Safety Assessment of Nonoxynols as Used in Cosmetics

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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]

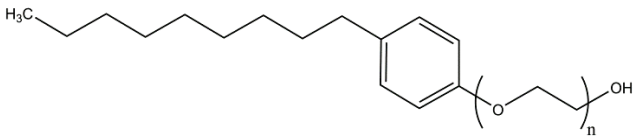

Figure 1. Chemical formula for Nonoxynols. The value of **n** can vary from 1 to 120.

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 ($\approx$ 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]

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.

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]

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

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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