in a fairly narrow range, usually in the 290 to 390. But, the next step then, according to what I got from Dan, was to measure a molar absorptivity value.

DR. BELSITO: Right.

DR. RETTIE: The question there being how big is it? Is it just above baseline, or is it a number that's really considered relevant? And, if it was considered relevant, their third course of action is to do an OECD cytotoxicity test. I forget the number, but I thought that was nicely laid out.

There's obvious places to go once you have one check. And I wondered whether that's something we should work up as a method for this group to follow to kind of delve just that little bit deeper into phototoxicity rather than it being binary at UV absorbency. Is it or isn't it?

DR. BELSITO: Right. Yeah, because we don't have UV absorbance, and we have that in vitro test suggesting that it's Nonoxynol was phototoxic. But we don't have any data on the Octoxynols. So, I'm just wondering if it's insufficient for UV absorption, and if absorbed, additional studies would be necessary.

DR. RETTIE: Yeah. I agree with that, insufficient for the Octoxynols phototoxicity.

DR. BELSITO: And I think, in terms of UV spectra, any of the lower-chain units would be fine.

MS. FIUME: So, then an IDA versus a Tentative Amended Report?

DR. BELSITO: Yeah. That was my thought.

DR. SNYDER: Okay.

DR. BELSITO: Sorry I didn't add that in earlier.

MS. FIUME: So, then can I ask? Just trying to look ahead to some of the conversations. If those other ingredients aren't okay for read-across, would it be proposed to separate them out? Or would an IDA be listed for those as part of this IDA looking for information specific on the ingredients that you feel don't read across?

DR. BELSITO: Yeah. I would have to ask Allan. I mean, are you okay reading across UV absorption of Octoxynols to Nonoxynols?

DR. RETTIE: Yeah, as long as we stay with the same chain length. Things get really out of whack once you get up to the large chain lengths. Above 20, 25, these things are really quite water soluble. So, read-across for distribution which would not be appropriate, I think, going from Octoxynol-3 to Octoxynol-40. But, what we're discussing here, I think it's okay. And, to follow up on that carboxylic acid question you asked earlier, Don, Table 2 has a lot of relevant information that I'm looking at here that I've overlooked.

I'm kind of surprised. Well, I guess I'm not really. Octoxynol-20 and Octoxynol-20 Carboxylic Acid are in the same ballpark for their log P. So, I think that's what we would take as decent evidence that it would be a okay read-across, certainly at the level of disability distribution.

DR. BELSITO: Okay.

DR. RETTIE: No harm asking the other team, though, if they have different opinions.

DR. KLAASSEN: The molecular weight would decrease the absorption. I mean, that's C20. What's the molecular weight of it or C24?

DR. RETTIE: We're up about a thousand into this.

DR. KLAASSEN: Yeah. It's pretty hard to get absorption at that molecular weight. So, while I agree that the distribution probably isn't so dramatically different, the absorption could be, but it would be less the larger.

DR. RETTIE: Yeah.

DR. KLAASSEN: On the other hand, we do have smaller ones here, also not used that much.

DR. RETTIE: Well, in an ideal world, we'll get UV data on all of them. Right?

DR. SNYDER: Yeah.

DR. RETTIE: But we won't.

DR. SNYDER: We won't.

DR. KLAASSEN: No.

DR. SNYDER: We'll just have to deal with it when we get it.

DR. KLAASSEN: Hopefully, you get to get some good data on C9.

DR. RETTIE: That's our main read-across here.

DR. KLAASSEN: Right.

DR. RETTIE: Both for Octoxynol-9 and Nonoxynol-9, huge amount of data, of course, for both of those.

DR. BELSITO: Yeah. And then, one other point, on PDF Page 64 for the Octoxynol-12, Potassium Octoxynol-12 Phosphate, there's a flip in the maximum concentration of use. But 2022 was not recorded, and the 0.0008 to 0.5 was from 1999, 2001. Do you see where I am?

DR. RETTIE: So, Page 64 is the Table 3 data?

DR. SNYDER: Yes. Yes.

DR. BELSITO: Yeah. Table 3 for the Potassium Octoxynol-12 Phosphate, the range of concentrations given for 2022 should be the range for 1999, 2001. There were none reported for 2022.

DR. RETTIE: So, the columns are just flipped?

DR. BELSITO: Yeah. I mean the column headings for range of concentrations were flipped for years.

DR. SNYDER: You get that, Priya?

MS. CHERIAN: Uh-huh.

DR. SNYDER: Okay. Anything else?

DR. BELSITO: No. I think insufficient for UV absorption. But hopefully, if we can get a spectra, it should be so easy.

DR. SNYDER: Okay. You all clear, Priya?

MS. CHERIAN: Uh-huh.

DR. SNYDER: So, we're going to go with insufficient data analysis for UV absorption data on the Octoxynols but preferred for the lower ones of C9. We're going to add the heavy metal plates data. We're going to remove the HRIPT.

DR. BELSITO: No evidence of use in baby products or douches.

DR. SNYDER: Right. Yeah.

DR. BELSITO: Formulated to be nonirritant.

DR. SNYDER: Yep.

DR. BELSITO: But I think we can use a lot of the Discussion from the prior report.

DR. SNYDER: Okay. Everybody okay with that?

DR. KLAASSEN: Yes.

DR. RETTIE: So, I still have access to a double beam, a nice double beam with lovely quartz cadets. And I'd be willing to be contracted out by this group to take a (inaudible) spectra if you want.

DR. KLAASSEN: $10,000 each.

DR. RETTIE: My financial consultant will negotiate with you. Is that even allowed? You get submissions from designees?

DR. SNYDER: Oh yeah. Oh yeah.

DR. RETTIE: Do you get to designate the groups?

MS. FIUME: Yeah. We don't. We accept the data.

DR. KLAASSEN: Really? Just from anyone?

MS. FIUME: Because we don't contract any of the tests as CIR. I'm not sure if it's pertinent to this report because I'm trying to search back and forth, but the Nonoxynols report did have some UV absorption information in its properties table. But I haven't been able to go back and forth to see if it's in this report as well or if it's just the phototox section that had the information from the last report.

DR. ZHU: It's on PDF Page 76.

DR. RETTIE: Oh, it is there? Okay. Great.

DR. ZHU: There is some other data on previous report.

MS. FIUME: Thank you.

DR. ZHU: Yep.

DR. SNYDER: Are we okay?

DR. BELSITO: I didn't hear you completely, Monice. So, there's additional data from the Nonoxynol reports on phototox that can be brought in here?

MS. FIUME: Well, in the properties, data there were some UV absorption spectra in the nonoxynols report. And I was just trying to figure out if it's in the current Octoxynols. You said 76, Jinqiu?

DR. ZHU: 76.

MS. FIUME: So, that's the actual --

DR. ZHU: Octoxynol-9 will have data to clarify that. In UVA and UVB.

MS. FIUME: So for Octoxynol-9?

DR. BELSITO: It's the one where it said the maximum absorption was 270 but it didn't give the spectra. Is that because there's one like that?

DR. SNYDER: Did you see that, Don, on Page 76, that UV absorption for the Octoxynol-9?

DR. BELSITO: PDF 76?

DR. SNYDER: Yeah.

MS. FIUME: Yeah.

DR. SNYDER: UV absorption for 9.

MS. FIUME: And then, it gives some of the Nonoxynol information. Thank you, Jinqiu

DR. BELSITO: So, absorption maximum of 276 and slight absorbance at 290, as a tail on the peak at 276. No detectable absorbance observed above 295. Yeah. I mean, if this data was brought in, then essentially the same. So, I mean you don't have any absorption above 290, essentially. And, if you were concerned about the little bit of absorbance above 295 you could do Henry's law. I'm sure it's going to be below 1,000 molar prevalence. Yeah. I mean, if you bring this in, then I think you get rid of the need for photo.

DR. RETTIE: I agree.

DR. BELSITO: And then, I think we would go back to a safe-as-used conclusion with all those other caveats. Right?

DR. SNYDER: Yep.

DR. BELSITO: So, basically, it would be safe as use when formulated to be nonirritating. Is that correct?

DR. SNYDER: That's correct. Okay. Did you get that? So, you're going to bring in that data from that old report, and then we're going to go safe as used when formulated to be nonirritating. Good.

MS. FIUME: One other discussion point will be that the carboxylic compounder is similar to --

DR. SNYDER: Yes. Yeah.

DR. RETTIE: I'm not even sure we need that with the data that's in the table.

MS. FIUME: Okay. Okay.

DR. RETTIE: So, because the log P's are pretty similar.

MS. FIUME: Okay. So, I didn't know if that needed to be a discussion point or not.

DR. RETTIE: I don't see a need for it.

DR. SNYDER: Do you want to discuss it with the other team though or no?

DR. RETTIE: Sure.

DR. SNYDER: Okay. I'll just bring it up and see what they say.

DR. RETTIE: I think it's okay. Yes.

DR. SNYDER: Okay. Okay. Nice. Good discussion. Priya, any questions? You okay?

MS. CHERIAN: I'm okay.

DR. SNYDER: We move around a lot, you know. First we start with safe as used, nonirritating, IDA.

MS. CHERIAN: I know.

DR. SNYDER: And then, we go back to safe as used.

DR. BELSITO: So, I missed the photo, Paul. I'm a little bit spacey here, so bear with me.

DR. SNYDER: That's okay. It's all right. That's why we have a team. So, it works. We were talking about that during the break of how well it works, the teams, you know, catching things that the other team doesn't catch and things. All right. Thank you. Good discussion.

Cohen Team – March 13, 2025

DR. DAVID COHEN: Okay. Why don't we start? We open with octoxynols. So this is a draft-amended report on the safety of octoxynols. These function as surfactants. And the Panel first published this report on 25 ingredients in 2004 with the conclusion that they were safe as used in rinse-off and rinse-on cosmetic products. That is a large group of them that are listed in the report.

The Panel concluded specifically that Octoxynol-1, -3, -5, -6, -7, -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of less than or equal to 5 percent in leave-on cosmetic products.

At the June meeting, we decided to reopen the safety assessment to explore mucous membrane irritation due to some newly-reported use of Octoxynol-9 in baby products according to a 2022 concentration of use survey. In December, the Panel reviewed the draft-amended report and determined to table the assessment until the RLD of these ingredients were available. RLD collected in 2024 indicate Octoxynol-9 is used in formulations that may result in mucous membrane exposure, such as bath soaps, washes, and disposable wipes. These data, as far as I see, did not indicate these ingredients were in baby products.

According to the RLD, Octoxynol-9 is an ingredient with the highest number of uses. In 2023, the VCRP showed Octoxynol-11 was reported to be used in the highest number of formulations. And we had concentration of use at 1.5 percent for Octoxynol-12 in face and neck preparations, 3 percent in a rinse-off, and I think, Octoxynol-9 at 3 percent in one of the tables.

DR. ROSS: I thought it was 2 percent.

DR. DAVID COHEN: All right. I've got to go back. It was PDF 58, but I'll come back to that in a discussion.

DR. ROSS: Okay. Yeah, sure.

DR. DAVID COHEN: Thank you. Then we had this issue of Triton X-100 being assumed to be Octoxynol-9, and turns out it may represent other octoxynols.

Let's see. We have a fair amount to deliberate here, also, with irritation in reconstructed vaginal and cervical skin. We have the WVE discussion about HIV risk with Octoxynol-9, but I think much of this data is coming from Nonoxynol-9. And we have HRIPT at 8 percent.

So I'll just open it up. There was a lot in there, and I didn't want to just put this all in prose. But do you want to start Dave?

DR. ROSS: I get the honor of kicking this off. Okay.

DR. DAVID COHEN: Yeah. You're the lead.

DR. ROSS: So let's start then. My summary, as you commented, the RLD data has been obtained. And I didn't see any baby use in that data.

The RLD data also didn't show use in vaginal douches as far as I could see. But the WV did state in their Wave 2 that there was some possibilities of exposure to mucous membranes there with other products.

There was no ocular concentration of use stated. And we know from the previous report that these compounds have been used in ocular products. VRCP 2023 data, for example, showed Octoxynol-11, but there was no octoxynol use that I could see in the RLD data. And correct me if anyone picked it up, but I didn't see any ocular use there. So is that precautionary statement needed? Or do we need any ocular data? I think if you believe the RLD numbers, we probably don't.

The hormonal endocrine effect, it's on PDF Page 38. I mentioned that last review. There's nothing new on that. It's just something to bear in mind. And if anyone has concerns, we can discuss that.

My overall conclusion on this was, if we ignore the baby use, if we ignore the ocular use, we could probably get to safe as used. But we don't have concentrations of use there, and so we could go safe as used and formulated to be nonirritating.

DR. DAVID COHEN: Yeah.

DR. ROSS: That was my conclusion.

DR. DAVID COHEN: That was how I concluded.

DR. ROSS: And I have a couple of editorial things that I won't bog people down with now, but I would like to come back to on the mic when I get a chance.

DR. DAVID COHEN: Go ahead, Sam.

DR. SAMUEL COHEN: Yeah. I had a great deal of confusion in this one since this is one of the first ones I looked at. As far as the use levels and things is they seemed to be all over the place and very contradictory.

So which one do we actually accept because there were ones in the tables that were listed, and then there were ones in the descriptions that were different, like 2 percent versus 1.5 percent, versus 3 percent. Which one are we supposed to rely on as the one?

And then the baby use versus no baby use. One place it says it was used in baby products, and then another place it says it's not used in baby products, and then the table says it's not used in baby products. So which of the data are we to rely on? Which one is actually in force?

DR. HELDRETH: And I'll try to speak to that, and I agree, it is very confusing. It was confusing even a couple years ago.

DR. DAVID COHEN: Closer to the mics.

DR. HELDRETH: I'm sorry. It was confusing even a couple of years ago before our data sources changed. So up until February of last year, we received our frequency of use information through FDA's voluntary cosmetic registration program, or VCRP.

And you'll see in our Use table there, you'll see VCRP 2023. But the VCRP program was shelved in February of last year. And so now, because of some legislation that passed at the end of 2022, so called MoCRA Act, we now have a new database that is essentially mandatory for most manufacturers. And it's a much more robust database because, instead of just a few thousand entries, there's hundreds of thousands of entries in there. And so that's the database we're shifting to.

We're still including the VCRP in our tables because we don't have a baseline yet for trends. So oftentimes we're trying to determine is the use increasing, is the use decreasing. And typically, over the years we would compare VCRP data from 15 years ago to VCRP data from this year. But we don't have any baseline for the RLD yet, so that's why, at least for the next two or three years, you'll see both in there.

But as far as which one takes precedence, I would rely on the RLD. It's the most recent, and as I mentioned, it's the most robust because, for most manufacturers, it's mandatory.

DR. SAMUEL COHEN: So if we conclude that it's safe as in use, which is in use referring to?

DR. HELDRETH: The most recent.

DR. SAMUEL COHEN: Okay.

DR. HELDRETH: Yes, the RLD. And then the concentrations of use come separately through industry survey that the Council provides for us.

And so, when we're looking at, you can see, under max concentration of use, there's two sets of numbers there. We take the most recent version there. So you'll see that there was a survey resulting in 2022, and then there was actually two earlier surveys in 1999, 2001. The most recent is what we consider to supersede the previous. Yeah.

DR. EISENMANN: And they also asked me to do another concentration of use survey, so there's another ongoing. And in general, the use of these ingredients is going down because they're out of favor anymore because there are environmental issues. In the most recent survey that's underway now, there's even less use.

DR. SAMUEL COHEN: And then one of the other questions that I had was on the irritation data. In some places, it was saying that it was irritating, but it was almost all either in vitro or in animal studies. But the human studies seemed to say it wasn't irritating. So do you just ignore the in vitro and the animal studies if you have animal?

DR. DAVID COHEN: No. You have to take it in the context of how the use is going to be used. So sometimes we'll take those cues and indicate to formulate to not be irritating. And other points we'll see there's a preponderance of human data to suggest it's not irritating in the concentrations of use.

DR. SAMUEL COHEN: Okay.

DR. BERGFELD: So you've been asked to have a discussion. I would think that the nonirritating statement to be compounded to be nonirritating might go there. But, also, going to the vaginal toxicity that's been elucidated by the WVE, may be elucidating in the discussion that it's an OTC. It's not a cosmetic.

DR. DAVID COHEN: And it's discussed in -- I think, in the Use section.

DR. EISENMANN: It's no longer an OTC. There is no OTC vaginal contraceptive products anymore.

DR. BERGFELD: Okay. So that's gone.

DR. EISENMANN: Yes.

DR. BERGFELD: So the WVE is off base on that?

DR. EISENMANN: I don't remember exactly what they said.

DR. BERGFELD: Oh, yeah. But they were quoting that. There's a lot of discussion on that.

DR. DAVID COHEN: No. They were discussing the fact that these are irritating to vaginal mucosa. Irritation in the absence of barrier protection might increase your risk of viral transmission.

DR. BERGFELD: Or other.

DR. DAVID COHEN: But there's a disconnect in that they're not being used for those purposes now, and it was Nonoxynol-9, right, not Octoxynol-9, which in the past may have been used. And I think it was Don who thought that they were being used in these preps.

DR. ROSS: It was.

DR. DAVID COHEN: And it reopened something that I don't think we originally were going to reopen. And I think we're back to where we were, which is nonirritating.

DR. BERGFELD: Nonirritating.

DR. DAVID COHEN: But, Susan, what were your comments?

DR. TILTON: My comments were in line with yours and David's. We were previously under agreement that it was safe as used with some concern for certain products and certain uses that would have been insufficient. And generally for those, we now have no reported uses, vaginal use, eye products, or baby products. And so, I would agree with safe as used and including the nonirritating formulation.

DR. BERGFELD: Do you want to put that in the Discussion, Susan?

DR. DAVID COHEN: Which part?

DR. BERGFELD: The discussion regarding the eye and the vaginal track.

DR. DAVID COHEN: Yeah. And, I mean, notwithstanding the comments about their decreasing use, the fact that they're not used in baby products now does not preclude their use in the future, right? I mean, they can use it. It can be irritating. If it's in the baby product, it can get in the eyes and on the lips and in their groins.

So I don't know how much the Belsito team will dig in on irritating, but there's enough data here about it being irritating under certain circumstances. And how can we be surprised? It's a surfactant.

DR. BERGFELD: Right.

DR. DAVID COHEN: Right? They get irritated. You get irritated with certain surfactants under certain conditions.

DR. SAMUEL COHEN: But if we are approving it for the uses that are actually listed, we're not approving it for baby buttocks or ocular use though. So if somebody uses it for that, it's not with our blessing essentially.

DR. DAVID COHEN: I don't know if that's -- is that the case?

DR. ROSS: That's probably the case if it's not being used in baby products right now.

DR. HELDRETH: Right. Yeah. So when we conclude in all of our conclusions, it's always in the present practices of use and concentration as described in this report. So if we're going on that most recent datasets and it's not there, then the conclusion just isn't speaking to it. It's not saying it's not safe. It's not saying it's unsupported, it's just not speaking to it at all.

Now, you can speak to that in your Discussion section and say, hey, we don't see anybody's doing this, but we don't think anybody should unless they can provide some data to demonstrate the safety there.

DR. DAVID COHEN: Yeah. I've been here a couple of years now, and I take that as present practices of use.

First of all, we've changed the categories, right? So any category that's new that's in a report from five years ago, technically is not present practices of use, right? I think that's way too narrow a focus that, if in a voluntary survey, nobody mentioned that it was being used as an ankle rinse or a foot powder, that it's not approved for foot powders because the voluntary system didn't show it. But now we do the RLD, it's a 10X increase in usage and all of a sudden it changes the present practice.

So I kind of look at this as we're adjudicating this under certain concentrations of use and general types of use. So if someone didn't report it three years ago, but they're reporting it now, I mean, I don't think all the reports from before are no good anymore.

DR. HELDRETH: Right. And that's why the end of the conclusion is as described in this report. So I mean, these things will change. I think that, since we collected data from the RLD, if I'm remembering right, there's still hundreds of thousands of more entries in the RLD since we collected it.

DR. DAVID COHEN: Yeah.

DR. HELDRETH: So it's drastically changing. But we can't conclude on those that we don't have in front of us. We don't have the frequency of use data, the concentration of use data, all of the things we would need to do the assessed risk on those. We can only do what's in front of us. And that's why we always end the conclusion with as described in the report.

DR. BERGFELD: Are you speaking against putting it in the Discussion because everyone agrees to the conclusion?

DR. DAVID COHEN: Putting what in the -- which part?

DR. BERGFELD: Well, we --

DR. DAVID COHEN: No. We could put that in the Discussion.

DR. BERGFELD: Yeah.

DR. DAVID COHEN: No. All right. I think we're going as safe when formulated to be nonirritating. And the point I just made is not specific to octoxynols, so I don't think it needs any particular discussion point, right?

DR. BERGFELD: Even vaginal? For the irritation?

DR. DAVID COHEN: Well, the vaginal part needs to be in the Discussion because it's the reason we reopened.

DR. BERGFELD: Right.

DR. DAVID COHEN: Right? We reopened. And I think the Women's Voices of the Earth comments, to me, were reasonable taps on the shoulder to take a look at this further. And then, when we looked into it further, the bridge was not fully built, right? These weren't being used in vaginal products. And then, when they are, there were warnings. But now they're not doing it anymore anyway. So I think the manuscript as it is now covers the necessary caveats about the reason we reopened.

DR. SAMUEL COHEN: Unrelated to all the discussion we've just had, there's a minor point that I think needs to be included, not just in this one, regarding the genotoxicity description but in some others.

A few years back, OECD took UDS and SCE off the list of accepted assays for genotoxicity. So I think in this one we refer to UDS. In some of the others we have UDS and SCE. I think we should have a comment there saying that these are no longer accepted as measures of genotoxicity, even though they're negative and it supports our point, the reality is they're of no value.

DR. ROSS: At OECD, right? They're not accepted by OECD.

DR. SAMUEL COHEN: Right.

DR. ROSS: And I think that's fair enough. I think not everything we put in these dossiers is going to be OECD approved.

DR. SAMUEL COHEN: Right.

DR. ROSS: I don't know how they want to deal with that, but I think it's a good point. It would need to go there.

DR. SAMUEL COHEN: I think just in a simple comment saying this is no longer an accepted measure of genotoxicity by OECD. And I have a reference for that if you need it. I think I sent it to you.

DR. DAVID COHEN: If we have that data, right, if not recognized, so the value of negative or positive, it's irrelevant.

DR. SAMUEL COHEN: It's irrelevant. Exactly.

DR. ROSS: Well, I'm not sure it's --

DR. DAVID COHEN: What about in the absence of other data? Yeah, you just don't like it.

DR. SAMUEL COHEN: Yeah. Well, especially for SCE, it's useless data.

DR. DAVID COHEN: Okay.

DR. SAMUEL COHEN: It can be very misleading because there's a lot of things that are positive in the SCE assays that aren't positive in anything else, and you just have to dismiss it.

DR. DAVID COHEN: Yeah, it just sends people awry.

DR. BERGFELD: Shouldn't that be deleted then and then discussed in the Discussion?

DR. ROSS: I think you can show the data, but you can comment -- as Sam says, you can comment that it's not OECD approved.

DR. SAMUEL COHEN: I think it's listed under genotoxicity, and just wherever it says that, just put another statement saying this is no longer accepted, but the data should be listed. At least it's there.

DR. DAVID COHEN: And it was used for the original report in 2004, right?

DR. SAMUEL COHEN: Yes.

DR. DAVID COHEN: So this is a new report, but we can comment on why these were cleared from old reports, right? There's historical value there.

DR. ROSS: Yeah.

DR. SAMUEL COHEN: In this compound, there's other genotox data that's negative, so with or without the UDS data is almost irrelevant. But I think it's worthwhile including the data to say that the test was done, but it's no longer an accepted measure of genotoxicity.

DR. BERGFELD: I think that belongs in the Discussion a little bit, a sentence or so, just to call it out again.

DR. ROSS: Or you could asterisk in the tables, footnote it in the tables.

DR. SAMUEL COHEN: Or just in the genotoxicity section.

DR. ROSS: Or in that section. Yeah.

DR. SAMUEL COHEN: Yeah.

DR. ROSS: I had a couple of things. Can I comment on those now?

DR. DAVID COHEN: Sure.

DR. ROSS: Previously, in Octoxynol-0, which was a previous version of this, we had a statement regarding the loss of liability in the vaginal epithelial cell model. That was with nonoxynol-9. That was with the read-across at both 0.1 and 2 percent. And that was on PDF page 32.

That somehow disappeared. And I think that sentence should be reinstated. And that, basically, shows the loss of vaginal epithelial cell of liability at 0.1 and at 2 percent nonoxynol. So that was the first one. We had added new data with vaginal epithelial cells in there, but that previous data should be back in, I think.

And then I made some comments last time that we didn't really have any business dealing with low reliability estimates with these QSAR models, these VERMEER models. And I think we took the MOE values out of there.

I think with the first one, the one with moderate reliability, we can keep that MOE. I think that's been removed. It was previously 337. I didn't see it in there.

But the second estimate of poor reliability, I'm not sure why we even bother with poor reliability estimates. I don't think that has any business in our dossier. Those were my two comments.

DR. DAVID COHEN: Any other comments? Are we generally in agreement with safe as used when formulated to be nonirritating? All right. We are using some ex vivo data for that nonirritating component. We're doing some read-across from nonoxynol past use. And the question is, does the current use have potential for irritation?

I still think we'll go with it, and then we can have the discussion tomorrow. We'll debate it then. Wilma, you have anything else?

DR. BERGFELD: I just want to make sure that you're clear on the Discussion what you're going to put in it if anything.

DR. DAVID COHEN: We'll put in the past use in vaginal preparations, right?

DR. BERGFELD: Mm-hmm.

DR. DAVID COHEN: What else were we going to put in the Discussion from you guys, anything specific? Oh, the UDS comments for the genotox.

DR. BERGFELD: Were you going to put in the not use in babies, or?

DR. DAVID COHEN: Yeah, no use in babies.

DR. ROSS: Yeah, I think that has to be in there that the RLD indicated no uses ocular or baby products because, otherwise, we would ask for ocular concentrations and baby concentrations. And that's one of the reasons, just in case in the future, for the formulated to be nonirritating.

DR. DAVID COHEN: That's an important point. So if it's in the discussion specifically calling out no use in babies and no use by ocular, then I think the report clearly articulates present practices of use specifically doesn't include this. I just don't think our other reports are that clear like that.

I know the term present practices of use -- I don't want to spin back into that conversation, but you see the difference between voluntary reporting and mandatory reporting. Voluntary reporting is really just a tiny fraction of real use.

DR. BERGFELD: But, David, the RLD is formulations, and there's redundancy in that. It's not real.

DR. DAVID COHEN: Yeah. Well, it's real that it's in a lot of formulations.

DR. BERGFELD: Yeah.

DR. DAVID COHEN: Right? It depends on how you look at it. But I don't know. Is there an increase in actual use reporting from it?

DR. BERGFELD: Use? Who knows? Will the PCPC still do the surveys for concentrations?

DR. DAVID COHEN: Right. You're going to do the concentrations? But that's voluntary, too, right, or no?

DR. SRINIVASAN: Yes, it's voluntary.

MS. KOWCZ: It's not the entire marketplace, no.

DR. EISENMANN: I mean, I would love if more companies participate, but I don't know how to reach everybody.

DR. BERGFELD: Well, threaten them with RLD. Threaten them with that. It's not specific to them.

DR. DAVID COHEN: What's the threat going to be though?

DR. BERGFELD: The threat is that we may look at it closer because there's so many uses and look at the toxicity, possible toxicity.

DR. DAVID COHEN: Sometimes then, that might say, well, the lesson for me shouldn't (inaudible). I don't know. I doubt they would.

DR. EISENMANN: But for this one, the uses are actually going down.

DR. DAVID COHEN: Yeah. Yeah. No, it's a general conversation. It's not an octoxynol conversation. All right.

DR. BERGFELD: Beat that enough.

DR. DAVID COHEN: I think we've gotten what we got out of octoxynol.

DR. SAMUEL COHEN: For my own information, who writes these Discussions?

DR. DAVID COHEN: Usually, the newest member of the group. No, I'm kidding. No, they'll be incorporated by the writer.

DR. SAMUEL COHEN: Okay.

DR. DAVID COHEN: And then, this comes around. This is a draft -- I closed it. This was a --

DR. HELDRETH: Draft-amended report?

DR. BERGFELD: Amended safety.

DR. DAVID COHEN: Draft-amended, so it'll come --

DR. SAMUEL COHEN: One more time.

DR. DAVID COHEN: It'll come back again.

DR. HELDRETH: That's right. That's right. If you're coming to a conclusion now, it'll go out as a tentative amended report and then --

DR. DAVID COHEN: And then a final, right?

DR. HELDRETH: -- and then a final draft.

DR. DAVID COHEN: So this going to go through two more hits.

DR. BERGFELD: Iterations.

DR. DAVID COHEN: You'll get to see it.

DR. SAMUEL COHEN: Okay.

DR. BERGFELD: Goes out for a 60-day review to public.

DR. DAVID COHEN: It'll be very good.

DR. HELDRETH: I will also say, well, we are also trying to get more involvement with the survey. The Council has worked with us to include an advertisement in every issue of IJT that we put out that invites people to submit information to their surveys to try to get more into that survey.

And then on the topic of a better explanation of what present practices of use and concentration, I absolutely agree. We need something more in our reports. And our ears are open to hear what the Panel would like to see in our reports because it's something that has been, I think, a deficiency for a long time. I'll go and present about CIR, and nobody knows what we mean by present practices of use and concentrations.

DR. DAVID COHEN: Yeah.

DR. ROSS: Yeah, that's right.

DR. HELDRETH: I think if the Panel would like to see more verbiage in the report, every report that explains that in more detail, we're happy to include that.

DR. ROSS: Just a closer definition, a more accurate definition. I mean, I think I've argued two or three times at these meeting for it to be more specific in the conclusion. And usually, those arguments have gone nowhere.

DR. BERGFELD: You could put it in the Discussion you know, the range of use.

DR. DAVID COHEN: Yeah. The Conclusion is tricky.

DR. ROSS: The Conclusion is tricky. But I think if we could be more specific with respect to use concentrations. This one was a bit difficult to interpret, and so I would appreciate that. And per my editorial comments regarding additions and deletions will be in the returns. Okay?

DR. DAVID COHEN: Look, if something is approved in a face wash, and nobody's listed it as a shampoo, but they decide to put it in a shampoo, that's technically not present practices of use. It's a completely reasonable thing for people to do, right? So I don't want this to be overly restrictive, but you want to have some guardrails for certain areas like baby products use where --

DR. BERGFELD: Eye.

DR. DAVID COHEN: -- oral ingestion is possible, eyes. So I don't know. Maybe both the group and PCPC can recommend some other options for us.

DR. BERGFELD: In the past, we had put in the limitations into the conclusion. We got stuck with that. It didn't serve us well. It would be better put in the Discussion, just a discussion of the ranges used and what it's used in even if it's repetitive from the Summary to make that clarification, this is what we're talking about.

DR. ROSS: I'm happy to be guided by my more experienced colleagues, yeah. So that's a good idea.

DR. HELDRETH: I think having that limitation to only what's reported in the report, it is one of the few sticks that we have to manufacturers. It's, hey, do you want the CIR report to cover your product? Then respond to Carol's survey. Otherwise, you're going to have -- you know what I mean?

In the U.S., the responsibility of substantiating safety before product goes to market, hundred percent on manufacturers' shoulders, but an expert Panel report can help mitigate that burden. But you got to participate, or you don't get included in that nice, easy out for substantiating safety.

DR. DAVID COHEN: It may be that the emergence of the RLD could be sort of a cry out to members and nonmembers to, look, this is going to start getting more detailed, which means in some ways it's more helpful, and in other ways it can be very restrictive.

DR. BERGFELD: The other thing that we can do that we did years ago was to look at things that we restricted, in some fashion, to see what happened in the marketplace. We did see, in the past, five that we had called unsafe just gradually leave the marketplace. It might be another stick to say, we are not empowered. And the Department of Justice is the empowered piece. But we're not empowered, but we do have an impact.

DR. DAVID COHEN: Yeah. I would expect. Yeah, I don't know what the frequency of impact is.

DR. BERGFELD: Well, we could do another review and publish that, the impact of the CIR decisions.

DR. TILTON: And generally, we can't come to a conclusion on uses that we don't know about or concentrations that we don't know.

DR. DAVID COHEN: Yeah. No, no, no. Of course. I completely agree, but there's sort of off-report uses, and then there's kind of most of the uses that are not specifically outlined are very reasonable uses, right, because there's so many. All right. I think we got it as best we can.

DR. HELDRETH: Just to speak to that a smidge more. I think a CIR report doesn't become useless to manufacturers in that way. Let's say the Panel reviewed a shampoo, and the maximum concentration was 2 percent, so we said safe as used for present practices of use and concentration. If a manufacturer out there wants to put it in at 5 percent, they can still start with the CIR report to substantiate their safety and do the extra legwork to fill in, okay, this is why it's safe 2 percent more. And it becomes part of their picture.

It doesn't become useless at that point if somebody goes a little off-book there. It puts more burden on their shoulders if they didn't participate in Carol's survey.

DR. DAVID COHEN: But we're going safe as used to be formulated with nonirritating is a bit precautionary, right, because we are concerned about other uses. But again, they're using it less and less, so it may be less of an issue. And if you using it less and less for all kinds of tangential reasons, the last place you might want to put it is in a baby product.

DR. BERGFELD: Right. Right.

DR. DAVID COHEN: Right?

DR. BERGFELD: Highly visible.

DR. DAVID COHEN: But that's assuming people have good sense, and they don't always. Yeah.

DR. SRINIVASAN: Would the report include even when you had no reported uses? Like, how will you say -- like, if you say safe as intended, however you're putting that sentence. What about something like, I'm thinking of potassium Octoxynol-12 had no reported uses, will that capture that too?

DR. DAVID COHEN: Well, that's a good question, right? So it's not used. So in present practices of use it can't be used.

DR. HELDRETH: So it depends.

DR. DAVID COHEN: Well, there we go.

DR. HELDRETH: So if we simply have a conclusion that says, in the present practices of use and concentration as described in this report, and there are no reported uses in there, technically, yeah, the report's not speaking to that ingredient.

However, sometimes when we're looking at a group of ingredients, we will look at ingredients we know do not have any use reported, whether it be frequency of use, concentration of use, or both. And we'll add a caveat that there are some ingredients in this report that are not reported to be used, however the Panel felt that these could be deemed the same conclusion, safe as used or safe as used when formulated to be nonirritating when used in the same types of products and similar concentrations of use. So there's the option there to extend it to those ingredients in the report that don't have a use. But the statement needs to be made to make it clear.

But again, a manufacturer can still use this report and add their information to it when they're substantiating safety. The responsible person at X company, when they're making it part of their package, they can fill in with those pieces.

DR. DAVID COHEN: Yeah, but I don't think intentionally, but you somewhat backtracked a little bit with that. Because if there's no use of Octoxynol-12, yet our conclusion is that it is safe as used -- in the conclusion -- safe as used in present practices, and we are using read-across to the nonoxynols -- forget the octoxynols, we're using nonoxynols. I would read that and say, I'm clear to use Octoxynol-12 because you read across, you specifically didn't -- you could have excluded it. You could have just said data insufficient on -12. But we've said we're going to throw -12 in with all the other because we feel the read-across is adequate to drag them all across the finish line.

So it is not present practices of concentration and use. It's the whole report and everything in there.

DR. BERGFELD: That's a discussion item to put in the Discussion, the read-across?

DR. DAVID COHEN: Well, we haven't always done that, right? I mean, we rarely do that.

DR. ROSS: The read-across, yeah, the read-across was approved. I mean, we said that last time with the nonoxynols. But, I mean, I think this whole discussion gets back to the point that our reports are not regulatory in any way. What we conclude is guidelines, I suppose.

DR. DAVID COHEN: It's advisory.

DR. ROSS: Yeah. And so that, I think, gets to the root of the problem. But I agree if it's safe in present practices of use and the present practices are not in the report, then, as Susan said, we've not looked at it because either we don't know the concentration, or we don't --

DR. DAVID COHEN: Then why are we approving it?

DR. ROSS: Yeah.

DR. DAVID COHEN: And why are we concluding? Why are we including it in the conclusion?

DR. ROSS: Safe as present practices of use? Because it's a lot of different concentrations of these chemicals, of these ingredients in these different products, and we could go through in the conclusion and list every one singly, but I don't think that's going to help.

DR. DAVID COHEN: Well, I think we've recognized that when you group large numbers of chemicals in the same report, this is what we're going to run into, right? So if this was a plant, right, we might say, leaves, flowers, and bark, but not root. We call those out in the conclusion specifically, but we don't do that here, so we're reading across.

DR. BERGFELD: You can adapt and put it in the Discussion and say what you've done.

DR. DAVID COHEN: I don't think there's anything unique to octoxynol for this conversation. I think it's all of them, right? So maybe we just need to, in a future meeting, get our heads wrapped around it. It's not an octoxynol conversation necessarily.

DR. HELDRETH: But I think you're right. I mean, the Panel does have a couple of options for removing that ambiguity. You could say insufficient for that ingredient that doesn't have use, you could say insufficient for.

DR. DAVID COHEN: No, but we --

DR. HELDRETH: Or you could include that caveat that we often include of similar uses and concentrations of the other ingredients in the report as part of the whole read-across approach. You're thinking about the use of it if this ingredient were used in similar products and at concentrations of use, does the Panel feel comfortable with it? Then that conclusion covers those ingredients.

DR. DAVID COHEN: You're pretty comfortable with the read-across on these, aren't you?

DR. ROSS: Yeah.

DR. DAVID COHEN: Yeah, so, I mean, it seems rational to me. Okay. Wow. You see this is what we're talking about. The ones you think may --

DR. SAMUEL COHEN: Well, this one I didn't think was going to be easy.

DR. DAVID COHEN: Yeah.

DR. ROSS: I think you did that on purpose, David.

DR. DAVID COHEN: I don't have any control of the order here. Let's move on to Propylene Carbonate.

Full Panel – March 14, 2025

DR. SNYDER: So this is Octoxynols. We issued a Final Report in 2004. In June 2023, the Panel decided to reopen due to mucus membrane irritation and the uses of Octoxynol-9 at .1 percent in other baby products. We received updated RLD data, so we still have mucus membrane exposures possible.

A new RLD data did not report any uses in baby products. The highest leave-on concentration is 1.5 percent for Octoxynol-12. Let's see, we received Wave 2 comments, and a table with the CIR response. Our team thought those were all good proposed changes, and agree with those changes.

There's some things to discuss, but I guess the Belsito Team said we want to go safe as used when formulated to be nonirritating as a lotion.

DR. DAVID COHEN: Second.

DR. BERGFELD: All right, you have other things to discuss then? Don, you want to speak?

DR. BELSITO: Yes, we needed to bring in the photo spectrum from the Octoxynol paper because of questions about photo absorption. And, the spectrum from Octoxynol-9 clearly shows that that's not an issue if there were some iffy UV data here.

DR. SNYDER: And we gave that information to Priya already, Page 76 on that. And I think she understands how we want that wording. Okay?

DR. BELSITO: Yes.

DR. BERGFELD: Any other points?

DR. BELSITO: And, also, Paul, we pointed out that it was not only not used in baby products, but it's not used in douches according to the RLD, which it was previously reported to be used in.

DR. SNYDER: Yes, correct.

DR. DAVID COHEN: Right. We align that the Discussion should discuss the past use in vaginal preparations, and no use in baby formulations or ocular exposures. As far as genotox, we had some comments about UDS and SCE no longer further being recognized. Well, we can have it in there, we're going to comment on that?

DR. ROSS: Yes, I think there was a comment on the exposure assessment from the section that had a low reliability. There was a VERMEER which is a QSAR exposure assessment basically. And we didn't think the lower reliability data should be in there. But, we shouldn't be in the business of putting lower reliability data in here, at least lower reliability predictions.

DR. BERGFELD: It sounds like there is agreement. All right. I'll call the question then on safe to be nonirritating. All those in favor with that conclusion raise your hand. Again, unanimous, thank you. Moving on to Dr. Cohen, the Propylene Carbonate.

MAY 2000 PANEL MEETING – INITIAL REQUEST FOR DATA

Octoxynol-1, Octoxynol-3, Octoxynol-5, Octoxynol-6, Octoxynol-7, Octoxynol-8, Octoxynol-9, Octoxynol-10, Octoxynol-11, Octoxynol-12, Octoxynol-13, Octoxynol-16, Octoxynol-20, Octoxynol-25, Octoxynol-30, Octoxynol-33, Octoxynol-40, Octoxynol-70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, Sodium Octoxynol-6 Sulfate, and Sodium Octoxynol-9 Sulfate

The Panel agreed that the following Informal Data Request on this group of ingredients should be issued:

(1) Method of manufacture and impurities
(2) Octanol/water partition coefficient
(3) Dermal absorption for chain lengths below 9 (Octoxynol-3 would be the best to use), and if significantly absorbed, then dermal reproductive and developmental toxicity data may be needed
(4) Ultraviolet radiation absorption; if there is significant absorption in the UVB or UVA regions, then photosensitization and phototoxicity studies may be needed
(5) Dermal irritation and sensitization data at concentration of use
(6) Ocular irritation data at concentration of use, if available

SEPTEMBER 2000 MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

Octoxynol-1, Octoxynol-3, Octoxynol-5, Octoxynol-6, Octoxynol-7, Octoxynol-8, Octoxynol-9, Octoxynol-10, Octoxynol-11, Octoxynol-12, Octoxynol-13, Octoxynol-16, Octoxynol-20, Octoxynol-25, Octoxynol-30, Octoxynol-33, Octoxynol-40, Octoxynol-70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, Sodium Octoxynol-6 Sulfate, and Sodium Octoxynol-9 Sulfate

It was noted that the following informal data request on this group of ingredients was issued at the May 18-19, 2000 Panel meeting:

(1) Method of manufacture and impurities
(2) Octanol/water partition coefficient
(3) Dermal absorption for chain lengths below 9 (Octoxynol-3 would be the best to use), and if significantly absorbed, then dermal reproductive and developmentaltoxicity data may be needed
(4) Ultraviolet radiation absorption; if there is significant absorption in the UVB or UVA regions, then photosensitization and phototoxicity studies may be needed
(5) Dermal irritation and sensitization data at concentration of use
(6) Ocular irritation data at concentration of use, if available

Dr. Schroeter stated that the following data were received in response to the Panel's request for data: (1) Data sheet containing chemical and physical properties and impurities data on Octoxynol-11; (2) Material safety data sheet containing chemical and physical properties on Octoxynol-11; (3) Repeat insult patch test on a foot gel containing 8.0% Octoxynol-9 (humans); (4) Two primary skin irritation tests (single insult occlusive patch tests) on

peel-off mask products containing 0.25% Octoxynol-9 (rabbits); (5) Two ocular irritation tests on skin fresheners containing 0.25% Octoxynol-9 (rabbits); (6) Single-insult patch test results on formulations containing 2.0% Octoxynol-9 (animal species not stated); and (7) Repeated insult patch test on a formulation containing 0.5% Octoxynol-9.

Dr. Schroeter also stated that after reviewing all of the available data, his Team concluded that Octoxynols -9 and above are safe as used in cosmetic products. However, concerning Octoxynols -1 through -8, it was suggested that data from the Final Report on Nonoxynols -1 through -8 be incorporated into the present review for use in the Panel's safety assessment of these ingredients. It was agreed that this information would negate the Panel's list of data requests on Octoxynols, as it relates to Octoxynols -1 through -8.

DR. SHANK added that the Panel's 5% concentration limit on Nonoxynols-1 through -8 for leave-on products is also applicable to Octoxynols- 1 through -8, and that any restrictions on Nonoxynol impurities (ethylene oxide, 1,4-dioxane, and unreacted phenols) are applicable as well.

DR. BELSITO said that hormonal effects of alkylphenol ethoxylates that are mentioned in the report on Octoxynols should be addressed in the report discussion, taking into consideration **DR. KLAASEN**'s statement (at yesterday's Team meeting) indicating that the estrogenic effect that would be anticipated from cosmetic products containing Octoxynols would be of very low potency. **DR. BELSITO** added that the issue of estrogenic effects should also be considered a non-issue because of the relative lack of dermal absorption of the Octoxynols and the limitation of 5% for use of Octoxynols in leave-on products.

DR. BELSITO noted that another issue that could be mentioned in the report discussion relates to the aerosol use of Octoxynol-9 in a hair spray.

Dr. Andersen noted that the safety of Octoxynol-9 in these products could be addressed by indicating that the particle size (mass mean aerodynamic diameter) that is associated with hair sprays is considerably larger than what would be expected to be respirable.

Dr. McEwen said that the expected exposure to a hair spray should be indicated in the report discussion as well.

The Panel voted unanimously in favor of issuing a Tentative Report on the Octoxynols with the following conclusion: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concluded

that Octoxynols -9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, and -70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. The Panel also concluded that Octoxynols -1, -3, -5, -6, -7, and -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave-on cosmetic products.

In response to Dr. Schroeter's concern, Dr. Andersen indicated that references in the report text identified as anonymous (e.g., Anonymous, no date) that were received from FDA in response to an FOI request will now be referenced to indicate that FDA is the author (i.e., FDA, no date).

Dr. Schroeter questioned the relevance of a study on the comedogenicity of Octoxynol-9 that was reviewed by the Panel. He said that if this study remains in the report, it should be documented in the discussion that it probably is not relevant because Octoxynol-9 was tested under occlusion (which is not indicative of how cosmetic products are applied) and no skin irritation reactions were reported.

DR. BELSITO recalled that Octoxynol-9 served as the vehicle for sulfur in the human comedogenicity study.

DR. SHANK noted that though Octoxynol-9 served as the vehicle control, it was classified as comedogenic. He said that these results need to be explained in the report discussion.

Dr. Andersen said that if the comedogenicity study remains in the report, the Panel needs to explain why the study's test methodology is not appropriate for evaluating comedogenicity.

DR. BELSITO said that the study should remain in the report and is relevant because skin irritation was not observed. However, he said that it should be explained that the study does not constitute a standard comedogenicity assay because the application site was on the back, humans (instead of rabbits) were used, and the test substance was applied under occlusion. **DR. BELSITO** added that the comedogenic effect observed could have been due to the occlusive effect inducing a folliculitis-like event.

DR. BERGFELD said that she would also prefer that the comedogenicity study remain in the report and, also, that the report discussion contain an explanation of the study results and their relevance.

FEBRUARY 2001 MEETING – THIRD REVIEW/DRAFT FINAL REPORT

Octoxynol-1, Octoxynol-3, Octoxynol-5, Octoxynol-6, Octoxynol-7, Octoxynol-8, Octoxynol-9, Octoxynol-10, Octoxynol-11, Octoxynol-12, Octoxynol-13, Octoxynol-16, Octoxynol-20, Octoxynol-25, Octoxynol-30, Octoxynol-33, Octoxynol-40, Octoxynol-70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, Sodium Octoxynol-6 Sulfate, and Sodium Octoxynol-9 Sulfate

DR. BELSITO recalled that the Panel voted unanimously in favor of issuing a Tentative Report on the Octoxynols with the following conclusion: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concluded that Octoxynols -9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, and -70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. The Panel also concluded that Octoxynols -1, -3, -5, -6, -7, and -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave-on cosmetic products.

DR. BELSITO also stated that the published report (developmental toxicity study on Octoxynol-9 by Leung and Ballantyne, [1999]) that is associated with the report abstract summarized in an earlier report draft has been incorporated into the report text. Pregnant CD rats were dosed orally or cutaneously with Octoxynol-9 in this study. **DR. BELSITO** said that, at fairly high doses of Octoxynol-9 (1600 mg/kg and above), an increased number of supernumerary ribs was noted among the offspring of treated rats. After reviewing these data, his Team reasoned that the doses were much higher than those that would be anticipated for human exposure to a leave-on product containing 5.0% Octoxynol-9 or a rinse-off product. **DR. BELSITO**'s Team also noted that the finding of supernumerary ribs was an exaggeration of a very common birth defect in the rats that were tested. Therefore, in consideration of this common finding in rats along with the observation that the anticipated human exposure to Octoxynol-9 during use of leave-on or rinse-off cosmetic products would be much less than that reported in the developmental toxicity study, **DR. BELSITO**'s Team concluded that a change in the Panel's tentative conclusion is not necessary.

DR. BELSITO noted that the developmental toxicity study by Leung and Ballantyne (1999) should be addressed in the report discussion, explaining why the findings in this study were not of concern.

DR. SHANK said that it is mentioned in the report discussion that the Nonoxynols may contain trace amounts of ethylene oxide, and that the fact that these ingredients may also contain 1,4-dioxane should also be mentioned. He noted that ethylene oxide is a carcinogen and that 1,4-dioxane is also an animal carcinogen, and, possibly, a human carcinogen.

The Panel voted unanimously in favor of issuing a Final Report on the Octoxynol ingredient family with the conclusion indicated in the first paragraph of this section.

Amended Safety Assessment of Octoxynols as Used in Cosmetics

Status: Draft Final Amended Report for Panel Review
Release Date: August 15, 2025
Panel Meeting Date: September 8 – 9, 2025

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel M. Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Preethi Raj, M.S., former Senior Scientific Analyst/Writer, and Priya Ferguson, M.S., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

BVDV	back vertex distance variability
Caco2	human colon adenocarcinoma cell line
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CTFA	Cosmetic, Toiletry, and Fragrance Association
DTH	delayed-type hypersensitivity
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EC_{50}	half-maximal effective concentration
ELISA	enzyme linked immunosorbent assay
EPA	Environmental Protection Agency
EPP	ethylphenyl proprionate
ET_{50}	exposure time that reduces tissue viability to 50%
FCA	Freund's complete adjuvant
FDA	Food and Drug Administration
FOU	frequency of use
FSDC	fetal skin dendritic cells
GRASE	generally recognized as safe and effective
H4IIE	rat liver hepatoma cell line
HeLa	human cervical carcinoma cells
HepG2	human liver hepatoma cell line
HIV	human immunodeficiency virus
HRIPT	human repeat insult patch test
IgM	immunoglobulin M
IL	interleukin
IP-10	gamma interferon inducible protein 10
LDH	lactate dehydrogenase
LD	lethal dose
l.o.	leave-on
MDCK	Madin-Darby canine kidney
MDSS	maximal primary Draize irritation score
MIP-3α	macrophage inflammatory protein 3α
MMAD	mass mean aerodynamic diameter
MoCRA	Modernization of Cosmetics Regulation Act
MOS	margin of safety
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	molecular weight
NOEL	no-observed-effect-level
NOAEL	no-observed-adverse-effect-level
NoG	Notes of Guidance
NRU	neutral red uptake
OECD	Organisation for Economic Co-operation and Development
OTC	over-the-counter
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered solution
PEG	polyethylene glycol
PFC	plaque-forming cells
PII	primary irritation index
RLD	Registration and Listing Data
r.o.	rinse-off
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SRBC	sheep red blood cells
SLS	sodium lauryl sulfate
TG	test guideline
TK6	human lymphoblastoid cell line
TNF-α	tumor necrosis factor alpha
TPA	12-*O*-tetradecanoylphorbol-13-acetate

US	United States
UV	ultraviolet
VEC-100	reconstructed human vaginal-ectocervical epithelium
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of 25 octoxynol ingredients, which are reported to function as surfactants in cosmetics. The Panel reviewed the available data to determine the safety of these ingredients. Industry should minimize impurities that could be present in cosmetic formulations, such as heavy metals and ethylene oxide impurities, according to limits set by the US Food and Drug Administration (FDA) and the US Environmental Protection Agency (EPA). The Panel issued an amended report with a revised conclusion stating the octoxynols reviewed in this report are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating.

INTRODUCTION

This assessment reviews the safety of the following 25 octoxynol ingredients as used in cosmetic formulations:

Octoxynol-1	Octoxynol-12	Octoxynol-9 Carboxylic Acid
Octoxynol-3	Octoxynol-13	Octoxynol-20 Carboxylic Acid
Octoxynol-5	Octoxynol-16	Potassium Octoxynol-12 Phosphate
Octoxynol-6	Octoxynol-20	Sodium Octoxynol-2 Ethane Sulfonate
Octoxynol-7	Octoxynol-25	Sodium Octoxynol-2 Sulfate
Octoxynol-8	Octoxynol-30	Sodium Octoxynol-6 Sulfate
Octoxynol-9	Octoxynol-33	Sodium Octoxynol-9 Sulfate
Octoxynol-10	Octoxynol-40	
Octoxynol-11	Octoxynol-70	

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), these ingredients are reported to function in cosmetics as surfactants (Table 1).[1] The Panel first reviewed these octoxynol ingredients in a safety assessment that was published in 2004.[2] The Panel issued a final report with the conclusion that Octoxynol-9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, -70, and Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. The Panel also concluded that Octoxynol-1, -3, -5, -6, -7, and -8, and Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave on cosmetic products.

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years, and it has been at least 15 years since the original assessment was issued. At its June 2023 meeting, the Panel determined that this safety assessment should be reopened to explore the irritation potential of these ingredients in products which come in contact with mucous membranes (suspected use of Octoxynol-9 in vaginally applied products). Furthermore, the report was also reopened due to the newly reported use of Octoxynol-9 at 0.1% in baby products.[3]

Of note, the Panel has also published reviews on the safety of nonoxynols, which are structurally similar, slightly longer chain (1 carbon longer) ingredients in 1983, 1999, and in 2015, which are available on the Cosmetic Ingredient Review (CIR) website (https://cir-reports.cir-safety.org).[4-6] During the 2015 review, the Panel concluded that the nonoxynols are safe in the present practices of use and concentration in cosmetics as described in the safety assessment, when formulated to be non-irritating.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted January 2025. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website ((https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline)). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Summarized excerpts from the previous report on these octoxynol ingredients are included in this document, as indicated by *italicized text*. Because the original (2004) octoxynols report included supporting data from the 1983 and 1999 nonoxynols reports; accordingly, those data, as well as data from the final report on nonoxynols that was published in 2015,[6] are also disseminated throughout the text of this re-review document as proposed read-across sources, as appropriate, and are also identified by *italicized text*. (This information is not included in the tables or the summary section.)

Much of the published data in the literature has been identified under the name "Triton X-100." According to different sources, this name corresponds to several different octoxynol ingredients named in this report (e.g., Octoxynol-1, Octoxynol-9). Because it is unknown which octoxynol ingredient is being referred to, studies using Triton X-100 have been placed under the subheading "an octoxynol (number of ethoxy repeat units unknown)" and the test substance is referred to as "an octoxynol" throughout the study summaries. It should be noted, however, that during the previous review of this report, it was thought that Triton X-100 referred only to Octoxynol-9. Therefore, data on Triton X-100 was included in those reports as Octoxynol-9 (and thus are included in this report, in italicized text, also as Octoxynol-9). It should also be noted that all

octoxynols are mixtures with varying averages of ethoxy repeat units. Triton X-100 is generally considered to have an average of 9.5 ethoxy units.[7]

CHEMISTRY

Definition and Structure

According to the *Dictionary*, these octoxynols are ethoxylated alkyl phenols which generally conform to the structure in Figure 1.[1]

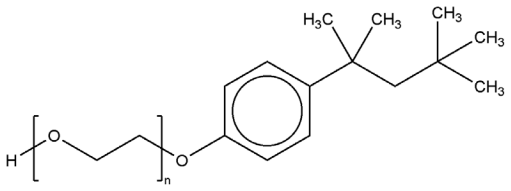


Figure 1. General formula for octoxynols, wherein "n" is the average number of ethoxy repeat units (e.g., n = 3 for Octoxynol-3)

These ingredients are mostly identified by the generic CAS Nos. 9002-93-1; 9036-19-5; and 9004-87-9. Specific CAS Nos. are assigned to several of the octoxynol ingredients. The definitions, idealized structures, and reported functions of the ingredients included in this review, as well as the CAS Nos., are provided in Table 1.[1]

Chemical Properties

Octoxynols, or polyoxyethylene octylphenyl ethers, are ethoxylated alkylphenols with the chemical formula, $C_8H_{17}C_6H_4(OCH_2CH_2)_nOH$, where n in the formula represents the number of moles of ethylene oxide, average value.[2] *The average value for n in chemicals of this class is evident in the ingredient name (e.g. Octoxynol-1, Octoxynol-3, etc.). For cosmetic ingredients, n can vary from 1 – 70. By contrast, the nonoxynols have the formula $C_9H_{19}C_6H_4(OCH_2CH_2)_nOH$.*

These ingredients are water white to light amber liquids.[2] Octoxynol-9 has a water solubility of 4.55 mg/l (at 20° C) and has an average molecular weight of 647 Da.[8] Chemical properties of the octoxynols included in this report are presented in Table 2.[9-20]

Method of Manufacture

Octoxynol-9 is reportedly prepared by reacting p-(1,1,3,3-tetramethylbutyl)phenol with ethylene oxide, at elevated temperature and under pressure, in the presence of sodium hydroxide.[2] *In general, the semi batch process is commonly used for the production of polyoxyethylated nonionic surfactants. A reaction vessel is charged with alkylphenol and an appropriate catalyst (not specified). The catalyzed alkylphenol is heated to reaction temperature and purged with nitrogen to reduce the water generated during the catalysis step; water removal is integral to minimize polyethylene glycol formation. After drying, ethylene oxide is added. When the alkylphenol has been polyoxyethylated to the desired extent, the reaction mixture is held at reaction temperature until the residual ethylene oxide concentration in the liquid product has been reduced to an acceptable level. The product is then neutralized, post-treated, and filtered for removal of insoluble salts formed during neutralization. The raw materials used in the production of Octoxynol-11 are exclusively from petrochemical origin.*

Impurities

At the time of the original report, Cosmetic, Toiletry, and Fragrance Association (CTFA [now known as the Personal Care Products Council (Council)]) specifications stated that Octoxynol-1 has a minimum purity of 99%, and that Ocyoxynol-5 and Octoxynol-9 contain sulfated ash (0.25% maximum) and water (0.5% maximum).[2] *The National Formulary stated Octoxynol-9 may contain arsenic (2 ppm), heavy metals (0.002%), and no more than 5 ppm ethylene oxide as impurities. A sample of Octoxynol-11 was reported to contain < 1% water; specifications for the following impurities included sulfated ashes (< 0.2%), heavy metals (< 10 ppm Pb), and arsenic (< 2 ppm). The percentage of volatiles in a sample of Octoxynol-13 was reported to be 0.5%, including < 0.0002% ethylene oxide.*

Ultraviolet (UV) Absorption

An ultraviolet (UV) spectral analysis of a 0.32 mM aqueous solution of Octoxynol-9 demonstrated an absorption maximum at 276 nm and slight absorbance at 290 nm, as a tail on the peak at 276 nm.[2] *No detectable absorbance was observed above 295 nm. It was concluded that Octoxynol-9 had no significant absorbance in the UVA and UVB regions of the spectrum.*

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US FDA the cosmetics industry on the expected use of these octoxynols in cosmetics. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (Council), and it is these values that define the present practices of use and concentration. Frequencies of use obtained from the FDA include data from the Voluntary Cosmetic Registration Program (VCRP) database as well as Registration and Listing Data (RLD). As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was discontinued in 2023 and, as of 2024, manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-year period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products are not included in this exemption.[21] Please note, at this time, it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting). Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to RLD submitted in 2024, Octoxynol-9 is reported to have the greatest number of uses (it is reported to be used in 38 formulations; Table 3).[22] VCRP (2023) data indicated Octoxynol-11 had the greatest reported frequency of use (8 total formulations).[23] In 2001, Octoxynol-9 was reported to have the highest number of uses (131 total formulations).[2] The results of the concentration of use survey conducted by the Council in 2022/2025 indicate Octoxynol-9 has the highest maximum reported concentration of use; it is used at 2% in skin cleansing preparations.[3,24] The highest concentration reported in leave-on products in 2022 was for Octoxynol-12; it is reported to be used at a maximum of 1.5% in face and neck products (not spray). Previous concentration of use data (1999/2001) indicated that Octoxynol-10 had the highest concentration of use (it was used at up to 25% Octoxynol-10 in hair bleaches). The ingredients not in use according to the VCRP, RLD, and industry survey are listed in Table 4.

VCRP (2023) data indicated that some of these ingredients may be used near the eye (e.g., Octoxynol-11 is used in eye lotions and other eye makeup preparations; concentrations not stated) and in products that may be incidentally ingested (Octoxynol-12 is used in lipsticks; concentration not stated). These uses, however, were not reported in RLD submitted to CIR in 2024. RLD indicate that mucous membrane exposure to Octoxynol-9 may occur, as it is used in bath soaps and body washes and disposable wipes (at 0.36%). According to 2022 concentration of use data, Octoxynol-9 is used at 0.1% in other baby products; however, this use is not reported in 2024 RLD.

In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays (e.g., Octoxynol-9 is used in cologne and toilet waters; concentration not stated) would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.

Some products containing these ingredients may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available, in some instances. Some of the reported product categories for these ingredients as listed in the RLD do require designation if airbrush application is used (e.g., Octoxynol-9 is used in indoor tanning preparations, but no airbrush use was indicated). Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available, when submitted. Please note that no concentration of use data were provided indicating airbrush application. Nevertheless, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The octoxynol ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[25]

Non-Cosmetic

Octoxynol-1, -3, -5, -7, -9, -8, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, and -70, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-2 Ethane Sulfonate have been approved for indirect food uses as surfactants in pesticide dilutions applied to crops (21CFR172.710); components of paper products that come in contact with dry food

(21CFR176.180); and components of defoaming agents (21CFR176.210) and emulsifiers (21CFR178.3400) used in the production of paper goods utilized for food transport. Octoxynol-30, -33, -40, and -70 are listed in 40CFR180.960 as polymers that are exempt from the requirement of tolerance.

In 2002 (67FR31123), the FDA issued a final rule stating that the use of Octoxynol-9 in over-the counter (OTC) drugs is not deemed generally recognized as safe or effective (GRASE), and therefore that any drug product containing Octoxynol-9 labeled for OTC use as a vaginal contraceptive or spermicide will be considered misbranded (and will require a drug application), which was reiterated in 21CFR310.545.

Octoyxnol-1 is commonly employed as a detergent in the manufacture of biotherapeutics, such as vaccines.[26,27] Octoxynol-40 has an FDA-approved drug use in ophthalmic solution drops at a maximum potency per unit dose of 0.05% w/v.[28]Additionally, Octoyxnol-40 is utilized in various (nanomicellar) ocular drug delivery formulations.[29-31]

In accordance with a 2020 Amendment to Article 56(1) of Regulation (EC) No. 1907/2006, uses of the substance group 4(1,1,3,3-tetramethylbutylphenol, ethoxylated (covering well-defined substances and substances of unknown or variable composition, complex reaction products or biological materials, polymers and homologues) require authorization for use in pharmaceuticals after January 2021.

TOXICOKINETIC STUDIES

Percutaneous Absorption

nonoxynols

The in vitro skin penetration of nonoxynol-2, -4, and -9 (10% w/w in isopropyl myristate) was evaluated using heat-separated human epidermal membranes in an experiment designed to mimic in-use conditions relative to ingredient use in "on-head" rinse-off products such as an oxidative hair color. Each nonoxynol solution (10 µl) was dispensed over the surface of the stratum corneum and rinsate samples (obtained with isopropyl myristate) were removed from the receptor medium at 2, 4, 6, 8, 25, and 48-h post application of the vehicle. Most of the applied nonoxynols were recovered in the 1 and 48-h rinsates and no quantifiable amounts were present in the receptor phase, indicating that none of the nonoxynols permeated through the skin to any great extent. In a third experiment, the in vitro skin penetration of nonoxynol-2, -4, and -9 (10% w/w in isopropyl alcohol per solution; volume = 15 µl) was evaluated in heat-separated human epidermal membranes (n =3) to mimic the in-use conditions relative to nonoxynols in leave-on products. Solutions remained in contact with the skin for 48 h, after which the entire receptor media was analyzed by high performance liquid chromatography. The total skin permeation for the nonoxynols was as follows 6.17 µg/cm^2, corresponding to 0.57% of the applied dose for nonoxynol-2, 7.10 µg/cm^2, corresponding to 0.66% of applied dose for nonoxynol-4, and 4.73 µg/cm^2, corresponding to 0.49% of the applied dose for nonoxynol-9. Based on these data, the researchers stated that the total skin penetration for nonoxynol-9 was slightly lower than that for nonoxynol-2, and -4, and, that the levels of nonoxynols absorbed followed a brief exposure period would be very low. Therefore, the potential for systemic exposure to the lower molecular weight nonoxynols was considered to be extremely low under conditions of rinse-off application to the scalp (500 – 750 cm^2) in products such as hair dyes.

The percutaneous absorption of nonoxynol-4 and nonoxynol-9 was studied in vitro using human, pig, and rat skin samples in flowthrough diffusion cells.[32] Topical solutions of 0.1, 1, or 10% ^{14}C-nonoxynol-4 (each in polyethylene glycol (PEG-400)) and 0.1, 1, or 10% aqueous ^{14}C-nonoxynol-9 were applied, and radioactivity in the perfusate was monitored over an 8-h period. Skin penetration was generally less than 5% of the applied dose, most of which was found in the stratum corneum. For both ^{14}C-nonoxynols in all skin samples, the fraction of dose absorbed was highest for the lowest applied concentration. Dermal absorption was similar across all concentrations. In rat skin, penetration, but not absorption, was greater when water was used as the vehicle compared to PEG-400 as the vehicle. The results of the study suggested that ^{14}C-nonoxynol-9 and ^{14}C-nonoxynol-4 were minimally absorbed across the skin.

Absorption, Distribution, Metabolism, and Excretion

Dermal

Octoxynol-9 was administered at doses ranging from 5 to 20 ml/kg to 3 guinea pigs in an acute dermal toxicity study.[2] No evidence of dermal absorption was observed. No further details were provided.

Oral

The absorption, distribution, and excretion of Octoxynol-40 was evaluated using 4 rats and 2 dogs.[2] Tritium-labelled Octoxynol-40 ([^{3}H]Octoxynol-40; specific activity = 5.85 mC/g) was fed, via gavage, to 4 rats; 2 additional rats served as controls. Feces and urine were collected and analyzed in 2 rats and both dogs, whereas only urinalyses was performed for the other 2 rats. Essentially all of the radioactivity that was fed was recovered in the feces of rats (up to 92.2%) and dogs (up to 86.4%). Urine (2 dogs and 2 rats) and carcass (2 rats) were said to contain minor amounts of radioactivity. The percent recovery of radioactivity in the urine was 0.59 – 2% (4 rats) and 1.17% and 1.46% (2 dogs, respectively).

Intravaginal

Octoxynol-9 was stated to be rapidly and quantitatively absorbed from the vaginal wall into the systemic circulation of rabbits and rats.[2] *This statement was based on a study in which nonoxynol-9 was absorbed through the vaginal wall of rabbits and rats and excreted by liver-bile-feces and kidney-urine routes (details not reported).*

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

The acute dermal toxicity of Octoxynol-9 was evaluated using 3 guinea pigs.[2] *Single doses of the test substance were administered via a cuff at doses ranging from 5 - 20 ml/kg. Slight to moderate edema and scattered erythema (at periphery) were observed 24 h post-application. At 1 wk, desquamation and slight alopecia were observed. There was no evidence of dermal absorption; the LD_{50} was greater than 20 ml/kg.*

an octoxynol (number of ethoxy repeat units unknown)

The acute dermal toxicity of a leather cream was evaluated in Wistar albino rats (3/sex/group) according to Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 402.[33] The cream comprised of white beeswax, carnauba wax, and distilled water, as well as an octoxynol, silicone oil, linseed oil, Sudan black dye, and nigrosine black dye (amounts not specified). Animals received either no treatment (controls), wax base, laboratory-based sample of the leather cream, or the marketed leather cream on a shaved area of the back and were observed for signs of irritation, general signs of toxicity, and mortality for 14 d; animals were necropsied on day 15 and treated tissue underwent histopathological examination. No mortality, signs of erythema or edema, significant changes in body weights, or food consumption was observed. No damage in skin tissue was observed in the treated groups, compared to controls, indicating that no dermal toxicity was caused by the leather cream samples. No further details were provided.

Oral

Several acute oral toxicity studies were performed in rats using short-chain octoxynols.[2] *A mean acute oral LD_{50} value of 7.1 ± 0.1 ml/kg was reported for rats (number not stated) dosed orally with Octoxynol-1. Following the single oral administration of Octoxynol-3 to rats (number not stated), a mean acute oral LD_{50} of 4.0 ± 0.2 ml/kg was reported. A mean acute oral LD_{50} of 3.8 ± 0.2 ml/kg was reported for rats (number and strain not specified) that received a single oral dose of Octoxynol-5. No further details were provided for these studies.*

The acute oral toxicity of undiluted Octoxynol-9 was evaluated using a total of 10 mice.[2] *A single dose of the test substance was administered at doses ranging from 200 – 3200 mg/kg. Weakness and diarrhea were observed; the LD_{50} was determined to be approximately 1600 mg/kg. In another acute oral toxicity study, groups of 10 Charles River SCD rats were administered a single oral dose of undiluted Octoxynol-9 at doses ranging from 0.678 – 1.86 ml/kg. The mortality rate per group was dose-dependent; 9 out of 10 of the animals administered the highest dose died. The acute oral LD_{50} was determined to be 1.06 ml/kg (confidence limits = 0.989 – 1.29 ml/kg). Ten adult rats were given a single oral dose of 200 – 3200 mg/kg Octoxynol-9. Slight to moderate weakness, diarrhea, ataxia, and prostration were noted at the highest dose; the LD_{50} was determined to be in the 800 – 1600 mg/kg range.*

Four groups of 6 Wistar-derived albino rats (3/sex/group; weights = 150 – 300 g) were used to evaluate the acute oral toxicity of Octoxynol-13.[2] *The animals received a single graded dose (from 691 – 1400 mg/kg) by gavage and were then observed for signs of pharmacologic activity and toxicity at 1, 3, 6, and 24 h after dosing. Following a 14-d non-treatment period, the animals were killed and subjected to necropsy. Gross changes included reddening of the gastrointestinal mucosa and fibrous tissue encasing the heart or lungs. An LD_{50} of 985 mg/kg Octoxynol-13 was reported.*

Fasted male albino rats were administered a single dose of either Octoxynol-16 (30%), Octoxynol-16 (70%), Octoxynol-20 (70%), Octoxynol-30 (70%), or Octoxynol-40 (70%) via gavage.[2] *Ten animals were used per group and 4 groups were used per test article, with the exception of Octoxynol-40 (70%), for which only one group was used. Octoxynol-16 (30%) was administered at up to 6 g/kg, Octoxynol-16 (70%) and Octoxynol-20 (70%) at up to 7 g/kg, Octoxynol-30 (70%) at up to 28 g/kg, and Octoxynol-40 (70%) at 28 g/kg. Eight of the 10 rats dosed with 6 g/kg Octoxynol-16 (30%) and 7/10 rats dosed with 7 g/kg Octoxynol-16 (70%) died; the LD_{50} values for these groups were 2.68 and 2.78 g/kg, respectively. Only one rat dosed with 28 g/kg Octoxynol-40 (70%) died. The LD_{50} values for the Octoxynol-20 (70%) and Octoxynol-30 (70%) groups were 3.64 and 21.20 g/kg, respectively. Diarrhea was reported with the groups given Octoxynol-16 and Octoxynol-20. An analysis of variance test using the LD_{50} values for 70% Octoxynol-16, 70% Octoxynol-20, and 70% Octoxynol-30 indicated that the difference between these values was significant at the 5% level.*

Inhalation

Two Swiss mice were exposed, nose-only, to airborne concentrations of 4.4, 15, 36, or 38 mg/l Octoxynol-9 at a rate of 30 l/min.[2] *The airborne exposure resulted in a concentration-related decrease in respiratory rate; Octoxynol-9 was classified as a sensory irritant. In another study, the acute inhalation toxicity of Octoxynol-9 was evaluated using 50 Syrian hamsters that were exposed to aerosolized Octoxynol-9 with a mass mean aerodynamic diameter (MMAD) of 1.5 µm and a*

concentration of 2.8 mg/l (estimated lung burden: 203 – 835 µg/g lung), or by bronchopulmonary lavage with 0.01 – 0.10% Octoxynol-9 in isotonic saline (estimated lung burden: 302 – 3180 µg of Octoxynol-9). In the inhalation study, animals died from laryngeal obstruction, with moderate pulmonary edema and pneumonitis, and the LD_{50} was 501 µg/g lung. In the lavage study, animals died from pulmonary edema and acute pneumonia, and the LD_{50} was 2060 µg/g. The lungs of Syrian hamsters were treated with 0.05% Octoxynol-9 in 0.9% saline, or only saline, via lavage (80% lung volume). Lung cell [^{3}H]thymidine uptake was evaluated after animals received a 2-h pulse of the radioactive label before they were killed at 2, 18, 24, 48, or 72 h after lavage was initiated. The researchers stated that the increased [^{3}H]thymidine uptake into the alveolar macrophages of lungs lavaged with Octoxynol-9, compared to saline controls, was not attributed to an altered distribution of type I, type II, or endothelial cells, but to an increased incorporation of label into the alveolar macrophages and injured ciliated airways. Six male and 6 female Syrian hamsters (Sch:(SYR) strain) were treated by lavage (1 lung per animal; two consecutive washes) with 0.01, 0.05, 0.075, or 0.1% Octoxynol-9 (in saline) via bronchopulmonary lavage and anesthetized. Lactate dehydrogenase (LDH) release into the alveolar fluid during lavage was measured as an indication of immediate injury. The increase of LDH activity in the cell-free portion of the lavage fluid was correlated with increasing concentrations of Octoxynol-9 (correlation coefficient = 0.98). No deaths occurred in the control group or in groups dosed with 0.01 or 0.05% Octoxynol-9. All the animals treated with 0.075 or 0.1% Octoxynol-9 died anywhere from 7 h to 3 d post lavage. Atelectasis (focal and mild) and severe pulmonary edema were noted at microscopic examination. Histopathologic findings in animals that died at days 2 and 3 post lavage included focal necrosis associated with hemorrhagic areas of the lung and an acute generalized pneumonia with polymorphonuclear leukocyte and macrophage exudation. Tritiated Octoxynol-9 was administered to groups of male and female Syrian hamsters (4 – 8/group; 32 total), via lavage, at weight percentage concentrations of 0.01, 0.05, 0.06, 0.075, or 0.1% in isotonic saline. Twenty-four hamsters treated with isotonic saline were used as controls; none of the controls died. Mortality rates in test animals were as follows: 0.01% (0/4), 0.05% (1/8), 0.06% (4/8), 0.075% (8/8), and 0.1% Octoxynol-9 (4/4). Congested lungs, focal areas of peripheral atelectasis, and blood-tinged fluid in the trachea and large bronchi were noted at necropsy. Several pulmonary and bronchial histopathologic changes were observed and varied as a function of survival time; no evidence of residual injury was observed in animals which survived until necropsy. An LD_{50} of 2100 µg (estimated mean lung burden of Octoxynol-9) was reported. In another experiment, groups of 50 hamsters (95-d or 419-d old) were exposed, nose-only, to an Octoxynol-9 aerosol. The 95 d-old hamsters were exposed to a nebulized aerosol of Octoxynol-9 with an MMAD of 1.47 µm while 419-d-old hamsters were exposed to a nebulized aerosol of Octoxynol-9 with an MMAD of 1.51 µm; in each group a mass concentration of 3 mg/l was produced by nebulization of 10% solution of Octoxynol-9 (in ethanol). Groups of 10 animals were removed from the exposure chamber at different time intervals (not specified) in order to provide initial respiratory tract burdens, which ranged from 800 – 3100 µg. Ten hamsters from each age group which were exposed to aerosolized ethanol for 37 min served as controls. Death was attributed to obstructive asphyxia; laryngeal and epiglottic edema were the most prominent gross features. No abnormalities were observed in the lower trachea, major bronchi, lungs, or in the large or small bronchi. Upon microscopic examination, mucosal ulcerations with necrotic bases were observed in laryngeal secretions and were present in single alveoli.

Short-Term Toxicity Studies

Dermal

Multiple octoxynols were applied to the skin of rabbits (strain and number not specified) over a period of 4 wk (20 applications total).[2] *Ingredients were applied at the following concentrations: 1% Octoxynol-1, 1% Octoxynol-3, 0.1% Octoxynol-9, and 0.1% Octoxynol-13. No histopathologic changes were noted for each ingredient tested. No further details were provided.*

Inhalation

In a short-term inhalation toxicity study, Sprague-Dawley CD rats (5/sex) were exposed to an ethoxylated para-tert-octyl phenol (an octoxynol, number of moles of ethylene oxide not stated; target concentration: 10 mg/m^3) in an inhalation chamber for 5 d/wk (6 h/d) for 2 wk.[2] *The MMAD of the test substance was 1.8 µm. None of the animals died. Lung-to-body weight ratios in test animals were significantly greater when compared to controls. Reddening of the lung was observed grossly in 4 males and 3 females. Upon histopathologic examination, inflammatory changes in the alveolar walls/perivascular space were noted. Compared to air-exposed controls, both the incidence and severity of this finding were greater. Alveolar/bronchiolar epithelial hyperplasia was observed only in treated animals, and therefore, was considered treatment-related.*

Intravaginal

<u>nonoxynols</u>

Groups of 6 Sprague-Dawley female rats were treated intravaginally with nonoxynol-9 in a short-term toxicity study.[2] *Instillations of 5 mg of nonoxynol-9/100 g bw, in saline, were made to the upper aspect of the vagina daily for 5, 10, 15, or 20 d, after which blood samples were also obtained. Controls were intravaginally injected with saline. Animals were exsanguinated at 5-d intervals and the liver, kidneys, and lungs were removed. Total hydroxyproline and deoxyribonucleic (DNA) content were determined in hepatic and renal tissues. Lesions of nonspecific inflammation with destruction of normal lobule architecture, increased density of rough endoplasmic reticulum, and a significant increase in serum glutamic*

oxaloacetic transaminase activity were observed in liver specimens after 15 injections. DNA content and total hydroxyproline were significantly increased in kidneys after 15 d.

Subchronic Toxicity Studies

Oral

Male and female rats (15/sex) received 5% Octoxynol-40, in the diet daily for 3 mo.[2] *Another group of 15 male and 15 female rats served as controls. Three test animals (all males) and 2 controls (1 male and 1 female) died. Test animal deaths were not related to dosing with Octoxynol-40. No effects on growth or food consumption were noted and urinary concentrations of sugar and protein were comparable between test and control animals. Results of hematologic evaluations indicated no definite effects of Octoxynol-40 dosing. No statistically significant differences between the organ-to-body weight ratios of heart, spleen, kidney, liver, and testes were observed between test and control animals. Mean testes/body weight ratios x 10-3 were 8.7 ± 1.1 g (test animals) and 9.2 ± 1.1 g (controls). No test substance-related lesions were observed at histopathologic examination.*

In another study, groups of young albino rats (30/sex/group) were administered 0.035, 0.35, or 1.4% Octoxynol-40 in daily diet for 3 mo. Controls received basic diet only. Compared to controls, no adverse effects on the testes/body weight ratio were noted at any of the 3 administered doses. In another study, Octoxynol-40 was administered to groups of 4 purebred Beagle dogs (2/sex/group) at concentrations of 0.35 or 5%, in the diet for 3 mo. An additional group of 4 dogs served as controls. No adverse effects on body weight, food consumption, hematocrit, hemoglobin, total and differential white cell counts, urinary concentrations of sugar and protein, organ-to-body weight ratios (including testes/body weight ratios), or test substance-related lesions were observed.

Chronic Toxicity Studies

Oral

The chronic oral toxicity of Octoxynol-40 was evaluated in groups of young albino rats (30/sex/group).[2] *Octoxynol-40 was administered at concentrations of 0.035, 35, or 1.4% in the daily diet for up to 2 yr. Controls received basic diet only. After the third month of dosing, 5 males and 5 females from each dose group were killed, and tissues (heart, lung, liver, kidney, and gonads + other tissues) were subjected to histopathologic examination. The remaining animals (20/group) continued to receive treatment till the end of the 2-yr study, after which surviving animals were killed and necropsied. No adverse effects on survival, growth, food consumption, hematocrit, hemoglobin, total and differential leukocyte counts, urinary concentrations of sugar and protein, organ-to-body weight ratios, or pathological lesions were observed.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Dermal

Groups of 25 Sprague-Dawley CD rats were dermally dosed with 530, 1600, or 4270 mg/kg/d Octoxynol-9, at a constant dose volume of 4 ml/kg, from gestation day 6 to day 15.[2] *Controls received dermal applications of deionized and filtered water. Each test article application was made under occlusion to a clipped, 20 cm^2 area of the back for 6 h. One rat in the highest dose group was found dead on gestation day 7; the cause of death was not determined. Body weight gain over the entire gestational period was reduced only in the highest dose group. No statistically significant differences in lung, liver, or kidney weights were noted between test (all dose groups) and control groups. No dams aborted or delivered early and no effects on gravid uterine weights, number of ovarian corpora lutea, number of total, viable, or nonviable implantations/litter, or preimplantation loss were observed, compared to controls. The incidence of atelectasis (lung collapse) was significantly increased in dams in the 1600 and 4270 mg/kg/d groups. A significant decrease in the incidence of dilated renal pelvis was noted in the 530 mg/kg/d group. An increased incidence of vestigial fourteenth thoracic rib was noted in pups from all 3 dose groups. The following statistically significant skeletal variations were observed only in pups from the highest dose group: poorly ossified lumbar arches, unossified and poorly ossified sternebra, unossified cervical centrum, rudimentary bone island, poorly ossified hyoid, poorly ossified zygomatic arch, and poorly ossified supraoccipital. The researchers concluded that dermal exposure to Octoxynol-9 produced a low order of maternal toxicity, while having a pronounced effect on fetal skeletal development. The toxicological significance of these abnormalities seen in this study were unclear; the increased incidence of supernumerary thoracic ribs was considered a common developmental variation. The no-observed-effect-level (NOEL) for Octoxynol-9 related to maternal toxicity was 1600 mg/kg/d, while the NOEL related to developmental toxicity was determined to be 70 mg/kg/d.*

Oral

No signs of maternal or fetal toxicity were observed in 50 female CD-1 mice that received 800 mg/kg/d Octoxynol-9, via gavage, on days 6 through 13 of gestation.[2] *In another developmental toxicity study, groups of 27 Sprague-Dawley CD rats received 0, 70, or 340 mg/kg/d Octoxynol-9, in the diet, from days 6 through 16 of gestation. A control group received untreated feed. On gestation day 17, the test diet was withdrawn and replaced with the control diet. None of the animals died, and no clinical signs were reported. No effects on gravid uterine weights were noted in any dosage group. When corrected for gravid uterine weight, body weight gains over the entire gestational period were reduced in the 70 mg/kg/d group; these results were not considered toxicologically significant. No effect on the number of ovarian corpora lutea, the number of total, viable, or nonviable implantations per litter, or preimplantation loss were observed, compared to controls.*

However, a statistically significant increase in the incidence of displaced testes in fetuses was noted in the 340 mg/kg/d group. Statistically significant skeletal variations observed only in the 340 mg/kg/d group included: vestigial fourteenth rib, accessory ribs on cervical vertebra 7, and both cervical and fourteenth thoracic rib, and decrease in the incidence of poorly ossified hyoid. The authors concluded that oral exposure to Octoxynol-9 produced a low order of maternal toxicity, while having a pronounced effect on fetal skeletal development. The toxicological significance of these abnormalities seen in this study was unclear; the increased incidence of supernumerary thoracic ribs was considered a common developmental variation.

Intravaginal

In a developmental and reproductive toxicity study, groups of pregnant Sprague-Dawley COBS CD rats were intravaginally administered either 0.5 or 5 mg/kg/d Octoxynol-9 (in contraceptive jelly) from gestation day 6 to gestation day 15.[2] *Three additional groups of 25 rats served as untreated controls, sham controls, and vehicle controls (contraceptive jelly excipients). Statistically significant reductions in body weight were observed in sham controls ($p = 0.05$) and the 5 mg/kg/d group ($p = 0.01$) on gestation day 6 to 16. The biological significance of the reduced body weight was questionable, given that body weights were comparable for all groups after the treatment period and for the entire duration of the observation period. Malformations were observed in 2 female fetuses from 2 different litters of dams dosed with 0.5 mg/kg/d. These malformations consisted of a threadlike tail in one fetus and the following in the other fetus: cleft palate, cleft lip, misplaced pinna, open eye lid, brachygnathia, and aglossia. Skeletal malformations were not observed. The incidence of developmental variations ranged from 70 (untreated controls) to 114 (sham controls) per group and consisted of the following: malaligned sternebrae, variations in the number of ribs, and, mainly, ossification retardation of the skull, hyoid, os coxae, sternebra, and vertebral centra. These variations were considered to be evenly distributed among test and control groups; visceral variations were not observed. One nonviable fetus from the 5 mg/kg/d group was examined. No malformations or developmental variations were noted and no other dead fetuses or late resorptions were observed. It was concluded that Octoxynol-9 was not embryotoxic or teratogenic when administered intravaginally to rats during organogenesis.*

GENOTOXICITY STUDIES

In Vitro

Octoxynol-1 was not mutagenic in an Ames test using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 at test concentrations ranging from 0.0031 – 0.1 µl/plate with metabolic activation and from 0.0063 – 0.1 µl/plate without metabolic activation.[2] *The mutagenic effect of several known mutagens in combination with Octoxynol-9 was tested using S. typhimurium strain TA100. Concentrations of the following mutagens, which were known to produce 500 – 1000 revertants/plate, were added to top agar: sodium azide in water (0.5 µg/plate); N-aminomorpholine in water (5.2 µmol/plate); ethyl methanesulfonate in dimethyl sulfoxide (DMSO) (42.3 µmol/plate); benzo(a)pyrene in DMSO (3 µg/plate, with metabolic activation); 2-aminoanthracene in DMSO (2 µg/plate); and styrene oxide in DMSO (4 µmol/plate). Octoxynol-9 (unspecified amount) was applied directly to the hardened agar, as crystals, or as a liquid to sterile, filter paper discs. Octoxynol-9 caused toxicity (background lawn appeared less dense compared to control plates) in the presence of sodium azide, styrene oxide, or N-aminomorpholine; the addition of Octoxynol-9 did not affect the mutagenicity of ethyl methylsulfonate, benzo(a)pyrene, or 2-aminoanthracene.*

Two successive treatments with Octoxynol-9 (to remove cytoplasmic contamination) preserved the integrity of DNA in a rat liver cell suspension.[2] *Three successive treatments resulted in DNA breakage and further decrease in ribonucleic acid and protein content. In a study evaluating the effect of Octoxynol-9 on chromatin in rat liver, thymus, and ascites hepatoma cells, treated cells had rough nuclear structure compared to controls and some compaction of chromatin was seen; no changes in DNA content were observed. Unscheduled DNA synthesis in a nontumorigenic adult rat hepatocyte cell line exposed to 10, 25, or 50 µg/ml Octoxynol-9 and 5µCi/ml [^{3}H] for 18 h was evaluated in a DNA repair assay; Octoxynol-9 did not induce DNA damage. No increases in single strand DNA were observed in mouse lymphoma L5178Y/TK $^{+/-}$ cells treated with 3, 10, 25, 30, or 100 µl/l Octoxynol-9 in an DNA alkaline unwinding test. The induction of DNA double-strand breaks in cultured human lung epithelial cells treated with 5% Octoxynol-9 only occurred after cell viability reduced to < 60% and was considered extragenomic damage.*

Octoxynol-9 was not mutagenic when tested in a nontumorigenic T51B rat hepatocyte cell line at up to 40 µg/ml in a hypoxanthine guanine phosphoribosyl transferase mutation assay and at up to 50 µg/ml in a malignant transformation assay.[2] *In a chromosomal aberration assay, Octoxynol-9 enhanced the induction of abnormalities in Chinese hamster ovary cells, when tested in conjunction with known clastogens, dimethylnitrosamine, benzo[a]pyrene, and aniline, but was not clastogenic alone. No significant mutagenic activity was observed in mouse lymphoma LT178Y TK $^{+/-}$ 3.7.2.C cells treated with 1 – 45 ug/l Octoxynol-9 in a mouse lymphoma thymidine kinase forward mutation assay.*

an octoxynol (number of ethoxy repeat units unknown)

An octoxynol (6.25, 12.5, 25, 50, 100 and 200 µg/ml) was used as a known non-genotoxic agent in a comet and micronucleus assay (assays performed using human lymphoblastoid cell line TK6).[34] In the comet assay, no significant

increase in the comet tail was observed at up to 100 µg/ml (irrelevant positive responses observed at 200 µg/ml). In the micronucleus assay, no increase in the frequency of micronucleated cells was observed at any dose level.

CARCINOGENICITY STUDIES

Oral

nonoxynols

Groups of 50 B6C3F$_1$ mice received concentrations of 500, 1500, or 4500 ppm nonoxynol-10 in the diet for 104 wk.[32] *The mean daily intakes of nonoxynol-10 were 81.5, 254, and 873 mg/kg/d, respectively. A fourth group was fed a control diet. No pathological or microscopic changes were attributable to nonoxynol-10 upon examination and an increase in neoplastic or non-neoplastic lesions was not observed. It was concluded that nonoxynol-10 did not cause any increase in the incidence of neoplastic lesions in mice; nonoxynol-10 was not considered a carcinogen.*

Intravaginal

nonoxynols

In a lifetime exposure study, rats (number and strain not specified) were dosed with 6.7 or 33.6 mg/kg nonoxynol-9, intravaginally, 3 times per wk for a total of 24 mo.[2] *The low and high doses represented approximately 4 times and 20 times the clinical dose, respectively. Two groups of rats served as sham and untreated controls. No significant differences were observed between experimental and control groups. This was true for all of the measured parameters, which included palpable masses and mortality, with the exception of histopathologic tissue examination. Any positive findings observed in the experimental group at necropsy were considered related to the process of aging and were not related to the test substance.*

OTHER RELEVANT STUDIES

Effect on Stratum Corneum

The effect of Octoxynol-9 on intercellular adhesion was evaluated in stratum corneum samples obtained from the back of guinea pigs.[2] *Samples (10 mm^2) were immersed in 10 ml of Octoxynol-9 solution (0.1 M and 0.1%) for 1 – 30 d without mechanical stimulation. There was no splitting of the stratum corneum into fragments; only rolling or curling. Corneocytes were rarely observed and differences in elasticity values between distilled water controls and Octoxynol-9-treated samples were slight. In another study, in vitro damage to the stratum corneum following exposure to 1% Octoxynol-9 was evaluated. Three suction blisters were obtained from the volar forearms of young adult males and viable epidermis was removed from the blister roofs with a saline-moistened cotton swab. Discs of stratum corneum were agitated in a 1% solution of Octoxynol-9 in distilled water for up to 6 h. Octoxynol-9 caused slight swelling, vacuolization, and moderate loss of staining intensity. Corneocytes which released into the distilled water had no discernable changes in size or shape and stained well with rhodamine.*

Comedogenicity

Octoxynol-9 was used as the vehicle control in two studies evaluating the comedogenicity of sulfur.[2] *Subjects had severe acne and a pronounced propensity for comedo formation. In the first study, an occlusive patch containing 0.25% Octoxynol-9 was applied to the back of 6 subjects 3 times per wk for 6 wk. A blank, dry occlusive patch was applied to an additional 6 subjects that served as controls. Comedones were observed in 3 of the 6 subjects tested with Octoxynol-9 and in 1 of the 6 controls. Two of 6 biopsy specimens from the Octoxynol-9-treated sites contained definite comedones; 1 of 6 biopsy specimens from the control sites contained definite comedones. In a separate study, 40 subjects were treated in a similar fashion. Twenty subjects had a history of acne but were free of active disease; the remaining 20 had active acne on their backs, either comedonal or comedonal with some small pustules. Comedones were observed in 2 out of 20 subjects, both tested with, or without, Octoxynol-9. Four out of 20 biopsy specimens from the Octoxynol-9-treated sites contained definite comedones, while 2 out of 20 control biopsy specimens contained definite comedones. The authors concluded that Octoxynol-9 was comedogenic.*

Immune System Effects

The effect of Octoxynol-9 dosing on humoral and cell-mediated immune responses and autoimmune response was evaluated using 129/Ao Boy strain mice.[2] *Mice were administered 0.125% Octoxynol-9, in drinking water, for 4 wk, and in vitro and in vivo effects were evaluated. For the humoral response, mice were immunized with intraperitoneal (i.p). injection of 0.2 ml of 10% sheep red blood cells (SRBCs) in phosphate buffered solution (PBS). The number of anti-SRBC plaque-forming cells (anti-SRBC PFCs) in the spleen was determined after 4 d; Octoxynol-9 was shown to enhance the production of anti-SRBC PFCs.*

For determination of the cellular response, anti-SRBC delayed type hypersensitivity (DTH) was evaluated.[2] *After 4 wk of dosing, mice were sensitized intravenously with 1 x 10^5 SRBCs in 0.1 ml PBS and after 4 d the reaction was elicited by intradermal introduction of 1 x 10^8 SRBCs into the left hind foot pad; Octoxynol-9 stimulated the cellular immune response to SRBCs. Octoxynol-9 did not affect the development of anti-SRBC DTH in mice that were dosed for 1 wk. In the in vivo*

study, Octoxynol-9 was shown to cause significantly greater stimulation of anti-hemoglobin plaque-forming cells (anti-Hb PFCs) in B lymphocytes isolated from treated mice, in the presence of thymocytes or T lymphocytes from control mice or from mice treated with Octoxynol-9. The immunotoxicity of Octoxynol-9 was evaluated in a double-blind study using 10 outbred CF-1 female mice. The animals received an i.p. injection of 0.2 ml Octoxynol-9 (concentration not stated), in sterile saline, for 24 d. Ten mice were dosed with saline (vehicle controls) and 5 mice were used as untreated controls. All mice were subcutaneously immunized with 0.05 ml of 5% SRBCs on day 11; immunization was repeated with 0.05 ml of 10% SRBCs on day 18. Animals were bled by caudal incision prior to treatment on days 16 and 25. No changes in organ or body weight, or changes in hemacrit, white blood cell counts, anti-red blood cell responses, or serum immunoglobin patterns were noted in treated animals, compared to saline-treated controls. Compared to the untreated controls, immunoglobin M (IgM) concentrations were significantly higher in the group injected with Octoxynol-9 and in the saline controls on day 16. The authors concluded that Octoxynol-9 had no significant effect on the immune or hematological system, and, thus, was nontoxic.

Hormonal/Endocrine Effects

Alkylphenols, which include octoxynols, and related compounds have been reported to be estrogenic, both in vivo and in vitro because they mimic the effects of estradiol (concentrations at which effects seen not stated).[2] *In rats, nonoxynol-9 can be metabolized to para-nonylphenol, which has been described as estrogen-like because it mimicked the effects of estradiol (i.e., induction of the progesterone receptor and cellular proliferation) in the MCF-7 (estrogen-dependent breast cancer) cell line. Results from several studies indicate that several alkylphenols and related nonylphenol ethoxylate degradation products (4-nonylphenol, 4-tert-octylphenol, 4-tert-butylphenol, 4-nonylphenoldiethoxylate, nonoxynol-9, and 4-nonylphenoxycarboxylic acid) also can mimic the effect of estradiol.*

Barrier Disruption

nonoxynols

Cadaver epidermal membranes (n = 12) were placed between two halves of horizontal Franz-type glass diffusion cells and pretreated with nonoxynol-2, -4, and -9 (20% w/w solutions in isopropyl myristate; dose per nonoxynol = 10 ul/cm^2) for 60 min prior to rinsing with water.[2] *Water ([^{3}H]$_2$O) permeation rates were determined over an 8 h period; membranes treated only with isopropyl myristate served as controls. The permeability coefficients (cm/h) for each nonoxynol, in isopropyl myristate were as follows: 2.26 x 10^{-3} for nonoxynol-2, 2.40 x 10^{-3} for nonoxynol-4, 3.37 x 10^{-3} for nonoxynol-9 (compared to 1.34 x 10^{-3} for controls and 0.5 – 1.5 x 10^{-3} in normal skin). Four of the 12 nonoxynol-treated skin samples were compromised, while barrier disruption was reported in 2/12 controls. Based on these findings, nonoxynols were considered to minimally influence the skin barrier to water; however, it was not possible to assign a definite surfactant-induced damage claim.*

Age and Ocular Damage

an octoxynol (number of ethoxy repeat units unknown)

The effect of bovine age on the susceptibility to ocular damage was evaluated using lenses from calves (8 – 18 mo; n = 6) and cows (2 - 3 yr; n = 10).[35] Lenses were isolated aseptically and studied for 96 h following treatment with an octoxynol (tested at 1%). Control lenses were left untreated (n = 55 adult control lenses; n = 24 calf control lenses). Optical damage was evaluated via calculation of back vertex distance variability (BVDV). There was a significant difference in BVDV in the treated group, with calf lenses showing greater optical damage compared to adult cows ($p \leq 0.05$; this effect was not observed in control lenses). BVDV values were similar among control calf and adult lenses and adult lenses treated with the octoxynol (approximately 0.5 mm). The BVDV value of calf lenses treated with the octoxynol was approximately 3 mm.

Octoxynol-Induced Changes in Inflammatory Mediators in Ex Vivo Cervicovaginal Epithelium Model

Octoxynol-9

The impact of an Octoxynol-9 solution and a vaginal cleansing film (containing 1 and 3% Octoxynol-9, respectively) on inflammatory mediators (interleukin-1α (IL-1α) and IL-1β, evaluated with both substances; IL-6, tumor-necrosis factor alpha (TNF-α), IL-8, gamma interferon inducible protein 10 (IP-10) and macrophage inflammatory protein 3α (MIP-3α), evaluated only with the vaginal cleansing film) was studied.[36] Assays were performed using VEC-100 (reconstructed human vaginal-ectocervical epithelium) tissue equivalents. A significant increase ($p < 0.001$) in both IL-1α and IL-1β levels were observed in tissues treated with the Octoxynol-9 solution and the vaginal cleansing film compared to untreated controls. The vaginal cleansing film caused a significant several-fold increase ($p < 0.05$) of IL-8 and IP-10 compared to the untreated control. Significant changes were not observed regarding MIP-3α, IL-6, and TNF-α levels compared to the untreated control.

Cytotoxicity

An in vitro growth inhibition assay was performed using Octoxynol-9, sodium lauryl sulfate (SLS), phenol, ethylphenyl proprionate (EPP), and 12-O-tetradecanoylphorbol-13-acetate (TPA) in human epidermal keratinocytes.[2] *Each chemical was added to keratinocyte growth medium containing standard antimicrobials; no growth factors were added. Test substance concentrations were produced by 10-fold dilutions (volume = 10 µl) and ranged from 10^{-10} to 10^{-2} M. Morphological changes in the keratinocytes included marked rounding and shrinkage of cells. Growth inhibition induced by*

Octoxynol-9 occurred within less than an hour of exposure. The rank order for morphological changes was SLS > Octoxynol-9 >phenol >EPP > TPA, while the rank order for growth inhibition was TPA > EPP > SLS > Octoxynol-9 > phenol. TPA was considered the most potent irritant. The skin irritation potential of Octoxynol-9 and other surfactants (not specified) was evaluated in primary rat keratinocytes. Leaking of LDH into the medium, MTT reduction, and lysosomal uptake of neutral red dye were measured after treatment for 1 h, and after 24 h. Compared to controls, Octoxynol-9 caused less than a 2-fold increase in LDH release at 24 h. A dose-related increase in cellular LDH leakage in the medium was observed at concentrations of 10 – 100 µg/ml Octoxynol-9; most of the enzyme leakage occurred during the 1-h treatment period. Results from the MTT and NR assays were comparable to the LDH leakage results. An EC_{50} value was not calculated because the response to Octoxynol-9 treatment was below 50% of the maximal response. The cytotoxic potential of Octoxynol-9 was considered equivalent to that of the other tested surfactants.

an octoxynol (number of ethoxy repeat units unknown)

The cytotoxic potential of an octoxynol (approximately 0.0001 – 2.7 mM) was evaluated in cell types that model the most vulnerable cells in human cervicovaginal mucosa (fully polarized columnar epithelial cells (Madin-Darby canine kidney (MDCK) and Caco-2 cells), human cervical non-polarized cells (HeLa), and dendritic cells (fetal skin dendritic cells (FSDC)).[37] Cytotoxicity was measured via a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, with cells exposed to the test substance for 20, 60, 180, and 540 min. The octoxynol was toxic to all evaluated cell types in a time- and concentration-dependent manner. Toxicity was observed at concentrations around the critical micelle concentration of the octoxynol (0.2 mM), which suggests a non-selective mode that involves destabilizing and/or damage to the cell membrane.

An octoxynol (0.002 – 0.16%) was used as a model irritant/cytotoxic agent in several assays evaluating cytotoxicity in cancer cell lines (rat liver hepatoma cell line (H4IIE), human colon adenocarcinoma cell line (Caco2), a human liver hepatoma cell line (HepG2)), and human melanoma cell lines (WM164, WM1366, and D24).[38,39] Cytotoxicity was observed in all evaluated cell lines.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

an octoxynol (number of ethoxy repeat units unknown)

In several studies, an octoxynol was used as a known dermal irritant, either as a positive control or as a well-defined model irritant to validate new in vitro dermal irritation/cytotoxicity models.[39-45] The test substance was evaluated in EpiDerm tissues (concentration not stated), an EpiDerm full thickness model (at 1%), reconstructed human epidermis (at 0.2 and 1%), immortalized human epidermal keratinocytes (at 0.005 and 0.1%), neonatal human epidermal keratinocytes (at 0.03 – 1%), and living skin equivalents (cultured human skin model; at 1 and 10%). In all studies, the octoxynol yielded expected (positive) results.

Animal

A peel-off mask product containing 0.25% Octoxynol-9 was classified as minimally irritating and non-irritating in 2 separate single-insult occlusive patch tests using rabbits (primary irritation index = 0 for both tests).[2] *A single dose of Octoxynol-9 (10% w/w aq.; 0.15 ml) was occlusively applied to shaved rabbit skin for 24 h and average values for skin irritation 1 and 24 h post-patch removal were utilized to obtain a maximal primary Draize irritation score (MDSS) score of 0.2 (scale = 0 – 8). In a developmental toxicity study, groups of 25 outbred Sprague-Dawley CD rats received dermal applications of Octoxynol-9 at doses of 530, 1600, or 4270 mg/kg/d, at a constant dose volume of 4 ml/kg from day 6 to 15 of gestation. Controls received applications of deionized and filtered water. Exfoliation/desquamation, excoriation, and erythema were observed in the 4270 mg/kg/d group. Only excoriation and erythema were observed in the low- and mid-dose groups.*

An aqueous solution of 20% Octoxynol-11 was classified as a moderate skin irritant.[2] *No further details were provided. An unspecified concentration of Octoxynol-13 (0.5 ml) was applied under an occlusive patch to intact or abraded, shaved rabbit skin. The average primary irritation index for reactions scored at 24 and 72 h was 0.50; Octoxynol-13 was not considered a primary dermal irritant.*

an octoxynol (number of ethoxy repeat units unknown)

The dermal irritation potential of a leather cream (laboratory and marketed) containing an octoxynol, white beeswax, carnauba wax, distilled water, silicone oil, linseed oil, Sudan black dye, and nigrosine black dye (concentrations of ingredients within cream not stated) was evaluated in rabbits (6/group; sex and strain not stated) according to OECD TG 404.[33] Creams were applied to the shaved back for 72 h and sites were evaluated 24, 48, and 72 h after exposure. Neither laboratory nor marketed creams were considered to be irritating.

Human

The skin irritation potential of Octoxynol-1, -3, -5, -9, and -13 (each undiluted) was evaluated in a 48-hr skin irritation test using 50 subjects.[2] *None of the test substances induced skin irritation. The skin irritation potential of 2 pairs of identical formulations (with and without 2% Octoxynol-9) was evaluated in 24-h single-insult occlusive patch tests. A PII of 0.55 (moderately irritating; with 2% Octoxynol) and 0.13 (minimally irritating; without 2% Octoxynol-9) were reported for the first pair of formulations. For the second pair of formulations (same composition except for presence or absence of 2% Octoxynol-9), a PII of 0.11 (minimally irritating; presence of Octoxynol-9 not indicated) was reported. These results were attributed to differences in the skin penetrability of Octoxynol-9 in one formulation compared to the other. Nine healthy female volunteers were tested with a daily application of 200 µl of 1% Octoxynol-9 in a polypropylene chamber for 4 d; Octoxynol-9 was classified as a nonirritant.*

Sensitization

Animal

nonoxynols

The skin sensitization potential of nonoxynol-6 was evaluated in a guinea pig maximization test.[2] *Groups of albino Hartley-Dalkin guinea pigs (5/group) were tested with 1.7, 3, 9, or 27 g % nonoxynol-6 (w/w) in propylene glycol during the induction phase. One animal in the 9% nonoxynol-6 group did not complete the study. On day 1 of induction, animals in each of the 4 groups received 3 pairs of injections of the following chemicals: (1) 0.1 ml nonoxynol-6, (2) 0.1 ml nonoxynol-6 mixed (50:50) with Freund's complete adjuvant (FCA), and (3) 0.1 ml FCA. On day 7, each injection site was shaved and an occlusive 48-h application of 100% nonoxynol-6 was made. During the challenge phase, an occlusive 24-h application of nonoxynol-6 (2.7% in petrolatum) was made and sites were scored at 48 h. A control group of 40 guinea pigs (20 exposed to deodorized kerosene and 20 exposed to tetraethylene glycol diacrylate during induction) were not exposed to nonoxynol-6 during the induction phase and were challenged with 2.7% nonoxynol-6. Challenge reactions in experimental animals were as follows: 2/5 (1.7% induction group), none in the 3% induction group, 1/4 (9% induction group), and 2/5 (27% induction group). The proportion of challenge reactions to 2.7% nonoxynol-6 in experimental groups was not significantly different from that in the control group; nonoxynol-6 was considered a non-sensitizer.*

an octoxynol (number of ethoxy repeat units unknown)

The dermal sensitization potential of a leather cream (laboratory and marketed; neat application over 25 cm^2 area) containing an octoxynol, white beeswax, carnauba wax, distilled water, silicone oil, linseed oil, Sudan black dye, and nigrosine black dye (concentrations of ingredients within cream not stated) was evaluated in rabbits (6/group; sex and strain not stated) according to OECD TG 406 (Buehler method).[33] Animals were treated on day 0 with 0.1% 1-chloro-2,4-dinitrobenzene (also used as positive control). No details regarding test substance application were provided. The test substance was considered to be non-sensitizing, and the positive control gave expected results.

Human

The skin sensitization potential of 0.1% Octoxynol-9 was evaluated in an assay using 84 men and 122 women.[2] *The test material was applied using a 1 in^2 cotton twill patch, and secured with adhesive tape, for 6 d to the arms of the men and to the arms and legs of the women. After a 2-wk nontreatment period, a 48-h challenge application was made. No reactions to the fabric treated with 0.1% Octoxynol-9 were observed. In a different sensitization assay, 9 consecutive, 24-h semi-occlusive applications of a foot gel containing 8% Octoxynol-9 (0.2 ml) were made to 20 males and 92 females over 3 wk. A challenge application was made after a 10 -14 d nontreatment period, which was scored 24 and 48 h post-application; no adverse reactions were observed and the foot gel containing 8% Octoxynol-9 was not considered to be a primary irritant or a sensitizer. A formulation containing 0.5% Octoxynol-9 was tested in an occlusive HRIPT using 102 subjects. Induction applications were made over 3 wk and reactions were scored 48 or 72 h post-application; after an unspecified nontreatment period, a 24-h challenge application was made and scored at 48 and 96 h post application. Seven subjects had a score of 1 or greater during induction and 1 subject had a score of 1 during the challenge phase; the test substance was not considered a sensitizer.*

Phototoxicity

In Vitro

nonoxynols

Photohemolysis of human red blood cell suspensions containing nonoxynol-9 (2 x 10^{-5} M) occurred after irradiation with ultraviolet light under aerobic conditions.[32] *Nonoxynol-9 was irradiated for 70 min under an oxygen and argon-enriched atmosphere in a photochemical reactor equipped with phosphorus lamps (emission maximum at 300 nm). Lysis was not observed after the red blood cells were irradiated for 80 min in the absence of 2 x 10^{-5} M nonoxynol-9 or when the cells were incubated with 2 x 10^{-5} M nonoxynol-9 in the dark. The researchers considered nonoxynol-9 was phototoxic in vitro.*

OCULAR IRRITATION STUDIES

In Vitro

The ocular irritation potential of Octoxynol-9 was evaluated in an in vitro cytotoxicity assay, at concentrations ranging from 0.005 – 0.1%, using corneal cells from the fetal pig.[2] *Three corneal cell types were cultured (epithelial, endothelial, and stromal) and the mitochondrial capacity of these cells was assessed by monitoring the reduction of MTT reagent. Octoxynol-9 caused 50% reduction of MTT at a concentration of 0.006% (EC_{50} = 0.006%), which was said to correlate well with in vivo Draize test data (Draize score = 5, severe or extreme irritation). Concentrations higher than 0.01% completely inhibited the reduction of MTT.*

Animal

Several ocular irritation assays were performed to evaluate Octoxynol-9, mostly using the Draize method in rabbits.[2] *Octoxynol-9 (10%) was instilled in 1 eye of 6 rabbits (contralateral eyes served as controls); treated eyes were rinsed in 3 rabbits. Discrete to translucent areas of the cornea had not cleared in 2 of the 3 rabbits with unrinsed eyes; rinsed eyes were normal within 4 d. In a second study, Octoxynol-9 was instilled in 1 eye of each of 2 rabbits (and unrinsed). Moderate to severe erythema, slight to moderate edema, slight corneal opacity, and iridial injection were observed in the unrinsed eye; similar symptoms had cleared in the rinsed eye by 14 d post instillation. Signs of slight pannus and slight erythema on the nictitating membrane persisted in the unrinsed eye up to 14 d post-instillation; Octoxynol-9 was classified as a moderate permanent ocular irritant. A skin freshener formulation containing 0.25% Octoxynol-9 was instilled, and remained unrinsed, in rabbit eyes in 2 separate ocular irritation studies; the product was classified as minimally irritating. An unspecified concentration of Octoxynol-9 was instilled into the conjunctival sac (right eye; left eye served as control) in 2 young adult, male New Zealand white rabbits. Treated and untreated eyes were not rinsed until approximately 20 s post instillation. Moderate iritis, moderate conjunctival redness and chemosis, and copious blood-tinged discharge were observed in both treated eyes. Conjunctival redness had cleared by day 21 and corneal opacity and iritis persisted beyond day 21 post-instillation. Biomicroscopic examinations indicated moderate to severe corneal injury, which was evident from day 1 to day 3 post-instillation. Mild and moderate corneal opacity were observed in rinsed and unrinsed eyes, respectively; Octoxynol-9 was classified as a moderate ocular irritant. The maximum average Draize scores reported for rabbits (4 – 6/group) which had up to 10% Octoxynol-9 instilled in the conjunctival sac of 1 eye (unrinsed) were: 2 (minimally irritating) for 1% Octoxynol-9; 32 (moderately irritating) for 5% Octoxynol-9; 59 (severely irritating) for 10% Octoxynol-9. These results were correlated with mild, moderate, and severe corneal swelling, respectively. Octoxynol-9 (10% aq.) was classified as an ocular irritant when applied directly to the cornea and yielded a Draize eye irritation score of 55 when instilled directly in the eyes of rabbits (eyes remained unrinsed in both studies). A single, unrinsed instillation of 100 µl Octoxynol-9 (unspecified concentration) into the conjunctival sac of rabbit eyes was reported as being slightly irritating.*

The highest test concentrations of Octoxynol-1 (15%), -3 (15%), -5 (5%), -9 (0.5%), and -13 (1%) did not induce irritation in the eyes of 3 or more, rabbits from test groups comprising 5 animals.[2] *An aqueous solution of 20% Octoxynol-11 was classified as "very badly tolerated" in an ocular irritation test. No further details were provided. Three male and 3 female New Zealand white rabbits had 0.1 ml Octoxynol-13 instilled into the right eye; untreated eyes served as controls. Eyes remained unrinsed and reactions were scored at 1, 2, 3, and 7 d post-instillation (Draize scale: 0 – 110). Draize ocular irritation scores were 30.2 on day 1, 28 on day 2, 34.3 on day 3, 28.8 on day 4, and 33.8 on day 7; Octoxynol-13 was classified as severely irritating.*

an octoxynol (number of repeat ethoxy units unknown)

In several studies, an octoxynol was used as a known ocular irritant, either as a positive control or as a well-defined model irritant to validate new in vitro ocular irritation/cytotoxicity models.[46-49] Studies were performed using immortalized human corneal cells (0.0025 – 0.1 %), SV40T-transformed human corneal epithelial cells (at 0.005 – 0.1%), reconstructed human cornea-like epithelium (at 0.3%), and a reconstructed corneal epithelial model prepared from primary-cultured human limbal epithelial cells (at 5%). In all assays, the octoxynol gave expected (positive) results.

MUCOUS MEMBRANE IRRITATION STUDIES

In Situ

The effect of Octoxynol-9 on the rat jejunum and colon was evaluated in a single-pass, in situ perfusion model using the release of LDH and solubilized mucus into luminal perfusate as potential markers of intestinal damage.[2] *Isolated jejunal and colonic segments of male Sprague-Dawley rats (4 -9/group) were perfused with 1% Octoxynol-9, polysorbate 80 (0.1 – 10% w/v in isotonic saline), or isotonic saline (controls) for 6 h. The LDH release rate was greatest in the Octoxynol-9 group and approximately 3 times lower in the colon than in the jejunum. Compared to controls, the release rate of LDH in the jejunum increased 2-fold after perfusion with 1% polysorbate, and 7-fold after perfusion with 1% Octoxynol-9. Mucous release rates for Octoxynol-9 and polysorbate 80 were similar and greater than in controls. The mucous and LDH release rates for Octoxynol-9-perfused rat colon segments returned to baseline values, suggesting that these effects were reversible. The following morphological changes which were observed after perfusion with 1% Octoxynol-9, were considered moderate: denudation of villous tips, desquamation of the epithelial surface, necrosis of the mucosal lamina propria, and intervillous adhesion. These changes were observed to a minimal degree after perfusion with saline or 1% polysorbate 80.*

an octoxynol (number of repeat ethoxy units unknown)

An octoxynol (tested at 1%) was used as a positive control in 2 studies evaluating the irritation/cytotoxic potential in oral tissues models.[50,51] Application of the octoxynol to tissues yielded expected (positive) results. An octoxynol (1%) was used as a positive control in an in vitro assay evaluating the irritation potential of spermicides and feminine care products.[52] Vaginal tissue samples (n = 2) were obtained from healthy women undergoing hysterectomies for benign indications. An octoxynol (83 µl; 1% concentration) was applied to the samples for 0.5, 1, and 2 h, and the exposure times that reduced tissue viability to 50% (ET_{50}) was determined. The average ET_{50} was determined to be 1.25 h. Water, the negative control used in this assay, resulted in an ET_{50} of 18 h. Additionally, a full thickness VEC tissue model (VEC-100-FT) was exposed to a lubricant doped with 0.1 or 2% nonoxynol-9 for 18-h. Tissue viability and cytokine release of the VEC-100-FT model were evaluated via an MTT and enzyme linked immunosorbent assay (ELISA); 2 commercial lubricants were used as negative controls. Loss of tissue viability in the VEC-100-FT model was greater in the tissue treated with nonoxynol-9 (2% nonoxynol-9 > 0.1% nonoxynol-9 > lubricant 1 > lubricant 2); IL-α and interleukin-1β (IL-1β) concentrations increased as structural damage increased while tumor necrosis factor-α release decreased as structural damage and loss in tissue viability increased.

Animal

nonoxynols

In a mucous membrane irritation study, female Wistar rats (n= 9 -10) received a single dose of aqueous nonoxynol-9 (pH = 2; 5 mg/100 g) intravaginally; groups of 5 controls received distilled water.[2] Animals were killed over a period of 6 wk. Primary mucosal damage was observed for up to 24 h post administration, which included epithelial degeneration, necrosis and sloughing. A secondary acute inflammatory response, involving the entire vaginal wall and perivaginal tissues, was observed. The severity of vaginal wall inflammation was time-dependent; areas with minimal mucosal damage eventually returned to normal and areas with severe mucosal damage healed abnormally. In another study, a contraceptive cream containing 5% nonoxynol-9 was administered intravaginally (dose = 0.1 g/100 g body weight) to groups of female Wistar rats (3 – 8/group); controls received distilled water. The resulting lesions were not as severe as induced by exposure to aqueous nonoxynol-9 (5 mg/100 g); however, acute cervicovaginitis was observed in some of the rats. Groups of Sprague-Dawley rats (7/group) were administered 5, 12.5, 25, 50, or 75% nonoxynol-9, in distilled water, via vaginal lavage; 2 control groups received distilled water. Minimal irritation and inflammatory-cell infiltrate were observed in the vaginal mucosa of animals in the 5 and 12.5% groups. Mild irritation and epithelial exfoliation were observed in the 25% group. Epithelial exfoliation was more severe and persistent in animals that received 50 and 75% nonoxynol-9 concentrations; edema was noted in both groups. The inflammatory cell-infiltrate was the most severe and persistent in the 75% nonoxynol-9 group. Groups of New Zealand white female rabbits (3 – 4/group) had a collagen sponge containing 2.5, 5, 20, or 50 mg nonoxynol-9 in aqueous solution inserted into the vagina for 10 d. Six controls received just a collagen sponge. Moderate inflammatory changes were observed in the vaginas of rabbits in the 2.5 mg group. The most striking finding was a pronounced infiltration of polymorphonuclear leucocytes on the inserted sponge. Minimal changes were observed in 2 of the 6 controls. A dose-dependent increase in inflammatory changes, including cellular inflammatory infiltrate, edema of the connective tissue of the submucosal layer, and denudation of the mucosal epithelium were observed. No epithelial lining was observed in the 50 mg group, except in areas that were far removed from the medicated sponge. Concentration-dependent irritation of vaginal mucosa was observed in groups of New Zealand white rabbits (6/group) that received 2.5, 5. 12.5, or 25% nonoxynol-9 in 20 ml water, via vaginal lavage, once daily for 4 d. Lesions that were observed included epithelial exfoliation, submucosal edema, and inflammatory cell infiltrate; mild irritation was observed in the 2.5 and 5% dose groups, while moderate to severe irritation was observed in the 12.5 and 25% groups.

Female mice of the CF-1 strain were exposed to a spermicide containing 3.5% nonoxynol-9, either intravaginally or through intrauterine exposure.[32] Both modes of administration, with various exposure times, resulted in disruption of the uterine epithelium. Following intrauterine injection, the nonoxynol-9 spermicide caused rapid focal, uterine epithelial sloughing and complete epithelial loss within 24; regeneration of the uterine epithelium began 48 h after exposure and was completely restored within 72 h. However, the new epithelial layer was composed of cuboidal cells instead of the columnar cells that are normally present. The researchers concluded that nonoxynol-9 had a deleterious effect on uterine epithelium. The intravaginal dosing of female BALB/c mice with a commercial spermicide containing 3.5% nonoxynol-9 for 14 d induced an inflammatory response that was characterized by increased levels of cytokines and chemokines, the recruitment of neutrophils and monocytes into the genital tract, and the activation of the transcription factors nuclear factor kappa light chain enhancer of activated B cells and activator protein-1. Vaginal irritation, epithelial exfoliation, vascular congestion, and leukocyte infiltration were reported in a study on the toxicity of liposomal gels, in which 5 New Zealand white rabbits received 4% nonoxynol-9 (positive control) intravaginally at a dosage of 1 g/rabbit/d for 10 d.

CLINICAL STUDIES

Sixty women were instructed to use (in conjunction with a diaphragm) a spermicidal jelly containing 1% w/w Octoxynol-9 for 6 mo.[2] Twenty-seven women did not complete the study; 2 withdrew because of side effects. Of the 33

subjects who completed the study, vaginal irritation and excessive discharge were reported by 3 and 2 women, respectively. These side effects were described as minor and reversible in nature. No further details were provided.

nonoxynols

A clinical trial of nonoxynol-9 (in gel form) was performed using 40 healthy female volunteers.[32] *Twenty women received the gel (20 mg/ml nonoxynol-9) and 20 received a placebo for 7 d; examinations were made on day 0, 7, and 14. Genital irritation, erythema, and histologic inflammation were observed in both the treatment and placebo groups. Inflammatory changes were characterized by patchy infiltration of the lamina propria, predominantly with CD^{8+} lymphocytes and macrophages; epithelial disruption was absent. The long-term effects of 5 spermicidal formulations containing nonoxynol-9, including 3 gels (52.5, 100, or 150 mg/dose), a film (100 mg/dose), and a suppository (100 mg/dose), were studied in groups of 30 women over 7 mo (subset of study performed in 1536 women summarized below). Overall, there was no increased risk for any new colposcopic lesion in any of the nonoxynol-9 groups, when compared to controls. However, women who had used any nonoxynol-9 product were more likely than controls to have genital lesions characterized by erythema or edema. A total of 34 serious adverse events occurred in 31 study participants either during or after spermicide use, but none was attributed to spermicide use. Seven month probability data for vulvar or vaginal irritation did not differ between test groups; the researchers concluded that all 5 spermicide products were safe as used by the study participants. Histological findings of inflammation, a statistically significant increase in IL-IRA, and deep epithelial disruption were reported for 4 out of 20 women that applied 4% nonoxynol-9 spermicide gel twice a day for 13.5 consecutive days. The collective results of 2 separate clinical studies in which women applied a spermicide containing 3.5% nonoxynol-9 for 14 d (n = 179 subjects) or a vaginal suppository containing 150 mg nonoxynol-9 for 2 wk suggested that nonoxynol-9 does not elevate the incidence of lesions with epithelial disruption when these products are used no more than once per day. The incidence of lesions that were attributable to the use of these products were associated with an increased frequency of use.*

Twelve contact dermatitis patients were patch tested with ingredients of a topical antiseptic preparation.[2] *Ten of the patients had previously used various antiseptic preparations that contained nonoxynol-9. The remaining 2 patients had used antiseptic preparations that contained nonoxynol-8.3 and nonoxynol-10. Nonoxynol-8.3, -9, and -10 were patch tested at 2% in water. Patches remained in place for 48 h and reactions were scored at 48 h and at 72 or 96 h. All of the patients had ++ (strong, edematous or vesicular reaction) positive reactions either at 72 or 96 h. Epicutaneous test results for other ingredients of antiseptic preparations were negative, with the exception of 1 patient reaction to iodine. When 6 of the 12 patients in the study were tested with 2% aqueous nonoxynol-6, -8.3, -9, -10, -14, and -18 several months later, most of the reactions observed at 72 or 96 h were ++ reactions. However, in a couple of instances, a + (weak, non-vesicular), negative, or doubtful reaction was observed.*

A multicenter study in Sweden was performed to evaluate the human sensitization potential of oxidized ethoxylated surfactants.[32] *The 528 participants (196 males; 332 females) were identified as consecutive dermatitis patients with suspected allergic contact dermatitis. Patients were patch tested with aqueous solutions of nonoxynol-10 (20%) and air-oxidized nonoxynol-10 (20%). None of the participants had reactions to nonxynol-10. Erythema was observed in 1 participant patch tested with oxidized nonoxynol-10, on day 7, which was noted as a non-allergic reaction.*

A randomized trial was conducted in 1536 women across the US to evaluate the safety of 5 nonoxynol-9 spermicides.[32] *The spermicides, used for a period of 7 mo, included 3 gels that contained nonoxynol-9 at doses of 52.5, 100, and 150 mg, respectively, and a film and suppository that each contained 100 mg nonoxynol-9. Papanicolaou smears and cervical cytology samples were obtained during follow-up visits done at 4, 17, and 30 wk after study initiation. Results for 640 women were included in a Papanicolaou smear analysis. No differences in the rates of cervical alterations among the women using different amounts or different formulations of nonoxynol-9 were found and no statistically significant evidence of a dose-response relationship between nonoxynol-9 and changes in cervical cytology was observed. Furthermore, duration, frequency, and total number of spermicide uses were not associated with any statistically significant changes in cervical cytology. Although a noted study limitation was the exclusion of more than half of the trial participants due to missing Papanicolaou smear data, there was no evidence that these exclusions were biased by spermicide group, and the group comparisons were deemed credible. The researchers concluded that exposure to different formulations and doses of spermicides containing nonoxynol-9 for 30 wk is unlikely to affect cervical cytology.*

Case Reports

A patch test was performed in a 58-yr old uranium mill maintenance worker that used a waterless hand cleanser at work, containing 0.5% Octoxynol-9 and nonoxynol-6, in petrolatum.[2] *Occlusive application of "A1 Test" strips were made to the upper back and sites were scored 48-h after application. No reaction to 0.5% Octoxynol-9 was observed. (Results for nonoxynol-6 were not provided.)*

nonoxynols

A 72-yr-old male and 71-yr-old female presented with symptoms of photosensitization after being treated with an antiseptic preparation containing nonoxynol-10.[2,32] *A follow-up photosensitization study was conducted with 2 of the affected subjects and 32 controls (13 males and 19 females). Controls were suspected of having photodermatosis and had not used the antiseptic preparation. The 2 affected subjects and controls were patch tested with the antiseptic preparation,*

undiluted nonoxynol-10, 2% nonoxynol-10 in petrolatum, and 0.2 and 2% nonoxynol-10 in water. The 2 affected subjects were also patch tested with 1% nonoxynol-10% in water. The male affected subject exhibited photosensitization reactions to the antiseptic preparation and to 0.2, 1, and 2% aqueous nonoxynol-10. The female affected subject exhibited photosensitization reactions to the antiseptic preparation and to 2% nonoxynol-10 in petrolatum. No other reactions were observed in any of the remaining photopatch or nonirradiated sites. Of the 32 control subjects, 13 had photosensitization reactions to the antiseptic preparation and 4 had photosensitization reactions to aqueous nonoxynol-10. Undiluted nonoxynol-10 did not elicit photosensitization reactions in either affected subject or in controls.

A woman (domestic cleaner) with a 5-mo history of acute severe dermatitis and a past history of atopic eczema was patch tested with nonoxynol-12, an ingredient of a polish utilized during work.[53] *The patient had severe dermatitis on the dorsa of the hands, forearms, and face. Positive patch test reactions to the following concentrations of nonoxynol-12 in petrolatum were reported: 0.01, 0.1, 0.5, and 1%. The reactions were classified as + on day 2 and ++ on day 4. Negative patch test results were reported for 30 control subjects.*

EXPOSURE ASSESSMENT

The diameters of anhydrous hair spray particles and pump hair spray particles were determined to be 60 – 80 µm and ≥ 80 µm, respectively, compared to respirable particles with a reported mean aerodynamic diameter of 4.25 ± 1.5 µm.[2] *Thus, the use of Octoxynol-9 in hair sprays was not expected to result in inhalation exposure.*

CIR staff applied the in silico tool, VERMEER Cosmolife (Ver. 0.24), previously named SpheraCosmolife[54] to estimate the daily exposure to octoxynols from cosmetic use. According to the Council's 2022 survey, the maximum reported concentration of use for this ingredient group is 2% (in skin cleansing formulas (rinse-off; reported for Octoxynol-9)).[3] As indicated by VERMEER Cosmolife, the following exposure parameters are sourced from the Scientific Committee on Consumer Safety (SCCS) Notes of Guidance (NoG)[55] and relevant published literature,[56-59] using 90th percentile exposure values:

Octoxynol-9 at 2% in skin cleansing formulas (e.g., makeup remover)

To utilize VERMEER Cosmolife for exposure estimation, a product category should be specified. Assuming the product type for "skin cleansing formulas (rinse-off)" is makeup remover:

Relative daily exposure of makeup remover: 5000 mg/d (8.33 mg/kg bw/d)
Body weight used for the product exposure: adult (60 kg)

Type of exposure: rinse-off
Retention factor applied: 0.1
Surface area involved: 565 cm^2 (½ area head - female)
Skin surface exposure: [5000 mg/d × 0.1 (retention factor) × 2% (use concentration)] ÷ 565 cm^2 = 0.018 mg/cm^2

External exposure of makeup remover for dermal uptake: 8.33 mg/kg bw/d × 2% (use concentration) = 0.17 mg/kg bw/d

Systemic Exposure Dose (SED) assuming 10% dermal absorption: 0.0017 mg/kg bw/d. (An acute dermal toxicity study involving three guinea pigs showed no evidence of dermal absorption of Octoxynol-9.[2]) As the data suggest poor dermal bioavailability, a value of 10% dermal absorption has been considered here for a conservative estimation.

SUMMARY

The 25 octoxynol ingredients being reviewed in this report are reported to function in cosmetics as surfactants. The Panel first reviewed these octoxynol ingredients in a safety assessment that was published in 2004. At that time, the Panel issued a final report with the conclusion that Octoxynol-9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, -70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. Additionally, the Panel concluded that Octoxynol-1, -3, -5, -6, -7, and -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave on cosmetic products. In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. At its June 2023 meeting, the Panel determined that this safety assessment should be reopened to explore the irritation potential of these ingredients in products which come in contact with mucous membranes and due to the newly reported use of Octoxynol-9 at 0.1% in baby products.

According to 2023 VCRP survey data, Octoxynol-11 had the greatest reported frequency of use, in 8 formulations; frequency of use reduced from 131 uses reported in 2001. According to RLD submitted to CIR in 2024, Octoxynol-9 is reported to have the greatest number of uses (38 total formulations). Results from a 2022/2025 concentration of use survey conducted by the Council indicate that Octoxynol-9 has the highest reported maximum concentration of use, at 2% in skin

cleansing preparations; in 2001, the highest reported concentration of use was Octoxynol-10 at 25% in hair lighteners with color.

The acute dermal toxicity of a leather cream comprised of white beeswax, carnauba wax, distilled water, an octoxynol, silicone oil, linseed oil, Sudan black dye, and nigrosine black dye was evaluated in Wistar albino rats (3/sex/group) according to OECD TG 402. No mortality, signs of erythema or edema, significant changes in body weights, or food consumption was observed, compared to controls.

An octoxynol (tested at up to 200 µg/ml) was used as a known non-genotoxic agent in a comet and micronucleus assay. The octoxynol gave expected results in both studies (positive results observed at the highest concentration in the comet assay; however, these results were considered irrelevant).

The effect of age on the ocular damage (from an octoxynol tested at 1%) susceptibility of bovine lenses was evaluated using calf and cow lenses. Ocular damage was statistically significantly greater in calf lenses compared to cow lenses.

The impact of an Octoxynol-9 solution and a vaginal cleansing film (containing 1 and 3% Octoxynol-9, respectively) on inflammatory mediators was evaluated in VEC-100 tissue equivalents. A statistically-significant increase in several of these inflammatory mediators were observed following application of the Octoxynol-9 solution (increase in IL-1α and IL-1β) and the cleansing film (increase in IL-1α and IL-1β, IL-8, and IP-10).

The cytotoxic potential of an octoxynol approximately (0.02 – 2.7 mM) was evaluated in cell types that model the most vulnerable cells in human cervicovaginal mucosa. Cytotoxicity was observed in all cell types in a time- and concentration-dependent manner. An octoxynol was used as a model irritant/cytotoxic agent in several cancer cell lines. Cytotoxicity was observed in all evaluated cell lines.

In several studies, an octoxynol was used as a known dermal irritant, either as a positive control or as a well-defined reference substance to validate new in vitro dermal irritation/cytotoxicity models. In all assays, the octoxynol gave expected (positive) results. A laboratory and marketed version of a cream containing an octoxyol (concentration of ingredient in cream not stated) was not considered to be irritating in a dermal irritation assay performed in rabbits. These creams were also considered to be non-sensitizing in an assay performed using guinea pigs.

In several studies, an octoxynol was used as a known ocular irritant, as either as a positive control or as a well-defined reference substance to validate new in vitro ocular irritation/cytotoxicity models. In all assays, the octoxynol gave expected (positive) results.

An octoxynol (tested at 1%) resulted in cytotoxicity to oral tissue models when used as a positive control in 2 assays. An octoxynol (tested at 1%) resulted in an average ET_{50} of 1.25 h when it was used as a positive control in an in vitro assay evaluating the irritation potential of vaginal products. A full thickness VEC tissue model was exposed to 0.1 or 2% nonoxynol-9 or 2 commercial lubricants for 18 h. Loss of tissue viability was from highest loss to lowest loss were as follows: 2% nonoxynol-9 > 0.1% nonoxynol-9 > lubricant 1 > lubricant 2.

A SED of 0.0017 mg/kg bw/d was determined in an exposure assessment based on the use of Octoxynol-9 at 2% in skin cleansing preparations (e.g., makeup remover). The estimate was prepared using the in silico tool, VERMEER Cosmolife (Ver. 0.24).

DISCUSSION

In accordance with its Procedures, the Panel re-evaluates the conclusion of previously issued reports every 15 years. In 2004, the Panel published a final report on these octoxynols and concluded that Octoxynol-9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, -70, and Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. The Panel also concluded that Octoxynol-1, -3, -5, -6, -7, and -8, and Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of ≤ 5% in leave-on cosmetic products. In June 2023, the Panel considered a re-review of these ingredients and re-opened this report to explore the irritation potential of these ingredients in vaginal douches, and a newly reported use of Octoxynol-9 in baby products. However, it should be noted that these ingredients are no longer reported to be used in categories/products of concern (baby products and vaginal douches) according to 2024 RLD.

After evaluation of previous and new data (including 2024 RLD), and in accordance with the product categories and concentrations of use identified in the Use section and Use table, the Panel issued a revised conclusion stating these ingredients are safe in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating. The Panel was concerned that the potential exists for irritation due to evidence of dermal and ocular irritation in assays summarized in this report.

The Panel expressed concern regarding heavy metals that may be present in these ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to minimize impurities in cosmetic formulations according to limits set by the US FDA and EPA. Furthermore, because some of these ingredients are ethoxylated, the Panel

was also concerned about 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 limit these impurities from these octoxynols before blending them into cosmetic formulations.

It should be noted that although data on an in vitro unscheduled DNA synthesis assay have been provided herein (in italicized text, as this was reported in the original report), this assay is no longer considered reliable and should not be used to determine the genotoxic potential of an ingredient. The lack of genotoxic potential for this ingredient group was supported by other more reliable assay types (e.g., Ames assay, micronucleus assay).

It has been reported that alkylphenol ethoxylates (including octoxynols) may be estrogenic. However, because octoxynol ingredients are used at low concentrations in cosmetics and dermal absorption is minimal, concern for octoxynol-induced estrogenic effects was mitigated.

In addition, the Panel noted the incidence of increased supernumerary ribs observed in fetuses of rats given ≥1600 mg/kg Octoxynol-9 in a developmental and reproductive toxicity assay. This effect was not considered to be of concern as this finding is a common finding in rat teratology assays and is not necessarily a manifestation of a teratogenic effect. In addition, concern for developmental and reproductive toxicity was further mitigated as these effects were observed at doses much higher than what would be used in cosmetics.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients, and the acute/short-term inhalation assays indicating pulmonary edema, pneumonitis, and alveolar/bronchiolar hyperplasia in animals following inhalation exposure to Octoxynol-9 (MMAD = 1.5 or 1.8 μm). The Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern. Although frequency and/or concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following octoxynols are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating:

Octoxynol-1	Octoxynol-12	Octoxynol-9 Carboxylic Acid*
Octoxynol-3	Octoxynol-13*	Octoxynol-20 Carboxylic Acid*
Octoxynol-5	Octoxynol-16*	Potassium Octoxynol-12 Phosphate*
Octoxynol-6*	Octoxynol-20*	Sodium Octoxynol-2 Ethane Sulfonate
Octoxynol-7*	Octoxynol-25*	Sodium Octoxynol-2 Sulfate*
Octoxynol-8*	Octoxynol-30	Sodium Octoxynol-6 Sulfate*
Octoxynol-9	Octoxynol-33*	Sodium Octoxynol-9 Sulfate*
Octoxynol-10	Octoxynol-40	
Octoxynol-11	Octoxynol-70	

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

TABLES

Table 1. Definitions, idealized structures, and reported functions[1, CIR Staff]

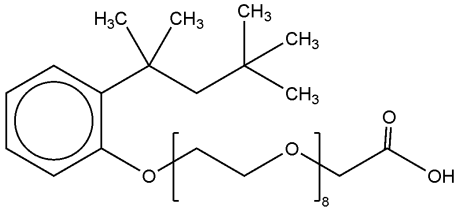
Ingredient/CAS No.	Definition	Function(s)
Octoxynol-1 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 2315-67-5	Octoxynol-1 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 1.	Surfactants – emulsifying agents
Octoxynol-3 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 27176-94-9 2315-62-0	Octoxynol-3 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 3.	Surfactants – emulsifying agents
Octoxynol-5 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 2315-64-2 27176-99-4	Octoxynol-5 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 5.	Surfactants – emulsifying agents
Octoxynol-6 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-6 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 6.	Surfactants- emulsifying agents
Octoxynol-7 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 27177-02-2	Octoxynol-7 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 7.	Surfactants – emulsifying agents
Octoxynol-8 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 3520-90-9 2638-43-9	Octoxynol-8 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 8.	Surfactants – emulsifying agents
Octoxynol-9 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 42173-90-0	Octoxynol-9 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 9.	Surfactants – emulsifying agents
Octoxynol-9 Carboxylic Acid 25338-58-3	Octoxynol-9 Carboxylic Acid is the organic acid that conforms generally to the following structure, where n has an average value of 8. 	Surfactants – emulsifying agents
Octoxynol-10 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 2315-66-4 27177-07-7	Octoxynol-10 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1 where n has an average value of 10.	Surfactants – emulsifying agents
Octoxynol-11 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 108437-62-3	Octoxynol-11 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 11.	Surfactants – emulsifying agents
Octoxynol-12 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-12 is the ethoxylated alkyl phenol that conforms generally to chemical structure depicted in Figure 1, where n has an average value of 12.	Surfactants – emulsifying agents

Table 1. Definitions, idealized structures, and reported functions[1, CIR Staff]

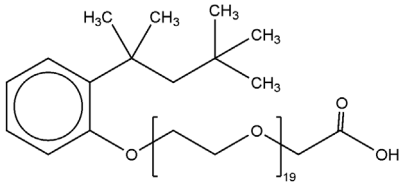
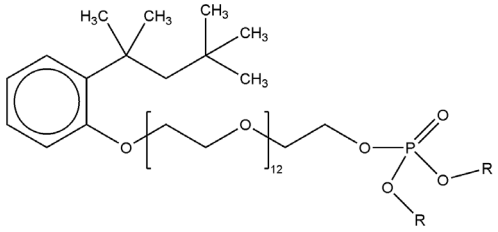
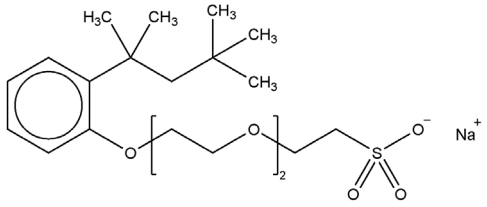
Ingredient/CAS No.	Definition	Function(s)
Octoxynol-13 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-13 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 13.	Surfactants – emulsifying agents
Octoxynol-16 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-16 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 16.	Surfactants – cleansing agents; Surfactants – emulsifying agents
Octoxynol-20 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-20 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 20.	Surfactants – emulsifying agents Surfactants – solubilizing agents
Octoxynol-20 Carboxylic Acid	Octoxynol-20 Carboxylic Acid is the organic acid that conforms generally to the following structure, where n has an average value of 19: 	Surfactants – cleansing agents
Octoxynol-25 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-25 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 25.	Surfactants – cleansing agents; Surfactants – solubilizing agents
Octoxynol-30 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-30 is the ethoxylated alky phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 30.	Surfactants – cleansing agents; Surfactants – solubilizing agents
Octoxynol-33 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-33 is the ethoxylated alky phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 33.	Surfactants – cleansing agents; Surfactants – solubilizing agents
Octoxynol-40 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-40 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 40.	Surfactants – cleansing agents; Surfactants – solubilizing agents
Octoxynol-70 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-70 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 70.	Surfactants – cleansing agents
Potassium Octoxynol-12 Phosphate	Potassium Octoxynol-12 Phosphate is the potassium salt of a complex mixture of esters of phosphoric acid and Octoxynol-12. This ingredient conforms to the following structure wherein R, in case, is hydrogen or potassium: 	Surfactants – cleansing agents; Surfactants – emulsifying agents; Surfactants – hydrotropes
Sodium Octoxynol-2 Ethane Sulfonate 2917-94-4 55837-16-6 67923-87-9	Sodium Octoxynol-2 Ethane Sulfonate is the organic compound that conforms generally to the following structure: 	Surfactants – cleansing agents

Table 1. Definitions, idealized structures, and reported functions[1, CIR Staff]

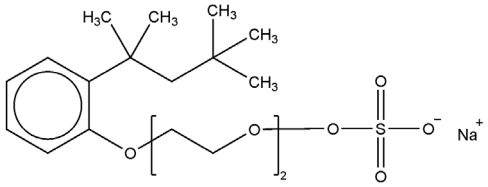
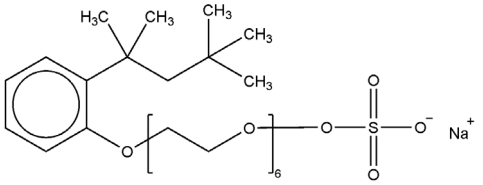
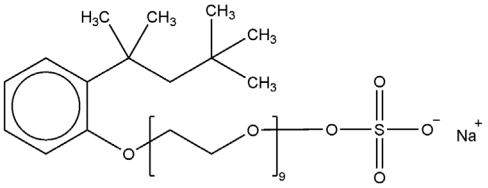
Ingredient/CAS No.	Definition	Function(s)
Sodium Octoxynol-2 Sulfate	Sodium Octoxynol-2 Sulfate is the sodium salt of the sulfuric acid ester of Octoxynol-2 that conforms generally to the following structure, where n has an average value of 2: 	Surfactants – cleansing agents
Sodium Octoxynol-6 Sulfate	Sodium Octoxynol-6 Sulfate is the sodium salt of the sulfuric acid ester of Octoxynol-6 that conforms generally to the following structure, where n has an average value of 6: 	Surfactants – cleansing agents
Sodium Octoxynol-9 Sulfate	Sodium Octoxynol-9 Sulfate is the sodium salt of the sulfuric acid ester of Octoxynol-9 that conforms generally to the following structure, where n has an average value 9: 	Surfactants – cleansing agents

Table 2. Chemical properties

Property	Value	Reference
	Octoxynol-1	
Physical Form	slightly hazy, viscous liquid	2
Color	light amber	2
Molecular Weight (g/mol)	250.38	60
Specific Gravity (@ 25ºC)	0.980 – 0.990	2
Viscosity (CPS @ 25ºC)	740 – 840	2
Solubility	Soluble in organic solvents; insoluble in water	2
log P (@ 25 ºC)	4.73 (estimated)	11
	Octoxynol-3	
Molecular Weight (g/mol)	338.5	61
log K_{ow} (@ 25 ºC)	4.42 (estimated)	11
	Octoxynol-5	
Physical Form	slightly hazy, free-flowing liquid	2
Color	water white to light amber	2
Molecular Weight (g/mol)	426.59	11
Specific Gravity (@ 25ºC)	1.030 – 1.040	2
Solubility	Soluble in organic solvents; insoluble in water	2
log P (@ 25 ºC)	4.25 (estimated)	11
	Octoxynol-6	
Molecular Weight (g/mol)	470.65	11
log P (@ 25 ºC)	3.95 (estimated)	11
	Octoxynol-7	
Molecular Weight (g/mol)	514.70	11
log P (@ 25 ºC)	3.95 (estimated)	11

Table 2. Chemical properties

Property	Value	Reference
	Octoxynol-8	
Molecular Weight (g/mol)	558.75	11
Specific Gravity (@ 25ºC)	1.054	2
Viscosity (CPS @ 25ºC)	260	2
log P (@ 25 ºC)	3.64 (estimated)	11
	Octoxynol-9	
Physical Form	free-flowing liquid	2
Color	water white to light amber	2
Average Molecular Weight (Da)	647	2
Molecular Weight (g/mol)	602.81	11
Specific Gravity (@ 25ºC; water = 1)	1.057 – 1.069	2
Vapor pressure (mmHg @ 20ºC)	< 1	2
Vapor Density (air = 1)	> 1	2
Melting Point (ºC)	6	2
Boiling Point (ºC)	> 200	2
Solubility (mg/l at 20° C)	4.55	8
log P (@ 25 ºC)	3.70 (estimated)	62
	Octoxynol-9 Carboxylic Acid	
Molecular Weight (g/mol)	616.79	11
log P (@ 25 ºC)	3.34 (estimated)	11
	Octoxynol-10	
Molecular Weight (g/mol)	646.86	11
log P (@ 25 ºC)	3.53 (estimated)	11
	Octoxynol-11	
Physical Form	viscous liquid	2
Color	Gardner scale < 3	2
Odor	Faint	2
Molecular Weight (g/mol)	690.91	63
Specific Gravity (@ 25ºC)	1.05 – 1.07	2
Solubility	Soluble in ethanol (96 °C, water, and vegetable oils); insoluble in water	2
log P (@ 25 ºC)	3.35 (estimated)	62
	Octoxynol-12	
Molecular Weight (g/mol)	734.96	11
log P (@ 25 ºC)	3.18 (estimated)	11
	Octoxynol-13	
Physical Form	free-flowing, viscous liquid	2
Odor	Aromatic	2
Molecular Weight (g/mol)	779.02	62
Specific Gravity (@ 25ºC; water =1)	1.06 -1.07	2
Vapor pressure	not volatile	2
Vapor Density	not volatile	2
Boiling Point (ºC)	200	2
Solubility	Soluble in water	2
log P (@ 25 ºC)	3.00 (estimated)	62
	Octoxynol-16	
Molecular Weight (g/mol)	911.18	64
Specific Gravity (@ 25ºC)	1.080	2
Viscosity (CPS @ 25ºC)	540	2
log P (@ 25 ºC)	2.48 (estimated)	62
	Octoxynol-20	
Molecular Weight (g/mol)	1086.89	62
Specific Gravity (@ 25 ºC)	1.088	2
Viscosity (kg/(CPS @ 25ºC)	420	2
log P (@ 25 ºC)	1.77 (estimated)	62
	Octoxynol-20 Carboxylic Acid	
Molecular Weight (g/mol)	1101.37	11
log P (@ 25 ºC)	3.26	11
	Octoxynol-25	
Molecular Weight (g/mol)	1307.65	11
log P (@ 25 ºC)	0.90 (estimated)	11

Table 2. Chemical properties

Property	Value	Reference
	Octoxynol-30	
Molecular Weight (g/mol)	1527.92	65
Specific Gravity (@ 25ºC)	1.095	2
Viscosity (CPS @ 25ºC)	470	2
log P (@ 25 ºC)	0.02 (estimated)	62
	Octoxynol-33	
Molecular Weight (g/mol)	1660.08	66
log P (@ 25 ºC)	-0.51 (estimated)	62
	Octoxynol-40	
Molecular Weight (g/mol)	1968.45	11
log P (@ 25 ºC)	-1.74 (estimated)	11
	Octoxynol-70	
Molecular Weight (g/mol)	3290.04	62
	Potassium Octoxynol-12 Phosphate	
Formula Weight (g/mol)	859.00 – 935.18	62
	Sodium Octoxynol-2 Ethane Sulfonate	
Formula Weight (g/mol)	424.5	67
	Sodium Octoxynol-2 Sulfate	
Formula Weight (g/mol)	440.5	68
	Sodium Octoxynol-6 Sulfate	
Formula Weight (g/mol)	572.7	69
	Sodium Octoxynol-9 Sulfate	
Formula Weight (g/mol)	704.8	70

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Octoxynol-1					**Octoxynol-3**				
Totals*	**1**	**1**	**57**	**NR**	**0.06 - 5**	**1**	**NR**	**1**	**NR**	**NR**
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*1*	*NR*	*NR*	*NR*	***	NR	1	NR	NR
Rinse-Off	***	*NR*	*57*	*NR*	*0.06 - 5*	***	NR	NR	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-Spray	***	NR	NR	NR	NR	***	NR	1[a]	NR	NR
Incidental Inhalation-Powder	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Dermal Contact	***	1	1	NR	NR	***	NR	1	NR	NR
Deodorant (underarm)	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Hair - Non-Coloring	***	NR	3	NR	1	***	NR	NR	NR	NR
Hair-Coloring	***	NR	53	NR	0.06 - 5	***	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
as reported by product category										
Baby Products										
Other Baby Products										
Bath Preparations										
Bath Oils, Tablets, and Salts										
Bubble Baths										
Eye Makeup Preparations (not children's)										
Eyebrow Pencil										
Eyeliner										
Eye Shadow										
Eye Lotion										
Eye Makeup Remover										
Mascara										
Other Eye Makeup Preparations										
Fragrance Preparations										
Cologne and Toilet Water										
Perfumes										
Other Fragrance Preparation										
Hair Preparations (non-coloring)						***1***				
Hair Conditioners	NR	NR	2	NR	1					
Hair Sprays (aerosol fixatives)										
Hair Straighteners										
Permanent Waves	NR	NR	1	NR	NR					
Rinses (non-coloring)										
Shampoos (non-coloring)						1	NR	NR	NR	NR
Tonics, Dressings, and Other Hair Grooming Aids										
Wave Sets										
Other Hair Preparations										

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Hair Coloring Preparations	***1***									
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	NR	53	NR	NR					
Hair Tints	1	NR	NR	NR	NR					
Hair Shampoos (coloring)										
Hair Lighteners with Color	NR	NR	NR	NR	5					
Hair Bleaches										
Other Hair Coloring Preparation	NR	NR	NR	NR	0.06 – 0.2					
Makeup Preparations (not eye; not children's)										
Blushers and Rouges (all types)										
Foundations										
Lipstick and Lip Glosses										
Makeup Bases										
Other Makeup Preparations										
Manicuring Preparations										
Other Manicuring Preparations										
Personal Cleanliness										
Bath Soaps and Body Washes										
Douches										
Disposable Wipes		NA	NA		NA		NA	NA		NA
Other Personal Cleanliness Products										
Shaving Preparations										
Aftershave Lotions										
Pre-shave Lotions (all types)										
Shaving Creams (aerosol, brushless, lather)										
Skin Care Preparations										
Cleansing	NR	NR	1	NR	NR					
Face and Neck (excluding shaving preparations)										
Body and Hand (excluding shaving preparations)										
Foot Powders and Sprays										
Moisturizing						NR	NR	1	NR	NR
Night										
Paste Masks (mud packs)										
Skin Fresheners										
Other Skin Care Preparations	NR	1	NR	NR	NR					
Suntan Preparations										
Suntan Gels, Creams, and Liquids										
Indoor Tanning Preparations										
Other Preparations (i.e., those preparations that do not fit another category)		NA	NA		NA		NA	NA		NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Octoxynol-5					**Octoxynol-6**				
Totals*	**2**	**NR**	**1**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*NR*	*NR*	*NR*	*NR*	***	*NR*	*NR*	*NR*	*NR*
Rinse-Off	***	*NR*	*1*	*NR*	*NR*	***	*NR*	*NR*	*NR*	*1*
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-Spray	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Incidental Inhalation-Powder	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Dermal Contact	***	NR	NR	NR	NR	***	NR	NR	NR	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	1	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
as reported by product category										
Baby Products										
Other Baby Products										
Bath Preparations (diluted for use)										
Bath Oils, Tablets, and Salts										
Bubble Baths										
Eye Makeup Preparations										
Eyebrow Pencil										
Eyeliner										
Eye Shadow										
Eye Lotion										
Eye Makeup Remover										
Mascara										
Other Eye Makeup Preparations										
Fragrance Preparations										
Cologne and Toilet Water										
Perfumes										
Other Fragrance Preparation										
Hair Preparations (non-coloring)										
Hair Conditioners										
Hair Sprays (aerosol fixatives)										
Hair Straighteners										
Permanent Waves										
Rinses (non-coloring)										
Shampoos (non-coloring)										
Tonics, Dressings, and Other Hair Grooming Aids										
Wave Sets										
Other Hair Preparations										

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Hair Coloring Preparations	***2***									
Hair Dyes and Colors (all types requiring caution statements and patch tests)	2	NR	NR	NR	NR					
Hair Tints										
Hair Shampoos (coloring)										
Hair Lighteners with Color										
Hair Bleaches	NR	NR	1	NR	NR					
Other Hair Coloring Preparation										
Makeup Preparations (not eye; not children's)										
Blushers and Rouges (all types)										
Foundations										
Lipstick and Lip Glosses										
Makeup Bases										
Other Makeup Preparations										
Manicuring Preparations (Nail)										
Other Manicuring Preparations										
Personal Cleanliness Products										
Bath Soaps and Body Washes										
Douches										
Disposable Wipes		NA	NA		NA		NA	NA		NA
Other Personal Cleanliness Products										
Shaving Preparations										
Aftershave Lotions										
Pre-shave Lotions (all types)										
Shaving Creams (aerosol, brushless, lather)										
Skin Care Preparations										
Cleansing										
Face and Neck (excluding shaving preparations)										
Body and Hand (excluding shaving preparations)										
Foot Powders and Sprays										
Moisturizing										
Night										
Paste Masks (mud packs)						NR	NR	NR	NR	1
Skin Fresheners										
Other Skin Care Preparations										
Suntan Preparations										
Suntan Gels, Creams, and Liquids										
Indoor Tanning Preparations										
Other Tattoo Preparations		NA	NA		NA		NA	NA		NA
Other Preparations (i.e., those preparations that do not fit another category)		NA	NA		NA		NA	NA		NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Octoxynol-9					**Octoxynol-10**				
Totals*	**38**	**5**	**131**	**0.1 – 2**	**0.08 - 5**	**5**	**1**	**NR**	**NR**	**25**
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*5*	*30*	*0.36*	*0.08 - 5*	***	*1*	*NR*	*NR*	*NR*
Rinse-Off	***	*NR*	*101*	*2*	*0.4 - 1*	***	*NR*	*NR*	*NR*	*25*
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-Spray	***	1[a]	4; 11[a]; 2[b]	NR	0.1 – 5; 0.08 – 1[a]; 3b	***	NR	NR	NR	NR
Incidental Inhalation-Powder	***	NR	2[b]; 1[c]	NR	1; 3b	***	NR	NR	NR	NR
Dermal Contact	***	5	21	0.1 – 2	0.5 - 5	***	1	NR	NR	NR
Deodorant (underarm)	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Hair - Non-Coloring	***	NR	48	NR	0.08 - 1	***	NR	NR	NR	NR
Hair-Coloring	***	NR	61	NR	0.4	***	NR	NR	NR	25
Nail	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Mucous Membrane	***	NR	5	0.36	0.5 – 0.9	***	NR	NR	NR	NR
Baby Products	***	NR	1	0.1	NR	***	NR	NR	NR	NR
as reported by product category										
Baby Products										
Other Baby Products	NR	NR	NR	0.1	NR					
Bath Preparations (diluted for use)										
Bath Oils, Tablets, and Salts	NR	NR	1	NR	NR					
Bubble Baths										
Eye Makeup Preparations										
Eyebrow Pencil										
Eyeliner	NR	1	NR	NR	NR					
Eye Shadow										
Eye Lotion										
Eye Makeup Remover										
Mascara										
Other Eye Makeup Preparations										
Fragrance Preparations	***1***									
Cologne and Toilet Water	1	NR	2	NR	5					
Perfumes	NR	NR	NR	0.7						
Other Fragrance Preparation	NR	NR	1	NR	NR					
Hair Preparations (non-coloring)	***3***									
Hair Conditioners	NR	NR	8	NR	0.4					
Hair Sprays (aerosol fixatives)	NR	NR	1	NR	0.1					
Hair Straighteners	NR	NR	1	NR	0.9					
Permanent Waves	NR	NR	17	NR	NR					
Rinses (non-coloring)										
Shampoos (non-coloring)	1 (r.o.)	NR	3	NR	0.7					
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	7	NR	0.08 – 1					
Wave Sets										

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Other Hair Preparations	2 (r.o.)	NR	11	NR	NR					
Hair Coloring Preparations	***3***									
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	NR	58	NR	NR					
Hair Tints	1	NR	NR	NR	NR					
Hair Shampoos (coloring)	NR	NR	1	NR	NR					
Hair Lighteners with Color										
Hair Bleaches	NR	NR	1	NR	NR	NR	NR	NR	NR	25
Other Hair Coloring Preparation	2 (l.o.)	NR	1	NR	0.4					
Makeup Preparations (not eye; not children's)	***1***									
Blushers and Rouges (all types)										
Foundations										
Lipstick and Lip Glosses										
Makeup Bases										
Other Makeup Preparations	1 (l.o.)	NR	NR	NR	NR					
Manicuring Preparations (Nail)	***1***									
Other Manicuring Preparations	1	NR	NR	NR	NR					
Personal Cleanliness Products	***4***									
Bath Soaps and Body Washes	3	NR	2	NR	NR					
Douches	NR	NR	1	NR	NR					
Disposable Wipes	1	NA	NA	0.36	NA		NA	NA		NA
Other Personal Cleanliness Products	NR	NR	2	NR	0.5 – 0.9					
Shaving Preparations	***1***									
Aftershave Lotions	NR	NR	1	NR	NR					
Pre-shave Lotions (all types)	1	NR	NR	NR	NR					
Shaving Creams (aerosol, brushless, lather)	NR	NR	NR	NR	1					
Skin Care Preparations	***23***					***5***				
Cleansing	3	NR	3	2	NR	2	NR	NR	NR	NR
Face and Neck (excluding shaving preparations)	18 (l.o.); 1 (r.o.)	NR	NR	0.22 (l.o.; not spray)	NR	1 (l.o.); 1 (r.o.)	NR	NR	NR	NR
Body and Hand (excluding shaving preparations)	NR	NR	2	NR	NR	1 (l.o.)	NR	NR	NR	NR
Foot Powders and Sprays	NR	NR	NR	NR	3					
Moisturizing	NR	1	2	NR	NR					
Night										
Paste Masks (mud packs)	NR	NR	3	NR	NR					
Skin Fresheners	NR	NR	2	NR	NR	1	NR	NR	NR	NR
Other Skin Care Preparations	3 (l.o.)	3	1	NR	NR	1 (l.o.); 1 (r.o.)	1	NR	NR	NR
Suntan Preparations	***3***									
Suntan Gels, Creams, and Liquids										
Indoor Tanning Preparations	3	NR	NR	NR	NR					
Other Preparations (i.e., those preparations that do not fit another category)	***1***	NA	NA	NR	NA		NA	NA		NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Octoxynol-11					**Octoxynol-12**				
Totals*	**1**	**8**	**19**	**NR**	**1**	**7**	**4**	**NR**	**1.5**	**NR**
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*8*	*14*	*NR*	*1*	***	*3*	*NR*	*1.5*	*NR*
Rinse-Off	***	*NR*	*5*	*NR*	*1*	***	*1*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	***	*NR*	*NR*	*NR*	*NR*	***	*NR*	*NR*	*NR*	*NR*
Exposure Type										
Eye Area	***	2	NR	NR	NR	***	NR	NR	NR	NR
Incidental Ingestion	***	NR	NR	NR	NR	***	2	NR	NR	NR
Incidental Inhalation-Spray	***	4[a]; 1[b]	1; 7[a]	NR	1a	***	1[b]	NR	NR	NR
Incidental Inhalation-Powder	***	1[b]	NR	NR	NR	***	1[b]	NR	1.5[c]	NR
Dermal Contact	***	8	15	NR	NR	***	2	NR	1.5	NR
Deodorant (underarm)	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Hair - Non-Coloring	***	NR	4	NR	1	***	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	***	2	NR	NR	NR
Baby Products	***	NR	NR	NR	NR	***	NR	NR	NR	NR
as reported by product category										
Baby Products										
Other Baby Products										
Bath Preparations (diluted for use)										
Bath Oils, Tablets, and Salts										
Bubble Baths										
Eye Makeup Preparations										
Eyebrow Pencil										
Eyeliner										
Eye Shadow										
Eye Lotion	NR	1	NR	NR	NR					
Eye Makeup Remover										
Mascara										
Other Eye Makeup Preparations	NR	1	NR	NR	NR					
Fragrance Preparations										
Cologne and Toilet Water										
Perfumes										
Other Fragrance Preparation	NR	NR	NR	NR	1					
Hair Preparations (non-coloring)						*3*				
Hair Conditioners										
Hair Sprays (aerosol fixatives)										
Hair Straighteners										
Permanent Waves										
Rinses (non-coloring)										
Shampoos (non-coloring)	NR	NR	NR	3	NR					
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	NR	NR	1					
Wave Sets										
Other Hair Preparations	NR	NR	NR	1	NR	3 (l.o.)	NR	NR	NR	NR

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Hair Coloring Preparations										
Hair Dyes and Colors (all types requiring caution statements and patch tests)										
Hair Tints										
Hair Shampoos (coloring)										
Hair Lighteners with Color										
Hair Bleaches										
Other Hair Coloring Preparation										
Makeup Preparations (not eye; not children's)										
Blushers and Rouges (all types)										
Foundations										
Lipstick and Lip Glosses						NR	2	NR	NR	NR
Makeup Bases	1	NR	NR	NR	NR					
Other Makeup Preparations										
Manicuring Preparations (Nail)										
Other Manicuring Preparations										
Personal Cleanliness Products						***2***				
Bath Soaps and Body Washes						2	NR	NR	NR	NR
Douches										
Disposable Wipes		NA	NA		NA		NA	NA		NA
Other Personal Cleanliness Products										
Shaving Preparations										
Aftershave Lotions										
Pre-shave Lotions (all types)										
Shaving Creams (aerosol, brushless, lather)										
Skin Care Preparations	***1***					***2***				
Cleansing	NR	NR	2	NR	1	NR	1	NR	NR	NR
Face and Neck (excluding shaving preparations)	NR	1	NR	NR	NR	NR	NR	NR	1.5 (not spray)	NR
Body and Hand (excluding shaving preparations)						NR	1	NR	NR	NR
Foot Powders and Sprays										
Moisturizing	NR	3	3	NR	NR	2	NR	NR	NR	NR
Night										
Paste Masks (mud packs)	NR	1	NR	NR	NR					
Skin Fresheners	NR	NR	2	NR	NR					
Other Skin Care Preparations	1 (r.o.)	1	4	NR	1					
Suntan Preparations										
Suntan Gels, Creams, and Liquids	NR	NR	2	NR	NR					
Indoor Tanning Preparations										
Other Preparations (i.e., those preparations that do not fit another category)		NA	NA		NA		NA	NA		NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Octoxynol-13					**Octoxynol-30**				
Totals*	**NR**	**NR**	**46**	**NR**	**0.1 - 2**	**NR**	**NR**	**NR**	**NR**	**1 - 2**
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*NR*	*30*	*NR*	*0.1*	***	NR	NR	NR	1 - 2
Rinse-Off	***	*NR*	*14*	*NR*	*2*	***	NR	NR	NR	NR
Diluted for (Bath) Use	***	*NR*	*2*	*NR*	*0.8*	***	NR	NR	NR	NR
Exposure Type										
Eye Area	***	NR	5	NR	2	***	NR	NR	NR	1 - 2
Incidental Ingestion	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Incidental Inhalation-Spray	***	NR	14[a]; 3b	NR	NR	***	NR	NR	NR	NR
Incidental Inhalation-Powder	***	NR	3b	NR	NR	***	NR	NR	NR	NR
Dermal Contact	***	NR	19	NR	0.8 - 2	***	NR	NR	NR	1
Deodorant (underarm)	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Hair - Non-Coloring	***	NR	24	NR	0.1	***	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	2	NR	0.8	***	NR	NR	NR	NR
Baby Products	***	NR	NR	NR	NR	***	NR	NR	NR	NR
as reported by product category										
Baby Products										
Other Baby Products										
Bath Preparations (diluted for use)										
Bath Oils, Tablets, and Salts										
Bubble Baths	NR	NR	2	NR	0.8					
Eye Makeup Preparations										
Eyebrow Pencil										
Eyeliner						NR	NR	NR	NR	1
Eye Shadow										
Eye Lotion										
Eye Makeup Remover	NR	NR	2	NR	2					
Mascara	NR	NR	3	NR	NR	NR	NR	NR	NR	2
Other Eye Makeup Preparations										
Fragrance Preparations										
Cologne and Toilet Water										
Perfumes										
Other Fragrance Preparation										
Hair Preparations (non-coloring)										
Hair Conditioners	NR	NR	4	NR	NR					
Hair Sprays (aerosol fixatives)										
Hair Straighteners										
Permanent Waves										
Rinses (non-coloring)	NR	NR	4	NR	NR					
Shampoos (non-coloring)	NR	NR	2	NR	NR					
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	10	NR	NR					
Wave Sets	NR	NR	2	NR	NR					
Other Hair Preparations	NR	NR	2	NR	0.1					

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Hair Coloring Preparations										
Hair Dyes and Colors (all types requiring caution statements and patch tests)										
Hair Tints										
Hair Shampoos (coloring)										
Hair Lighteners with Color										
Hair Bleaches										
Other Hair Coloring Preparation										
Makeup Preparations (not eye; not children's)										
Blushers and Rouges (all types)	NR	NR	1	NR	NR					
Foundations	NR	NR	1	NR	NR					
Lipstick and Lip Glosses										
Makeup Bases										
Other Makeup Preparations										
Manicuring Preparations (Nail)										
Other Manicuring Preparations										
Personal Cleanliness Products										
Bath Soaps and Body Washes										
Douches										
Disposable Wipes		NA	NA		NA		NA	NA		NA
Other Personal Cleanliness Products										
Shaving Preparations										
Aftershave Lotions	NR	NR	1	NR	NR					
Beard Softeners										
Pre-shave Lotions (all types)										
Shaving Creams (aerosol, brushless, lather)										
Skin Care Preparations										
Cleansing										
Face and Neck (excluding shaving preparations)										
Body and Hand (excluding shaving preparations)	NR	NR	3	NR	NR					
Foot Powders and Sprays	NR	NR	3	NR	NR					
Moisturizing										
Night										
Paste Masks (mud packs)										
Skin Fresheners	NR	NR	1	NR	NR					
Other Skin Care Preparations	NR	NR	5	NR	NR					
Suntan Preparations										
Other Suntan Preparations										
Other Preparations (i.e., those preparations that do not fit another category)		NA	NA		NA		NA	NA		NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Octoxynol-40					**Octoxynol-70**				
Totals*	**2**	**2**	**18**	**NR**	**0.007 – 0.02**	1	NR	NR	NR	NR
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*NR*	*2*	*NR*	*NR*	***	*NR*	*NR*	*NR*	*NR*
Rinse-Off	***	*2*	*16*	*NR*	*0.007 – 0.02*	***	*NR*	*NR*	*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-Spray	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Incidental Inhalation-Powder	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Dermal Contact	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Deodorant (underarm)	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Hair - Non-Coloring	***	2	10	NR	0.007 – 0.01	***	NR	NR	NR	NR
Hair-Coloring	***	NR	8	NR	0.02	***	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
as reported by product category										
Baby Products										
Other Baby Products										
Bath Preparations (diluted for use)	*1*									
Bath Oils, Tablets, and Salts	1	NR	NR	NR	NR					
Bubble Baths										
Eye Makeup Preparations										
Eyebrow Pencil										
Eyeliner										
Eye Shadow										
Eye Lotion										
Eye Makeup Remover										
Mascara										
Other Eye Makeup Preparations										
Fragrance Preparations										
Cologne and Toilet Water										
Perfumes										
Other Fragrance Preparation										
Hair Preparations (non-coloring)	*1*					*1*				
Hair Conditioners	NR	NR	5	NR	0.01					
Hair Sprays (aerosol fixatives)										
Hair Straighteners	NR	2	NR	NR	NR					
Permanent Waves	NR	NR	1	NR	NR					
Rinses (non-coloring)										
Shampoos (non-coloring)	NR	NR	1	NR	0.007					
Tonics, Dressings, and Other Hair Grooming Aids										

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Wave Sets	NR	NR	1	NR	NR					
Other Hair Preparations	1 (l.o.)	NR	2	NR	NR	1 (l.o.)	NR	NR	NR	NR
Hair Coloring Preparations										
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	NR	1	NR	0.02					
Hair Tints										
Hair Shampoos (coloring)										
Hair Lighteners with Color										
Hair Bleaches	NR	NR	6	NR	NR					
Other Hair Coloring Preparation	NR	NR	1	NR	NR					
Makeup Preparations (not eye; not children's)										
Blushers and Rouges (all types)										
Foundations										
Lipstick and Lip Glosses										
Makeup Bases										
Other Makeup Preparations										
Manicuring Preparations (Nail)										
Other Manicuring Preparations										
Personal Cleanliness Products										
Bath Soaps and Body Washes										
Douches										
Disposable Wipes		NA	NA		NA		NA	NA		NA
Other Personal Cleanliness Products										
Shaving Preparations										
Aftershave Lotions										
Pre-shave Lotions (all types)										
Shaving Creams (aerosol, brushless, lather)										
Skin Care Preparations										
Cleansing										
Face and Neck (excluding shaving preparations)										
Body and Hand (excluding shaving preparations)										
Foot Powders and Sprays										
Moisturizing										
Night										
Paste Masks (mud packs)										
Skin Fresheners										
Other Skin Care Preparations										
Suntan Preparations										
Suntan Gels, Creams, and Liquids										
Indoor Tanning Preparations										
Other Preparations (i.e., those preparations that do not fit another category)		NA	NA		NA		NA	NA		NA

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
	Potassium Octoxynol-12 Phosphate					**Sodium Octoxynol-2 Ethane Sulfonate**				
Totals*	**NR**	**NR**	**18**	**NR**	**0.0008 – 0.5**	**NR**	**NR**	**NR**	**NR**	**1**
summarized by likely duration and exposure**										
Duration of Use										
Leave-On	***	*NR*	*18*	*NR*	*0.0008 - 5*	***	*NR*	*NR*	*NR*	*NR*
Rinse-Off	***	*NR*	*NR*	*NR*	*NR*	***	*NR*	*NR*	*NR*	*1*
Diluted for (Bath) Use	***	*NR*	*NR*	*NR*	*NR*	***	*NR*	*NR*	*NR*	*NR*
Exposure Type										
Eye Area	***	NR	18	NR	0.002 – 0.5	***	NR	NR	NR	NR
Incidental Ingestion	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Incidental Inhalation-Spray	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Incidental Inhalation-Powder	***	NR	NR	NR	NR	***	NR	NR	NR	NR
Dermal Contact	***	NR	6	NR	NR	***	NR	NR	NR	1
Deodorant (underarm)	***	NR	NR	NR	0.0008 - 0.5	***	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	***	NR	NR	NR	NR
Baby Products	***	NR	NR	NR	NR	***	NR	NR	NR	NR
as reported by product category										
Baby Products										
Other Baby Products										
Bath Preparations (diluted for use)										
Bath Oils, Tablets, and Salts										
Bubble Baths										
Eye Makeup Preparations										
Eyebrow Pencil	NR	NR	NR	NR	0.05					
Eyeliner	NR	NR	6	NR	0.02 – 0.05					
Eye Shadow	NR	NR	NR	NR	0.002					
Eye Lotion										
Eye Makeup Remover										
Mascara	NR	NR	12	NR	0.01 – 0.05					
Other Eye Makeup Preparations										
Fragrance Preparations										
Cologne and Toilet Water										
Perfumes										
Other Fragrance Preparation										
Hair Preparations (non-coloring)										
Hair Conditioners										
Hair Sprays (aerosol fixatives)										
Hair Straighteners										
Permanent Waves										
Rinses (non-coloring)										
Shampoos (non-coloring)										
Tonics, Dressings, and Other Hair Grooming Aids										
Wave Sets										

Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*		*# of Uses*			*Max Conc of Use*	
	RLD (2024)[22]	**VCRP (2023)[23]**	**VCRP (2001)[2]**	**% (2022/ 2025)[3,24]**	**% (1999, 2001)[2]**	**RLD (2024)[22]**	**VCRP (2023)[23]**	**VCRP (1999, 2001)[2]**	**% (2022)[3]**	**% (1999, 2001)[2]**
Other Hair Preparations										
Hair Coloring Preparations										
Hair Dyes and Colors (all types requiring caution statements and patch tests)										
Hair Tints										
Hair Shampoos (coloring)										
Hair Lighteners with Color										
Hair Bleaches										
Other Hair Coloring Preparation										
Makeup Preparations (not eye; not children's)										
Blushers and Rouges (all types)										
Foundations										
Lipstick and Lip Glosses										
Makeup Bases										
Other Makeup Preparations										
Manicuring Preparations (Nail)										
Other Manicuring Preparations										
Personal Cleanliness Products										
Bath Soaps and Body Washes										
Douches										
Disposable Wipes		NA	NA		NA		NA	NA		NA
Other Personal Cleanliness Products										
Shaving Preparations										
Aftershave Lotions										
Pre-shave Lotions (all types)										
Shaving Creams (aerosol, brushless, lather)										
Skin Care Preparations										
Cleansing										
Face and Neck (excluding shaving preparations)										
Body and Hand (excluding shaving preparations)										
Foot Powders and Sprays										
Moisturizing										
Night						NR	NR	NR	NR	1
Paste Masks (mud packs)										
Skin Fresheners										
Other Skin Care Preparations										
Suntan Preparations										
Suntan Gels, Creams, and Liquids	NR	NR	NR	NR	0.0008					
Indoor Tanning Preparations										
Other Preparations (i.e., those preparations that do not fit another category)		NA	NA		NA		NA	NA		NA

NR – not reported; NA – not applicable (this category was not part of the VCRP)
l.o. – leave-on; r.o. – rinse-off
*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple product categories. For data supplied via the VCRP or by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple exposure types.
**Likely duration and exposure are derived from VCRP and survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

***In the RLD, each ingredient may be reported under several product categories, making a summation of RLD misleading in comparison to VCRP data. Accordingly, RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] 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

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 4. Octoxynol ingredients not reported to be in use [3,23]

Octoxnyol-6
Octoxynol-7
Octoxynol-8
Octoxynol-13
Octoxynol-16
Octoxynol-20
Octoxynol-25
Octoxynol-33
Octoxynol-9 Carboxylic Acid
Octoxynol-20 Carboxylic Acid
Potassium Octoxynol-12 Phosphate
Sodium Octoxynol-2 Sulfate
Sodium Octoxynol-6 Sulfate
Sodium Octoxynol-9 Sulfate

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Final Report on the Safety Assessment of Octoxynol-1, Octoxynol-3, Octoxynol-5, Octoxynol- 6, Octoxynol-7, Octoxynol-8, Octoxynol-9, Octoxynol-10, Octoxynol-11, Octoxynol-12, Octoxynol-13, Octoxynol-16, Octoxynol-20, Octoxynol-25, Octoxynol-30, Octoxynol-33, Octoxynol-40, Octoxynol-70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, Sodium Octoxynol-6 Sulfate, and Sodium Octoxynol-9 Sulfate[1]

Octoxynols are ethoxylated alkylphenols in which the size of the molecule is related to the number of moles of ethylene oxide used in synthesis. Reactions are performed at elevated temperature, under pressure, and in the presence of NaOH. It is possible that the synthesis may leave trace amounts of ethylene oxide, 1,4-dioxane, and unreacted C_9 phenols. Octoxynols of various chain lengths as well as octoxynol salts and organic acids function in cosmetics either as surfactants—emulsifying agents, surfactants—cleansing agents, surfactant—solubilizing agents, or surfactants—hydrotropes in a wide variety of cosmetic products at concentrations ranging from 0.0008% to 25%, with most less than 5.0%. The octoxynols are chemically similar to nonoxynols, the safety of which were previously considered. Long-chain nonoxynols (9 and above) were considered safe as used, whereas short-chain nonoxynols (8 and below) were considered safe as used in rinse-off products and safe at concentrations less than 5% in leave-on formulations. Acute exposure of hamsters to Octoxynol-9 by bronchopulmonary lavage produced pneumonia, pulmonary edema, and intra-alveolar hemorrhage. Octoxynol-9 at doses over 1 g/kg was toxic in rats and in mice in acute oral toxicity studies. No significant effects were noted in short-term oral studies of Octoxynol-9 in rats, in subchronic oral studies of Octoxynol-40 in rats and dogs, or in chronic oral studies of Octoxynol-40 in rats. The intraperitoneal LD_{50} of Octoxynol-9 in rats and mice was around 100 mg/kg. In skin irritation studies, octoxynols ranged from nonirritating to moderately irritating. Octoxynols were not ocular irritants in one rabbit study, but in others there was ocular irritation. No immune system toxicity in CF-1 female mice was noted following the intraperitoneal injection of Octoxynol-9 followed by subcutaneous immunization with sheep red blood cells (SRBCs). Octoxynol-9 produced no humoral and cell-mediated immune responses, or autoimmune response in mice. In the Ames test, Octoxynol-1 was not mutagenic with and without metabolic activation nor was Octoxynol-9 clastogenic. Results for Octoxynol-9 were negative in the following assays: unscheduled DNA synthesis, hypoxanthine guanine phosphoribosyl transferase mutation assay, malignant transformation assay, DNA alkaline unwinding test, and mouse lymphoma thymidine kinase locus forward mutation assay. Ethoxylated alkylphenols are generally considered to be estrogenic in that they mimic the effects of estradiol. Dermal exposure at three dose levels of rats to Octoxynol-9 failed to induce any malformations by category (external, visceral, or skeletal) or by individual anatomical location that were different from controls at statistically significant level. An increased incidence of a vestigial thoracic rib was observed in all dose groups. Octoxynol-9 also did not induce developmental toxicity (number of viable litters, liveborn per litter, percentage survival, birth weight per pup, and weight gain per pup) in female specific pathogen–free CD-1 mice dosed daily by gavage on gestation days 6 through 13. No reproductive toxicity was seen in male albino rats which received 5% Octoxynol-40 in the diet daily for 3 months; however, in an in vitro test, Octoxynol-9 (0.24 mg/ml) totally immobilized all human spermatozoa within 20 s. Women who used Nonoxynol-9 or Octoxynol-9 as spermicides, but who did become pregnant, did not have an increase in the overall risk of fetal malformations. In a human skin irritation study, formulations containing 2.0% Octoxynol-9 were classified as moderately irritating and minimally irritating, respectively, in a 24-h single-insult, occlusive patch test. Octoxynol-9 (1.0%) was classified as a nonirritant in a clinical study of nine subjects patch tested for 4 consecutive days. The skin sensitization potential of Octoxynols-1, -3, -5, -9, and -13 was evaluated using 50 subjects. Octoxynol-1 induced sensitization in two subjects; all other results were negative. No sensitization was

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observed in the following studies: 8.0% Octoxynol-9 in 103 subjects, 0.5% Octoxynol-9 in 102 subjects, and 0.1% Octoxynol-9 in 206 subjects. Concerns about even trace levels of 1,4-dioxane, ethylene oxide, or unreacted C_9 led to the recommendation that levels be limited. Concerns about the ocular irritancy of short-chain octoxynols led to a recommendation that they should not be used in products that will be used in the area surrounding the eyes. A limitation on the use concentration for short-chain octoxynols (8 and below) arose from consideration of the skin sensitization potential of octoxynols and the recognition that the short-chain octoxynols could be absorbed into the skin more than the long-chain octoxynols. Overall, based on the available data, it was concluded that long-chain octoxynols (9 and above) are safe as used, whereas short-chain octoxynols (8 and below) are safe as used in rinse-off products and safe at concentrations less than 5% in leave-on formulations.

INTRODUCTION

The safety of octoxynols (*aka* ethylene glycol octyl phenyl ethers or ethoxylated alkyl phenols) of various chain lengths and their salts and carboxylic acids in cosmetics is reviewed in this report. In cosmetic products, these ingredients function mainly as surfactants—emulsifying agents, surfactants—solubilizing agents, and surfactants—cleansing agents.

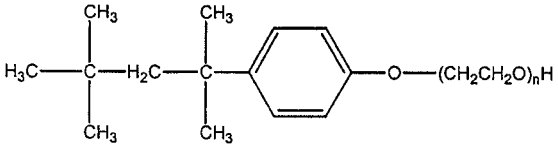
Octoxynols are chemically similar to nonoxynols. In its safety assessment of nonoxynols, the Cosmetic Ingredient Review (CIR) Expert Panel concluded that Nonoxynol-9, -10, -12, -14, -15, -30, -40, and -50 are safe as used (Elder 1983) and that Nonoxynol-1, -2, -3, -4, -5, -6, -7, and -8 are safe as used in rinse-off products and safe at concentrations of $\leq$5% in leave-on products (Andersen 1999).

There are sufficient data in this report to evaluate long-chain octoxynols, so the CIR Expert Panel did not consider the previous data on long-chain nonoxynols in any detail. The data on the short-chain nonoxynols, however, supplement the limited data on short-chain octoxynols and are summarized throughout the report.

CHEMISTRY

Chemical and Physical Properties

The Octoxynols, or polyoxyethylene octylphenyl ethers, are ethoxylated alkylphenols with the chemical formula, $C_8H_{17}C_6H_4$ $(OCH_2CH_2)_nOH$, where **n** in the formula represents the number of moles of ethylene oxide, average value. For cosmetic ingredients, **n** can vary from 1 to 70 (Wenninger, Canterbery, and McEwen 2000). By contrast, the nonoxynols have the formula $C_9H_{19}C_6H_4(OCH_2CH_2)_nOH$. The chemical structure that corresponds to the empirical formula for Octoxynols is:



The average value for **n** in chemicals of this class is evident in the ingredient name (e.g., Octoxynol-**1**, Octoxynol-**3**, etc).

The ingredients included in this review are listed below along with other chemical names/definitions. According to the Food and Drug Administration (FDA), Octoxynol-1 through -13 are supplied at 99% minimum active ingredient content, and Octoxynol-16 and -30 are supplied as 70% solutions in water (FDA 1999a).

Octoxynol-1 (CAS nos. 9002-93-1, 9036-19-5, 9004-87-9, and 2315-67-5) has four other names (Wenninger, Canterbery, and McEwen 2000):

- Ethanol, 2-[*p*-(1,1,3,3-Tetramethylbutyl)Phenoxy]-
- Ethylene Glycol Octyl Phenyl Ether
- PEG-1 Octyl Phenyl Ether
- 2-[*p*-(1,1,3,3,-Tetramethylbutyl)Phenoxy] Ethanol

Octoxynol-3 (CAS nos. 9002-93-1, 9036-19-5, 9004-87-9, 2315-62-0, and 27276-94-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- Ethanol, 2-[2-(Octylphenoxy)Ethoxy]Ethoxy]-
- Ethanol, 2-[2-[2-[*p*-(1,1,3,3-Tetramethylbutyl)Phenoxy] Ethoxy]Ethoxy]-
- 2-[2-[2-(Octylphenoxy)Ethoxy]Ethoxy]Ethanol
- PEG-3 Octyl Phenyl Ether
- Polyethylene Glycol (3) Octyl Phenyl Ether
- Polyoxyethylene (3) Octyl Phenyl Ether
- 2-[2-[2-[*p*-(1,1,3,3-Tetramethylbutyl) Phenoxy]Ethoxy] Ethoxy]Ethanol
- Triethylene Glycol Octylphenyl Ether

Octoxynol-5 (CAS nos. 9002-93-1, 9036-19-5, 9004-87-9, 2315-64-2, and 27176-99-4) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- 14-(Octylphenoxy)-3,6,9,12-Tetraoxatetradecan-1-ol
- PEG-5 Octyl Phenyl Ether
- Pentaethylene Glycol *p*-tert-Octylphenyl Ether; Polyethylene Glycol (5) Octyl Phenyl Ether
- Polyoxyethylene (5) Octyl Phenyl Ether
- 14-[4-(1,1,3,3,Tetramethylbutyl)Phenoxy]-3,6,9, 12-Tetraoxatetradecan-1-ol
- 3,6,9,12-Tetraoxatetradecan-1-ol, 14-(Octylphenoxy)-
- 3,6,9,12-Tetraoxatetradecan-1-ol, 14-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-

Octoxynol-6 is also known as (Wenninger, Canterbery, and McEwen 2000):

- Polyethylene Glycol (6) Octyl Phenyl Ether
- Polyoxyethylene (6) Octyl Phenyl Ether

Octoxynol-7 (CAS nos. 9002-93-1, 9036-19-5, 9004-87-9, and 27177-02-2) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- Heptaethylene Glycol Octylphenyl Ether
- 3,6,9,12,15,18-Hexaoxaeicosan-1-ol, 20-(Octylphenoxy)-

- 20-(Octylphenoxy)- 3,6,9,12,15,18-Hexaoxaeicosan-1-ol
- PEG-7 Octyl Phenyl Ether
- Polyethylene Glycol (7) Octyl Phenyl Ether
- Polyoxyethylene (7) Octyl Phenyl Ether

Octoxynol-8 (CAS nos. 9004-87-9, 9036-19-5, 9002-93-1, 3520-90-9, and 2638-43-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- 3,6,9,12,15,18,21-Heptaoxatricosan-1-ol, 23-(4-Octylphenoxy)-
- Octaethylene Glycol Octylphenyl Ether
- 23-(4-Octylphenoxy-3,6,9,12,15,18, 21-Heptaoxatricosan-1-ol
- PEG-8 Octyl Phenyl Ether
- Polyethylene Glycol 400 Octyl Phenyl Ether
- Polyoxyethylene (8) Octyl Phenyl Ether

Octoxynol-9 (CAS nos. 9004-87-9, 9036-19-5, 9010-43-9, 42173-90-0, and 9002-93-1) is most commonly known as Triton X-100; it also has the following other names (Wenninger, Canterbery, and McEwen 2000):

- Nonaethylene Glycol Octylphenyl Ether
- 3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol, 26-(Octylphenoxy)-
- 3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol, 26-(4-Octylphenoxy)-3,6,9,12,15,18,21, 24-Octaoxahexacosan-1-ol
- PEG-9 Octyl Phenyl Ether
- Polyethylene Glycol 450 Octyl Phenyl Ether
- Polyoxyethylene (9) Octyl Phenyl Ether

Octoxynol-10 (CAS nos. 9002-93-1, 9036-19-5, 9004-87-9, 2315-66-4, and 27177-07-7) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- Decaethylene Glycol Octylphenyl Ether
- 3,6,9,12,15,18,21,24,27-Nonaoxanonacosan-1-ol, 29-(Octylphenoxy)-
- 3,6,9,12,15,18,21,24,27-Nonaoxanonacosan-1-ol,
- 29-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-
- 29-(Octylphenoxy)-3,6,9,12,15,18,21,24, 27-Nonaoxanonacosan-1-ol
- PEG-10 Octyl Phenyl Ether
- Polyoxyethylene (10) Octyl Phenyl Ether
- Polyethylene Glycol 500 Octyl Phenyl Ether
- Polyoxyethylene (10) Octyl Phenyl Ether
- 29-[4-(1,1,3,3,-Tetramethylbutyl)Phenoxy]-3,6,9,12, 15,18,21,24,27-Nonaoxanonacosan-1-ol

Octoxynol-11 (CAS nos. 9004-87-9, 9036-19-5, 9002-93-1, and 108437-62-3) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-11 Octyl Phenyl Ether
- Polyethylene Glycol (11) Octyl Phenyl Ether
- Polyoxyethylene (11) Octyl Phenyl Ether
- 32-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-3,6,9,12,15, 18,21,24,27,30-Decaoxadotriacontan-1-ol

Octoxynol-12 (CAS nos. 9002-93-1, 9036-19-5, and 9004-87-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-12 Octyl Phenyl Ether
- Polyethylene Glycol 600 Octyl Phenyl Ether
- Polyoxyethylene (12) Octyl Phenyl Ether

Octoxynol-13 (CAS nos. 9002-93-1, 9036-19-5, and 9004-87-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- 3,6,9,12,15,18,21,24,27,30,33,36-Dodecaoxatriacontan-1-ol, 38-[4-(1,1,3,3,-Tetramethylbutyl)Phenoxy]-
- PEG-13 Octyl Phenyl Ether
- Polyethylene Glycol (13) Octyl Phenyl Ether
- Polyoxyethylene Glycol (13) Octyl Phenyl Ether
- 38-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-3,6,9,12, 15,18,21,24,27,30,33,36-Dodecaoxaoctatriacontan-1-ol

Octoxynol-16 (CAS nos. 9004-87-9, 9036-19-5, and 9002-93-1) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-16 Octyl Phenyl Ether
- Polyethylene Glycol (16) Octyl Phenyl Ether
- Polyoxyethylene (16) Octyl Phenyl Ether

Octoxynol-20 (CAS nos. 9002-93-1, 9036-19-5, and 9004-87-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-20 Octyl Phenyl Ether
- Polyethylene Glycol 1000 Octyl Phenyl Ether
- Polyoxyethylene (20) Octyl Phenyl Ether

Octoxynol-25 (CAS nos. 9002-93-1, 9036-19-5, and 9004-87-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-25 Octyl Phenyl Ether
- Polyethylene Glycol (25) Octyl Phenyl Ether
- Polyoxyethylene (25) Octyl Phenyl Ether

Octoxynol-30 (CAS nos. 9004-87-9, 9036-19-5, and 9002-93-1) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-30 Octyl Phenyl Ether
- Polyethylene Glycol (30) Octyl Phenyl Ether
- Polyoxyethylene (30) Octyl Phenyl Ether

Octoxynol-33 (CAS nos. 9002-93-1, 9036-19-5, and 9004-87-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-33 Octyl Phenyl Ether
- Polyethylene Glycol (33) Octyl Phenyl Ether
- Polyoxyethylene (33) Octyl Phenyl Ether

Octoxynol-40 (CAS nos. 9002-93-1, 9036-19-5, and 9004-87-9) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-40 Octyl Phenyl Ether
- Polyethylene Glycol 2000 Octyl Phenyl Ether
- Polyoxyethylene (40) Octyl Phenyl Ether

Octoxynol-70 (CAS nos. 9004-87-9, 9036-19-5, and 9002-93-1) has the following other names (Wenninger, Canterbery, and McEwen 2000):

- PEG-70 Octyl Phenyl Ether
- Polyethylene Glycol (70) Octyl Phenyl Ether
- Polyoxyethylene (70) Octyl Phenyl Ether

Octoxynol-9 Carboxylic Acid (CAS no. 25338-58-3) is the organic acid that conforms generally to the following formula (Wenninger, Canterbery, and McEwen 2000):

$$C_8H_{17}C_6H_4\ (OCH_2CH_2)_n\ OCH_2COOH$$

where **n** has an average value of 8. Other names for this chemical are as follows (Wenninger, Canterbery, and McEwen 2000):

- 3,6,9,12,15,18,21,24-Octaoxahexacosanoic Acid, 26-(Octylphenoxy)-
- 26-(Octylphenoxy)-3,6,9,12,15,18,21,24-Octaoxahexacosanoic Acid
- PEG-9 Octyl Phenyl Ether Carboxylic Acid
- Polyethylene Glycol 450 Octyl Phenyl Ether Carboxylic Acid
- Polyoxyethylene (9) Octyl Phenyl Ether Carboxylic Acid

Octoxynol-20 Carboxylic Acid is the organic acid that conforms generally to the formula (Wenninger, Canterbery, and McEwen 2000):

$$C_8H_{17}C_6H_4(OCH_2CH_2)_nOCH_2COOH$$

where **n** has an average value of 19. Other names for this chemical are as follows (Wenninger, Canterbery, and McEwen 2000):

- PEG-20 Octyl Phenyl Ether Carboxylic Acid
- Polyethylene Glycol 1000 Octyl Phenyl Ether Carboxylic Acid
- Polyoxyethylene (20) Octyl Phenyl Ether Carboxylic Acid

Potassium Octoxynol-12 Phosphate is the potassium salt of a complex mixture of esters of phosphoric acid and Octoxynol-12 (Wenninger, Canterbery, and McEwen 2000).

Sodium Octoxynol-2 Ethane Sulfonate (CAS No. 2917-94-4) is the organic compound that conforms to the formula (Wenninger, Canterbery, and McEwen 2000):

$$C_8H_{17}C_6H_4O(CH_2CH_2O)_2CH_2CH_2SO_3Na$$

Other names for this chemical are as follows (Wenninger, Canterbery, and McEwen 2000):

- Entsufon; 2-[2-[2-(Octylphenoxy)Ethoxy]Ethoxy] Ethanesulfonic Acid, Sodium Salt
- Sodium Octoxynol-3 Sulfonate
- Sodium Octylphenoxy Diethoxyethyl Sulfonate

Sodium Octoxynol-2 Sulfate is the sodium salt of the sulfuric acid ester of Octoxynol-2 that conforms generally to the formula (Wenninger, Canterbery, and McEwen 2000):

$$C_8H_{17}C_6H_4(OCH_2CH_2)_nOSO_3Na$$

where **n** has an average value of 2. Other names for this chemical are as follows (Wenninger, Canterbery, and McEwen 2000):

- PEG-2 Octyl Phenyl Ether Sulfate, Sodium Salt
- Polyethylene Glycol (2) Octyl Phenyl Ether Sulfate, Sodium Salt
- Polyethylene Glycol (2) Octyl Phenyl Ether Sulfate, Sodium Salt

Sodium Octoxynol-6 Sulfate is the sodium salt of the sulfuric acid ester of Octoxynol-6 that conforms generally to the formula (Wenninger, Canterbery, and McEwen 2000):

$$C_8H_{17}C_6H_4(OCH_2CH_2)_nOSO_3Na$$

where **n** has as an average value of 6. Other names for this chemical are as follows (Wenninger, Canterbery, and McEwen 2000):

- PEG-6 Octyl Phenyl Ether Sulfate, Sodium Salt
- Polyethylene Glycol 300 Octyl Phenyl Ether Sulfate, Sodium Salt
- Polyoxyethylene (6) Octyl Phenyl Ether Sulfate, Sodium Salt

Sodium Octoxynol-9 Sulfate is the sodium salt of the sulfuric acid ester of Octoxynol-9 (q.v.) that conforms to the following formula (Wenninger, Canterbery, and McEwen 2000):

$$C_8H_{17}C_6H_4(OCH_2CH_2)_nOSO_3Na$$

where **n** has an average value of 9. Other names for this chemical are as follows (Wenninger, Canterbery, and McEwen 2000):

- PEG-9 Octyl Phenyl Ether Sulfate, Sodium Salt
- Polyethylene Glycol 450 Octyl Phenyl Ether Sulfate, Sodium Salt
- Polyoxyethylene (9) Octyl Phenyl Ether Sulfate, Sodium Salt

Union Carbide Corporation (2000a) reported an estimated octanol/water partition coefficient of 1.9 for Octoxynol-9. Other available chemical and physical properties of octoxynols are provided in Table 1.

TABLE 1
Properties of Octoxynols

	Octoxynol-1	
Appearance	Light amber, slightly hazy, viscous liquid	Nikitakis and McEwen 1990a
Solubility	Soluble in typical organic solvents; insoluble in water	Nikitakis and McEwen 1990a
Specific gravity at 25°/25°C (water = 1)	0.980 to 0.990	Nikitakis and McEwen 1990a
Viscosity at 25°C	740 to 840 cps	Nikitakis and McEwen 1990a
pH	5.0 to 8.0 (5% solution—30/70 ethanol/water)	Nikitakis and McEwen 1990a
	Octoxynol-5	
Appearance	Water white to light amber, slightly hazy, free-flowing liquid	Nikitakis and McEwen 1990b
Solubility	Soluble in common polar organic solvents; insoluble in water	Nikitakis and McEwen 1990b
Specific gravity at 25°/25°C (water = 1)	1.030 to 1.040	Nikitakis and McEwen 1990b
Cloud point (°C)	39.4 to 44.8 (as mls H_2O)	Nikitakis and McEwen 1990b
Neutralization number (as mg KOH/g)	0.2 maximum	Nikitakis and McEwen 1990b
	Octoxynol-8	
Specific gravity (25°/25°C)	1.054	Food and Drug Administration (FDA) 1999a
Viscosity (CPS)	260	FDA 1999a
Cloud point (°C) of 1% aqueous solution	21	FDA 1999a
Pour point (°C)	−9	FDA 1999a
	Octoxynol-9	
Appearance	Water white to light amber, free-flowing liquid	Nikitakis and McEwen 1990b
Solubility	Soluble in common polar organic solvents and in water	Nikitakis and McEwen 1990b
Average molecular weight	647 Da	Gennaro 1990
Density	1.07 g/cm^3	Oxford University 2000
Specific gravity at 25°/25°C (water = 1)	1.057 to 1.069	Nikitakis and McEwen 1990b
Boiling point	>200°C	Oxford University 2000
Melting point	6°C	Oxford University 2000
Cloud point (°C)	63°C to 69°C (for 1% water solution)	Nikitakis and McEwen 1990b
Vapor density (air = 1)	>1	
Vapor pressure	<1 mm Hg at 20°C	
Flash point	251°C	Oxford University 2000
Residue on ignition	Not more than 0.4%	Committee of Revision of the United States Pharmacopeial Convention 1995
pH	6.0 to 8.0 (1 in 100 aqueous solution)	Gennaro 1990
Neutralization number (as mg KOH/g)	0.2 maximum	Nikitakis and McEwen 1990b
Hydroxyl value	Between 85 and 101	Committee of Revision of the United States Pharmacopeial Convention 1995

(Continued on next page)

TABLE 1
Properties of Octoxynols *(Continued)*

	Octoxynol-11	
Appearance	Viscous liquid; color (Gardner scale) <3	Gattefossé s.a. 1998
Odor	Faint	Gattefossé s.a. 1998
Solubility	Soluble in ethanol (96°C, water, and vegetable oils; insoluble in water)	Gattefossé s.a. 1998
Density	1.05 to 1.070	Gattefossé s.a. 2000
Specific gravity at 25°/25°C (water = 1)	1.050 to 1.070 (at 20°C)	Gattefossé s.a. 1998
Boiling point	>150 °C	Gattefossé s.a. 2000
Cloud point (°C)	67°C to 71°C	Gattefossé s.a. 1998
Flash point	>150 °C	Gattefossé s.a. 2000
pH	4.0 to 7.0 (at 10% in water)	Gattefossé s.a. 1998
Hydroxyl value	80 to 105 mg KOH/g	Gattefossé s.a. 1998
Acid value	<0.50 mg KOH/g	Gattefossé s.a. 1998
Refractive index	1.470 to 1.494 (at 20°C)	Gattefossé s.a. 1998
	Octoxynol-13	
Appearance	Free-flowing, viscous liquid	Rhone-Poulenc, Inc. 1992
Odor	Aromatic	Rhone-Poulenc, Inc. 1992
Solubility	Soluble in water	Rhone-Poulenc, Inc. 1992
Specific gravity at 25°/25°C (water = 1)	1.06 to 1.07	Rhone-Poulenc, Inc. 1992
Boiling point	200°C	Rhone-Poulenc, Inc. 1992
Vapor density (air = 1)	Not volatile	Rhone-Poulenc, Inc. 1992
Vapor pressure	Not volatile	Rhone-Poulenc, Inc. 1992
Evaporation rate	Not volatile	Rhone-Poulenc, Inc. 1992
Flash point	>200°F	Rhone-Poulenc, Inc. 1992
pH	5.0 to 8.0 (10% solution in distilled water)	Rhone-Poulenc, Inc. 1992
	Octoxynol-16	
Specific gravity (25°/25°C)	1.080	FDA 1999a
Viscosity (CPS)	540	FDA 1999a
Cloud point (°C) of 1% aqueous solution	>100	FDA 1999a
Pour point (°C)	13	FDA 1999a
	Octoxynol-20	
Specific gravity (25°/25°C)	1.088	FDA 1999a
Viscosity (CPS)	420	FDA 1999a
Cloud point (°C) of 1% aqueous solution	>100	FDA 1999a
Pour point (°C)	−1	FDA 1999a
	Octoxynol-30	
Specific gravity (25°/25°C)	1.095	FDA 1999a
Viscosity (CPS)	470	FDA 1999a
Cloud point (°C) of 1% aqueous solution	>100	FDA 1999a
Pour point (°C)	2	FDA 1999a

Methods of Production

Gennaro (1990) reported that Octoxynol-9 is prepared by reacting *p*-(1,1,3,3-tetramethylbutyl)phenol with ethylene oxide, at elevated temperature and under pressure, in the presence of NaOH.

According Weinheimer and Varineau (1998), the semibatch process is commonly used for the production of polyoxyethylated nonionic surfactants. In this procedure, a reaction vessel is charged with alkylphenol and catalyst (catalyst not identified). The catalyzed alkylphenol is heated to reaction temperature and purged with nitrogen to reduce the water generated during the catalysis step. The authors stated that water removal is important if polyethylene glycol formation is to be minimized.

After drying, ethylene oxide is added. When the alkylphenol has been polyoxyethylated to the desired extent, the reaction mixture is held at reaction temperature until the residual ethylene oxide concentration in the liquid product has been reduced to an acceptable level. The product is then neutralized and post-treated (high-molecular-weight products may require a multi-step manufacturing process). Finally, the product may be filtered to remove any insoluble salts formed during neutralization (Weinheimer and Varineau 1998).

Stability/Reactivity

Information was not available on the stability/reactivity of many of the octoxynols, including Octoxynol-1, Octoxynol-3, Octoxynol-6, Octoxynol-7, Octoxynol-8, Octoxynol-10, Octoxynol-12, Octoxynol-16, Octoxynol-20, Octoxynol-25, Octoxynol-30, Octoxynol-33, Octoxynol-40, Octoxynol-70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, Sodium Octoxynol-6 Sulfate, and Sodium Octoxynol-9 Sulfate. Available information on Octoxynol-5, Octoxynol-9, Octoxynol-11, and Octoxynol-13 follows.

The Cosmetic, Toiletry, and Fragrance Association (CTFA) specifications for Octoxynol-5 contain the following warning: "Material which appreciably exceeds 100 ppm free ethylene oxide may present an explosion hazard when stored in a closed container. This is due to the release of dissolved ethylene oxide to the container headspace where it may build up to a level which exceeds the explosive limit" (Nikitakis and McEwen 1990b).

Octoxynol-9 has been described as a stable compound, incompatible with strong oxidizing agents (Oxford University 2000). The CTFA specifications for Octoxynol-9 contain the following warning: "Material which appreciably exceeds 100 ppm free ethylene oxide may present an explosion hazard when stored in a closed container. This is due to the release of dissolved ethylene oxide to the container headspace where it may build up to a level which exceeds the explosive limit" (Nikitakis and McEwen 1990b).

Gattefosseé s.a. (2000) states that Octoxynol-11 is not a self-igniting chemical compound. It reacts with strong acids and oxidizing agents, and incomplete combustion leads to the release of monoxyd carbon and dioxyd carbon.

Rhone-Poulenc, Inc. (1992) describes Octoxynol-13 as a stable compound and states that hazardous polymerization will not occur. Octoxynol-13 is incompatible with strong oxidizing or reducing agents; acrid smoke and fumes are emitted when it is heated to decomposition.

Analytical Methods

Nikitakis and McEwen (1990b) stated that nuclear magnetic resonance (NMR) and infrared spectroscopy (IR) have been used to identify Octoxynol-5.

Octoxynol-9 has been analyzed using the following methods: ion-exchange chromatography (Landi et al. 1979a); affinity chromatography (Landi et al. 1979b); high-performance liquid chromatography (HPLC) (Holt et al. 1986); thin-layer chromatography (Whitmore and Wheeler 1980); solid-phase extraction, liquid chromatography, and liquid chromatography–mass spectrometry (Scullion et al. 1996); a simple turbidimetric method (Yoshida et al. 1980); spectrophotometry (Tôei, Motomizu, and Tôru 1982; Terada et al. 1985); and IR and NMR spectroscopy (Nikitakis and McEwen 1990b).

Ultraviolet Absorption

Union Carbide Corporation (2000a, 2000b) stated that a ultraviolet (UV) spectral analysis of a 0.32 mM (200 ppm) aqueous solution of Octoxynol-9 demonstrated an absorption maximum at 276 nm (molar absorptivity = 1600 cm/M) and slight absorbance at 290 nm, as a tail on the peak at 276 nm. No detectable absorbance was observed above 295 nm. Extinction coefficients at 290 nm and 295 nm were 225 cm/M and 30 cm/M, respectively. It was concluded that Octoxynol-9 had no significant absorbance in the UVA and UVB regions of the spectrum.

UV spectral analyses of the structurally similar nonoxynols were available. Nonoxynol -2, -4, and -9 were diluted with water and 10% isopropanol, respectively. The UV absorption spectra for Nonoxynol-2, -4, and -9 were essentially the same; absorption was noted in the UVC band (200- to 290-nm range). All three nonoxynols show only a tail of absorption above the 290-nm range to a similar degree (Clairol, Inc./Rhone-Poulenc, Inc. 1994).

Composition/Impurities

Octoxynols

In the process for manufacturing polyoxyethylene alkylphenols, the removal of water from the catalyzed alkylphenol prior to polyoxyethylation is important if polyethylene glycol (PEG) formation is to be minimized. In addition to PEG, small amounts (ppm levels) of acetaldehyde, formaldehyde, and 1,4-dioxane are formed (Weinheimer and Varineau 1998).

CTFA specifications state that Octoxynol-1 has a minimum purity of 99% and that Octoxynol-5 and Octoxynol-9 contain

TABLE 2
Quantitative analyses of impurities in Octoxynol-9 (Triton X-100) (Ashanti and Catravas 1980)

	Oxidation			Heavy metal concentration (ppm) in 1% detergent solution			
Detergent	Oxidation of ArSH[a] (μmol/min in 2% solution)	Oxidation of Fe^{2+} (meq/100g)[b]	Carbonyl compound (meq/100 g)	Fe	Cu	Zn	Al
Triton X-100 (I)	1.2 (0.19)[c]	1.6 (0.027)[d]	0.17	0.1	0.09	0.06	0.1
Purified Triton X-100 (I)	<0.05	<0.02	0.14	—	—	—	—
Triton X-100 (II)	0.4 (0.06)	0.6 (0.01)	0.12	0.1	0.08	NM[e]	NM
Triton X-100 (III)	0.25 (0.04)	0.5 (0.0085)	0.14	0.1	0.08	NM	NM
Purified Triton X-100 (III)	<0.05	<0.02	0.12	—	—	—	—

[a]Reduced form of 5,5′-dithiobis(2-nitrobenzoic acid). Addition of 2 mM EDTA reduced rate by 10% to 20%.
[b]Based on stoichiometric oxidation to ferric ion, completed within 10 min.
[c]H_2O_2, percentage equivalent in neat detergent that will oxidize ArSH at same rate as detergent.
[d]H_2O_2, percentage equivalent calculated, assuming equivalent weight of 17.
[e]NM, not measured.

sulfated ash (0.25% maximum) and water (0.5% maximum) (Nikitakis and McEwen 1990a).

According to the National Formulary (Committee of Revision of the United States Pharmacopeial Convention 1995), Octoxynol-9 may contain arsenic (2 ppm), heavy metals (0.002%), and not more than 5 ppm ethylene oxide as impurities.

The results of impurities analyses by Ashani and Catravas (1980), summarized in Table 2, indicate that Octoxynol-9 from three different chemical suppliers (I, II, and III) contains strong oxidizing impurities (0.04% to 0.19% H_2O_2 equivalents), carbonyl compounds, and heavy metal impurities. The results for samples of purified Triton X-100 are also included. In the assay of oxidizing impurities (method A), the oxidation of 2-thio-5-nitrobenzoic acid, the reduced form of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), was followed spectrophotometrically. Ferrous thiocyanate solution was used as a reducing agent to assay quantitatively the amount of oxidizing materials present in the detergent (method B). Dilute solutions of H_2O_2 and $FeCl_3$ were used to calibrate methods A and B, respectively. In the assay of carbonyl groups, stemming either from ketones or aldehydes, quantitative estimation was based on the colorimetric method, whereby 2,4-dinitrophenylhydrazine was used as the coupling agent. Additionally, content of carbonyl groups was estimated quantitatively from the IR absorption spectra.

Gattefossé s.a. (1998) reports that Octoxynol-11 contains <1.0% water. Specifications for the following impurities include sulfated ashes (<0.2%), heavy metals (<10 ppm Pb), and arsenic (<2 ppm). The raw materials used in the production of Octoxynol-11 are exclusively from petrochemical origin.

Rhone-Poulenc, Inc. (1992) reported that the percentage of volatiles in Octoxynol-13 is 0.5%, including <0.0002% ethylene oxide.

Nonoxynols

Other data on residues in octoxynols were not available, but data were available on the structurally similar nonoxynols. Nonoxynol-1 may contain up to 20 ppm ethylene oxide (CTFA 1989a), and Nonoxynol-6, up to 35 ppm ethylene oxide (CTFA 1989b).

Clairol, Inc./Rhone-Poulenc, Inc. (1994) analyzed samples of Nonoxynol-2, -4, and -9 for the presence of nonylphenol (unreacted C_9) using a gas chromatography flame ionization test (solvent = methanol; nonylphenol detection limit = 500 ppm). Nonylphenol was detected at concentrations of <500 ppm.

Assays for 1,4-dioxane and ethylene oxide were also performed on samples of Nonoxynol-2, -4, and -9 using the same technique. Neither 1,4-dioxane nor ethylene oxide was detected in triplicate samples of Nonoxynol-2. However, Nonoxynol-4 (five samples) contained 4.5 to 20 ppm 1,4-dioxane and 7.9 to 67 ppm ethylene oxide. Triplicate samples of Nonoxynol-9 contained <4.5 to 5.9 ppm 1,4-dioxane and <3.6 to 12.2 ppm ethylene oxide. The limits of detection for 1,4-dioxane and ethylene oxide in these assays were 4.5 ppm and 3.6 ppm, respectively (Clairol, Inc./Rhone-Poulenc, Inc. 1994).

The International Agency for Research on Cancer (IARC) has concluded, on the basis of epidemiological, experimental, and other relevant data, that ethylene oxide is "probably carcinogenic to humans." With respect to degrees of evidence of carcinogenicity, IARC stated that there is "limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals" (IARC 1987). In 1994, the IARC Working Group (IARC 2001a) upgraded its conclusion to indicate that ethylene oxide is "carcinogenic to humans."

The IARC Working Group (IARC 2001b) also concluded that 1,4-dioxane is "possibly carcinogenic to humans."

Given the possibility that ethylene oxide and 1,4-dioxane are impurities in Nonoxynols and may also be present in octoxynols,

these IARC conclusions regarding ethylene oxide and 1,4-dioxane were taken into consideration in this evaluation.

USE

Purpose in Cosmetics

According to the *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger, Canterbery, and McEwen 2000), Octoxynol-1, -3, -5, -6, -7, -8, -9, -10, -11, -12, and -13 function as surfactants—emulsifying agents in cosmetics. Additional functions are associated with the following other Octoxynols: Octoxynol-16 (surfactant—emulsifying agent; surfactant—cleansing agent); Octoxynol-20 (surfactant—emulsifying agent; surfactant—solubilizing agent); Octoxynol-25, -30, -33, and -40 (surfactant—cleansing agent; surfactant—solubilizing agent); and Octoxynol-70 (surfactant—cleansing agent).

The following Octoxynol acids/salts function as surfactants—cleansing agents in cosmetics: Octoxynol-20 Carboxylic Acid, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, Sodium Octoxynol-6 Sulfate, and Sodium Octoxynol-9 Sulfate. Additionally, Octoxynol-9 Carboxylic Acid functions as a surfactant—emulsifying agent, and the functions of Potassium Octoxynol-12 Phosphate in cosmetics are as follows: surfactant—cleansing agent, surfactant—emulsifying agent, and surfactant—hydrotrope (Wenninger Canterbery, and McEwen 2000).

Scope and Extent of Use in Cosmetics

Table 3 gives the frequency of use data as a function of product category as reported by manufacturers to FDA in 2001 (FDA 2001). Collectively, Octoxynol-1, -3, -5, -9, -11, -13, -40, and Potassium Octoxynol-12 Phosphate are reportedly used in 294 cosmetic products.

Current concentration of use data received from the cosmetics industry in 1999 (CTFA 1999) and updated in 2001 (CTFA 2001) are also shown in Table 3. In some cases, concentrations of use are provided for product categories in which there were no reports to FDA of uses, but it is reasonable to assume there is at least one use of the particular octoxynol in that product category. Based on these data, Octoxynols are used in cosmetics at concentrations ranging from 0.0008% to 25%, but most are $<5\%$.

The Octoxynols included in this review are not included among the substances listed as prohibited from use in cosmetic products marketed in the European Union (European Economic Community 2001). They do not appear in the *List of Japanese Cosmetic Ingredients* (Rempe and Santucci 1997).

Noncosmetic Use

Octoxynols are used as nonionic detergents, emulsifiers, dispersing agents, and spermaticides (Budavari, O'Neil, and Smith 1989).

FDA (1995) issued a proposed rule that would require clinical testing to support the effectiveness of Octoxynol-9 and Nonoxynol-9 in over-the-counter (OTC) drug products. FDA acknowledged the ability of these chemicals to kill sperm in vivo and in vitro, but concluded that the resulting effectiveness in an OTC drug product could not be separated from the products vehicle and use.

The Code of Federal Regulations (CFR) contains the following five direct/indirect food additive uses approved by FDA for Octoxynol-5, -7, -8, -10, -11, -12, and -13: surfactant for addition to pesticide dilutions prior to application to the growing crop (21 CFR 172.710); components of adhesives present in articles used to hold or transport food (21 CFR 175.105); components of paper and paperboard in contact with dry food (21 CFR 176.180); components of defoaming agents used in the manufacture of paper and paperboard for use in holding or transporting food (21 CFR 176.210); and emulsifiers and/or surface-active agents used in the manufacture of articles for use in holding or transporting food (21 CFR 178.3400).

Some of the preceding five approved direct/indirect food uses are also applicable to the following other Octoxynols: Octoxynol-1 (21 CFR 172.710; 175.105; 176.180); Octoxynol-3 (21 CFR 175.105; 176.180; 176.210); Octoxynol-9 (21 CFR 175.105; 176.180; 176.210; 178.3400); Octoxynol-16, -20, and -25 (21 CFR 175.105; 176.180); Octoxynol-30, -33, and -40 (21 CFR 172.710; 175.105; 176.180; 178.3400); Octoxynol-70 (21 CFR 172.710; 176.180); Potassium Octoxynol-12 Phosphate (21 CFR 175.105); and Sodium Octoxynol-2 Ethane Sulfonate (21 CFR 176.180).

BIOLOGICAL PROPERTIES

Absorption, Distribution, and Excretion

Octoxynol-9

Gossell (1983) states that Octoxynol-9 is rapidly and quantitatively absorbed from the vaginal wall into the systemic circulation of rabbits and rats. This statement is apparently based on a study by Chvapil et al. (1980a), which indicated that Nonoxynol-9 is absorbed through the vaginal wall of rabbits and rats, and is excreted by liver-bile-feces and kidney-urine routes.

Other Octoxynols

Larson and Lyman (1960) evaluated the absorption, distribution, and excretion of Octoxynol-40 using six rats and two dogs. [^{3}H]Octoxynol-40 (specific activity = 5.85 mC/g) was fed by stomach tube to four rats, and two additional rats served as controls. Feces and urine were collected separately. Complete analyses were done on two rats, whereas only urinalyses were done on the remaining two. Feces and urine collected from the two dogs dosed orally with ^{3}H-Octoxynol-40 were also analyzed.

Essentially all of the radioactivity that was fed was recovered in the feces of rats (up to 92.2%) and dogs (up to 86.4%). The urine (two dogs and two rats) and carcass (two rats) were said to contain minor amounts of radioactivity. The percent recovery of

TABLE 3
Product formulation data on Octoxynols

Product category (number of formulations reported to FDA) (FDA 2001)	Number of formulations containing ingredient (FDA 2001)	Current concentration of use (CTFA 1999, 2001)
Octoxynol-1		
Hair conditioners (630)	2	1%
Permanent waves (211)	1	—
Hair dyes and colors (1588)	53	—
Hair lighteners with color (5)	—	5%
Other hair-coloring preparations (59)	—	0.06%–0.2%
Cleansing skin care preparations (creams, lotions, powder, sprays) (733)	1	—
2001 total uses/ranges for octoxynol-1	**57**	**0.06%–5%**
Octoxynol-3		
Moisturizing skin care preparations (creams, lotions, powders, and sprays) (881)	1	—
2001 total uses/ranges for Octoxynol-3	**1**	—
Octoxynol-5		
Hair bleaches (115)	1	—
2001 total uses/ranges for Octoxynol-5	**1**	—
Octoxynol-6		
Paste masks (mud packs) (269)	—	1%
2001 total uses/ranges for Octoxynol-6	—	**1%**
Octoxynol-9		
Bath oils, tablets, and salts (140)	1	—
Colognes and toilet waters (683)	2	5%
Perfumes (227)	—	0.7%
Other fragrance preparations (173)	1	—
Hair conditioners (630)	8	0.4%
Hair sprays (aerosol fixatives) (267)	1	0.1%
Hair straighteners (63)	1	0.9%
Permanent waves (211)	17	—
Shampoos (noncoloring) (851)	3	0.7%
Tonics, dressings, and other hair-grooming aids (577)	7	0.08%–1%
Other hair preparations (276)	11	—
Hair dyes and colors (1588)	58	—
Hair shampoos (coloring) (31)	1	—
Hair bleaches (113)	1	—
Other hair-coloring preparations (59)	1	0.4%
Bath soaps and detergents (405)	2	—
Douches (5)	1	—
Other personal cleanliness products (307)	2	0.5%–0.9%
Aftershave lotion (230)	1	—
Shaving cream (aerosol, brushless, and lather) (133)	—	1%
Cleansing skin care preparations (creams, lotions, powder, and sprays) (733)	3	—
Body and hand (excluding shaving) skin care preparations (creams, lotions, powder, and sprays) (827)	2	—

(Continued on next page)

TABLE 3
Product formulation data on Octoxynols *(Continued)*

Product category (number of formulations reported to FDA) (FDA 2001)	Number of formulations containing ingredient (FDA 2001)	Current concentration of use (CTFA 1999, 2001)
Foot powders and sprays (35)	—	3%
Moisturizing skin care preparations (creams, lotions, powder, and sprays) (881)	2	—
Paste masks (mud packs) (269)	3	—
Skin fresheners (181)	2	—
Other skin care preparations (creams, lotions, powder, and sprays) (715)	1	—
2001 total uses/ranges for Octoxynol-9	**132**	**0.08%–5%**
Octoxynol-10		
Hair bleaches (115)	—	25%
2001 total uses/ranges for Octoxynol-10	**—**	**25%**
Octoxynol-11		
Other fragrance preparations (173)	1	—
Shampoos (noncoloring) (851)	3	—
Tonics, dressings, and other hair-grooming preparations (577)	—	1%
Other hair preparations (276)	1	—
Makeup bases (136)	1	—
Cleansing skin care preparations (creams, lotions, powder, and sprays) (733)	2	1%
Moisturizing skin care preparations (creams, lotions, powder, and sprays) (881)	3	—
Skin fresheners (181)	2	—
Other skin care preparations (creams, lotions, powder, and sprays) (715)	4	1%
Suntan gels, creams, and liquids (131)	2	—
2001 total uses/ranges for Octoxynol-11	**19**	**1%**
Octoxynol-13		
Bubble baths (209)	2	0.8%
Eye makeup remover (99)	2	2%
Mascara (187)	3	—
Hair conditioners (630)	4	—
Rinses (noncoloring) (41)	4	—
Shampoos (noncoloring) (851)	2	—
Tonics, dressings, and other hair-grooming aids (577)	10	—
Wave sets (53)	2	—
Other hair preparations (276)	2	0.1%
Blushers (all types) (243)	1	—
Foundations (287)	1	—
Aftershave lotion (230)	1	—
Body and hand (excluding shaving) skin care preparations (creams, lotions, powder, and sprays) (827)	3	—
Moisturizing skin care preparations (creams, lotions, powder, and sprays) (881)	3	—
Skin fresheners (181)	1	—

(Continued on next page)

TABLE 3
Product formulation data on Octoxynols *(Continued)*

Product category (number of formulations reported to FDA) (FDA 2001)	Number of formulations containing ingredient (FDA 2001)	Current concentration of use (CTFA 1999, 2001)
Other skin care preparations (creams, lotions, powder, and sprays) (715)	5	—
2001 total uses/ranges for Octoxynol-13	**46**	**0.1%–2%**
Octoxynol-30		
Eyeliner (533)	—	1%
Mascara (187)	—	2%
2001 total uses/ranges for Octoxynol-30	—	**1%–2%**
Octoxynol-40		
Hair conditioners (630)	5	0.01%
Permanent waves (211)	1	—
Shampoos (noncoloring) (851)	1	0.007%
Wave sets (53)	1	—
Other hair preparations (276)	2	—
Hair dyes and colors (1588)	1	0.02%
Hair bleaches (115)	6	—
Other hair-coloring preparations (59)	1	—
2001 total uses/ranges for Octoxynol-40	**18**	**0.007%–0.02%**
Potassium Octoxynol-12 Phosphate		
Eyebrow pencil (99)	—	0.05%
Eyeliner (533)	6	0.02%–0.05%
Eye shadow (551)	—	0.002%
Mascara (187)	12	0.01%–0.05%
Suntan gels, creams, and liquids (131)	—	0.0008%
2001 total uses/ranges for Octoxynol-12 Phosphate	**18**	**0.0008%–0.05%**
Sodium Octoxynol-2 Ethane Sulfate		
Paste masks (mud packs) (269)	—	1%
2001 total uses/ranges for Sodium Octoxynol-2 Ethane Sulfonate	**—**	**1%**

radioactivity in the urine was 0.59% to 2.0% (4 rats) and 1.17% and 1.46% (two dogs, respectively). Radioactivity equivalent to 2% to 4% (two rats) was detected in the carcass (exclusive of the gastrointestinal [GI] tract).

Values for the percent recovery of radioactivity in livers from the two rats were 0.02% and 0.06%, respectively. The authors stated that the data indicating no storage of ^{3}H-Octoxynol-40 in the liver and the detection of radioactivity, 2% to 4%, in the carcass may be indicative of the metabolism of Octoxynol-40, with ^{3}H appearing as water. The authors concluded that Octoxynol-40 was not absorbed to any substantial degree.

Because the molecular weights of the Octoxynol-40 fractions follow a Poisson distribution, the authors considered that the lower molecular weight fraction may have contributed unduly to the small amount of ^{3}H that was found in the urine. Thus, a portion of the Octoxynol-40 was diluted to a concentration of 70% and extracted with isooctane. Specific activity indicated that the fraction removed was Octoxynol-2 or lower. Portions of the extracted Octoxynol-40 were fed to two dogs, after which urinalyses were performed. Urinalysis results (two dogs) were consistent with the results for two dogs in the preceding experiment (approximately 1% recovery in the urine) (Larson and Lyman 1960).

Percutaneous Absorption

Octoxynol-9

The E. K. Company Laboratory of Industrial Medicine (1969) reported no evidence of dermal absorption of Octoxynol-9 in an acute dermal toxicity study involving three guinea pigs. The test substance was administered at doses ranging from 5 to 20 cc/kg.

Nonoxynols

An-eX analytical Services Ltd. (1995a), prior to initiation of an in vitro skin penetration study, performed a study to determine whether the rinse-off exposure protocol would alter skin integrity.

Epidermal membranes (cadaver skin) were placed between the two halves of horizontal Franz-type glass diffusion cells and pretreated with Nonoxynol-2, -4, and -9 (20% *w/w* solutions in isopropyl myristate; dose per Nonoxynol = 10 μl/cm^2). The Nonoxynols were rinsed from the skin after 60 min of exposure, and water ([^{3}H]$_2$O) permeation rates were determined over an 8-h period. Epidermal membranes treated only with isopropyl myristate served as negative controls. Twelve replicates for each surfactant and 12 replicates for isopropyl myristate (total of 48 cells) were run.

The permeability coefficients (cm/h) for each Nonoxynol (in isopropyl myristate) and isopropyl myristate are as follows: Nonoxynol-2 ([2.26 $\pm$ 0.30] $\times$ 10^{-3}), Nonoxynol-4 ([2.40 $\pm$ 0.29] $\times$ 10^{-3}), Nonoxynol-9 ([3.37 $\pm$ 0.9] $\times$ 10^{-3}), and isopropyl myristate ([1.34 $\pm$ 0.18] $\times$ 10^{-3}). Water permeability coefficients for normal skin range from 0.5 $\times$ 10^{-3} to 1.5 $\times$ 10^{-3} cm/h. The investigators noted that the number of skin samples with signs of damage was of concern. For Nonoxynol-treated skin, data from four samples of each group of 12 cells ($\sim$33%) suggested that the skin membranes were compromised.

Evidence of barrier disruption was reported for 2 of 12 samples ($\sim$17%) of the isopropyl myristate–treated skin. However, based on these findings, it was not possible to assign a definite surfactant-induced damage claim. The investigators also stated that if the anomalous "damaged" skin samples are discounted, it is apparent that the Nonoxynols influenced the skin barrier to water, but not to any great degree (An-eX analytical Services Ltd. 1995a).

An-eX analytical Services Ltd. (1995c) evaluated the in vitro skin penetration of Nonoxynol-2, -4, and -9 using heat-separated, human epidermal membranes. An HPLC analysis–UV detection method was used to determine the distribution of homologues with varying ethylene chain lengths in commercial samples of Nonoxynol-2, -4, and -9, respectively, prior to application to epidermal membranes. The distribution of homologues was as follows: Nonoxynol-2 (Nonoxynol-1, -2, -3, and -4 homologues present) , Nonoxynol-4 (Nonoxynol-1, -2, -3, -4, -5, -6, -7, -8 homologues), and Nonoxynol-9 (Nonoxynol-2, -3, -4, -5, -6, -7, -8, -9, -10, and -11 homologues).

The experiment was designed to mimic in-use conditions relative to ingredient use in rinse-off products. Female human skin was obtained either at autopsy or following cosmetic reduction surgery. Six different individual donors were used. Epidermal membranes (comprising stratum corneum and viable epidermis) were placed between the two halves of horizontal Franz-type glass diffusion cells; the stratum corneum faced the donor chamber. The area available for diffusion in each diffusion cell was approximately 1.1 cm^2 (range = 0.92 to 1.37 cm^2). Receptor chamber volume varied from 2.24 to 3.45 ml. The nonoxynols were dissolved in isopropyl myristate to generate 10% (*w/w*) solutions of Nonoxynol -2, -4, and -9, respectively. The 10% *w/w* concentration was representative of "on-head" exposures from an oxidative hair color base mixed with an equal volume of peroxide.

TABLE 4
Maximum cumulative flux and total absorption at 48 hours (An-eX Analytical Services 1995c)

Nonoxynols	Maximum amount permeated (μg/cm^2)	Maximum % applied dose permeated
Nonoxynol-2	$<1.44 \pm 0.10$	$<0.19 \pm 0.01$
Nonoxynol-4	$<7.85 \pm 0.35$	$<1.04 \pm 0.04$
Nonoxynol-9	$<10.46 \pm 0.49$	$<1.33 \pm 0.03$

Each Nonoxynol solution (10 μl) was dispensed over the surface of the stratum corneum. Following 1 h of exposure to each solution, epidermal membrane surfaces were rinsed with isopropyl myristate. The rinsates per individual cell were pooled and submitted for HPLC analysis (UV detection method). Samples (200 μl per sample) were removed from the receptor medium at 2, 4, 6, 8, 24, and 48-h post application of the vehicle, using a digital pipette, and then submitted for HPLC analysis. After removal of the 48-h sample, epidermal membrane surfaces were rinsed again with isopropyl myristate, and the rinsates were submitted for HPLC analysis.

No quantifiable levels of either Nonoxynol homologue were detected in the receptor chambers. Therefore, the skin permeation data are expressed as maximum cumulative permeation (based on detection limits and diffusion cell parameters) in Table 4.

The investigators stated that the values in Table 4 refer to the total amount of commercial Nonoxynol that was applied and that no attempts were made to define values for individual homologues. It was also stated that the maximum values given can be gross exaggerations of the actual amount of Nonoxynol that permeated (An-eX Analytical Services, Ltd. 1995c).

The sponsors of this study made the observation that the actual amounts of Nonoxynols permeated may have been substantially below the detection limits stated in Table 4 (Clairol, Inc./Rhone-Poulenc, Inc. 1995).

In the An-eX Analytical Services, Ltd. (1995c) study, data relating to the quantities of Nonoxynol that were rinsed from the skin at 1 h and 48 h post application were provided only for Nonoxynol-9. These data are included in Table 5. The

TABLE 5
Recovery of Nonoxynol-9 in rinses (An-eX Analytical Services, Ltd. 1995c)

Homologue	% applied dose (1 h)	% applied dose (48 h)	Total
N5	88.7	9.8	98.5
N6	80.1	13.3	93.4
N7	80.3	11.4	91.7
N8	73.9	16.9	90.8
N9	58.6	14.6	73.2

investigators stated that given the quantity of Nonoxynol that was recovered in the 1-h and 48-h rinses, it is not surprising that no quantifiable amounts were present in the receptor phase. It was also stated that, overall, the data in this study indicate that none of the Nonoxynols permeated through the skin to any great extent (An-eX Analytical Services, Ltd. 1995c).

In a second experiment, the in vitro skin penetration of Nonoxynol-2, -4, and -9 was also evaluated using heat-separated, human epidermal membranes. Skin samples were obtained from three individual donors. This experiment was designed to mimic in-use conditions relative to Nonoxynols in leave-on products, and was conducted to maximize the potential for quantifying the relative permeability of the various Nonoxynols and their constituent homologues.

Each of three test solutions of Nonoxynol-2, -4, and -9, respectively (10% *w/w* in isopropyl alcohol per solution; volume = 15 μl), was applied to epidermal membranes according to a modification of the procedure in the preceding experiment. Solutions remained in contact with the skin for 48 h, after which the entire receptor media were analyzed by HPLC. The HPLC analysis employed a fluorescence detection method (An-eX Analytical Services Ltd. 1995d).

This experiment includes data from three of the six replicate permeation experiments that were conducted for each Nonoxynol. The results indicate that the mean total amount of Nonoxynol permeated decreased with chain length from 7.21 μg of Nonoxynol-2 to 2.77 μg of Nonoxynol-9. Additionally, the lower Nonoxynol homologues permeated to a greater extent than the higher oligomers (An-eX Analytical Services Ltd. 1995e).

According to the sponsors of the preceding experiment (Clairol, Inc./Rhone-Poulenc, Inc. 1995), the total permeation for Nonoxynols was as follows: 6.17 ± 0.94 μg/cm^2, corresponding to $0.57\% \pm 0.07\%$ of applied dose (Nonoxynol-2); 7.10 ± 1.47 μg/cm^2, $0.66\% \pm 0.14\%$ of applied dose (Nonoxynol-4); and 4.73 ± 2.33 μg/cm^2, $0.49\% \pm 0.27\%$ of applied dose (Nonoxynol-9). Based on these data, it was stated that the total skin penetration for Nonoxynol-9 was slightly lower than that for Nonoxynol-2 and -4. The sponsors also stated that the levels of nonoxynols absorbed following an abbreviated exposure period (1 h) would be anticipated to be very low (0.13, 0.15 and 0.10 μg/cm^2 for nonoxynol-2, -4, and -9, respectively), based on simple linear extrapolation of the 48-h data. Therefore, the potential for systemic exposure to the lower molecular weight nonoxynols is extremely low under conditions of rinse-off application to the scalp (500 to 750 cm^2) in products such as hair dyes.

Hormonal Effects

Octoxynols

Nimrod and Benson (1996) stated that alkylphenol ethoxylates (which includes the Octoxynols) and related compounds have been reported to be estrogenic, both in vivo and in vitro, because they mimic the effects of estradiol.

Nonoxynols

In rats, Nonoxynol-9 can be metabolized to *para*-nonylphenol (Knaak, Eldridge, and Sullivan 1966; Walter, Agha, and Digenis 1988), which has been described as estrogen-like because it mimicked the effects of estradiol (i.e., induction of the progesterone receptor and cellular proliferation) in the MCF-7 (estrogen-dependent breast cancer) cell line. According to Jobling and Sumpter (1993), the results of studies using cultured rainbow trout hepatocytes have indicated that several alkylphenols and related nonylphenol ethoxylate degradation products (4-nonylphenol, 4-*tert*-octylphenol, 4-*tert*-butylphenol, 4-nonylphenoldiethoxylate, nonoxynol-9, and 4-nonylphenoxycarboxylic acid) mimicked the induction of vitellogenesis, which is an effect that is associated with estradiol. In a study by Nimrod and Benson (1996), *para*-nonylphenol induced the production of vitellogenin in male fish. Vitellogenin, produced under the control of estradiol, is a protein that is usually found only in sexually mature females.

Effects on Enzymes/Other Proteins

Stimulatory Effects of Octoxynol-9

Maiorino et al. (1986) reported that the addition of 0.5 to 1 mM Octoxynol-9, concentrations greatly above the critical micellar concentration, to a reaction mixture containing phospholipid hydroperoxides (in liposomal form) dramatically stimulated phospholipid hydroperoxide glutathione peroxidase (PHGPX) activity. In the presence of a much higher concentration of Octoxynol-9, the reaction was inhibited (unpublished observation). PHGPX was originally named peroxidation inhibiting protein on the basis of its dramatic inhibition of microsomal lipid peroxidation.

Dygas and Zborowski (1989) stated that Octoxynol-9 (5.4 mM) stimulated the activity of rat liver phosphatidylserine decarboxylase in mitochondrial membranes. The decarboxylation of phosphatidylserine in vitro was monitored by measuring $^{14}CO_2$ production.

Grabow, Chakraborty, and Ledeen (1996) reported significant activation of the enzyme guanylyl cyclase, isolated from myelin in the rat brain, in the presence of Octoxynol-9. Optimal activation was noted at a concentration of 0.5% to 1.0%.

Gils and Declerck (1997) stated that Octoxynol-9 accelerated the conversion of active plasminogen activator inhibitor 1 (PAI-1) into latent PAI-1 via an "induced" substrate-like conformation. The half-life of active PAI-1 decreased significantly in the presence of increasing concentrations of Octoxynol-9 (0.005%, 0.01%, 0.02%, and 0.2%). PAI-1, a member of the serine proteinase inhibitors superfamily, controls the plasminogen activator (t-PA). This plasminogen activator system regulates many physiological processes, including fibrinolysis.

Stimulatory/Inhibitory Effects of Octoxynol-9

Barbero et al. (1984) evaluated the effect of Octoxynol-9 on the activity of the succinate:coenzyme Q reductase complex (complex II); ubiquinol:cytochrome *c* reductase (complex III);

and cytochrome *c* oxidase (complex IV) from rat liver mitochondria. Succinate dehydrogenase, the mitochondrial enzyme that oxidizes succinate, is a component of the succinate:coenzyme Q reductase complex (complex II).

The specific activities of complexes II, III, and IV were not inhibited in the presence of Octoxynol-9 concentrations up to 5×10^{-3} M. At a concentration of 5×10^{-4} M Octoxynol-9, an increase in complex II activity was observed. Complex IV activity was enhanced in the presence of 10^{-3} M. When respiratory complexes were assayed in groups of two or three, the activity of complexes II + III and complexes II + III + IV disappeared completely in the presence of 2.5×10^{-3} M Octoxynol-9 (Barbero et al. 1984).

Tóth, Gimes, and Hertelendy (1987) reported the effect of Octoxynol-9 on phospholipid metabolism in human decidua and in the primordial placenta (chorion frondosum). Octoxynol-9 (0.05%) enhanced markedly the rate of incorporation of [^{32}P]Pi into phosphatidic acid in the decidua and chorion frondosum. Compared to controls, the maximal increase in the rate of incorporation was fivefold in the chorion frondosum and threefold in the decidua. The increase in [^{32}P]Pi incorporation into phosphatidic acid occurred in spite of an Octoxynol-9–induced decrease in the labelling of ATP. Inhibition of ATP synthesis by Octoxynol-9 (0.01% and 0.05%) was dose-related.

The authors noted that phosphate is incorporated into phosphatidic acid via the reaction catalyzed by diacylglycerol kinase acting on ATP and diacylglycerol as substrates or via the reduction and subsequent acylation by specific fatty acyltransferases of the glycolytic intermediate, dihydroxyacetone 3-phosphate. They suggested that the increase in the rate of [^{32}P]Pi incorporation into phosphatidic acid that was induced by Octoxynol-9 may have been due to some effect of Octoxynol-9 on the enzyme, diacylglycerol kinase. Octoxynol-9 (0.05%) had only a slight stimulatory effect on the incorporation of [^{32}P]Pi into phosphatidylinositol. The incorporation of [^{32}P]Pi into phosphatidylcholine decreased markedly in the presence of 0.05% Octoxynol-9. It was noted that CTP cytidylyltransferase is the regulatory enzyme for phosphatidylcholine synthesis, and that this enzyme may be involved in the inhibitory action of Octoxynol-9 on this process (Tóth, Gimes, and Hertelendy 1987).

Inhibitory Effects of Octoxynol-9

Sharma and Wang (1981) stated that 14 μM Octoxynol-9 was required for 50% inhibition of the activity of calmodulin-activated cyclic nucleotide phosphodiesterase in a standard reaction containing 40 ng calmodulin (from bovine brain).

Yu (1981) stated that rat liver monoamine oxidase (MAO) activity on different substrates (*p*-tyramine, serotin, and B-phenylethylamine) was inhibited (strong inhibition) by Octoxynol-9 in the 0.1%-1% concentration range. Enzyme activity decreased with increasing concentrations of Octoxynol-9. Complete inhibition of MAO activity was not achieved, even at a concentration of 5% Octoxynol-9.

McIntosh and Davidson (1984) reported that, at concentrations in the range of 0.02% to 0.05% *w/v*, Octoxynol-9 altered several properties of the Ca^{2+}-ATPase of sarcoplasmic reticulum vesicles (e.g., inhibition of Ca^{2+} transport resulted). Sarcoplasmic reticulum was obtained from rabbit back and hind limb white muscles. The concentrations at which effects were observed (0.02% to 0.05% *w/v*) were considered below those required for solubilization of membranes. Many of the effects observed were reversed at higher concentrations.

According to Boutin (1986), Octoxynol-9 inhibited the spontaneous oxidation of NADH and NADPH that is associated with rat liver microsomes. The incubation of microsomal proteins (15 mg/ml) with 0.05% *v/v* Octoxynol-9 caused a decrease in the NADH oxidation rate to 78% of that noted in the control (detergent-free buffer). At a concentration of 5% Octoxynol-9, the NADH oxidation rate was 97% of that noted in the control. Similar results were reported for the oxidation rate of NADPH; however, reaction rates were slower.

Hardy et al. (1987) stated that Octoxynol-9 induced a time-dependent inactivation of the enzyme, hexosaminidase C (from human brain) at concentrations up to 10 g/L of solution. Additionally, this inactivation was pH dependent (pH range: 4 to 7). The enzyme was rapidly inactivated in the presence of 0.1% Octoxynol-9.

Gimes and Tóth (1993) reported that Octoxynol-9 (0.05%) almost completely inhibited the conversion of [^{3}H]diacylglycerols to [^{3}H]triacylglycerols in human placenta fragments incubated with [^{3}H]glucose, indicating that the activity of diacylglycerol acyltransferase was inhibited. However, 0.05% Octoxynol-9 did not have any effect on the appearance of label in the sum of acylglycerols (mono-, di-, and triacylglycerol) and phosphatidylcholine, meaning that no effect on phosphatidate phosphohydrolase (key enzyme in cellular synthesis of new triacylglycerols and phosphatidylcholine) was demonstrated.

Effect on Muscle Contraction

Octoxynol-9

Gülden (1993) evaluated the effect of Octoxynol-9 on muscle using cultured cells from skeletal muscle tissue of the hind legs of 6- to 10- week-old male Sprague-Dawley rats. The following three end points were assessed: (1) Spontaneous contractility was determined after exposure to 3% *v/v* Octoxynol-9 for 1 and 24 h. (2) Gross structural damage to cell membranes (cell death) after 1 h of exposure was assessed by measuring the release of creatine kinase (CK) into the medium. After 24 h of exposure, lethal damage to cells was monitored by measuring the depletion of intracellular CK. (3) Consumption of glucose in the medium during the 24-h exposure period was measured to assess alterations in energy metabolism. An EC_{50} value for each parameter in question was determined. The EC_{50} is the concentration of an agent that decreases the parameter in question to 50% of the control value. Additionally, phase-contrast microscopy was used to screen cultures for morphological alterations.

Octoxynol-9 inhibited contractility at concentrations (1 h: $EC_{50} = 0.925$ μM ; 24 h: $EC_{50} = 2.3$ μM) much less than those that induced loss of cellular CK following exposure for 1 h ($EC_{50} = 214$ μM) and 24 h ($EC_{50} = 56.2$ μM), or those that caused inhibition of glucose consumption ($EC_{50} = 95.7$ μM). However, glucose consumption was stimulated to 170% ± 21% (mean ± *SD*, $n = 3$) of the control at intermediate concentrations of Octoxynol-9 ($EC_{50} = 9.4$ μM). No prominent morphological alterations were observed at a concentration of 33.2 μM (0.002%, *v/v*). Practically all of the myotubules were destroyed at a concentration of 82.9 μM Octoxynol-9 (0.005%, *v/v*). After 1 h of exposure to the 82.9 μM Octoxynol-9, blebbing was observed. At a higher concentration of 165.8 μM (0.01%), the myotubules were destroyed (Gülden 1993).

Kellermayer (1997) evaluated the effect of Octocynol-9 on the motility of actin filaments over heavy meromyosin (HMM) in vitro. Octoxynol-9 had no effect on motility at concentrations <0.004%. At concentrations >0.007%, actin filaments became dissociated from HMM and motility was not observed. Octoxynol-9 induced the dissociation of sliding actin filaments from HMM at concentrations ranging from 0.004 to 0.007%. It was stated that a discrepancy exists between the dramatic effects of low concentrations of Octoxynol-9 in the in vitro motility assay and the lack of such activity in muscle fiber experiments.

Effect on Histamine Release

Octoxynol-9

Ennis, Lorenz, and Gerland (1986) studied the effect of Octoxynol-9 on histamine release from mast cells. Mixed peritoneal mast cells (cell suspension) were obtained from female Sprague-Dawley rats. Secretory agents were added, with or without Octoxynol-9, to the prewarmed cellular suspension and histamine release was allowed to proceed for 10 min. The reactions were then terminated and the cells were recovered by centrifugation. Histamine was determined in both the supernatants and cells using the combined fluorometric-fluoroenzymatic assay. The results were expressed as percent histamine release, which was not corrected for the spontaneous release.

Octoxynol-9 (0.01 μl/ml) caused 3.8% ± 0.6% histamine release ($n = 8$) and Octoxynol-9 (0.02 μl/ml) caused 3.3 ± 0.6% histamine release ($n = 8$).

The authors suggested that the results indicated that Octoxynol-9 did not act as a histamine releaser at these concentrations. In the presence of the histamine releaser, compound 48/80, both concentrations of Octoxynol-9 potentiated the release of histamine. Octoxynol-9 significantly inhibited the histamine release that was induced by either concanavalin A or the calcium ionophore A 23187 (Ennis, Lorenz, and Gerland 1986).

Pharmacologic Activity

Octoxynol-9

Pavlik and Rutledge (1980) evaluated the effect of Octoxynol-9 on cytoplasmic and nuclear estrogen receptors from the rat uterus. Specific binding was determined as the difference between total binding that was measured in the presence of [^{3}H]-estradiol alone and unsaturable, nonspecific binding measured in the presence of [^{3}H]-estradiol and excess competitor. Octoxynol-9 (0.04%) increased the rate of ligand dissociation from cytoplasmic estrogen receptor approximately two-fold and increased the rate of ligand dissociation from nuclear receptor by approximately 4.5-fold.

Paczkowska and Szadujkis-Szadurski (1992) reported that the treatment of perfused arteries (in the rat tail) with Octoxynol-9 resulted in a large decrease in the potency of the following drugs in causing an increase in perfusion pressure and a decrease in maximal response: norepinephrine, phenylephrine, and clonidine.

Effect on Respiration

Octoxynol-9

Barbero et al. (1983) studied the effect of Octoxynol-9 on coupled and uncoupled respiration using rat liver mitochondria. At surfactant concentrations below 10^{-5} M, no effect on oxygen consumption by coupled or uncoupled mitochondria was observed. However, a slight decrease (~20%) in coupled respiration was noted at 10^{-5} M Octoxynol-9. At a higher concentration of 10^{-4} M, oxygen consumption of coupled and uncoupled mitochondria was decreased greatly and was virtually zero at 2×10^{-4} M.

Antimicrobial/Antiviral Activity

Octoxynol-9

According to Nadir and Gilbert (1979), the addition of Octoxynol-9 (30 to 40 μM) during the exponential phase had an inhibitory effect on the growth of *Bacillus megaterium* KM$^-$ cultures. This effect of Octoxynol-9 was greatly enhanced in the presence of KCl.

Podoplekina, Shutova, and Fyodorov Yu (1986) stated that the sensitivity of lymphocytic choriomeningitis virus (LCMV) and Tacaribe virus to Octoxynol-9 has been demonstrated. According to these authors, the effect of Octoxynol-9 is directed against the protein-lipid virus envelope. Formalin and hydrogen peroxide, but not Octoxynol-9, were among the chemicals that resulted in the rapid inactivation of both viruses.

Ukkonen et al. (1988) noted complete inactivation of the human immunodeficiency virus (i.e., no residual infectious virus detected, >7 log reduction of virus titre) after incubation of the virus with 0.2% Octoxynol-9 and 50% serum for 1 h (at 37°C).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Octoxynol-9

The Procter & Gamble Company (1964a) administered undiluted Octoxynol-9 orally to groups of ten Charles River SCD rats (weights = 200–300 g) at doses ranging from 0.678 to 1.86 ml/kg. The acute oral LD_{50} was 1.06 ml/kg (confidence

limits = 0.989–1.29 ml/kg). The mortality rate per group was dose related. At the highest dose administered, 9 of 10 animals died.

The E. K. Company Laboratory of Industrial Medicine (1969) evaluated the acute oral toxicity of Octoxynol-9 using 10 adult rats (strain and weights not stated). The test substance was administered at doses ranging from 200 to 3200 mg/kg. The LD_{50} was in the 800 to 1600 mg/kg range. Slight to moderate weakness, diarrhea, ataxia, and prostration were noted at the high dose. Octoxynol-9 was classified as slightly toxic.

In another study, the acute oral toxicity of undiluted Octoxynol-9 was evaluated using 10 mice (strain and weights not stated). The test substance was administered at doses ranging from 200 to 3200 mg/kg. The LD_{50} was approximately 1600 mg/kg. Weakness and diarrhea were observed. Octoxynol-9 was classified as slightly toxic (E. K. Company Laboratory of Industrial Medicine 1969).

Other Octoxynols

The Consumer Product Testing Company Inc. (1978a) evaluated the acute oral toxicity of Octoxynol-13 using four groups of six Wistar-derived albino rats (three males, three females; weights = 150–300 g). The animals were dosed individually (graded doses) by gavage and then observed for signs of pharmacologic activity and drug toxicity at 1, 3, 6, and 24 h post dosing. Dosing was followed by a 14-day nontreatment period, after which animals were killed and subjected to gross necropsy. An LD_{50} of 985 (691 to 1400) mg/kg was reported. Gross changes included reddening of the gastrointestinal mucosa and fibrous tissue encasing the heart or lungs.

A mean acute oral LD_{50} of 7.1 ± 0.1 cc/kg was reported for rats (number and strain not stated) dosed orally with Octoxynol-1 (FDA 1999a).

Following the oral administration of Octoxynol-3 to rats (number and strain not stated), a mean acute LD_{50} of 4.0 ± 0.2 cc/kg was reported (FDA 1999a).

A mean acute oral LD_{50} of 3.8 ± 0.2 cc/kg was reported for rats (number and strain not stated) dosed orally with Octoxynol-5 (FDA 1999a).

Data provided by FDA (1999b) included a study in which Octoxynol-16 (30%), Octoxynol-16 (70%), Octoxynol-20 (70%), Octoxynol-30 (70%), and Octoxynol-40 (70%) each were administered orally (stomach tube) to groups of 10 fasted, young male albino rats (average weight = 120 g). Except for animals dosed with Octoxynol-40 (70%) (one group), each test substance was administered to four groups of rats (different groups per test substance). In all of the groups tested, death was usually preceded by depression and the findings at necropsy were essentially negative.

Octoxynol-16 (30%) and Octoxynol-16 (70%) were administered at doses up to 6.0 and 7.0 g/kg, respectively. Eight of the 10 rats dosed with 6.0 g/kg (30% Octoxynol-16) and 7 of the 10 rats dosed with 7.0 g/kg (70% Octoxynol-16) died. The total number of deaths after dosing with 30% Octoxynol-16 (all dose groups combined) was 17, and the same was true after dosing with 70% Octoxynol-16.

The LD_{50} values for 30% Octoxynol-16 and 70% Octoxynol-16 were 2.68 ± 0.56 g/kg and 2.78 ± 0.95 g/kg, respectively. Diarrhea was associated with both concentrations of Octoxynol-16.

Octoxynol-20 (70%) was administered at doses up to 7.0 g/kg. Seven of the 10 rats receiving this dose died. The total number of deaths after dosing (all dose groups combined) was 16, and an LD_{50} of 3.64 ± 1.33 g/kg was reported. Diarrhea was associated with some of the animals tested.

Octoxynol-30 (70%) was administered at doses up to 28.0 g/kg. Again, 7 of the 10 rats in this dose group died. The total number of deaths after dosing (all dose groups combined) was 16, and an LD_{50} of 21.20 ± 2.0 g/kg was reported. None of the animals had diarrhea. An analysis of variance test using the LD_{50} values for 70% Octoxynol-16, 70% Octoxynol-20, and 70% Octoxynol-30 indicated that the difference between these values is significant at the 5% level.

One of 10 rats dosed with 70% Octoxynol-40 (28.0 g/kg) died. None of the animals had diarrhea (FDA 1999b).

Nonoxynols

The Consumer Product Testing Company (1978b) stated that, in a study involving 30 male and female rats (weights and strain not stated), the LD_{50} for Nonoxynol-6 was 1.98 g/kg. Doses ranging from 1.45 to 2.67 g/kg were administered by gavage.

In acute oral toxicity studies involving rats (numbers, weights, and strain not stated), the LD_{50} for Nonoxynol-5 ranged from 3500 to 4500 mg/kg (CTFA 1979a).

Acute Intraperitoneal Toxicity

Octoxynol-9

The E. K. Company Laboratory of Industrial Medicine (1969) evaluated the acute intraperitoneal toxicity of Octoxynol-9 (undiluted and 10% aqueous) using 20 rats (strain and weights not stated). The test substance was administered at doses ranging from 25 to 3200 mg/kg. Animals dosed with undiluted Octoxynol-9 died within 0.5 h post dosing. The LD_{50} was approximately 100 mg/kg. Moderate to extreme weakness (with ataxia and tremor), cyanosis, and initial prostration were observed in animals dosed with 10% Octoxynol-9. Octoxynol-9 was classified as moderately toxic.

In another study, the acute intraperitoneal toxicity of undiluted Octoxynol-9 was evaluated using 20 mice (strain and weights not stated). The test substance was administered at doses ranging from 50 to 3200 mg/kg. The LD_{50} was in the 50- to 100-mg/kg range. Weakness and rough coats were observed. Octoxynol-9 was classified as moderately toxic (E. K. Company Laboratory of Industrial Medicine 1969).

Acute Dermal Toxicity

Octoxynol-9

The acute dermal toxicity of Octoxynol-9 was evaluated using three guinea pigs (strain and weights not stated). The test

substance was administered (cuff = method of administration) at doses ranging from 5 to 20 cc/kg. The LD_{50} was greater than 20 cc/kg. Slight to moderate edema and scattered erythema (at periphery) were observed at 24 h post application. At 1 week, desquamation and slight alopecia were observed. Slight alopecia was observed at 2 weeks post application. There was no evidence of dermal absorption (E. K. Company Laboratory of Industrial Medicine 1969).

Nonoxynols

Although no details were available, an acute dermal toxicity study involving rabbits failed to achieve an LD_{50} for Nonoxynol-5 at a dose of 2.0 g/kg (CTFA 1979a). Likewise, an LD_{50} was not achieved at a dose of 3.0 g/kg when Nonoxynol-6 was tested in a dermal toxicity study involving rabbits (CTFA 1979b).

Acute Inhalation Toxicity

Octoxynol-9

Damon et al. (1978) evaluated the inhalation toxicity of Octoxynol-9 in dose-response studies using Syrian hamsters. The method of exposure was either inhalation or bronchopulmonary lavage. Fifty animals were exposed to an Octoxynol-9 aerosol with a mass mean aerodynamic diameter (MMAD) of 1.5 μm and a concentration of 2.8 mg/L. Estimated lung burdens ranged from 203 to 835 μg/g lung. The animals were also treated by lavage with Octoxynol-9 concentrations ranging from 0.01% to 0.10% in isotonic saline. Lung burdens after lavage ranged from 302 to 3180 μg of Octoxynol-9. LD_{50} values (with 95% confidence limits [CL]) were obtained by probit analysis of the 7-day mortality data.

An LD_{50} of 501 μg/g lung (CL = 372–676 μg/g) was reported for the inhalation experiment, and an LD_{50} of 2060 μg/g (CL = 1860–2700 μg/g) was reported for the lavage experiment. Animals in the inhalation experiment died from laryngeal obstruction, with moderate pulmonary edema and pneumonitis. In the lavage experiment, animals died from pulmonary edema and acute pneumonia (Damon et al. 1978).

Hackett and Henderson (1978) treated the lungs of Syrian hamsters by lavage (80% lung volume) with 0.05% Octoxynol-9 in 0.9% saline. Lung cell [^{3}H]thymidine uptake was evaluated after animals received a 2-h pulse of label before they were killed at 2, 18, 24, 48, and 72 h after lavage was initiated.

An assay of the lactate dehydrogenase (LDH) that was released into the alveolar fluid during lavage indicated immediate injury. Compared to saline-lavage controls, whole lung tissue uptake of [^{3}H]thymidine was increased significantly in animals lavaged with Octoxynol-9. Though [^{3}H]thymidine uptake into alveolar macrophages was greater in lungs lavaged with Octoxynol-9 (35%) than in saline-lavaged controls (20%), lavage with Octoxynol-9 did not alter the population distribution of type II cells or alveolar macrophages at 18 h post lavage.

The authors stated that the exposure of lungs to Octoxynol-9 (0.05%) causes increased uptake of [^{3}H]thymidine that is not attributed to type I, type II, or endothelial cells, but to increased incorporation of label into alveolar macrophages and injured ciliated airways (Hackett and Henderson 1978).

Henderson, Damon, and Henderson (1978) exposed 12 1-year-old Syrian hamsters (Sch:(SYR) strain; six, males, six females) to Octoxynol-9 (in saline) by bronchopulmonary lavage. The animals were anesthetized with halothane in oxygen and intubated intratracheally. Groups of four animals were treated by lavage (one lung per animal; two consecutive washes) with 0.01%, 0.05%, 0.075%, or 0.10% Octoxynol-9 in saline. Eighteen control animals received two consecutive washes with approximately 4 ml of 0.15 M saline. The volume of the lavage fluid was measured and centrifuged.

LDH activity in the cell-free supernatant and the iron content of the lavage fluid were determined. LDH activity was monitored by a decrease in absorbance at 340 nm in the presence of NADH and pyruvate. The presence of extracellular LDH activity in the airways served as an indicator of early pulmonary damage. Iron content of the lavage fluid (indicative of lysed red blood cells) was determined by atomic absorption spectroscopy.

LDH activity in the cell-free portion of the lavage fluid increased with increasing concentrations of Octoxynol-9 (correlation coefficient = 0.98). LDH activity (expressed as International Units per milliliter [IU/ml] of lavage fluid) ranged from 0.045 $\pm$ 0.008 (0.01% Octoxynol-9) to 0.337 $\pm$ 0.080 (0.10% Octoxynol-9). The mean value for LDH activity in the control group was 0.017 $\pm$ 0.008. All of the animals treated by lavage with 0.075% or 0.1% Octoxynol-9 died anywhere from 7 h to 3 days post lavage. Atelectasis (focal and mild) and severe pulmonary edema were noted at microscopic examination. Histopathologic findings in animals that died at days 2 and 3 post lavage included focal necrosis associated with hemorrhagic areas of the lung and an acute generalized pneumonia with polymorphonuclear leukocyte and macrophage exudation.

No deaths occurred in the control group or in groups dosed with 0.01% or 0.05% Octoxynol-9. The results of a second experiment indicated that, most likely, the sources of LDH activity were damaged epithelial cells in the airways and/or lysed red blood cells that may have entered the airways through damaged capillaries. The fact that the iron content of the cell-free lavage fluid of test animals was indistinguishable from that of control animals indicated that lysed red blood cells were not the major source of LDH in the lavage fluid (Henderson, Damon, and Henderson 1978).

Damon et al. (1982) evaluated the acute inhalation toxicity of Octoxynol-9 using male and female Syrian hamsters (*Mesocricetus auratus,* Sch:SYR Sprague-Dawley; 374 days old). Tritiated Octoxynol-9 was adminstered via bronchopulmonary lavage (see procedure in preceding study) to a total of 32 animals. Five groups of animals (4 to 8 per group; 32 animals total) received Octoxynol-9 at weight percentage concentrations of 0.01%, 0.05%, 0.06%, 0.075%, and 0.10% in isotonic saline, respectively. Twenty-four hamsters (controls) were treated by lavage with isotonic saline. The tritium activity and volume of

recovered lavage fluid were measured to determine the amount of Octoxynol-9 that was deposited in the lungs. Necropsy was performed on all animals that died. Surviving animals were killed by lethal injection and necropsy was performed at day 7 post exposure.

An LD_{50}, determined by probit analysis, of 2100 μg (estimated mean lung burden of Octoxynol-9) with 95% confidence limits of 1900 to 2700 μg was reported. Mortality rates were as follows: 0.01% Octoxynol-9 (0/4), 0.05% Octoxynol-9 (1/8), 0.06% Octoxynol-9 (4/8), 0.075% Octoxynol-9 (8/8), and 0.10% Octoxynol-9 (4/4). None of the control animals died. Congested lungs, focal areas of peripheral atelectasis, and blood-tinged fluid in the trachea and large bronchi were noted at necropsy.

The following histopathologic changes, which varied as a function of survival time, were observed: severe intraseptal and peribronchial congestion (with only occasional intraalveolar fibrin strands), fibrinous exudate (with large numbers of neutrophils, macrophages, and cellular debris) in terminal bronchioles and alveoli, and focal necrosis of intraalveolar septa and intraalveolar hemorrhage. Fibrin strands in the larynx, trachea, and major bronchi were also noted. No evidence of residual injury was observed in animals that were available for examination on day 7 (Damon et al. 1982).

In another experiment reported by these authors, 50 95-day-old hamsters and 50 419-day-old hamsters were exposed (nose-only) to an Octoxynol-9 aerosol. The 95-day-old animals were exposed to aerosol with an MMAD of 1.47 ± 0.06 μm and a geometric standard deviation of 1.84 ± 0.07. The 419-day-old animals were exposed to an Octoxynol-9 aerosol with an MMAD of 1.51 ± 0.07 μm and a standard deviation of 1.91 ± 0.08. In each group, a mass concentration of 3.0 mg/L was produced by nebulization of a 10% solution of Octoxynol-9 (in ethanol) in a Retec nebulizer. Groups of 10 animals were removed from the exposure chamber at different time intervals in order to provide initial respiratory tract burdens, which ranged from 800 to 3100 μg. Ten control 95-day-old hamsters and 10 control 419-day-old hamsters were exposed to aerosolized ethanol for 37 min.

An LD_{50} (determined by probit analysis) of 1700 μg with 95% confidence limits of 1300 to 2100 μg was reported. Death was attributed to obstructive asphyxia. Laryngeal and epiglottic edema were the most prominent gross features. The mucosa overlying the epiglottis and vocal folds was focally desquamated and hemorrhagic ulcerations were observed.

No gross abnormalities were observed in the lower trachea, major bronchi, or lungs. At microscopic examination, mucosal ulcerations with necrotic bases were observed in laryngeal sections. The ulcerations contained neutrophils and macrophages, and, occasionally, small clusters of neutrophils and fibrin were present in single alveoli. No abnormalities were observed in large or small bronchi (Damon et al. 1982).

Dorato (1990) exposed two Swiss mice to increasing airborne concentrations of Octoxynol-9. The mice (weight = 24–26 g) were exposed, nose-only to concentrations of 4.4, 15.0, and 36.0 or 38.0 mg/L at a rate of 30 L/min. An animal's respiratory movements in the plethysmograph alternately created a positive and negative pressure during inspiration and expiration, respectively. Pressure changes, sensed by a pressure transducer, were recorded on an oscillograph. If no decrease in respiratory rate was observed, a chemical was classified as a nonirritating material. Exposure resulted in a concentration-related decrease in respiratory rate. Octoxynol-9 was classified as a sensory irritant by this author.

Short-Term Oral Toxicity

Octoxynol-9

A developmental toxicity study by Leung and Ballantyne (1999) provided short-term oral toxicity results. The study will be further presented in the section on Reproductive and Developmental Toxicity. Three groups of 27 outbred, Sprague-Dawley CD rats (10 to 11 weeks old at time of mating) were used. Two groups received dietary concentrations of 0.06% Octoxynol-9 (dose = 70 mg/kg/day) and 0.3% Octoxynol-9 (dose = 340 mg/kg/day), respectively, on gestation days (GDs) 6 through 16. The third group (control) received untreated rat chow. On GD 17, the test diet was withdrawn and replaced with the control diet. The dams were killed on GD 20 by nitrogen asphyxiation.

None of the animals died, and no clinical signs were reported. The dams were not subjected to gross or microscopic examination (Leung and Ballantyne, 1999).

Short-Term Dermal Toxicity

Octoxynol-9

The developmental toxicity study by Leung and Ballantyne (1999) also provided short-term dermal toxicity information. Three groups of 25 outbred, Sprague-Dawley CD rats (10 to 11 weeks old at time of mating) received dermal applications of Octoxynol-9 at concentrations of 12.5% (dose = 530 mg/kg/day), 37.5% (1600 mg/kg/day), and 100.0% (4270 mg/kg/day), respectively, each at a constant dose volume of 4 ml/kg on GDs 6 through 15. Control animals received deionized and filtered water.

One rat in the highest dose group (undiluted Octoxynol-9, 4270 mg/kg/day) was found dead on GD 7. The cause of death was not determined. The following clinical signs were observed in the highest dose group: urine stains, audible respiration, and perinasal encrustation. Perinasal encrustation, but not audible respiration or urine stains, was observed in the remaining two lower dose groups.

Clinical signs relating to skin changes at the application site are included in the section on Skin Irritation later in this report. When corrected for gravid uterine weight, body weight gain over the entire gestational period was reduced only in the highest dose group. No statistically significant differences in lung, liver, or kidney weights were noted between test (all dose groups) and control groups. Increases in relative liver and kidney weights were considered to be associated with reductions in maternal weight gain instead of a direct effect of dosing on these organs.

For maternal toxicity, the no-observable-effect level (NOEL) for Octoxynol-9 was 1600 mg/kg/day (Leung and Ballantyne 1999).

Other Octoxynols

In a study evaluating systemic effects provided by FDA (1999a), mixed octoxynols were applied to the skin of rabbits over a period of 4 weeks (20 applications total). Ingredients were applied at the following concentrations: 1% Octoxynol-1; 1% Octoxynol-3; 0.1% Octoxynol-9; and 0.1% Octoxynol-13. Neither the age range nor strain of the animals tested was stated. For each ingredient tested, no abnormal changes were noted at histopathologic examination.

Short-Term Parenteral Toxicity

Nonoxynols

Chvapil et al. (1986) evaluated the toxicity of Nonoxynol-9, in saline using female Sprague-Dawley rats (weights ≈200 g). Ten rats were intraperitoneally injected with 5 mg Nonoxynol-9/100 g body weight daily for a total of 5 days. Control rats were injected intraperitoneally with saline according to the same procedure. The animals were exsanguinated, and the livers were infused in situ. The liver, kidneys, and lungs were then removed from each animal. An increase in serum glutamyl oxaloacetic transaminase (SGOT) activity was detected after a single intraperitoneal injection of Nonoxynol-9. SGOT activity reached a maximum (900 IU) between 4 and 8 h. The administration of Nonoxynol-9 for 5 days caused a significant increase ($p < .001$; 2.27 ± 0.12 mg/liver) in the content of collagen in the liver. Total collagen content as well as the density of collagenous hydroxyproline in the liver were increased by approximately 100%. The cellularity of the liver, based on the amount of DNA, was also significantly increased. Compared to saline-treated controls, transmission electron micrographs of randomly selected cubes of liver tissue from experimental animals indicated a dramatic increase in the amount of rough endoplasmic reticulum. Changes in all of the preceding parameters were not observed in the lungs. The investigators concluded that the intraperitoneal administration of Nonoxynol-9 produced morphological and biochemical changes in the liver.

In another short-term toxicity test by these authors, 5 mg of Nonoxynol-9/100 g body weight (in saline) were instilled into the upper aspect of the vagina of four groups of six Sprague-Dawley female rats (weights ≈200 g). Injections were made daily for 5, 10, 15, and 20 days, respectively; blood samples were also taken on these days. Control rats (four groups of three) were intravaginally injected with saline. The animals were exsanguinated at 5-day intervals and the liver, kidneys, and lungs were removed from each animal. Total hydroxyproline and DNA content were determined in hepatic and renal tissues. Fifteen days post administration, a significant increase in hepatic collagen ($p < .01$; 339 ± 46.4 μg/g) was noted. No effect on DNA was observed during the 15-day post administration period. When liver specimens were examined by light microscopy, lesions of nonspecific inflammation with destruction of normal lobule architecture were observed (after 15 injections). Liver specimens examined by transmission electron microscopy had an increased density of rough endoplasmic reticulum (after 15 injections of Nonoxynol-9) primarily in the vicinity of the cell nucleus. In the kidneys, both DNA content and total hydroxyproline were significantly increased after 15 days ($p < .01$) and 20 days ($p < .05$) of Nonoxynol-9 administration. A significant increase in SGOT activity was also noted during each of the four time periods at which blood samples were taken ($p < .001$ on days 5 and 15; $p < .01$ on days 10 and 20). The researchers concluded that the intravaginal administration of Nonoxynol-9 produced morphological and biochemical changes in the liver and biochemical changes in the kidneys (Chvapil et al. 1986).

Short-Term Inhalation Toxicity

Other Octoxynols

Bio/dynamics, Inc. (1992) evaluated the short-term inhalation toxicity of an ethoxylated *para*-tert-octyl phenol (an octoxynol). The authors did not state the number of moles of ethylene oxide. The study used five male (weights = 230–250 g) and five female (weights = 145–175 g) Sprague-Dawley CD rats. The animals were exposed to the test substance (target concentration in inhalation chamber = 10 mg/m^3) 5 days per week (6 h/day) for 2 weeks. The MMAD of the test substance was 1.8 μm.

Reddening of the lung was observed grossly in four males and three females. At histopathologic examination, inflammatory changes in the alveolar walls/perivascular space were noted. Compared to air-exposed controls, both the incidence and severity of this finding were greater.

Alveolar/bronchiolar epithelial hyperplasia was observed only in animals exposed to the test substance, and, therefore, was considered treatment related. Lung-to-body weight ratios in test animals were significantly greater when compared to controls. None of the animals died (Bio/dynamics, Inc. 1992).

Subchronic Oral Toxicity

Other Octoxynols

Larson (1961a) evaluated the subchronic oral toxicity of Octoxynol-40 using young albino rats (15 males, 15 females). Mean body weights for male and female rats were 71 and 79 g, respectively. The test substance was administered at a concentration of 5% in the diet daily for 3 months. Another group of 15 male and 15 female rats served as the control. Three test animals (all males) and two controls (one male, one female) died. The death of test animals was not related to dosing with Octoxynol-40. No effects on growth and food consumption were noted. Urinary concentrations of sugar and protein were comparable between test and control animals, and the results of hematologic evaluations indicated no definite effects of Octoxynol-40 dosing.

Data on organ-to-body weight ratios (heart, spleen, kidney, liver, testes) indicated no differences between test and control animals that were statistically significant. Mean testes/body weight

ratios $\times 10^{-3}$ were 8.7 $\pm$ 1.1 g (test animals) and 9.2 $\pm$ 1.1 g (controls), and these results are also included in the section on Reproductive and Developmental Toxicity later in this report. No test substance–related lesions were observed at histopathologic examination (Larson 1961a).

In a study by Larson et al. (1963), Octoxynol-40 was administered to two groups of four (two males, two females/group) purebred Beagle dogs at concentrations of 0.35% and 5.0% in the diet, respectively. An additional group of four dogs served as the control. The animals were between 6 and 7 months of age and weights ranged from 4.9 to 10.25 kg. The animals were killed at the end of the study and tissues subjected to histopathologic examination.

No adverse effects on the following parameters were noted: body weight, food consumption, hematocrit, hemoglobin, total and differential white cell counts, urinary concentrations of sugar and protein, or organ-to-body weight ratios. No test substance–related lesions were observed (Larson et al. 1963). Study results relating to testes/body weight ratios are included in the section on Reproductive and Developmental Toxicity later in this report.

Chronic Oral Toxicity

Other Octoxynols

Larson (1961b) evaluated the chronic oral toxicity of Octoxynol-40 using groups of young albino rats (30 males, 30 females/group). Mean body weights for male and female rats were 63 and 58 g, respectively. Octoxynol-40 was administered to the groups at dietary concentrations of 0.035%, 0.35%, and 1.4%, respectively, daily for 3 months or 2 years. The control group (30 males, 30 females) received basic diet only.

At the end of the third month of dosing, five males and five females from each dose group were killed and tissues (heart, lung, liver, kidney, and gonads + other tissues) subjected to histopathologic examination. Dosing of the remaining rats (20 per dose group) proceeded to the end of the 2-year study, after which surviving animals were killed and tissues subjected to histopathologic examination.

No adverse effects on the following measured parameters were observed at either of the administered doses: survival, growth, food consumption, hematologic values (hematocrit, hemoglobin, total and differential leucocyte counts), urinary concentrations of sugar and protein, organ-to-body weight ratios, or kind, incidence, and degree of pathologic lesions (Larson 1961b). Data on the testes/body weight ratio at each dose administered are included in the section on Reproductive and Developmental Toxicity later in this report.

Ocular Irritation

Octoxynol-9

The Procter & Gamble Company (1964b) evaluated the ocular irritation potential of Octoxynol-9 (10%) using six rabbits. Only three rabbits were subjected to ocular rinsing. By day 35 post instillation, discrete to translucent areas of the cornea had not cleared in two of three rabbits that were not subjected to ocular rinsing. In the remaining three rabbits (ocular rinsing), all eyes were normal within four days.

In a study by E. K. Company Laboratory of Industrial Medicine (1969), a single drop of undiluted Octoxynol-9 was instilled into one eye of each of two rabbits. The eye of one animal was rinsed after instillation. Reactions were scored at 1 h, 24 h, 48 h, and 14 days post instillation.

Moderate to severe erythema, slight to moderate edema, slight corneal opacity, and iridial injection were observed in the unrinsed eye. Signs of ocular irritation (slight pannus and slight erythema on the nictitating membrane) persisted to 14 days post instillation (unrinsed eye).

In the rinsed eye, slight to moderate erythema, slight edema, slight corneal opacity, and iridial injection were observed. Reactions (rinsed eye) had cleared by 14 days post instillation. Octoxynol-9 was classified as a moderate permanent ocular irritant. It was stated that permanent damage may be prevented by prompt irrigation (E. K. Company Laboratory of Industrial Medicine 1969).

The ocular irritation potential of a skin freshener was reported by CTFA (1986c). The formulation, containing 0.25% Octoxynol-9, was instilled into the eyes of six rabbits (strain not stated; single instillation). Eyes were not rinsed after instillation, and reactions were scored according to the Draize scale (maximum score = 110) on days 1 through 7 post instillation.

A total Draize score of 5 was reported on day 1 and had decreased to 2 by day 7 post instillation. The product was classified as minimally irritating (CTFA 1986c).

In a second ocular irritation study (three rabbits; same procedure) of a skin freshener containing 0.25% Octoxynol-9, total Draize scores of 1 and 0 were reported on days 1 and 3 post instillation, respectively. Reactions were not scored beyond day 3. The product was classified as minimally irritating (CTFA 1986d).

E. I. du Pont de Nemours and Company, Inc. (1987) provided the results of a study in which the ocular irritation potential of Octoxynol-9 was evaluated using two young adult, male New Zealand white rabbits. The test substance (concentration not stated) was instilled into the right conjunctival sac of each animal. The left eyes served as controls. Both the treated and control eye of one animal were not rinsed until approximately 20 s post instillation. The animals were examined for signs of ocular irritation according to the following schedule: 1 h, 4 h, and 1, 2, 3, 7, 14, and 21 days post instillation. Reactions were scored according to the Draize scale.

The following reactions were observed in treated eyes of both rabbits: moderate iritis, moderate conjunctival redness and chemosis, and copious, blood-tinged discharge. Conjunctival redness had cleared by day 21. Mild and moderate corneal opacity were observed in rinsed and unrinsed eyes, respectively. The

results of biomicroscopic examinations indicated moderate to severe corneal injury in both treated eyes. Corneal injury, as determined by fluorescein stain examinations, was evident in both treated eyes on days 1 to 3 post instillation. Corneal opacity and iritis (in unrinsed eye) persisted beyond day 21 post instillation. Octoxynol-9 was classified as a moderate ocular irritant (E. I. du Pont de Nemours and Company, Inc. 1987).

Kennah et al. (1989) evaluated Octoxynol-9 (up to 10 vol %) in the Draize test using four to six rabbits. The test substance (0.1 ml) was instilled into the conjunctival sac of one eye. The untreated eye served as the control. The cornea, iris, and conjunctiva were scored according to the following schedule: 24 h, 48 h, and 72 h post instillation and at days 7, 10, 14, and 21, if irritation persisted. At each observation time, a Draize score was computed by averaging the total scores of all rabbits tested. The maximum average score observed was primarily the 24 h Draize score. The following Draize scores were reported: 59 = severely irritating (for 10% Octoxynol-9); 32 = moderately irritating (for 5% Octoxynol-9); and 2 = minimally irritating (for 1% Octoxynol-9). Draize scores were correlated with corneal swelling induced by the three test concentrations (10% = severe corneal swelling; 5% = moderate; 1% = mild). Results indicated that changes in corneal thickness can be used to quantitate total ocular irritation.

Tachon et al. (1989) evaluated Octoxynol-9 (10% *w*/*v* in aqueous solution) in the Draize test using six albino rabbits. The test substance (0.1 ml) was applied directly to the cornea of each rabbit using a syringe. The maximum ocular Draize irritation score (IO max) was recorded at 1 or 2 h and the Draize ocular irritation score (IO-J7) was also recorded at day 7 post instillation. Draize IO max and IO-J7 values of 40.33 and 9.33, respectively, were reported.

Octoxynol-9 was classified as an ocular irritant. In the same study, these test results were said to have correlated well with the results of an in vitro cytotoxicity assay using Chinese hamster lung fibroblasts. It was suggested that this test could be a reliable alternative to the Draize ocular irritation test (Tachon et al. 1989).

Joller et al. (1994) instilled Octoxynol-9 (100 μl; concentration not stated) into the conjunctival sac of one eye of each rabbit (number not stated). The upper and lower eyelids were then held together for 1 s to prevent loss of the test substance from the eye. Eyes were not rinsed after instillation, and the contralateral eye served as the control. Reactions were scored at 1 h, 24 h, 48 h, 72 h, and 94 h post instillation. Using a scale of minimally irritating (0–15), slightly irritating (>15–25), moderately irritating (>25–50), severely irritating (>50–80), and extremely irritating (>80–110), the authors reported an ocular irritation score of 25.

Kojima et al. (1995) evaluated the ocular irritation potential of Octoxynol-9 (10% *w*/*w* in distilled water) using three Japanese white female rabbits. The test solution was instilled directly into the left eye of each animal. Eyes were not rinsed after instillation, and untreated eyes served as negative controls. Reactions in the cornea, iris, and conjunctiva were scored at 1, 3, 6, 24, 48, 72, 96, and 168 h post application according to the Draize scale. The average ocular irritation score (three rabbits) was calculated at each observation period and the maximum value for the eight time periods was considered the maximal Draize rabbit eye irritation score (MDES; scale: 0 to 110). An MDES of 55.0 was reported.

In a study by Schneider, Maier-Reif, and Graeve (1997), the ocular irritation potential of Octoxynol-9 was evaluated in an in vitro cytotoxicity assay using corneal cells from the fetal pig. Three corneal cell types were cultured (epithelial, endothelial, and stromal), and test concentrations in the culture medium ranged from 0.1% to 0.005%. For cytotoxicity testing, the focus was assessing the mitochondrial capacity of corneal cells, which was accomplished by monitoring the reduction of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) reagent. Results for each test concentration were plotted as percent of untreated control versus percent concentration of test substance using a log scale. EC_{50} values (effective concentration of test substance that inhibits 50% of the mitochondrial capacity) were interpolated directly from the graph.

Octoxynol-9 caused 50% reduction of MTT at a concentration of 0.006% (EC_{50} = 0.006%). This EC_{50} value was said to correlate well with in vivo Draize test data (Draize score = 5, severe or extreme irritation). Concentrations higher than 0.01% completely inhibited the reduction of MTT (Schneider, Maier-Reif, and Graeve 1997).

Other Octoxynols

The Consumer Product Testing Company, Inc. (1978a) evaluated the ocular irritation potential of Octoxynol-13 using a group of six New Zealand rabbits (males and females). The test substance (0.1 ml; concentration not stated) was instilled into the right eye of each animal. Untreated eyes served as controls. Eyes were not rinsed and reactions were scored at 1, 2, 3, and 7 post instillation according to the Draize scale (0 to 110). Octoxynol-13 was classified as severely irritating. Draize ocular irritation scores were as follows: 30.2 (day 1), 28.0 (day 2), 34.3 (day 3), 28.8 (day 4), and 33.8 (day 7).

In a study provided by FDA (1999a), the ocular irritation potential of Octoxynol-1, -3, -5, -9, and -13 was evaluated using groups of five rabbits. The highest test concentrations that did not induce ocular irritation in three, or more, of five rabbits per group were as follows: 15% Octoxynol-1, 15% Octoxynol-3, 5% Octoxynol-5, 0.5% Octoxynol-9, and 1% Octoxynol-13.

Gattefossé s.a. (2000) classified an aqueous solution of 20% Octoxynol-11 as "very badly tolerated" in an ocular irritation test. Details concerning the animal species tested, the test protocol, or study results were not stated.

Nonoxynols

The Consumer Product Testing Company, Inc. (1978b) evaluated the ocular irritation potential of Nonoxynol-6 in a Draize test using six rabbits; the eyes were not rinsed. The test substance was classified as a severe ocular irritant. The average

Draize scores (scale = 0–110) on days 1 and 7 post instillation were 28.8 and 16.0, respectively.

CTFA (1979a) reported that severe ocular irritation reactions were observed in animals tested with Nonoxynol-5. An ocular irritation score of 55 persisted through day 7. CTFA (1979b) reported that Nonoxynol-6 also induced severe ocular irritation reactions in animals. Growth of blood vessels onto the cornea was observed. Irritation reactions persisted to day 21. In neither report were the experimental procedure or the animal species stated.

In Vivo Skin Irritation

Octoxynol-9

In a study provided by CTFA (1986a), the skin irritation potential of a peel-off mask product containing 0.25% Octoxynol-9 was evaluated in a single-insult occlusive patch test using nine rabbits (strain not stated). Reactions were scored at 2 h and 24 h post application (grading scale not stated), and a primary irritation index (PII) was calculated.

At 2 h post application, a score of 1 (for erythema) was reported for eight rabbits and a score of 2 (for erythema) was reported for the remaining rabbit; edema was not observed. No reactions were observed at 24 h. The PII was 0 out of a maximum possible score of 8. The product was classified as minimally irritating (CTFA 1986a).

No reactions were observed in another study (CTFA 1986b) in which six rabbits were tested (same procedure) with another peel-off mask product containing 0.25% Octoxynol-9. The PII was 0 out of a maximum possible score of 8. The product was classified as a non-irritant.

Kojima et al. (1995) evaluated the skin irritation potential of Octoxynol-9 using six Japanese white female rabbits. The trunk and lateral areas on each animal were shaved, and the test substance (10% *w*/*w* in distilled water; volume = 0.15 ml) was applied under gauze patches to intact skin. Patches were removed at 24 h post application, and reactions scored for erythema and edema at 1 and 24 h post removal. Average values for skin irritation at each observation period were calculated, and the maximum value for both time periods was considered the maximal primary Draize skin irritation score (MDSS; scale = 0–8.0). An MDSS of 0.2 was reported.

A developmental toxicity study by Leung and Ballantyne (1999) provided data on skin irritation. Three groups of 25 outbred, Sprague-Dawley CD rats (10 to 11 weeks old at time of mating) received dermal applications of Octoxynol-9 at concentrations of 12.5% (dose = 530 mg/kg/day), 37.5% (1600 mg/kg/day), and 100.0% (4270 mg/kg/day), respectively, each at a constant dose volume of 4 ml/kg on GDs 6 through 15. Control animals received deionized and filtered water.

At the highest dose tested, skin changes at the site of application included exfoliation/desquamation, excoriation, and erythema. Excoriation and erythema, but not desquamation/exfoliation, were observed in the remaining two dose groups (Leung and Ballantyne 1999).

Other Octoxynols

The Consumer Product Testing Company, Inc. (1978a) evaluated the skin irritation potential of Octoxynol-13 using six New Zealand albino rabbits (males and females). The test substance (0.5 ml under occlusive patch; concentration not stated) was applied to intact and abraded skin sites that had been clipped free of hair. Occlusive patches were secured with adhesive tape, and the trunk of each animal was wrapped with an impermeable occlusive wrapping. At 24 and 72 h post application, reactions (erythema and edema) were scored according to the following scales: 1 (very slight erythema) to 4 (severe erythema [beet redness] to slight eschar formation [injuries in depth]) and 1 (very slight edema) to 4 (severe edema [raised more than 1 mm and extending beyond the area of exposure]). The PII was calculated by averaging the mean scores that were recorded at 24 and 72 h.

At 24 h, very slight erythema and edema were observed at intact and abraded sites. Reactions were not observed at 72 h post application. It was concluded that Octoxynol-13 was not a primary dermal irritant (PII = 0.50). The potential for slight irritation was also noted (Consumer Product Testing Company, Inc. 1978a).

An aqueous solution of 20% Octoxynol-11 was classified as a moderate skin irritant. Details concerning the animal species tested, the test protocol, or study results were not stated (Gattefossé s.a. 2000).

Nonoxynols

The Consumer Product Testing Company, Inc. (1978a) evaluated the skin irritation potential of Nonoxynol-6 in a study involving six rabbits. The test substance (0.5 ml) was applied under occlusive patches to clipped intact and abraded skin. Reactions (erythema and edema) were scored at 24 and 72 h, and the mean scores were averaged in order to determine the PII. Nonoxynol-6 was classified as severely irritating to the skin of rabbits (PII = 3.0).

CTFA (1979a) reported that severe skin irritation reactions were observed in animals tested with Nonoxynol-5. The reactions observed included reddening, cracking, and drying. CTFA (1979b) stated that Nonoxynol-6 was classified as a severe skin irritant in animals in another study; primary irritation score = 6.6. In neither case were the experimental procedure or the animal species provided.

Nethercott and Lawrence (1984) evaluated the skin irritation potential of Nonoxynol-6 using six New Zealand white rabbits. The test substance was applied to clipped skin of the back at concentrations of 25, 50, 75, and 100 g % (*w*/*w*) in petrolatum. The test sites were then covered with patches ("Al Test" strips) secured with tape and a bandage. The bandages were removed at 24 h and sites were scored for the presence of irritation at 48 h. No effort was made to determine the severity of individual reactions observed. Nonoxynol-6 concentrations of 25%, 50%, and 75% each induced skin irritation in four of six rabbits. Nonoxynol-6 (100%) induced skin irritation in five of six rabbits.

In Vitro Skin Irritation

Octoxynol-9

Bloom et al. (1993) evaluated Octoxynol-9 and four known skin irritants (sodium lauryl sulfate [SLS], phenol, ethylphenyl propionate [EPP], and tetradecanoyl phorbol acetate [PMA]) in an in vitro growth inhibition assay using human epidermal keratinocytes.

This research is based on the premise that the measurement of in vitro qualitative differences between irritants would help to develop a more reasonable and physiologically accurate in vitro test for evaluating skin irritation potential in animals and humans. Each chemical was added to keratinocyte growth medium containing the standard antimicrobials; no growth factors were added.

Test substance concentrations (produced by 10-fold dilutions; volume = 10 μl) ranged from 10^{-10} to 10^{-2} M. It is important to note that the chemicals were diluted with ethanol prior to serial dilutions in the medium. The final ethanol concentration was $\leq$1%. (Ethanol (1%) did not induce significant growth inhibition or morphological change.) The test concentration that was required to induce 50% inhibition of cell function (I_{50}) was calculated after 1 h and 18 h of exposure. Growth inhibition was noted after both periods of exposure; I_{50} values at 1 and 18 h were 7.7×10^{-5} M and 3.4×10^{-3} M, respectively.

The growth toxicity induced by Octoxynol-9 occurred rapidly (onset <1 h). Morphological changes in the keratinocytes included marked rounding and shrinkage of cells. The rank order for morphological changes was SLS, Octoxynol-9 > phenol > EPP, PMA. The rank order for growth inhibition was PMA > EPP > SLS, Octoxynol-9 > phenol. The authors noted that in vivo studies indicate that PMA is the most potent of the five irritants.

According to the authors, each chemical induced markedly different morphological changes, and it was possible, knowing the length of exposure and concentration, to distinguish one irritant from another when photographs were compared (Bloom et al. 1993).

In a study by Giridhar and Acosta (1993), the skin irritation potential of Octoxynol-9 and other surfactants was evaluated in vitro using primary rat keratinocyte cultures. Three-day-old confluent cultures were treated with the test substance and cytotoxicity was measured based on the following: (1) monitoring leakage of cytosolic enzyme LDH into the medium; (2) mitochondrial reduction of MTT; and (3) lysosomal uptake of the dye neutral red (2-amino-3 methyl-7-dimethyl-amino-phenazonium chloride) (NR). Measurements were made at the end of the 1 h treatment period and after 24 h.

Compared to controls, Octoxynol-9 caused less than a two-fold increase in LDH release during the 24-h period. The release of cytosolic enzyme LDH into the medium from control cultures was approximately 10% of the total LDH present in cells. An EC_{50} value was not calculated because the response to Octoxynol-9 treatment was below 50% of the maximal response. Changes in cell morphology were evaluated using light microscopy. The cytotoxicity potential of Octoxynol-9 was said to have been equal to that of the anionic surfactants that were tested. Damage to cells continued after the 1-h treatment period and removal of the test substance.

Octoxynol-9 caused a dose-related increase in cellular LDH leakage into the medium at concentrations of 10 to 100 μg/ml. Most of the enzyme leakage occurred during the 1-h treatment period. The results of MTT and NR assays were comparable to the LDH leakage results. The authors concluded that primary rat keratinocytes serve adequately as an in vitro model in the screening of surfactants for skin irritancy potential (Giridhar and Acosta 1993).

Skin Sensitization

Nonoxynols

Nethercott and Lawrence (1984) evaluated the skin sensitization potential of Nonoxynol-6 using the guinea pig maximization test (Magnusson and Kligman 1970). Four groups of five albino, guinea pigs of the Hartley-Dalkin strain (weights = 300–500 g) were tested with Nonoxynol-6 concentrations of 1.7, 3, 9, and 27 g % (*w*/*w*) in propylene glycol, respectively, during the induction phase. One animal in the 9% Nonoxynol-6 treatment group did not complete the study. On day 1 of induction, animals in each of the four groups received three pairs of injections (unshaved shoulder region) of the following chemicals: (1) 0.1 cc Nonoxynol-6, (2) 0.1 cc Nonoxynol-6 mixed (50:50 mixture) with Freund's complete adjuvant, and (3) 0.1 cc Freund's complete adjuvant. On day 7, each injection site was shaved and 100% Nonoxynol-6 was applied for 48 h under an occlusive patch secured with a bandage.

During the challenge phase, Nonoxynol-6 (2.7% in petrolatum) was applied via occlusive patches to shaved skin of the flanks on day 21. Each patch was secured with a bandage for 24 h, and sites were scored at 48 h. The test results from a pretest control group of 10 guinea pigs established a nonirritant concentration of 2.7% Nonoxynol-6 in petrolatum for use during the challenge phase.

A control group of 40 guinea pigs (20 exposed to deodorized kerosene and 20 exposed to tetraethylene glycol diacrylate during induction) was not exposed to Nonoxynol-6 during the induction phase, but was challenged with 2.7% Nonoxynol-6.

The incidence of challenge reactions in experimental groups was as follows: 1.7% Nonoxynol-6 induction group (2/5 guinea pigs), 3% group (0/5), 9% group (1/4), and 27% group (2/5). Five of the 40 control animals had challenge reactions to 2.7% Nonoxynol-6. The proportion of challenge reactions to 2.7% Nonoxynol-6 in experimental groups was not significantly different from that in the control group. It was concluded that Nonoxynol-6 did not induce sensitization in guinea pigs (Nethercott and Lawrence 1984).

Effect on Stratum Corneum

Octoxynol-9

Takahashi et al. (1987) evaluated the effect of Octoxynol-9 on intercellular adhesion using stratum corneum removed from the

backs of guinea pigs. Stratum corneum samples (10 × 10 mm) were immersed in 10 ml of Octoxynol-9 solution (0.1 M and 0.1%) and allowed to stand for 1 to 30 days without mechanical stimulation. The extent of stratum corneum decomposition was observed directly. The number of corneocytes dispersed in test solution was counted using a hemocytometer and phase-contrast microscopy (without staining).

There was no splitting of the stratum corneum into fragments; only rolling or curling was noted. Corneocytes were rarely observed. Differences in elasticity values between controls (distilled water treatment) and Octoxynol-9–treated stratum corneum were slight. It was noted that the intercellular region of the stratum corneum and the adhesion between corneocytes are of great interest because they are closely related to desquamation and disease states such as ichthyosis (Takahashi et al. 1987).

In a study by Shukuwa, Kligman, and Stoudemayer (1997), damage to the stratum corneum following exposure to 1% Octoxynol-9 in vitro was evaluated. Suction blisters were raised on the volar forearms of young adult males using a hand-held vacuum pump. Three blisters were obtained from each forearm. Blister roofs were removed and the under surface of each rubbed with a saline-moistened cotton swab in order to remove the viable epidermis. Discs of stratum corneum were agitated in a 1% solution of Octoxynol-9 in distilled water for up to 6 h. One-microliter samples were removed, placed on a glass slide, and stained. Morphologic changes in the corneocytes were evaluated using conventional microscopy.

Octoxynol-9 caused slight swelling, vacuolization, and moderate loss of staining intensity. Corneocytes released into distilled water had no discernible changes in size or shape and stained well with rhodamine (Shukuwa, Kligman, and Stoudemayer 1997).

Effect on Mucous Membranes

Octoxynol-9

Oberle, Moore, and Krummel (1995) studied the effect of Octoxynol-9 on the rat jejunum and colon in a single-pass, in situ perfusion model using the release of LDH and solubilized mucus into lumenal perfusate as potential markers of intestinal damage.

Enzyme leakage in this model, especially cytosolic LDH, has been proposed as a sensitive measure of minor damage or disruption of the cell membrane, and the authors stated that studies support the measurement of mucus secretion as an indicator of irritation.

Jejunal and colonic segments in male Sprague-Dawley rats were isolated and cannulated. The isolated jejunal or colonic segments of male rats were perfused with 1% Octoxynol-9, the surfactant polysorbate 80 (0.1–10.0% *w/v* in isotonic saline), and isotonic saline for 6 h in a single-pass, in situ perfusion model. Isolated jejunal or colonic segments of control animals were perfused with isotonic saline. The number of animals per treatment group ranged from four to nine.

At the end of the experiment, the length of the intestinal segment was determined after removal. Selected segments of unperfused jejunum and colon were frozen in saline for later analysis of total LDH release. The jejunum and colon were assessed using light microscopy and scanning electron microscopy.

The rate of release of LDH increased in the order of saline <1% polysorbate 80 < 1% Octoxynol-9 in both the jejunum and colon. The LDH release rate was approximately three times lower in the colon than in the jejunum. Compared to saline controls, the release rate of LDH in the jejunum increased twofold after perfusion with 1% polysorbate 80 and sevenfold after perfusion with 1% Octoxynol-9. The mucus release rate was greater in the presence of 1% polysorbate 80 or Octoxynol-9 than in the presence of saline. Mucus release rates for Octoxynol-9 and polysorbate 80 were similar. When perfusion with Octoxynol-9 was followed by saline perfusion, mucus and LDH release rates returned to baseline values, suggesting that damage was reversible.

The following morphological changes, described by the authors as moderate, were observed in the jejunum and colon following perfusion with 1% Octoxynol-9: denudation of villous tips, desquamation of the epithelial surface, necrosis of the mucosal lamina propria, and intervillous adhesion. These changes were observed to a minimal degree after perfusion with saline or 1% Polysorbate 80 (Oberle, Moore, and Krummel 1995).

Nonoxynols

Chvapil et al. (1980b) conducted a mucous membrane irritation test using 19 New Zealand white female rabbits (weights between 4 and 5 kg). A collagen sponge containing a known amount of Nonoxynol-9 (2.5, 5.0, 20.0, and 50.0 mg in aqueous solution) was inserted into the vagina of each animal. Each treatment group consisted of three or four rabbits, and, in each group, the sponges remained in place for ten days. A collagen sponge was inserted into the vagina of each of six control rabbits. The magnitude of vaginal irritation was evaluated.

Moderate inflammatory changes were observed in the vaginas of rabbits exposed to 2.5 mg Nonoxynol-9. The most striking finding was a pronounced infiltration of polymorphonuclear leucocytes on the inserted sponge. Minimal changes were observed in two of the six control rabbits. Increasing the amount of Nonoxynol-9 in the sponge resulted in more pronounced inflammatory changes. Increased cellular inflammatory infiltrate, more edema of the connective tissue of the submucosal layer, and denudation of the mucosal epithelium were observed. At the highest dose of Nonoxynol-9 tested (50 mg), no epithelial lining was observed, except in areas that were far removed from the medicated sponge (Chvapil et al. 1980b).

Tryphonas and Buttar (1982) administered aqueous Nonoxynol-9 (pH 2; single dose = 5 mg/100 g) intravaginally to groups of 9 to 10 female Wistar rats. Groups of five control rats received distilled water according to the same procedure. The animals were killed over a period of 6 weeks.

At 24 h post administration, primary mucosal damage, including epithelial degeneration, necrosis, and sloughing, was observed. Mucosal damage was complicated by a secondary acute inflammatory response that eventually involved the entire vaginal wall and the perivaginal tissues. Inflammation of the vaginal wall was time dependent, having increased in severity within 24 h. Areas of the vagina with minimal mucosal damage eventually returned to normal. However, areas with severe mucosal damage healed abnormally (Tryphonas and Buttar 1982).

When a contraceptive cream containing 5% Nonoxynol-9 was administered intravaginally (dose = 0.1 g/100 g body weight) to groups of three to eight Wistar rats, the lesions observed were not as severe as those induced by aqueous Nonoxynol-9 in the preceding study. However, acute cervicovaginitis was observed in some of the rats (Tryphonas and Buttar 1984).

Kaminsky et al. (1985) administered Nonoxynol-9 at concentrations of 2.5%, 5.0%, 12.5%, and 25.0% in 20 ml of water by vaginal lavage to four groups of six New Zealand white rabbits, respectively, once daily for 4 days. Distilled water was administered to a control group of six rabbits according to the same procedure.

Irritation of the vaginal mucosa was dependent on concentration. Concentrations of 2.5% and 5.0% induced mild irritation, whereas, 12.5% and 25.0% concentrations induced moderate to severe irritation. The lesions that were observed included epithelial exfoliation, submucosal edema, and inflammatory-cell infiltrate.

In additional experiments in this report, Nonoxynol-9 at concentrations of 5.0%, 12.5%, 25.0%, 50.0%, and 75.0% , in distilled water, was administered by vaginal lavage to five groups of seven Sprague-Dawley rats. Distilled water was administered to two groups of control rats.

Concentrations of 5.0% and 12.5% Nonoxynol-9 induced minimal irritation, and inflammatory-cell infiltrate was observed. Nonoxynol-9 (25.0%) induced mild irritation and epithelial exfoliation. Epithelial exfoliation was more severe and persistent in animals that received 50.0% and 75.0% concentrations, and edema was also noted in these two groups. The inflammatory-cell infiltrate became more severe and persistent only in the 75.0% Nonoxynol-9 treatment group (Kaminsky et al. 1985).

Immune System Effects

Octoxynol-9

In a study by Szymaniec, Zimecki, and Wieczorek (1980), the effect of Octoxynol-9 dosing on humoral and cell-mediated immune responses and the autoimmune response was evaluated using 129/Ao Boy strain mice (6 to 8 weeks old). The following parameters were studied: numbers of anti-sheep red blood cell plaque-forming cells (anti-SRBC PFCs) in the spleen (for humoral response), anti-SRBC delayed type hypersensitivity (DTH) (for cellular response), and anti-hemoglobin (Hb) PFCs producing antibodies against an autologous red blood cell antigen (for autoimmune response).

Octoxynol-9 was administered to mice (129/Ao Boy strain, 6 to 8 weeks old) in drinking water at a concentration of 0.125%. The mice drank the solution readily, and it was estimated that a mouse would drink approximately 2 mg of Octoxynol-9 within 24 h. Control mice received drinking water only.

For determination of the humoral response, two experimental procedures were used. In the first procedure, groups of mice drank the test solution throughout the duration of the experiment. After 4 weeks of dosing, the mice were immunized by intraperitoneal injection of 0.2 ml of 10% SRBCs in phosphate-buffered saline (PBS). The number of anti-SRBC PFCs in the spleen was determined after 4 days. Octoxynol-9 enhanced the production of anti-SRBC PFCs.

For determination of the cellular response, DTH was evaluated in mice that drank the test solution throughout the duration of the experiment. The mice were sensitized intravenously with 10^5 SRBCs in 0.1 ml PBS, and, after a 4-day period, the reaction (foot pad swelling) was elicited by intradermal introduction of 10^8 SRBCs into the left hind foot pad. Octoxynol-9 stimulated the cellular immune response to SRBCs.

The effect of short-term treatment of mice with Octoxynol-9 on the humoral anti-SRBC and anti-Hb antibody response and the cellular immune response was also evaluated. Octoxynol-9 (concentration not stated) was administered in drinking water for 1 week, after which the mice were immunized according the same procedures (for cellular and humoral response determinations) described in the preceding paragraphs. The magnitude of the immune response was determined after 4 days. Octoxynol-9 did not affect the development of anti-SRBC DTH.

The autoimmune response both in vivo and in vitro was determined using erythrocytes from heparinized syngeneic mouse blood. Peritoneal cells from control mice and mice treated with Octoxynol-9 (concentration not stated) were collected by washing the peritoneal cavity. The number of cells that produced antibodies against autologous red blood cell antigen was determined using the technique of local hemolysis in gel.

In the in vivo study, the effect of Octoxynol-9 on lymphocytes involved in the immune response was evaluated using the following two systems: (1) B lymphocytes from control mice in the presence of thymocytes or T lymphocytes from control mice or from mice treated with Octoxynol-9 and (2) B lymphocytes from mice treated with Octoxynol-9 in the presence of thymocytes or T lymphocytes from control mice or from the mice treated with Octoxynol-9. Octoxynol-9 caused significantly greater stimulation in system number 2 (i.e., greater number of anti-Hb PFCs in B cells isolated). In the in vitro experiment, Octoxynol-9 resulted in significant stimulation of the autoimmune response. Lymph node cells were not affected (Szymaniec, Zimecki, and Wieczorek 1980).

Caren and Brunmeier (1987) evaluated the immunotoxicity of Octoxynol-9 in a double-blind study using 10 outbred CF-1 female mice. The animals were injected intraperitoneally with 0.2 ml of Octoxynol-9 (concentration not stated) in sterile saline daily for 24 days. A control group of 10 mice was dosed with

saline according to the same procedure. A group of five mice served as the untreated control. On day 11, all mice were immunized subcutaneously with 0.05 ml of 5% SRBCs. Immunization (0.05 ml 10% SRBCs) was repeated on day 18. The mice were bled by caudal incision prior to treatment and on days 16 and 25. Values for the following were determined: hematocrits, leucocyte (white blood cell, WBC) counts, anti-SRBC titers, and serum immunoglobulin (Ig)M and IgG concentrations. At the end of the study, the animals were killed and organ-to-body weight ratios determined.

The animals injected with Octoxynol-9 remained healthy and active and did not experience weight loss. Furthermore, no changes in hematocrit, WBC counts, or anti-RBC responses were noted, and serum immunoglobulin patterns were the same as those noted in saline-treated controls. Serum IgG and IgM concentrations were similar in mice injected with Octoxynol-9 or saline. Compared to the untreated control group, IgM concentrations were significantly higher in the group injected with Octoxynol-9 and in the saline control group on day 16.

The authors stated that this observation could either reflect the stimulatory effect of daily injections or the possibility that saline and Octoxynol-9 were contaminated with a contaminant such as bacterial lipopolysaccharide. No changes in size were observed in the following organs: spleen, liver, kidneys, heart, lungs, or thymus. It was concluded that Octoxynol-9 had no significant effect on the immune or hematological system, and, thus, was nontoxic (Caren and Brunmeier 1987).

In a subchondral bone model system, Rodrigo et al. (1996) studied the inhibition of immune response by cytotoxic agents. Entire knee joints (with marrow and endosteal bone removed) were obtained from 31 Lewis rats. Knee joint complexes (11 specimens total) were placed onto a frame in order to provide continuous irrigation with a 15% Octoxynol-9 solution for 36 h. Control femurs (10 specimens total) were irrigated with Ringer's lactate solution. At the end of the irrigation period, the knee joints were removed and the left distal femur of each pair was transplanted into rats of a different strain (Brown Norway rats).

In order to determine the immune response, serum samples were obtained from the recipient Brown Norway rats preoperatively and 4 weeks postoperatively. Serum samples were assayed for the presence of cytotoxic antibodies against donor (Lewis) spleen cells using a fluorescein release lymphocytotoxicity assay. None of the recipient rats had an antibody response against donor antigens preoperatively.

Sixty percent (6 of 10) of the control rats had a positive antibody response at 4 weeks post transplantation. Of the 11 rats irrigated with 15% Octoxynol-9, 18% (2 of 11) developed an antibody response. Based on chi-square analysis, the immunogenicity of Octoxynol-9–irrigated grafts was significantly less ($p = .026$) than the irrigated controls. Irrigation with Octoxynol-9 for 36 h killed most metaphyseal cells, but cells within the epiphyses were viable. Bone from femurs treated with Octoxynol-9 did not grow cells in culture, but 50% (5 of 10) of the metaphyseal samples from control femurs grew bone cells in tissue culture after irrigation (Rodrigo et al. 1996).

Nonoxynols

Caren and Brunmeier (1987) studied the immunotoxicity of Nonoxynol-9 in a double-blind study, using outbred CF-1 female mice (weights = 26–33 g each). In the experimental group, 10 mice were injected intraperitoneally with 0.2 ml of 0.2% Nonoxynol-9 in sterile saline daily for 24 days, with the exception of days on which the animals were bled. Mice were bled by caudal incision before dosing and on days 16 and 25. On days 11 and 18, all of the mice were immunized subcutaneously with 0.05 ml of 5% SRBCs and 0.05 ml of 10% SRBCs, respectively. The 10 negative-control mice were injected with 0.2 ml of saline according to the same procedure, and another group of 5 mice received no treatment, but was immunized and bled. The animals were weighed prior to treatment and on days 3, 10, 17, and 28.

Significant weight loss was noted in experimental animals on days 10, 17, and 28 (day 28: $p < .02$; mean weight change = 1.9 g). In conjunction with the weight loss, the livers of mice dosed with 0.2% Nonoxynol-9 were somewhat reduced in size compared to saline-treated controls ($p < .05$; mean weight change = 0.0065 g). Spleens in the experimental animals were larger than those in the saline control group ($p < .05$; mean weight change = 0.001 g) or in the untreated control group ($p < .02$; mean weight change = 0.002 g). On day 16, hematocrits of the experimental mice were lower than those in the saline-treated control mice ($p < .05$; difference of 2); an increase in the hematocrits of untreated mice was noted between days 16 and 25 ($p < .01$; difference of 5).

However, even when considering these variations, all hematological values were within normal range. There were no significant differences between saline-treated and experimental groups with respect to the following: sizes of organs other than the liver or spleen, leucocyte counts, primary and secondary anti-SRBC titers, and serum IgM and IgG concentrations. It was concluded that Nonoxynol-9 induced only minor deleterious effects in mice, which included decreased body weight, reduction in liver size, and enlargement of the spleen (Caren and Brunmeier 1987).

Hemolytic Activity

Octoxynol-9

Duck-Chong (1983) demonstrated an effect of Octoxynol-9 on the alkaline denaturation of hemoglobin in maternal and fetal blood. The conversion of hemoglobin to alkaline hematin was determined by the increase in absorbance at 375 nm. The denaturation of hemoglobin by NaOH (20 mmol/L) was accompanied by a marked increase in absorbance between 340 and 390 nm.

In the presence of Octoxynol-9 (0.3 g/L), the maximum change in absorbance occurred at 377 nm. Hemoglobin (fetal and maternal) was denatured rapidly in the presence of Octoxynol-9, compared to the results for NaOH alone. In the absence of

Octoxynol-9, the maximum change in absorbance for maternal and cord blood occurred at 372 nm. The overall result was the same, whether Octoxynol-9 was added before the alkali or 1 min later (Duck-Chong 1983).

Sugiyama et al. (1985) incubated samples of dog erythrocytes in isotonic saline containing 0.008%, 0.010%, and 0.012% Octoxynol. The degree of hemolysis was 31.6% ± 3.9% (mean ± *SD*) in 0.008% Octoxynol-9, 84.6% ± 5.6% in 0.010% Octoxynol-9, and 90.1% ± 8.9% in 0.012% Octoxynol-9.

Bielawski (1990) reported that Octoxynol-9 (concentrations of ~ 0.003% to 0.008%) induced swelling, followed by hemolysis, of erythrocytes suspended in 160 mM KCl.

Rodeghiero et al. (1990) reported that incubation of a human platelet suspension with 1/40 *v/v* of 20% Octoxynol-9 in distilled water for 1 h also resulted in lysis.

Duncan et al. (1994) classified Octoxynol-9 as highly lytic to rat red blood cells at pH 5.5, 7.4, and 8.0. It caused 100% hemoglobin release at a concentration of approximately 100 μg/ml. Data (mean values) were expressed as hemoglobin release as a percentage of the control. The IC_{50} for Octoxynol-9 was 1 μg/ml.

Chernitsky and Senkovich (1997) evaluated the hemolytic activity of Octoxynol-9 in vitro using donor erythrocyte suspensions (hematocrit = 0.062% to isotonic NaCl solution, 20°C). Hemolytic activity increased over the range of concentrations tested (100 to 200 μM).

Other Octoxynols

Trägner and Csordas (1987) reported that Octoxynol-8, -9, and -13 interacted with human erythrocyte membranes in vitro in a biphasic manner. At low concentrations (0.0001% to 0.01% *v/v*), they stabilized the erythrocytes against hypoosmotic hemolysis. At the upper limit of this concentration range, these Octoxynols became hemolytic. Conversely, Octoxynol-5 did not exhibit this biphasic behavior, but protected against osmotic rupture up to saturating concentrations. Octoxynol-5 did not induce hemolysis, even at a concentration as high as 1%. Thus, a critical chain length of octylphenoxy polyethylene ethers (Octoxynols) is required for the hemolytic effect.

Nonoxynols

Freisleben et al. (1989) evaluated the hemolytic activity of Nonoxynol-9 using blood samples from rabbits. Nonoxynol-9 was tested at concentrations ranging from 0.006% to 0.1% in saline. Each cell suspension-test material mixture was incubated at 37°C for 15 min, centrifuged, and then observed for hemolytic activity. Complete hemolysis was defined as the absence of cell sedimentation. The control solution, for detection of spontaneous hemolysis, consisted of 1 ml of the diluted rabbit blood in 1 ml of saline.

Nonoxynol-9 caused complete hemolysis at concentrations of 0.006% to 0.12% (Dolan 1981). In a more recent study, it was concluded that Nonoxynol-9 destabilizes the erythrocyte cell membrane. In the range of 0.2 to 2.0 mg of membrane lyophylisate per milliliter of suspension, Nonoxynol-9 was incorporated into the erythrocyte membrane at a ratio of 1 mol per 40 mol of phospholipids. Additionally, Nonoxynol-9 reduced phase transition breaks of the membrane, particularly in the temperature range of 16°C to 20°C (Freisleben et al. 1989).

Cytotoxicity

Octoxynol-9

Schappert and Khachatourians (1984) determined the effect of Octoxynol-9 on growth reduction caused by T-2 toxin in the yeast *Saccharomyces carlsbergensis*. T-2 toxin is a mycotoxin that inhibits cell growth. At concentrations below 1% (*v/v*), Octoxynol-9 sensitized the yeast cells to T-2 toxin. However, the cells were protected from T-2 toxin at Octoxynol-9 concentrations greater than 1% (*v/v*). Octoxynol-9 concentrations greater than 5% (*v/v*) were toxic to yeast. The growth that occurred in the presence of T-2 toxin served as the measurement of toxicity.

Buttar, Swierenga, and Matula (1986) evaluated the cytotoxicity of Octoxynol-9 using a nontumorigenic, rat liver cell line (T51B cells). The cells were treated with concentrations of Octoxynol-9 up to 100 μg/ml for 24 h. Colony formation was estimated 7 days after plating. The mean effective concentration that was required to reduce the number of viable cells by 50% (LC_{50}) was 43 μg/ml.

Grando et al. (1993) studied the cytotoxicity of Octoxynol-9 using epidermal keratinocyte (EK) cell lines, EKL-4 and EKL-11, that were obtained from normal human neonatal foreskins. Cultures were grown for 72 h and then exposed to Octoxynol-9 at concentrations ranging from 0.001% to 1.0%. The number of cells per well, assessed after initial proliferation of EK inoculated into the microplate wells (pretreatment control), was conditionally termed cell count after initial proliferation (CCIP).

Exposure to the test concentrations for 30 min significantly diminished the number of cells, compared to their CCIP values ($p < .05$). The percentage of cells killed by each concentration of Octoxynol-9 was similar in both cell lines ($p > .05$). In order for 0.1% and 1% *v/v* Octoxynol-9 solutions to kill more than 90% of the epidermal keratinocytes, incubation for 30 min was required (Grando et al. 1993).

Borner et al. (1994) reported that Octoxynol-9 induced a pattern of death in human carcinoma cell lines (PC-3, SW-620, and HT-29) that resembled cytotoxic lymphocyte-induced apoptosis. Treatment of cell cultures with Octoxynol-9 at a concentration of 0.01% (*w/v*) resulted in death of 100% of the cells. A mixture of typical apoptotic (10% to 15% of total cells) and necrotic cells was observed using transmission electron microscopy. Apoptotic features, such as condensation of chromatin or cytoplasm, were not noted in control cells. Additionally, 0.01% Octoxynol-9 induced internucleosomal DNA fragmentation that was typical of apoptosis within 1 h of treatment. The percent of total DNA that was fragmented after 1 h was 21% ± 1.6%.

Nagoshi et al. (1994) reported that cell death was significantly enhanced in hepatocyte cultures, compared to controls,

dosed with Octoxynol-9 (0.5 μl). Cells were obtained from male Sprague-Dawley rats. The percent of dead cells was 3.6% $\pm$ 0.1% in untreated cultures and 29.0% $\pm$ 0.8% in cultures dosed with Octoxynol-9.

In a study by Carson (1996), immunofluorescent and phase contrast microscopy were used to evaluate the effects of Octoxynol-9 on cell morphology and tissue factor (an integral membrane protein) distribution on the cell surface of cultured fibroblasts. The cells were from the human fibroblast cell lines GM05659 and GM05758.

Nonlytic concentrations of the test substance resulted in the formation and release of membrane blebs and vesicles. Specifically, 0.01% Octoxynol-9 caused the release of vesicles from the cells into the buffer. The vesicles did not contain any detectable tissue factor antigen. Antigen annulus, or collar, was noted in the remainder of the plasma membrane. The formation of vesicles and the antigen annulus at the vesicle-cell interface was also noted in cells treated with 0.025% Octoxynol-9. It was noted that nonlytic concentrations of Octoxynol-9 increasingly solubilized cell phospholipids, with no apparent effect on tissue factor activity. The formation of vesicles and blebs was not observed after treatment with 0.1% Octoxynol-9, which dissolved the cell membrane (Carson, Kuszynski, and Pirruccello 1996). Another study by one of the authors indicated that the manifestation of tissue factor activity coincided with breakdown of the plasma membrane (Carson 1996).

Ahn et al. (1997) reported that DNA ladder formation, considered a hallmark of apoptosis, was noted in DNA extracted from human hepatoma cell lines treated with 0.01% Octoxynol-9. The induction of apoptosis was assessed by DNA integrity analysis with agarose gel electrophoresis. DNA fragmentation was observed within an hour of treatment. Apoptotic bodies and chromatin condensation were observed using hematoxylin and eosin stain.

Apoptosis was induced in more than 90% of the cells that had been treated with 0.01% Octoxynol-9 for 150 min. Fragmented nucleosome was detected using the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) test, thereby confirming Octoxynol-9–induced fragmentation of DNA. Microscopically, scattered tumor cells had strong, brown positive staining in their nuclei and some of them had fragmented nuclei. Results strongly suggest that Octoxynol-9 induces apoptosis in human hepatoma cell lines (Ahn et al. 1997).

Nonoxynols

Buttar, Swierenga, and Matula (1986) evaluated the cytotoxicity of Nonoxynol-9 using rat liver cells (T51B cells) from a nontumorigenic cell line. T51B cells were plated at a density of 3.2×10^3 per cm^2, maintained for 24 h in complete medium, and then treated with various concentrations of Nonoxynol-9 for an additional 24 h. At the end of the 24-h period, the cells from each treated culture were replated at a density of 80 cells (viable and nonviable) per cm^2. Colony formation of survivors was estimated at 7 days after plating. Nonoxynol-9 was cytotoxic to T51B rat liver cells at concentrations of >10 μg/ml to 50 μg/ml; the degree of cytotoxicity was concentration dependent. These results are based upon the dose-response curve on Nonoxynol-9 cytotoxicity that was generated.

Neurotoxicity

Octoxynol-9

In a study by Fox, Epstein, and Bass (1983), the neurotoxicity of Octoxynol-9 was evaluated using rat (male rats) jejunal segments in vivo. A portion of the jejunum was moved outside of the peritoneal cavity, and various concentrations of Octoxynol-9 were applied to a 2- to 3-cm segment of the serosal surface. Octoxynol-9 (1% in saline) was applied every 5 min for 30 min (six applications). Saline (0.9%) was applied as a nondrug treatment according to a similar procedure. Tissue samples of treated and untreated (control) portions of the gut were evaluated 20 days after application. At the end of the application period, the serosa of the bowel was thoroughly rinsed with 0.9% saline and then returned to the peritoneal cavity.

Octoxynol-9 (1%) caused significant reduction in the number of ganglion cells in the myenteric plexus. In the myenteric plexus, the mean number of ganglion cells/mm jejunum was 0.47, compared to 4.04 for the control (untreated jejunum) (Fox, Epstein, and Bass 1983).

Effect on Cardiac Tissue

Octoxynol-9

Lee et al. (1994) reported the in vitro effect of Octoxynol-9 on the electromechanical activity of human endocardial endothelium and on twitch force and action potentials in guinea pig cardiac tissues. Human atrial tissues were obtained from the hearts of nine patients during corrective cardiac surgery. Ventricular tissues were excised from five patients undergoing cardiac transplantation. Additionally, sinoatrial tissues and ventricular papillary muscles were obtained from 10 guinea pigs. After perfusion in a tissue bath, transmembrane potentials for strands of atrial or ventricular muscle fibers were recorded.

The treatment of a guinea pig sinoatrial preparation with 1% Octoxynol-9 (20 μl) immediately caused a decrease in twitch force that was 25% less than the control value. A steady-state value for twitch force, 10% less than the control value, was achieved at 10 min. The results for five experiments indicated that the twitch force was moderately decreased (29% $\pm$ 7.6%, $p < .05$), but that the spontaneous cycle length was not affected. Similar results were reported for a ventricular papillary muscle preparation (five guinea pigs) treated with 1% Octoxynol-9 (up to 100 μl).

Compared to the guinea pig, human atrial tissues (five atrial preparations) were much more sensitive to Octoxynol-9 treatment. Octoxynol-9 (1%; volume = 20 μl) caused a progressive decrease in the amplitude of phase-0 depolarization, the action potential plateau, and the twitch force of human atrial trabeculae.

The action potential changed from a fast response to a slow response within 8 min. At a concentration of 0.25% Octoxynol-9 (five atrial preparations), depression of the upstroke of the slow response and a marked decrease in twitch force were observed.

In human ventricular tissue (which was more sensitive than guinea pig tissue), 1% Octoxynol-9 briefly suppressed the fast response action potential and decreased the twitch force by one-half after the tissue resumed excitability. When human ventricular tissues were exposed to a lower concentration of Octoxynol-9 (0.25%) in four experiments, the decrease in twitch force was smaller (compared to 1% Octoxynol-9), but statistically significant.

The preceding results for human atrial and ventricular tissues indicated that brief exposure to Octoxynol-9 (0.25 to 1 vol %) caused endocardial damage and depressed the excitability of fast and slow response action potentials (Lee et al. 1994).

Vascular Effects

Octoxynol-9

Verrecchia et al. (1986) reported that tests performed on rat pial arteries perfused with 0.1% Octoxynol-9 indicated that maximal dilator responses to intraluminal and extraluminal acetylcholine were significantly reduced. Based on scanning electron microscopic results, the endothelial layer of perfused arteries was partially stripped off. Only minor damage to the internal elastic lamina was noted.

GENOTOXICITY

Bacterial Cell Assays

Octoxynol-9

Zeiger and Pagano (1984) studied the effect of known mutagens in combination with Octoxynol-9 using *Salmonella typhimurium* strain TA100. Doses of the following mutagens that would produce 500 to 1000 revertants per plate were added to the top agar: sodium azide (NaN_3) in water (0.5 μg/plate); *N*-aminomorpholine (AM) in water (5.2 μmol/plate); ethyl methanesulfonate (EMS) in DMSO (42.3 μmol/plate); benzo(a)pyrene (BaP) in DMSO (3 μg/plate, with metabolic activation); 2-aminoanthracene (2-AA) in DMSO (2 μg/plate); and styrene oxide (SO) in DMSO (4.0 μmol/plate).

After the agar hardened, Octoxynol-9 was applied either directly, as crystals, or as a liquid to sterile, filter paper discs. After incubation for 48 h, the plates were examined for zones of revertant colony inhibition that were found outside of the toxic zones (if present). Octoxynol-9 caused toxicity (background lawn appeared less dense compared to control plates) in the presence of NaN_3, SO, or AM. There was no effect on the mutagenicity of EMS, BaP, or 2-AA (Zeiger and Pagano 1984).

Other Octoxynols

The Procter & Gamble Company (1979) evaluated the mutagenicity of Octoxynol-1 using *S. typhimurium* strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538 (with and without metabolic activation). Test concentrations ranged from 0.0031 to 0.1 μl/plate with activation, and 0.0063 to 0.1 μl/plate without activation. EMS, 9-aminoacridine, and 2-nitrofluorene served as positive controls with and without metabolic activation. The positive controls for tests with and without metabolic activation were mutagenic, but not Octoxynol-1.

Mammalian Cell Assays

Octoxynol-9

Buttar, Swierenga, and Matula (1986) performed mutation and transformation assays using T51B rat hepatocyte cells from a nontumorigenic line. The cells were maintained in complete medium and then treated with Octoxynol-9 (concentrations up to 40 μg/ml) for 24 h. In one set of experiments, the cells were exposed to Octoxynol-9 for 11 days, washed, and then maintained in fresh medium until they became confluent. Cells were subsequently replated into selective media containing 8-azaguanine to determine HGPRT (hypoxanthine guanine phosphoribosyl transferase) mutants or into low-calcium medium to determine transformation frequency.

Octoxynol-9 was not mutagenic (no HGPRT mutants) at concentrations up to 40 μg/ml. In the low-calcium assay (test concentration = Octoxynol-9 at 50 μg/ml), no malignant transformation response was observed (Buttar, Swierenga, and Matula 1986).

Matsuoka, Sofuni, and Ishidate (1986) measured the induction of chromosomal aberrations by clastogens in combination with Octoxynol-9 in Chinese hamster ovarian cells. Cells were treated with the following three carcinogens (with metabolic activation): dimethylnitrosamine (DMN), BaP, or aniline. The induction of chromosomal aberrations was enhanced remarkably after the addition of Octoxynol-9, although Octoxynol-9 itself was not found to be clastogenic. Another assay indicated that Octoxynol-9 enhanced the enzyme activity of the S9 fraction that was used. It was concluded that the enhancement of chromosomal aberrations was due, in part, to the modification of metabolic activity that was induced by Octoxynol-9.

Wangenheim and Bolcsfoldi (1988) reported that Octoxynol-9 did not produce significant mutagenic activity in another mouse lymphoma thymidine kinase (TK) locus forward mutation assay using heterozygous L5178Y $TK^{+/-}$ 3.7.2.C cells. Octoxynol-9 was tested at concentrations ranging from 1 to 45 μg/L.

DNA Assays

Octoxynol-9

Carlo, Martelli, and Bignone (1981) demonstrated that Octoxynol-9 (0.75% *v/v*) could preserve the integrity of the DNA in a procedure (two successive Octoxynol-9 treatments) to remove cytoplasmic contamination from a rat liver cell suspension. Three successive treatments resulted in DNA breakage and a further decrease in RNA and protein content. In this analysis, DNA integrity was estimated by using an oscillating crucible viscometer to determine the viscosity of DNA.

Erenpreisa and Zaleskaya (1983) studied the effect of Octoxynol-9 on chromatin in liver, thymus, and ascites hepatoma cells from rats. Intact Zajdela hepatoma cells were treated with 0.05% Octoxynol-9. Smears of normal rat liver and thymus were treated similarly. Isolated rat thymus nuclei were treated with 0.5% to 1% Octoxynol-9. Ascites hepatoma cells washed in cold buffered saline were treated with Octoxynol-9, monitored by phase contrast microscopy, and smeared on slides for DNA histochemistry. Cells were also analyzed uisng DNA histochemistry and electron microscopy.

Phase-contrast microscopy of Octoxynol-9–treated hepatoma cells revealed a distinct and rough nuclear structure, compared to control cells. DNA histochemistry results indicated nuclei of coarse structure and enlarged roundish nucleoli. Identical changes were observed in smears of liver and thymus cell nuclei. In ultrathin sections, some compact of chromatin and its margination were observed in hepatoma cells that had been treated with Octoxynol-9. Octoxynol-9 did not cause any change in the average DNA content of hepatoma cells.

Electron microscopy results on rat thymus nuclei treated with Octoxynol-9 revealed chromatin fibers that were settled tighter and more orderly than those of control specimens. The authors noted that the change in the chromatin fibers may have resulted from a decrease in surface tension that was caused by Octoxynol-9 (Erenpreisa and Zaleskaya 1983).

In a DNA repair assay, Buttar, Swierenga, and Matula (1986) evaluated unscheduled DNA synthesis using an adult rat hepatocyte, nontumorigenic cell line (T51B) that had been exposed to Octoxynol-9 and 5 μCi/ml [^{3}H]thymidine (specific activity = 25 Ci/mmol) for 18 h and subjected to autoradiography. Octoxynol-9 was tested at concentrations of 10, 25, and 50 μg/ml. Methyl methane sulfonate (MMS) and saline served as positive and negative controls, respectively. DNA repair was expressed as grains over the nucleus minus grains over a similar-sized area in the cytoplasm. Results (net grains per nucleus) were as follows: highest concentration of Octoxynol-9, 50 μg/ml (1.42 $\pm$ 2.06), saline (1.7 $\pm$ 2.1), and MMS (21.5 $\pm$ 7.8). Octoxynol-9 did not induce DNA damage.

Garberg, Akerblom, and Bolcsfoldi (1988) studied the genotoxicity of Octoxynol-9 in a DNA alkaline unwinding test (without metabolic activation) using mouse lymphoma L5178Y/TK$^{+/-}$ cells. The results of this test were compared with those of the mouse lymphoma TK locus forward mutation assay, without metabolic activation. The DNA alkaline unwinding assay was based on measurement of the proportion of single- to double-stranded DNA (ssDNA) by alkaline unwinding and hydroxyapatite elution (using chromatography) in cells treated with the following concentrations of Octoxynol-9: 3.0, 10.0, 25.0, 30.0, and 100.0 μl/L. The two techniques (alkaline unwinding and hydroxapatite chromatography) were used to detect DNA-strand breaks, which are indicative of DNA damage. Results were expressed as the difference between the viability of treated and control cultures and between the fraction of DNA found to be single-stranded in control and treated cultures. By expressing these results as percentages, a direct numerical comparison was made between the increase in toxicity and the increase in ssDNA. This provided a measurement of the DNA-damaging affinity of Octoxynol-9. A 6.5% increase in the relative fraction of ssDNA at a relative toxicity of $<$5% was considered positive.

The results for Octoxynol-9 (without metabolic activation) were negative in the DNA alkaline unwinding test and in the mouse lymphoma TK locus forward mutation assay (Garberg, Akerblom, and Bolcsfoldi 1988).

Vock et al. (1998) reported that the induction of DNA double-strand breaks by 5% Octoxynol in cultured human lung epithelial cells (A549) was observed only after cell viability was reduced to less than $\sim$60%. These results indicated that DNA double-strand breaks resulted from extragenomic damage.

Nonoxynols

In a study by Long, Warren, and Little (1982), the effect of Nonoxynol-9 on malignant transformation was evaluated in an in vitro transformation assay involving mouse BALB/3T3 fibroblasts and mouse 10T1/2 fibroblasts. For each experimental group, data were pooled from three experiments.

When BALB/3T3 cells were treated with 0.0001% or 0.001% Nonoxynol-9 (final concentrations in cell medium) for 11 days or with 0.00001% Nonoxynol-9 for 3 weeks, a significant number of transformed foci was induced. The amount of transformation was not significantly elevated over background in cultures treated with 0.00001% Nonoxynol-9 when treatment was discontinued at 11 days.

When 0.00001% Nonoxynol-9 was added to mouse 10T1/2 fibroblast cultures once per week for 5 weeks, the number of transformed foci was significantly enhanced over background. However, the incubation of these cultures with 0.001% Nonoxynol-9 for 48 h produced minimal toxicity and no significant increase in transformation.

The authors concluded that the results of this study indicate that Nonoxynol-9 can induce transformation in two mouse cell transformation systems, and that this induction was dependent on dose as well as duration of exposure.

These authors also evaluated the promotional effects of Nonoxynol-9 using mouse 10T1/2 fibroblast cultures. After a single x-ray exposure (100 rad) the cells were incubated with 0.00001% Nonoxynol-9 for 5 weeks and 0.001% Nonoxynol-9 for 48 h, respectively. Cultures were also exposed to x-rays (100 rad) only, and to x-rays (100 rad) plus 0.1 μg/ml 12-0-tetradecanoylphorbol-13-acetate TPA and incubated for 5 weeks. Untreated cultures served as negative controls. In each experimental group, data were pooled from two separate experiments.

For cultures exposed to x-rays and incubated with either 0.00001% or 0.001% Nonoxynol, the transformation response was no greater than the added responses of cells exposed to x-rays only plus those exposed to either concentration of Nonoxynol-9. The results of a statistical analysis of the data indicated p values of $<.05$ and $>.09$ for irradiated cultures treated with 0.00001% and 0.001% Nonoxynol-9, respectively. For cultures exposed to x-rays alone and x-rays plus TPA, the

p values were >.7 and <.01, respectively (Long, Warren, and Little 1982).

Buttar, Swierenga, and Matula (1986) measured unscheduled DNA synthesis in freshly isolated adult rat hepatocytes treated with Nonoxynol-9. The cells were exposed to test concentrations of 5, 10, and 25 μg/ml Nonoxynol-9, respectively, along with 5 μCi/ml [^{3}H]thymidine (specific activity 25 Ci/mmol) for 18 h, and processed for autoradiography. Grains were counted, and repair was expressed as grains over the nucleus minus grains over a similar-sized area in the cytoplasm. Nonoxynol-9 did not induce unscheduled DNA synthesis at any of the test concentrations. MMS (positive control) induced unscheduled DNA synthesis and negative results were reported for the saline negative control.

These authors also evaluated the effect of Nonoxynol-9 on rat liver cells (T51B cells) from a nontumorigenic cell line. T51B cells were plated at a density of 6.7×10^3 per cm^2, maintained for 24 h in complete medium, and then treated with 5, 10, 15, and 25 μg/ml Nonoxynol-9, respectively, for an additional 24 h. In one set of experiments, the cells were exposed to Nonoxynol-9 for 11 days, with regular medium changes. After exposure, the cells were washed twice with PBS and maintained in fresh medium until the cells became confluent.

Cells were plated in appropriate media to determine HGPRT mutants and transformation frequency. Nonoxynol-9 was not mutagenic, nor did it induce malignant transformations. HGPRT mutants were induced in the positive-control 7,12-dimethylbenzathracene (DMBA) culture. Neither HGPRT mutants nor malignant transformations were observed in negative control cultures (Buttar, Swierenga, and Matula 1986).

Meyer et al. (1988) evaluated the mutagenicity of Nonoxynol-9 in the Ames test. *S. typhimurium* strains TA1535, TA1537, TA100, and TA98 were tested with Nonoxynol-9 (in sterile water) concentrations of 40, 200, 1000, 5000, and 25000 μg/plate both with and without metabolic activation. Negative control cultures were exposed to sterile water. In tests without metabolic activation, sodium azide was the positive control for strains TA1535 and TA100 and 2-nitrofluorene was the positive control for strains TA1537 and TA98. In metabolic activation tests, 2-anthramine was the positive control for all strains.

Without metabolic activation, Nonoxynol-9 was not mutagenic. With metabolic activation, the number of revertants was elevated 30% in strain TA98 cultures exposed to Nonoxynol-9 at a concentration of 1000 μg/plate. This was not considered a clear-cut mutagenic response, because the increase in the number of revertants was considerably less than 100%. Mutagenic effects also were not noted in any of the remaining metabolically activated cultures. It was concluded that Nonoxynol-9 was not mutagenic in the Ames test, either with or without metabolic activation (Meyer et al. 1988).

Sheu et al. (1988) studied the induction of malignant transformation in vitro by Nonoxynol-9 (in distilled water) in another study using BALB/3T3 cells. Nonoxynol-9 was tested at concentrations ranging from 0.08 to 10 μg/ml. In each assay, 20 cultures per test concentration were incubated for 48 h. Distilled water and 3-methylcholanthrene served as solvent and positive controls respectively. 1,4-Dioxane, a known carcinogen, was tested at concentrations ranging from 0.25 to 4 mg/ml according to the same test procedure.

Of the 20 cultures examined per test concentration, the number of type III foci ranged from 0 to 3 in the solvent control, 0 to 2 in Nonoxynol-treated cultures, and 1 to 44 in cultures treated with 1,4-dioxane. A positive response to 3-methylcholanthrene was observed in all assays. BALB/3T3 cell cultures were also exposed to the same test and control compounds for 13 days. Of the 20 cultures examined per test concentration, the numbers of type III foci were as follows: 5 and 7 (solvent control), 0 to 4 (Nonoxynol-treated cultures), and 7 to 42 (dioxane-treated cultures). There were 19 and 45 foci per 20-positive-control cultures.

Similar results for Nonoxynol-9 were reported when this test was repeated. The results of 48-h and 13-day exposures indicated that the responses to Nonoxynol-9 in BALB/3T3 cells were comparable to those observed in solvent control cultures. However, 1,4-dioxane was effective in the induction of morphological transformation in BALB/3T3 cells (Sheu et al. 1988).

Table 6 summarizes the genotoxicity studies on Octoxynol-9.

CARCINOGENICITY

Studies on the carcinogenicity of Octoxynols were not identified in the published literature.

Nonoxynols

Malyk (1984) conducted a lifetime exposure study in rats to evaluate the carcinogenicity of Nonoxynol-9. The animals (no details on the animals were provided) were dosed intravaginally with 6.7 mg/kg and 33.6 mg/kg Nonoxynol-9 three times per week for a total of 24 months. The low and high doses represented approximately 4 times and 20 times the clinical dose, respectively. Two groups of rats served as sham and untreated controls, respectively.

No significant differences were observed between the experimental and control groups. This was true for all of the measured parameters, which included palpable masses and mortality, but not histopathologic examination of tissues. Any positive findings observed in experimental groups at necropsy were considered related to changes associated with the process of aging and not related to test substance administration. The author concluded that Nonoxynol-9 was neither toxic nor carcinogenic in this lifetime exposure study, even at a dose that was 20 times that recommended for humans (Malyk 1984).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Dermal Application

Octoxynol-9

Leung and Ballantyne (1999) conducted a developmental toxicity study of Octoxynol-9 using four groups of 25 outbred,

TABLE 6
Genotoxicity of Octoxnol-9

Test system	Protocol and dose	Results	Reference
	Bacterial cell assays		
Salmonella typhimurium strain TA100	Ames spot test to evaluate effect of Octoxynol-9 (crystals or liquid) on mutagenicity of:		Zeiger and Pagano 1984
	styrene oxide (4.0 μmoles/plate)	Background lawn on Octoxynol-9 treated plates appeared less dense compared to control plates	
	sodium azide (0.5 μg/plate)	Background lawn on Octoxynol-9 treated plates appeared less dense compared to control plates	
	N-aminomorpholine (5.2 μmoles/plate)	Background lawn on Octoxynol-9 treated plates appeared less dense compared to control plates	
	ethylmethanesulfonate (42.3 μmoles/plate)	No effect of Octoxynol-9	
	benzo(a)pyrene (3 μg/plate)	No effect of Octoxynol-9	
	2-aminoanthracene (2 μg/plate)	No effect of Octoxynol-9	
	Mammalian cell assays		
Chinese hamster cells	Chromosomal aberrations assay (with metabolic activation) of Octoxynol-9 clastogenicity with dimethylnitrosamine, benzo[a]pyrene, and aniline	Octoxynol-9 enhanced the induction of chromosomal aberrations by these known carcinogens, but was not clastogenic alone	Matsuoka, Sofuni, and Ishidate 1986
Rat hepatocytes (T51B cells from nontumorigenic cell line)	Unscheduled DNA synthesis assay. Octoxynol-9 test concentrations up to 50 μg/ml	No DNA damage with Octoxynol-9	Buttar, Swierenga, and Matula 1986
T51B cells	Octoxynol-9 test concentrations up to 40 μg/ml in hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutation assay and 50 μg/ml in malignant transformation assay	Octoxynol-9 not mutagenic and no malignant transformations	Buttar, Swierenga, and Matula 1986
Mouse lymphoma L5178Y/TK$^{+/-}$ cells	Mouse lymphoma thymidine kinase (TK) locus forward mutation assay without metabolic activation; Octoxynol-9 test concentrations up to 100 μl/L	Octoxynol-9 not mutagenic	Garberg, Akerblom, and Bolcsfoldi 1988
Mouse lymphoma L5178Y TK$^{+/-}$ 3.7.2.C cells	Mouse lymphoma TK locus forward mutation assay; Octoxynol-9 test concentrations up to 45 μg/L	No significant mutagenic activity with Octoxynol-9	Wangenheim and Bolcsfoldi 1988

(Continued on next page)

TABLE 6
Genotoxicity of Octoxnol-9 *(Continued)*

Test system	Protocol and dose	Results	Reference
	DNA assays		
Rat liver cells	Octoxynol-9 (0.75% *v*/*v*) used to treat cell suspension during DNA isolation	Two treatments did not damage DNA, but three caused DNA breakage	Carlo, Martelli, and Bignone 1981
Rat thymus, ascites hepatoma, and normal liver cells	Cell smears treated with 0.05% Octoxynol-9; isolated thymus cell nuclei treated with 0.5% to 1% Octoxynol-9	Octoxynol-9 treated cells had rough nuclear structure compared to controls; some compaction of chromatin seen, but no change in DNA content/cell	Erenpreisa and Zaleskaya 1983
Rat hepatocyte	DNA damage as measured by unscheduled DNA synthesis in cells treated with Octoxynol-9 compared to positive control—methyl methane sulfonate	Octoxynol-9 did not induce DNA damage	Buttar, Swierenga, and Matula 1986
Mouse lymphoma L5178Y/TK$^{+/-}$ cells	DNA ss versus ds after alkaline unwinding; DNA from cells treated with Octoxynol-9 at 5 concentrations from 3 to 100 μg/L	No increase in ss DNA	Garberg, Akerblom, and Bolcsfoldi 1988
Human lung epithelial cells (A549)	DNA double-strand breaks assay. 5% Octoxynol-9 tested	Double-strand breaks induced only after cell viability reduced to <60%. Positive results due to extragenomic damage	Vock et al. 1998

Sprague-Dawley CD rats (10 to 11 weeks old at time of mating). Three groups received dermal applications of 12.5% (dose = 530 mg/kg/day), 37.5% (1600 mg/kg/day), and 100.0% (4270 mg/kg/day), respectively, each at a constant dose volume of 4 ml/kg on GDs 6 through 15. The morning at which successful mating was observed was designated as GD 0.

Each test solution was applied directly to skin in the interscapular area (20 cm^2, clipped free of hair) using a disposable syringe, after which the test site was occluded with a gauze square and polyethylene film attached to a specially designed Lycra-Spandex jacket with Velcro closures. The jacket and gauze were removed at approximately 6 h after dosing and the test site was blotted dry. Deionized and filtered water (dose volume = 4 ml/kg) was applied to the control group according to the same test procedure. The dams were killed by carbon dioxide asphyxiation on GD 21, and maternal lung, liver, kidney, and uterine weights measured at necropsy.

No dams aborted or delivered early. Fetal mortalities and resorption sites were recorded, and fetuses were examined for variations and malformations. Study results are included below. Results relating to maternal deaths (gross findings included) and skin irritation potential are included in the sections on Short-term Dermal Toxicity and Skin Irritation, respectively, earlier in this report.

Compared to controls, no effects on gravid uterine weight were noted in either of the three dose groups. Dosing with Octoxynol-9 also had no effect on the number of ovarian corpora lutea, the number of total, viable, or nonviable implantations per litter, or preimplantation loss. No statistically significant difference in the incidence of any individual external variation was observed between test and control groups. However, the incidence of atelectasis was significantly increased ($p < .05$) in two of the three dose groups (1600 and 4270 mg/kg/day), and a significant decrease in the incidence of dilated renal pelvis was reported for the 530-mg/kg/day dose group. Concerning skeletal variations, an increased incidence of vestigial 14th thoracic rib ($p < .01$) was noted in all three dose groups (79% to 100% of the litters). Concurrent and historical control incidences of thoracic extra ribs were 30% and 0% to 22%, respectively.

The remaining statistically significant skeletal variations, observed only in the highest dose group, were poorly ossified lumbar arches ($p < .01$), unossified sternebra 6 ($p < .05$), poorly ossified sternebra 6 ($p < .01$), unossified cervical centrum 5 ($p < .05$), unossified cervical centrum 6 ($p < .05$),

rudimentary bone island (cervical arch 7) ($p < .01$), poorly ossified hyoid ($p < .01$), poorly ossified zygomatic arch ($p < .01$), and poorly ossified supraoccipital ($p < .05$). Although no malformations by category (external, visceral, or skeletal) or by individual anatomical location were statistically significantly different from controls, the percentage of litters with any malformations was increased in the highest dose group (4270 mg/kg/day).

The authors concluded that Octoxynol-9 produced a low order of maternal toxicity and had no adverse effect on any of the gestational parameters. However, it had a pronounced effect on fetal development, producing a number of skeletal abnormalities, notably an increase in the incidence of supernumerary ribs arising from the lumbar and cervical regions. The authors noted that the toxicological significance of the skeletal abnormalities observed in this study was unclear. However, it was agreed that one of the reasons why the increased incidence of supernumerary ribs was not considered serious is because supernumerary thoracic ribs are common developmental variations and generally result in no impairment of physiological functions. The NOEL for Octoxynol-9 was 1600 mg/kg/day for maternal toxicity and 70 mg/kg/day for developmental toxicity (Leung and Ballantyne 1999).

Nonoxynols

Meyer et al. (1988) applied Nonoxynol-9, in distilled water, to the skin of 19 and 24 female mated rats (11-week-old, outbred SPF rats) in doses of 50 and 500 mg/kg/day, respectively. A porous dressing, which had been impregnated with the test substance at the dose levels specified, was applied to shaved skin. The dressing was secured with tape, and the application period was from day 6 to day 15 of gestation. The negative control group (19 rats) received water on GDs 6 to 15.

Compared to the control group, a concomitant decrease in feed consumption was observed in dams dosed with 500 mg/kg Nonoxynol-9. However, all rats given epicutaneous doses, including the control group, had a marked decrease in body weight and weight gain during treatment. Increased litter size and decreased postimplantation loss ($p < .05$ for both) were observed in the 500 mg/kg dose group. No dose-related effects on skeletal and soft tissues were observed; however, an increased incidence of extra ribs was observed in the 50 mg/kg dose group ($p < .02$), but not in the 500 mg/kg dose group (Meyer et al. 1988).

Oral Dosing

Octoxynol-9

Hardin et al. (1987) reported a study in which the developmental toxicity of Octoxynol-9 was evaluated using 50 female, specific pathogen–free CD-1 mice (6 weeks old). The test substance was administered by gavage once daily, 800 mg/kg/day, on days 6 through 13 of gestation; none of the dams died. A negative-control group of 50 mice was dosed with corn oil. One control animal and one animal from the test group died. Compared to the negative-control group, no significant differences were found in any of the following results: number of viable litters, liveborn per litter, percentage survival, birth weight per pup, and weight gain per pup. The authors concluded that Octoxynol-9 did not induce developmental toxicity in mice.

Leung and Ballantyne (1999) evlauated the developmental toxicity of Octoxynol-9 in an oral study using three groups of 27 outbred, Sprague-Dawley CD rats (10 to 11 weeks old at time of mating). Two groups received dietary concentrations of 0.06% Octoxynol-9 (dose = 70 mg/kg/day) and 0.3% Octoxynol-9 (dose = 340 mg/kg/day), respectively, on GDs 6 through 16. The third group (control) received untreated rat chow. On GD 17, the test diet was withdrawn and replaced with the control diet. The dams were killed on GD 20 by nitrogen asphyxiation. Fetal mortalities and resorption sites were recorded, and fetuses were examined for variations and malformations.

Compared to controls, no effects on gravid uterine weight were noted in either of the three dose groups. When corrected for gravid uterine weight, body weight gains over the entire gestational period were reduced in the 70 mg/kg/day dose group. However, these results were not considered to be toxicologically significant because of their very small magnitude and the lack of a similar effect in the 340 mg/kg/day dose group.

Dosing with Octoxynol-9 also had no effect on the number of ovarian corpora lutea, the number of total, viable, or nonviable implantations per litter, or preimplantation loss. No statistically significant difference in the incidence of any individual external variation was observed between test and control groups. However, a statistically significant increase ($p < .05$) in the incidence of displaced testes in fetuses was noted in the 340 mg/kg/day dose group. Statistically significant increases in skeletal variations, observed only in the 340 mg/kg/day dose group, were as follows: vestigial 14th thoracic rib ($p < .01$), accessory ribs on cervical vertebra 7 ($p < .01$), and both cervical and 14th thoracic rib ($p < .01$). Concurrent and historical control incidences of thoracic extra ribs were 22% and 5% to 33%, respectively. A statistically significant decrease ($p < .05$) in the incidence of poorly ossified hyoid was also reported for the 340 mg/kg/day dose group.

As with the dermal exposure study, the authors concluded that Octoxynol-9 produced a low order of maternal toxicity and had no adverse effect on any of the gestational parameters. However, it had a pronounced effect on fetal development, producing a number of skeletal abnormalities, notably an increase in the incidence of supernumerary ribs arising from the lumbar and cervical regions. The authors noted that the toxicological significance of the skeletal abnormalities observed in this study was unclear. However, it was agreed that one of the reasons why the increased incidence of supernumerary ribs was not considered serious is because supernumerary thoracic ribs are common developmental variations and generally result in no impairment of physiological functions. The NOEL for Octoxynol-9 was 1600 mg/kg/day for maternal toxicity and 70 mg/kg/day for developmental toxicity (Leung and Ballantyne 1999).

Other Octoxynols

In a subchronic oral toxicity study, Larson (1961a) administered Octoxynol-40 to young albino rats (15 males, 15 females) at a dietary concentration of 5% daily for 3 months. Mean body weights for male and female rats were 71 and 79 g, respectively. Another group of 15 male and 15 female rats served as the control.

Data on organ-to-body weight ratios indicated no differences between test and control animals that were statistically significant. Mean testes/body weight ratios $\times 10^{-3}$ were 8.7 ± 1.1 g (test animals) and 9.2 ± 1.1 g (controls). Additional results for this subchronic study are included in the section on Subchronic Oral Toxicity earlier in this report (Larson 1961a).

Larson (1961b) evaluated the chronic oral toxicity of Octoxynol-40 using groups of young albino rats (30 males, 30 females/group). Mean body weights for male and female rats were 63 and 58 g, respectively. Octoxynol-40 was administered to the groups at dietary concentrations of 0.035%, 0.35%, and 1.4%, respectively, daily for 3 months or 2 years. The control group (30 males, 30 females) received basic diet only. At the end of the third month of dosing, five males and five females from each dose group were killed and tissues (gonads and other tissues) were examined microscopically. Dosing of the remaining rats (20 per dose group) proceeded to the end of the 2-year study, after which surviving animals were killed and tissues were examined microscopically. Compared to controls, no adverse effects on the testes/body weight ratio were noted at either of the three administered doses. Testes/body weight ratios $\times 10^{-3}$ were as follows: 9.6 ± 0.6 (controls), 9.0 ± 0.8 (0.035% Octoxynol-40), 8.8 ± 0.4 (0.35% dose), and 9.6 ± 1.3 (1.4% dose). Additional results from this chronic study are included in the section on Chronic Oral Toxicity earlier in this report.

Larson et al. (1963) reported testes/body weight ratios in a 3-month study in which Octoxynol-40 was administered to two groups of four (two males, two females/group) purebred Beagle dogs daily at concentrations of 0.35% and 5.0% in the diet, respectively. An additional group of four dogs served as the control. The animals were between 6 and 7 months of age and weights ranged from 4.9 to 10.25 kg. No adverse effect on testes/body weight ratios $\times 10^{-3}$ was noted at either of the doses administered. The mean testes/body weight ratios $\times 10^{-3}$ were as follows: 1.95 (controls), 1.75 (0.35% Octoxynol-40), and 1.45 (5.0% dose). Additional results for this subchronic study are included in the section on Subchronic Oral Toxicity earlier in this report.

Nonoxynols

Hardin et al. (1987) studied the developmental toxicity of Nonoxynol-10 using 49 female, specific pathogen–free CD-1 mice (6 weeks old). The test substance was administered by gavage once daily, 600 mg/kg/day, on days 6 through 13 of gestation; none of the dams died. A negative-control group of 50 mice was dosed with corn oil. Compared to the negative-control group, no significant differences were found in the number of viable litters, liveborn per litter, percentage survival, birth weight per pup, and weight gain per pup. The authors concluded that Nonoxynol-10 did not induce developmental toxicity in mice.

Meyer et al. (1988) evaluated the teratogenicity of Nonoxynol-9 (in distilled water) using 11-week-old, outbred SPF rats. The rats were maintained in stainless steel wire cages and fed powdered chow (chow 101) prior to mating. Three groups of 22 to 25 mated female rats then received oral doses of 50, 250, and 500 mg/kg/day, respectively, on days 6 to 15 of gestation. In the fourth experimental group, 21 rats were dosed orally with Nonoxynol-9 (500 mg/kg/day) on days 1 to 20 of gestation. Twenty-five control rats were dosed with water (5 ml/kg/day) on gestation days 6 to 15; a positive control was not used in the study. On day 21, the rats were killed by exsanguination under CO_2 anesthesia and necropsied. Half of the fetuses were examined for skeletal anomalies and the remaining fetuses were fixed and sectioned.

The 50 mg/kg dose group was the only treatment group for which a statistically significant decrease in weight gain was not observed. Slightly lower average litter sizes that were considered statistically significant ($p < .05$; number affected not stated) were observed in groups of mice that received 250 and 500 mg/kg/day doses on days 6 through 15 of gestation; litter sizes per group were not stated. A statistically significant ($p < .05$; number affected not stated) increase in preimplantation loss was also observed in these two groups.

A statistically significant dose-related increase in extra ribs and rudiments of ribs was observed in rats dosed orally with Nonoxynol-9. The incidence of statistically significant skeletal anomalies for the litters was as follows: 250 mg/kg/day (24 of 25 with rudiments of ribs; $p < .02$), 500 mg/kg/day (10 of 20 with extra ribs, $p < .05$; 10 of 20 with rudiments of ribs, $p < .01$) and 500 mg/kg/day on days 1 to 20 of gestation (12 of 21 with extra ribs, $p < .01$; 21 of 21 with rudiments of ribs). An increased incidence of fetuses (500 mg/kg/day dose group; dosing on GDs 1 to 20) with a slightly dilated pelvic cavity was also reported. The incidence was 12 of 21 litters ($p < .05$) compared to 5 of 25 litters in the control group. The investigators concluded that the no-effect level for Nonoxynol-9 in this teratogenicity study was 50 mg/kg/day (GDs 9 to 15) when the test substance was administered orally (Meyer et al. 1988).

In a study provided by the Environmental Protection Agency (1992), the developmental toxicity of a test material containing 14.0% Nonylphenoxy polyethoxy ethanol (a Nonoxynol; number of moles ethylene oxide not stated), 64% Tallow fatty acid amine ethoxylate, and 22% Butoxyethanol was evaluated using groups of 25 female Crl:CD BR nulliparous rats (approximately 64 days old). The test substance was administered by gavage in doses of 3, 8, 20, and 50 mg/kg body weight on days 7 to 16 of gestation; the control group (25 rats) was dosed with deionized water. Maternal toxicity was induced at doses of 20 and 50 mg/kg/day. No significant differences in fetal malformations were observed between experimental and control groups at any of the doses tested. The investigators concluded that the

no-observable-adverse-effect level (NOAEL) was 8 mg/kg/day for the dam and greater than 50 mg/kg/day for the conceptus.

Parenteral Administration

Octoxynol-9

Saad et al. (1984) administered Octoxynol-9 (in contraceptive jelly) intravaginally to two groups of 25 pregnant Sprague Dawley COBS CD rats (weight range = 215–333 g) at dosages of 0.5 mg/kg/day and 5 mg/kg/day, respectively, on GDs 6 to 15. Three additional groups of 25 rats served as untreated controls, sham controls, and vehicle controls, respectively. The vehicle control consisted of contraceptive jelly excipients. The animals were killed by carbon dioxide inhalation on GD 20. Statistically significant reductions in body weight were observed in sham controls ($p = .05$) and the 5 mg/kg/day dose group ($p = .01$) on GDs 6 to 16. The biological significance of reduced body weight gains was questionable. It was also stated that body weight gains were comparable for all groups after the treatment period and for the entire duration of the observation period (GDs 0 to 20).

The number of viable fetuses, implantations, and mean fetal body weights was comparable for all groups. Of the 118 litters examined, the number of viable fetuses was 1691. Malformations (considered spontaneous in origin and unrelated to treatment) were observed in two female fetuses from two different litters of dams dosed with 0.5 mg/kg/day. These malformations consisted of a threadlike tail in one fetus and another fetus with the following: cleft palate, cleft lip, misplaced pinna, open eye lid, brachygnathia, and aglossia. Skeletal malformations were not observed. The incidence of developmental variations ranged from 70 (untreated control) to 114 (sham control) per group and consisted of the following: malaligned sternebrae, variations in the number of ribs, and, mainly, ossification retardation of the skull, hyoid, os coxae, sternebra, and vertebral centra. These variations were said to have been distributed uniformly among test and control groups. Visceral variations were not observed.

One nonviable fetus from the 5.0 mg/kg/day dose group was examined. Neither malformations (external or soft tissue) nor developmental variations were noted. No other dead fetuses or late resorptions were observed in the study. It was concluded that Octoxynol-9 was not embryotoxic or teratogenic when administered intravaginally to rats during organogenesis (Saad et al. 1984).

Nonoxynols

Abruytyn, McKenzie, and Nadaskay (1982) conducted a study to determine the teratogenicity of a contraceptive cream containing Nonoxynol-9 (50 mg/ml). Five groups of 30 female, Long-Evans hooded rats (body weights = 242–317 g) were used. In the two experimental groups, pregnant rats were dosed intravaginally with 0.08 ml/kg cream (4 mg/kg Nonoxynol) and 0.8 ml/kg cream (40 mg/kg Nonoxynol), respectively, on days 6 through 15 of gestation. Animals of the vehicle control group were dosed intravaginally with 0.8 ml/kg cream base (no Nonoxynol-9), and the two remaining groups of rats were untreated controls and sham controls, respectively. On day 20 of gestation, the dams were killed with carbon dioxide and necropsy was performed; viable fetuses were examined for external malformations. One third of the fetuses from each litter were fixed and visceral examination was performed. The remaining two thirds were examined for gross visceral anomalies; skeletal malformations were also determined.

None of the dams died and no adverse clinical signs were observed during the study. No differences were observed between experimental and control groups with respect to the following: number of corpora lutea per dam, number of implants per dam, percentage of reabsorption per litter, or litter size. Statistically significant differences in mean fetal weight, crown to rump length, and sex distribution between experimental and control groups also were not noted, and no test substance–related major or minor visceral malformations were found.

The following spontaneous malformations were observed among 1824 fetuses from 139 litters examined: absence of urinary bladder and ureters (1); kinky tail (1); abnormally shaped eye (1); small testes (1); undescended testes (1); small kidneys (1); pouchlike cheek (1); pale fetus (3); and hydroureter and/or hydronephrosis (94). Hydroureter and hydronephrosis, observed in 5.5% of the fetuses, were uniformly distributed between experimental and control groups. This percentage was said to compare favorably with the spontaneous incidence of 6.3% in a comprehensive study of 2075 Long-Evans rats.

Of the 1219 fetuses that were examined for skeletal malformations, the fetal and litter incidences of major and minor skeletal malformations were comparable between experimental and control groups. Delayed closure of cranial sutures and delayed ossification were observed in fetuses of all groups, including controls. Additionally, relative to delayed ossification, the fetal incidence in untreated and high-dose (40 mg/kg Nonoxynol-9) groups was significantly greater ($p < .01$) than that in sham and/or low-dose (4 mg/kg Nonoxynol-9) groups. The litter incidence in the untreated control group was also statistically greater ($p < .05$) than that in the sham and low-dose groups.

It was concluded that intravaginally administered Nonoxynol-9 was not embryotoxic or teratogenic in rats at dosages up to 40 mg/kg/day, which is equivalent to approximately 20 times the clinical application (Abruytyn, McKenzie, and Nadaskay 1982).

Buttar (1982) administered single doses (2.5 mg/100 g body weight) of Nonoxynol-9 intravaginally to groups of pregnant Wistar rats (number of animals and weights not stated) on days 1 through 10 of gestation; uterine contents were observed on day 21. Control rats were dosed with distilled water. The incidences of nonpregnancies and resorptions were greatest in dams dosed on days 3, 4, 5, and 6 of gestation. Additionally, the number of live fetuses was significantly reduced in dams dosed on gestation days 4, 5, and 9. The average litter size for dams treated on day 10 of gestation was similar to that for control animals. For dams dosed on day 5 of gestation, fetal weights were

significantly reduced. Neither visceral nor skeletal abnormalities were observed in any of the treatment groups. Nonoxynol-9 was embryolethal and fetocidal, but was not teratogenic.

Buttar, Swierenga, and Matula (1986) studied the reproductive toxicity of Nonoxynol-9 in an in vivo sperm abnormality assay. Two separate experiments, several months apart, were performed; similar doses were tested. Nonoxynol-9, in distilled water, was injected intraperitoneally into groups of five F_1 male mice (C57Bl/6 × C3H/He) in doses of 20, 40, 50, or 60 mg/kg, respectively, once daily for 5 days. The mice were 9 to 10 weeks old and weights ranged from 28 to 32 g. Mice in the negative-control group were dosed with distilled water (10 ml/kg/day) according to the same procedure. Positive-control mice were intraperitoneally injected with aqueous cyclophosphamide (100 mg/kg/day). At 35 days post injection, cervical dislocation was performed and sperm from the cauda epididymis were suspended in physiological saline and stained with eosin-Y. In both experiments, at least 300 spermatozoa from each mouse were examined microscopically.

There were no deaths at doses up to 60 mg/kg. However, following the injection of 100 mg/kg/day, a few mice (number not stated) died after the third or fourth injection. The percentage of abnormal sperm observed in the positive control (cyclophosphamide) group was significantly different ($p < .05$) from the vehicle-control group and all treatment groups. It was concluded that data from the two experiments indicated that systemic administration of Nonoxynol-9 did not increase the frequency of morphologically abnormal sperm over that observed in the control group. The investigators also stated that whether the lack of genotoxic response was due to low affinity of the male germinal cells for Nonoxynol-9 and its metabolites, or to the existence of a blood-testicular barrier in adult mice was not known (Buttar, Swierenga, and Matula 1986).

Tryphonas and Buttar (1986) evaluated the embryotoxicity of Nonoxynol-9 using groups of nulliparous female Wistar rats (five per group; weights = 180–200 g). Each rat was dosed intravaginally with 5 mg Nonoxynol-9/100 g (0.1 ml Nonoxynol/100g) on GDs 3 and 7. The concurrent control rats (five per group) received a per vaginam application of physiological saline (0.1 ml/100g). The groups of treated animals were killed by CO_2 inhalation on GDs 6, 9, 12, and 15, and 8, 9, 10, 12, and 15, respectively. Gross and microscopic examinations were performed.

Ulcerative vaginitis and perivaginal edema, which occasionally extended to the rectal wall and the pelvic connective and adipose tissues, were observed in the treated dams. The severity of vaginal and perivaginal lesions decreased throughout the course of the study, and, on day 15, no lesions were observed.

Other common findings included a decrease in the number of embryos and a concomitant increase in the number of resorption sites. The frequency of these alterations was indirectly proportional to the duration of pregnancy at which Nonoxynol-9 was administered. For dams dosed on day 3 of gestation, the mean number of normal implantation sites was reduced to one or less per uterus. For dams dosed on day 7, 9.2 normal implantation sites per uterus and 4.8 resorption sites per uterus were found. Compared to the saline-treated control group, the number of normal implantation sites was smaller and the number of resorption sites was greater in experimental groups; the difference was significant ($p < .01$) (Tryphonas and Buttar 1986).

In Vitro Studies

Octoxynol-9

Furuse, Ishizeki, and Iwahara (1983) reported that the effective concentration of Octoxynol-9 for totally immobilizing all spermatozoa (human) within 20 s in vitro was 0.12 mg/ml.

Mummery et al. (1984) performed a short-term screening test for teratogenicity to evaluate the potential for Octoxynol-9 to interfere with morphological differentiation in mouse N1E-115 neuroblastoma cells in vitro. Neuroblastoma is a malignant neoplasm of early childhood, probably originating from neural crest cells. Mouse N1E-115 neuroblastoma cells can be induced to differentiate by the removal of serum from the culture medium. The cells then begin to acquire many of the differentiated neuronal properties, including the formation of neurites. Results were positive for Octoxynol-9, and the lowest effective dose was 0.00001%. Eighty-six percent of the compounds screened using this assay were correctly identified as teratogens.

Nonoxynols

Furuse, Ishizeki, and Iwahara (1983) reported that the effective concentration of Nonoxynol-9 for totally immobilizing all spermatozoa (human) within 20 s in vitro was 0.24 mg/ml.

Buttar, Moffatt, and Bura (1985) reported a study in which 2-day-old Swiss-Webster mouse embryos were cultured for 72 h in media containing 0.25 to 10 μg/ml Nonoxynol-9. The 10 μg/ml concentration was lethal to all embryos within 24 h. Viability was reduced in a concentration-dependent manner. In some instances, embryos failed to divide beyond the 8- to 16-cell stage and disintegrated within 48 h.

CLINICAL ASSESSMENT OF SAFETY

Antiplaque Activity

Octoxynol-9

Giertsen, Scheie, and Rölla (1989) conducted antiplaque tests using 10 dental hygienist students with full dentitions and healthy gingival conditions. Antiplaque activity was evaluated using the bacterial strains *Streptococcus sobrinus* strain OMZ 176 and *Streptococcus sanguis*. Over a 4-day period, the subjects rinsed twice daily (morning and evening for 1 min) with 10 ml of an unbuffered solution of 11.6 mM Octoxynol-9 (pH 5.95). The subjects also rinsed with deionized water (placebo) according to the same procedure.

Octoxynol-9 solutions produced 15.8% plaque-free surfaces, whereas the placebo produced 21.4% plaque-free surfaces. The

authors concluded that Octoxynol-9 (11.6 mM) had no inhibitory effect on plaque accumulation. The authors noted that, in bacteriologic tests, Octoxynol-9, inhibited the growth of *S. sobrinus* strain OMZ at a concentration of 0.16 mM and, the growh of *S. sanguis* 10556, at a concentration of 0.18 mM (Giertsen, Scheie, and Rölla 1989).

Clinical Trials

Octoxynol-9

Sixty women were instructed to use (in conjunction with a diaphragm) a spermicidal jelly containing 1% *w/w* Octoxynol-9 for 6 months. Twenty-seven women did not complete the study; two withdrew because of side effects. Of the 33 subjects who completed the study, vaginal irritation and excessive discharge were reported by three and two women, respectively. These side effects were described as minor and reversible in nature (Black and Houghton 1983).

Nonoxynols

Malyk (1981) evaluated the effect of intravaginal application of a cream containing 5.0% Nonoxynol-9 on serum chemistry values in 30 nonpregnant, premenopausal women between the ages of 19 and 39 years. Twelve women applied the cream (2.5 g) daily for 14 days. In vehicle (cream without Nonoxynol-9) and untreated control groups, 11 and 7 women, respectively, applications were made according to the same procedure. Blood samples, obtained before the first application and on days 8 and 15, were analyzed for proteins, lipids, triglycerides, and serum enzymes. The results indicated no significant differences between blood tests conducted before and after application. Neither evidence of alterations in hepatic function nor increased metabolic activity in hepatic cells was observed.

Chvapil, Droegemueller, and Earnest (1982) studied the effects of intravaginal application of Nonoxynol-9 in 10 women. Hematologic parameters, routine liver function biochemistry, and serum lipids were evaluated. The test substance (150 mg) was applied daily for 14 consecutive days. Four women withdrew from the study; two complained of vaginal irritation and itching and the remaining two had candidiasis and a urinary tract infection, respectively. The only significant finding in the study was a reduction in serum cholesterol. No effects of Nonoxynol-9 on either liver function or hematologic parameters were observed.

Niruthisard, Roddy, and Chutivongse (1991) conducted a study involving 20 female subjects (ages not stated) in order to determine if frequent use of Nonoxynol-9 affected the vaginal or cervical mucosa. Each of the remaining 15 women inserted a suppository containing 150 mg Nonoxynol-9 into the vagina hourly for 4 consecutive hours each day; washing or douching was initiated 1 h later. This procedure was repeated for a total of 14 consecutive days. The remaining five women were given a placebo such that the examining physician and women did not know whether the Nonoxynol-9 product was being used.

Of the 14 women (Nonoxynol-9 group) who returned for follow-up examinations, physical findings, which included disruption of the epithelium and/or bleeding, were observed in 6 subjects. Breaks in the cervical epithelium that were observed in four women appeared to have resulted from the sloughing of a thin layer of cells. Additionally, one subject had a severe edematous reaction (with bleeding) of the cervix, and bleeding and sloughing of the vaginal mucosa were noted in another subject. All physical findings that had been noted were not apparent within one week after use of the product had been discontinued. There were no abnormal findings in subjects who received the placebo (Niruthisard, Roddy, and Chutivongse 1991).

In a study by Roddy et al. (1993), the irritation potential of Nonoxynol-9 was evaluated using four groups of 35 normal female subjects (18 to 45 years old). The groups were instructed to insert suppositories containing Nonoxynol-9 (190 mg) into the vagina according to the following schedules: one every other day for 2 weeks (group 1), one daily for 2 weeks (group 2), two daily for 2 weeks (group 3), and four daily for 2 weeks (group 4). Each of 35 control subjects inserted four placebo suppositories daily for 2 weeks. Celibacy and refraining from vaginal douching during the study were mandatory. The women were examined for erythema and epithelial disruption by colposcopy.

For women of group 1, the rate of epithelial disruption was essentially the same as that for control subjects. Women of group 2 and group 3 had rates of epithelial disruption that were 2.5 times that of controls, and the rate was even greater (factor of 5) in group 4 women. In each experimental group, erythema was the major alteration noted in the vagina; the cervix was the site of most of the erythema and epithelial disruption (Roddy et al. 1993).

Skin Irritation

Octoxynol-9

In a study provided by CTFA (1987), the skin irritation potential of four formulations (two with and two without 2.0% Octoxynol-9) was evaluated in human subjects using 24-h single-insult, occlusive patch tests. The number and age range of the subjects tested were not stated. PIIs were determined.

For the first pair of formulations (same composition except for presence or absence of 2.0% Octoxynol-9), PIIs of 0.55 (moderately irritating, with Octoxynol-9) and 0.13 (minimally irritating, without Octoxynol-9) were reported. For the second pair of formulations (same composition except for presence or absence of 2.0% Octoxynol-9), a PII of 0.11 (minimally irritating) was reported. It was suggested that the difference in results between the two formulations containing 2.0% Octoxynol-9 was due to differences in the skin penetrability of Octoxynol-9 in one formulation versus the other. Data supporting this suggestion indicate that the addition of 20% glycol acrylic polymer to both formulations resulted in a slower rate of Octoxynol-9 skin penetration in one formulation versus the other (CTFA 1987).

Harvell et al. (1994) evaluated the skin irritation potential of 1% Octoxynol-9 using nine healthy female volunteers (mean

age = 52 years; age range = 43–72 years). Patches containing 200 μl of the test substance were applied to the interscapular area of the back. The type of patch used was described as a large-sized polypropylene chamber. Patches were applied to the same sites on the back for 4 consecutive days. Reactions were scored on the fifth day according to the following scales: erythema (1+ = slight redness, spotty or diffuse to 4+ = fiery, with edema); scaling (1+ = fine to 3+ = severe with large flakes); and fissures (1+ = fine cracks to 3+ = wide cracks with hemorrhage or exudation). Octoxynol-9 was classified as a nonirritant.

Skin Sensitization—Predictive Tests

Octoxynol-9

In a study provided by E. I. du Pont de Nemours and Company (1956), the skin sensitization potential of a cotton twill (1 square inch) treated with 0.1% Octoxynol-9 was evaluated using 84 men and 122 women. The test material was applied to the arms of men and to the arms and legs of women. The patches were secured with adhesive tape and remained in place for 6 days. After a 2-week nontreatment period, new patches were applied and then removed 48 h later. Test sites were examined 2 and 6 days after the initial application and 48 h after the final application. No reactions to fabric treated with 0.1% Octoxynol-9 were observed.

AMA Laboratories, Inc. (1996) evaluated the skin irritation and sensitization potential of a foot gel containing 8.0% Octoxynol-9 using 112 subjects (20 males, 92 females; 16 to 76 years old), 103 of whom completed the study. Withdrawal from the study was unrelated to test substance administration. A semiocclusive patch containing 0.2 ml or 0.2 g of the test substance was applied to the infrascapular region of the back (at right or left of midline) for 24 h. Patch removal was followed by application of another patch containing the test substance to the same site.

Reactions were scored prior to subsequent patch applications according to the following scale: 0 (no evidence of any effect) to 4 (severe—deep red erythema with vesiculation or weeping with or without edema). This procedure was repeated for a total of nine consecutive 24 h applications on Mondays, Wednesdays, and Fridays for 3 weeks. The induction phase was followed by a 10- to 14-day nontreatment period, after which each subject was challenged (new test site) with 0.2 ml or 0.2 g of the test substance. Challenge reactions were scored at 24 and 48 h post application.

No adverse reactions were observed in any of the subjects during the induction or challenge phase. The foot gel containing 8.0% Octoxynol-9 was neither a primary irritant nor a sensitizer (AMA Laboratories, Inc. 1996).

Hill Top Research, Inc. (1986) evaluated the skin sensitization potential of a formulation containing 0.5% Octoxynol-9 using 106 subjects (males and females; age range = 18–65 years), 102 of whom completed the study. Three subjects withdrew for reasons unrelated to the test substance. One subject was released because of a preexisting dermatological disorder. During the induction phase, the test substance was applied to the back of each subject and the test site covered with an occlusive patch for 24 h. Induction applications (to same site) were made on Mondays, Wednesdays, and Fridays over a period of 22 days.

Reactions were scored at 48 or 72 h post application according to the following scale: 0 (no evidence of any reaction) to 5 (vesicular/bullous eruption). The induction phase was followed by a nontreatment period (duration not stated). Challenge patches were then applied for 24 h and reactions scored at 48 and 96 h post application.

Seven subjects had a score of 1 or greater during induction. One subject had a score of 1 during the challenge phase. The formulation containing 0.5% Octoxynol-9 did not induce reactions that are indicative of contact sensitization (Hill Top Research, Inc. 1986).

Other Octoxynols

Information provided by FDA (1999a) indicated that the skin irritation and sensitization potential of Octoxynol-1, -3, -5, -9, and -13 (each undiluted) was evaluated in a 48 h skin irritation test using 50 subjects. None of the test substances induced skin irritation. Octoxynol-1 induced sensitization in two subjects, the only sensitization reactions that were observed. Details concerning the challenge test procedure were not included.

Nonoxynols

In a series of studies, Jordan (1994, 1995a, 1995b) evaluated Nonoxynol-2 (5% in mineral oil), Nonoxynol-2 (10% in mineral oil), and Nonoxynol-4 (10% in mineral oil) in skin irritation/sensitization studies according to the same experimental procedure. In each test, the subjects were free of interfering systemic or dermatologic disorders, visible skin diseases, active atopic dermatitis, or psoriasis.

Jordan (1994) evaluated the skin irritation/sensitization potential of Nonoxynol-2 (5% in mineral oil) using 110 volunteers (9 males, 101 females; 19 to 61 years old). Eight of the original 110 withdrew from the study for reasons that were unrelated to administration of the test substance. During induction, 0.2 ml of the test substance was applied, under occlusive patches, to the scapular region of the back three times per week for 3 weeks (nine induction applications). Patches were removed, and sites evaluated, at 48-h intervals. Patches applied on Friday were removed, and sites evaluated, on the following Monday (72 h post application).

The induction phase was followed by a 14-day nontreatment period. During the challenge phase, initiated at week 6, two consecutive 48-h patches were applied to new sites in the scapular region of the back. Challenge reactions were scored after 48 and 96 h. During induction and challenge phases, reactions were scored according to the following scale: 0 (no reaction) to 4 (bullae or extensive erosions involving at least 50% of the test area).

Isolated evidence of faint to moderate erythema was observed in three subjects during the induction phase. Three subjects also had reactions during the challenge phase; however, no evidence of allergic contact dermatitis was found (Jordan 1994).

Jordan (1995a) evaluated the skin irritation/sensitization potential of Nonoxynol-2 (10% in mineral oil) using 111 volunteers (15 males, 96 females; 18 to 64 years old). Eight of the original 111 withdrew from the study for reasons that were unrelated to administration of the test substance. The experimental procedure is described above.

During the induction phase, isolated evidence of slight to moderate erythema was observed in 15 subjects, and, in an additional subject, strong, infiltrated erythema was observed after removal of the last induction patch. The subject with the strong induction reaction also had allergic reactions during the challenge phase. A total of 23 subjects had reactions during the challenge phase; however, 9 of the 23 had reactions that were classified as allergic contact dermatitis.

Seven of the nine subjects with contact allergic dermatitis were retested according to a different procedure. The test substance was applied under a semiocclusive patch for 30 min, after which the test site was rinsed with warm water. Reactions were scored at 24 h post application (seven subjects) and at 24 and 48 h post application (one subject). In the retest, discernible, mild allergic responses were observed in two of seven subjects; reactions were not observed in the remaining five.

The investigator concluded that Nonoxynol-2 (10% in mineral oil) induced allergic contact sensitization in the initial experiment. However, when exposure was limited to 30 min, evidence of a mild allergic response was observed in two of the seven subjects with allergic contact sensitization who were retested (Jordan 1995a).

Jordan (1995b), using the same technique described above, evaluated the skin irritation/sensitization potential of Nonoxynol-4 (10% in mineral oil) using 111 volunteers (10 males, 101 females; 19 to 62 years old). Four of the original 111 withdrew from the study for reasons that were unrelated to administration of the test substance.

During the induction phase, isolated evidence of faint to moderate erythema was observed in 36 subjects. A total of 31 subjects had reactions during the challenge phase; however, only 3 of the 36 had reactions that were classified as allergic contact dermatitis. The three subjects with allergic contact dermatitis were retested according to the retest procedure included in the preceding paragraph. In the retest, a discernible mild allergic response was observed in one of the three subjects; reactions were not observed in the remaining two.

The investigator concluded that Nonoxynol-4 (10% in mineral oil) induced allergic contact sensitization in the initial experiment. However, when exposure was limited to 30 min (retest), evidence of a mild allergic response was observed in one of the three subjects with allergic contact dermatitis (Jordan 1995b).

Skin Sensitization—Provocative Tests

Nonoxynols

Dooms-Goossens et al. (1989) patch tested a total of 12 contact dermatitis patients with the ingredients of a topical antiseptic preparation. Ten of the patients had used antiseptic preparations that contained Nonoxynol-9; all 10 had not used the same antiseptic preparation. The remaining two patients had used antiseptic preparations that contained Nonoxynol-8.3 and Nonoxynol-10, respectively. Nonoxynol-8.3, -9, and -10 were tested at concentrations of 2.0% in water. The patches remained in place for 48 h and reactions were scored at 48 h and at 72 or 96 h.

All of the patients had positive reactions to 2.0% aqueous Nonoxynol solutions either at 72 or 96 h; reactions classified as ++ (strong, edematous or vesicular, reaction) were observed in all patients. Epicutaneous test results for other ingredients of antiseptic preparations were negative, with the exception of one patient who reacted to the antiseptic, iodine.

When 6 of the 12 patients in the above study were tested with 2.0% aqueous Nonoxynol-6, -8.3, -9, -10, -14, and -18 several months later, most of the reactions observed at 72 or 96 h were ++ reactions. However, in some instances, a + (weak, nonvesicular, reaction), negative, or doubtful reaction was observed. Subjects 1, 2, and 7 each had a + reaction to Nonoxynol-18 at 72 h. Additionally, subject 5 had a + reaction to Nonoxynol-6 at 6 h and subject 4 had a + reaction to Nonoxynol-8.3 at 96 h. Subjects 4 and 6 each had negative reactions to Nonoxynol-18 at 96 h and 72 h, respectively. Finally, subject 5 had what was classified as a doubtful reaction to Nonoxynol -8.3, -10, -14, and -18. This subject did not return for retesting (Dooms-Goossens et al. 1989).

Comedogenicity

Strauss et al. (1978) used Octoxynol-9 as the vehicle control in two studies evaluating the comedogenicity of sulfur. In the first study, an occlusive patch containing 0.25% Octoxynol-9 was applied to one area on the back of each of six subjects. Patches were held in place with impermeable plastic tape. The subjects had severe acne and a pronounced propensity for comedo formation. Test sites were clinically free of comedones. Patches were replaced three times per week for 6 weeks. A blank (dry) occlusive patch applied to each of six additional subjects served as an additional control. At the end of the study, the sites were evaluated clinically for the presence or absence of papules or comedones. A biopsy specimen was obtained from each site. Comedones were observed in three of six subjects tested with Octoxynol-9 and in one of six subjects that received the occlusive patch only. Two of six biopsy specimens from Octoxynol-9–treated sites contained definite comedones. One of six biopsy specimens from sites with an occlusive patch only contained definite comedones.

In the second study, 40 subjects were tested according to the procedure in the preceding paragraph. Twenty subjects had a history of acne, but were free of active disease. The remaining 20

had active acne on their backs, either comedonal or comedonal with some small pustules. Comedones were observed in 2 of 20 subjects tested with Octoxynol-9 and in 2 of 20 subjects that received the occlusive patch only. Four of 20 biopsy specimens from Octoxynol-9–treated sites contained definite comedones. Two of 20 biopsy specimens from sites with an occlusive patch only contained definite comedones. The authors concluded that Octoxynol-9 was comedogenic (Strauss et al. 1978).

Photosensitization

Nonoxynols

Michel et al. (1994) observed photosensitization in sun-exposed areas of two patients (72-year-old male; 71-year-old female) who had been treated with an antiseptic preparation that contained Nonoxynol-10. Based on these case reports, a follow-up photosensitization study involving the two patients and 32 control subjects was initiated. The 13 male and 19 female control subjects, all suspected of having photodermatosis, had a mean age of 42 years and had never used the antiseptic preparation that induced photosensitization in the two elderly patients. The control subjects and two patients were patch tested with the antiseptic preparation, undiluted Nonoxynol-10, 2% Nonoxynol-10 in petrolatum, and 0.2% and 2% Nonoxynol-10 in water. The two patients were also patch tested with 1% Nonoxynol-10 in water. Three series of patch tests (Finn chambers) were placed on the backs of all subjects, with the exception of one subject (72-year-old patient) who received an additional (fourth) series. Test sites (two series of patch tests only) were exposed to a suberythemal dose of UVA (330 to 460 nm; 35 mW/cm^2) or UVB (285 to 350 nm; 1.5 mW/cm^2) light at 24 h post application. Test sites (irradiated and nonirradiated series) were evaluated at 72 h post application.

Results for each UV exposure and each chemical were not reported. One male patient had photosensitization reactions to the antiseptic preparation and to 0.2%, 1%, and 2% aqueous Nonoxynol-10. Undiluted Nonoxynol-10 did not induce photosensitization. Reactions were not observed in any of the remaining photopatch tests or at nonirradiated sites.

One female patient had photosensitization reactions to the antiseptic preparation and to 2% Nonoxynol-10 in petrolatum. Again, undiluted Nonoxynol-10 did not induce photosensitization. Reactions were not observed in any of the remaining photopatch tests or at nonirradiated sites.

Of the 32 control subjects, 13 had photosensitization reactions to the antiseptic preparation and four had photosensitization reactions to aqueous Nonoxynol-10. There were no photosensitization reactions to undiluted Nonoxynol-10 (Michel et al. 1994).

Case Reports

Octoxynol-9

Nethercott and Lawrence (1984) patch tested a 58-year-old uranium mill maintenance worker with allergic contact dermatitis who used a waterless hand cleanser containing Nonoxynol-6 at work with 0.5% Octoxynol-9 in petrolatum. "Al Test" strips occluded with "Scanpor" tape were applied to the upper back. At 48 h post application, sites were scored using the scoring system recommended by the International Contact Dermatitis Group (Fisher 1973). No reaction to 0.5% Octoxynol-9 was observed.

Nonoxynols

Nethercott and Lawrence (1984) patch tested the same 58-year-old uranium mill worker (who used a waterless hand cleanser containing Nonoxynol-6) with 0.5% Nonoxynol-6 in petrolatum. The patient had an allergic contact reaction (1+ reaction) at 48 and 96 h. Reactions to Nonoxynol-6 (0.5% in petrolatum) were not observed in eight control subjects.

Meding (1985) observed dermatitis on the hands and forearms of a 64-year-old worker in the metal industry who regularly immersed metal objects into a fluid containing Nonoxynol-6. Patch test results indicated weak, nonvesicular reactions (score = +) to 0.001%, 0.01%, and 0.1% aqueous Nonoxynol-6 and strong edematous or vesicular reactions (score = ++) to 1.0% and 5.0% Nonoxynol-6. Reactions were not observed in a control group of 165 patients patch tested with 1.0% Nonoxynol-6.

Kabasawa and Kanzaki (1989) diagnosed allergic contact dermatitis in a 61-year-old female patient with rheumatoid arthritis who had recently had foot surgery. Hibitane (cleanser) had been applied to the surgical wound daily for 6 days, and there was no evidence of dermatitis after applications were discontinued. The patient had positive patch test reactions to 0.04% aqueous Nonoxynol, the surfactant in Hibitane.

EPIDEMIOLOGY

Jick et al. (1981) evaluated the relationship between the use of vaginal spermicides and congenital disorders in 4772 pregnant females (4,655 women whose pregnancies terminated in a live birth and 107 women whose pregnancies terminated in a nonvoluntary abortion that resulted in hospitalization). All of the women were members of the Group Health Cooperative medical plan. Approximately 80% of the spermicide use at Group Health involved products containing Octoxynol (available at Group Health pharmacy). Use of spermicides containing nonoxynol-9 accounted for 20% of the spermicide use at Group Health. Of the 4772 pregnant females, 790 (17%) had filled a prescription for a vaginal spermicide within 600 days prior to delivery (or abortion). These women did not subsequently fill a prescription for other contraceptives.

Of the 4665 infants who were born alive, 56 (1.2%) had one malformation/abnormality. The frequency of this occurrence in infants whose mothers had used a spermicide was 2.2% (17/763), and 1.0% (39/3902) for the remainder (controls). It is important to note that 18 infants were excluded from the group with malformations because they had anomalies that were generally considered as familial, minor, or positional. An excess of the

following categories of anomalies was reported for infants whose mothers were exposed to spermicides: (1) limb reduction deformities, (2) neoplasms, (3) chromosomal abnormalities, and (4) hypospadias.

The results for the 107 women whose pregnancies terminated in a nonvoluntary abortion indicated that 27 of the 107 pregnant females had vaginal spermicide prescribed prior to becoming pregnant. The data presented in this study show a positive association between vaginal spermicide use and certain congenital disorders, namely, limb reduction deformities, neoplasms, chromosomal abnormalities, and hypospadias (Jick et al. 1981).

Mills et al. (1982) reported malformation rates in offspring of 34,660 women using spermicides. Spermicide use by the study participants was categorized as follows: 3146 had used spermicides before, but not after, their last menstrual period; 2282 were exposed to spermicides after their last menstrual period; 13,148 had used other forms of birth control before their last menstrual period only; 2831 were exposed after their last menstrual period.

For women practicing contraception only before the last menstrual period or after the last menstrual period, the rate of malformations in the offspring of spermicide users was no greater than that for women who used other methods of contraception. For those exposed to spermicides before the last menstrual period, the relative risk of major malformations was 0/97 (95% confidence intervals of 0.71/97 to 1.33/97). For those exposed to spermicides after the last menstrual period, the relative risk of malformations was 0.75/97 (95% confidence intervals of 0.49/97 to 1.15/97). Adjusting for maternal age, education, race, smoking, alcohol use, and previous malformed infants by multiple logistic regression did not change the estimates of relative risk.

No significant differences in malformation rates in any organ system were noted in the group exposed only before the last menstrual period or in the group that used spermicides or other methods of contraception after the last menstrual period. Additionally, no significant associations between spermicide use and anomalies were found when the 60 individual malformations, grouped by organ system, were examined individually based on use of spermicides or other contraceptive methods before and after the last menstrual period. The results (before and after the last menstrual period) that were reported when the spermicide users were subdivided by active ingredient are as follows: The malformation rates in females who used spermicides containing Oxtoxynol were 105.3 per 1000 (use before last menstrual period) and 22.2 per 1000 (use after last menstrual period).

Whether or not the spermicides contained Octoxynol-9 was not stated. In females who used spermicides containing Nonoxynol-9, the malformation rates were 129.2 per 1000 (use before last menstrual period) and 127.3 per 1000 (use after last menstrual period). No group exposed to spermicides had significantly poorer outcomes when compared to users of other methods of contraception (Mills et al. 1982).

Shapiro et al. (1982) studied the 50,282 pregnancies in a cohort study (subjects recruited between 1958 and 1965), in which 462 pregnant women used nonmercurial spermicides. Use of Nonoxynol-9 (74% of the spermicides) and Octoxynol (84% of the spermicides) spermicides predominated. Whether or not the Octoxynol spermicides contained Octoxynol-9 was not stated. The 954 pregnancies that were terminated before week 20 of gestation were not considered. Four-hundred thirty-eight of the 462 pregnant women had also used spermicides during the month that preceded the last menstrual period.

Of the 462 mother-child pairs exposed to spermicides in the first four lunar months of pregnancy, malformations were observed in 31 children (6.7%). The corresponding frequency among the 49,820 nonexposed pairs was 3217 (6.5%). Major malformations accounted for ten exposed (2.2%) and 1383 nonexposed (2.8%) children. The only specific deformity that occurred in more than one child was atrial septal defect (two children). The estimated rate ratio for major malformations was 0.9 (95% confidence limits, 0.6 to 1.6).

No excess of limb reduction deformities, neoplasms, Down syndrome or hypospadias occurred in children exposed to spermicides. None of the offspring of the 25 pregnant women who used the nonmercurial spermicides only during the month prior to the last menstrual cycle was malformed. The evidence in this study suggests that the nonmercurial spermicides used did not cause an increase in the overall risk of malformations (Shapiro et al. 1982).

Louik et al. (1987) studied the relationship between maternal exposure to spermicides (active ingredient, Octoxynol or nonoxynol) and specific birth defects. Five separate groups of infants were evaluated: 265 with Down syndrome, 396 with hypospadias, 146 with limb reduction defects, 116 with neoplasms (benign and malignant), and 215 with neural tube defects. The remaining 3442 infants with other defects comprised the control group. Infants with malformations were used as controls because of the possibility that mothers of such babies recall or report their contraceptive histories differently, compared to the mothers of normal infants.

The authors concluded that the risks of Down syndrome, hypospadias, limb reduction defects, neoplasms, and neural tube defects were not increased by exposure to spermicide contraceptives in the first four months of pregnancy, at the time of conception, or at any time prior to conception. Overall, the relative-risk estimates for the cases were all close to 1.0 (Louik et al. 1987).

Folb and Graham Dukes (1990) reported that, for women who had used a vaginal spermicide during the 10 months prior to conception, the frequency of major congenital anomalies in the 763 infants who were born alive was 2.2%. The incidence in the control group of 3902 infants was 1.0%. The difference between the two groups was attributed to an excess of limb-reduction defects, neoplasms, syndromes associated with chromosomal anomalies, and hypospadias in the infants of mothers who were suspected of having used spermicides. Approximately 80% of the spermicide use in this study involved products containing Octoxynol. Whether or not the Octoxynol spermicides contained Octoxynol-9 was not stated. Products containing Nonoxynol-9 accounted for 20% of the spermicide use.

Pray (1992) offered the following critique of the preceding study: (1) A woman was considered a user if a prescription for a spermicide was filled 600 days or less before delivery. Thus, many users may not have actually used a spermicide. (2) Evidence that the control group of "unexposed" females had not used spermicides was lacking. (3) The proportion of malformations in the "control" group (1%) was far below the national average of 2% to 5%. Thus, the 2.2% incidence seen in the "exposed" group was actually within normal estimates for all females. (4) The identified malformations lacked a common basis of teratogenesis (as phocomelia with thalidomide), making it unlikely that spermicides were the shared risk factor for all of them.

AEROSOL INHALATION EXPOSURE ASSESSMENT

Octoxynol-9 is used in hair sprays. Jensen and O'Brien (1993) reported that the mean aerodynamic diameter of respirable particles is $4.25 \pm 1.5\ \mu$. Bower (1999) stated that the mean aerodynamic diameter of $4.25 \pm 1.5\ \mu$ of respirable particles above may be compared with diameters of anhydrous hair sprays particles of 60 to 80 μ (typically, $<1\%$ are below 10 μ) and pump hair sprays with particle diameters of $\geq 80\ \mu$, suggesting that Octoxynol-9 in hair sprays would not result in inhalation exposures.

SUMMARY

Octoxynols

The Octoxynols, or polyoxyethylene octylphenyl ethers, are ethoxylated alkylphenols with the chemical formula, $C_8H_{17}C_6H_4(OCH_2CH_2)_nOH$. The average value of number of moles of ethylene oxide, **n**, can vary from 1 to 70.

Octoxynols of various chain lengths as well as Octoxynol salts and organic acids function either as surfactants—emulsifying agents, surfactants—cleansing agents, surfactants—solubilizing agents, or surfactants—hydrotropes in cosmetic products. Frequency of use data provided by FDA in 2001 indicate that, collectively, Octoxynol-1, -3, -5, -9, -11, -13, -40, and Potassium Octoxynol-12 Phosphate are being used in 294 cosmetic products. Concentration of use data received from the cosmetics industry in 1999 and updated in 2001 indicate that the Octoxynols (their salts and organic acids included) are used in cosmetics at concentrations ranging from 0.0008% to 25%, and that most of the use concentrations are less than 5.0%.

Octoxynol-9 and Nonoxynol-9 are recognized by FDA as effective spermicides, but FDA has proposed that OTC products containing these ingredients be required to submit clinical data on the effectiveness of products containing these ingredients.

Octoxynols (up to Octoxynol-70), Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-2 Ethane Sulfonate have been approved by FDA for use as direct/indirect food additives.

The results of a UV spectral analysis of a 0.32 mM (200 ppm) aqueous solution of Octoxynol-9 indicated no significant absorbance in the UVA and UVB regions of the spectrum. The highest energy wavelength at which an absorption maximum was observed was at 276 nm (molar absorptivity = 1600 cm/M). Slight absorbance at 290 nm, as a tail on the peak at 276 nm, was also noted.

An octanol/water partition coefficient of 1.9 has been estimated for Octoxynol-9.

Essentially all of the radioacitivity administered (oral feeding of [^{3}H]Octoxynol-40) to six rats and two dogs was excreted in the feces, indicating that Octoxynol-40 was not absorbed to any significant degree. The values for recovery in the feces were up to 92.2% (rats) and up to 86.4% (dogs).

Alkylphenol ethoxylates (which includes the Octoxynols) and related compounds have been reported to be estrogenic, based on the in vivo and in vitro demonstration that they mimic the effects of estradiol.

Octoxynol-9 has been associated with stimulatory/inhibitory effects on various enzymes. The inhibition of monoamine oxidase activity in the presence of Octoxynol-9 (0.1% to 1.0%), and of diacylglycerol acyltransferase activity in the presence of 0.05% Octoxynol-9 have been reported. Optimal activation of guanylyl cyclase in the presence of 0.5% to 1.0% Octoxynol-9 has also been reported.

Octoxynol-9 was classified as a sensory irritant in a study in which two mice were exposed (nose-only) to concentrations up to 36.0 or 38.0 mg/L. A concentration-related decrease in respiratory rate was noted.

An acute inhalation LD_{50} of 501 μg/g lung (confidence limits = 376–676 μg/g) was reported for a group of 50 Syrian hamsters exposed to an aerosol (MMAD = 1.5 μm) containing Octoxynol-9. The cause of death was laryngeal obstruction, with moderate pulmonary edema and pneumonitis. Pneumonia, pulmonary edema, and intra-alveolar hemorrhage were observed in hamsters exposed to Octoxynol-9 (0.01 to 0.10%) by bronchopulmonary lavage.

Alveolar/bronchiolar epithelial hyperplasia was observed in 10 Sprague-Dawley CD rats exposed to Octoxynol-9 (MMAD = 1.8 μm) over a period of 2 weeks. None of the animals died.

The following acute oral LD_{50} values (rats) have been reported for Octoxynol-1, -3, and -5: Octoxynol-1 ($LD_{50} = 7.1 \pm 0.1$ cc/kg), Octoxynol-3 (4.0 ± 0.2 cc/kg), and Octoxynol-5 (3.8 ± 0.2 cc/kg).

Octoxynol-9 was classified as slightly toxic in rats (LD_{50} = between 800 and 1600 mg/kg, 10 rats) and in mice (1600 mg/kg, 10 mice) in an acute oral toxicity study. In another study, a dose-related increase in mortality was noted in groups of 10 Charles River SCD rats that received doses of Octoxynol-9 ranging from 0.678 to 1.86 ml/kg. An acute oral LD_{50} of 1.06 ml/kg (confidence limits = 0.989–1.29 ml/kg) was reported.

Acute oral LD_{50} values (albino rats) for Octoxynols were Octoxynol-13 (985 [691 to 1400] mg/kg), 30% Octoxynol-16 (2.68 ± 0.56 g/kg), 70% Octoxynol-16 (2.78 ± 0.95 g/kg), 70% Octoxynol-20 (3.64 ± 1.33 g/kg), and 70% Octoxynol-30 (21.20 ± 2.0 g/kg). One of 10 albino rats dosed with 70% Octoxynol-40 (dose = 28.0 g/kg) died. Determination of an LD_{50} value was not possible.

No deaths were reported following the short-term oral administration of Octoxynol-9 to female Sprague-Dawley rats in a developmental toxicity study. Two groups of 27 animals were fed dietary concentrations of 0.06% Octoxynol-9 (dose = 70 mg/kg/day) and 0.3% Octoxynol-9 (dose = 340 mg/kg/day), respectively, on GDs 6 through 16.

The subchronic oral toxicity of Octoxynol-40 was evaluated using 30 young albino rats. The test substance was administered at a concentration of 5% in the diet daily for 3 months. No statistically significant differences in organ-to-body weight ratios (heart, spleen, kidney, liver, and testes) were noted between test and negative-control rats. At microscopic examination, no test substance–related lesions were observed. Similar results were reported for purebred Beagle dogs (4 dogs/dose group) fed Octoxynol-40 at dietary concentrations of 0.35% and 5.0%.

In a chronic oral toxicity study, three groups of 60 young albino rats were fed Octoxynol-40 at concentrations of 0.035%, 0.35%, and 1.3% in the diet, respectively, for 3 months to 2 years. After 3 months, 10 animals per group were killed and tissues examined microscopically. The remaining animals were killed at the end of the 2-year study. No adverse effects on the following parameters were observed in either of the three groups: survival, growth, food consumption, hematologic values, urinary concentrations of sugar and protein, organ-to-body weight ratios, or kind, incidence, and degree of pathologic lesions.

An acute dermal LD_{50} of >20 cc/kg was reported in a study involving three guinea pigs. Slight to moderate edema and scattered erythema were observed at 24 h post application.

In a short-term dermal toxicity study, the following Octoxynols were applied to the skin of rabbits over a period of 4 weeks (20 applications total): 1% Octoxynol-1, 1% Octoxynol-3, 0.1% Octoxynol-9, and 0.1% Octoxynol-13. No abnormal changes were observed at histopathologic examination.

In a short-term study in which the developmental toxicity of Octoxynol-9 was evaluated, three groups of 25 Sprague-Dawley rats received dermal applications of Octoxynol-9 (under occlusion) at concentrations of 12.5% (dose = 530 mg/kg/day), 37.5% (1600 mg/kg/day), and 100.0% (4270 mg/kg/day), respectively, on GDs 6 through 15. No statistically significant differences in lung, liver, or kidney weights were noted between test and control dams. One dam in the highest dose group died. The cause of death was not determined.

Octoxynol-9 was classified as moderately toxic in rats ($LD_{50} \approx$ 100 mg/kg) and mice (LD_{50} = between 50 and 100 mg/kg dose range) dosed intraperitoneally.

The ocular irritation potential of short- and long-chain Octoxynols in rabbits was evaluated. In one study, 15% Octoxynol-1, 15% Octoxynol-3, 5% Octoxynol-5, 0.5% Octoxynol-9, and 1% Octoxynol-13 were classified as nonirritants. However, in other studies, Octoxynol-13 (concentration not stated) was classified as a severe ocular irritant and another long-chain Octoxynol (20% Octoxynol-11) was classified as very badly tolerated. It is also important to note that a skin freshener containing 0.25% Octoxynol-11 was classified as minimally irritating.

Additional study results on Octoxynol-9 indicated that this ingredient was a moderate to severe ocular irritant in undiluted form or at a concentration of 10% in distilled water. Ocular rinsing reduced the ocular irritation potential of undiluted Octoxynol-9 from moderate to severe to slight to moderate.

At the highest dose of Octoxynol-9 tested in a developmental toxicity study (4270 mg/kg/day, dermal exposure), skin changes at the site of application included exfoliation/desquamation, excoriation, and erythema. In this study, three groups of 25 Sprague-Dawley rats received dermal applications of Octoxynol-9 at concentrations of 12.5% (dose = 530 mg/kg/day), 37.5% (1600 mg/kg/day), and 100.0% (4270 mg/kg/day) on GDs 6 through 15.

The skin irritation potential of Octoxynol-9 (10% *w*/*w* in distilled water) was evaluated in a 24-h occlusive patch test using six rabbits. A Draize irritation score of 0.2 (maximum score = 8) was reported. No reactions were reported in two 24-h occlusive patch test (nine rabbits) in which the skin irritation potential of a peel-off mask product containing 0.25% Octoxynol-9 was evaluated.

A 20% aqueous solution of Octoxynol-11 was classified as a moderate skin irritant. Details concerning the test protocol and study results were not provided. Octoxynol-13 was not classified as a primary dermal irritant in a 24-h occlusive patch test involving six rabbits. However, the potential for slight irritation was noted.

In an in vitro study, rolling or curling, but not fragmentation, was observed in guinea pig corneocytes immersed in Octoxynol-9 solution (0.1 M and 0.1%) over a period of 30 days. The following morphological changes were observed in human corneocytes exposed to 1% Octoxynol-9 (in distilled water) in vitro for up to 6 h: swelling, vacuolization, and moderate loss of staining intensity.

Denudation of villous tips, desquamation of the epithelial surface, necrosis of the mucosal lamina propria, and intervillous adhesion were observed in the rat jejunum and colon following perfusion with 1% Octoxynol-9.

No significant effect on the immune system (i.e, no toxicity) of CF-1 female mice was noted following the intraperitoneal injection of Octoxynol-9 (concentration not stated) followed by subcutaneous immunization with SRBCs. The following values were determined: hematocrits, leucotye counts, anti-SRBC titers, and serum concentrations of IgM and IgG.

In another study, the effect of oral dosing of Octoxynol-9 (4 weeks) on humoral and cell-mediated immune responses and the autoimmune response was evaluated using 129/Ao Boy strain mice. Octoxynol-9 enhanced the production of anti-RBC PFCs (humoral response) and also stimulated the cellular immune response. When the duration of oral dosing was reduced to 1 week, no effect on development of the cell-mediated immune response was observed. The autoimmune response was determined using erythrocytes from heparinized syngeneic mouse

blood. Stimulation of the autoimmune response was demonstrated both in vivo and in vitro.

The hemolytic activity of Octoxynol-8, -9, and -13, but not Octoxynol-5, was demonstrated in vitro using human erythrocytes. Octoxynol-9 also caused the hemolysis of rat blood cells in vitro. In another study, hemoglobin (fetal and maternal) was denatured rapidly in the presence of Octoxynol-9, compared to the results for NaOH alone.

Octoxynol-9 has also been found to be cytotoxic to rat hepatocytes, human epidermal keratinocytes, and human fibroblasts in vitro.

Complete inactivation of the human immunodeficiency virus in the presence of Octoxynol-9 has been reported.

In a study evaluating the neurotoxicity of Octoxynol-9, application of the test substance to the serosal surface of the rat jejunum (moved outside of peritoneal cavity, not excised) caused a significant reduction in the number of ganglion cells in the myenteric plexus.

Treatment of a guinea pig sinoatrial preparation in vitro with Octoxynol-9 induced a decrease in twitch force that was 25% below the control value. Human atrial and ventricular tissues were even more sensitive to Octoxynol-9 treatment. In these experiments, Octoxynol-9 caused endocardial damage and depressed the excitability of fast and slow response action potentials.

In the Ames test, Octoxynol-1 was not mutagenic to *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation.

Octoxynol-9 was not clastogenic in the chromosomal aberrations assay (with metabolic activation), but remarkably enhanced the induction of chromosomal aberrations induced by dimethylnitrosamine, BaP, or aniline. In another assay (DNA double-strand breaks assay), double-strand breaks were induced only after cell viability was reduced to <60%.

Results for Octoxynol-9 were negative in the following other mammalian assays: unscheduled DNA synthesis, HGPRT mutation assay, malignant transformation assay, DNA alkaline unwinding test, and mouse lymphoma TK locus forward mutation assay.

The treatment of human carcinoma cell cultures (PC-3, SW-620, and HT-29) with Octoxynol-9 induced internucleosomal DNA fragmentation that was typical of apoptosis. Apoptosis was also observed in human hepatoma cell lines treated with Octoxynol-9.

Three groups of female CD rats received dermal applications (under occlusion) of 12.5% (dose = 530 mg/kg/day), 37.5% (1600 mg/kg/day), and 100% (4270 mg/kg/day) Octoxynol-9, respectively, on GDs 6 through 15. Octoxynol-9 had a pronounced effect on fetal development, producing a number of skeletal abnormalities, notably an increase in the incidence of supernumerary ribs arising from the lumbar and cervical regions. Compared to controls, the only statistically significant increases in skeletal variations occurred in the 4270 mg/kg/day dose group. Statistically significant increases in the incidence of atelectasis were noted in 1600 and 4270 mg/kg/day dose groups.

In the same study, two groups of 27 Sprague-Dawley rats received dietary concentrations of 0.06% Octoxynol-9 (dose = 70 mg/kg/day) and 0.3% Octoxynol-9 (dose = 340 mg/kg/day), respectively, on GDs 6 through 16. Octoxynol-9 had a pronounced effect on fetal development, producing a number of skeletal abnormalities. Statistically significant increases in skeletal variations were observed only in the 340 mg/kg/day dose group. Regarding the preceding two experiments (oral and dermal administration), the authors noted that the toxicological significance of the skeletal abnormalities observed was unclear. Furthermore, in both experiments, the NOEL for Octoxynol-9 was 1600 mg/kg/day for maternal toxicity and 70 mg/kg/day for developmental toxicity.

Octoxynol-9 also did not induce developmental toxicity in female specific pathogen–free CD-1 mice dosed daily (gavage, 800 mg/kg/day) on GDs 6 through 13. The following parameters were evaluated: number of viable litters, liveborn per litter, percentage survival, birth weight per pup, and weight gain per pup.

The intravaginal administration of Octoxynol-9 to female Sprague Dawley COBS CD rats at doses up to 5.0 mg/kg/day on GDs 6 through 15 did not induce teratogenicity or embryotoxicity. No statistically significant differences in testes weight/body weight ratios were noted between male albino rats that received 5% Octoxynol-40 in the diet daily for three months and controls. Similar results were reported for male albino rats in another study in which dietary concentrations of Octoxynol-40 up to 1.4% were administered daily for 3 months or 2 years. In the same study, no adverse effect on testes weight/body weight ratios were noted in Beagle dogs that received concentrations of Octoxynol-40 up to 5.0% in the diet daily for 3 months.

In an in vitro test, Octoxynol-9 (0.24 mg/ml) totally immobilized all human spermatozoa within 20 sec.

In one study (462 pregnant women), the use of Nonoxynol-9 or Octoxynol spermicides by female subjects did not result in an increase in the overall risk of malformations. The results of another study indicated a positive association between vaginal spermicide use and the following congenital disorders: limb reduction deformities, neoplasms, chromosomal abnormalities, and hypospadias. The number of pregnant females involved in the study that used a spermicide was 4772. Octoxynol spermicides accounted for 80% of the spermicide use, and, Nonoxynol-9, 20%. It is important to note that the latter study was considered flawed and that an FDA Fertility and Maternal Health Advisory Committee concluded that no increased risk of birth defects is associated with the use of spermicides.

In a human skin irritation study, two formulations containing 2.0% Octoxynol-9 were classified as moderately irritating and minimally irritating, respectively, in a 24-h single-insult, occlusive patch test. The different results were attributed to differences in the skin penetration of Octocynol-9 in one formulation versus the other. Octoxynol-9 (1.0%) was classified as a nonirritant in a study in which nine subjects were patch tested (polypropylene chamber) for four consecutive days.

In comedogenicity studies, comedones were observed in 3 of 6 subjects with severe acne patch tested with 0.25% Octoxynol-9 and in 2 of 20 subjects (with acne or history of acne) patch tested with 0.25% Octoxynol-9.

The skin sensitization potential of Octoxynol-1, -3, -5, -9, and -13 was evaluated using 50 subjects. Neither test substance induced irritation, and only Octoxynol-1 induced sensitization (2 subjects). A foot gel containing 8.0% Octoxynol-9 induced neither skin irritation nor sensitization in a repeated insult patch test (semiocclusive patches) involving 103 subjects. In a repeat-insult patch test (occlusive patches) involving 102 subjects, 0.5% Octoxynol-9 was not classified as a sensitizer. However, reactions with a score of 1 or greater were observed in seven subjects during induction. In a study evaluating the sensitization potential of 0.1% Octoxynol-9 in 206 subjects, occlusive patches containing the test substance remained in place for 6 days, and 48-h challenge patches were applied after a 2-week nontreatment period. No sensitization reactions were observed.

In a case report on a uranium mill maintenance worker with allergic contact dermatitis who was found to be sensitive to Nonoxynol-6, no reaction to 0.5% Octoxynol-9 was observed after 48 h of contact.

Nonoxynols

Data on the safety of nonoxynols were included throughout the report because of the close chemical structure relationship with octoxynols and the belief that safety data on nonoxynols would be applicable to octoxynols.

Nonoxynols absorb ultraviolet radiation, but only at wavelengths below 290 nm, with an absorption tail above 290 nm. These ingredients may contain residues of ethylene oxide, nonylphenol (unreacted C_9), and 1,4-dioxane.

The skin penetration of nonoxynols varies inversely as a function of the chain length, but the levels actually absorbed are low (0.13, 0.15, and 0.10 $\mu g/cm^2$ for Nonoxynol-2, -4, and -9, respectively).

The LD_{50} of Nonoxynol-5 in an acute oral toxicity study in rats ranged from 3500 to 4500 mg/kg, but the dermal LD_{50} was not reached in an acute dermal toxicity study at 2 g/kg.

The LD_{50} of Nonoxynol-6 in an acute oral toxicity study in rats was 1.98 g/kg, but the dermal LD_{50} was not reached in an acute dermal toxicity study at 3 g/kg.

In rats, morphological and biochemical changes in the liver, e.g., increase in the amount of rough endoplasmic reticulum, were found with intraperitoneal injections of 50 mg/kg Nonoxynol-9 daily for 5 days. Intravaginal placement of the same dose produced similar results in the liver, and biochemical changes in the kidney.

Nonoxynols are severe ocular irritants in test animals. Nonoxynol-5 and -6 were skin irritants in test animals, but Nonoxynol-6 was not a skin sensitizer. Irritation of the vaginal mucosa in rabbits by Nonoxynol-9 is a function of concentration; concentrations of 5% or less produced only mild irritation.

In a double-blind immunotoxicity study, mice were given intraperitoneal injections of 0.2 ml of 0.2% Nonoxynol-9 for 24 days. No effect was found in treated animals on leucocyte counts, primary and secondary anti-SRBC titers, and serum IgM and IgG concentrations. Decreased body weight, reductions in liver size, and enlargement of the spleen were found.

Nonoxynol-9 was cytotoxic to rat liver cells in culture and to sperm, but was not mutagenic in the Ames test. Nonoxynol-9 did induce cell transformation in two mouse cell transformation systems as a function of concentration and duration of exposure. Another study failed to demonstrate malignant transformation. Nonoxynol-9 was not carcinogenic in a lifetime study involving rats.

The intraperitoneal administration of Nonoxynol-9 at doses ranging from 20 to 60 mg/kg for 5 days did not cause an increase in the frequency of morphologically abnormal sperm over that observed in the control group. Intravaginal doses of Nonoxynol-9 (5 mg/100 g body weight) on GDs 3 and 7 caused significant differences in the number of normal implantation sites and the number of resorption sites between experimental and control groups. Nonoxynol-10 (600 mg/kg/day) did not induce developmental toxicity in female mice dosed orally on days 6 through 13 of gestation.

Nonoxynol-9 was embryolethal and fetocidal, but not teratogenic, when administered intravaginally (2.5 mg/100 g body weight) to groups of pregnant rats on days 1 through 10 of gestation. In another study, the no-effect level for Nonoxynol-9 in an oral teratogenicity study was 50 mg/kg/day (GDs 9 to 15); doses up to 500 mg/kg/day were administered. Nonoxynol-30 induced neither reproductive nor teratogenic effects on the skeleton and soft tissues of female rats at doses of 50, 250, and 1000 mg/kg/day.

When doses of 50 and 500 mg/kg/day Nonoxynol-9 (GDs 6 to 15) were administered dermally to female rats, no dose-related effects on skeletal and soft tissues were observed; however, a significant increase in extra ribs was observed only in the 50 mg/kg dose group. In another study, it was concluded that Nonoxynol-9 (in a contraceptive cream) was neither embryotoxic nor teratogenic when administered intravaginally to female rats at doses up to 40 mg Nonoxynol/kg/day on days 6 through 15 of gestation.

The oral administration of a product containing 14.0% Nonylphenoxy polyethoxy ethanol (a Nonoxynol; number of moles ethylene oxide not stated) on days 7 to 16 of gestation did not result in any significant differences in fetal malformations between experimental and control groups. Doses up to 50 mg/kg/day were tested.

Individual patients enrolled in clinical tests of the spermicidal use of Nonoxynol-9 reported vaginal irritation and itching and/or disruption of the epithelium and bleeding. One patient reported severe edematous reaction of the cervix. All symptoms resolved within 1 week after the treatment was discontinued. The only hematologic parameter reported was a reduction in serum cholesterol. No liver function changes were seen.

Nonoxynol-2 (5% in mineral oil), Nonoxynol-2 (10% in mineral oil), and Nonoxynol-4 (10% in mineral oil) were tested in three separate human repeat-insult patch tests. There was no evidence of allergic contact dermatitis in any of the 102 subjects patch tested with 5% Nonoxynol-2 in mineral oil. Allergic contact dermatitis was observed in 9 of 103 subjects patch tested with 10% Nonoxynol-2 in mineral oil and in 3 of 107 subjects patch tested with 10% Nonoxynol-4 in mineral oil.

Strong sensitization reactions were observed in each of twelve contact dermatitis patients patch tested with a 2.0% aqueous solution of Nonoxynol-8.3, Nonoxynol-9, or Nonoxynol-10. Ten of the patients had used antiseptic preparations containing Nonoxynol-9, and the remaining 2, antiseptic preparations containing Nonoxynol-8.3 and Nonoxynol-10, respectively. Six of the 12 patients were retested several months later, and each of the 6 had a sensitization reaction to 2% aqueous Nonoxynol-6, -8.3, -9, -10, -14, or -18.

Photosensitization reactions to diluted Nonoxynol-10 (concentrations up to 2% aqueous), but not to undiluted Nonoxynol-10, were reported in two patients who had been treated with an antiseptic preparation that contained Nonoxynol-10. Follow-up studies on 32 control subjects who had never used this antiseptic preparation showed an inconsistent pattern of reactions.

Allergic reactions to Nonoxynol-6 were noted in two case reports. However, no reactions were observed when 8 and 165 control subjects were patch tested with 0.5% and 1.0% Nonoxynol-6, respectively.

DISCUSSION

The CIR Expert Panel considered that octoxynols and nonoxynols are sufficiently similar in chemical structure and effects that safety test data on nonoxynols are applicable to octoxynols. Previously, the Panel concluded that the long chain-length nonoxynols are safe as used. These data, combined with the available data on long-chain octoxynols, support the safety of long chain octoxynols.

There are several impurities that were found in nonoxynols that raise concerns regarding their possible presence in octoxynols. For example, nonoxynols may contain trace amounts of ethylene oxide and 1,4-dioxane. The IARC has concluded that ethylene oxide is carcinogenic to humans and that 1,4-dioxane is possibly carcinogenic to humans. Nonoxynol-1 may contain up to 20 ppm ethylene oxide, and, Nonoxynol-6, up to 35 ppm. The Panel had previously concluded that the ethylene oxide content of nonoxynols in cosmetic products should not result in ethylene oxide exposures that approach 0.1 mg/day. The same admonition applies to octoxynols in cosmetic products. The Panel also had previously expressed concern over unreacted C_9 phenols that can be present in nonoxynols, and noted that such impurities should not be present at toxic concentrations; the same applies to octoxynols.

Again considering the safety test data on nonoxynols, the CIR Expert Panel had previously noted the potential for these ingredients as skin sensitizers. In human repeat-insult patch tests, there was no evidence of allergic contact dermatitis in any of the 102 subjects patch tested with 5% Nonoxynol-2 in mineral oil. However, allergic contact dermatitis was observed in 9 of 103 subjects patch tested with 10% Nonoxynol-2 in mineral oil and in 3 of 107 subjects patch tested with 10% Nonoxynol-4 in mineral oil. In in vitro skin penetration studies using cadaver skin (rinse-off and leave-on protocols), the total skin penetration of Nonoxynol-2, -4, and -9 was less than 1% over a period of 48 h. Based on the human repeat-insult patch test data and the results of in vitro skin penetration studies, the Panel had previously determined that cosmetic use concentrations of Nonoxynol-2 and -4 and other low-molecular-weight Nonoxynols (not greater than Nonoxynol-8) should be limited to $\leq$5% in leave-on products. The available clinical safety test data on octoxynols is consistent with that finding, so the Panel concluded that the same limitation applies to octoxynols.

Due to the severity of ocular irritation reactions that was observed in animal studies, the Panel had previously concluded that products containing certain short-chain-length Nonoxynols, Nonoxynol-1, -5, and -6, and, perhaps, other low-molecular-weight Nonoxynols, should not be used in the area surrounding the eyes. Again, the ocular toxicity data available for octoxynols are consistent with a concern about ocular damage and the admonition to avoid using in products intended for use in the area surrounding the eyes is repeated for octoxynols.

In comedogenicity studies, comedones were observed in 3 of 6 subjects with severe acne patch tested with 0.25% Octoxynol-9 and in 2 of 20 subjects (with acne or history of acne) patch tested with 0.25% Octoxynol-9. The Panel concluded that the results do not suggest a safety concern because the test substance was applied to the back (an atypical site for comedogenicity testing) and under occlusion (which is not indicative of how cosmetic products are generally applied), and because skin irritation was not reported. The Panel noted that the positive findings may be attributed to folliculitis that resulted from occlusion.

Reportedly, alkylphenol ethoxylates (which include the octoxynols) and related compounds are estrogenic. The Panel concluded, however, that the octoxynol-induced estrogenic effect anticipated from a cosmetic product would be of very low potency and that any effect would be further minimized given the relative lack of dermal absorption of the octoxynols and the proposed concentration limit of 5% for Octoxynols-1 through -8 in leave-on cosmetic products.

The Panel is aware of acute/short-term inhalation studies indicating moderate pulmonary edema, pneumonitis, and alveolar/bronchiolar epithelial hyperplasia in animals after exposure to an aerosol containing Octoxynol-9 (MMAD = 1.5 or 1.8 μm). The Panel determined, however, that Octoxynol-9 can be used safely in hair sprays, because the particle size associated with these products is not respirable. The Panel reasoned that the median aerodynamic diameter of 4.25 $\pm$ 1.5 μm for a respirable particulate mass was small compared to the particle

sizes of anhydrous hair sprays (60 to 80 μ and pump hair sprays (>80 μm).

After reviewing reproductive and developmental toxicity data indicating an increased number of supernumerary ribs among fetuses of Sprague-Dawley CD rats that received relatively high doses of Octoxynol-9 (1600 mg/kg and above), the Panel reasoned that these doses are much higher than those anticipated for human exposure to a rinse-off or leave-on cosmetic product containing Octoxynols at concentrations less than 5.0% (typical use concentrations). Furthermore, the Panel did not consider the increased incidence of supernumerary ribs to be problematic, noting that this finding was an exaggeration of a very common birth defect that is found in some strains of mice (e.g., CD-1 mice) and that supernumerary ribs is a common finding in rat teratology studies that is not necessarily a manifestation of a teratogenic effect.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concluded that Octoxynol-9, -10, -11, -12, -13, -16, -20, -25, -30, -33, -40, and -70, Octoxynol-9 Carboxylic Acid, Octoxynol-20 Carboxylic Acid, Potassium Octoxynol-12 Phosphate, and Sodium Octoxynol-9 Sulfate are safe as used in rinse-off and leave-on cosmetic products. The Panel also concluded that Octoxynol-1, -3, -5, -6, -7, and -8, Sodium Octoxynol-2 Ethane Sulfonate, Sodium Octoxynol-2 Sulfate, and Sodium Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations of $\leq$5% in leave-on cosmetic products.

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Concentration of Use by FDA Product Category[1]*

Octoxynol-1
Octoxynol-3
Octoxynol-5
Octoxynol-6
Octoxynol-7
Octoxynol-8
Octoxynol-9
Octoxynol-10
Octoxynol-11
Octoxynol-12
Octoxynol-13
Octoxynol-16
Octoxynol-20
Octoxynol-25
Octoxynol-30
Octoxynol-33
Octoxynol-40
Octoxynol-70
Octoxynol-9 Carboxylic Acid
Octoxynol-20 Carboxylic Acid
Potassium Octoxynol-12 Phosphate
Sodium Octoxynol-2 Ethane Sulfonate
Sodium Octoxynol-2 Sulfate
Sodium Octoxynol-6 Sulfate
Sodium Octoxynol-9 Sulfate

Ingredient	Product Category	Maximum Concentration of Use
Octoxynol-9	Disposable wipes	0.36%
Octoxynol-9	Face and neck products (not spray) Leave-on	0.22%

*The 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 2025
Table prepared: March 27, 2025

[1] The new FDA cosmetic product categories under MoCRA were used for this survey.