Amended Safety Assessment of Octoxynols as Used in Cosmetics

Status:	Draft Amended Report for Panel Review
Release Date:	November 9, 2023
Panel Meeting Date:	December 4-5, 2023

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.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, 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. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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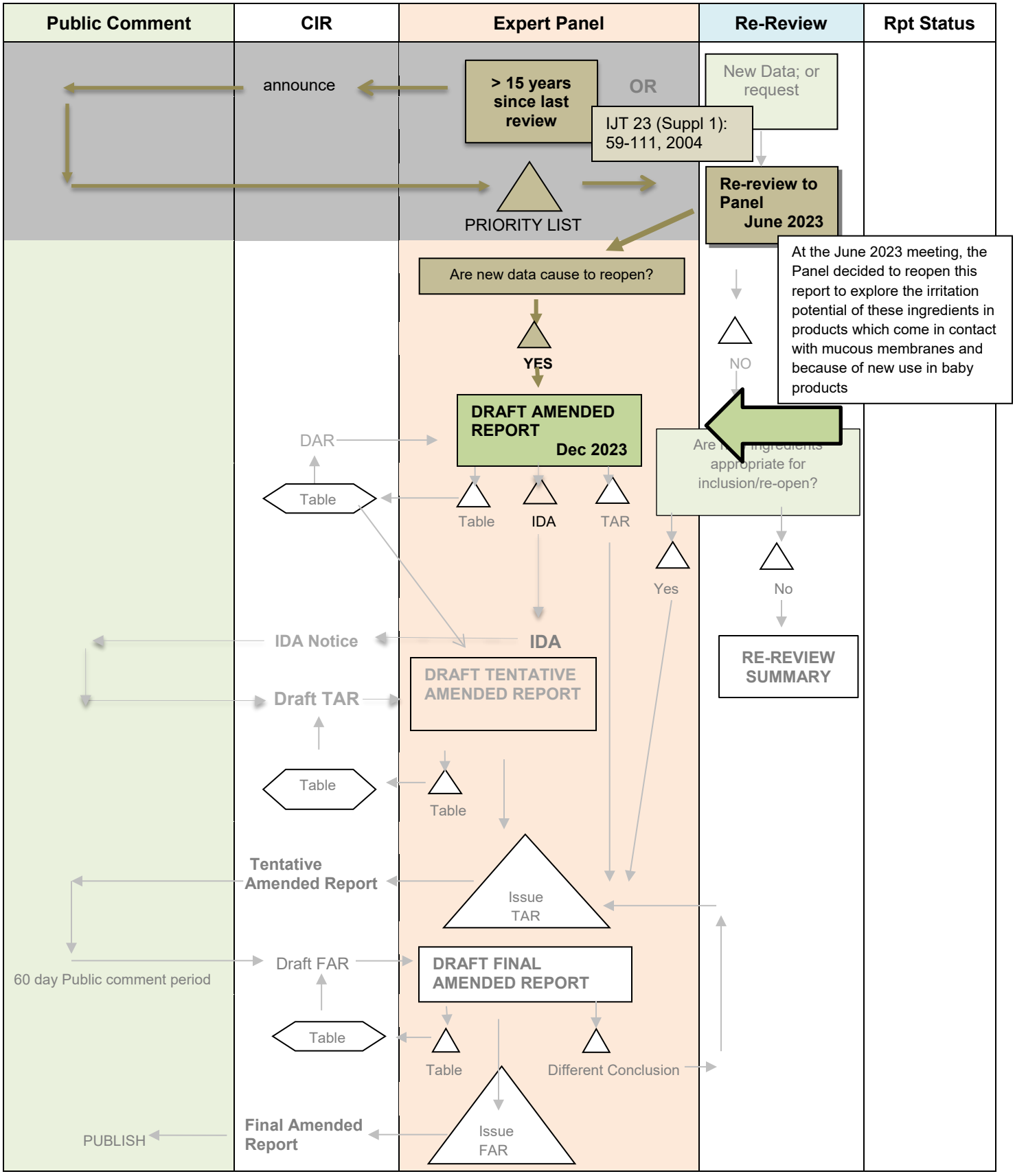
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Octoxynols

MEETING December 2023



Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Preethi Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR
Date: November 9, 2023
Subject: Amended Safety Assessment of Octoxynols as Used in Cosmetics

Enclosed is the Draft Amended Report on the Safety Assessment of Octoxynols as Used in Cosmetics. (It is identified as *report_Octoxynols_122023* in the pdf document). The Panel first published a final report on these 25 ingredients in 2004, with the conclusion that based on the animal and clinical data included in the report, 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 ≤ 5% in leave on cosmetic products (*originalreport_Octoxynols_122023*). At its June 2023 meeting, the Panel decided to reopen this safety assessment to explore the mucous membrane irritation potential of these ingredients and due to newly reported use of Octoxynol-9 at 0.1% in other baby products.

Since the last review, reported frequencies and concentrations of use for these ingredients have decreased greatly. According to 2023 VCRP data, Octoxynol-11 is reported to have the greatest frequency of use, in 8 formulations; in 2021, it was reported to have 19 uses. In 2001, Octoxynol-9 was reported to be used in 131 formulations; however, according to 2023 VCRP data, it is only reported to now be used in 5 formulations. At the time of the original review, Octoxynol-10 had the greatest concentration of use, with a maximum concentration of use of 25% in hair lighteners with color; currently, no concentrations of use are reported for this ingredient. Results of the concentration of use survey conducted by the Council in 2022 indicate that Octoxynol-9 has the highest reported maximum concentration of use, at up to 2% in skin cleansing preparations; in 2001, Octoxynol-9 was reported to be used at up to 5% in cologne and toilet water formulations. The highest currently reported concentration of use resulting in leave-on dermal exposure is 1.5% Octoxynol-12 in face and neck preparations. Risk assessments for both 2% Octoxynol-9 in skin cleansing preparations and .5% Octoxynol-12 in face and neck preparations have been prepared by Dr. Zhu and are included in the report for the Panel's consideration.

The Panel has published reviews on the safety of nonoxynols in 1983, 1999, and in 2015. In the original safety assessment of octoxynols, the Panel relied on the chemical similarity of these ingredients (1 carbon longer) to support the safety of octoxynols. Therefore, when data on octoxynols are absent, supporting data on nonoxynols has been included, as was done in the previous safety assessment of octoxynols; data from the 2015 final amended report on nonoxynols (*nonoxynols2015_Octoxynols_122023*) have also been included for potential read-across sources, as appropriate.

Additional supporting documents for this report package include: a flow chart (*flow_Octoxynols_122023*), report history (*history_Octoxynols_122023*), search strategy (*search_Octoxynols_122023*), a data profile (*dataprofile_Octoxynols_122023*), the minutes from past meetings at which Octoxynols was originally discussed (*originalminutes_Octoxynols_122023*), concentration of use data (*data_Octoxynols_122023*), and transcripts from the previous meeting at which the rereview of octoxynols was discussed (*transcripts_Octoxynols_122023*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

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).

Octoxynols Data Profile* – December 2023 – Preethi Raj

				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					X	O		O						O						X	O		X	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

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

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
total # of hits/total # useful

Search Strategy

General Search

octoxynol-9 vaginal tissue damage – 333,000 hits/1 useful
triton x-100 estrogeno-mimetic – 568,000 hits/2 useful

estrogenic effects alkylphenol ethoxylates – 250,000/6 useful
vaginal irritation; mucous membrane irritation – 2/0 useful

PubMed – last search performed 10/2023 - *total # of hits / # hits that were useful*]
Octoxynol-9 vaginal use – 18 hits/5 hits useful (old)
Octoxynol douche – 6 hits/1 useful
Octoxynol endocrine disruption – 12 hits/0 useful
Triton x-100 AND (2000:2024[pdat]) – 6,872 hits/ 7 useful
Triton-x-100 endocrine disruption – 19 results/0 useful
Triton x-100 douche- 16 results/2 useful
Triton x-100 irritation – 39 results/ 6 useful
Triton x-100 endothelial disruption – 24 results/ 0 useful
Triton x-100 mimic estrogen – 2 hits/ 0 useful
Triton x-100 endocrine disruption – 19 results/0 useful
Nonoxynol estrogen – 11 results/0 useful
Nonoxynol endocrine disruption – 3 results/0 useful
Estrogen octylphenols cancer – 77 hits/6 useful
Estrogen octoxynol cancer – 10 hits/ 0 useful

Octoxynol-1: ((((((((((octoxynol-1) OR (9002-93-1)) OR (9036-19-5)) OR (9004-87-9)) OR (2315-67-5)) OR (2-[p-(1,1,3,3-Tetramethylbutyl)Phenoxy]Ethanol)) OR (Ethanol, 2-[p-(1,1,3,3-Tetramethylbutyl)Phenoxy]- Ethylene Glycol Octyl Phenyl Ether)) OR (octoxinol)) OR (Octoxynol)) OR (PEG-1 Octyl Phenyl Ether)) OR (Polyoxyethylene Octylphenyl Ether)) OR (Igepal CA-210**)** – 6,071 hits/3 useful

Octoxynol-3: ((((((((((((octoxynol-3) OR (9002-93-1)) OR (9036-19-5)) OR (9004-87-9)) OR (27176-94-9)) OR (2315-62-0)) OR (2-[2-[2-(Octylphenoxy)Ethoxy]Ethoxy]Ethanol)) OR (2-[2-[2-[p-(1,1,3,3- Tetramethylbutyl)Phenoxy]Ethoxy]Ethoxy]Ethanol)) OR (Ethanol, 2-[2-[2-(Octylphenoxy)Ethoxy]Ethoxy]-)) OR (Ethanol, 2-[2-[2-[p-(1,1,3,3- Tetramethylbutyl)Phenoxy]Ethoxy]Ethoxy]-)) OR (PEG-3 Octyl Phenyl Ether)) OR (Polyethylene Glycol (3) Octyl Phenyl Ether)) OR (Polyoxyethylene (3) Octyl Phenyl Ether)) OR (Triethylene Glycol Octylphenyl Ether)) OR (Igepal CA-420) AND (1999:2023[pdat]) – 2960 hits/3 useful

Octoxynol-5: ((((((((((((octoxynol-5) OR (2315-64-2)) OR (27176-99-4)) OR (14-(Octylphenoxy)-3,6,9,12-Tetraoxatetradecan-1-ol)) OR (14-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-3,6,9,12- Tetraoxatetradecan-1-ol)) OR (3,6,9,12-Tetraoxatetradecan-1-ol, 14-(Octylphenoxy)-)) OR (3,6,9,12-Tetraoxatetradecan-1-ol, 14-[4-(1,1,3,3- Tetramethylbutyl)Phenoxy]- octoxinol)) OR (PEG-5 Octyl Phenyl Ether)) OR (Pentaethylene Glycol p-tert-Octylphhenyl Ether)) OR (Polyethylene Glycol (5) Octyl Phenyl Ether)) OR (Polyoxyethylene (5) Octyl Phenyl Ether)) OR (Igepal CA-520)) OR (Norfox OP-45) AND (1999:2023[pdat]) – 79 hits/1 useful

Octoxynol-6: (((((((octoxynol-6) OR (octoxinol)) OR (Octoxynol)) OR (Polyethylene Glycol (6) Octyl Phenyl Ether)) OR (Polyoxyethylene (6) Octyl Phenyl Ether)) OR (9002-93-1)) OR (9036-19-5)) OR (9004-87-9) AND (1999:2023[pdat]) – 2878 hits/2 useful

Octoxynol-7: (((((((octoxynol-7) OR (27177-02-2)) OR (20-(Octylphenoxy)-3,6,9,12,15,18-Hexaoxaeicosan-1-ol)) OR (3,6,9,12,15,18-Hexaoxaeicosan-1-ol, 20-(Octylphenoxy)-)) OR (Heptaethylene Glycol Octylphenyl Ether)) OR (PEG-7 Octyl Phenyl Ether)) OR (Polyethylene Glycol (7) Octyl Phenyl Ether)) OR (Polyoxyethylene (7) Octyl Phenyl Ether)) OR (Igepal CA-620) – 1 hit/0 useful

Octoxynol-8: (((((((octoxynol-8) OR (3520-90-9)) OR (2638-43-9)) OR (23-(4-Octylphenoxy-3,6,9,12,15,18,21-Heptaoxatricosan-1-ol)) OR (3,6,9,12,15,18,21-Heptaoxatricosan-1-ol, 23-(4-Octylphenoxy)-)) OR (Octaethylene Glycol Octylphenyl Ether)) OR (PEG-8 Octyl Phenyl Ether)) OR (Polyethylene Glycol 400 Octyl Phenyl Ether)) OR (Polyoxyethylene (8) Octyl Phenyl Ether) AND (1999:2023[pdat]) – 35 hits/0 useful

Octoxynol-9: ((((((((((((octoxynol-9) OR (9010-43-9)) OR (42173-90-0)) OR (59935-87-4)) OR (2315-65-3)) OR (26-(4-Octylphenoxy)-3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol)) OR (26-(Octylphenoxy)-3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol)) OR (3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol, 26-(4-Octylphenoxy)-)) OR (3,6,9,12,15,18,21,24-Octaoxahexacosan-1-ol, 26-(Octylphenoxy)-)) OR (Nonaethylene Glycol Octylphenyl Ether)) OR (PEG-9 Octyl Phenyl Ether)) OR (Polyethylene Glycol 450 Octyl Phenyl Ether)) OR (Polyoxyethylene (9) Octyl Phenyl Ether)) OR (Igepal CA-630)) OR (Jeechem OP-9)) OR (Protachem OP-9) AND (1999:2023[pdat]) – 3,034 hits/4 useful

Octoxynol-10: ((((((((((((octoxynol-10) OR (2315-66-4)) OR (27177-07-7)) OR (29-(Octylphenoxy)-3,6,9,12,15,18,21,24,27-Nonaoxanonacosan-1-ol)) OR (29-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-3,6,9,12,15,18,21,24,27-Nonaoxanonacosan-1-ol)) OR (3,6,9,12,15,18,21,24,27-Nonaoxanonacosan-1-ol, 29-(Octylphenoxy)-)) OR (3,6,9,12,15,18,21,24,27-Nonaoxanonacosan-1-ol, 29-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-)) OR (Decaethylene Glycol Octylphenyl Ether)) OR (PEG-10 Octyl Phenyl Ether)) OR (Polyethylene Glycol 500 Octyl Phenyl Ether)) OR (Polyoxyethylene (10) Octyl Phenyl Ether)) OR (Jeechem OP-10)) OR (Norfox OP-100) AND (1999:2023[pdat]) -119 hits/0 useful

Octoxynol-11: (((((octoxynol-11) OR (108437-62-3)) OR (32-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-3,6,9,12,15,18,21,24,27,30-Decaoxadotriacontan-1-ol)) OR (PEG-11 Octyl Phenyl Ether)) OR (Polyethylene Glycol (11) Octyl Phenyl Ether)) OR (Polyoxyethylene (11) Octyl Phenyl Ether) – 1 hit/0 useful

Octoxynol-12: (((octoxynol-12) OR (PEG-12 Octyl Phenyl Ether)) OR (Polyethylene Glycol 600 Octyl Phenyl Ether)) OR (Polyoxyethylene (12) Octyl Phenyl Ether) – 4 hits/ 0 useful

Octoxynol-13: (((((((octoxynol-13) OR (3,6,9,12,15,18,21,24,27,30,33,36-Dodecaoxatriacontan-1-ol, 38-[4-(1,1,3,3,-Tetramethylbutyl)Phenoxy]-)) OR (38-[4-(1,1,3,3-Tetramethylbutyl)Phenoxy]-3,6,9,12,15,18,21,24,27, 30,33,36-Dodecaoxaoctatriacontan-1-ol)) OR (PEG-13 Octyl Phenyl Ether)) OR (Polyethylene Glycol (13) Octyl Phenyl Ether)) OR (Polyoxyethylene (13) Octyl Phenyl Ether)) OR (Igepal CA-720)) OR (Igepal CA-730)) OR (Protachem OP-13) – 11 hits/ 0 useful

Octoxynol-16: (((octoxynol-16) OR (PEG-16 Octyl Phenyl Ether)) OR (Polyethylene Glycol (16) Octyl Phenyl Ether)) OR (Polyoxyethylene (16) Octyl Phenyl Ether) – 1 hit/0 useful

Octoxynol-20: (((octoxynol-20) OR (PEG-20 Octyl Phenyl Ether)) OR (Polyethylene Glycol 1000 Octyl Phenyl Ether)) OR (Polyoxyethylene (20) Octyl Phenyl Ether) – 1 hit/0 useful

Octoxynol-25: (((octoxynol-25) OR (PEG-25 Octyl Phenyl Ether)) OR (Polyethylene Glycol (25) Octyl Phenyl Ether)) OR (Polyoxyethylene (25) Octyl Phenyl Ether) – 1 hit/0 useful

Octoxynol-30: (((octoxynol-30) OR (PEG-30 Octyl Phenyl Ether)) OR (Polyethylene Glycol (30) Octyl Phenyl Ether)) OR (Polyoxyethylene (30) Octyl Phenyl Ether) – 1 hit/0 useful

Octoxynol-33: (((octoxynol-33) OR (PEG-33 Octyl Phenyl Ether)) OR (Polyethylene Glycol (33) Octyl Phenyl Ether)) OR (Polyoxyethylene (33) Octyl Phenyl Ether) – 20 hits/ 0 useful

Octoxynol-40: (((octoxynol-40) OR (PEG-40 Octyl Phenyl Ether)) OR (Polyethylene Glycol 2000 Octyl Phenyl Ether)) OR (Polyoxyethylene (40) Octyl Phenyl Ether) – 11 hits/ 0 useful

Octoxynol-70: (((octoxynol-70) OR (PEG-70 Octyl Phenyl Ether)) OR (Polyethylene Glycol (70) Octyl Phenyl Ether)) OR (Polyoxyethylene (70) Octyl Phenyl Ether) – 1 hit/0 useful

Octoxynol-9 Carboxylic Acid: ((((((octoxynol-9 carboxylic acid) OR (25338-58-3)) OR (26-(Octylphenoxy)-3,6,9,12,15,18,21,24-Octaoxahexacosanoic Acid)) OR (3,6,9,12,15,18,21,24-Octaoxahexacosanoic Acid, 26-(Octylphenoxy)-)) OR (PEG-9 Octyl Phenyl Ether Carboxylic Acid)) OR (Polyethylene Glycol 450 Octyl Phenyl Ether Carboxylic Acid)) OR (Polyoxyethylene (9) Octyl Phenyl Ether Carboxylic Acid) AND (1999:2023[pdat]) – 256 hits/ 0 useful

Octoxynol-20 Carboxylic Acid: (((octoxynol-20 carboxylic acid) OR (PEG-20 Octyl Phenyl Ether Carboxylic Acid)) OR (Polyethylene Glycol 1000 Octyl Phenyl Ether Carboxylic Acid)) OR (Polyoxyethylene (20) Octyl Phenyl Ether Carboxylic Acid) – 1 hit/0 useful

Potassium Octoxynol-12 Phosphate: potassium octoxynol-12 phosphate – 1 hit/0 useful

Sodium Octoxynol-2 Ethane Sulfonate: (((((((sodium octoxynol-2 ethane sulfonate) OR (2917-94-4)) OR (55837-16-6)) OR (67923-87-9)) OR (2-[2-[2-(Octylphenoxy)Ethoxy]Ethoxy]Ethanesulfonic Acid, Sodium Salt)) OR (Sodium Octoxynol-3 Sulfonate)) OR (Sodium Octylphenoxy Diethoxyethyl Sulfonate)) OR (entsufon) – 1 hit/0 useful

Sodium Octoxynol-2 Sulfate: (((sodium octoxynol-2 sulfate) OR (PEG-2 Octyl Phenyl Ether Sulfate, Sodium Salt)) OR (Polyethylene Glycol (2) Octyl Phenyl Ether Sulfate, Sodium Salt)) OR (Polyoxyethylene (2) Octyl Phenyl Ether Sulfate, Sodium Salt) – 1 hit/0 useful

Sodium Octoxynol-6 Sulfate: (((Sodium Octoxynol-6 Sulfate) OR (PEG-6 Octyl Phenyl Ether Sulfate, Sodium Salt)) OR (Polyethylene Glycol 300 Octyl Phenyl Ether Sulfate, Sodium Salt)) OR (Polyoxyethylene (6) Octyl Phenyl Ether Sulfate, Sodium Salt) – 1 hit/0 useful

Sodium Octoxynol-9 Sulfate: (((sodium octoxynol-9 sulfate) OR (PEG-9 Octyl Phenyl Ether Sulfate, Sodium Salt)) OR (Polyethylene Glycol 450 Octyl Phenyl Ether Sulfate, Sodium Salt)) OR (Polyoxyethylene (9) Octyl Phenyl Ether Sulfate, Sodium Salt) – 299 hits/0 useful

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/
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JUNE 2023 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – June 12, 2023

DR. BELSITO: Octoxynols. This was a Wave 2, again, from Women's Voice for the Earth. And this has to do with -- I mean, their biggest complaint was -- I mean, we dealt with this with nonoxynol-9 as well as vaginal epithelial disruption. And the data that they sent were -- some of it was on triton X and some of it was on octoxynol-9. But I'm not sure, Preethi, in your search, I didn't see that you were searching for triton X-100. Did you do that?

Because otherwise you should've picked up their report. No?

MS. RAJ: I may have come across it but most of the stuff that I came across was for ocular uses. But it was more like injecting the eye in various kind of ocular uses, which were not relevant to cosmetic safety.

DR. BELSITO: If you didn't search using triton X-100 -- triton X-100, when I looked at the old report, we looked at that. So, triton X-100 is laboratory grade and octoxynol-9 is pharmaceutical grade of this same product. It's not clear to me how pure laboratory grade is versus the purity of pharmaceutical grade. But then in at least one of the studies on octoxynol it said triton X, it then said octoxynol-9 was the ingredient that was used.

So, I just think we need to reopen this document as well because the data showed vaginal irritation at 2 percent. We don't know the concentration in 3 products. And I think they do have a good concern because after reading this I took a stroll to the CVS, which is one block from my apartment, and walked down an isle I never walk down, feminine hygiene. And literally Summer's Eve and CVS's equivalent to Summer's Eve dominated those shelves.

And Summer's Eve clearly doesn't report in to PCPC I don't think. And octoxynol-9 is listed as the second ingredient after water. It doesn't tell us what the concentration is. I mean, these -- the douche could be 99 percent water and 0.5 percent, but we need more information on that category in particular.

And there's new product use category I have, baby products it's used in, which is another reason to reopen it since it's used in baby products and I don't know -- 0.1 percent in baby products. So, I thought this report needs to be reopened, particularly, looking at information on the concentration and mucosally-applied products and really focusing on irritation.

I mean, I think that's the major issue here from what I could read. We could punt and say formulate to not be irritating, but I think we just need to look at all the new data, is my personal opinion.

DR. SNYDER: I concur with that.

DR. RETTIE: And I think the lab grades are all pretty pure. The one's I was using years ago were better than 95 percent.

DR. BELSITO: Yeah.

DR. RETTIE: I'm pretty sure it's pretty good.

DR. BELSITO: So, we're going to reopen. Make sure your searches include triton X-100.

MS. FIUME: So, it should've hit because it's the same CAS number as some of the CAS numbers in the monograph.

DR. BELSITO: So, searching using monograph. At least based on my google search it says the 9036-19-5, which is one of the generic CAS numbers for octoxynol-9.

DR. BELSITO: Okay.

MS. FIUME: It's the same CAS numbers. But we will make sure we include it, and we find all the data associated with it.

DR. BELSITO: Great, thank you. Okay.

Cohen Team – June 12, 2023

DR. COHEN: Okay. Octoxynols. Is that right, Octoxynols?

DR. HELDRETH: Octoxynols.

DR. COHEN: Okay. So, the Panel published a review of 25 Octoxynols ingredients in 2004, with a conclusion that the ones listed here are safe as used in rinse off and leave-on cosmetics. The Panel also concluded that Octoxynol-1, -3, -5, -6, -7, -8, Sodium Octoxynol-2 Ethane Sulfonate and some of the others listed here are safe as used in rinse off cosmetics and safe as concentration of less than 5 percent in leave on.

Octoxynol-40 has been FDA approved as an inactive ingredient in ophthalmic solutions at 0.05 percent. No new relevant tox data were found. We have 2023 uses in eight formulations. Highest concentration of use was 5 percent more recently, but in

2001 was 25 percent. In 2022, the highest concentration was 2 percent of number -9 in skin cleansing preparations. It's now reported to be used at 0.1 percent of baby products, a previously unreported use category.

We've also published on the safety of nonoxynol, which has a similar, slightly longer chain ingredient in 1983, 1999, and 2015. So, their conclusion was safe when formulated to be not irritating. So, we also had some Wave 2 comments. So, what's the group think? These are ethoxylated alkyl phenols surfactants. What would the group like to do? Tom?

DR. SLAGA: Do not reopen, same conclusion?

DR. BERGFELD: Yay.

DR. ROSS: No new data. decreased use, decreased concentration. And there was a discussion with WVE and the staff. But yeah, that's what I came down to.

DR. TILTON: Yeah, I just noted that concentrations in the use category did not warrant reopening.

DR. COHEN: Looks like we have an unanimous, I had it as a do not reopen. I don't like to color it by just saying that in the introduction.

DR. COHEN: Any other comments or questions? That's ours for tomorrow.

Full Panel – June 13, 2023

DR. COHEN: The Panel published a review of the safety of 25 Octoxynols ingredients and concluded a large number of them listed in the report were safe as used in rinse-off and leave-on cosmetics. The Panel also concluded that numbers 1, 3, 5, 6, 7, 8 and Sodium Octoxynol-2 Ethane Sulfonate, and Octoxynol-2 Sulfate, and Octoxynol-6 Sulfate are safe as used in rinse-off cosmetic products and safe at concentrations less than five percent in leave-on.

We received no new toxicologic data. In 2001, the ingredient that had the greatest frequency of use was Octoxynol-9 with 131 uses and that is now down for the majority of them. And max use also came down quite a bit. Of note, the Panel published reviews on the safety of nonoxynols, slightly longer chain ingredient, in 1983, 1999 and 2015, that they were safe in present practices of use when formulated to be nonirritating. Our motion is to not reopen.

DR. BELSITO: We did receive new data from Women Voices for the Earth, which showed vaginal irritation.

DR. COHEN: Yeah.

DR. BELSITO: And we have products out there where Octoxynol-9 is the second lead ingredient after water. Now that may be that water is 99 percent of that vaginal douche, but we don’t know that. We thought we needed to reopen it and look at the data.

It also appears that Triton X-100 is the laboratory grade of Octoxynol-9, and that may not have been adequately searched for. So, there may be additional data out there that we need, so we wanted to reopen it.

DR. COHEN: Okay, given that information you just mentioned, I’ll revise my motion to reopen.

DR. BELSITO: Second.

DR. BERGFELD: Any further discussion? I’ll call the question, all those in favor of a reopening? Unanimous.

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 Amended Report for Panel Review
Release Date: November 9, 2023
Panel Meeting Date: December 4-5, 2023

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.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, 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. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CTFA	Consumer, Toiletry, Fragrance Association
DTH	delayed type hypersensitivity
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
EPP	ethylphenyl proprionate
ET_{50}	exposure time that reduces tissue viability to 50%
FCA	Freund's complete adjuvant
FDA	Food and Drug Administration
GRASE	generally recognized as safe and effective
HIV	human immunodeficiency virus
HRIPT	human repeat insult patch test
IgM	immunoglobulin M
IL-α	interleukin-1α
IL-β	interleukin-1β
LDH	lactate dehydrogenase
LD	lethal dose
MDSS	maximal primary Draize irritation score
MMAD	mass mean aerodynamic diameter
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
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
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SRBC	sheep red blood cells
SLS	sodium lauryl sulfate
TG	test guideline
TPA	12-*O*-tetradecanoylphorbol-13-acetate
US	United States
VEC	vaginal-ectocervical tissue model
VEC-100-FT	full thickness vaginal-ectocervical tissue model
VCRP	Voluntary Cosmetic Registration Program
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)

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 (*wINCI; *Dictionary)*, these ingredients are reported to function in cosmetics as surfactants (Table 1).[1] The Expert Panel for Cosmetic Ingredient Safety (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 yr, and it has been at least 15 yr 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 (e.g., Octoxynol-9 in spermicides and vaginally applied products) and due to the newly reported use of Octoxynol-9 at 0.1% in baby products.

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.[3-5] 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 October 2023. 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 Cosmetic Ingredient Review (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*. 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,[5] 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.)

CHEMISTRY

Definition and Structure

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

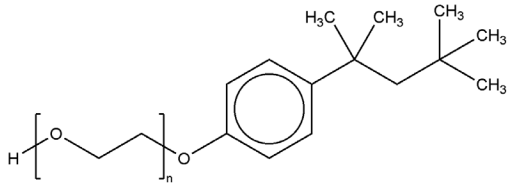


Figure 1. General formula for octoxynols, wherein "n" equals the number of ethoxy repeat units (e.g., n = 3 for Octoxynol-3)

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] While Octoxynol-1, -5, and -11 are soluble in polar organic solvents and insoluble in water, Octoxynol-9 is soluble in organic solvents and in water and has a an average molecular weight of 647 Da. Octoxynol-1 has a specific gravity of 0.980 – 0.990 while Octoxynol-30 has a specific gravity of 1.095 (both at 25 °C). Octoxynol-1 has a molecular weight of 250.38 g/mol.[6] Chemical properties of the octoxynols included in this report are presented in Table 2.

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

Specifications state 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] According to the National Formulary, 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.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, 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.

According to the 2023 VCRP survey data, Octoxynol-11 has the greatest reported frequency of use, in 8 formulations, all of which are leave-on products (Table 3).[7] Five other octoxynols are reported to the VCRP as in-use (5 formulations or less). The results of the concentration of use survey conducted by the Council in 2022 indicate Octoxynol-9 has the highest maximum reported concentration of use; it is used at 2% in skin cleansing preparations.[8] The highest reported concentration of use resulting in leave-on dermal exposure is 1.5% Octoxynol-12, in face and neck preparations; no concentration of use data were submitted for any other octoxynols. In 2001, Octoxynol-9 was the ingredient with the highest reported frequency of use (131); 25% Octoxynol-10 in hair lighteners with color, and 5% Octoxynol-9 in cologne and toilet water, were the maximum reported concentrations of use in 2001.[2] The ingredients not in use according to the VCRP and industry survey are listed in Table 4.

Octoxynol-9 and Octoxynol-11 have reported uses in products used near the eye, such as an eyeliner, eye lotion, and other eye makeup preparation; Octoxynol-12 has 2 reported uses in lipstick, a product that can be incidentally ingested. (Concentrations of use were not reported for any of these uses.) Several of the octoxynols are used in cosmetic formulations

that could possibly be in spray or powder form. 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 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. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients (and without consumer habits and practices data or particle size data related to this use technology), 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.[9]

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. Additionally, 21CFR201.325 states that when nonoxynol-9 is used in OTC vaginal contraceptives and spermicides, labeling requirements include a warning that the products do not protect against human immunodeficiency virus (HIV) or other sexually transmitted diseases, of the potential for vaginal/ rectal irritation, and of potentially increased risk of HIV transmission from an infected partner.

Octoyxnol-1 is commonly employed as a detergent in the manufacture of biotherapeutics, such as vaccines.[10,11] Octoxynol-40 has an FDA-approved drug use in ophthalmic solution drops at a maximum potency per unit dose of 0.05% w/v.[12] Additionally, Octoyxnol-40 is utilized in various (nanomicellar) ocular drug delivery formulations.[13-15]

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-tetramethylbutylpheonol, 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

Three guinea pigs were administered doses of Octoxynol-9, ranging from 5 – 20 ml/kg in a dermal absorption test.[2] No evidence of dermal absorption was observed.

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. 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.[16] 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 (ADME)

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.

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.

Octoxynol-1

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.[17] The cream comprised bees white wax, carnauba wax, and distilled water, as well as Octoxynol-1, 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 7 g/kg Octoxynol-20 (70%) and 28 g/kg 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 g) 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% Ocotoxnynol-9 (0/4), 0.05% Octoxynol-9 (1/8), 0.06% Octoxynol-9 (4/8), 0.075% Octoxynol-9 (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

nonoxynols

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.

Differences in organ-to-body weight ratios were not statistically significant in young male and female albino rats (15/sex) that received 5% Octoxynol-40, in the diet for 3 mo.[2] In another study, groups of young albino 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 kind, incidence, and degree of pathologic 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 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 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. 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 tested 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. 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. 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.

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.[16] 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 species 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. 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.

In a subchondral bone model system, knee joint complexes of Lewis rats (n =31) were continuously irrigated with 15% Octoxynol-9 solution for 36 h. Control femurs (n =10) 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. Sixty percent (6 of 10) of the control rats had a positive antibody response at 4-wk post transplantation. Based on Chi-square analysis, the immunogenicity of Octoxynol-9-irrigated grafts was significantly less (p =0.026) than irrigated controls.

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.[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.

Several metabolites of alkylphenols have been shown to mimic estrogen and may exhibit endocrine disruption effects. For instance, 4-*tert*-octylphenol has been shown to disrupt estrous cycles in rats[18] and promote changes in human breast cancer cells.[19,20] An estrogen-like response was also exhibited when human first trimester placenta and trophoblast-derived choriocarcinoma cells were exposed to *para*-nonylphenol.[21]

Cytotoxicity

Octoxynol-1

The reproducibility of an in vitro skin irritation test using a 3-D reconstructed skin model, EpiDerm™, was evaluated in repeated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and interleukin-1α (IL-1α) expression

assays.[22] Two test articles, both containing a mixture of anionic, amphoteric, and non-ionic surfactants, were included as benchmark materials (no further details provided). Octoxynol-1 was used as the assay positive control to assess the quality of the tissue lots in the experiments. Octoxynol-1-treated tissue demonstrated a reduced cell viability of 18.9 ± 10.6% after 4 h and 82.5 ± 10% after 8 h of exposure (compared to the mean percentages of viable tissues treated with the benchmark materials: 100.1 ± 7.1% and 96.9 ± 6.4%, respectively). Mean values for IL-α release after exposure to Octoxynol-1 for 4 and 8 h were 100 ± 44 pg/ml and 320 ±124 pg/ml (compared to 69 ± 25 pg/ml and 149 ± 53 pg/ml for the benchmark materials).

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

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.

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.

Octoxynol-1

The dermal irritation potential of a leather cream was evaluated in rabbits (6/group) in accordance with OECD TG 404.[17] The cream comprised bees white wax, carnauba wax, and distilled water, as well as silicone oil, linseed oil, Sudan black dye, nigrosine black dye, and Octoxynol-1 (amounts not specified). Animals received applications of either distilled water (controls), 0.8% w/v aq. formaldehyde (positive control), a laboratory-based sample of the leather cream, or the marketed leather cream to a shaved, 25 cm^2 area of the back (amount not specified). After 72 h of exposure, the test materials were removed from the test site and rinsed with distilled water. Test sites were evaluated using the Draize scoring system at 24, 48, and 72 h; primary irritation indexes (PII) were calculated. Positive controls produced expected results (PII = 9.99). The PII was calculated as 0 in the control, sample cream, and marketed cream group; the test article was not considered a dermal irritant.

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

Octoxynol-1

The sensitization potential of a leather cream was evaluated in guinea pigs (6/group) in accordance with OECD TG 406, and was modified as per the Buehler method.[17] The cream comprised bees white wax, carnauba wax, and distilled water, as well as silicone oil, linseed oil, Sudan black dye, nigrosine black dye, and Octoxynol-1 (amounts not specified). The animals were divided into negative controls, positive controls (treated with 0.1% w/v 1-chloro-2,4-dinitrobenzene (CDNB) in 10% propylene glycol), a group treated with a laboratory based sample of the leather cream, and a group tested with the marketed leather cream. On day 0, 0.1% w/v of CDNB was evenly spread over shaved skin. (Further details describing the application of test materials was not provided). Reactions were assessed 24 h after application. Positive controls produced expected results. No sensitization was observed in controls or in animals treated with either leather cream sample and no erythema or edema was observed.

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.

Human

The skin sensitization potential of 0.1% Octoxynol-9 was evaluated in a human repeat insult patch test (HRIPT) 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 another HRIPT, 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 (2×10^{-5} M) occurred after irradiation with ultraviolet light under aerobic conditions.[16] 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 2×10^{-5} M nonoxynol-9 or when the cells were incubated with 2×10^{-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.

Octoxynol-1

Ezrin expression was evaluated as a potential biomarker of eye irritation in an in vitro eye irritation test.[23] Octoxynol-1 and other well-known eye irritants, such as cetylpyridinium bromide, cyclohexanol, ethanol, 2-methyl-1-pentanol, and sodium hydroxide, were shown to significantly increase ezrin expression in immortalized human corneal cells. Induction of the ezrin promoter in irritant-treated cells was confirmed by a luciferase gene reporter assay, indicating that ezrin expression may be utilized as a biomarker for detecting ocular irritation in vitro.

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.[2] 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.

MUCOUS MEMBRANE IRRITATION STUDIES

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.

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.[16] 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.

A clinical trial of nonoxynol-9 (in gel form) was performed using 40 healthy female volunteers.[16] 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. 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 interleukin 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.

Octoxynol-1

An in vitro vaginal-ectocervival (VEC) tissue model was used to evaluate the irritation potential of several spermicides and feminine care products.[24] Vaginal tissue was obtained from healthy women undergoing hysterectomies for benign indications. Tissues (n=2) were exposed to 1% Octoxynol-1 and water (used as positive and negative controls, respectively) in quality control testing. After exposure the test article was rinsed from the tissue using PBS and tissue viability was assessed via an MTT viability assay and exposure times that reduced tissue viability to 50% (ET_{50}). Based on the ET_{50} values, the categorization of test materials according to their irritation potential was as follows: feminine washes > spermicides > anti-itch creams > anti-fungal agents, douche, lubricant. Materials which had lower ET_{50} values exhibited more histological damage at shorter exposure times. Douches and personal lubricants had the longest ET_{50} values,

considering a lack of active ingredients with significant pharmacological or surfactant properties. 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.

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

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.[16] 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.[16] 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.[2,16] 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.[16] 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.

RISK ASSESSMENT

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

Based on the Council's 2022 survey, the maximum reported concentration of use is Octoxynol-9 at 2% in skin cleansing formulas (rinse-off).[8] Octoxynol-12 is reported to be used at 1.5% in face and neck products (leave-on).

i) Octoxynol-9 at 2% in skin cleansing formulas

According to the user guide, VERMEER Cosmolife (Ver. 0.24) defines the product types based on Tables 2 and 3 of the Scientific Committee on Consumer Safety (SCCS) Notes of Guidance (NoG; 10th revision). A suitable product category for "skin cleansing formulas (rinse-off)" appears to be make-up remover. Consequently, the risk assessment is carried out based on this specific use.

The outputs from VERMEER Cosmolife indicate that certain parameters are sourced from the SCCS NoG (11th revision):[25]

Body weight used for the product exposure: adult (60 kg)
Surface area involved: 565 cm^2 (½ area head -female)
Type of exposure: rinse-off
Time of exposure: 0.5 h
Relative daily exposure for the selected body weight: 8.33 mg/kg bw/d
Systemic Exposure Dose (SED) with 50% absorption (dermal absorption): 0.0833 mg/kg bw/d

Using the CORAL no-observed-adverse-effect-level (NOAEL) model implemented in VEGA software (NOAEL (IRFMN-CORAL) v.1.0),[26,27] VERMEER Cosmolife predicts a NOAEL of 28.11 mg/kg bw/d, while the it also indicates this prediction is considered to have a moderate reliability level. When experimental data on dermal absorption is lacking, a conservative 50% default value is applied.[25] Consequently, the margin of safety (MOS) is computed to be 337.5 when assuming 50% absorption. This figure is greater than 100. A default MOS value of 100 is generally accepted for considering a cosmetic ingredient safe for use.

ii) Octoxynol-12 at 1.5% in face and neck products

Within VERMEER Cosmolife, two distinct product categories exist: face cream and face cream-applied on neck. Accordingly, the risk assessment is conducted by separately considering the parameters associated with these two specific usage types.

Body weight used for the product exposure: adult (60 kg)
Surface area involved: 565cm^2 (½ area head-female-face cream); 320cm^2 (face cream-applied on neck)
Type of exposure: leave-on
Time of exposure: 24 h
Relative daily exposure for the selected body weight: 24.14 mg/kg bw/d (face cream); 13.67 mg/kg bw/d (face cream-applied on neck)
SED with 50% absorption (dermal absorption): 0.181 mg/kg bw/d (face cream); 0.10 mg/kg bw/d (face cream-applied on neck)

Using the IRFMN/CORAL model,[26,27] VERMEER Cosmolife predicts the NOAEL to be 33.62 mg/kg bw/d. It is important to note that this prediction has a low reliability, as indicated by the software. Consequently, the resulting MOS is 185.7 for face cream, and 336.2 for face cream-applied on neck, assuming a 50% absorption. Both figures are greater than 100, indicating the ingredient is considered safe for use in this product category.

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 has the greatest reported frequency of use, in 8 formulations; the 5 other octoxynols that are in use are reported to be used in 5 formulations or less; frequency of use reduced from 131 uses reported in 2001. Results from a 2022 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, comprising bees white was, carnauba wax, distilled water, Octoxynol-1, 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.

Several metabolites of alklylphenols, such as 4-*tert*-octylphenol, have been shown to mimic estrogen by exhibiting endocrine disruption effects and promoting changes in cancer cells. Octoxynol-1 treated tissue demonstrated a reduced cell viability of 18.9% after 4 h and 82.5% after 8 h of exposure in an in vitro skin irritation test using the EpiDerm™ skin model. Mean values for IL-α after exposure to Octoxynol-1 for 4 and 8 h were 100 and 320 pg/ml, respectively (compared to 69 and 149 pg/ml for the benchmark materials).

Octoxynol-1 was used as a positive control to assess tissue quality in an in vitro skin irritation test of various surfactant-based cleansers using the EpiDerm™ skin model. Octoxynol-1-treated tissue demonstrated a reduced cell viability of 18.9 ± 10.6% after 4 h and 82.5 ± 10% after 8 h of exposure (compared to the mean percentages of viable tissues treated with benchmark materials: 100.1 ± 7.1% and 96.9 ± 6.4%, respectively). Mean values for IL-α release after exposure to Octoxynol-1 for 4 and 8 h were 100 ± 44 pg/ml and 320 ±124 pg/ml (compared to 69 ± 25 pg/ml and 149 ± 53 pg/ml for benchmark materials).

The dermal irritation potential of a leather cream containing Octoxynol-1 (along with bees white wax, carnauba wax, distilled water, silicone oil, linseed oil, Sudan black dye, and nigrosine black dye) was evaluated in rabbits (6/group) according to OECD TG 404. Animals received applications of either distilled water (controls), 0.8% w/v aq. formaldehyde (positive control), a laboratory-based sample of the leather cream, or the marketed leather cream to a shaved, 25 cm^2 area of the back (amount not specified). The PII was calculated as 0 in the control, sample cream, and marketed cream group; the test article was not considered a dermal irritant. No sensitization, erythema, or edema reactions were observed in groups of guinea pigs (n = 6) that were tested with the same cream formulation in accordance with OECD TG 406.

An in vitro VEC tissue model was used to evaluate the irritation potential of several spermicides and feminine care products; 1% Octoxynol-1 served as positive control and produced expected results. Based on the ET_{50} values, the categorization of test materials according to their irritation potential was as follows: feminine washes > spermicides > anti-itch creams > anti-fungal agents, douche, lubricant. Materials which had lower ET_{50} values exhibited more histological damage at shorter exposure times. Additionally, a full thickness VEC tissue model was exposed to a lubricant doped with 0.1 or 2% nonoxynol-9 for 18-h, compared to 2 other lubricant samples, in an MTT and ELISA assay. 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 IL-1β concentrations increased as structural damage increased while tumor necrosis factor-α release decreased as structural damage and loss in tissue viability increased.

Using the IRFMN/CORAL model, VERMEER Cosmolife predicts an NOAEL of 28.11 mg/kg bw/d and a computed MOS of 337.5 (assuming 50% absorption) for the reported use of Octoxynol-9 at 2% in skin cleansing formulas. Additionally, using the IRFMN/Coral model, VERMEER Cosmolife predicts a NOAEL of 33.62 mg/kg bw/d for the reported use of Octoxynol-12 at 1.5% in face and neck products, with MOS values of 185.7 for face cream and 336.2 for face cream-applied on neck (assuming 50% absorption).

PREVIOUS (2004) 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 C9 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 ≤ 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 use 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 ± 1.5 μm for a respirable particulate mass was small compared to the particle sizes of anhydrous hair sprays (60 – 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.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

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

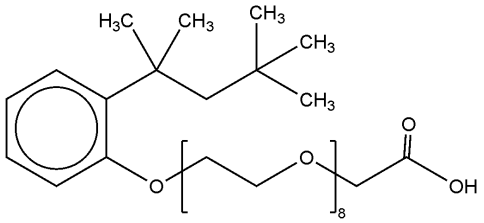
Ingredient/CAS No.	Definition	Function(s)
Octoxynol-1 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic) 2315-67-5	Octoxynol-1 is the ethoxylated alkyl phenol that conforms generally to the chemical structure depicted in Figure 1, where n has an average value of 1.	Surfactants – emulsifying 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

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

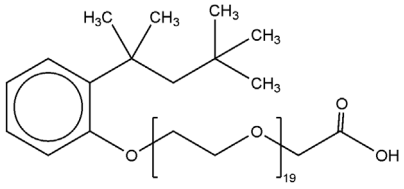
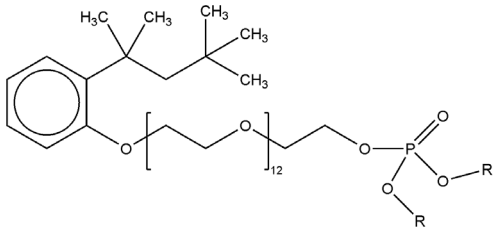
Ingredient/CAS No.	Definition	Function(s)
Octoxynol-12 9002-93-1 (generic) 9036-19-5 (generic) 9004-87-9 (generic)	Octoxynol-12 is the ethoxylated alkyl phenol that conforms generally to chemical structure depicted in Figure 1, where n has an average value of 12.	Surfactants – emulsifying agents
Octoxynol-13 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

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

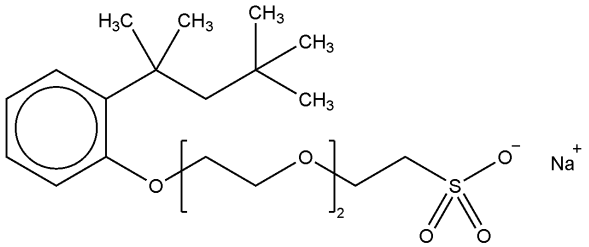
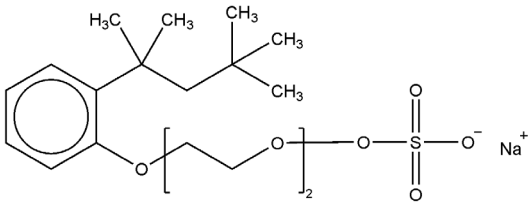
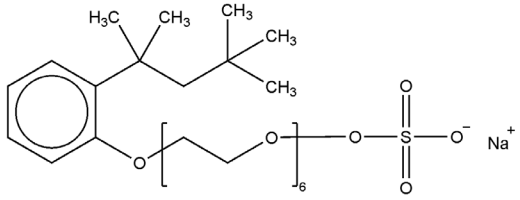
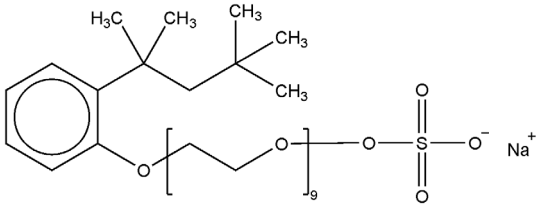
Ingredient/CAS No.	Definition	Function(s)
Sodium Octoxynol-2 Ethane Sulfonate 2917-94-4 55837-16-6 67923-87-9	Sodium Octoxynol-2 Ethane Sulfonate is the organic compound that conforms generally to the following structure: 	Surfactants – cleansing agents
Sodium Octoxynol-2 Sulfate	Sodium Octoxynol-2 Sulfate 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	[6]
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)	[28]
	Octoxynol-3	
Molecular Weight (g/mol)	338.5	[29]
log K_{ow} (@ 25 ºC)	4.42 (estimated)	[28]
	Octoxynol-5	
Physical Form	slightly hazy, free-flowing liquid	[2]
Color	water white to light amber	[2]
Molecular Weight (g/mol)	426.59	[28]
Specific Gravity (@ 25ºC)	1.030 – 1.040	[2]
Solubility	Soluble in organic solvents; insoluble in water	[2]
log P (@ 25 ºC)	4.25 (estimated)	[28]
	Octoxynol-6	
Molecular Weight (g/mol)	470.65	[28]
log P (@ 25 ºC)	3.95 (estimated)	[28]
	Octoxynol-7	
Molecular Weight (g/mol)	514.70	[28]
log P (@ 25 ºC)	3.95 (estimated)	[28]
	Octoxynol-8	
Molecular Weight (g/mol)	558.75	[28]
Specific Gravity (@ 25ºC)	1.054	[2]
Viscosity (CPS @ 25ºC)	260	[2]
log P (@ 25 ºC)	3.64 (estimated)	[28]
	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	[28]
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	Soluble in organic solvents and in water	[2]
log P (@ 25 ºC)	3.70 (estimated)	[28]
	Octoxynol-9 Carboxylic Acid	
Molecular Weight (g/mol)	616.79	[28]
log P (@ 25 ºC)	3.34 (estimated)	[28]
	Octoxynol-10	
Molecular Weight (g/mol)	646.86	[28]
log P (@ 25 ºC)	3.53 (estimated)	[28]
	Octoxynol-11	
Physical Form	viscous liquid	[2]
Color	Gardner scale < 3	[2]
Odor	Faint	[2]
Molecular Weight (g/mol)	690.91	[30]
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)	[28]
	Octoxynol-12	
Molecular Weight (g/mol)	734.96	[28]
log P (@ 25 ºC)	3.18 (estimated)	[28]

Table 2. Chemical properties

Property	Value	Reference
	Octoxynol-13	
Physical Form	free-flowing, viscous liquid	[2]
Odor	Aromatic	[2]
Molecular Weight (g/mol)	779.02	[28]
Specific Gravity (@ 25ºC; water =1)	1.06 -1.07	[2]
Vapor pressure (mmHg@ ºC)	not volatile	[2]
Vapor Density (mmHg)	not volatile	[2]
Boiling Point (ºC)	200	[2]
Solubility	Soluble in water	[2]
log P (@ 25 ºC)	3.00	[28]
	Octoxynol-16	
Molecular Weight (g/mol)	911.18	[31]
Specific Gravity (@ 25ºC)	1.080	[2]
Viscosity (CPS @ 25ºC)	540	[2]
log P (@ 25 ºC)	2.48 (estimated)	[28]
	Octoxynol-20	
Molecular Weight (g/mol)	1086.89	[28]
Specific Gravity (@ 25 ºC)	1.088	[2]
Viscosity (kg/(CPS @ 25ºC)	420	[2]
log P (@ 25 ºC)	1.77 (estimated)	[28]
	Octoxynol-20 Carboxylic Acid	
Molecular Weight (g/mol)	1101.37	[28]
log P (@ 25 ºC)	3.26	[28]
	Octoxynol-25	
Molecular Weight (g/mol)	1307.65	[28]
log P (@ 25 ºC)	0.90 (estimated)	[28]
	Octoxynol-30	
Molecular Weight (g/mol)	1527.92	[32]
Specific Gravity (@ 25ºC)	1.095	[2]
Viscosity (CPS @ 25ºC)	470	[2]
log P (@ 25 ºC)	0.02 (estimated)	[28]
	Octoxynol-33	
Molecular Weight (g/mol)	1660.08	[33]
log P (@ 25 ºC)	-0.51 (estimated)	[28]
	Octoxynol-40	
Molecular Weight (g/mol)	1968.45	[28]
log P (@ 25 ºC)	-1.74	[28]
	Octoxynol-70	
Molecular Weight (g/mol)	3290.04	[28]
	Potassium Octoxynol-12 Phosphate	
Formula Weight (g/mol)	859.00 – 935.18	[28]
	Sodium Octoxynol-2 Ethane Sulfonate	
Formula Weight (g/mol)	424.5	[34]
	Sodium Octoxynol-2 Sulfate	
Formula Weight (g/mol)	440.5	[35]
	Sodium Octoxynol-6 Sulfate	
Formula Weight (g/mol)	572.7	[36]
	Sodium Octoxynol-9 Sulfate	
Formula Weight (g/mol)	704.8	[37]

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**
	Octoxynol-1				**Octoxynol-3**				**Octoxynol-5**				**Octoxynol-6**			
Totals*	**1**	**57**	**NR**	**0.06 - 5**	**NR**	**1**	**NR**	**NR**	**NR**	**1**	**NR**	**NR**	**NR**	**NR**	**NR**	**1**
summarized by likely duration and exposure**																
Duration of Use																
Leave-On	*1*	*NR*	*NR*	*NR*	*NR*	*1*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*NR*	*57*	*NR*	*0.06 - 5*	*NR*	*NR*	*NR*	*NR*	*NR*	*1*	*NR*	*NR*	*NR*	*NR*	*NR*	*1*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type																
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	1[a]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	1	1	NR	NR	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR	1
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	3	NR	1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	53	NR	0.06 - 5	NR	NR	NR	NR	NR	1	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	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 Conditioner	NR	2	NR	1												
Hair Spray (aerosol fixatives)																
Hair Straighteners																
Permanent Waves	NR	1	NR	NR												
Rinses (non-coloring)																
Shampoos (non-coloring)																
Tonics, Dressings, and Other Hair Grooming Aids																
Wave Sets																

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**
Other Hair Preparations																
Hair Coloring Preparations																
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	53	NR	NR												
Hair Shampoos (coloring)																
Hair Lighteners with Color	NR	NR	NR	5												
Hair Bleaches									NR	1	NR	NR				
Other Hair Coloring Preparation	NR	NR	NR	0.06 – 0.2												
Makeup Preparations																
Blushers (all types)																
Foundations																
Lipstick																
Makeup Bases																
Personal Cleanliness Products																
Bath Soaps and Detergents																
Douches																
Feminine Deodorants																
Other Personal Cleanliness Products																
Shaving Preparations																
Aftershave Lotion																
Shaving Cream																
Skin Care Preparations																
Cleansing	NR	1	NR	NR												
Face and Neck (exc shave)																
Body and Hand (exc shave)																
Foot Powders and Sprays																
Moisturizing																
Paste Masks (mud packs)													NR	NR	NR	1
Skin Fresheners																
Other Skin Care Preparations	1	NR	NR	NR												
Suntan Preparations																
Suntan Gels, Creams, and Liquids																

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**
	Octoxynol-9				**Octoxynol-10**				**Octoxynol-11**				**Octoxynol-12**			
Totals*	**5**	**131**	**0.1 – 2**	**0.08 - 5**	**1**	**NR**	**NR**	**25**	**8**	**19**	**NR**	**1**	**4**	**NR**	**1.5**	**NR**
summarized by likely duration and exposure**																
Duration of Use																
Leave-On	*5*	*30*	*0.1*	*0.08 - 5*	*1*	*NR*	*NR*	*NR*	*8*	*14*	*NR*	*1*	*3*	*NR*	*1.5*	*NR*
Rinse-Off	*NR*	*101*	*2*	*0.4 - 1*	*NR*	*NR*	*NR*	*25*	*NR*	*5*	*NR*	*1*	*1*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type																
Eye Area	1	NR	NR	NR	NR	NR	NR	NR	2	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	2	NR	NR	NR
Incidental Inhalation-Spray	1[a]	4; 11[a]; 2[b]	NR	0.1 – 5; 0.08 – 1[a]; 3[b]	NR	NR	NR	NR	4[a]; 1[b]	1; 7[a]	NR	1[a]	1[b]	NR	NR	NR
Incidental Inhalation-Powder	NR	2[b]; 1[c]	NR	1; 3[b]	NR	NR	NR	NR	1[b]	NR	NR	NR	1[b]	NR	1.5[c]	NR
Dermal Contact	5	21	0.1 – 2	0.5 - 5	1	NR	NR	NR	8	15	NR	NR	2	NR	1.5	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	48	NR	0.08 - 1	NR	NR	NR	NR	NR	4	NR	1	NR	NR	NR	NR
Hair-Coloring	NR	61	NR	0.4	NR	NR	NR	25	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	5	NR	0.5 – 0.9	NR	NR	NR	NR	NR	NR	NR	NR	2	NR	NR	NR
Baby Products	NR	1	0.1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products																
Other Baby Products	NR	NR	0.1	NR												
Bath Preparations (diluted for use)																
Bath Oils, Tablets, and Salts	NR	1	NR	NR												
Bubble Baths																
Eye Makeup Preparations																
Eyebrow Pencil																
Eyeliner	1	NR	NR	NR												
Eye Shadow																
Eye Lotion									1	NR	NR	NR				
Eye Makeup Remover																
Mascara																
Other Eye Makeup Preparations									1	NR	NR	NR				
Fragrance Preparations																
Cologne and Toilet Water	NR	2	NR	5												
Perfumes	NR	NR	NR	0.7												
Other Fragrance Preparation	NR	1	NR	NR					NR	1	NR	NR				
Hair Preparations (non-coloring)																
Hair Conditioner	NR	8	NR	0.4												
Hair Spray (aerosol fixatives)	NR	1	NR	0.1												
Hair Straighteners	NR	1	NR	0.9												
Permanent Waves	NR	17	NR	NR												
Rinses (non-coloring)																
Shampoos (non-coloring)	NR	3	NR	0.7					NR	3	NR	NR				
Tonics, Dressings, and Other Hair Grooming Aids	NR	7	NR	0.08 -1					NR	NR	NR	1				
Wave Sets																

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**
Other Hair Preparations	NR	11	NR	NR					NR	1	NR	NR				
Hair Coloring Preparations																
Hair Dyes and Colors (all types requiring caution statements and patch tests)	NR	58	NR	NR												
Hair Shampoos (coloring)	NR	1	NR	NR												
Hair Lighteners with Color					NR	NR	NR	25								
Hair Bleaches	NR	1	NR	NR												
Other Hair Coloring Preparation	NR	1	NR	0.4												
Makeup Preparations																
Blushers (all types)																
Foundations																
Lipstick													2	NR	NR	NR
Makeup Bases									NR	1	NR	NR				
Personal Cleanliness Products																
Bath Soaps and Detergents	NR	2	NR	NR												
Douches	NR	1	NR	NR												
Other Personal Cleanliness Products	NR	2	NR	0.5 - 0.9												
Shaving Preparations																
Aftershave Lotion	NR	1	NR	NR												
Shaving Cream	NR	NR	NR	1												
Skin Care Preparations																
Cleansing	NR	3	2	NR					NR	2	NR	1	1	NR	NR	NR
Face and Neck (exc shave)									1	NR	NR	NR	NR	NR	not spray: 1.5%	NR
Body and Hand (exc shave)	NR	2	NR	NR									1	NR	NR	NR
Foot Powders and Sprays	NR	NR	NR	3												
Moisturizing	1	2	NR	NR					3	3	NR	NR				
Paste Masks (mud packs)	NR	3	NR	NR					1	2	NR	NR				
Skin Fresheners	NR	2	NR	NR												
Other Skin Care Preparations	3	1	NR	NR	1	NR	NR	NR	1	4	NR	1				
Suntan Preparations																
Suntan Gels, Creams, and Liquids									NR	2	NR	NR				

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2002[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**
	Octoxynol-13				**Octoxynol-30**				**Octoxynol-40**				**Potassium Octoxynol-12 Phosphate**			
Totals	**NR**	**46**	**NR**	**0.1 - 2**	**NR**	**NR**	**NR**	**1 - 2**	**2**	**18**	**NR**	**0.007 – 0.02**	**NR**	**18**	**NR**	**0.0008 – 0.5**
summarized by likely duration and exposure*																
Duration of Use																
Leave-On	*NR*	*30*	*NR*	*0.1*	*NR*	*NR*	*NR*	*1 - 2*	*NR*	*2*	*NR*	*NR*	*NR*	*18*	*NR*	*0.0008 - 5*
Rinse-Off	*NR*	*14*	*NR*	*2*	*NR*	*NR*	*NR*	*NR*	*2*	*16*	*NR*	*0.007 – 0.02*	*NR*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*2*	*NR*	*0.8*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*																
Eye Area	NR	5	NR	2	NR	NR	NR	1 - 2	NR	NR	NR	NR	NR	18	NR	0.002 – 0.5
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	14[a]; 3[b]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	3[b]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	19	NR	0.8 - 2	NR	NR	NR	1	NR	NR	NR	NR	NR	6	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	0.0008 - 0.5
Hair - Non-Coloring	NR	24	NR	0.1	NR	NR	NR	NR	2	10	NR	0.007 – 0.01	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	8	NR	0.02	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	2	NR	0.8	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	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	NR	1	NR	NR												
Bubble Baths	NR	2	NR	0.8												
Eye Makeup Preparations																
Eyebrow Pencil													NR	NR	NR	0.05
Eyeliner					NR	NR	NR	1					NR	6	NR	0.02 – 0.05
Eye Shadow													NR	NR	NR	0.002
Eye Lotion																
Eye Makeup Remover	NR	2	NR	2												
Mascara	NR	3	NR	NR	NR	NR	NR	2					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 Conditioner	NR	4	NR	NR					NR	5	NR	0.01				
Hair Spray (aerosol fixatives)																
Hair Straighteners									2	NR	NR	NR				
Permanent Waves									NR	1	NR	NR				
Rinses (non-coloring)	NR	4	NR	NR												
Shampoos (non-coloring)	NR	2	NR	NR					NR	1	NR	0.007				
Tonics, Dressings, and Other Hair Grooming Aids	NR	10	NR	NR												
Wave Sets	NR	2	NR	NR					NR	1	NR	NR				
Other Hair Preparations	NR	2	NR	0.1					NR	2	NR	NR				

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2002[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**	**2023[7]**	**2001[2]**	**2022[8]**	**1999, 2001[2]**
Hair Coloring Preparations																
Hair Dyes and Colors (all types requiring caution statements and patch tests)									NR	1	NR	0.02				
Hair Shampoos (coloring)																
Hair Lighteners with Color																
Hair Bleaches									NR	6	NR	NR				
Other Hair Coloring Preparation									NR	1	NR	NR				
Makeup Preparations																
Blushers (all types)	NR	1	NR	NR												
Foundations	NR	1	NR	NR												
Lipstick																
Makeup Bases																
Personal Cleanliness Products																
Bath Soaps and Detergents																
Douches																
Other Personal Cleanliness Products																
Shaving Preparations																
Aftershave Lotion	NR	1	NR	NR												
Shaving Cream																
Skin Care Preparations																
Cleansing																
Face and Neck (exc shave)																
Body and Hand (exc shave)	NR	3	NR	NR												
Foot Powders and Sprays																
Moisturizing	NR	3	NR	NR												
Paste Masks (mud packs)																
Skin Fresheners	NR	1	NR	NR												
Other Skin Care Preparations	NR	5	NR	NR												
Suntan Preparations																
Suntan Gels, Creams, and Liquids													NR	NR	NR	0.0008

Table 3. Frequency (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*															
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**														
	Sodium Octoxynol-2 Ethane Sulfonate																	
Totals	**NR**	**NR**	**NR**	**1**														
summarized by likely duration and exposure*																		
Duration of Use																		
Leave-On	*NR*	*NR*	*NR*	*NR*														
Rinse-Off	*NR*	*NR*	*NR*	*1*														
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*														
Exposure Type*																		
Eye Area	NR	NR	NR	NR														
Incidental Ingestion	NR	NR	NR	NR														
Incidental Inhalation-Spray	NR	NR	NR	NR														
Incidental Inhalation-Powder	NR	NR	NR	NR														
Dermal Contact	NR	NR	NR	1														
Deodorant (underarm)	NR	NR	NR	NR														
Hair - Non-Coloring	NR	NR	NR	NR														
Hair-Coloring	NR	NR	NR	NR														
Nail	NR	NR	NR	NR														
Mucous Membrane	NR	NR	NR	NR														
Baby Products	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 Conditioner																		
Hair Spray (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 (2023/2001) and concentration (2022/1999, 2001) of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[7]	**2001[2]**	**2022[8]**	**1999, 2001[2]**
Hair Coloring Preparations				
Hair Dyes and Colors (all types requiring caution statements and patch tests)				
Hair Shampoos (coloring)				
Hair Lighteners with Color				
Hair Bleaches				
Other Hair Coloring Preparation				
Makeup Preparations				
Blushers (all types)				
Foundations				
Lipstick				
Makeup Bases				
Personal Cleanliness Products				
Bath Soaps and Detergents				
Douches				
Other Personal Cleanliness Products				
Shaving Preparations				
Aftershave Lotion				
Shaving Cream				
Skin Care Preparations				
Cleansing				
Face and Neck (exc shave)				
Body and Hand (exc shave)				
Foot Powders and Sprays				
Moisturizing				
Paste Masks (mud packs)	NR	NR	NR	1
Skin Fresheners				
Other Skin Care Preparations				
Suntan Preparations				
Suntan Gels, Creams, and Liquids				

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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

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

[b] 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[7,8]

Octoxynol-8	Octoxynol-9 Carboxylic Acid
Octoxynol-16	Octoxynol-20 Carboxylic Acid
Octoxynol-20	Potassium Octoxynol-12 Phosphate
Octoxynol-25	Sodium Octoxynol-2 Sulfate
Octoxynol-33	Sodium Octoxynol-6 Sulfate
Octoxynol-70	Sodium Octoxynol-9 Sulfate

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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

Received 18 July 2003; accepted 30 October 2003.

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International Journal of Toxicology, 23(Suppl. 1):59–111, 2004

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810490274306

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.

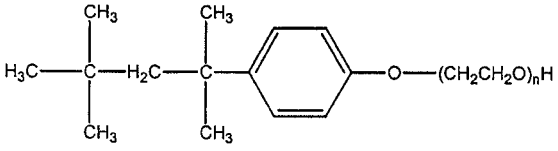
Octoxynols are chemically similar to nonoxynols. In its safety assessment of nonoxynols, the Cosmetic Ingredient Review (CIR) Expert Panel concluded that Nonoxynol-9, -10, -12, -14, -15, -30, -40, and -50 are safe as used (Elder 1983) and that Nonoxynol-1, -2, -3, -4, -5, -6, -7, and -8 are safe as used 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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Safety Assessment of Nonoxynols as Used in Cosmetics

Status: Final Amended Report
Release Date: February 2, 2016
Panel Date: December 14-15, 2015

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ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) reviewed the safety of 27 Nonoxynols which function mostly as surfactants - emulsifying agents in cosmetic products. The Panel reviewed relevant data relating to the safety of these ingredients, and concluded that these Nonoxynols are safe in the present practices of use and concentration in cosmetics as described in this safety assessment, when formulated to be non-irritating. This conclusion pertains to Nonoxynols in which the average number of ethylene oxide uints (n) per molecule ranges from 1 to 120 (i.e., Nonoxynols-1 to -120 range), and supersedes the Panel's previous conclusions on Nonoxynols-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -12, -14, -15, -30, -40, and -50.

INTRODUCTION

The safety of Nonoxynols, listed below, in cosmetics is reviewed in this safety assessment. These 27 ingredients function mostly as surfactants-emulsifying agents in cosmetic products.

- Nonoxynol-1
- Nonoxynol-2
- Nonoxynol-3
- Nonoxynol-4
- Nonoxynol-5
- Nonoxynol-6
- Nonoxynol-7
- Nonoxynol-8
- Nonoxynol-9
- Nonoxynol-10
- Nonoxynol-11
- Nonoxynol-12
- Nonoxynol-13
- Nonoxynol-14
- Nonoxynol-15
- Nonoxynol-18
- Nonoxynol-20
- Nonoxynol-23
- Nonoxynol-25
- Nonoxynol-30
- Nonoxynol-35
- Nonoxynol-40
- Nonoxynol-44
- Nonoxynol-50
- Nonoxynol-70
- Nonoxynol-100
- Nonoxynol-120

The Panel has evaluated the safety of Nonoxynols-2, -4, -8, -9, -10, -12, -14, -15, -30, -40, and -50 in cosmetics and issued a final report (published in 1983) with the following conclusion: "On the basis of the available information presented in this report, the Panel concludes that Nonoxynols-2, -4, -8, -9, -10, -12, -14, -15, -30, -40, and -50 are safe as cosmetic ingredients in the present practices of concentration and use".[1] The Panel reevaluated the safety of Nonoxynols (Nonoxynols-1, -2, -3, -4, -5, -6, -7, and -8 included) in cosmetics and issued an amended final report (published in 1999) with the following conclusion:[2] "On the basis of the available animal and clinical data included in this report, the CIR Expert Panel concludes that Nonoxynols-1, -2, -3, -4, -5, -6, -7, and -8 are safe as used in rinse-off products and safe at concentrations ≤ 5% in leave-on products [Note: this conclusion modifies a previous conclusion for Nonoxynols-2, -4, and -8, which had been considered safe as used in both rinse-off and leave-on products]".

The final safety assessments of Nonoxynols were reopened to determine whether data that have become available since the publication of the two prior final safety assessments warrant a change in either of the two conclusions previously issued by the Panel. The following ingredients were not previously reviewed, and their safety in cosmetics is being evaluated in this safety assessment: Nonoxynols-13, -18, -20, -23, -25, -35, -44, -70, -100, and -120 (listed above with previously reviewed Nonoxynols). Appropriate data from the 1983 and 1999 published safety assessments on Nonoxynols are included in this safety assessment to fill data gaps in the safety assessment of Nonoxynols-13, -18, -20, -23, -25, -35, -44, -70, -100, and -120 (See Table 5). The published safety assessments should be consulted for additional information (http://www.cir-safety.org/ingredients).

CHEMISTRY

Definition and Structure

The Nonoxynols, or nonylphenoxy polyethoxyethanols, are ethoxylated alkylphenols with the chemical structure shown in Figure 1.; n can vary from 1 to 120.[3] Nonoxynols are nonionic surfactants; the nonpolar alkyl chain has lipophilic properties, and the polar polyoxyethylene portion of the molecule has hydrophilic properties. The Nonoxynols with short chains are liquids (n = 14 to 15); those with longer chains are waxes (n > 20). Liquid Nonoxynols are generally sold as 50 to 70% aqueous solutions.[1]

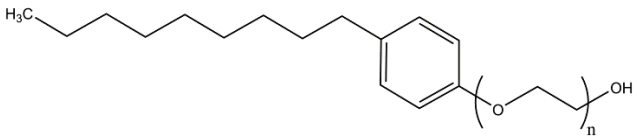

Figure 1. Chemical formula for Nonoxynols. The value of **n** can vary from 1 to 120.

The definitions and functions of the Nonoxynols reviewed in this safety assessment are presented in Table 1.[3]

Chemical and Physical Properties

Nonoxynol-9

The absorption spectrum of Nonoxynol-9 (in buffered aqueous medium) has two bands that are centered at 225 nm and 276 nm, with a tail extending to 300 nm.[4] Ultraviolet (UV) absorbance and other properties of the Nonoxynols are presented in Table 2.

USE

Cosmetic

The safety of the Nonoxynols is evaluated based on the expected use of these ingredients in cosmetics. The Panel utilizes data received from the Food and Drug Administration (FDA) and the cosmetics industry to determine the cosmetic use. Data on the use frequencies of individual ingredients in cosmetics are submitted by manufacturers and organized by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted in response to surveys of maximum reported use concentrations, by product category, which are conducted by the Personal Care Products Council (Council).

According to the 2015 VCRP data, the greatest use frequency was reported for Nonoxynol-4 (90 formulations, all rinse-off), followed by Nonoxynol-6 (65 formulations, all rinse-off) (Table3).[5] Lower use frequencies were reported for the remaining Nonoxynols, mostly relating to use in rinse-off products. The results of a concentration of use survey conducted by the Council in 2014 indicate that Nonoxynol-12 has the highest reported maximum concentration of use; it is used at concentrations up to 8.33% in rinse-off products (on-head concentration in hair dyes and colors) (Table 3).[6] The highest maximum concentration of use reported for products resulting in leave-on dermal exposure is 0.42% (Nonoxynol-12, in aerosol hair sprays). Addtionally, according to the Council's survey, Nonoxynol-9 was reported to be used in other personal cleanliness products (hand cleanser, a rinse-off product) at a maximum concentration of 2.5%. Only use concentration data on nononxynol-9 and Nonoxynol-12 were reported in this survey. In some cases, reported uses appear in the VCRP database, but concentration of use data were not provided. For example, Nonoxynol-4 was reported to be used in 90 cosmetic formulations, but use concentration data were not reported.

It should be noted that the distributor of a body wash (cosmetic feminine wash - intended for use on the vaginal area) stated that this product contains Nonoxynol-9 at a concentration of 3%, and that it is in the process of removing this ingredient from the formula due to the company's awareness of potential safety issues.[7]

Historical use concentration data on Nonoxynols-1 to -30 from published CIR final safety assessments are also presented in Table 3.[1,2] The use concentrations of Nonoxynols have decreased; Nonoxynols were used in cosmetics at concentrations up to ~50% (Nonoxynol-6, in rinse-off and leave-on products). Nonoxynols -1 and -2 were used at concentrations up to ~10% and ~20%, respectively, in rinse-off products. The frequency of use of Nonoxynols has also decreased. For example, in 1999, the reported frequency of use was 575 for Nonoxynol-4, as opposed to 90 reported uses of Nonoxynol-4 in 2015.

Nonoxynols are being used in products that could possibly be inhaled. Specifically, Nonoxynol-9 is used in cologne and toilet waters and in hairspray products (use concentrations not available), and Nonoxynol-12 is used in hairspray products at a maximum use concentration of 0.42%. In practice, 95% to 99% of the droplets/particles released from cosmetic

sprays have aerodynamic equivalent diameters >10 μm, with propellant sprays yielding a greater fraction of droplets/particles below 10 μm, compared with pump sprays.[8,9,10,11] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[8,9]

Nonoxynols-1 to -120 are not included in the list of ingredients prohibited from use in cosmetic products marketed in the European Union [EU]).[12] However, in the EU, nonylphenol and nonylphenol ethoxylates (another name for Nonoxynols) shall not be placed on the market, or used, as substances or in mixtures, in concentrations equal to or greater than 0.1% by weight for various purposes, including cosmetic products and other personal care products (except spermicides).[13] The need for restrictons on nonylphenol and nonylphenol ethoxylates in industrial products is based on the premise that European water bodies are at risk from the combined effects of nonylphenol ethoxylate (NPEO) degradation products, i.e., nonylphenol, short-chain NPEOs, and nonylphenol ethoxycarboxylates (NPECs), including effects arising from their potential endocrine disrupting properties.[14] Though Nonoxynols are not included on the EU's list of ingredients prohibited from use in cosmetic products, it should be noted that nonylphenol and 4-nonylphenol are included on the list of prohibited ingredients.[12]

The Environmental Protection Agency (EPA) has noted the following:[15] Nonylphenol (NP) is persistent in the aquatic environment, moderately bioaccumulative, and extremely toxic to aquatic organisms. Furthermore, NP has also been shown to exhibit estrogenic properties in *in vitro* and *in vivo* assays. NP's main use is in the manufacture of nonylphenol ethoxylates (NPEs). Under the Toxic Substances Control Act (TSCA), in 2014, EPA proposed a significant new use rule (SNUR) to require Agency review before a manufacturer begins or resumes use of 15 NPs and NPEs. When finalized, this SNUR will provide EPA with the opportunity to review and evaluate any intended new or resumed uses of these chemicals and, if necessary, take action to limit those uses. The public comment period for this proposal closed on January 15, 2015.

Noncosmetic

Nonoxynols-1, -5, -6, -9, and -10

The Code of Federal Regulations (CFR) describes indirect food additive uses of Nonoxynol-1 as a component of adhesives and a component of paper and paperboard in contact with dry food. Nonoxynols-5 and -6 have the following indirect food additive uses: components of adhesives, components of resinous and polymeric coatings, components of paper and paperboard in contact with aqueous and fatty food, components of paper and paperboard in contact with dry food, defoaming agents in the manufacture of paper and paperboard, and emulsifiers and/or surface-active agents for articles that contact food.[16]

Sulfosuccinic acid 4-ester with polyethylene glycol nonyl phenyl ether is permitted for use in adhesives, which are used as components of articles intended for use in packaging, transporting, or holding food. The alcohol moiety of this chemical is produced by the condensation of 1 mole of nonylphenol and an average of 9-10 moles of ethylene oxide.[17]

The FDA Advisory Panel on OTC Contraceptives and Other Vaginal Drug Products issued a proposed rule classifying Nonoxynol-9 as safe and effective and not misbranded for use as a vaginal contraceptive active ingredient.[18] According to the final rule issued by the FDA, the warnings and labeling information will advise consumers that use of vaginal contraceptives and spermicides containing Nonoxynol-9 irritate the vagina.[19] The FDA also determined that, based on its history of safety and effectiveness, Nonoxynol-9 should remain on the market until FDA has completed its review of data relating to the efficacy of Nonoxynol-9 containing spermicides. These data are from a clinical trial that compares the effectiveness and safety of 5 spermicides. A summary of this clinical trial appears in the last paragraph of the section on Mucous Membrane Irritation.

TOXICOKINETICS

Nonoxynol-9 (in saline) was administered intravaginally to 3 female New Zealand white rabbits at doses of 100 mg/kg and 300 mg/kg (dose volume = 3 ml/2.5 kg).[20] Blood samples were collected from the marginal ear vein for up to 8 h post-dosing. Plasma was separated by centrifuging the blood samples. The mean peak concentration (C_{max}) of 4.87 ± 0.3 ng/ml (300 mg/kg dose) was achieved at 1 h post-dosing. A C_{max} of 3.10 ± 0.79 ng/ml was reported for the 100 mg/kg dose. The concentration of Nonoxynol-9 in the plasma decreased rapidly and was eliminated from the plasma, with a terminal half-life of 1.45 ± 0.07 h.

Percutaneous Absorption

Nonoxynols-4 and -9

The percutaneous absorption of Nonoxynol-4 and Nonoxynol-9 *in vitro* was studied using human, pig, and rat skin samples in flowthrough diffusion cells.[21] Topical solutions of 0.1%, 1%, or 10% ^{14}C-nononxynol-4 (each in 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. The dermal absorption of ^{14}C-nonxynol-4 and ^{14}C-Nonoxynol-9 was similar across skin from 3 species at less than 1% of the applied dose. 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. Absorption, expressed as mass absorbed over 8 h was similar (≈ 0.3 $\mu g/cm^2$) across all concentrations. Particularly in rat skin, skin penetration, but not absorption, was greater when water was used as the vehicle compared to PEG-400 as the vehicle. The results of this study suggest that, in skin samples from all 3 species, ^{14}C-Nonoxynol-9 and ^{14}C-nonxynol-4 were minimally absorbed across the skin.

TOXICOLOGY

Appropriate data from the 1983 and 1999 published safety assessments on Nonoxynols are included in this safety assessment (See Tables 4 and 5) to provide a safety profile on the Nonoxynols that have been reviewed and fill data gaps in the safety assessment of the Nonoxynols that have not been reviewed.

Acute (Single Dose) Toxicity

Acute oral toxicity data on Nonoxynols-2, -4, -6, -7, -9, -10, -12, -13, 15, -30, and -40 are presented in Table 4. The data indicate that these Nonoxynols are, at most, slightly toxic.

Repeated Dose Toxicity

Oral

Nonoxynol-10

Groups of 50 female B6C3F$_1$ mice (4 weeks old) received nononxynol-10 at concentrations of 500 ppm, 1500 ppm, or 4500 ppm in the diet, respectively, for 104 weeks.[22] The estimated mean dose rates of Nonoxynol-10 were 81.5 mg/kg/day, 254 mg/kg/day, and 873 mg/kg/day, respectively. A fourth group was fed control diet. There were no differences in mortality among the 3 dose groups, and mortalities did not exceed thecontrol group. Additionally, there were no signs observed that were attributable to feeding with Nonoxynol-10, and hematological examination results were negative. Lower absolute liver and kidney weights and higher relative (to body weight) weights of the brain, liver, and kidney were observed in the 4500 ppm group. These findings were considered changes associated with the suppression of body weight gain observed only in this group. Microscopic examination of major organ systems did not identify any changes that were attributable to Nonoxynol-10 in the diet. Additional results are presented in the section on Carcinogenicity.

Parenteral

Non-Human

Nonoxynol-10

The following doses of nononxynol-10 (in isotonic saline) were administered subcutaneously (s.c.) to 4 groups of female Jcl:Wistar rats in a developmental toxicity study: 5 mg/kg/day (20 rats), 20 mg/kg/day (22 rats), and 80 mg/kg/day (21 rats).[23] Nonoxynol-10 (pH 6.3) was dissolved in saline, and the test solution was administered to the subcutis of the dorsal regions. Doses (dose volume = 3 ml/kg body weight) were administered daily from the date of birth to day 21 after the birth of F$_1$ offspring. The negative control group receiving isotonic saline consisted of 21 rats. Scab formation and hair loss at the application site were observed in F$_0$ dams of all dose groups. Induration at the application site was observed in 20 mg/kg/day and 80 mg/kg/day dose groups; this finding was also reported during necropsy at the end of the dosing period.

Necropsy findings, in all dose groups, also included hemorrhage and what was described as a whitish change of the subcutis at the application site.

Other necropsy findings (at the application site) for the 20 mg/kg/day and 80 mg/kg/day dose groups included adhesion of the somatic muscles and granulation tissue in the subcutis. Swelling of the adrenal glands and spleen were also observed in the 80 mg/kg/day dose group. Either reduction or a tendency for reduction in feed consumption from the initial day of dosing (day 0) to day 17 after the birth of F_1 offspring was reported for the 80 mg/kg/day dose group. However, body weight gains, based on the weights at 4 weeks after birth, in the 80 mg/kg/day dose group did not differ from those of the control group. Neither body weights nor necropsy findings on the day after birth and day of weaning were indicative of any test substance-related effects. The "noneffective dose level" was considered to be 20 mg/kg/day for general toxicity to the dams and their offspring. Additional study results are presented in the section on Reproductive and Developmental Toxicity.[23]

In another developmental toxicity study, groups of 40 female Jcl:Wistar rats (5weeks old) were injected s.c.with Nonoxynol-10 at doses of 2 mg/kg/day and 20 mg/kg/day (dose volume = 3 ml/kg body weight) for 15 weeks.[24] The negative control group also consisted of 40 rats. Nonoxynol-10, dissolved in saline, was administered daily according to the procedure in the preceding study. The high dose of 20 mg/kg/day was selected based on the results of a preliminary study that involved daily applications of 20 mg/kg/day or ≥ 40 mg/kg/day for 5 weeks. Results from the preliminary study are as follows: Swelling at the application site (≥ 20 mg/kg/day) and desquamation at the application site (≥ 40 mg/kg/day). At necropsy, subcutaneous hemorrhage and a whitish change at the application site were observed in all dose groups. However, swelling of the spleen was observed in groups dosed with 20 mg/kg/day or greater, and adhesion of the subcutis and trunk muscle was observed in groups dosed with 40 mg/kg/day or greater.

Effects in the F_0 dams exposed to 2 mg/kg/day and 20 mg/kg/day were described as scab formation and hair loss at the application site. Induration of the skin was also observed in animals that received 20 mg/kg/day. Collectively, these skin changes were classified as local irritation. Increases in body weight and food consumption were reported for the 20 mg/kg/day dose group. Necropsy findings included what were described as whitish changes of the subcutis in the 2 mg/kg/day and 20 mg/kg/day dose groups and adhesion of abdominal organs in the 20 mg/kg/day dose group. Additional study results are presented in the section on Reproductive and Developmental Toxicity.[24]

Estrogenic Activity

A 2001 *Environment Canada and Health Canada Priority Substances List and Assessment Report on Nonylphenol and its Ethoxylates* is available.[25] According to this report, Nonoxynol-9 (and higher ethoxylates) has lower estrogenic activity when compared to Nonoxynol-1 and Nonoxynol-2. The report also references unpublished rodent uterotrophic assays that include negative studies on Nonoxynol-4 and Nonoxynol-9, both at doses up to 1000 mg/kg/day.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Non-Human

Nononxynol-10

The following doses of nononxynol-10 (in isotonic saline) were injected subcutaneously into 4 groups of female Jcl:Wistar rats in a lactational exposure study: 5 mg/kg/day (20 rats), 20 mg/kg/day (22 rats), and 80 mg/kg/day (21 rats).[23] Nonoxynol-10 (pH 6.3) was dissolved in saline, and the test solution was administered to the subcutis of the dorsal regions. Doses (dose volume = 3 ml/kg body weight) were administered daily from the date of birth to day 21 after the birth of F_1 offspring. The negative control group consisted of 21 rats. A significant decrease in food consumption from the initial day of dosing (day 0) to day 17 after the birth of F_1 offspring was reported for the 80 mg/kg/day dose group. There was a tendency for decreased body weight in the 80 mg/kg/day group from day 0 to day 14 after the delivery of F_1 offspring, compared to the control. This was not true for the 5 and 20 mg/kg/day groups. Physical development and behavioral test results, observations at cesarean section and external examination of F_2 fetuses, skeletal examinations, and necropsy findings did not reveal any Nonoxynol-10-related effects. Histopathological findings for males or females that did not achieve successful gestation also were not indicative of a Nonoxynol-10-related effect. However, it was noted that Nonoxynol-10 affected the growth of offspring from dams treated only during lactation. The "noneffective dose level" was considered to be 20 mg/kg/day for general toxicity to the dams and their offspring.

In another study, groups of 40 female Jcl:Wistar rats were injected s.c.with Nonoxynol-10 at dose rates of 2 mg/kg/day and 20 mg/kg/day (dose volume = 3 ml/kg body weight) for 15 weeks.[24] The negative control (isotonic saline) group also consisted of 40 rats. Nonoxynol-10, dissolved in saline, was administered daily according to the procedure in the preceding study. The animals were observed once per day from the day that dosing was initiated to day 20 of gestation (most of the animals) or to day 22 after delivery, the weaning day of F_1 pups. The observations at cesarean section or external, visceral, and skeletal examinations were not indicative of any test substance-related effects on F_1 fetuses. Physical development and behavioral test results, observations at cesarean section and external examinations, skeletal examinations, and necropsy findings for the F_1 and F_2 offspring did not reveal any test substance-related effects. It was concluded that Nonoxynol-10 had no effect on the reproductive ability of females or on the fetal development or growth, behavior, and functions of their offspring.

The effects of Nonoxynol-treated sperm on fetal development were studied using Japanese white rabbits of the Kbl:JW strain (20 males, 66 females).[26] Prior to this study, rabbits (20 males, 43 females) were used in a preliminary test to determine the test concentrations of Nonoxynol-10 for the main study. Based on these test results, the following concentrations were tested in the main study: 0.04% (caused severe sperm impairment), 0.01% (sperm affected, but conception was observed), and 0.0025% (lowest concentration, set at one-fourth the medium concentration of 0.01%).

Female rabbits were artificially inseminated with semen mixtures, and killed on day 28 of gestation. Pregnant dams were evaluated for the following: number of corpora lutea, number of implantations, fetal losses, number of viable fetuses, placental weight, and placental abnormalities. Gestation was not observed after insemination with semen treated with 0.04% Nonoxynol-10. After insemination with semen treated with 0.01% Nonoxynol-10 or 0.0024% Nonoxynol-10 for 1 h, gestation occurred without impairing the viability, organogenesis, or growth of embryos or fetuses. The authors noted that these results may indicate that gestation may not be possible with sperm severely impaired by exposure to Nonoxynol-10, but there was no untoward effect on embryonic or fetal development when conception resulted from semen exposed to Nonoxynol-10.[26]

Human

Nonoxynol-9

A multicenter, randomized, double-masked trial of two spermicidal gels was performed. One of the gels was a commercially available Nonoxynol-9 spermicide, and the other gel was a mixture of 2 surfactants (unnamed).[27] Healthy females participated in the study, and 633 women used the Nonoxynol-9 spermicide; 932 women used the other spermicide. The women were followed for 12 months. One case of hypersensitivity and one case of pelvic inflammatory disease were reported as definitely and probably related to Nonoxynol-9 product use, respectively. Also, in the group of 633 women, 2 of 46 viable fetuses (4.3%) had congenital anomalies. One infant was born with cardiac anomalies and the other was born with gastroschisis. These 2 serious adverse events were said to have been potentially related to Nonoxynol-9 use. In the group of 932 women (other spermicide), one pregnancy resulted in a fetus with renal and cardiac malformations. However, these abnormalities were deemed unrelated to this spermicide. No post-study safety concerns were reported. Because women were followed for only 12 months in this study, it was noted that data relating to adverse events that may occur with longer term use are lacking.

In Vitro

Nonoxynol-9

A study was performed to determine the effects of Nonoxynol-9 on the human endometrium.[28] Human endometrial biopsies were cultured and incubated with various concentrations of Nonoxynol-9 (0.03%, 0.3% and 3%) for 6 h or 24 h. Endometrial histology was assessed by light microscopy. Inflammatory response was determined by analyzing proinflammatory cytokines with an enzyme-linked immunosorbent assay, and endometrial mucin was assessed by immunohistochemistry analysis and real-time polymerase chain reaction. Histological changes consistent with focal coagulative necrosis were seen after 6 h and 24 h of culture. All cytokines (interleukin 1β, tumor necrosis factor α, and interleukin 8) decreased at all concentrations of Nonoxynol-9 after 24 h of incubation. The expression of Mucin1 was inhibited in a dose-dependent manner at both the protein and messenger RNA levels. These results demonstrated that Nonoxynol-9 has multiple, potential deleterious effects on the human endometrium, characterized by necrosis, alteration of proinflammatory cytokines and inhibition of Mucin1 expression. These *in vitro* findings also suggest that Nonoxynol-9 can interrupt the functional barrier provided by the endometrium.

GENOTOXICITY

In Vitro

Nonoxynol-9

The genotoxicity of 3 OTC spermicide gels was evaluated in an Ames test using *Salmonella typhimurium* strains TA1535 and TA1538 with and without metabolic activation.[29] Spermicides A and B (100 ml each) contained 3% Nonoxynol-9, and Spermicide C (100 ml) contained 2% Nonoxynol-9. With metabolic activation, Spermicide B was genotoxic in strain TA1535 and Spermicide C was genotoxic in strain TA1538. Spermicide A was genotoxic in strain TA1535 with and without metabolic activation. All 3 spermicides were classified as strongly genotoxic. Because all 3 spermicides tested contained Nonoxynol-9, it was noted that other substances in the gels may explain the differences observed in the genotoxicity of the different formulations. Whether or not the spermicides were cytotoxic was not stated.

CARCINOGENICITY

Non-Human

Nonoxynol-10

The carcinogenicity of Nonoxynol-10 was evaluated using groups of 50 female $B6C3F_1$ mice.[22] As reported in the Repeated Dose Toxicity section, groups of mice received concentrations of 500 ppm, 1500 ppm, or 4500 ppm in the diet, of Nonoxynol-10 for 104 weeks. The mean intakes of Nonoxynol-10 were 81.5 mg/kg/day, 254 mg/kg/day, and 873 mg/kg/day, respectively. A fourth group was fed control diet. At pathological or microscopic examination, there were no changes that were attributable to Nonoxynol-10. Additionally, at histological examination, there were no neoplastic or non-neoplastic lesions in any of the dietary groups that were unequivocally observed to have increased in occurrence. It was concluded that Nonoxynol-10 did not cause any increase in the incidence of neoplastic lesions in mice exposed orally to Nonoxynol-10 in the diet for 2 years, and, Nonoxynol-10 was thus classified as noncarcinogenic in this study.

Human

Nonoxynol-9

A randomized trial was conducted, between June 1998 and August 2002, at 14 sites in the United States to evaluate the safety of five nononxynol-9 spermicides.[30] A total of 1,536 women participated in the study, 640 of which were included in a Papanicolaou smear analysis. The spermicides, used for a period of 7 months, 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. Follow-up visits were done 4, 17, and 30 weeks after study initiation. A Papanicolaou smear was performed routinely, and cervical cytology samples were interpreted and results were classified as either normal or abnormal. Abnormalities ranged from low-grade squamous intraepithelial lesion and atypical squamous cells of undetermined significance (Subcategory I) to results suggestive of invasive cancer (Subcategory III).

No differences in the rates of cervical alterations among the women using different amounts or different formulations of Nonoxynol-9 were found. There also was no statistically significant evidence of a dose-response relationship between Nonoxynol-9 and changes in cervical cytology. Furthermore, duration, frequency, and total number of spermicide uses were not associated with any statistically significant changes in cervical cytology. The most serious limitation of this study was that more than half of the original trial participants were excluded from the analysis because of missing Papanicolaou smear data. Thus, the analysis may have underestimated the absolute proportion of Nonoxynol-9 users with progression of cytologic abnormalities. However, there was no evidence that the exclusions were biased by spermicide group, and it was expected that the group comparisons were credible. The authors concluded that exposure to different formulations and doses of spermicides containing Nonoxynol-9 for 30 weeks is unlikely to influence cervical cytology.[30]

IRRITATION AND SENSITIZATION

Skin Irritation and Sensitization

Human

Provocative Tests

Nonoxynol-10

A multicenter study in Sweden was performed to address the question of human sensitization to oxidized ethoxylated surfactants.[31] The 528 participants (196 males, 332 females) were identified as consecutive dermatitis patients with suspected allergic contact dermatitis. The patients were patch tested with aqueous solutions of Nonoxynol-10 (20%) and air-oxidized Nonoxynol-10 (20%). Each test solution (15 ml) was applied using Finn chambers, with occlusion, for 48 h. The area and location of the application site were not stated. Reactions were scored at days 3 and 7 according International Contact Dermatitis Research Group criteria. None of the patients had reactions to Nonoxynol-10. Erythema was observed in 1 patient, only at day 7, patch tested with oxidized Nonoxynol-10. It was noted that this was not an allergic reaction.

Phototoxicity

In Vitro

Nonoxynol-9

Photohemolysis of human red blood cell suspensions containing Nonoxynol-9 (2×10^{-5} M) occurred after irradiation with UV light under aerobic conditions.[4] Nonoxynol-9 was irradiated for 70 minutes under 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 minutes in the absence of 2×10^{-5} M Nonoxynol-9 or when the cells were incubated with 2×10^{-5} M Nonoxynol-9 in the dark. It was concluded that Nonoxynol-9 was phototoxic *in vitro*.

Case Reports

Nonoxynol-12

A woman (domestic cleaner) with a 5-month history of acute severe dermatitis and a past history of atopic eczema was patch tested with Nonoxynol-12, an ingredient of the polish that was used on the job.[32] 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: 1%, 0.5%, 0.1%, and 0.01%. The reactions were classified as + on day 2 and ++ on day 4. Negative patch test results were reported for 30 control subjects.

Mucous Membrane Irritation

Non-Human

Nonoxynol-9

Female mice of the CF-1 strain were exposed to a spermicide containing 3.5% Nonoxynol-9. The method of exposure was either intravaginal or intrauterine.[33] After various exposure times, the animals were killed and uterine tissue sections were subjected to histological examination. Three mice were used for each time point. Both modes of administration resulted in disruption of the uterine epithelium. Intravaginal exposure resulted in histological findings that were consistent with uterine epithelial disruption. Following intrauterine injection, the Nonoxynol-9 spermicide caused rapid focal, uterine epithelial sloughing and complete epithelial loss within 24 h. Regeneration of the uterine epithelium began 48 h after exposure and the epithelium 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 authors concluded that Nonoxynol-9 had a deleterious effect on the uterine epithelium.

The intravaginal dosing of female BALB/c mice with a commercial spermicide containing 3.5% Nonoxynol-9 for 14 days 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 NF-kB and activator protein-1.[34] The concentrations of cytokines and chemokines in vaginal washes pooled from at least 10 mice at each time point were measured at baseline (day 0) and 3, 7, 14, and 21 days after 14 daily applications of the Nonoxynol-9 spermicide.

Five New Zealand white rabbits received 4% Nonoxynol-9 intravaginally at a dosage of 1 g/rabbit/day for 10 days.[35] vaginal irritation, epithelial exfoliation, vascular congestion, and leukocyte infiltration were reported. These results were reported in a study on the toxicity of liposomal gels, and 4% Nonoxynol-9 served as the positive control.

Human

A clinical trial of Nonoxynol-9 (in gel form) was performed using 40 healthy female volunteers. Twenty women received the gel and 20 received a placebo.[36] The women were instructed to use the gel (Nonoxynol-9 concentration = 20 mg/ml [100 mg/dose]) on each of 7 consecutive days. Examinations occurred on days 0, 7, and 14. Genital irritation was observed in 10 women who received Nonoxynol-9 and in 5 women in the placebo group. Colposcopy revealed erythema in 9 women in the Nonoxynol-9 group and in 2 women in the placebo group. Histologic inflammation was reported in 7 women who received Nonoxynol-9 and in 2 women who received the placebo. Inflammatory changes were characterized by patchy infiltration of the lamina propria, predominantly with CD^{8+} lymphocytes and macrophages; epithelial disruption was absent.

To better understand the colposcopic appearance of the genital epithelium after typical long-term spermicide use, a long-term study of women who used spermicides that contain Nonoxynol-9 was performed.[37] A subset of participants from a multicenter randomized clinical trial that compared the contraceptive effectiveness of 5 Nonoxynol spermicide formulations (summarized in the Carcinogenicity section) was used. Each study group consisted of 30 participants. The control group consisted of 31 women. The genital epithelium was evaluated by colposcopic and naked eye examination at baseline and during follow-up at weeks 4, 17, and 30. Five spermicidal formulations containing Nonoxynol-9 were studied, including 3 gels, a film, and a suppository. The 3 gels were described as follows: gel containing 52.5 mg Nonoxynol-9 at a concentration of 3.5% per dose (Gel A), gel containing 100 mg Nonoxynol-9 at a concentration of 4.0% per dose (Gel B), and Gel B at a dose of 150 mg Nonoxynol-9 per application. Each dose of film contained 100 mg Nonoxynol-9 at a concentration of 28% per sheet. Each suppository contained 100 mg Nonoxynol-9 at a concentration of 3.0% per dose. Overall, there was no increased risk for any new colposcopic lesion in any of the nononxynol-9 groups, when compared to the control group. However, when compared to the control, women who had used any Nonoxynol-9 product were more likely to have genital lesions characterized by erythema or edema ($p = 0.01$).

Twenty women applied 4% Nonoxynol-9 spermicide gel twice daily for 13.5 consecutive days.[38] An additional 20 women applied a placebo. Biopsies and endocervical cytobrush specimens were obtained at visits 2 (baseline) and 5. Histological findings of inflammation and deep epithelial disruption after product use were reported for 4 women. Dosing with Nonoxynol-9 caused astatistically significant increase in interleukin IL-1RA at visit 5.

The safety of a vaginal spermicidal gel containing 52.5 mg Nonoxynol-9 (3.5% Nonoxynol-9 in product), applied by 179 healthy women once daily for 14 days, was evaluated.[39] Also, in a randomized parallel study of nonxynol-9 local toxicity, groups of 35 healthy women (140 women total) used a vaginal suppository containing 150 mg Nonoxynol-9 for 2 weeks; the most frequent use reported was 4 times per day.[40] Collectively, the results of these studies suggest that Nonoxynol-9 does not elevate the incidence of lesions with epithelial disruption when these products are used no more than approximately once per day. The other types of lesions observed were minor/minimal. The incidences of lesions that are attributable to the use of these products tend to increase as the frequency of use increases to greater than once per day.

As stated earlier in the Noncosmetic Use section, the FDA is reviewing data from a clinical trial in which the effectiveness and safety of 5 spermicides, described as follows, were compared:[19] (1) 3 gels containing 52.5 mg, 100 mg, or 150 mg Nonoxynol-9 per dose and (2) both a film and a suppository, each containing 100 mg Nonoxynol-9 per dose. Vulvar or vaginal irritation, without evidence of concurrent vulvar or vaginal infection, was one of the focus areas of primary safety analyses. Vulvar or vaginal irritation was defined as follows: vaginal or vulvar itching, pain, discharge, dryness, loss of sensitivity; or puffiness, unspecified vaginitis or cervicitis, allergy to spermicide, or specified genital lesions or signs of irritation. The enrollment in this study was 1485 women total, which included trials conducted at 14 sites in the United States and approximately 300 women per group (5 groups total and 5 spermicides, respectively) .[41] Each participant provided data for safety analyses through the earlier of 7 months or 1 week after the date that use of the spermicide stopped. A total of 34 serious adverse events occurred in 31 study participants either during or after spermicide use, but none was felt to have been related to spermicide use. Seven-month probability data for vulvar or vaginal irritation, without current infection were: 13.6% (52.5 mg Nonoxynol-9 [in gel]; N = 296), 15% (100 mg Nonoxynol-9 [in gel]; N = 295), 13.8% (150

mg nonxynol-9 [in gel]; N = 300), 11.3% (100 mg Nonoxynol-9 [in film]; N = 295), and 13.9% (100 mg Nonoxynol-9 [in suppository]; N = 299). The 7-month probability of vulvar or vaginal irritation without infection did not differ among groups. None of the data for vulvar or vaginal irritation differed materially when the analysis was repeated, including only women who had not used any Nonoxynol-9 product within the week before admission to the study. The authors concluded that all 5 spermicide products were safe as used by the study participants.[41]

In Vitro

Nonoxynol-9

A study was performed to determine whether Nonoxynol-9 could induce genital tract inflammation by measuring levels of the following in normal human peripheral blood mononuclear cells (PBMCs) and macrophages: tumor necrosis factor (TNF-α), interleukin 1β (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8).[42] Ingredient dilutions that yielded culture viabilities ≥ 60%, compared to control cultures, were considered nontoxic. The nontoxic dilutions of Nonoxynol-9 were 1:1000 in both PBMCs and macrophages. Nonoxynol-9 (nontoxic dilution) was associated with relatively low levels of IL-1β, TNF- α, and IL-6. These results were indicative of the low toxicity in terms of the release of cytokines.

SUMMARY

The Nonoxynols are ethoxylated alkylphenols. Collectively, data on use frequency from FDA and use concentration from a Council survey indicate that the following 12 Nonoxynols (of the 27 reviewed in this safety assessment) are used in cosmetic products:

Nonoxynol-1	Nonoxynol-6	Nonoxynol-14
Nonoxynol-2	Nonoxynol-9	Nonoxynol-15
Nonoxynol-4	Nonoxynol-10	Nonoxynol-23
Nonoxynol-5	Nonoxynol-12	Nonoxynol-30

According to the 2015 VCRP, the greatest reported use frequency of these ingredients is for Nonoxynol-4 (90 formulations, all rinse-off), followed by Nonoxynol-6 (65 rinse-off formulations). Lower use frequencies are reported for the remaining Nonoxynols, most of which are used in rinse-off products. The results of a survey conducted in 2014 indicate that Nonoxynol-12 has the highest reported maximum concentration of use; it is used at concentrations up to 8.33% in rinse-off products (in hair dyes and colors). The highest maximum concentration of use reported for leave-on products is 0.42% (Nonoxynol-12 in hair spray).

The distributor of a body wash (cosmetic feminine wash - intended for use on the vaginal area) stated that this product contains Nonoxynol-9 at a concentration of 3%, and that it is in the process of removing this ingredient from the formula due to the company's awareness of potential safety issues.

The UV-light absorption spectrum of Nonoxynol-9 in water has two bands that are centered at 225 nm and 276 nm, with a tail extending to 300 nm.

Nonoxynol-9 was administered vaginally to 3 female New Zealand white rabbits at doses of 100 mg/kg and 300 mg/kg, and blood samples were collected from the marginal ear vein. With 300 mg/kg Nonoxynol-9, the mean peak plasma concentration (C_{max}) of 4.87 ± 0.3 ng/ml was achieved at 1 h post-dosing.

The percutaneous absorption of Nonoxynol-4 and Nonoxynol-9 *in vitro* was studied using human, porcine, and rat skin samples in flowthrough diffusion cells. In skin samples from all 3 species, ^{14}C-Nonoxynol-9 and ^{14}C-nonxynol-4 were minimally absorbed across the skin.

Three groups of 50 female B6C3F$_1$ mice received nononxynol-10 at concentrations up to 4500 ppm in the diet for 104 weeks. The estimated mean dose rate of Nonoxynol-10 was 873 mg/kg/day at 4500 ppm. There were no pathological or histological evidence of toxicity attributable to Nonoxynol-10 in the diet.

Nononxynol-10 was administered s.c. to female Jcl:Wistar rats in a developmental toxicity study at dose rates up to 80 mg/kg/day. The dams received daily s.c. injections from the date of the birth to 21 days after the birth of their offspring. The "noneffective dose level" was considered to be 20 mg/kg/day for general toxicity to the dams and their offspring.

Female Jcl:Wistar rats were injected s.c. daily with Nonoxynol-10 for 15 weeks. Effects in the F_0 dams exposed to 2 mg/kg/day and 20 mg/kg/day were described as scab formation and hair loss at the application site. Induration of the skin was also observed in animals that received 20 mg/kg/day. Necropsy findings included whitish changes of the subcutis in both dose groups and adhesion of abdominal organs in the 20 mg/kg/day dose group.

In rats exposed s.c. to Nonoxynol-10 at dose rates up to 80 mg/kg/day in a lactational exposure study, there were no reproductive effects in the offspring of the female rats that were treated during lactation, and there were no teratogenic effects in the F_2 generation. Following the insemination of rabbits with sperm treated with 0.01% or 0.0024% Nonoxynol-10, gestation occurred without impairing the viability, organogenesis, or growth of embryos or fetuses.

In a multicenter study on a Nonoxynol-9 spermicide, 2 of 49 viable fetuses in a group of 633 women had congenital anomalies. These events may be related to Nonoxynol-9 use. Results for the Nonoxynol-9 spermicide were compared with another group of 932 women who used another spermicide (mixture of 2 unnamed surfactants). In this group, one pregnancy resulted in a fetus with renal and cardiac malformations. However, these abnormalities were deemed unrelated to this spermicide.

The genotoxicity of 3 over-the-counter spermicide gels was evaluated in an Ames test using *S. typhimurium* strains TA1535 and TA1538 with and without metabolic activation. Spermicides A and B contained 3% Nonoxynol-9, and Spermicide C contained 2% Nonoxynol-9. With metabolic activation, Spermicide B was genotoxic in strain TA1535 and Spermicide C was genotoxic in strain TA1538. Spermicide A was genotoxic in strain TA1535 with and without metabolic activation. All 3 spermicides were classified as strongly genotoxic; however, it was noted that other substances in the gels may explain the differences in genotoxicity that were observed.

No evidence of carcinogenicity was observed in a 2-year study in which mice were fed Nonoxynol-10 daily at concentrations up to 4500 ppm in the diet.

There was no statistically significant evidence of a dose-response relationship between Nonoxynol-9 and changes in cervical cytology in a 7-month Nonoxynol-9 spermicide trial involving 640 women. There also were no differences in the rates of cervical alterations among the women using different amounts or different formulations of Nonoxynol-9. The spermicides 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.

Dermatitis patients (196 men and 332 women) with suspected allergic contact dermatitis were patch tested with aqueous solutions of Nonoxynol-10 (20%) and air-oxidized Nonoxynol-10 (20%). None of the patients had allergic reactions to Nonoxynol-10.

Photohemolysis of human red blood cell suspensions containing Nonoxynol-9 occurred in vitro after UV irradiation, indicating the potential for phototoxicity.

Female mice were exposed (intravaginal or intrauterine) to a spermicide containing 3.5% Nonoxynol-9. The spermicide was placed intravaginally (single application) or administered by intrauterine injection (single injection). Intravaginal exposure resulted in histological findings that were consistent with uterine epithelial disruption. Following intrauterine injection, the Nonoxynol-9 spermicide caused rapid focal, uterine epithelial sloughing and complete epithelial loss within 24 h. Regeneration of the epithelium was complete within 72 h. The intravaginal dosing of a spermicide containing 3.5% Nonoxynol-9 for 14 days induced an inflammatory response that was characterized by increased levels of cytokines and chemokines and the recruitment of neutrophils and monocytes into the genital tract.

Irritation, epithelial exfoliation, vascular congestion, and leukocyte infiltration were observed in a study in which rabbits received 4% Nonoxynol-9 intravaginally (1 g/day) for 10 days.

In a study in which 40 women used a spermicide a gel that contained Nonoxynol-9 (20 mg/ml) for 7 consecutive days, histologic inflammation was observed in 7 women; epithelial disruption was absent. Histological findings of inflammation were also reported for 4 of 20 women who applied a 4% Nonoxynol-9 spermicide gel twice daily for 13.5 consecutive days. The following spermicidal formulations were evaluated using groups of 30 women: gel containing 52.5 mg Nonoxynol-9 at a concentration of 3.5% per dose (Gel A), gel containing 100 mg Nonoxynol-9 at a concentration of 4.0% per dose (Gel B), and Gel B at a dose of 150 mg Nonoxynol-9 per application. Each dose of film contained 100 mg

Nonoxynol-9 at a concentration of 28% per sheet. Each suppository contained 100 mg Nonoxynol-9 at a concentration of 3.0% per dose. When compared to a control group of 31 women, women who had used any Nonoxynol-9 product were more likely to have genital lesions characterized by erythema or edema ($p = 0.01$).

DISCUSSION

The Panel has evaluated the safety of Nonoxynols in cosmetics and issued a final report published in 1983 with the following conclusion: "On the basis of the available information presented in this report, the Panel concludes that Nonoxynols-2, -4, -8, -9, -10, -12, -14, -15, -30, -40, and -50 are safe as cosmetic ingredients in the present practices of concentration and use". The Panel reevaluated the safety of Nonoxynols in cosmetics and issued an amended final report published in 1999 with the following conclusion: "On the basis of the available animal and clinical data included in this report, the CIR Expert Panel concludes that Nonoxynols-1, -2, -3, -4, -5, -6, -7, and -8 are safe as used in rinse-off products and safe at concentrations ≤ 5% in leave-on products [Note: this conclusion modifies a previous conclusion for Nonoxynols-2, -4, and -8, which had been considered safe as used in both rinse-off and leave-on products]". The following ingredients were not previously reviewed, and their safety in cosmetics is evaluated in this safety assessment: Nonoxynols-13, -18, -20, -23, -25, -35, -44, -70, -100, and -120

The Panel discussed the ≤5% concentration limit specified for Nonoxynols-1, -2, -3, -4, -5, -6, -7, and -8 in leave-on products in 1999, in light of the ≤ 0.1% limit of the EU on the concentrations of Nonoxynols and nonylphenols in cosmetic and many other commercial and industrial products. The EU's restriction is based on the premise that the ecologies of European water bodies are at risk from environmental releases of Nonoxynols and their environmental degradation products, because these compounds are persistent in the environment and have the potential to cause endocrine disruption in ecological species. The Panel determined these environmental issues are not relevant for assessing the consumer safety of Nonoxynols as used in cosmetic products.

In addition, the Panel discussed the 5% concentration limit in the context of correspondence indicating that a feminine wash product (intended for use on the vaginal area) containing 3% Nonoxynol-9 is on the market in the United States, for sale through the Internet. After considering evidence of the irritation potential of spermicidal products containing Nonoxynol-9 as well as the irritation potential of Nonoxynol-9, the Panel removed the 5% concentration limit specified in its previous conclusion and agreed that cosmetic products containing Nonoxynols should be formulated to be non-irritating. Though the review of Nonoxynol-9 as a spermicide (noncosmetic use) is not within the Panel's purview, data indicating that Nonoxynol-9 spermicides cause mucous membrane irritation in animals and in human subjects were considered in the Panel's safety evaluation of Nonoxynols. However, the Panel does not expect that irritation or spermicidal activity would be associated with the intended use of cosmetic products containing Nonoxynol-9.

CONCLUSION

The CIR Expert Panel concluded that the following 27 Nonoxynols listed below are safe in the present practices of use and concentration in cosmetics as described in this safety assessment, when formulated to be non-irritating:

Nonoxynol-1	Nonoxynol-10	Nonoxynol-25*
Nonoxynol-2	Nonoxynol-11*	Nonoxynol-30
Nonoxynol-3*	Nonoxynol-12	Nonoxynol-35*
Nonoxynol-4	Nonoxynol-13*	Nonoxynol-40*
Nonoxynol-5	Nonoxynol-14	Nonoxynol-44*
Nonoxynol-6	Nonoxynol-15	Nonoxynol-50*
Nonoxynol-7*	Nonoxynol-18*	Nonoxynol-70*
Nonoxynol-8*	Nonoxynol-20*	Nonoxynol-100*
Nonoxynol-9	Nonoxynol-23	Nonoxynol-120*

*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.

Table 1. Names, CAS Registry Numbers, Definitions, and Functions of the Nonoxynols[3]
(Items in brackets were added by CIR Staff)

Ingredient & CAS No.	Definitions and Functions
Nonoxynol-1 26027-38-3 (generic) 27986-36-3 37205-87-1 (generic)	Nonoxynol-1 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1, wherein n has an average value of 1]. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-2 26027-38-3 (generic) 27176-93-8 (generic) 37205-87-1 (generic) 9016-45-9 (generic)	Nonoxynol-2 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1], where n has an average value of 2. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-3 26027-38-3 (generic) 27176-95-0 (generic) 37205-87-1 (generic) 51437-95-7 (generic) 84562-92-5 (generic) 9016-45-9 (generic)	Nonoxynol-3 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1], where n has an average value of 3. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-4 26027-38-3 (generic) 27176-97-2 37205-87-1 (generic) 68412-54-4 7311-27-5 9016-45-9 (generic)	Nonoxynol-4 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1], where n has an average value of 4. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-5 20636-48-0 26027-38-3 (generic) 26264-02-8 37205-87-1 (generic) 9016-45-9 (generic)	Nonoxynol-5 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 5. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-6 26027-38-3 (generic) 27177-01-1 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-6 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 6. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-7 26027-38-3 (generic) 27177-03-3 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-7 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure]1 where n has an average value of 7. **Function**: Surfactants - Emulsifying Agents

Table 1. Names, CAS Registry Numbers, Definitions, and Functions of the Nonoxynols[3]
(Items in brackets were added by CIR Staff)

Ingredient & CAS No.	Definitions and Functions
Nonoxynol-8 26027-38-3 (generic) 26571-11-9 27177-05-5 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-8 is the ethoxylated alkyl phenol that conforms generally to the formula in [Figure 1] where n has an average value of 8. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-9 14409-72-4 26027-38-3 (generic) 26571-11-9 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-9 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 9. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-10 26027-38-3 (generic) 27177-08-8 27942-26-3 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-10 is the ethoxylated alkyl phenol that conforms generally to the formula[in Figure 1] where n has an average value of 10. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-11 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-11 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 11. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-12 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-12 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 12. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-13 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 (generic) 9016-45-9 (generic)	Nonoxynol-13 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 13. **Function**: Surfactants - Emulsifying Agents
Nonxynol-14 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-14 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 14. **Function**: Surfactants - Emulsifying Agents
Nonxynol-15 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-15 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 15. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-18 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-18 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 18. **Function**: Surfactants - Emulsifying Agents
Nonoxynol-20 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-20 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 20. **Functions**: Surfactants - Cleansing Agents; Surfactants - Emulsifying Agents; Surfactants - Solubilizing Agents
Nonoxynol-23 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-23 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 23. **Functions**: Surfactants - Cleansing Agents; Surfactants - Solubilizing Agents
Nonoxynol-25 9016-45-9 (generic)	Nonoxynol-25 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 25. **Functions**: Surfactants - Cleansing Agents; Surfactants - Solubilizing Agents
Nononxynol-30 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-30 is the ethoxylated alkyl phenol that conforms generally to the formula[in Figure 1] where n has an average value of 30. **Functions**: Surfactants - Cleansing Agents; Surfactants - Solubilizing Agents

Table 1. Names, CAS Registry Numbers, Definitions, and Functions of the Nonoxynols[3]
(Items in brackets were added by CIR Staff)

Ingredient & CAS No.	Definitions and Functions
Nonoxynol-35 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-35 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 35. **Functions**: Surfactants - Cleansing Agents; Surfactants - Solubilizing Agents
Nonoxynol-40 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-40 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 40. **Functions**: Surfactants - Cleansing Agents; Surfactants - Solubilizing Agents
Nonoxynol-44 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-44 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 44. **Function**: Surfactants - Cleansing Agents
Nonoxynol-50 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-50 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 50. **Function**: Surfactants - Cleansing Agents
Nonoxynol-70	Nonoxynol-70 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 70. **Function**: Surfactants - Solubilizing Agents
Nonoxynol-100 26027-38-3 (generic) 37205-87-1 (generic) 68412-54-4 9016-45-9 (generic)	Nonoxynol-100 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 100. **Function**: Surfactants - Cleansing Agents
Nonoxynol-120 26027-38-3 (generic) 37205-87-1 (generic) 9016-45-9 (generic)	Nonoxynol-120 is the ethoxylated alkyl phenol that conforms generally to the formula [in Figure 1] where n has an average value of 120. **Function**: Surfactants - Cleansing Agents

Table 2. Properties of Nonoxynols.[1,2]

Ingredient	Property	Value
Nonoxynol-1		
	Physical Form	Colorless to light yellow liquid
	Odor	Very slight
	Specific Gravity	0.98 @ 20/20˚C
	Vapor Density	Greater than air
	Boiling Point	> 400˚F
	Solubility	Slightly soluble/insoluble in water; soluble in oil
Nonoxynol-2		
	Physical Form	Liquid
	Specific Gravity	0.984-0.986 @ 25/25˚C
	Solubility	Soluble in oil
	UV Absorption	200-290 nm
Nonoxynol-4		
	Physical Form	White to light amber liquid
	Specific Gravity	1.020-1.030 @ 25/25˚C
	Solubility	Soluble in oil and common organic solvents
	UV Absorption	200-290 nm
Nonoxynol-5		
	Physical Form	Clear light-colored liquid
	Odor	Slightly aromatic
	Specific Gravity	1.024-1.034
	Viscosity	240 cps
	Vapor Pressure	Nil @ 20˚C
	Solubility	Soluble in oil; dispersible in water
Nonoxynol-6		

Table 2. Properties of Nonoxynols.[1,2]

Ingredient	Property	Value
	Physical Form	Colorless to light amber liquid
	Odor	Very slight
	Specific Gravity	1.030-1.050 @ 25/25˚C
	Viscosity	150-250cps @ 25˚C
	Vapor Pressure	Nil @ 20˚C
	Vapor Density	Greater than air
	Boiling Point	Greater than 400˚C
	Solubility	Soluble in oil, water, and common organic solvents
Nonoxynol-7		
	Physical Form	Liquid
	Specific Gravity	1.055 at 20/20˚C
	Solubility	Soluble in aromatic solvents
Nonoxynol-8		
	Physical Form	Liquid
	Specific Gravity	1.05 @ 25/25˚C
Nonoxynol-8		
	Solubility	Soluble in water
Nonoxynol-8.5		
	Physical Form	Pale yellow liquid
	Specific Gravity	1.040-1.060 @ 25/25˚C
	Viscosity	200-300 @ 25˚C
	Solubility	Soluble in water and polar organic solvents
Nonoxynol-9		
	Physical Form	Liquid
	Solubility	Soluble in aromatic solvents
	UV Absorption	200-290 nm
Nonoxynol-9.5		
	Physical Form	Colorless to light amber liquid
	Specific Gravity	1.040-1.060 @ 25/25˚C
	Viscosity	175-250 cps @ 25˚C
	Solubility	Soluble in water and polar organic solvents
Nonoxynol-10		
	Physical Form	Liquid
	Solubility	Soluble in water and aromatic solvents
Nonoxynol-11		
	Physical Form	Liquid
	Solubility	Soluble in water
Nonoxynol-12		
	Physical Form	Liquid
	Specific Gravity	1.07 @ 25/25˚C
Nonoxynol-13		

Table 2. Properties of Nonoxynols.[1,2]

Ingredient	Property	Value
	Physical Form	Liquid
	Specific Gravity	1.07 @ 20/20˚C
	Solubility	Soluble in water
Nonoxynol-14		
	Physical Form	Viscous liquid
	Solubility	Soluble in water
Nonoxynol-15		
	Physical Form	Opaque viscous liquid
	Specific Gravity	1.060-1.080 @ 25/25˚C
	Viscosity	500-600 cps @ 25˚C
	Solubility	Soluble in water and polar organic solvents
Nonoxynol-20		
	Physical Form	Wax
	Solubility	Soluble in water
Nonoxynol-30		
	Physical Form	Pale yellow to light amber viscous liquid
Nonoxynol-30		
	Specific Gravity	1.080-1.100 @ 25/25˚C
	Solubility	Soluble in water
Nonoxynol-40		
	Physical Form	Wax
	Viscosity	1.082 @ 58/25˚C
	Solubility	Soluble in water
Nonoxynol-50		
	Physical Form	Wax
	Solubility	Soluble in water
Nonoxynol-100		
	Physical Form	Wax
	Specific Gravity	1.08 @ 57/20˚C
	Solubility	Soluble in water

Table 3. Current and Historical Frequency and Concentration of Use According to Duration and Type of Exposure.[1,2,6,5]

	Nonoxynol-1 (2015)		Nonoxynol-1 (1999)		Nonoxynol-2 (2015)	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	2	NR	58	10	58	NR
Duration of Use						
Leave-On	2	NR	NR	NR	NR	NR
Rinse off	NR	NR	58	10	58	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	2**	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	2**	NR	NR	NR	NR	NR
Dermal Contact	2	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	58	10	58	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Nonoxynol-2 (1999)		Nonoxynol-4 (2015)		Nonoxynol-4 (1999)	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	219	20	90	NR	575	10
Duration of Use						
Leave-On	NR	NR	NR	NR	10	NR
Rinse off	219	20	90	NR	555	10
Diluted for (bath) Use	NR	NR	NR	NR	6	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	4	NR
Incidental Ingestion	1	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	1**	NR
Incidental Inhalation- Powders	NR	NR	NR	NR	1**	NR
Dermal Contact	NR	NR	1	NR	13	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	4	NR
Hair-Coloring	218	20	89	NR	554	10
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	1	NR	1	NR	7	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Nonoxynol-5 (2015)		Nonoxynol-5 (1999)		Nonoxynol-6 (2015)	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	1	NR	2	1	65	NR
Duration of Use						
Leave-On	1	NR	NR	NR	NR	NR
Rinse off	NR	NR	NR	NR	65	NR
Diluted for (bath) Use	NR	NR	1	1	NR	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	1*	NR	1*	1	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	1	1	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	65	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	1	1	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[1,2,6,5]

	Nonoxynol-6 (1999)		**Nonoxynol-9 (2015)**		**Nonoxynol-9 (1983)*** **	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	10	50	10	2.5	140	1-50
Duration of Use						
Leave-On	NR	50	5	NR	5	1-25
Rinse off	10	50	4	2.5	99	1-50
Diluted for (bath) Use	NR	NR	NR	NR	12	1-25
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	50*	4**	NR	3*	25*
Incidental Inhalation- Powders	NR	NR	4	NR	NR	NR
Dermal Contact	3	50	8	2.5	27	1-25
Deodorant (underarm)	NR	NR	NR	NR	2	5
Hair - Non-Coloring	NR	NR	1	NR	27	1-10
Hair-Coloring	7	50	NR	NR	58	50
Nail	NR	NR	NR	NR	1	1
Mucous Membrane	3	NR	1	NR	21	1-25
Baby Products	NR	NR	NR	NR	NR	NR
	Nonoxynol-10 (2015)		**Nonoxynol-10 (1983)*** **		**Nonoxynol-12 (2015)**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	25	NR	42	1-25	12	0.42-8.33
Duration of Use						
Leave-On	5	NR	1	1	7	0.42
Rinse off	20	NR	41	1-25	5	8.33
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	2	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	5*	NR	NR	NR	1*	0.42
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	7	NR	7	1-10	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	18	NR	5	1	6	0.42
Hair-Coloring	NR	NR	30	25	2	8.33
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	4	NR	2	5-10	NR	NR
Baby Products	1	NR	NR	NR	NR	NR
	Nonoxynol-12 (1983)* **		**Nonxynol-14 (2015)**		**Nonoxynol-15 (2015)**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	9	1-5	1	NR	1	NR
Duration of Use						
Leave-On	NR	1	1	NR	1	NR
Rinse off	7	1-5	NR	NR	NR	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	1	1	1**	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	1**	NR	NR	NR
Dermal Contact	2	1	1	NR	1	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	5	1-5	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	4	NR	NR	NR	NR	NR
Baby Products	1	NR	NR	NR	NR	NR

Table 3. Current Frequency and Concentration of Use According to Duration and Type of Exposure.[1,2,6,5]

	Nonoxynol-15 (1983)* **		**Nonoxynol-23 (2015)**		**Nonoxynol-30 (2015)**	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Totals/Conc. Range	2	0.1	1	NR	1	NR
Duration of Use						
Leave-On	NR	NR	NR	NR	NR	NR
Rinse off	2	0.1	1	NR	1	NR
Diluted for (bath) Use	NR	NR	NR	NR	NR	NR
Exposure Type						
Eye Area	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Sprays	NR	NR	NR	NR	NR	NR
Incidental Inhalation- Powders	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	2	0.1	1	NR	1	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Nonoxynol-30 (1983)* **	
	# of Uses	Conc. (%)
Totals/Conc. Range	1	0.1
Duration of Use		
Leave-On	NR	NR
Rinse off	1	0.1
Diluted for (bath) Use	NR	NR
Exposure Type		
Eye Area	NR	NR
Incidental Ingestion	NR	NR
Incidental Inhalation- Sprays	NR	NR
Incidental Inhalation- Powders	NR	NR
Dermal Contact	NR	NR
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	NR	NR
Hair-Coloring	1	0.1
Nail	NR	NR
Mucous Membrane	NR	NR
Baby Products	NR	NR

NR = Not Reported; Totals = Rinse-off + Leave-on + Diluted for (bath) Product Uses.
*It is possible that these products may be sprays, but it is not specified whether the reported uses are sprays.
**Not specified whether a powder or spray, so this information is captured for both categories of incidental inhalation.
***Because use concentrations per ingredient were reported as a range and not as individual values in this published report, the upper limit of each range is presented. The upper limit of a range may or may not have actually been a reported use concentration.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

Table 4. Acute Oral Toxicity Studies on Nonoxynols from Prior Safety Assessments.[1,2]

Ingredients	Animals	LD_{50}s
Nonoxynol-2	30 rats (males and females)	3.55 g/kg (slightly toxic)
Nonoxynol-4	25 male rats	7.4 g/kg (practically nontoxic)
Nonoxynol-5	Rats (number not stated)	3.5 to 4.5 g/kg (slightly toxic)
Nonoxynol-6	30 rats (males and females)	1.98 g/kg (slightly toxic)
Nonoxynol-7	20 male rats	3.67 ml/kg (slightly toxic)
Nonoxynol-9	80 rats	3 g/kg (slightly toxic)
Nonoxynol-9	80 guinea pigs	2 g/kg (slightly toxic)
Nonoxynol-9	12 rabbits	4.4 g/kg (slightly toxic)
Nonoxynol-9	20 mice	4.29 ml/kg (slightly toxic)
Nonoxynol-10	20 male rats	1.3 g/kg (slightly toxic)
Nononxynol-10	20 female rats	1.3 g/kg (slightly toxic)
Nonoxynol-12	34 rats (males and females)	5.10 ml/kg (slightly toxic)
Nonoxynol-13	15 male rats	3.73 ml/kg (slightly toxic)
Nonoxynol-15	50 rats	2.5 g/kg (slightly toxic)
Nonoxynol-30	50 rats	Relatively harmless (doses up to 64 ml/kg). LD_{50} not determined
Nonoxynol-40	25 rats	Relatively harmless (doses up to 26.01 g/kg). LD_{50} not determined
Nonoxynol-40	10 rats	Relatively harmless (up to 64 ml/kg). LD_{50} not determined

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

Properties -UV Absorption

Nononxynols-2, -4, and -9

UV spectral analyses of Nonoxynol-2, -4, and -9 were conducted in two sets of experiments in which samples of each chemical were diluted with water and 10% isopropanol, respectively. The results of these experiments indicated that 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). The following comments were made by the investigator: "UV absorption is not affected by addition of ethylene oxide formation of ethylene glycol linkages, since they are not part of the chromophore. However, molecular weight does inversely influence absorption for the Nonoxynols. Thus, Nonoxynol-9 with 9 moles of ethylene oxide has less UVC absorption than a Nonoxynol with 4 moles of ethylene oxide. All three Nonoxynols show only tail absorption above the 290 ml range to a similar degree. Thus, there does not appear to be any significantly greater UVA or UVB absorption for either Nonoxynol-2 or -4 as compared to Nonoxynol-9"[2]

Method of Manufacture

Nonoxynols

Alkylphenols [such as these Nonoxynols] are synthesized commercially by Friedal-Crafts alkylation of a phenol to an olefin. The resulting monoalkylphenol product is purified by distillation and ethoxylated with the appropriate number of moles of ethylene oxide to produce the desired alkylphenol ethoxylate.[1]

Composition

Nonoxynols-2, -4, and -9

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. The distribution of homologues was as follows: Nonoxynol-2 (Nonoxynol- 1, -2, -3, and -4 homologues present), Nonoxynol-4 (Nonoxynols- 1, -2, -3, -4, -5, -6, -7, and -8 homologues), and Nonoxynol-9 (Nonoxynol-2, -3, -4, -5, -6, -7, -8, -9, -10, and -11 homologues).[2]

Impurities

Nonoxynols -1, -2, -4, -6, and -9

Nonoxynol-1 may contain up to 20 ppm ethylene oxide and, Nonoxynol-6, up to 35 ppm ethylene oxide. Assays for 1,4-dioxane and ethylene oxide were also performed on samples of Nonoxynol-2, -4, and -9. Neither 1,4- dioxane nor ethylene oxide was detected in triplicate samples of Nonoxynol-2. However, Nonoxynol-4 (5 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. Samples of Nonoxynol-2, -4, and -9 were analyzed 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.[2]

Toxicokinetics

Nonoxynols-7, -10, -12, and -15

The metabolism of Nonoxynols takes place by shortening the ethylene oxide chain and some carboxylation of the alkyl chain by omega-oxidation. No metabolic formation of free phenolic groups has been reported. Ethylene-14C-oxide-labeled Nonoxynol-7, -10, -12, or -15 (dose = 67 mg/kg) was fed to groups of four rats each. Seven days cumulative ^{14}C levels in urine, feces, and expired air were determined. With increasing ethoxy chain length, urinary and pulmonary excretion of radiocarbon decreased and fecal excretion of label increased, indicating decreased intestinal absorption.[2]

Nonoxynol-9

It has been reported that Nonoxynol-9 is absorbed through the vaginal wall of rabbits and rats, and is excreted by liver-bile-feces and kidney-urine routes.[1]

Four rats were injected intravenously with ^{14}C-Nonoxynol-9 (single dose = 5.2 mg/kg; 3.08 µCi/mg). At 6 and 24 hours, the tissues and organs contained 44.4 and 48.1% of the administered radioactivity, respectively. Again, the largest counts of radioactivity were reported in the small and large intestines (contents included). In these tissues, 38.7% and 43.3% of the administered dose were detected at 6 and 24 hours, respectively. At 48 hours, 60.8% and 42.8% of the administered radioactivity were excreted in the feces and urine, respectively. Radiomonitored HPLC analysis of urine (pooled from 4 rats at 24 hours) and bile (pooled from an additional 3 rats at 6 hours) collected after intravenous administration of ^{14}C-Nonoxynol-9 indicated that no intact Nonoxynol-9 was present in the urine or bile, and that Nonoxynol-9 was metabolized to highly polar species. Urinary metabolites that were neutral and acidic in character were detected.[2]

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

The transplacental transfer and disposition of ^{14}C-Nonoxynol-9 were evaluated using female rats (number, strain not stated). Each animal received a single dose of the test substance (dose = 0.1 mg/100 g) intravaginally on day 15 of gestation. The animals were killed at 24,48, or 96 hours post-administration. At 24 and 48 hours, the concentrations of ^{14}C in the amniotic fluid, placenta, and fetus were similar to those in the plasma; however, at 96 hours, concentrations of ^{14}C in these three tissues were two to five times greater than that detected in the maternal plasma. The analysis of blood samples obtained from the tail vein indicated that maximal concentrations of ^{14}C in the blood were detected at 45 to 60 minutes, and that blood concentrations decreased gradually thereafter. Approximately 86% and 96% of the administered dose were absorbed from the vagina after 24 and 48 hours, respectively. The greatest uptake of ^{14}C occurred in the following organs: maternal liver, cecum, duodenum, bladder, kidneys, adrenal and thyroid glands, and uterus. The lowest uptake of ^{14}C occurred in the brain. The combined recoveries of ^{14}C in the urine and feces were 34,46, and 54% of the administered dose at 24,48, and 96 hours, respectively.[2]

Percutaneous Absorption

Nonxynols-2, -4, and -9

The in vitro skin penetration of Nonoxynol-2, -4, and -9 was 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 (10% w/w in isopropyl alcohol per solution; volume = 15 ~1) was applied to epidermal membranes. Solutions remained in contact with the skin for 48 hours, after which the entire receptor media were analyzed by HPLC. The HPLC analysis employed a fluorescence detection method. 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. The total permeation for Nonoxynols was as follows: 6.17 ± 0.94 µg/cm^2, corresponding to 0.57 ± 0.07% of applied dose (Nonoxynol-2); 7.10 ± 1.47 µg/cm^2, 0.66 ± 0.14% of applied dose (Nonoxynol-4); and 4.73 ± 2.33 µg/cm^2, 0.49 ± 0.27% of applied dose (Nonoxynol-9).[2]

Based on preceding data, it was stated that the total skin penetration for Nonoxynol-9 was slightly lower than that for Nonoxynol-2 and -4. The following comments were also made: "Based on the leave-on application data, the levels of Nonoxynols absorbed following an abbreviated exposure period (1 hour) 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-hour 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 (500-750 cm2) in products such as hair dyes"[2]

Single Dose (Acute) Toxicity - Inhalation

Nonoxynols -4, -7, and -9

Groups of six male rats were placed in inhalation chambers and exposed once to Nonoxynol-4, -7, or -9 for either four or eight hours. Animals were observed for 14 days following exposure. Inhalation of these Nonoxynols did not cause toxic effects in rats (normal weight gains and no mortalities).[1]

Single Dose (Acute) Toxicity - Oral

Nonoxynols 2 through -40

Oral LD_{50} values are included in Table 4.

Single Dose (Acute) Toxicity - Dermal

Nonoxynols-4, -7, -9, -10, -13, and -40

One diluted and five undiluted Nonoxynols were tested in rabbits for dermal toxicity. In each study, the sample was applied once under occlusion to shaved, abraded skin. The patches were removed at 24 h, the exposed sites rinsed, and the animals observed for 14 days. The LD_{50} values resulting from these studies indicate that Nonoxynols-4, -7, -9, -10, and -13 ranged from 1.8 ml/kg to 4.4 g/kg. A 50% Nonoxynol-40 applied in a similar manner was reported to have an LD_{50} of greater than 10 g/kg. The following toxic effects were observed after dosing: Nonoxynol-4 (5 rabbits tested: erythema and necrosis of skin; lung congestion and hemorrhages in dead animals); Nonoxynol-7 (5 rabbits tested: erythema and necrosis of skin; lung congestion, hemorrhages, and liver congestion), Nonoxynol-9 (12 rabbits tested: diarrhea, liver lesions, and erythema at 8 and 50 g/kg), Nonoxynol-9 (5 rabbits tested: skin necrosis), Nonoxynol-13 (8 rabbits tested: lung hemorrhages and mottled liver and kidneys in dead animals), Nonoxynol-40 (6 rabbits tested: erythema and necrosis of skin).[1]

Nonoxynols-5 to 11.5

Nonoxynols-5 to -11.5, when applied topically to rabbits, resulted in minimum lethal doses of 2 to 10 g/kg. Toxicity decreased as ethoxylation increased.[1]

Nonoxynols-5 and -6

In an acute dermal toxicity study involving rabbits (number and strain not stated), the LD_{50} for Nonoxynol-5 was not achieved at a dose of 2.0 g/kg. The procedure for test substance administration was not stated. Nonoxynol-5 was slightly toxic in rabbits. The LD_{50} also was not achieved at a dose of 3.0 g/kg when Nonoxynol-6 was tested in a dermal toxicity study involving rabbits (number and strain not stated). The procedure for test substance administration was not stated. Nonoxynol-6 was slightly toxic in rabbits.[2]

Single Dose (Acute) Toxicity - Parenteral

Nonoxynol-9

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

Three groups of female rats were injected with Nonoxynol-9, intraperitoneally (undiluted), subcutaneously (undiluted), or intravenously (1% solution in saline). The corresponding LD_{50} values determined were 210 mg/kg, 1000 mg/kg, and 44 mg/kg.[1]
Repeated Dose Toxicity - Oral
Nonoxynol-6
The repeated dose toxicity of Nonoxynol-6 was evaluated using four groups of 20 (10 males and 10 females per group) weanling Sprague-Dawley rats. The test substance was fed to three experimental groups at doses of 0.040, 0.20, and 1.0 g/kg/day for 90 days. The control group was fed a standard pulverized rat stock diet. The only deaths reported were two rats (1 male, 1 female) of the 1.0 g/kg/day group and one female rat of the 0.20 g/kg/day group; deaths were due to respiratory failure. Neither microscopic changes nor significant gross pathologic changes that were related to administration of the test substance were observed. Statistically significant differences in weight gain between experimental and control groups were noted only in male and female rats of the 1.0 g/kg/day dose group. However, the results of a special paired feeding study indicated that this growth effect was due to poor diet palatability. Data from hematologic and urine analyses were similar between experimental and control groups. Increased liver weights and liver-to-body weight ratios were noted in female rats that received 1.0 g/kg/day and in male rats that received 0.20 and 1.0 g/kg/day. These increases were dose-correlated and, consequently, directly related to ingestion of the test material. Liver-to-body weight ratios were also significantly increased in female rats that received 0.20 g/kg/day. However, at this dose, absolute liver weights were not significantly increased over those of the control group, and significant body weight reduction was not noted. The researchers concluded that the importance of increased liver-to-body weight ratios in relation to the ingestion of Nonoxynol-6 was questionable.[2]
In another study, the repeated dose toxicity of Nonoxynol-6 was evaluated using four groups of four (2 males, 2 females) pure-bred beagle dogs. The test substance was fed to three experimental groups at doses of 0.040, 0.20, and 1.0 g/kg/day for 90 days. The control group was fed a stock diet. None of the animals died. However, during the first two weeks of testing, emesis was noted daily in dogs that received 0.2 and 1.0 g/kg/day. After the first two weeks, occasional emesis was noted only in the 1.0 g/kg/day group. The results of gross and histopathologic examinations did not indicate any changes that were related to test substance administration. Significant abnormalities also were not observed in hematologic studies, clinical blood chemistry analyses, urinalyses, and liver function tests. Compared to the control group, there was a slight increase in the liver-to-body weight ratio in female dogs that received 1.0 g/kg/day; this finding was not considered significant.[2]
Nonoxynols-4 and -9
Nonoxynols-4 and -9 were administered to rats (0.20 and 0.14 g/kg/day) and dogs (0.04 and 0.03 g/kg/day) in two two-year feeding studies. Body weight and hematologic parameters were monitored in all studies. A number of rats at each dose level were sacrificed and necropsied after 12 months; all remaining rats were sacrificed and necropsied after 24 months. All dogs were sacrificed and necropsied after 720 days. The results of these tests at the dose levels tested indicate that these Nonoxynols have a low chronic toxicity.[1]
Two four-week feeding studies were conducted individually on four rats (2 males, 2 females). Animals were placed on diets containing 0.025%-2.5% Nonoxynol-9. At the end of the test, animals at the 2.5% dose level had scanty body fat deposits; carcasses were moderately thin to emaciated. The rats on a diet containing 0.025% Nonoxynol-9 were unaffected.[1]
Nonoxynols-4, -6, -9, -15, -20, -30, and -40
Rats and dogs were fed diets containing either 0.04 to 5.0 g/kg or 0.01% -1 % Nonoxynol-4, -6, -9, -15, -20, -30, or -40 for 90 days. After 90 days, 1 or 5 g/kg/day Nonoxynol-20 caused focal myocardial necrosis in dogs but not in rats. In other studies with Nonoxynol-20 at 1 g/kg/day, six of eight dogs died between 4 and 14 days. Overall, dogs and guinea pigs showed evidence of cardiac lesions, but not rabbits, rats, and cats. In a 90-day study, Nonoxynol-20 at 0.04 g/kg/day produced cardiac lesions in dogs, whereas 5.00 g/kg/day had no effect in rats. Dosing with Nonoxynols-4 to -9 frequently increased liver weights in these studies. This finding was not reported for Nonoxynols-15 to -40.[1]
Repeated Dose Toxicity - Parenteral
Nonoxynol-9
The toxicity of Nonoxynol-9, in saline, was evaluated 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 glutamyloxalacetic transaminase (SGOT) activity was detected after a single intraperitoneal injection of Nonoxynol-9. SGOT activity reached a maximum (900 IU) between 4 and 8 hours. The administration of Nonoxynol-9 for 5 days caused a significant increase ($p < 0.001$; 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. None of the changes observed in the liver were observed in the lungs. The investigators concluded that the intraperitoneal administration of Nonoxynol-9 produced morphologic and biochemical changes in the liver.[2]
Cytotoxicity
Nonoxynol-9

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

The cytotoxicity of Nonoxynol-9 was evaluated using rat liver cells (T5 1B cells) from a non-tumorigenic cell line. T5 IB cells were plated at a density of 3.2×10^3 per cm^2, maintained for 24 hours in complete medium, and then treated with various concentrations of Nonoxynol-9 for an additional 24 hours. At the end of the 24-hour 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 T5 1B rat liver cells at concentrations of 11 to 50 pg/ml; the degree of cytotoxicity was concentration-dependent.[2]

Hematotoxicity

Nonoxynol-9

The hemolytic activity of Nonoxynol-9 was evaluated 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 minutes, 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 0.1%. In another 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 ml 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 to 20°C.[2]

Immunotoxicity

Nonoxynol-9

The immunotoxicity of Nonoxynol-9 was evaluated in a double- blind study, using outbred CF-I female mice. 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% sheep red blood cells (SRBC) and 0.05 ml of 10% SRBC, respectively. The 10 negative control mice were injected with 0.2 ml of saline according to the same procedure, and another group of five 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: $p < 0.02$; mean weight change = 1.9 g).[2]

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 ($p < 0.05$; mean weight change = 0.0065 g). Spleens in the experimental animals were larger than those in the saline control group ($p < 0.05$; mean weight change = 0.001 g) or in the untreated control group ($p < 0.02$; mean weight change = 0.002 g). On day 16, hematocrits of the experimental mice were lower than those in the saline-treated control mice ($p < 0.05$; difference of 2); an increase in the hematocrits of untreated mice was noted between days 16 and 25 ($p < 0.01$; difference of 5). However, even when considering these variations, all hematologic 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.[2]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY- Non-Human

Nonoxynol-9

The toxicity of Nonoxynol-9 was evaluated in the 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 x C3H/He) in doses of 20, 40, 50, or 60 mg/kg 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 physiologic saline and stained with Eosin-Y. In both experiments, at least 300 spermatozoa from each mouse were examined microscopically. Results indicated 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 ($p < 0.05$) 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.[2]

The embryotoxicity of Nonoxynol-9 was evaluated using groups of nulliparous female Wistar rats (5 per group; weights = 180 to 200 g). Each rat was dosed intravaginally with 5 mg Nonoxynol-9/100 g (0.1 ml Nonoxynol/100 g) on gestation day 3 or 7. The concurrent control rats (5 per group) received a per vaginam application of physiologic saline (0.1 ml/l00 g). The groups of treated animals were killed by CO_2 inhalation on gestation days 6,9, 12, and 15, or 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 ($p < 0.01$).[2]

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

Two-day old Swiss-Webster mouse embryos were cultured for 72 hours in media containing 0.25 to10 pg/ml Nonoxynol-9. The 10 pg/ml concentration was lethal to all embryos within 24 hours. 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 hours.[2]

Single doses (2.5 mg/100 g body weight) of Nonoxynol-9 were administered intravaginally to groups of pregnant Wistar rats (number of animals 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 non-pregnancies 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.[2]

The teratogenicity of Nonoxynol-9 (in distilled water) was evaluated using 11-week-old, outbred SPF rats. The rats were maintained in stainless steel wire cages and fed powdered chow prior to mating. Three groups of 22 to 25 mated female rats then received oral doses of 50, 250, or 500 mg/kg/day 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 in Bouin's solution 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 ($p < 0.05$; number affected not stated) were observed in groups of mice that received 250 or 500 mg/kg/day doses on days 6 through 15 of gestation; litter sizes per group were not stated. A statistically significant ($p < 0.05$; 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.[2]

The incidence of statistically significant skeletal anomalies for the litters was as follows: 250 mg/kg/day (24 of 25 with rudiments of ribs; $p < 0.02$), 500 mg/kg/day (10 of 20 with extra ribs, $p < 0.05$; 10 of 20 with rudiments of ribs, $p < 0.01$), and 500 mg/kg/day on days 1 to 20 of gestation (12 of 21 with extra ribs, $p < 0.01$; 21 of 21 with rudiments of ribs). An increased incidence of fetuses (500 mg/kg/day dose group; dosing on gestation days 1-20) with a slightly dilated pelvic cavity was also reported. The incidence was 12 of 21 litters ($p < 0.05$), 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 (gestation days 9 to 15) when the test substance was administered orally. In this study, Nonoxynol-30 (in distilled water) was also administered orally to three groups of 21 to 25 mated female rats (same weights and strain) in doses of 50, 250, or 1,000 mg/kg/day on days 6 to 15 of gestation. In a fourth experimental group, 19 rats were dosed orally with Nonoxynol-9 (1,000 mg/kg/day) on gestation days 1 to 20. In all treatment groups, none of the dams had signs of any adverse effects, and neither reproductive effects nor teratogenic effects on the skeleton and soft tissues were observed.[2]

In a second experiment by the above investigators, Nonoxynol-9, in distilled water, was applied to the skin of 19 and 24 female mated rats (same weights and strain) in doses of 50 and 500 mg/kg/day, respectively. The procedure for dosing involved the application of a porous dressing, which had been impregnated with the test substance, to shaved skin. The dressing was secured with tape, and the application period was from days 6 to 15 of gestation. The negative control group (19 rats) received water on gestation days 6 to 15. With the exception of the method of administration, the experimental procedure was identical to that stated above. 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 post-implantation loss ($p < 0.05$ 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 ($p < 0.02$), but not in the 500 mg/kg dose group.[2]

The teratogenicity of a contraceptive cream containing Nonoxynol-9 (50 mg/ml) was evaluated using five groups of 30 female, Long-Evans Hooded rats. 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) 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 in Bouin's solution, 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.[2]

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); pouch-like 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 ($p < 0.01$) 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 ($p < 0.05$) 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.[2]

Nonoxynol-10

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

The developmental toxicity of Nonoxynol-10 was evaluated 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 any of the following results: number of viable litters, liveborn per litter, percentage survival, birth weight per pup, and weight gain per pup. Nonoxynol- 10 did not induce developmental toxicity in mice.[2]

GENOTOXICITY - In Vitro

Nonoxynol-9

The genotoxicity of Nonoxynol-9 was evaluated in the Salmonella/mammalian microsome test. *S. typhimurium* strains TA1535, TA1537, TA100, and TA98 were tested with Nonoxynol-9 (in sterile water) at 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.[2]

The induction of unscheduled DNA synthesis was evaluated using freshly isolated adult rat hepatocytes. The cells were exposed to test concentrations of 5, 10, and 25 kg/ml Nonoxynol-9 along with 5 µg/ml [^{3}H]thymidine (specific activity: 25 Ci/mmol) for 18 hours, 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. Methyl methane sulfonate (positive control) induced unscheduled DNA synthesis and negative results were reported for the saline negative control.[2]

The genotoxicity of Nonoxynol-9 was evaluated in mutagenicity and transformation assays involving rat liver cells (T5 1B cells) from a non-tumorigenic cell line. T5 1B cells were plated at a density of 6.7 x 10^3 per cm^2, maintained for 24 hours in complete medium, and then treated with 5, 10, 15, and 25 µg/ml Nonoxynol-9 for an additional 24 hours. 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 phosphate-buffered saline and maintained in fresh medium until the cells became confluent. In order to determine HGPRT mutants, the cells were replated (density = 8 x10^3 cells per cm^2) into selective media containing 10 pg/ml 8- azaguanine. The cells were replated (density = 80 cells per cm^2) in the presence of a low concentration of calcium (0.02 mM) in order to determine transformation frequency. Nonoxynol-9 was not mutagenic at any of the concentrations tested and did not induce malignant transformations in the low calcium assay. HGPRT mutants were induced in the positive control (DMBA) culture. Neither HGPRT mutants nor malignant transformations were observed in negative control cultures.[2]

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 0.0000 1% 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 hours produced minimal toxicity and no significant increase in transformation. 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.[2]

The induction of malignant transformation in vitro by Nonoxynol-9 (in distilled water) was evaluated in another study using BALB/3T3 cells. In the cell transformation assay (repeated three times), 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 hours. 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-hour 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 morphologic transformation in BALB/3T3 cells.

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

Promotional effects of Nonoxynol-9 were also evaluated 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 hours, respectively. Cultures were also exposed to X-rays (100 rad) only, and to X-rays (100 rad) plus 0.1 µg/ml IZO-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 0.0000 1 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 <0.05 and >0.09 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 >0.7 and ~0.01, respectively.[2]

Nonoxynol-10

The genotoxicity of Nonoxynol-10 in strains TA1537, TA100, and TA98 of S. typhimurium was evaluated in a histidine reversion test, according to a modification of the Ames test procedure. Nonoxynol-10 was tested at concentrations of 100 to 10,000 pg/plate with and without metabolic activation. Mutagenic effects were not observed in any of the bacterial strains tested.

Nonoxynol-40

The effect of Nonoxynol-40 on malignant transformation in vitro was evaluated using BALB/3T3 fibroblasts. Cultures were incubated with 0.00001, 0.0001, and 0.001% Nonoxynol-40 for 48 hours and with 0.00001% Nonoxynol-40 for 3 weeks. Untreated cultures served as controls. In each experimental group, data were pooled from two separate experiments. For each concentration of Nonoxynol-40 that was tested, no increase was observed in the frequency of transformed cultures over that noted in control cultures. This was true even after incubation with 0.00001% Nonoxynol-40 for 3 weeks.[2]

CARCINOGENICITY- Non-Human

Nonoxynols-4, -7, -9, -10

Nonoxynols -4 and -9 were not carcinogenic when fed for two years to rats at doses of 0.20 and 0.14 and to dogs at 0.04 and 0.03 g/kg/day.[1]

Nonoxynol (-7 and/or -10), along with other surfactants, was tested at 2 g/L as a potential co-carcinogen with N-methyl-N'-nitro-N-nitrosoguanidine (NG) (0.1 g/l); both were supplied concurrently with the drinking water to 15 rats for 36 weeks. NG alone was supplied to 13 control animals. The overall incidence of stomach adenocarcinoma was 12 of 15 in the experimental group, and 8 of 13 for the controls. Neither negative control data nor a statistical analysis of the data were available. The author suggested that the surfactants may have a promoter effect because of their surfactant nature, which enables the NG to penetrate the gastric barrier and come into contact with the gastric mucosa or to penetrate mucosal cells.[1]

Nonoxynol-9

The carcinogenicity of Nonoxynol-9 was evaluated in a lifetime exposure study involving rats (numbers and strain not stated). The animals were dosed intravaginally with 6.7 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. 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 authors 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.

Ethylene Oxide (***detected as impurity in Nonoxynols -1, -4, -6, and -9 samples***)

The International Agency for Research on Cancer (IARC) has concluded, on the basis of epidemiologic, 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". Additionally, an IARC expert working group upgraded IARC's conclusion on ethylene oxide to "carcinogenic to humans" at its February 1994 meeting in Lyon, France. In evaluating the carcinogenicity of ethylene oxide, the working group took into consideration the very strong supporting evidence for genotoxicity, including the fact that it is a powerful mutagen and clastogen at all phylogenetic levels, induces gene mutations and heritable translocations in germ cells, and induces persistent dose-related increases in the frequency of chromosomal aberrations and sister chromatid exchanges in exposed workers.[2]

Skin Irritation and Sensitization - Non-Human

Nonoxynol-5

Severe skin irritation reactions were observed in animals tested with Nonoxynol-5. The reactions observed included reddening, cracking, and drying. Neither the experimental procedure nor the animal species was stated.[2]

Nonoxynol-6

The skin irritation potential of Nonoxynol-6 was evaluated 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 grams % (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 hours and sites were scored for the presence of irritation at 48 hours. 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.[2]

Nonoxynol-6 (0.5 ml) was applied under occlusive patches to clipped intact and abraded skin of 6 rabbits. Reactions (erythema and edema) were scored at 24 and 72 hours, and the mean scores were averaged in order to determine the Primary Irritation Index (PII). Nonoxynol-6 was classified as severely irritating to the skin of rabbits (PII = 3.0).[2] Nonoxynol-6 was classified as a severe skin irritant in animals in another study (primary irritation score = 6.6). Neither the experimental procedure nor the animal species was stated.[2]

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

The skin sensitization potential of Nonoxynol-6 was evaluated using the guinea pig maximization test. Four groups of five albino guinea pigs of the Hartley-Dalkin strain (weights = 300 to 500 g) were tested with Nonoxynol-6 concentrations of 1.7,3,9, and 27 grams % (w/w) in propylene glycol 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 hours 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 day21. Each patch was secured with a bandage for 24 hours, and sites were scored at 48 hours.[2]

The test results from a pretest control group of ten guinea pigs established a non-irritant 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.[2]

Nonoxynols-9 and -10

Nonoxynols -9 and -10 were applied, under occlusion, to the abraded and intact skin of the rabbit abdomen and ear. Ten applications to intact areas were made over a period of 14 days, insuring continuous contact with the sample for14 days. Three applications to abraded areas were made over three days. Five ml per exposure of l%, 5%, or 25% aqueous preparation were used. All concentrations caused very slight erythema.[1]

Nonoxynols -5 to -11.5

Nonoxynols -5 to -11.5 were evaluated for skin irritancy according to the Draize procedure. Irritation scores ranged from 2.0 to 4.3 (indicating mild to moderate irritation) after 24 h; no irritation remained after 120 h.[1]

Nonoxynols-2, -4, -6, -7, -9, -10, -12, -13, -15, -30, and -40

Eleven Nonoxynols were tested for skin irritation in rabbits according to the following protocols: (A) 0.01 ml of the test substance was applied undiluted to the clipped intact skin of each rabbit and examined 24 h later; (B) 0.5 ml of the test material was applied under occlusion to clipped intact and abraded skin. The sites were individually examined at 24 h and scored separately for erythema and edema at 24 and 72 h. The mean scores for 24- and 72-h gradings were averaged to determine the Primary Irritation Index (PII). The results indicated that Nonoxynols -7, -9, -10, -12, -13, -15, -30, and -40 were non-irritating to mildly irritating, whereas Nonoxynols -2, and -6 were moderately to severely irritating to the skin. Undiluted Nonoxynol-4 was reported to be non-irritating in one study but was found to be a primary irritant in another. In the latter study, well-defined to severe erythema and slight to severe edema, which in most cases worsened by 72 h, were observed in all animals at both intact and abraded sites. Primary irritation index (PII) values for these Nonoxynols, determined from these skin irritation tests on rabbits, ranged from 0.45 to 5.58. [1]

Skin Irritation and Sensitization - Human (Predictive Tests)

Nonoxynols-2 and -4

Nonoxynol-2 (5% in mineral oil), Nonoxynol-2 (10% in mineral oil), and Nonoxynol-4 (10% in mineral oil) were evaluated in three separate skin irritation/sensitization studies, respectively, 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. The results of the three studies, along with the experimental procedure, are summarized below.[2]

The skin irritation/sensitization potential of Nonoxynol-2 (5% in mineral oil) was evaluated 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 (9 induction applications). Patches were removed, and sites evaluated at 48-hour intervals. Patches applied on Friday were removed, and sites evaluated on the following Monday (72 hours post-application). The induction phase was followed by a 14-day non-treatment period. During the challenge phase, initiated at week 6, two consecutive 48-hour patches were applied to new sites in the scapular region of the back. Challenge reactions were scored after 48 and 96 hours. 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.[2]

The skin irritation/sensitization potential of Nonoxynol-2 (10% in mineral oil) was evaluated 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 and grading scale for this study were as stated in the preceding paragraph. 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.[2]

Seven of the nine subjects with allergic contact dermatitis were retested according to a different procedure. The test substance was applied under a semiocclusive patch for 30 minutes, after which the test site was rinsed with warm water. Reactions were scored at 24 hours post-application (seven subjects) and at 24 and 48 hours 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 investigators concluded that Nonoxynol-2 (10% in mineral oil) induced allergic contact sensitization in the initial experiment. However, when exposure was limited to 30 minutes, evidence of a mild allergic response was observed in two of the seven subjects with allergic contact sensitization who were retested.[2]

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

The skin irritation/sensitization potential of Nonoxynol-4 (10% in mineral oil) was evaluated 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. The experimental procedure and grading scale are referred to in the preceding paragraph. 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 investigators concluded that Nonoxynol-4 (10% in mineral oil) induced allergic contact sensitization in the initial experiment. However, when exposure was limited to 30 minutes (retest), evidence of a mild allergic response was observed in one of the three subjects with allergic contact dermatitis.[2]

Undiluted Nonoxynol-4 was tested on 25 men and 25 women in a repeated insult patch test. Discs (1.25″ diameter) saturated with sample were applied to the backs of the volunteers. The primary application was left in place for 48 h; the subsequent 14 induction patches were applied for 24 h each. After a two-week non-treatment period, challenge patches were applied for 24 h. None of the subjects showed immediate or delayed reactions to either the induction or challenge patches. It was noted that Nonoxynol-4 appeared to be neither a primary irritant, a sensitizer, nor a fatiguing agent.

Nonoxynols-4, -9, and -12

Cosmetic formulations containing Nonoxynol-4 (5%), -9 (1.75 to 4%), or -12 (20%) were tested for cumulative skin irritation. The test material was applied to the volar forearm surface and/or the inner aspect of the arm of the 20 test subjects, and held under occlusive patches for 24 h. After patch removal and test site grading, fresh patches were reapplied to the same site. This procedure was repeated for a total of 10 applications. The results showed a range of effects on the skin, ranging from slightly to mildly irritating. The formulations containing Nonoxynol-9 caused reactions ranging from slightly irritating to nonirritating. The formulation containing nonxynol-4 and the formulation containing Nonoxynol-12 were classified as slightly irritating.[1]

Two cosmetic gels containing 2% and/or 4% Nonoxynol-9 were separately tested for irritation on 25 subjects. The gel was applied under an occlusive patch for 48 h before scoring. All sites received a score of zero.[1]

A gel containing 4% Nonoxynol-9 was tested on 212 subjects. The material was applied 11 times under an occlusive patch. Neither the time interval between patch testing nor the quantity of gel applied was stated. A score of 11 out of a maximum possible score of 804 was reported. The investigator concluded that the product showed no evidence of primary skin irritation or allergic sensitization.[1]

Undiluted Nonoxynol-9 was tested on 50 men and 50 women for skin irritation/sensitization potential. A single induction patch, applied to the back of each subject, was held in contact with the skin for five days. After a three-week non-treatment period, a challenge patch was applied to each subject for 48 h. There were no reactions to either patch. Undiluted Nonoxynol-9 was neither a primary irritant nor a sensitizer [1]

Nonoxynols-15 and -50

A repeated insult patch test was performed on 168 subjects (115women, 53 men) using 0.1 ml of a 50% aqueous solution of Nonoxynol-15 and/or Nonoxynol-50. The test material was applied at 48 -h intervals, three times per week for three weeks, to the backs of the subjects. The test area was occluded for 24 h before removal, and washed with distilled water. The test sites were read at 48 h, after which fresh test material and the occlusive patch were reapplied. After a three-week non-treatment period, the test area, as well as an untreated site, were challenged with the test material. The sites were scored for sensitization at 24, 48, and 72 h. The investigator noted that only transient reactions were observed during the test, and that neither Nonoxynol-15 nor Nonoxynol-50 was an irritant or sensitizer.

Skin Irritation and Sensitization -Human (Provocative Tests)

Nonoxynols-6, -8.3, -9, -10, -14, and -18

A total of twelve contact dermatitis patients was patch tested with the ingredients of a topical antiseptic preparation according to the International Contact Dermatitis Research Group's (ICDRG) patch test procedure. 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 hours and reactions were scored at 48 hours and at 72 or 96 hours. All of the patients had positive reactions to 2.0% aqueous Nonoxynol solutions either at 72 or 96 hours; 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.[2]

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 according to the ICDRG patch test procedure, most of the reactions observed at 72 or 96 hours 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 hours. Additionally, Subject 5 had a + reaction to Nonoxynol-6 at 96 hours and Subject 4 had a + reaction to Nonoxynol-8.3 at 96 hours. Subjects 4 and 6 each had negative reactions to Nonoxynol-18 at 96 and 72 hours, 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.[2]

Phototoxicity/Photosensitization- Human

Nonoxynol-10

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

Photosensitization was observed 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 2 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 hours post-application. Test sites (irradiated and non-irradiated series) were evaluated at 72 hours post-application.[2]

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 non-irradiated 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.[2]

Nonoxynols-15 and -50

Twenty-eight of the 168 subjects tested for Nonoxynol-15 and Nonoxynol-50 irritation and sensitization potential in the section on Skin Irritation and Sensitization (Predictive Tests) were randomly selected to test the ability of Nonoxynol-15 and Nonoxynol-50 to induce a phototoxic or photosensitization reaction following ultraviolet light exposure. The test protocols were the same except that the forearm was used as a test site. The 28 subjects were divided into two groups, 19 received only UVA and nine received both UVA and UVB. The UVA (320-400 nm) light was applied for 15 min to the 19 subjects (4.4 $\mu W/cm^2$ at the skin surface, measured at the 360 nm wave length peak). The UVB light was applied, at twice the Mean Erythema Dose (MED), to nine subjects (light source: 150 watt Xenon Arc Solar Simulator emitting at 280-320 nm). The subjects receiving the UVB exposure were also exposed for 5 min to UVA. The investigator noted that only transient reactions were observed, and that Nonoxynol-15 and Nonoxynol-50 were not photosensitizers.

Case Reports

Nonoxynol-6

Scaling, redness, vesiculation, and fissuring of the dorsal hands and forearms, associated with a transverse dystrophy of the fingernails, was observed in a 58-year-old uranium mill worker who used a waterless hand cleanser containing Nonoxynol-6 at work. The patient had an allergic contact reaction (1+ reaction) to 0.5% Nonoxynol-6, in petrolatum, at 48 and 96 hours. Reactions to Nonoxynol-6 (0.5% in petrolatum) were not observed in eight control subjects.[2]

Dermatitis was observed 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, non-vesicular 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.[2]

Ocular Irritation - Non-Human

Nonoxynol-5

Severe ocular irritation reactions were observed in animals tested with Nonoxynol-5. An ocular irritation score of 55 persisted through day 7. Neither the experimental procedure nor the animal species was stated.[2]

Nonoxynol-6

Nonoxynol-6 induced severe ocular irritation reactions in animals; growth of blood vessels onto the cornea was observed. Irritation reactions persisted to day 21. Neither the experimental procedure nor the animal species was stated.[2]

Nonoxynols -9 and -10

A 20% solution of Nonoxynol-9 (0.1 ml) at pH 6.1 was applied directly onto the cornea of one eye of each of 10 rabbits, 14 guinea pigs, 8 rats, and 11 mice. Corneal changes and lesions were evaluated at 1, 4, 7, and 30 h; scores were 34.4, 41.4, 30.8, and 70.7 (maximum score = 100) for rabbits, guinea pigs, rats, and mice, respectively. In rabbits, the effect of rinsing the treated eye with 20 ml of water 4 seconds after instillation of the sample was also studied. The results of this study indicated that Nonoxynol-9 is a moderate to severe eye irritant.[1]

Two drops of 1%, 5%, or 25% Nonoxynols -9 and -10 were instilled into both eyes of each of three rabbits per concentration. Studies were performed with and without immediate irrigation. The lowest concentration tested caused very slight conjunctivitis; the middle concentration caused slight conjunctivitis and moderate corneal injury; the highest concentration caused moderate to severe cornea1 injury. Washing the eye lowered the average irritation index by 36.8%.[1]

Nonoxynols -4, -9, and -12

Two shampoos, two bath oils, and one moisturizer containing 1.75%-2% Nonoxynols -4, -9, or -12 were tested for eye irritation potential according to the method of Draize. Results of these tests indicate that these products are minimally to moderately irritating when instilled in the eyes of rabbits.[1]

Table 5. Chemistry and Safety Test Data on Nonoxynols from Prior CIR Safety Assessments.[1,2]

Nonoxynols-2, -4, -6, -7, -9, -10, -12, -13, -15, -30, and -40

Five Nonoxynols were tested in rabbits for ocular irritation according to the Draize method. Six other Nonoxynols were tested according to the following protocol: single doses of 0.005, 0.02, 0.10, or 0.5 ml of undiluted Nonoxynol or 0.5 ml of 40%, 15%, 5%, or 1% dilutions were placed in the conjunctival sacs of five rabbits per group. Eyes were examined within 1 h unstained and at 24 h after fluorescein staining and were scored. The results indicated that Nonoxynols -2 (undiluted), -15 (10% and 15%), -30 (25%), and -40 (undiluted) were nonirritating to minimally irritating, and that undiluted Nonoxynols -4, -6, -7, -9, -10, -12, -13, and -15 were severely irritating to the eyes of rabbits.[1]

Mucous Membrane Irritation - Non-Human

Nonoxynol-9

Concentrations of 2.5, 5.0, 12.5, and 25.0% aqueous Nonoxynol-9 (volume = 20 ml) were administered by vaginal lavage to four groups of six New Zealand rabbits 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 concentration-dependent. 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.[2]

In additional experiments, 5.0, 12.5, 25.0, 50.0, and 75.0% Nonoxynol-9 concentrations, in distilled water, were 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.[2]

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Concentration of Use by FDA Product Category – Octoxynols*

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	Other baby products	0.1%
Octoxynol-9	Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	2%
Octoxynol-12	Face and neck products Not spray	1.5%

*Ingredients found 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 2022
Table prepared: July 7, 2022