International Journal of Toxicology, 27(Suppl. 2):93–128, 2008

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810802244595

Safety Assessment of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, Poloxamer 105 Benzoate, and Poloxamer 182 Dibenzoate as Used in Cosmetics[1]

Poloxamers are polyoxyethlyene, polyoxypropylene block polymers. The impurities of commercial grade Poloxamer 188, as an example, include low-molecular-weight substances (aldehydes and both formic and acetic acids), as well as 1,4-dioxane and residual ethylene oxide and propylene oxide. Most Poloxamers function in cosmetics as surfactants, emulsifying agents, cleansing agents, and/or solubilizing agents, and are used in 141 cosmetic products at concentrations from 0.005% to 20%. Poloxamers injected intravenously in animals are rapidly excreted in the urine, with some accumulation in lung, liver, brain, and kidney tissue. In humans, the plasma concentration of Poloxamer 188 (given intravenously) reached a maximum at 1 h, then reached a steady state. Poloxamers generally were ineffective in wound healing, but were effective in reducing postsurgical adhesions in several test systems. Poloxamers can cause hypercholesterolemia and hypertriglyceridemia in animals, but overall, they are relatively nontoxic to animals, with LD_{50} values reported from 5 to 34.6 g/kg. Short-term intravenous doses up to 4 g/kg of Poloxamer 108 produced no change in body weights, but did result in diffuse hepatocellular vacuolization, renal tubular dilation in kidneys, and dose-dependent vacuolization of epithelial cells in the proximal convoluted tubules. A short-term inhalation toxicity study of Poloxamer 101 at 97 mg/m^3 identified slight alveolitis after 2 weeks of exposure, which subsided in the 2-week postexposure observation period. A short-term dermal toxicity study of Poloxamer 184 in rabbits at doses up to 1000 mg/kg produced slight erythema and slight intradermal inflammatory response on histological examination, but no dose-dependent body weight, hematology, blood chemistry, or organ weight changes. A 6-month feeding study in rats and dogs of Poloxamer 188 at exposures up to 5% in the diet produced no adverse effects. Likewise, Poloxamer 331 (tested up to 0.5 g/kg day^{-1}), Poloxamer 235 (tested up to 1.0 g/kg day^{-1}), and Poloxamer 338 (at 0.2 or 1.0 g/kg day^{-1}) produced no adverse effects in dogs. Poloxamer 338 (at 5.0 g/kg day^{-1}) produced slight transient diarrhea in dogs. Poloxamer 188 at levels up to 7.5% in diet given to rats in a 2-year feeding study produced diarrhea at 5% and 7.5% levels, a small decrease in growth at the 7.5% level, but no change in survival. Doses up to 0.5 mg/kg day^{-1} for 2 years using rats produced yellow discoloration of the serum, high serum alkaline phosphatase activity, and elevated serum glutamicpyruvic transaminase and glutamic-oxalacetic transaminase activities. Poloxamers are minimal ocular irritants, but are not dermal irritants or sensitizers in animals. Data on reproductive and developmental toxicity of Poloxamers were not found. An Ames test did not identify any mutagenic activity of Poloxamer 407, with or without metabolic activation. Several studies have suggested anticarcinogenic effects of Poloxamers. Poloxamers appear to increase the sensitivity to anticancer drugs of multidrug-resistant cancer cells. In clinical testing, Poloxamer 188 increased the hydration of feces when used in combination with a bulk laxative treatment. Compared to controls, one study of angioplasty patients receiving Poloxamer 188 found a reduced myocardial infarct size and a reduced incidence of reinfarction, with no evidence of toxicity, but two other studies found no effect. Poloxamer 188 given to patients suffering from sickle cell disease had decreased pain and decreased hospitilization, compared to controls. Clinical tests of dermal irritation and sensitization were uniformly negative. The Cosmetic Ingredient Review (CIR) Expert Panel stressed that the cosmetic industry should continue to use the necessary purification procedures to keep the levels below established limits for ethylene oxide, propylene oxide, and 1,4-dioxane. The Panel did note the absence of reproductive and developmental toxicity data, but, based on molecular weight and solubility, there should be little skin penetration and any penetration of the skin should be slow. Also, the available data demonstrate that Poloxamers that are introduced into the body via routes other than dermal exposure have a rapid clearance from the body, suggesting that there would be no risk of reproductive and/or developmental toxicity. Overall, the available data do not suggest any concern about carcinogenesis. Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used, and at what concentration, indicates a pattern of use. Based on these safety test data and the information that the manufacturing process can be controlled to limit unwanted impurities, the Panel concluded that these Poloxamers are safe as used.

INTRODUCTION

The generic CAS No. 9003-11-6 applies to all Poloxamers. Poloxamer 105 Benzoate and Poloxamer 182 Dibenzoate have no CAS numbers. These polymers are synthetic block copolymers of ethylene oxide and propylene oxide. In medical and research applications, they commonly are referred to as Pluronic® block polymers, a Poloxamer trade name. Available

[1]Reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. Subhashni D. Singh-Joy, former scientific analyst, and Valerie C. McLain, CIR scientific analyst, prepared this report. Address correspondence to Ms. Valerie C. McLain, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

data demonstrating safety in medical uses of Poloxamers are included in this report, but this safety assessment offers a conclusion only regarding the safety of the use of these ingredients in cosmetic formulations.

CHEMISTRY

Definition and Structure

As described in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004) Poloxamers are defined as polyoxyethylene, polyoxypropylene block polymers. Wout et al. (1992) stated that Poloxamer 407 is made up of 70% polyoxyethylene and 30% polyoxypropylene.

Poloxamers generally conform to the formula shown in Figure 1, where the average values of x, y, and z are defined in Table 1.

Several Poloxamers are known by trade name Pluronic® followed by a letter and number, either L, F, or P, which refers to liquid, flake, or paste physical forms, respectively (Anonymous 1997).

Poloxamer 105 Benzoate is an ester of Poloxamer 105 and benzoic acid and Poloxamer 182 Dibenzoate is the diester of Poloxamer 182 and benzoic acid (Gottschalck and McEwen 2004).

Physical and Chemical Properties

As described by Leaf (1967), many poloxamers dissolve in water and form gels when a concentrated aqueous solution is made. These reversible gels revert to liquid when the temperature is lowered, and then reform as gels when the temperature is increased. This reversibility is temperature dependent and can occur limitless times. Viscosity increases in relationship to increasing percent of polyoxypropylene hydrophobe and percent of polyoxyethylene hydrophile.

Table 2 gives the available information on the physical and chemical properties of poloxamers.

According to Moghimi et al. (2000), Poloxamers are also known as Pluronic® macromolecules. Covering a range of liquids, pastes, and solids, they are a family of over 50 various amphiphilic nonionic block polymers of hydrophobic propylene oxide (PO) and hydrophilic ethylene oxide (EO). Polymers consist of a central polyoxyethylene (POE) molecule, which is flanked on both sides by two hydrophilic POE chains. These surfactants were first introduced in the 1950s and since then have found a wide range of diverse applications as shown in Table 3.

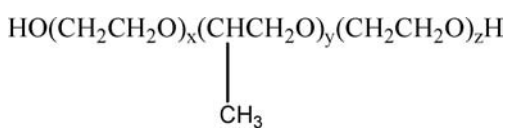

FIGURE 1
Chemical formula for Poloxamers, with average values of x, y, and z defined in Table 1 (Gottschalck and McEwen, 2004).

TABLE 1
Average values for x, y, and z for Poloxamers shown in Figure 1 (Gottschalck and McEwen 2004), with Pluronic trade name designation (Anonymous 1997).

Poloxamer	x[a]	y[a]	z[a]	Pluronic no.[b]
101	2	16	2	L-31
105	11	16	11	L-35
108	46	16	46	F-38
122	5	21	5	—
123	7	21	7	L-43
124	11	21	11	L-44
181	3	30	3	L-61
182	8	30	8	L-62
183	10	30	10	—
184	13	30	13	—
185	19	30	19	—
188	75	30	75	F-68
212	8	35	8	—
215	24	35	24	—
217	52	35	52	F-77
231	6	39	6	L-81
234	22	39	22	P-84
235	27	39	27	P-85
237	62	39	62	F-77
238	97	39	97	F-87
282	10	47	10	L-92
284	21	47	21	—
288	122	47	122	F-98
331	7	54	7	L-101
333	20	54	20	P-103
334	31	54	31	P-104
335	38	54	38	P-105
338	128	54	128	F-108
401	6	67	6	L-121
402	13	67	13	—
403	21	67	21	P-123
407	98	67	98	F-127

[a]See chemical structure in Figure 1.
[b]L, F, or P refer to liquid, flake, or paste physical forms.

Method of Manufacture

In a review article, Schmolka (1994) stated that Poloxamers are generally prepared at high temperature and pressure. Propylene oxide, followed by ethylene oxide, is added to propylene glycol in the presence of an alkaline catalyst, such as sodium or potassium hydroxide. This catalyst is neutralized and the neutral salt becomes part of the final product.

Analytical Methods

Wang and Stern (1975) determined the purity of Poloxamer 108 with the use of thin-layer chromatography on silica

TABLE 2
Physical and chemical properties of Poloxamers.

	Poloxamer				
Property	124	188	237	338	407
Average molecular weight	2090–2360[a]	7680–9510[a]	6840–8830[a]	12700–17400[a]	9840–14600[a]
Description	Colorless liquid[b]	White solid[a]	Solid[a]	Solid[a]	White solid[a]
pH, 2.5% aqueous	5.0–7.5[c]	5.0–7.5[c]	5.0–7.5[c]	5.0–7.5[c]	5.0–7.5[c]
Unsaturation (mEq/g)	0.020 ± 0.008[a]	0.026 ± 0.008[a]	0.034 ± 0.008[a]	0.031 ± 0.008[a]	0.048 ± 0.017[a]
Weight % oxyethylene	46.7 ± 1.9[c]	81.8 ± 1.9[c]	72.4 ± 1.9[c]	83.1 ± 1.7[c]	73.2 ± 1.7[c]
Melting point (°C)	16[b]	52[b]	49[b]	57[b]	56[b]
Cloud point, 10%	71–75°C[c]	>100°C[c]	>100°C[c]	>100°C[c]	>100°C[c]
Solubility	Soluble in water, 95% ethanol, propylene glycol, and propan-2-ol[b]	Soluble in water and 95% ethanol[b]	Soluble in water and 95% ethanol, but sparingly soluble in propan-2-ol[b]	Soluble in water and 95% ethanol but sparingly soluble in propylene glycol[b]	Soluble in water, 95% ethanol, and propan-2-ol[b]

[a]Committee of Revision of the United States Pharmacopeial Convention 1995.
[b]Anonymous 1997.
[c]BASF 2002.

TABLE 3
Poloxamer descriptions (Moghimi et al. 2000).

Poloxamer	Average molecular weight[a]	EO units	PO units	HLB[b]	Suggested applications
188	8400	2×52	30	29	Antithrombotic, hemorheological activities, cell membrane sealing, phagocyte activation (stimulations of phagocytosis and superoxide anion production), and neutrophil degranulation.
401	2000	2×5	67	7	Nanoparticle engineering (lymphotrophic particles), inhibition of multidrug resistance and adjuvant activities.
402	2500	2×11	67	22	See Poloxamer 401.
407	12600	2×98	67	22	Long circulating particles, slow release gels, macrophage stimulation, stimulating EGF[c] production.

[a]BASF (NJ, USA).
[b]HLB, hydrophile-lipophile balance.
[c]EGF, epidermal growth factor.

gel. Grendel et al. (2002) used gel-permeation chromatography (GPC) and a refractive index detector to detect Poloxamer 188 in plasma samples from Sprague-Dawley rats, Beagle dogs, humans, and New Zealand albino rabbits.

Impurities

Synthesis of these block polymers involves the use of ethylene oxide, which may remain as an impurity unless removed (Schmolka 1994).

Wang and Stern (1975) used thin-layer chromatography to determine that ^{14}C-labeled Poloxamer 108 contained less than 2% impurities; although, the impurities were not stated. Kentley et al. (1988) stated that the impurities of commercial grade Poloxamer 188 are low-molecular-weight substances, including aldehydes and both formic and acetic acids. The authors were able to purify Poloxamer 188 by filtering it through a silica-Amberlite resin column. A 4% (*w*/*v*) solution of Poloxamer 188 in distilled water was purified in a 30 × 20-cm^2 column packed with either silica 60–120 mesh, alone, or in combination with Amberlite MB-1 16–50 mesh. The unpurified solution had an absorption band with a peak at 278 to 280 nm, strong fluorescent emission bands at 315 and 330 nm, and also tested positive for Fehling's test for reducing agents. After one filtration through the column, the ultraviolet band was not detectable and the fluorescene emission was reduced by five times that of the unpurified. Also, the purified solution gave a negative reaction to Fehling's test. The impurities filtered out were not identified.

Edwards et al. (1999) purified Poloxamer 188 with a silica gel resin (SGR) or by supercritical fluid fractionation (SFF). They tested both purified and unpurified Poloxamer 188 on human polymorphonuclear leukocyte (PMNL) chemiluminescence in vitro. Commercial Poloxamer 188 stimulated chemiluminescence by 26% whereas purified Poloxamer stimulated chemiluminescence by up to 53%. The authors state that these results reinforce previous suggestions that trace impurities in commercial Poloxamer 188 may be responsible for transient inhibition of polymorphonuclear leucocyte function.

The United States Pharmacopeia National Formulary (USP National Formulary 2004) established the maximum limits of ethylene oxide, propylene oxide, and 1,4-dioxane in Poloxamers at 1, 5, and 5 ppm, respectively.

USE

Cosmetic

As described in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), most of the Poloxamers function as surfactants, emulsifying agents, cleansing agents, and/or solubilizing agents in cosmetics. Poloxamer 188 is also an antimicrobial agent and Poloxamer 182 dibenzoate is listed as a skin conditioning agent and emollient.

Table 4 lists the Poloxamers reported by industry to the Food and Drug Administration (FDA) to be in use as a function of product type (FDA 2002). For example, of 651 hair conditioner products reported to FDA, 5 contained Poloxamer 105. Current concentration of use data from an industry survey (CTFA 2004a) are also provided. Overall, 141 uses were reported, over a concentration range from 0.005% to 20%. In some cases; e.g., Poloxamer 334, industry reports a current concentration of use, but no reported uses were reported to FDA. In other cases; e.g., Poloxamer 335, uses were reported to FDA, but no current concentration of use data were provided. Some Poloxamers were not reported used in data provided to FDA, nor were use concentrations reported in the Cosmetic, Toiletry, and Fragrance Association (CTFA) survey.

Noncosmetic

There are numerous non-cosmetic uses of Poloxamer 188 as a food additive, stool softener, wetting agent, antimicrobial carrier, topical wound cleanser, emulsifying agent in intravenous fat emulsions, precipitant of plasma proteins, and an additive in cardiopulmonary bypass perfusion solutions (Jewell et al. 1997). Additional noncosmetic applications are given in Table 3 (Moghimi et al. 2000).

Medical

Poloxamer 188 has also been tested as a protective agent for mammalian and insect cells during sparging. Sparging is a method for supplying oxygen to a culture medium in large-scale animal cell bioreactors that can damage cells (Murhammer and Goochee 1990). Poloxamer 407 has been tested for its use as a bacterial abhesive (antiadhesive) for hydrogel contact lenses (Portoles et al. 1994). El-Kamel (2002) evaluated Poloxamers as a vehicle for ocular drug delivery of timolol maleate.

In a review article describing the use of Poloxamers in drug delivery, Kabanov and Alakhov (2002) noted that Poloxamers are recognized pharmaceutical excipients. They may be used in micelle form to increase both solubility and stability of drugs. In addition to recounting the history of the use of Poloxamers in drug delivery, which includes the applications cited in this section, these authors also described the use of Poloxamers (1) as sensitizers of drug-resistant cancers to doxorubicin; (2) in increasing transport of drugs across the blood-brain barrier; and (3) as enhancers of oral bioavailability of drugs.

Kabanov et al. (2002) described the use of Pluronic® block copolymers in drug delivery, from miscellar nanocontainers to biological response modifiers. Pluronic® block copolymers are used extensively in experimental medicine and pharmaceutics, and are acknowledged pharmaceutical excipients listed in the U.S. and British Pharmacopoeia.

Tissue Engineering

Cao et al. (1998) expanded on earlier work on cartilage regeneration in mice by using a similar approach in an immunocompetent porcine animal model. Chondrocytes were isolated and seeded onto a polyglycolic acid carrier, suspended in calcium

TABLE 4
Frequency and concentration of use of Poloxamers.

Product category (Total number of formulations) (FDA 2002)	Number of formulations containing ingredient (FDA 2002)	Maximum concentration of use (%) (CTFA 2004a)
	Poloxamer 105	
Hair conditioners (651)	5	—
Shaving cream (134)	2	3
Total uses/ranges for Poloxamer 105	**7**	**3**
	Poloxamer 181	
Moisturizers (905)	—	0.005
Other skin care preparations (725)	—	6
Total uses/ranges for Poloxamer 181	—	**0.005– 6**
	Poloxamer 182	
Other bath preparations (196)	1	
Eyeliners (548)	1	
Lipsticks (962)	1	
Shaving cream (134)	1	
Paste masks/mud packs (271)	—	3
Other skin care preparations (725)	—	0.2
Suntan gels, creams, and liquids (131)	—	6
Total uses/ranges for Poloxamer 182	**4**	**0.2–6**
	Poloxamer 184	
Other bath preparations (196)	11	0.4
Eye makeup remover (100)	9	0.03–0.04
Hair conditioners (651)	6	—
Noncoloring shampoos (884)	5	10
Makeup fixatives (20)	—	3
Other makeup preparations (201)	1	0.9
Skin cleansing creams, lotions, etc. (775)	27	3–6
Body and hand creams, lotions, etc. (840)	1	0.4
Moisturizers (905)	—	0.3
Other skin care preparations (725)	1	3
Suntan gels, creams, liquids (131)	—	2
Indoor tanning preparations (71)	1	—
Total uses/ranges for Poloxamer 184	**62**	**0.03–10**
	Poloxamer 185	
Eye Lotion (25)	—	0.5–9
Eye makeup remover (100)	—	2
Other Eye Makeup Preparations (125)	1	—
Paste Masks (mud packs) (271)	8	—
Other skin care preparations (725)	—	0.5
Total uses/ranges for Poloxamer 185	**9**	**0.5–9**
	Poloxamer 188	
Eye lotion (25)	1	—
Permanent waves (207)	6	—
Skin cleansing creams, lotions, etc. (775)	3	1–2
Night creams, lotions, etc. (200)	1	—
Other skin care preparations (725)	1	2
Indoor tanning preparations (71)	—	0.8
Total uses/ranges for Poloxamer 188	**12**	**0.8–2**

(Table continued on next page)

TABLE 4
Frequency and concentration of use of Poloxamers *(Contiued)*.

Product category (Total number of formulations) (FDA 2002)	Number of formulations containing ingredient (FDA 2002)	Maximum concentration of use (%) (CTFA 2004a)
	Poloxamer 212	
Other skin care preparations (725)	—	2
Total uses/ranges for Poloxamer 212	—	**2**
	Poloxamer 217	
Other personal hygiene products (308)	2	—
Total uses/ranges for Poloxamer 217	**2**	—
	Poloxamer 234	
Noncoloring shampoos (884)	—	0.1
Hair tonics, dressings, etc. (598)	—	0.3–2
Skin cleansing creams, lotions, etc. (775)	—	0.5–10
Face and neck creams, lotions, etc. (310)	—	0.5–1
Body and hand creams, lotions, etc. (840)	—	0.01
Moisturizers (905)	—	0.5
Other skin care preparations (725)	—	0.5
Other suntan preparations (38)	—	0.01
Total uses/ranges for Poloxamer 234	—	**0.01–10**
	Poloxamer 237	
Eye makeup remover (100)	1	—
Dentifrices (40)	1	—
Mouthwashes and breath fresheners (46)	2	—
Total uses/ranges for Poloxamer 237	**4**	—
	Poloxamer 238	
Other bath preparations (196)	1	—
Body and hand creams, lotions, etc. (840)	2	—
Foot powders and sprays (35)	1	—
Total uses/ranges for Poloxamer 238	**4**	—
	Poloxamer 333	
Body and hand creams, lotions, etc. (840)	—	1
Total uses/ranges for Poloxamer 333	**—**	**1**
	Poloxamer 334	
Hair tonics, dressings, etc. (598)	—	0.3
Total uses/ranges for Poloxamer 334	**—**	**0.3**
	Poloxamer 335	
Mouthwashes and breath fresheners (46)	2	—
Total uses/ranges for Poloxamer 335	**2**	—
	Poloxamer 338	
Mouthwashes and breath fresheners (46)	2	—
Total uses/ranges for Poloxamer 338	**2**	—
	Poloxamer 401	
Body and hand creams, lotions, etc. (840)	2	—
Total uses/ranges for Poloxamer 401	**2**	—
	Poloxamer 407	
Eye makeup remover (100)	—	2
Permanent waves (207)	2	—
Other manicuring preparations (55)	—	5
Dentifrices (40)	—	12–20
Mouthwashes and breath fresheners (46)	18	0.3–1

(Table continued on next page)

TABLE 4
Frequency and concentration of use of Poloxamers *(Continued)*.

Product category (Total number of formulations) (FDA 2002)	Number of formulations containing ingredient (FDA 2002)	Maximum concentration of use (%) (CTFA 2004a)
Other oral hygiene products (6)	1	—
Underarm deodorants (247)	6	—
Other personal hygiene products (308)	1	—
Skin cleansing creams, lotions, etc. (775)	—	9
Moisturizers (905)	—	1
Skin fresheners (184)	—	3
Other skin care preparations (725)	3	—
Total uses/ranges for Poloxamer 407	**31**	**0.3–20**

alginate, or suspended in Poloxamer gel (F127) and implanted or injected into the pigs from which the cells had been isolated. The Poloxamer gel produced histologic features that most resembled native elastic cartilage.

Cao et al. (2002) cultured primary keratinocytes and fibroblasts isolated from swine skin, mixed them with Poloxamer 407, and seeded the mixture onto a polyglycolic acid carrier. This tissue-engineered complex was then used effectively to repair full-thickness skin defects in swine.

Gene Transfer

Van Belle et al. (1998) reported that Poloxamer 407 as a vehicle increases percutaneous adenovirus-mediated gene delivery and reduces the time required for transfection. They also demonstrated that gene transfer could be done during stent implantation using a Poloxamer vehicle. These studies were undertaken as part of the development of new strategies to prevent restenosis of vessels through introduction of antiproliferative genes into arterial smooth muscle cells at angioplasty sites.

In their review article, Kabanov and Alakhov (2002) also noted that Poloxamers significantly increased expression of plasmid DNA in skeletal muscle in mice, raising the possibility that these block copolymers may be useful for gene therapy.

Kuo (2003) expanded the study of Poloxamers for in vivo gene delivery in a study of serum-mediated inhibition of gene transfer of DNA complexes in NIH/3T3 cells. The higher the hydrophillic to lipophillic balance in the Poloxamer, the more improvement in gene transfer was seen.

GENERAL BIOLOGY

Hematologic Effects

There are several in vitro studies examining the effect of Poloxamers, especially Poloxamer 188, on whole blood, neutrophils, as well as red blood cell (RBC)-induced platelet aggregation.

Xiao et al. (1989) studied the effect of Poloxamer 188 (concentration not clearly stated) on neutrophil phagocytic function and intracellular cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) concentration. Chemiluminescence (CL) was used to determine neutrophil phagocytic function. After 1 h of incubation, the CL of neutrophils exposed to Poloxamer 188 was 41.8% lower than that of controls. After 2 h of incubation, the CL increased, but was still 34.1% lower than controls. After 1 h, the cAMP concentration was 15.5 times greater than that of the control and after 2 h, the cAMP concentration had dropped, but was still 3.7 times the control value. All treatment values were statistically significantly different from controls.

Carr et al. (1991) examined the effect of Poloxamer 188 on fibrin assembly and structure in purified and plasma systems. The Poloxamer was tested at a concentration range from 0.1 to 20 mg/ml. As the concentration increased to 8 mg/ml, fibrin assembly was accelerated in both the plasma and purified systems; however, beyond 8 mg/ml, precipitation of fibrinogen was observed.

Poloxamer 188 was tested on RBC-induced platelet aggregation at plasma concentrations of 0.05 to 5 mg/ml (Armstrong et al. 1995). The Poloxamer inhibited platelet aggregation at $>95\%$ and 41% at concentrations of 1 and 0.05 mg/ml, respectively.

In a similar study, Carr et al. (1996) investigated the effect of Poloxamer 407 on fibrin assembly and structure in platelet-rich plasma (PRP), platelet-poor plasma (PPP), and a purified protein system. At concentrations up to 20 mg/ml, Poloxamer 407 increased fiber size in all three test systems; although, precipitation was noted in the purified systems at concentrations of Poloxamer $\geq$20 mg/ml.

Edwards et al. (1996) investigated the effect of Poloxamer 188 on adenosine diphosphate (ADP)-induced platelet aggregation in vitro. Solutions of Poloxamer 188, at concentrations ranging from 0 to 2.0 mg/ml in saline, were incubated with blood from healthy volunteers and were found to enhance platelet disaggregation in a concentration-dependent manner, compared to saline controls.

Edwards et al. (1998) investigated the relationship between Poloxamer 188 and platelet aggregation agonists

phorbol-myristate acetate (PMA) and collagen (COLL) or the antibiotic, ristocetin (RIST) in human whole blood in vitro. At 0.04% (*w*/*v*), Poloxamer 188 significantly inhibited platelet aggregation caused by 0.05 and 0.1 μg/ml PMA, but was less effective when 0.15 μg/ml PMA was used. Poloxamer 188 (0.04%) had no significant effect on platelet aggregation caused by COLL at 0.25 or 0.5 μg/ml but did inhibit aggregation with COLL at 0.125 μg/ml. When Poloxamer 188 (0.04%) was added to blood treated with 0.3 or 0.6 μg/ml RIST, the Poloxamer virtually abolished the aggregation but had a lesser effect on blood treated with 1.2 μg/ml RIST.

An in vitro study testing blood from two healthy volunteers evaluated the effect of 0, 0.75, 3.75, and 18.75 mg/ml Poloxamer 188 on plasma and whole blood viscosity (Lechmann and Reinhart 1998). Only the highest concentration of Poloxamer caused an increase in the high and low shear viscosity.

Toth et al. (2000) investigated the ability of Poloxamer 188 to decrease RBC aggregation caused by a variety of water-soluble polymers. Poloxamer 188 was tested at a concentration range of 0.5 to 5 mg/ml and caused a concentration-dependent inhibition in the extent and strength of aggregation.

Armstrong et al. (2001) investigated the effects of coating RBCs with Poloxamer 188, 238, 288, or 338 and found that the Poloxamers reduced RBC aggregation.

Absorption, Distribution, Metabolism, and Excretion

General

Batrakova et al. (2001a; 2001b) and Batrakova et al. (2003) reported that Poloxamers increase the permeability of some drugs across the blood-brain barrier.

Poloxamer 108

Wang and Stern (1975) investigated the pharmacokinetics of [^{14}C]Poloxamer 108 in rats. Excretion studies involved administering an intravenous injection of 7 mg/kg of a solution of 0.28% or 4.0% Poloxamer in saline. Young and mature (exact ages not specified) Holtzman rats were tested; their urine and feces were collected for 24 h after administration.

At both concentrations, the [^{14}C]Poloxamer 108 was excreted rapidly in the urine and virtually all of the Poloxamer was excreted by 4 days after injection. The total percentage of the administered dose excreted by young and mature rats after 4 days was 99.4 $\pm$ 2.4% and 96.4 $\pm$ 1.8%, respectively.

Further studies involved administering an intravenous injection of 100 mg/kg of the same Poloxamer 108 solutions in saline to male rats. At 3 min and 20 h after administration, some rats were anesthetized and blood was collected for analysis. At 3 min following injection, the highest concentration of Poloxamer 108 was found in the kidneys (10.66 mg/g tissue), followed by lungs (1.491 mg/g tissue). At 20 h after administration, the Poloxamer concentration in the kidneys was 0.0177 mg/g tissue and in the lungs was 0.0013 mg/g tissue. The authors concluded that Poloxamer 108 was rapidly excreted, mainly by renal but a small amount by biliary excretion (Wang and Stern 1975).

Port et al. (1978) reported a study in which 80 male Sprague-Dawley rats (142 to 232 g) were divided into five groups. Poloxamer 108 in NaCl was injected into the tail vein with: 0.9% NaCl solution alone (I); doses of 4, 2, 1, or 0.150 g/kg Poloxamer 108 for groups II, III, IV, and V, respectively. Test solutions were administered at 3 ml/min at a volume of 40ml/kg body weight daily, 5 days a week for 2 weeks. Body weights were monitored before and during the experimental period. Four rats from each group were killed at 15 min and 24 h after the 1st, 3rd, 5th, 7th, and 10th injections as well as at 3, 7, 10, and 14 days after the last injection. At each of these periods, blood and urine samples were collected. Tissue and organ samples were collected after necropsy. Analysis of serum and urine at various intervals indicate rapid secretion of Poloxamer 108 into the urine. Poloxamer was not detectable in the serum. Concentration of Poloxamer in the lung, liver, and kidneys showed a dose-dependent response, although considerable variability was noted.

Poloxamer 188

Willcox et al. (1978) studied Poloxamer 188 distribution in dogs after an intravenous injection. The Poloxamer was purified by recrystallization from an aqueous solution and 1.5g was randomly labeled with ^{3}H by catalytic exchange of hydrogen; the concentration of the final solution was 50 mg/ml Poloxamer 188. Eight mongrel dogs (4.2 to 15.7 kg) were fasted and anesthetized. One group of five dogs remained under anesthesia for the entire 24-h period. The three dogs in group II were under general anesthesia for 2 h and then became fully awake. At the end of the 24-h period, blood and urine samples were collected from the dogs in group II and then a rinse out of the circulating blood was carried out by perfusion with 2500 ml of Ringer's lactate. Other than these differences, all dogs were treated the same. After anesthesia was induced, the dogs were cannulated and a catheter was placed in the bladder. Baseline samples were collected and then all animals intravenously received 1.0 ml of labeled Poloxamer 188 and 50 mg/kg unlabeled Poloxamer 188. Blood samples were collected at 3, 6, 10, 15, 20, and 30 min, and at 1, 2, and 24 h after injection.

In all dogs, about 40% of the administered dose was recovered in the urine after the 24-h period, 20% of which was excreted in the first hour. Analysis of the urine from group II demonstrates that Poloxamer 188 did not cause an osmotic diuretic effect at any time during the experimental period. Blood samples from this group contained no significant radioactivity. The concentration of ^{3}H-labeled Poloxamer 188 was determined in several tissues, including brain, kidney, liver, and bile. Labeled Poloxamer was found in all tissues, with the highest concentration in the bile. High levels were also found in the liver in group I and in the lung in group II at the end of the 24-h period. Little Poloxamer was found in the fat or brain tissue and the authors hypothesize that Poloxamer 188 is not fat soluble. Poloxamer 188 was found to be transported in the plasma; about

26% was attached to the albumin fraction and 74% circulated free (Willcox et al. 1978).

Jewell et al. (1997) evaluated the pharmacokinetics of Poloxamer 188 in male volunteers aged 19 to 35 and weighing between 122 and 230 pounds. Three groups of 12 underwent two dosing periods at least 3 weeks apart, receiving a single intravenous (i.v.) infusion of either Poloxamer 188 (150 mg/ml in water) or placebo (vehicle alone) during each period. Six doses were studied: 10, 30, or 45 mg/kg/h for 72 h; 60 mg/kg/h for 43.3 to 72 h; and 60 or 90 mg/kg/h for 24 h. Plasma and urine samples were collected during and up to 36 h after the end of the infusions. These were assayed for Poloxamer 188 by gel-permeation chromatography. Twenty volunteers, nine treated with Poloxamer and 11 given placebo, completed the two dosing periods.

Eight subjects suffered from adverse effects of Poloxamer 188, such as back pain, leg pain, headache, or nausea. Three subjects given Poloxamer and one administered placebo had persistent elevated alanine aminotransaminase (ALT) and aspartate transaminase (AST) after the first dosing period and did not complete the second period.

The most frequent adverse reactions were pain, injection site abnormalities such as redness or swelling, and nausea. Monitoring of clinical chemistry, hematology, urinalysis, and electrocardiographic results showed only mild to moderate, reversible elevations in hepatic enzymes (primarily ALT and AST) more often in Poloxamer-treated individuals compared to those given placebo.

Most of the Poloxamer 188 was eliminated by renal excretion. Across all dosing periods, 72% to 94% of the administered dose was eliminated in the urine. Although the mechanism for renal clearance was not determined, the authors hypothesized that Poloxamer 188 was cleared via glomerular filtration. With increasing infusion rate values up to 90 mg/kg/h, steady-state plasma concentrations increased in a linear fashion, whereas plasma clearance levels (mean clearance of 1.06 ml/min/kg) were independent of the infusion rate. These and other parameters studied suggest that the pharmacokinetics of Poloxamer 188 were independent of infusion rate (Jewell et al. 1997).

Grindel et al. (2002) investigated the pharmacokinetics of purified Poloxamer 188 in Sprague-Dawley rats, pregnant albino New Zealand rabbits, purebred Beagle dogs, and six volunteers. Rats were tested with 0, 15, 45, or 150 mg/kg/h. Groups of 12 male and 12 female rats per dose were administered a 30-day continuous i.v. infusion of Poloxamer 188 via the femoral vein. Each group was then divided (three animals/sex/dose) into four subsets, each of which was bled on different days throughout the trial, as well as various times on day 30 just before and after the termination of infusion. For each sample, the plasma was separated and analyzed by gel-permeation chromatography (GPC) for Poloxamer 188. Poloxamer 188 plasma levels of all rats, except those in the 150 mg/kg/h group, reached a steady state by 46 h after the beginning of infusion. The 150 mg/kg/h group reached a steady state by day 7. The steady-state plasma concentrations were dose dependent. No differences were seen between the genders.

Twenty-nine pregnant rabbits were i.v. administered Poloxamer 188 at a dose of 0, 125, 415, 830, or 1250 mg/kg/day for 15 min on days 6 to 18 of gestation. Plasma samples from each dose group were taken at the end of days 6 and 18 and were compared; there were no differences in the maximum concentration of Poloxamer 188. Comparison across doses showed a dose-dependent increase in overall Poloxamer 188 concentration, but no change in plasma $t_{1/2}$.

Twenty male and female dogs were given 30, 100, or 300 mg/kg/h (720, 2400, or 72,000 mg/kg/day) Poloxamer 188 via a continuous i.v. infusion for 30 days. Plasma samples were taken during and after infusion. A steady state for all dose groups was achieved by day 7 and remained stable throughout administration. Analysis of plasma showed a dose-proportional concentration of Poloxamer 188 and the half-life ranged from 17.5 to 19.6 h but did not vary across doses or genders. Mean plasma clearance ranged from 49.4 to 87.9 ml/h and also showed no dose or gender effect. A single metabolite, which the authors labeled HW1, was detected in the plasma of all dogs at 10% to 12% of the parent compound at steady state. HW1 had a molecular weight of 1600 daltons and mass spectrometry showed it to be a block copolymer structure.

Five male and one female volunteers received an i.v. infusion of Poloxamer 188 at a dose of 100 mg/kg/h $\times$ 1 h plus 30 mg/kg/h $\times$ 47 h (1510 mg/kg total). Blood was collected over ethylenediamine tetraacetate (EDTA) at 0, 1, 6, 12, 18, 24, 36, 48, 48.5, 49, 49.5, 50, 52, 54, 56, 60, 62, 68, and 72 h. Urine and feces were collected for 96 h after the start of infusion. GPC analysis was used to determine the Poloxamer 188 concentration in the plasma, urine, and feces. Poloxamer 188 was well tolerated, and volunteers suffered from a few minor adverse effects including frequent urination, headache, leg and lower back pain, and lethargy. The mean concentration of Poloxamer 188 in the plasma at steady state was 522 $\pm$ 118 mg/L and the mean maximum concentration, which occurred at the end of the 1-h infusion, was 909 $\pm$ 165 mg/L. The total mean body clearance, when estimated using the plasma concentration data only and when using the plasma concentrations at the mid-point of urine collections, was 4.40 $\pm$ 0.77 and 5.40 $\pm$ 1.241 L/h, respectively. At steady state, all six volunteers had HW1 in their samples at approximately 40% of the parent compound (Grindel et al. 2002).

Poloxamer 407

Li et al. (1996) investigated the kinetics of a single injection of Poloxamer 407 using male Sprague-Dawley rats weighing 263 to 481 g. Rats were divided into groups of six and intraperitoneally (i.p.) injected with 300 mg of a solution of 30% Poloxamer 407 in saline. Blood was collected from each group at either 4, 6, 12, 18, 24, or 48 h after the injection; the rats were then killed. The plasma concentration of Poloxamer 407 steadily increased to a maximum concentration of 13.5 mg/ml

at 12 h after injection. To determine the urinary excretion rate of Poloxamer 407, 10 rats were intraperitoneally injected with 300 mg of a 30% Poloxamer 407 solution and placed in metabolic cages. Urine was collected in 24-h intervals for 96 h.

During the first 24 h, the mean amount of Poloxamer 407 excreted was 76.3 ± 1.8 mg. No Poloxamer 407 was detected in the urine samples taken from 24 to 96 h after the injection. The authors also investigated the distribution of Poloxamer 407 in hepatic and renal tissue after administering i.p. injections of 300 mg of a 30% Poloxamer 407 solution to 12 rats. Twenty-four hours after the injection, the animals were killed, their livers and kidneys excised and homogenized, then the concentration of Poloxamer 407 in each was determined. The mean amounts in the kidney and liver homogenates were 3.1 ± 0.26 and 15.9 ± 1.6 mg, respectively (Li et al. 1996).

Multidrug Resistance–Associated Protein and Poloxamers

Miller et al. (1999) described the key role of drug efflux transport proteins (adenosine triphosphate [ATP]-binding cassette proteins) that function to pump selected agents out of mammalian cells, including P-glycoprotein (Pgp), the activity of which is inhibited by Poloxamers. In this study, the authors identify multidrug resistance–associated protein (MRP) that actively transports chemotherapeutic agents out of cells. The study further demonstrated that Poloxamers can inhibit MRP activity. The authors suggest the possibility that Poloxamer-induced changes in the plasma membrane that would affect both Pgp and MRP as a mechanism of action.

Batrakova et al. (1999) examined the fundamental relationships between Poloxamer composition and their effects on multiple drug resistance (MDR) cancer cells. They reported that Poloxamer concentrations below those needed to form micelle structures were effective in increasing the activity of anticancer cytotoxic agents. When the concentration increases to the point where micelle structures form, the Poloxamer molecules were less effective.

This laboratory elaborated on the mechanism of Poloxamer sensitization of MDR cancer cells (Batrakova et al. 2001c). They tested the effect of Poloxamer 235 at 10 concentrations ranging from 0.0001% to 5% *w/w* on a human breast carcinoma cell line (MCF-7) and its MDR subline, MCF-7/ADR. Cells were incubated with Poloxamer 235 at various concentrations for 2 h, then washed twice with phosphate-buffered saline (PBS) and solubilized in a 1% solution of Triton X-100 in PBS. Lysates were collected and frozen for subsequent ATP quantification.

In the MCF-7/ADR (resistant) cells, concentrations greater than 0.01% Poloxamer 235 caused up to a 3.8% decrease in ATP levels. In the sensitive (MCF-7) cells, ATP levels did not significantly drop until concentrations greater than 1% Poloxamer 235 were applied, at which point, ATP levels decreased by as much as 15% of the initial value. Having demonstrated a decrease in ATP levels selectively in MDR cells associated with exposure to Poloxamers, these authors suggest that a successful strategy for treating MDR cancers could be based on selective energy depletion in MDR cancer cells, including the use of Poloxamers (Batrakova et al. 2001c).

Antimicrobial Activity

Reports of antimicrobial activity of Poloxamers (primarily of Poloxamer 188) are outlined in Table 5. Activity was seen against *Mycobacterium avium* and *Streptomyces coelicolor*, but not against *Staphalococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus epidermidis, Salmonella typhimurium, Salmonella worthington, Escherichia coli, Listeria monocytogenes, Listeria innocua,* and *Saccharomyces cerevisiae*.

Immune Function

Moghimi and Murray (1996) divided male lipopolysaccharide nonresponder C3H/HeJ mice into groups of three and administered an i.v. injection of 15 mg/kg body weight of a 5.0% Poloxamer 188 solution. Control rats were injected with saline. Four days later, all animals received an i.v. dose of 0.3 mg radiolabeled phagocyte-resistant particles, and were killed 1 or 5 h later. Radioactivity in the blood, liver, and spleen was measured. In comparing the percentage of the administered radioactive dose in the blood at 1 h, the test mice had 20% less of the given dose than control mice. The test mice had almost 20% and 3% higher levels in the liver and spleen, respectively. At 5 h, concentrations in the blood of test mice were almost half those of control mice. Liver and spleen concentrations were higher by 30% and 4% of the administered dose, respectively. The authors concluded that i.v. injections of Poloxamer 188 stimulates phagocytic activity in the liver and spleen macrophages.

Wound Healing

Wahl and Butterfield (1976) reported that Poloxamer 188 has an inhibitory effect on both gastric acid concentration and ulcer formation in rats.

Rodeheaver et al. (1976) suggested that Pluronic® block copolymers are safe for intravenous use in humans. Pluronic F-68 (also known as Poloxamer 188), a Pluronic® polyol with a high ethylene oxide component (80%) and a molecular weight of 8350 Da was the detergent used in the experiment. This detergent had no antibacterial activity and at high concentrations, topical application did not impair tissue defenses or wound healing. It was discovered that scrubbing contaminated wounds with sponges soaked in this detergent resulted in bacterial removal without tissue injury and aided against infection. Poloxamer 188 has also been administered to human subjects as a wound cleanser without side effects. The benefit is that this nontoxic surfactant can solubilize elemental iodine, which is relatively insoluble, to form an iodophor. Iodophors are effective germicidal agents that have lengthy antiseptic activity in contaminated wounds.

TABLE 5
Antimicrobial activity of Poloxamers.

Microbe	Protocol and Dose	Results	Reference
Escherichia coli and Saccharomyces cerevisia	Poloxamer was sterilized using a Millipore filter (0.22 μ) and 50 ml aliquots of 1.5–5.0% (*w/v*) Poloxamer solution in distilled water was added to cultures of both cell lines. Distilled water was used as a control. Cultures were maintained in a 37°C water bath for 6 h and samples were removed throughout the experimental period to determine cell growth.	Poloxamer 188 did not affect the growth rate of *E. coli*, compared to control values, but did inhibit growth of the yeast at all concentrations tested in a dose -dependent fashion. A concentration of 5% Poloxamer caused growth inhibition of about 50%, compared to control values.	Chandler et al. 1987
Streptococcus pyogenes or *Staphylococcus aureus*	Four dorsal lacerations (3 cm) were made on each of 22 albino guinea pigs. After hemostasis, 0.3 ml of bacterial inoculum was placed on each laceration. Ten animals were inoculated with *Streptococcus pyogenes* (2.6×10^9 CFU/ml) and 12 with *Staphylococcus aureus* (3×10^9 CFU/ml). Four hours later, lacerations were either scrubbed (SCR) with 1 ml of 20% Poloxamer 188, irrigated with 180 ml of 1% povidone iodine (PI) in NaCl, or both (SCR-PI). Some lacerations were left as controls. 96 h after inoculation, wounds were excised, ground, then diluted with 0.9% saline solution and plated on 5% sheep's blood agar. Colonies were incubated at 37°C, counted at 24 h, and recorded as colonies per g of tissue.	The mean bacterial counts of the control and treated tissues were not significantly different. The mean bacterial counts of the *S. pyogenes* infected tissues for the control, PI, SCR-PI, and SCR treated samples were 7.00 ± 0.65, 5.23 ± 1.33, 5.81 ± 1.32, 6.60 ± 1.56, respectively. SCR, PI, and SCR-PI decreased bacterial counts in *S. pyogenes*, but scrubbing with Poloxamer 188 alone did not reduce counts significantly.	Howell et al. 1993a
Staphylococcus aureus	Lacerations were made as described above in 12 albino guinea pigs and inoculated with 0.4 ml of *S. aureus*. One laceration on each of six guinea pigs was left as a control, while the other three were irrigated with cefazolin (CZ) (2 g in 1 L saline), normal saline, or 1% PI solution. Lacerations on the other six guinea pigs were: SCR with 5 ml of 20% Poloxamer 188, scrubbed and SCR-PI, or both (SCR-CZ). Bacteria were counted 2, 7, and 12 h after irrigation.	Treatment with PI, CZ, and normal saline resulted in bacterial counts comparable to those of controls. SCR-PI significantly decreased bacterial counts at 2, 7, and 12 h. SCR-CZ also significantly decreased counts, but only at 7 and 12 h. SCR with Poloxamer 188, alone, caused significantly lower levels at 7 h only.	Howell et al. 1993b

(Table continued on next page)

TABLE 5
Antimicrobial activity of Poloxamers *(Continued)*.

Microbe	Protocol and Dose	Results	Reference
Streptomyces coelicolor A3(2)	In part of an investigation of perfluorocarbon in which Poloxamer 188 was used as an emulsifier, Poloxamer 188 was tested alone with *Streptomyces coelicolor*. Poloxamer 188 was tested at various concentrations ranging from 0 to 30 g/L.	Poloxamer 188 caused a decrease in bacterial growth to levels below that of the control cultures. Concentrations ranged from 0% to 80%.	Elibol and Mavituna 1995
Mycobacterium avium complex (MAC) isolates from patients with active clinical infections	Poloxamers 181, 182, 183, 184, 185, 331, 333, 335, 338, 122, 182, 282, and 402 were tested at a concentration of 1.0 mg/ml. Poloxamers were incubated with MAC isolates at 36°C without shaking and tested daily for growth index. Controls received no Poloxamer. Poloxamer 331 was tested on 14 isolates at 1.0 mg/ml, in the same manner.	The % inhibition for each Poloxamer was P181, 91%; P182, 82%; P183, 80%; P184, 60%; P185, 25%; P331, 96%; P333, 90%; P335, 86%; P338, 14%; P122, 56%; P182, 82%; P282, 88%; and P402, 90%. P331 inhibited three isolates between 98.9% and 97.8%. It inhibited seven isolates between 83.0% and 54.7%, as well as three isolates between 42.0% and 29.0%, and one 0%.	Hunter et al. 1995
Staphylococcus aureus	New Zealand albino rabbits were anesthetized and clipped of dorsal hair. The skin was cleaned and four wounds were symmetrically made on each side of the vertebral column. To three of the wounds, Poloxamer 188 solution (10%, 20%, and 40% [w/w]) was applied. The fourth (control) wound received 0.9% NaCl solution. After 10 min of application, wounds were blotted and animals were divided into two treatment groups. The two groups were administered 0.1 ml of NaCl solution with either 1.1×10^5 or 3.3×10^6 organisms of *S. aureus* to all four wounds. Wounds were closed with microporous tape 5 min after contamination. On day 4, inflammatory responses were measured; each wound was opened and examined and an estimate was made of the number of bacteria in each wound. Each wound was excised, weighed, homogenized, and the number of bacteria in the suspension was determined.	The incidence of infection in the Poloxamer-treated and control groups was comparable. Also, the mean bacterial counts of wounds treated with Poloxamer were similar to those of the control wounds.	Rodeheaver et al. 1980

(Table continued on next page)

TABLE 5
Antimicrobial activity of Poloxamers *(Continued).*

Microbe	Protocol and Dose	Results	Reference
Pseudomonas aeruginosa, Micrococcus luteus, Staphylococcus epidermidis, Salmonella typhimurium, Salmonella worthington, Escherichia coli, Listeria monocytogenes, and *Listeria innocua*	A 30% (w/v) Poloxamer solution in tryptone soy broth (Oxoid CM129) was made and refrigerated overnight. Then, 3 ml of chilled Poloxamer was inoculated with a 1/100 dilution, in fresh TSB, of an overnight (16 h) stationary phase culture (300 μl) to give 10^{4-5} CFU/ml. Drops of each culture solution (200 μl) were placed in triplicate onto prewarmed sterile stainless steel discs and incubated for 5 h at 30°C.	All strains grew well, to between 10^6 and 10^7 cfu/ml.	Harkonene et al. 1999

Rodeheaver et al. (1980) undertook a series of experiments to test the wound healing capacity of Poloxamer 188. In the first in vitro study, fresh human blood was divided into tubes containing EDTA, then combined with equal volumes of test solution and maintained at 37°C for 30 min. The test solutions included 0.9% sodium chloride and 20% w/w Poloxamer 188. Aliquots were then removed from each tube and the numbers of intact white and red blood cells were counted and the amount of hemoglobin in the plasma was determined.

In the second phase of the in vitro study, fresh venous blood was collected in heparinized tubes and combined with an equal volume of 3% dextran and the erythrocytes were allowed to settle. The supernatant was centrifuged to create buttons of leukocytes, which were then resuspended in either 20% *w/w* Poloxamer or Hanks' balanced salt solution (HBSS). The suspensions were rotated and then recentrifuged. The leukocyte pellets were washed twice in HBSS and equal numbers of white blood cells from the treated and control tubes were added separately to tubes with 1.4 ml of HBSS and 0.4 ml of autologous serum. Then, 0.2 ml of *Staphylococcus aureus* (10^7 organisms) was added to each tube, which were then rotated and maintained at 37°C for 2 h. Fluid was then removed from all tubes and the number of bacteria were counted.

Results from the first study show that Poloxamer 188 did not alter the white or red blood cell counts and no hemoglobin was noted in the plasma. Also, it had no adverse effects on the function of white blood cells; it did not limit the ability of the cells to phagocytize and kill bacteria compared to controls.

In the second in vivo experiment, male Hartley guinea pigs (300 to 350 g) were anesthetized and two standard paravertebral incisions were made in each guinea pig. One wound was treated with 0.2 ml of 10%, 20%, or 40% Poloxamer solution whereas the other served as a control and received 0.1 ml of 0.9% NaCl solution. After 10 min, the wounds were closed with microporous tape. Fourteen days later, the animals were killed and the breaking strength of the wounds was determined. Poloxamer did not have any affect on the healing of the wounds. The breaking strength of the treated wounds was comparable to that of the control wounds.

In the last part of the investigation, the authors determined the therapeutic value of Poloxamer 188 in New Zealand albino rabbits. Three wounds were made as described above and all three were contaminated with 10^7 *S. aureus*. One wound was scrubbed with a polyurethane foam sponge soaked in 0.9% saline, whereas the other wound was scrubbed with a sponge soaked with 20% Poloxamer 188. The third wound was not scrubbed and served as a control. After scrubbing, the wounds were closed and 4 days later, the animals were killed; the inflammatory responses were measured.

Scrubbing wounds with Poloxamer 188 prevented infection. No scrubbing and scrubbing with NaCl resulted in 100 and 83% gross infection, whereas wounds scrubbed with Poloxamer had 0% ($p < .01$) gross infection. The authors suggest that Poloxamer 188 is an effective wound cleanser; although, it exhibits no antibacterial activity (Rodeheaver et al. 1980).

Nalbandian et al. (1987) investigated the use of Poloxamer 407 with additives for use as a treatment of third-degree burns in pigs (40 to 50 pounds). Pigs were anesthetized and three separate third-degree thermal burns were induced with a branding iron on an approximately 1600-mm^2 area of their shaved backs. One site was left untreated and served as a control, whereas areas of the other two sites received treatment with one of four combinations: Poloxamer + propylene glycol (P + PG), Poloxamer + propylene glycol + garamycin (P + PG + GAR), Poloxamer +

propylene glycol + piracetam (P + PG + PIR), or Poloxamer + propylene glycol + piracetam + garamycin (P + PG + PIR + GAR). Solutions were made up of a final concentration of 19.0 g% w/v Poloxamer 407, 3.0 g% propylene glycol, 100 M piracetam, and 0.016% garamycin. Biopsies were taken and pigs were followed for 30 days.

Compared to controls, P + PG, P + PG + GAR, P + PG + PIR, and P + PG + PIR + GAR reduced the burn damage by 39.2%, 38.2%, 0.03%, and 32.1%, respectively. The authors did not offer an explanation for the 0.003% result but concluded that Poloxamer 407 did significantly increase the rate of wound healing, and although the mechanism is unknown, they suggest it is by stimulation of epithelial growth factor (Nalbandian et al. 1987).

Leach and Henry (1990) determined the effect of Poloxamer 407 on postoperative adhesions using 22 female Sprague-Dawley rats (300 to 400 g). The rats underwent bilateral surgical injury to the uterine horn and the parietal peritoneum. Each rat served as its own control, with treatment applied to one side in which a 30% Poloxamer 407 solution was cooled and applied to the uterine horn and parietal peritoneal defect by a syringe. The Poloxamer rapidly gelled and was smoothed over the surface. Approximately 0.5 and 1.5 ml of solution was used to cover the uterine horn and peritoneal defect, respectively. The abdominal wall was closed after application; rats were killed 21 days later and examined for adhesion formation. A 0 to 4 grading system was used to evaluate both the prevalence and character of the adhesions.

Two animals died within 72 h of surgery but the remaining 20 lived until the end of the experimental period. Nineteen (95%) of the 20 animals developed adhesions on the untreated control side, which had an average score of 3.21 ± 1.1. Comparatively, only 8 of the 20 rats developed adhesions on the treated side and among these 8, the adhesion score was an average of 1.6 ± 0 (Leach and Henry 1990).

Steinleitner et al. (1991) evaluated the use of Poloxamer 407 for the prevention of postsurgical adhesion formation and reformation in 6-week-old female Golden hamsters and New Zealand white rabbits. Hamsters were subjected to a standardized traumatic lesion by devascularization of the left uterine horn. Two ml of a Poloxamer 407 solution in saline was applied to the injured horn at concentrations of 15%, 20%, 25%, 30%, or 35% to groups of ten hamsters for each concentration. Hamsters were killed 14 days after surgery and assessed, on a cumulative scale from 0 to 6, for location, thickness, and extent of adhesion formation. Control hamsters underwent the same surgery but were untreated and they developed severe adhesions with an average score of 4.5 ± 0.5.

Poloxamer 407, at concentrations of 15% and 20%, had little effect on postsurgical adhesion formation; however, the 25% solution significantly reduced the location and thickness of induced lesions. At concentrations of 30% and 35%, Poloxamer 407 significantly inhibited adhesion formation with mean total adhesion scores of 2.3 ± 0.8 and 2.2 ± 0.9, respectively. Five and 7 of the 10 animals in each group tested with 30% and 35% Poloxamer, respectively, had no adhesions at all.

In the second experiment, rabbits were subjected to three sequential laparotomies, each 14 days apart, for placement of the adhesion-producing lesion, evaluation (prescore), surgical lysis of induced adhesions, and evaluation of adhesion reformation (postscore). The control group received no treatment while nine animals were treated (as described above) with 3 ml of a 35% Poloxamer 407 solution immediately after lysis of adhesions. Two weeks after the last surgery, all animals were killed and adhesion scoring was carried out.

The mean pre-score for all rabbits was 5.4 ± 0.3, which was similar to the postscore of control rabbits, 5.1 ± 0.3. The rabbits in the treated groups had postscore values that were approximately 50% less than the corresponding prescores for location, thickness, and extent of adhesions. Poloxamer 407 significantly reduced adhesion reformation (Steinleitner et al. 1991).

Naim et al. (1993) experimented with the effect of three different adhesion barriers on bowel adhesion formation in rats. Polypropylene mesh (Marlex) is used to reinforce the abdominal wall during hernia repair but can cause significant bowel adhesions. Sixty-one male Harlan Sprague-Dawley rats (300 g) were anesthetized and a midline incision was made in the abdominal cavity of all rats. They were then divided into four groups and received the following treatment: group 1, sterile Marlex mesh was placed lateral to the midline using staples; group 2, Gore-Tex was placed over the Marlex; group 3, Interceed was placed over the Marlex; and group 4, 2 ml of 30% Poloxamer 407 was placed over the Marlex. All incisions were closed and sutured. Animals were killed 6 weeks after the operation and the adhesions were scored on a scale from 0 to 4.

Rats in the Marlex alone group and Marlex + Gore-Tex group had the highest adhesion scores (2.7 ± 0.6 and 3.0 ± 0, respectively) and also had adhesions with the highest tensile strength. Rats in the Poloxamer group had adhesion scores of 2.0 ± 0.6, with the lowest tensile strength. The Marlex + Interceed group had adhesion scores of 1.6 ± 0.6 but the adhesions had a greater tensile strength than those in the Poloxamer group. Poloxamer 407 significantly reduced tensile strength and Marlex mesh incorporation (Naim et al. 1993).

In a series of three studies, Rice et al. (1993) compared the effect of Poloxamer 407 and Interceed (TC7) on postoperative adhesion formation in the rat uterine model. Adult female Sprague-Dawley rats (200 to 300 g) were anesthetized and underwent laparotomies. Each uterine horn and ipsilateral sidewall was denuded.

The first experiment involved applying either 1.0 ml of a 28% aqueous solution of Poloxamer 407, Interceed (absorbable adhesion barrier), or no treatment (to serve as control) to the sidewall defect of the left or right side. The abdominal wall was closed.

For animals in the second experiment involving the same surgical procedure, each side was treated with either Poloxamer

407 or no treatment. After abdominal wall closure, 10 ml of lactated Ringer's solution mixed with methylene blue was injected into the abdominal cavity.

In the final experiment, the only change in the surgical procedure was that hemostasis was controlled. Then, the rats' sides were randomized to receive either Poloxamer or Interceed. During the excising of the parietal peritoneum, a ventral sidewall vessel was incised to ensure that a thrombus was present over the injured area before application of Poloxamer or Interceed. The rest of the procedure was the same as above.

Animals were killed 14 days later. Adhesions were graded according to a modified Linsky Grading System from 0 to 3. The adhesion scores for Poloxamer-treated animals were significantly lower than those of controls, approximately 0.9 versus 1.6. When tested with lactated Ringer's solution, Poloxamer 188 caused total adhesion scores similar to those of controls. The presence of blood also reduced the adhesion-reducing properties of Poloxamer 407. Interceed did not reduce adhesion formation. The authors concluded that Poloxamer 407 is more effective than Interceed (TC7) in preventing postoperative adhesions; however, the presence of blood decreases its effectiveness and complete hemostasis would be required before Poloxamer application (Rice et al. 1993).

Reigel et al. (1993) investigated the effect of Poloxamer 407 on the prevention of leptomeningeal adhesion formation, which frequently causes complications after operations of the central nervous system. Twelve male New Zealand white rabbits were used, eight for the first experiment and four for the second. In the first part of the study, the lumbar spinal roots of the rabbits were surgically isolated under magnification and one root sleeve axilla was opened and closed with a 10-0 suture to serve as a control. A second root sleeve was opened and a 23.1% (*w*/*w*) solution of Poloxamer (dose not specified) was injected into the root sleeve, which was subsequently closed with a 10-0 nylon suture. Half of the rabbits were killed and examined 1 to 21 days after the operation and the rest 21 to 42 postoperative days.

Of the seven rabbits treated with Poloxamer, five showed no arachnoidal adhesions at the level of the nerve root. Of the two with lesions, the average adhesion grade was 1.25 compared to the grade for all controls, which was 2.5. In the second study, four rabbits had their lamina removed, the dura over the spinal cord was opened at two sites separated by one to two lumbar segments. One site was treated by inserting Poloxamer 407 under the dura following durotomy, whereas the other site was opened and closed similarly to serve as a control. Rabbits were killed 21 to 42 days after the operation. A 50% reduction in leptomeningeal adhesion formation was found in the Poloxamer-treated sites (Reigel et al. 1993).

West and Hubbell (1995) carried out a study comparing covalently and physically cross-linked polyethylene glycol-based hydrogels in the prevention of postoperative adhesions in female Sprague-Dawley rats (250 to 300 g). Rats were anesthetized and underwent a low midline laparotomy. The uterine horns were isolated and two spots on the antimesenteric surface of each horn were cauterized. The animals were then divided into three groups of seven animals. One group received no treatment, whereas in another group, 2 ml of 35% Poloxamer 407 (physically cross-linked) solution, in saline, was applied to the uterine horns. The third group was treated with a polyethylene glycol-co-lactic acid diacrylate (covalently cross-linked hydrogel). The uterine horns were replaced in the peritoneum and the skin was closed. Seven days after the operation, all animals were killed and the extent, as well as severity, of adhesion formation was evaluated.

Rats in the control group exhibited $75 \pm 10\%$ adhesion formation, whereas those treated with the covalent or physically linked hydrogels had formations at $16 \pm 6\%$ and $38\% \pm 19\%$, respectively. A continuous barrier was apparent for 4 days with the covalently linked hydrogel but for only 2 days with the physically cross-linked hydrogel, which may explain the difference in efficacy (West and Hubbell 1995).

Cardiovascular Effects

Colbassani et al. (1989) investigated the effect of Poloxamer 188 on the cerebral blood flow in rabbits following focal cerebral ischemia. Twenty-two New Zealand white rabbits (3.5 to 4.5 kg) underwent a retro-orbital craniectomy. Then, a bipolar current was used to occlude the parietal branch of the middle cerebral artery. Thirty minutes after occlusion, 10 rabbits were given an intravenous injection of Poloxamer 188 in saline at 0.6 mg/ml blood volume, followed by an infusion of 0.6 mg/ml blood volume/h until the end of the experiment for a total of 50 mg/kg Poloxamer 188. The 12 rabbits in the control group received an equal volume of saline. Cerebral blood flow was measured 1, 2, 3, and 4 h after occlusion.

Poloxamer 188 increased blood flow 4 h after occlusion by a mean of 69%; it improved blood flow in areas of moderate to severe initial ischemia by $>120\%$. The authors hypothesized that this improvement may be due to Poloxamer 188 inhibiting adhesive interactions among proteins and cells in the microcirculation but was not due to hemodilution or a change in blood viscosity (Colbassani et al. 1989).

Gosselin and Biro (1990) investigated the effect of Poloxamer 188 on the cardiovascular system of adult mongrel dogs of both sexes (14 to 27 kg). Poloxamer was filtered through activated coconut charcoal filters and administered as a 20% w/v solution in NaCl. Dogs were anesthetized and intubated. The arteries and veins were annulated and a thoracotomy was performed on the left fourth intercostal space. Catheters were placed in the left ventricle and pulmonary artery. Measurements were taken to serve as control levels before the dogs were intravenously administered hydrocortisone sodium succinate via a 10-min infusion (5 mg/kg). Then, the test material was intravenously injected over a 15-min period (0.66 g/kg). This was followed by a 15- min pause period before a second infusion of Poloxamer 188 (1.11 g/kg) over 15 min.

Poloxamer 188 caused dose-dependent increases in cardiac filling pressures and in systemic and pulmonary arterial blood

pressure but had no affect on heart rate or contractility. Cardiac output increased with increasing doses of Poloxamer 188 and blood flow to the heart, kidney cortex, and lung increased significantly. The authors suggest that Poloxamer 188, when purified and after steroid prophylaxis, possesses significant hemorheologic and cardiovascular effects (Gosselin and Biro 1990).

The effect of Poloxamer 188 and mannitol on myocardial infarct size was studied in 60 adult mongrel dogs weighing 20 to 30 kg (Justicz et al. 1991). The dogs were divided into four groups: saline-treated control dogs, dogs intravenously treated with 48 mg/kg Poloxamer 188, those intravenously administered 0.5 mg/kg mannitol, and dogs receiving both Poloxamer 188 and mannitol at the same doses as when administered individually. All dogs were anesthetized with 25 mg/kg sodium thiopental and underwent surgery to cause a left anterior descending coronary artery (LAD) occlusion. Seventy-five minutes later, dogs received the appropriate drug treatment for 15 min of LAD occlusion and for an additional 45 min of reperfusion. Animals were killed 24 h after surgery and areas of myocardial infarction (MI) and risk of infarction (R) were determined. Infarct data were determined in terms of the ratio of area of infarct over area at risk or total area of left ventricular (LV) myocardium in slices.

Fifteen animals died during perioperative procedures and of the 45 hearts examined at the end of the procedure, data for analysis was collected from 41 hearts (10 control, 10 Poloxamer-treated, 11 mannitol-treated, 10 Poloxamer and mannitol–treated).

The MI/R ratios for control, Poloxamer-treated, mannitol-treated, and combination-treated dogs were 25.6 $\pm$ 12.8%, 12.7 $\pm$ 2.0%, 10.6 $\pm$ 2.5%, and 8.0 $\pm$ 4.1%, respectively. The MI/LV ratios or control, Poloxamer-treated, mannitol-treated, and combination-treated dogs were 9.7 $\pm$ 0.6%, 5.3 $\pm$ 1.0%, 5.1 $\pm$ 1.3%, and 3.8 $\pm$ 1.9%, respectively.

Both sets of data show a statistically significant reduction in the infarct size of all treated groups, when compared to the control group. Poloxamer 188 and mannitol both reduced infarct size by about 50% and the combination of the two caused a slightly greater but statistically nonsignificant reduction as compared to the effects of the individual agents. No detectable signs of toxicity were seen in any animals treated with Poloxamer or mannitol (Justicz et al. 1991).

Mezrow et al. (1992) investigated the neurological outcome of Poloxamer 188–treated dogs after hypothermic circulatory arrest. Thirteen adult mongrel dogs (20 to 25 kg) were cooled (perfusion and surface) to 10°C. The heart was then arrested for 150 min, after which the dogs were rewarmed, weaned from bypass, and evaluated for 1 week. The treatment group was divided into three regimens of a loading dose before cardiopulmonary bypass (CPB) and drug infusion for 6 h after deep hypothermic circulatory arrest (DHCA). The loading doses for two, two, and three animals were 260, 300, and 500 mg/kg/h, respectively, and infusion doses of 60, 140, and 275 mg/kg/h, respectively. Six dogs in the control group received a loading dose and continuous infusion of saline (dose not reported). During the week-long examination period, neurological outcome was graded on a scale of 1 to 6: grade 1, death within the observation period; grade 2, comatose; grade 3, hold head up; grade 4, sits up; grade 5, stands; and grade 6, normal in both behavior and gait.

Three of the six dogs in the control group died, whereas the lowest score in the treated group was grade 4 for one dog. Four of the treatment dogs scored a 5.5 and two scored a 6. The remaining three dogs in the control group all scored a 3. There were no apparent differences between dogs of different regimens in the treatment group (Mezrow et al. 1992).

Mayer et al. (1994) investigated the effect of Poloxamer 188 on hemorrhagic shock induced in mature male New Zealand white rabbits. Rabbits were anesthetized, stabilized, and blood was withdrawn over a 5-min period to reduce arterial pressure. All rabbits were then administered a 60-min shock period. The animals were then divided into five groups of eight; 1, shock with no retransfusion; 2, retransfusion of autologous shed blood; 3, retransfusion with autologous blood and infusion of an additional volume of saline equivalent to the volume of Poloxamer 188 administered in the test groups; 4, low-dose drug; and 5, high-dose drug (intravenous injection of 200 mg/kg Poloxamer 188 in saline over 5 min at retransfusion plus a continuous infusion of Poloxamer at 150 or 200 mg/kg/h for the low- and high-dose groups, respectively).

No rabbits in the shock alone group (group 1) survived the 3-h monitoring period following shock. In the treated groups, six and seven of the rabbits in the low- and high-dose groups, respectively, survived to the end of the monitoring period. Four of the eight in group 2 and two of the eight in group 3 survived (Mayer et al. 1994).

Schaer et al. (1994) tested adult male mongrel dogs (20 to 30 kg) to assess the effect of Poloxamer 188 on the reduction in reperfusion-induced myocardial necrosis. Three groups of dogs underwent 90 min of left anterior descending coronary artery occlusion produced by using an angioplasty balloon followed by 72 h of reperfusion. Poloxamer 188 was administered as a sterile solution of 140.4 mg/ml Poloxamer, 4.5 mg/ml NaCl, and 1.8 μg/ml butylated hydroxytoluene. The Poloxamer (75 mg/kg) was intravenously administered 15 min before reperfusion and at 150 mg/kg/h in a continuous i.v. infusion for either 4 (n = 13) or 48 (n = 13) h. The control group (n = 12) received saline for 48 h.

The dogs that received Poloxamer 188 for 48 h showed improved left ventricular function and a 42% reduction in infarct size, as compared to dogs in the control group. However, those in the 4-h treatment group had only a 25% reduction in infarct size. The authors suggest that the 4-h infusion of Poloxamer 188 was not as beneficial as the 48-h because additional reperfusion injury occurred between 4 and 48 h; thus, the 48-h treatment is clearly more effective (Schaer et al. 1994).

Male Sprague-Dawley rats were tested by Follis et al. (1995) to assess the effects of Poloxamer 188 before and after aortic cross-clamping. The animals were divided into two groups

and received an intravenous injection of either placebo ($n = 11$) or Poloxamer 188 ($n = 12$) at a dose of 200 mg/kg. All animals underwent occlusion of the thoracic aorta and both subclavian arteries for 13 min. Animals were then connected to an intravenous pump and received either placebo or 250 mg/kg/h Poloxamer 188 at a rate of 0.942 ml/h for 48 h. The health of the animals was scored every day for 4 weeks after which, the animals were killed and the spinal cords were processed for histology.

Neurological recovery was initially faster in animals treated with Poloxamer 188; however, after the 30-day test period, the neurological scores did not differ between the test and placebo groups. Histologically there were no histopathological differences in the spinal cords between the groups (Follis et al. 1995).

Liu et al. (1996) examined the effect of Poloxamers on thrombus formation. A group of 30 domestic pigs were anesthetized and divided into test and saline control groups. A left angiogram was performed via the femoral artery and the proximal left anterior descending and the circumflex arteries were dilated three times in all animals. Beginning 30 min before angioplasty and continuing for 24 h, 15 animals received intravenous infusions of Poloxamer 188 (50 mg/kg bolus followed by 100 mg/kg/h constant infusion) or 0.45% saline. Animals were killed 2 weeks after angioplasty and histological studies of the arteries were carried out.

No differences were seen between the groups in terms of presence of intimal proliferation, medial dissection, and disruption of internal or external elastic lamina. The treated group had significantly less thrombus material in the neointima as compared to the control group (0.013 ± 0.001 versus 0.029 ± 0.006 mm^2) but the neointimal and luminal areas were comparable. The authors concluded that Poloxamer 188 may decrease mural thrombus formation but did not decrease neointima formation (Liu et al. 1996).

Neurological Effects

Clark et al. (1999) carried out a series of experiments to determine the effect of Poloxamer 188 on neurotransmitter uptake and release, as well as learning and memory in rats. The initial brain penetration study involved determining the amount of Poloxamer 188 that would penetrate the brain from a peripheral injection. Four rats (no details provided) were given an intraperitoneal dose of 2400 μg/kg of ^{14}C-labeled Poloxamer 188 in PBS. Rats were anesthetized 30 min after injection and perfused transcardially with 100 ml of PBS. The brains were then removed; two were dissected and homogenates from each were measured for radioactivity 4 h later. The moles of labeled Poloxamer 188/mg wet tissue were back-calculated from the DPM measured.

In order to be able to compare in vivo and in vitro Poloxamer 188 doses, 50 mg of wet brain tissue was repeatedly homogenized in 1 ml of 0.1% (*w/v*) sodium dodecyl sulfate (SDS), centrifuged, and assayed for total protein content. The amount of Poloxamer was measured in terms of moles of ^{14}C-labeled Poloxamer 188 per mg of SDS-extractable protein. Poloxamer 188 was detectable at similar concentrations in homogenates from both the whole brain and from several brain regions. Concentrations ranged from 0.11 to 0.15 nmole/mg SDS-extractable protein. A dose of 0.3 nmoles of ^{14}C-labeled Poloxamer 188 per mg of SDS extractable protein in the cell pellet was used in an [^{3}H]NE (norepinephrine) uptake assay.

Poloxamer 188 significantly ($p < .005$) decreased NE uptake from tissue culture medium, thus indicating that endocytosis of the neurotransmitter substance was inhibited by Poloxamer 188 in the membrane. In PC-12 cells previously loaded with [^{3}H]NE prior to Poloxamer exposure, the Poloxamer significantly ($p < .03$) reduced the amount of NE released as a consequence of nicotine exposure. A similar reduction was observed when the fluorescent dye, FM I-43, was loaded into the depolarization sensitive intracellular vesicle population of PC-12 cells, instead of NE. The authors suggest that these data show that the presence of Poloxamer in the plasma membrane affects the ability of the neural acetylcholine receptor (nAChR) to bring about neurotransmitter release upon nicotine stimulation.

Male Wistar rats (45 days old) were used for the behavioral experiments with Poloxamer 188. Thirty minutes before testing, rats were intraperitoneally injected with a saline control or 200, 800, or 2400 μg/kg Poloxamer 188 in PBS. Rats were tested as to whether they would repeatedly go into an "unsafe" chamber where they received a shock. The latency to enter the unsafe chamber (step-through latency) and learning frequencies (number of rats not crossing into the unsafe side) during the trial were used as measures of memory.

Poloxamer caused a dose-dependent decrease in step-through latencies and increased the frequencies of crossing into the unsafe side of the chamber. In a similar test, using the Morris water maze, Poloxamer 188 increased the latencies and swim distances required to locate a hidden platform and reduced the time spent and distance swam in previous trials. However, at doses of 200, 800, and 2400 mg/kg, Poloxamer did not impair performance of rats in a memory task, the rat delayed stimulus discrimination task (Clark et al. 1999).

Cytotoxicity

Mizrahi (1975) tested Poloxamers 188, 217, 238, and 338 in two human lymphocyte cell lines. These cell lines were the Roswell Park Memorial Institute (RPMI) cell line no. 8432 from a patient with acute lymphatic leukemia and the Israel Institute for Biological Research (IIBR) no. 1 cell line from the peripheral blood of a healthy individual. Cells were maintained and grown in RPMI 1640 medium supplemented with 5% (*v/v*) heat inactivated fetal cow serum plus 100 U/ml of sodium-G-penicillin and 100 μg/ml of dihydrostreptomycin. All Poloxamer solutions were made in deionized water and were sterilized. All Poloxamers were tested at concentrations of 0.5%, 0.10%, and 0.20% on both cell lines and the growth of each line was compared to that on basal medium.

In the RPMI 8432 line, the addition of 0.05% or 0.1% Poloxamer increased growth, compared to that on basal medium. With 0.2% Poloxamer, growth was similar or significantly lower than that on basal medium. In the IIBR 1 cell line, only Poloxamers 188 and 238, at concentrations of 0.05% or 0.1%, increased cell counts above those in the basal medium. At 0.2%, all Poloxamers caused poor growth as well as at 0.05% of Poloxamers 217 and 338. No detrimental effects on cell growth or cell morphology were noted. The authors hypothesized that cell growth effects of different Poloxamers may be cell line specific (Mizrahi 1975).

Lane and Krukonis (1988) tested the cytotoxicity of Poloxamer 188 in human and animal cells. Five different lots of Poloxamer 188 were used and a portion of four lots was fractionated using supercritical fluid fractionation (SFF). SFF utilizes gasses, such as carbon dioxide, to dissolve the parent compound under pressure. This is followed by precipitation and recovery of the dissolved material. Early fractions of SFF extraction (XT) as well as the residual partially purified (RES) material was tested. All lots and fractions of each were dissolved in minimum essential culture medium (MEM) with 10% calf serum and sterilized with 0.2-micron filters.

HeLa cells and B16 mouse melanoma cells were suspended with 0.5–1 $\times$ 10^5 cells/ml in MEM with or without Poloxamer 188 or its SFF fractions at concentrations ranging from 0 to 50 mg/ml. Cells were grown for 4 days, harvested, and counted. Poloxamer 188 displayed a dose-dependent inhibition of cell growth in both cell lines. A concentration of 30 $\pm$ 10 mg/ml of Poloxamer 188 was required to inhibit 50% of HeLa cell growth, whereas only 2 to 5 mg/ml was required to inhibit 50% of B16 growth. Testing with the unfractionated Poloxamer 188, XT (16.9 $\pm$ 3.9% of the parent compound), and RES involved testing each part at a concentration of 20 mg/ml.

The XT fractions were 77 $\pm$ 25% more toxic than the parent compound, whereas the RES fractions were 30 $\pm$ 11% less toxic. Fractionation of Poloxamer 188 significantly decreased Poloxamer-induced inhibition of granulocyte chemotaxis. The authors conclude that Poloxamer 188 causes a dose-dependent toxicity to human and animal cell growth but SFF separates out a highly toxic fraction of the parent compound and removal of this fraction significantly decreases the cytotoxicity and chemotaxis-inhibiting properties of Poloxamer 188 (Lane and Krukonis 1988).

Clarke and McNeil (1992) tested the cytotoxicity of 2% (*w*/*v*) aqueous Poloxamer 188 on bovine aortic endothelial cells (BAECs). BAECs were suspended in culture medium and seeded at 10,000 cells/well in 24-well plates and were allowed to attach overnight. The medium was removed and replaced with 1 ml of fresh medium with or without 2% Poloxamer 188. The number of cells was counted at 0, 1, 2, and 3 days. Poloxamer 188 did not cause a significant change in the growth rate of BAECs, as compared to those grown in medium, alone.

Kier et al. (1995) measured the cytotoxicity of Poloxamer 101 in a Chinese hamster ovary cell line (AS52), a rat lung epithelial cell line (LEC), and in freshly isolated rat alveolar macrophages (RAM) in vitro. Concentrations of 10, 20, 100, 200, 1000, 2000, 10000, and 20000 μg/ml Poloxamer 101 were incubated with cells for 24 h at 37°C in a humidified 5% CO_2 atmosphere. Lactate dehydrogenase (LDH) release was determined and employed as a measure of cytotoxicity. The no observed effect level (NOEL) found in the LEC, RAM, and AS52 cell lines was 2000, 200, and 2000 μg/ml Poloxamer 101, respectively. The estimated half maximal response (EC_{50}), the estimated concentration to give half-maximal LDH release, for the LEC, RAM, and AS52 was 4000, 3000, and 4000 μg/ml Poloxamer, respectively.

Hypercholesterolemia and Hypertriglyceridemia

Poloxamer 407

Wout et al. (1992) demonstrated that Poloxamer 407 causes hypercholesterolemia and hypertriglyceridemia in male Sprague-Dawley rats. Rats were divided into five dose groups of at least three rats in each. The concentrations tested included 0.1% (1 mg), 1.5% (15 mg), 7.0% (70 mg), 15.0% (150 mg), and 30.0% (300 mg) Poloxamer 407 in deionized water. Rats were injected intraperitoneally and blood samples were taken 25 h after administration. Triglyceride (TRIG) and cholesterol (CHOL) levels were determined. Plasma concentrations of both TRIG and CHOL were significantly elevated in a dose-dependent manner. Triglyceride levels showed a sharp increase with the 15% and 30% concentrations, whereas cholesterol levels increased significantly with the 7%, 15%, and 30% concentrations. The authors hypothesized, based on extrapolation, that cholesterol concentration levels would plateau at approximately 550 mg/dl at doses greater than or equal to 150 mg.

Further studies investigated the influence of food and i.p. versus intramuscular (i.m.) administration of 30% Poloxamer 407 on the levels of triglycerides and cholesterol after a single injection. Rats were divided into five groups, four rats in each. The first two groups served as controls; one group was fasted for 48 h and the other fed. The third and fourth groups were fasted for 48 h but one group received an i.p. injection of Poloxamer 407, whereas the other received an i.m. injection. Group five was fed and intraperitoneally injected with Poloxamer solution. Blood was collected 24 and 48 h after injection, as well as 96 h later for the rats in group five. The fasted and fed control groups had similarly low cholesterol levels. However, rats injected with either i.p. or i.m. injections of Poloxamer 407 had increased cholesterol levels at 24 and 48 h. These levels decreased in the fasted i.m. group and the fasted i.p. group, although the fasted i.p. group had significantly higher levels than the fasted i.m. group at 24 and 48 h. The fed i.p. group, however, had the highest levels 24, 48, and 96 h after injection, suggesting that food intake helped to sustain hypercholesterolemia and hypertriglyceridemia.

In the last series of studies, animals were divided into three groups of five rats in each, one of which served as a control group and received neither lovastatin nor Poloxamer 407. The second group was orally dosed with 75 mg/kg/day of lovastatin on 3 consecutive days and was injected i.p. with a 30% Poloxamer 407 solution on the second day, an hour before the oral dose of lovastatin. The third group was given no lovastatin, only an i.p. injection of 30% Poloxamer 407 on the second day, at the same time as those rats of group two. Blood was collected from each rat at 24, 48, and 96 h after the i.p. injection. Levels of both CHOL and TRIG in the plasma of rats given lovastatin and Poloxamer 407 were very close to those of control rats at 24, 48, and 96 h. However, those rats injected with Poloxamer and not given lovastatin developed hypercholesterolemia and hypertriglyceridemia, signifying that lovastatin did reduce high CHOL and TRIG concentrations caused by Poloxamer 407 (Wout et al. 1992).

Johnston and Palmer (1993) carried out a series of tests on Male Wistar rats (300 to 400 g) to determine the mechanism for Poloxamer 407–induced hypertriglyceridemia. In the first study, rats (number not specified) were fasted overnight and given a 1 ml i.p. injection of 300 mg of 30% (*w*/*w*) Poloxamer 407 solution the following morning. Blood samples were collected from each animal immediately before the injection and 1, 2, 4, 6, 8, 12, and 24 h after. Poloxamer 407 caused an increase from a control value of 84 $\pm$ 10 to 3175 $\pm$ 322 mg/dl plasma TRIG concentration over the 24-h period. The greatest accumulation was observed between hours 2 and 4 at a rate of 5.74 mg/dl/min. In an extension of this study, a control group was administered a saline injection at the same time as the treatment group received Poloxamer 407. Blood was collected at 3, 6, 12, and 24 h following injection. Hyperlipidemic rats in the control and test groups were anesthetized and intravenously injected with 0.3 ml of 1000 IU heparin. Two minutes later, blood samples were taken and plasma was analyzed for lipoprotein lipase (LPL) activity.

Three hours after administration of Poloxamer 407, LPL activity was reduced by 95%, compared to that in control rats. Inhibition remained greater than 90% for treated rats throughout the 24-h period.

Another group of fasted rats was injected with 300 mg of Poloxamer 407 and, 24 h later, was anesthetized with 50 mg/kg sodium pentobarbital i.p. Various tissues were removed, weighed, buffered, homogenized, and assayed for LPL activity.

LPL activity was increased in homogenates of skeletal and cardiac muscle, but no change was seen in adipose, testes, or lung tissue. A 37%, 69%, and 66% increase in activity was seen in the myocardium, soleus, and gastrocnemius muscle, respectively, of the treated rats.

To determine the in vitro effect of Poloxamer 407 on LPL, two male rats were anesthetized with ether, injected i.v. with 100 IU of heparin, and blood samples were taken. Plasma was pooled and 5 μl of plasma was incubated with 95 μl of saline with Poloxamer 407 at concentrations ranging from 50 to 500 μM. Samples were incubated for 1 h and LPL levels were compared to those of control (saline alone) samples.

Poloxamer 407 caused a dose-dependent decrease in LPL activity. Fifty percent and 100% enzyme activity was inhibited at 24 and 350 μM (or above), respectively. In analyzing the results of the entire series of studies, the authors suggest that Poloxamer 407 inhibits heparin-releasable LPL, which causes a reduced rate of TRIG hydrolyzation and, thus, an increase in circulating TRIG (Johnston and Palmer 1993).

Nash et al. (1996) tested four groups of five male Wistar rats (300–350 g) to assess the mechanism by which Poloxamer 407 induces hyperlipidemia. Half of the animals (P) received i.p. an injection of 1 ml of 30% (*w*/*w*) Poloxamer 407 (300 mg/ml) in distilled water on the first day, while the other half (SAL) received 1 ml of saline as a control. Then, half of the animals in each group received an i.p. dose of 100 mg/kg nicotinic acid (PNA and NA). The remaining animals received an equal volume of saline (PSAL and SAL). Blood samples were collected before and 6 h after injection of Poloxamer or saline. Blood was also collected at 24, 48, 72, and 96 h, with subsequent administration of nicotinic acid or control. A final blood sample was taken at 120 h.

Poloxamer 407, without nicotinic acid treatment, caused a rapid and significant increase in triacylglycerol levels, which decreased towards normal at 120 h. Although hyperlipidemia was produced with hypertriglyceridemia, increasing from 53.4 $\pm$ 7.0 mg/dl at time 0 to 4029 $\pm$ 42.1 mg/dl by 24 h, the authors did not report any adverse side effects. The triacylglycerol concentration in the NA group was 1.5 times higher than that of the SAL group at both 72 and 96 h. The addition of nicotinic acid significantly attenuated the hypertriglyceridemia (HTG) response at 24 h; however, the average triglyceride concentration increased by 2.8 times from 72 to 120 h. Poloxamer 407 also causes hypercholesterolemia (HCHO), causing an increase from 47.5 $\pm$ 1.8 to 468.5 $\pm$ 27.9 mg/dl by 48 h. Nicotinic acid significantly reduced the cholesterol concentration from 364.4 $\pm$ 16.1 to 276.8 $\pm$ 16.4 mg/dl at 24 h. The authors suggest that Poloxamer 407 may stimulate the release of free fatty acids from the adipocyte for at least 24 h after injection since nicotinic acid reduced both plasma triacylglycerol and cholesterol concentrations at 24 h (Nash et al. 1996).

Johnston and Palmer (1997) carried out a series of experiments with Poloxamer 407 administration in male Sprague-Dawley rats (225 to 250 g). To determine the effect of Poloxamer 407 on plasma cholesterol, six rats were fasted overnight and injected with 1 ml of Poloxamer 407 solution (30% *w*/*w* in double-deionized water). At 0, 1, 2, 4, 6, 8, 12, and 24 h after injection, a 1-ml blood sample was analyzed.

Plasma cholesterol concentration was significantly ($p < .05$) elevated only 1 h after injection, as compared to controls administered normal saline. The plasma CHOL concentration reached 449 $\pm$ 57 mg/dl 24 h after administration, with the fastest rate accumulation from 1 to 12 h and the maximum concentration of

663 ± 91 mg/dl at 48 h. CHOL levels returned to normal about 96 h after injection.

In an *i*n vivo investigation of the effect of 0 to 5 mM Poloxamer 407 on hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase, 42 rats were given i.p. injections of 1 ml of 30% (*w*/*w*) Poloxamer 407 solution in deionized water at 2, 4, 6, 15, 24, 48, and 72 h before animals were killed. An additional 42 rats served as a control group and were administered normal saline at the same times. Livers were excised and homogenized. HMG-CoA reductase activity of the microsomal homogenates was assayed.

Poloxamer 407 did not significantly affect the activity of HMG-CoA reductase in vitro, as compared to values from the control group.

Forty-four rats were injected with either saline or a 30% (*w*/*w*) Poloxamer 407 solution in deionized water at 2, 4, 8, 15, 24, or 48 h before death. Rats were killed, hepatic tissue was obtained, and liver specimens were homogenized. Cholesterol levels were determined.

Compared to tissue from control rats, the CHOL concentration in the hepatic tissue of test rats was significantly higher at 2 and 4 h after Poloxamer 407 injection but was significantly reduced 15 h after. Hepatic CHOL content returned to normal control levels about 24 h after injection of Poloxamer 407 (Johnston and Palmer 1997).

Palmer et al. (1998) investigated whether Poloxamer 407 administration would cause atherogenic arterial lesions in mice due to its ability to produce hyperlipidemia in rats. Female C57BL/6 mice (17 g) were administered i.p. injections of varying doses (0.25 to 1.5 g/kg) of Poloxamer 407 in 0.5 ml saline to determine the dose of Poloxamer that would produce hyperlipidemia in mice. Blood was taken at various times following injection and analyzed.

Poloxamer 407 caused a dose-dependent increase in TRIG concentration at doses between 0.25 and 1.0 g/kg; the 1.5 g/kg dose caused TRIG levels similar to those caused by 0.5 g/kg Poloxamer. Plasma cholesterol levels increased dose-dependently at the 0.25 and 0.5 g/kg doses of Poloxamer; however, a successive decrease was seen in cholesterol levels with further increasing doses of Poloxamer. Hypercholesterolemia in mice peaked at 24 h but returned to control levels 96 h after treatment.

Chronic studies were then carried out on four groups of mice, two groups of which ate control chow, one group ate control chow containing cholic acid, and the fourth ate chow containing cholic acid and CHOL. The groups were treated as follows: C, control chow and i.p. injection with 0.5 ml saline every third day; P, control chow and 0.5 ml i.p. injection of 0.5 g/kg Poloxamer 407 every third day; PC, cholic acid chow and 0.5 ml i.p. injection of 0.5 g/kg Poloxamer 407 every third day; HF, CHOL diet without injections. Mice on the CHOL diet served as a positive control. At days 90, 145, 200, and 300 of treatment, blood samples were obtained and hearts were harvested at each time point while livers were removed at the 145- and 300-day sampling time points.

Mice in the C group maintained control levels of plasma CHOL (<100 mg/dl) and triglyceride throughout the study. The HF (positive control) group displayed CHOL levels that plateaued at about 225 mg/dl. CHOL concentrations of the P and PC mice were 600 and 1000 to 1500 mg/dl, respectively. No lesion formations were found in any of the C mice, but lesions were present in the other three groups by day 90. The lesions grew as the treatment continued, with the largest lesions found in the PC mice (Palmer et al. 1998).

ANIMAL TOXICOLOGY

Acute Toxicity

Multiple Poloxamers

Leaf (1967) investigated the acute toxicity of Poloxamers 124, 182, 188, and 235 in albino rats (no further details about animals provided). Single doses of the Poloxamers were administered by intubation. Poloxamers 124 and 182 were administered as neat liquids at doses ranging from 1 to 15 g/kg. Poloxamer 188 was dissolved in water or corn oil (not specified) and administered as a 50% (*w*/*v*) solution at a dose range of 2 to 15 g/kg. Poloxamer 235 was given as a 25% (*w*/*v*) solution in corn oil at a dose range of 10.2 to 34.6 g/kg. Animals were observed for 24 h before and for 7 days after dosing; any animals that died were necropsied.

At the lowest doses (not specified), the animals were asymptomatic but as dosage increased, the rats exhibited mild sedation, which increased in severity with time. In the animals that died, severe respiratory depression was observed and pulmonary edema with rales was a common sign. Necropsy revealed marked engorgement of the lungs, stomach distention, and massive vascular dilation of the intestines.

The oral LD_{50} values for Poloxamers 124, 182, 188, and 235 were determined as 5, 5.5, >15, and >34.6 g/kg, respectively. The author offered no further details (Leaf 1967).

Johnston and Miller (1985) investigated the affect of Poloxamers 238, 335, 403, and 407 CPK levels in the blood after intramuscular injection. All Poloxamers were tested at 25% (*w*/*w*) concentration in 0.9% (*w*/*v*) NaCl. Male New Zealand white rabbits (2.4 to 4.4 kg) were tested in single and multiple dose studies; in all studies, injections were made into the vastus lateralis muscle in the hind leg. The single dose studies involved injection of 1 ml of Poloxamer and blood samples were taken before the injection, as well as at regular intervals up to 70 h post injection.

Blood samples were analyzed for CPK and at the end of the study; all animals were killed. Poloxamers 335 and 403 caused maximum CPK concentrations of 12200 and 24100 U/L, respectively, compared to normal saline levels of 2620 U/L. These levels of CPK produced marked muscle damage. In contrast, Poloxamers 238 and 407 caused CPK levels comparable to control levels.

In the multiple dose studies, rabbits received 1 ml injections of Poloxamer into each hind leg at 24-h intervals for 5 days.

The Poloxamers were randomly assigned so animals did not necessarily receive an injection of the same Poloxamer in both legs. Animals were all killed 24 h after the last injection and the muscle masses were excised for examination.

These studies produced similar results to the single dose studies. Both Poloxamers 335 and 403 caused significantly higher CPK levels compared to rabbits injected with saline alone. Poloxamers 238 and 407 caused CPK levels similar to those of control rabbits. The authors determined that high CPK levels in the blood, which are indicative of muscular toxicity, were proportional to the lipophilicity of the Poloxamers. The more lipophilic the Poloxamer, the greater the elevation in plasma CPK (Johnston and Miller 1985).

As part of a study in which Poloxamers 181 and 407 were tested for their efficacy as vehicles, Alakhov et al. (1999) carried out experiments to establish the toxicity of these vehicles in rats and dogs. Precise details were not provided regarding the Poloxamers; they were referred to as the "carrier."

Using rats, groups of five males and five females were administered single i.v. doses of 2, 4, 8, 10, or 15 ml/kg/min of carrier. The acute toxicity was calculated at about 12 ml/kg/min and death was caused by hypervolemia and pulmonary edema. In the subacute study, in which groups of 7 rats/sex were dosed for 14 consecutive days, doses of 0.3, 3.0, and 6.0 ml/kg/min were administered. Five rats/sex were killed after the test period, whereas the rest were observed for 14 more days. Pink discoloration of the blood, appetite suppression, weight gain, and increase in liver, spleen, and adrenal weight were seen at the highest dose. Congestion and/or hemorrhage, vacuolation, and histiocytosis in major organs and tissues were also seen at the high dose. At the mid-dose, rats had similar reactions but at lower intensity and incidence and reactions were reversible and no deaths occurred. The low dose caused no adverse reactions.

In an acute i.v. toxicity study using two Beagle dogs, once every third day, dogs were given increasing doses of 0.2, 0.4, 1.2, 2.4, 4.8, and 9.6 ml/kg/min of the Poloxmer. No mortality occurred but clinical signs such as vomiting, tachycardia, and reduced activity were noted at the 9.6 ml/kg/min dose. Levels up to 4.8 ml/kg/min caused reddening of the gums but necropsy analysis revealed no other adverse changes in treated animals. The authors thus established the acute toxicity at 9.6 ml/kg/min (Alakhov et al. 1999).

Poloxamer 188

Magnusson et al. (1986) evaluated the toxicity of Poloxamer 188 administered to rats i.v. for one month in daily doses of 0, 10, 20, 50, 100, 200, 500, and 1000 mg/kg. Poloxamer 188 induced pulmonary foam cells at the dose levels of 500 and 1000 mg/kg and slight focal degenerative changes in the proximal tubules of the kidneys at the dose levels of 100, 200, 500, and 1000 mg/kg.

Bentley et al. (1989) tested purified and unpurified 4% Poloxamer 188 solution in male and female Wistar rats (150 to 330 g). Rats were separated into four groups of 24: group I, saline controls; group II, unpurified Poloxamer; group III, Poloxamer purified by silica only; and group IV, Poloxamer purified by silica and amberlite. All animals were anesthetized and injected i.v. with 10 ml/kg body weight test solution or saline. Animals were killed at either 24 or 72 h or 7 days after injection.

At 24 h, liver weights of rats administered unpurified Poloxamer 188 solution were up to 14% greater than the weights of rats given saline or either of the purified solutions. Spleen weights were comparable between groups. After 48 h, tissue and organ weights were similar between all groups. After 7 days, the spleen weights of female rats given unpurified Poloxamer solution were significantly increased ($p < .01$) compared to those of controls; however, all other tissue and organ weights were comparable (Bentley et al. 1989).

Poloxamer 407

Li et al. (1998) tested the acute toxicity of a solution of 25% Poloxamer 407 (*w*/*w*) in potassium dihydrogen phosphate and PBS. Eighty male Kunming mice (20 ± 2 g) were divided into four even groups and administered an i.p. injection of either 0.2 ml/mouse PBS or Poloxamer solution at a dose of 0.4, 0.2, or 0.1 ml/mouse.

No animals manifested any significant twitch reactions. Ten of the 20 mice given 0.4 ml/mouse (5.0 g/kg) Poloxamer 407 died within 8 weeks, thus signifying an LD_{50} at this dose; however, no mice died in any of the other groups.

In a second study, 54 Kunming mice were divided into three groups of 18 and administered an i.p. injection of either 0.2 ml/mouse PBS or 25% Poloxamer 407 solution at a dose of 0.2 or 0.1 ml/mouse. Six mice from each group were assessed on days 3, 7, and 14 following injection. Blood was collected from the eyeballs and serum was isolated, frozen, and analyzed for levels of ALT, blood urea nitrogen (BUN), CHOL, and TRIG.

Blood ALT levels among all groups were similar throughout the trial period. BUN levels were slightly lower in test groups and were 8.8 ± 5.4 and 11.7 ± 1.3 mmol/L at day 14, compared to the control value of 13.4 ± 2.9 mmol/L. At 3 days after injection, CHOL and TRIG levels in test mice were significantly lower than those of the control group. The CHOL levels for the control, 0.1, and 0.2 ml test groups were 4.7 ± 0.8, 14.4 ± 4.1, and 12.9 ± 1.8 mmol/L, respectively, at day 3. The TRIG levels for the control, 0.1, and 0.2 ml test groups were 1.2 ± 0.1, 5.1 ± 1.6, and 5.0 ± 0.6 mmol/L, respectively. By day 7, CHOL and TRIG levels among groups were similar except TRIG levels were still slightly higher in both test groups (Li et al. 1998).

In a dose-dependency study in rabbits by Blonder et al. (1999), the authors suggested that, although side effects were observed with high doses of Poloxamer 407, single injections of lower doses (≤27.5 mg/kg) neither induced hyperlipidemia nor altered other blood chemistries.

Short-Term Toxicity

Multiple Poloxamers

In a study by Leaf (1967), rats were divided into groups of 20 (10 males and 10 females). Test animals were fed increasing concentrations of Poloxamer 235 or 338 by intubation. Poloxamer 182 was administered to a larger group than the others and was given as a steady dose of 1 g/kg for 63 days. Poloxamer 235 was given at 1%, 5%, and 25% on days 1 to 7, 8 to 14, and 15 to 24, respectively. Poloxamer 338 was administered at 5%, 10%, 20%, and 30% on days 1 to 7, 8 to 14, 15 to 21, and 22 to 42, respectively.

Mortality for Poloxamers 182, 235, and 338 was 51%, 20%, and 15%, respectively, for the entire trial period. The authors stated that the cause of death in all rats was severe inanition. Results of further examination, including hematology and urine analysis, as well as gross and microscopic pathology were comparable to control animals. Female liver weights were slightly increased in groups fed Poloxamers 182 and 235, whereas male adrenal glands were slightly enlarged in the Poloxamer 338 group.

This author also intravenously administered 0.5 g/kg/day of Poloxamer 188 for 5 days a week for 7 weeks. No adverse reactions were observed during the test period; blood and urine analyses appeared normal. Necropsy at the end of the test period showed no significant effects. No further details were provided for either study (Leaf 1967).

Comai and Sullivan (1980) examined Poloxamers 188 and 331 for their antiobesity activity. Two weeks before experimentation, female Charles River CD strain rats (180 to 200 g) were fasted for 24 h and then trained to consume a high-fat control diet within 2 h. Rats were divided into groups of 10 animals and fed either the control high-fat diet, or diet with the same caloric density as the control diet but with Poloxamer 331 (1% or 3%) or Poloxamer 188 (3%), which was substituted for the cellulose component of the diet. Rats were fed their respective diet for 2 h daily for 42 days. Fecal-fat elimination and dietary fat absorption were determined throughout the study. On the final day, rats were killed and blood samples, as well as liver and adipose tissues, were taken.

A dose-dependent increase in fecal-fat elimination was observed in the groups given a diet with 1% and 3% Poloxamer 331. Fecal-fat elimination was increased by 50 and 125 mg/day compared to control for the 1% and 3% diet, respectively. The Poloxamer 331–treated rats also had decreased dietary fat absorption by 3.4% and 9.8% for the 1% and 3% diets, respectively. Poloxamer 188 caused no significant increases in fecal-fat elimination or dietary fat absorption. Rats in all four groups consumed similar amounts of food, but rats fed Poloxamer 331 gained significantly less weight than those in the other groups even though they all consumed the same amount of calories.

The Poloxamer 331–fed rats also had a decreased percentage of carcass fat as compared to controls but similar percentages of carcass protein. Liver wet weights were decreased significantly only with the 3% Poloxamer 331 diet. Serum levels of cholesterol, triglyceride, and glucose were similar across groups and no signs of toxicity were observed (Comai and Sullivan 1980).

In a subacute study by Alakhov et al. (1999), three male and three female Beagle dogs were injected with 0.3, 1.0, or 2.0 ml/kg of Poloxamers 181 and 407 (details were not provided regarding the Poloxamers; they were referred to as the "carrier") for 14 consecutive days. Animals were killed and examined at the end of the experimental period, except for one that was observed for 14 additional days.

No clinical signs were noted in the treated groups but the high dose did cause pink discoloration of the blood and reversible increases in protein, AST, ALT, bilirubin, and glucose levels. No abnormalities were found at necropsy. The mid and high doses caused liver weight increases and clear histological cell changes, as well as Kupffer cell pigmentation. The low dose only caused minor clear cell changes in the liver (Alakhov et al. 1999).

Poloxamer 101

A 2-week inhalation study on the effect of Poloxamer 101 on rats was carried out by Ulrich et al. (1992). The male Sprague-Dawley albino rats (271 to 306 g) were 8 weeks old and sorted into groups of 10. The negative control group was exposed to clean air, the positive control group to 55 mg/m^3 of UCON® 50-HB–5100 (an ethylene oxide/propylene oxide copolymer), and the test group to 97 mg/m^3 of Poloxamer 101. The rats underwent whole-body liquid droplet aerosol exposures for 6 h/day for 5 days a week over a 2-week period, after which five rats from each group were killed and examined, whereas the remaining rats were observed for a 2-week recovery period and then killed. The particle size distribution was not given.

After three exposures, 9 out of 10 rats in the positive control group died, whereas the remaining rat in this group was killed at the end of the first week. All rats were necropsied and congestion, consolidation, and red discoloration of the lungs was noted. In comparison, the group treated with Poloxamer 101 survived the full 10 exposures and showed no signs of toxicity. Body weights, organ weights, hematological evaluations, pharmacotoxic signs, as well as macroscopic and microscopic evaluations after necropsy were comparable to those of the negative-control group. The only adverse effect observed was slight alveolitis after 2 weeks of exposure, which subsided by the end of the 2-week postinhalation observation period (Ulrich et al. 1992).

Poloxamer 108

Port et al. (1978) divided 80 male Sprague-Dawley rats (142 to 232 g) into five groups and injected into the tail vein with solutions of Poloxamer 108 in NaCl (group I received 0.9% NaCl solution, alone. Groups II, III, IV, and V received doses of 4, 2, 1, or 0.150 g/kg Poloxamer 108, respectively. Rats were administered solutions at 3 ml/min at a concentration of 40 ml/kg body weight daily, 5 days a week for 2 weeks. Body weights were monitored before and during the experimental period. Four rats from each group were killed at 15 min and 24 h after the 1st,

TABLE 6
Exposure/dose information for short-term dermal toxicity study of Poloxamer 184 (CTFA 2004c).

Group no.	No. of rabbits and sex	Test material	Dose volume (ml/kg)	Dose (mg/kg)
1	F = 5; M = 3	Ethanol (control) 50%	2.0	—
2	F = 4; M = 4	Poloxamer 184 (50%) in ethanol	2.0	1000
3	F = 4; M = 4	Poloxamer 184 (15%) in ethanol	2.0	300
4	F = 4; M = 4	Poloxamer 184 (5%) in ethanol	2.0	100

3rd, 5th, 7th, and 10th injections as well as at 3, 7, 10, and 14 days after the last injection. At each of these time periods, blood and urine samples were collected. Tissue and organ samples were collected after necropsy.

There was no statistical difference between body weights of the control group and any of the dosage groups and, overall, there were few signs of toxicity. Lesions were found in the liver, lungs, and kidneys of rats receiving 1, 2, and 4 g/kg but not in those receiving 0.15 g/kg Poloxamer. Diffuse hepatocellular vacuolization was found 15 min after the first injection of the larger doses; vacuolization was dose dependent and increased as more injections were given. Tubular dilatation occurred in the kidneys of rats administered 4 g/kg at 15 min after the first injection and persisted throughout the treatment period.

Dose-dependent vacuolization of epithelial cells in the proximal convoluted tubules was found in all rats given doses of 1, 2, and 4 g/kg. Vacuolization appeared to decrease in the hepatocytes and epithelial cells over the 14-day postinjection observation period. Electron microscopy indicated that the vacuoles were most likely distended lysosomes. The authors suggest that Poloxamer 108 is rapidly phagocytized and is well tolerated in large doses (Port et al. 1978).

Poloxamer 184

CTFA (2004c) reported results of a 4-week subacute dermal toxicity study using 32 New Zealand strain rabbits. Rabbits were treated five days a week for 4 consecutive weeks with Poloxamer 184 at exposure levels of 5%, 15%, and 50% to yield the dose levels shown in Table 6. An untreated control group received only the ethanol vehicle. The hair was clipped off the back of each animal. The area of exposure was abraded at weekly intervals during exposure in four of the animals of each group; the skin of the remaining four animals was not abraded. The cut areas of skin were significantly deep enough to penetrate the stratum corneum, but not to disturb the dermis or cause bleeding. The test material was applied by gentle inunction to the skin of each rabbit daily five days a week for a total of 20 applications. Observations were made daily for any signs of dermal irritation and systemic toxicity.

The authors determined survival, body weight, general appearance and behavior; hematology, including erythrocryte count, hemoglobin, hematocrit, total and differential white blood cell count; blood chemistry, including blood urea nitrogen, blood glucose, serum alkaline phosphatase, and serum glutamic pyruvic transaminase activity; absolute and relative organ weights; and gross and histopathologic evaluation, as shown in Table 7.

The authors concluded that Poloxamer 184 produced skin changes characterized grossly by slight erythema.

TABLE 7
Short-term dermal toxicity of Poloxamer 184 in New Zealand rabbits (CTFA 2004c).

	Group no.			
Parameter	1	2	3	4
Dose (mg/kg)	0	1000	300	100
Survival (%)	100	75	100	62.5
Body weight gain (%)	12.5	12.3	24.6	35.6[a]
Hematology				
Hematocrit (%)	34.9	36.7	35.2	36.3
Hemoglobin (g%)	13.1	13.6	13.3	13.5
RBC ($\times 10^6$/cc)	5.98	5.94	6.15	6.35
WBC ($\times 10^3$/cc)	8.55	8.49	7.65	6.81
Clot time (s)	242	211	204	172
Neutrophil/lymphocyte ratio	39/59	34/62	31/65	40/57
Blood Chemistry				
Glucose (mg%)	136	122	112	121
BUN (mg%)	38	26	24	19
SGPT (I.U.)	32	55[a]	40	60[a]
SAP (K-A units)	13	16	15	15
Organ weights (g)				
Liver	124.60	153.17	143.59	120.06
Kidney	19.14	21.93	23.81	20.42
Spleen	1.42	1.43	1.40	1.32
Adrenal	0.31	0.32	0.39	0.35
Brain	9.41	9.48	9.84[a]	9.95[a]
Organ/body weight (%)				
Liver	4.55	4.40	4.78	2.19[a]
Kidney	0.71	0.57	0.80	0.41[a]
Spleen	0.05	0.04	0.05	0.03[a]
Adrenal	0.01	0.01	0.01	0.01
Brain	0.35	0.24	0.33	0.19[a]

[a]Statistically significant compared to controls at $p < 0.05$.

Histopathologically, the skin had comparable slight intradermal inflammatory responses, but no systemic effects were caused by Poloxamer 184 at the dosage employed (CTFA 2004c).

Poloxamer 407

Johnston et al. (1993) investigated the toxicity of Poloxamer 407 in male Sprague-Dawley rats (300 to 325 g). Rats were divided into three groups of five rats each. For 4 days, the first two groups received a daily i.p. injection of 1 ml/day of either 10% or 30% (*w*/*w*) Poloxamer 407 in saline, corresponding to a dose of 0.33 or 1.0 g/kg/day. The third group did not receive any injections and served as a control group. All rats were killed on the fifth day.

Compared to control animals, rats administered a daily dose of 0.33 g/kg/day of Poloxamer 407 did not have a significant ($p > .05$) increase or decrease in spleen, liver, or total body weights; however, they did have a significant ($p < .05$) increase in the percentage of monocytes (MO) in blood.

Administration of 1.0 g/kg/day of Poloxamer 407 resulted in distinct splenomegaly from red pulp expansion due to the infiltration of macrophages, which contained phagocytized lipids. These rats had a significant reduction in body weight and a significant decrease in the percentage of lymphocytes, red blood cells, hemoglobin, and hematocrit. The 1.0 g/kg/day dose group also had a significant increase in the number of white blood cells and the percentage of MO, compared to the control group. Other factors were assessed but were comparable to those found in the control group and included mean corpuscular volume, percentage of granulocytes, and liver to body weight ratio (Johnston et al. 1993).

Subchronic Toxicity

A 6-month feeding study was carried out on rats and dogs to test the effects of Poloxamer 188 administration (Leaf 1967). Groups of 45 rats were administered Poloxamer 188 at 0%, 3%, or 5% by weight in food. During the test period, 2 and 14 animals died in the 3% and 5% groups, respectively. Deaths were attributed to a combination of infection and inanition. Animals killed throughout the period for pathological examination had no adverse effects.Twelve dogs were divided into three groups and received 0, 0.05, or 0.1 g/kg Poloxamer 188 as a capsule before feeding. No differences were observed in the test and control dogs. Results of blood and urine analysis of test animals were comparable to those of control dogs. Gross and microscopic examinations were unremarkable. No further details were provided.

In a further series of studies, this author tested Poloxamers 331, 235, and 338 in 90-day feeding studies on rats and dogs. In the rat studies, groups of 50 rats (25 male and 25 female) were tested, whereas the studies with dogs tested groups of six dogs (three male and three female). Poloxamers were administered in the feed of rats and as a capsule before feeding for the dogs. Poloxamer 331 was administered at a dose of 0.04, 0.2, or 0.5 g/kg/day, whereas Poloxamer 235 was given at 0.04, 0.20, or 1.0 g/kg/day and Poloxamer 338 at 0.2, 1.0, or 5.0 g/kg/day. No deaths attributable to the Poloxamers occurred in any groups. The rats in the 5.0 g/kg group had slight transient diarrhea. No further details were provided (Leaf 1967).

Chronic Toxicity

Leaf (1967) carried out a two-year feeding study of Poloxamer 188 in rats at 0%, 3%, 5%, and 7.5% in food. Apart from rats at the two higher doses suffering from continuous moderate diarrhea, no other adverse reactions were observed. The mortality rate of the control animals was higher than that of the test groups. A small decrease in growth was seen in the rats fed 7.5% Poloxamer, but no pathological effects were observed in any of the rats. No further details were provided.

In another study, this author tested Poloxamer 182 in a 2-year feeding study with rats and dogs. Groups of 50 rats (25 male and 25 female) were tested, whereas the dogs were tested in groups of six dogs (three male and three female). Poloxamer was administered in the feed of rats and as a capsule before feeding for the dogs at a dose of 0.04, 0.2, or 0.5 g/kg/day for all animals. Deaths occurred in all groups of rats due to chronic respiratory infections unrelated to administration of Poloxamer 182. No abnormal symptoms were observed in rats. Blood and urine samples from all rat groups were comparable to controls. No gross pathological changes were noted in rats or dogs. In the 0.5 g/kg group, one male and all three female dogs died; females had frequent severe emesis prior to death. Dogs in the 0.2 g/kg group showed occasional emesis and salivation. Dogs in the 0.2 and 0.5 g/kg groups had yellow discoloration of the serum, high serum alkaline phosphatase activity, and elevated serum glutamic-pyruvic transaminase and serum glutamic-oxalacetic transaminase activities. No further details were provided (Leaf 1967).

Palmer et al. (1998) reported that the chronic administration of Poloxamer 407, injected i.p. into mice (0.5 g/kg every third day, 300 days), was shown to raise plasma cholesterol and triglyceride levels and resulted in the formation of atherosclerotic lesions regressed by administration of HMG-CoA inhibitor drugs, such as atorvastatin, which, the authors proposed, may be one way to decrease these side effects of the block copolymers.

In Vitro Toxicity

Lowe et al. (1995) tested the effects of purification of Poloxamer 188 versus commercial-grade Poloxamer on the hemolysis of blood from mice, rats, rabbits, or hamsters. Solutions of silica-purified and commercial grade Poloxamer in saline at concentrations of 4%, 6%, 8%, and 10% (*w*/*v*) were tested.

Neither commercial grade nor purified Poloxamer produced significant hemolysis in any blood at the 4% concentration. No concentration of purified or commercial Poloxamer caused significant hemolysis when incubated with mouse or hamster blood.

Incubation of rabbit blood with 10.0% commercial grade Poloxamer 188 caused 0.5% hemolysis. Slight hemolysis of rat blood occurred when treated with commercial grade Poloxamer above the 4% concentration. The greatest effect was noted in rat blood incubated with 10.0% Poloxamer, which caused 4.7 ± 1.5% mean hemolysis. With the same concentration (10.0%) of purified Poloxamer, the mean hemolysis was only 0.5 ± 0.3%. The authors suggest that purified Poloxamer may be preferable for formulations for intravascular applications (Lowe et al. 1995).

Ocular and/or Mucosal Irritation

Multiple Poloxamers

As reported by Leaf (1967), Poloxamers 124, 182, and 235 were instilled into the right eye of rabbits (details not provided), whereas the left eye served as a control. Poloxamers 124 and 182 were administered (dose volume not specified) at 100%, 75%, 50%, or 25% *w*/*v* in water. Poloxamer 235 was a paste and given as 100 mg neat. The rabbits were observed for 10 days and irritation was scored on the Draize scale. Poloxamer 124 at 25%, 50%, 75%, and 100% concentration caused initial irritation scores on day 1 of 6.5, 12.0, 20.2, and 20.4, respectively, and caused a score of 0 at days 2, 4, 8, and 10, respectively. Poloxamer 182 caused scores of 11.2, 13.8, 19.2, and 20.3 for concentrations of 25%, 50%, 75%, and 100% on day 1. Scores of 0 were obtained on days 6, 6, 9, and 10 for 25%, 50%, 75%, and 100% Poloxamer 182. Poloxamer 235, at 100%, caused a score of 4.0 on day 1 and a score of 0 by day 4.

Poloxamer 338

Kim et al. (1988) investigated a 25% *w*/*w* aqueous solution of Poloxamer 338 for potential use as an alloplastic keratorefractive material. Keratorefractive procedures involve surgical alteration of the cornea to change the refractive power and improve visual acuity; this study tested Poloxamer 338 in rabbits in hopes of its use in humans. Seventeen adult New Zealand albino rabbits were examined to determine corneal curvature and thickness. Surgery was performed involving dissection of a circular pocket within the axial cornea stroma of both eyes of the rabbits. Sterilized Poloxamer (0.03 ml) was injected into the prepared bed of one cornea whereas the other was left untreated to serve as a control. The wound was closed with sutures that were removed 3 to 4 weeks later. Three rabbits were killed at 1 week, as well as at 1, 2, and 3 months postoperatively to study the cornea under light and electron microscopy.

Both control and Poloxamer-treated eyes had evidence of conjunctival hyperemia, extensive cornea vascularization, and small epithelial defects at the onset. All eyes had an initial increase in cornea thickness, which peaked at 1 week but were normal at the end of 3 weeks. Corneal scarring was visible at the incision site and numerous punctate stromal opacities were found within the implant bed. Scarring and inflammation were resolved at the end of 3 months.

Scanning electron microscopy revealed little difference between treated and control eyes except for a decrease in dark young epithelial cells in treated eyes. The authors reported that, despite initial scarring and other issues, the Poloxamer was well tolerated by the end of 3 months and may be of use in keratorefractive surgery. They did not find a well-defined disc of material upon examination of treated eyes and suggest that they did not achieve adequate gelation of the material and the Poloxamer may have been phagocytized by keratocytes or resorbed throughout the stroma (Kim et al. 1988).

Poloxamer 407

Davidorf et al. (1990) evaluated pluronic polyol F-127 (Poloxamer 407) as a vitreous substitute and an intraocular drug delivery system. Commercial grade Poloxamer 407 was prepared in a 20% solution by the cold process for injection into the vitreous cavity after vitrectomy. Poloxamer 407 was slowly added to distilled-deionized water (5°C to 10°C) while constant agitation was maintained with a magnetic stirrer. The clear, viscous solution was refrigerated at 5°C until injection. The vitrectomy was performed on 18 New Zealand rabbits under sterile technique and general anesthesia. A three-port vitrectomy technique was used with an infusion line of balanced salt solution (BSS) in one 20-gauge sclerotomy incision. Poloxamer 407(20%) was injected into the vitreous cavity in nine eyes folowing total vitrectomy. In the control group, vitrectomies were performed in a similar manner, excluding the use of Poloxamer 407. Neosporin® ointment was applied topically at the end of the procedure.

The vitrectomized eyes were evaluated for 2 weeks with serial ophthalmoscopy/fundus photography, electroretinography (ERG), and tonometry. Indirect ophthalmoscopy and fundus photography were performed at postoperative hours 6, 12, and 24. Tonometry was performed after surgery on both control (BSS) and Poloxamer 407–treated eyes at postoperative hours 6, 12, and days 1, 2, 7, 8, and 10. For ERG study, the rabbits were anesthetized with 5 mg/kg xylosene. The rabbits were restrained by a stereotaxic unit within an electrically shielded, light-tight enclosure where they were allowed to dark adapt under deep red illumination for 15 min.

Retinal function was decreased following injection of very high concentrations of Poloxamer 407 (15% to 20%) into the vitreous cavity of experimental animals. There was a slight clinical difference between the eyes containing Poloxamer 407 and the control eyes. Both groups showed mild postoperative inflammation, with no differences in intraocular pressures. Histopathologic findings for the control group showed no significant retinal alteration, and serial ERG findings were within normal limits. The eyes containing Poloxamer 407 showed significant destruction of the retina by 2 weeks after surgery. The ERG amplitudes decreased dramatically to a flat tracing by 24 h after surgery. The authors concluded that Poloxamer 407 is not safe for human use in this application at the concentration used, although it is appealing as a potential vitreous substitute.

The authors also suggested that Pluronic® polyol F-127 has a number of characteristics that suggest it could be used as an intravitreal drug delivery system. It is visibly clear, at cool temperatures (<5°C) it is a liquid, and at body temperatures it forms a gel. It is water soluble, but due to its molecular weight, it is slowly absorbed from the vitreous cavity. It has been shown to slowly release drugs merged in the polymer gel and is considered to be nontoxic; therefore, it is essentially an inert ingredient. Pluronic® polyols are a family of high-molecular-weight block polymers consisting of polyoxypropylene-polyoxyethylene-polyoxypropylene moieties. Poloxamer 407 (molecular weight [MW] 11,500 Da) is one of this family of polymers (Davidorf et al. 1990).

Poloxamer 182 Dibenzoate

The Consumer Product Testing Company (1990) reported on primary ocular irritation of Poloxamer 182 Dibenzoate in rabbits. Six New Zealand white rabbits, each weighing about 2 kg and about 3 months old, were used in this study. The sex of the rabbits was not specified. Each received a single intraocular application of 0.1 ml of Poloxamer 182 Dibenzoate in one eye. The opposite eye remained untreated and served as a control. The eyes of all the animals remained unwashed for 24 h. Observations of corneal opacity, iritis, and conjunctivitis were recorded 24, 48, and 72 h following treatment, and at four and seven days if irritation persisted. The test article was used as received. The Average Draize Scores were 1.0, 0.3, and 0.0 at 24, 48, and 72 h, respectively, for Poloxamer 182 Dibenzoate.

Poloxamer 105 Benzoate

A study was conducted using Poloxamer 105 Benzoate by the Consumer Product Testing Company (1992), similar to that described above. Six New Zealand white rabbits, each weighing about 2 kg and about 3 months old, were used in this study. The sex of the rabbits was not referenced. A dose of 0.1 ml of the test material was placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test article was dropped. The eyelids were gently held together for 1 second. The opposite eye served as a control and did not undergo treatment. The remaining Poloxamer 105 Benzoate was washed out with distilled water after the 24-h reading. Observations of ocular irritation were recorded at 24, 48, and 72 h following administration of the Poloxamer 105 Benzoate. If irritation persisted, additional readings were made at 4 and 7 days following application. The Average Draize Scores were 3.7, 1.7, and 0.0 at 24, 48, and 72 h, respectively, for Poloxamer 105 Benzoate.

Dermal Irritation

Leaf (1967) tested the sensitization of Poloxamer 188 on dogs, rabbits, and guinea pigs (no details provided). Animals were clipped of hair over the test area and the product was applied, in paste form, every other day for a total of 10 applications. No animals showed any irritation. After 14 days of rest, a challenge patch was applied. There was no evidence of skin sensitization. Some tissue sections were removed from the test rabbits and microscopically examined; the epidermis appeared normal. No further details were provided.

CTFA (2004b) provided the results of a single insult patch test of Poloxamer 184 in six rabbits, completed in 1974. The primary irritation index (PII) was determined to be 0.37.

The Consumer Product Testing Company (1990) reported a primary dermal irritation study in six New Zealand white rabbits given a single dermal application of 0.5 ml of Poloxamer 182 Dibenzoate on two test sites, one abraded and one intact. The two test sites were each 2.5 cm^2 and were chosen on opposite sides of the vertebral column. The rabbits weighed about 2 kg and were around 3 months old. The sex of the rabbits was not specified. The test sites were occluded for 24 h and were observed individually for erythema, edema, and other effects 24 and 72 h following application. Mean scores from the 24- and 72-h readings were averaged to determine the PII. A score of 5 or more indicates a primary dermal irritant. The test article was used as received. The PII was 1.60. The scores for each animal are given in Table 8.

The Consumer Product Testing Company (1992) conducted a similar study using Poloxamer 105 Benzoate in which 6 New Zealand white rabbits were used, each weighing about 2 kg. The rabbits were approximately 3 months of age and their sexes were unspecified. The animals were prepared by close clipping the hair of the mid-dorsal area of the trunk. Two 2.5-cm^2 test sites were chosen on opposite sides of the vertebral column. A single application of 0.5 ml of the Poloxamer 105 Benzoate was made to each test site. After treatment of the two test sites,

TABLE 8
Primary dermal irritation of Poloxamer 182 Dibenzoate in rabbits (Consumer Product Testing Company 1990).

		Hours			
		24		72	
Rabbit number	Skin	Erythema	Edema	Erythema	Edema
1	Intact	1	0	1	0
	Abraded	1	0	1	0
2	Intact	2	1	1	0
	Abraded	2	1	1	0
3	Intact	2	2	1	0
	Abraded	2	2	1	0
4	Intact	2	1	1	0
	Abraded	2	1	1	0
5	Intact	2	0	0	0
	Abraded	2	0	0	0
6	Intact	2	0	0	0
	Abraded	2	0	0	0

TABLE 9
Primary dermal irritation of Poloxamer 105 Dibenzoate in rabbits (Consumer Product Testing Company 1992).

		Hours			
		24		72	
Rabbit number	Skin	Erythema	Edema	Erythema	Edema
1	Intact	0	0	0	0
	Abraded	0	0	0	0
2	Intact	1	0	0	0
	Abraded	1	0	0	0
3	Intact	1	0	0	0
	Abraded	1	0	0	0
4	Intact	1	0	0	0
	Abraded	1	0	0	0
5	Intact	2	0	0	0
	Abraded	2	0	1	0
6	Intact	1	0	0	0
	Abraded	1	0	0	0

the entire trunk of each animal was covered in an impermeable plastic occlusive wrapping. Twenty-four hours later, the wrapping and test article were removed. The left over Poloxamer 105 Benzoate was gently washed from the skin with water and the skin was dried. Each test site was then individually examined and scored at 24 and 72 h for erythema and edema. The PII was 0.55. The results for each animal are given in Table 9.

Dermal Sensitization

CTFA (2004e) reported a study in which Poloxamer 185 was evaluated for its allergy potential. The test was conducted using a guinea pig maximization procedure. Thirty young albino female guinea pigs, weighing between 250 and 300 g, were used in this experiment. Group I consisted of 15 animals (the control group). Group II included 15 animals and served as the test group. The Poloxamer 185 vehicle, Neobee M-5, was used for the control animals. Test animals received 0.1 ml intradermal injections at six sites on the upper dorsum; two sites received 50% Freund's adjuvant only; two sites received 5% Poloxamer 185 in Neobee M-5; and two sites received Poloxamer 185 in Neobee M-5 diluted with Freund's adjuvant to a concentration of 5%.

One week after the induction injection, all of the animals were given topical boosters of their respective test or control materials. This required applying full strength Poloxamer 185 or Neobee M-5 to a 1 × 2-inch Webril dressing placed over the appropriate injection sites. The dressings were occluded and held in place for 48 h.

Two weeks later, the animals were topically challenged on prior untreated sites on the left flank with Poloxamer 185 (25% in Neobee M-5). The challenge (0.5 ml) was applied to a 1 × 1-inch Webril Pad. An occlusive wrapping was again used; contact with the skin was for 24 h. The patches were removed and the sites were scored for erythema at 24 and 48 h after patch removal. A week following the occlusive patch challenge, all animals were topically challenged on preshaven virgin skin sites located on the right flank. Four to five doses of a 25.0% concentration of Poloxamer 185 in Neobee M-5 were applied to the test and the control groups. The sites were then scored for erythema 24 and 48 h after dosing.

The results indicated that Poloxamer 185 failed to sensitize any guinea pigs under occlusive or open patch conditions. Therefore, Poloxamer 185 is considered a non-sensitizer (CTFA 2004e).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No reproductive or developmental toxicity data were available on any of the Poloxamers.

GENOTOXICITY

Marino (1987) reported the results of an Ames test using Poloxamer 407 and *Salmonella* strains TA97, TA98, TA100, TA1535, and TA1537. A solution of 50% Poloxamer in absolute ethanol was added at a volume of 0.4 ml to a 0.4-ml culture with and without 2 ml of S9 mix and mixed for 20 min at 37°C. Selective top agar (10 ml) was added to each container; then samples were mixed, plated (3 ml/plate), and incubated for 48 h at 37°C. Poloxamer 407 was not mutagenic in any strain, with or without metabolic activation.

CARCINOGENICITY

No data suggesting carcinogenicity of Poloxamers were found, but data on anticarcinogenicity and cancer treatment were found.

Anticarcinogenicity

Silk and Sigman (1972) investigated the effect of Poloxamer 188 on the development of tumor metastasis. Sixty-five adult male rats (150 to 200 g) were tested (strain not specified). One milliliter of Walker 256 ascitic tumor (100,000 cells/ml) was injected into the tail vein of all rats. Thirty-one rats received, intravenously, 4 mg/100 g body weight of Poloxamer 188 every day for 7 days prior to and 7 days following tumor inoculation. Any animals that died during the experimental period were necropsied and the remaining animals were killed after 8 weeks and also underwent necropsy.

The control group of 34 rats had an 85.3% incidence of pulmonary metastasis, whereas the test group had a 16.1% incidence ($p > 0.01$). Also, 80% of the rats of the test group survived the 8-week trial period compared to 23% of the control group (Silk and Sigman 1972).

Parnaud et al. (2001) conducted in vivo studies on the effect of Poloxamer 188 on colon cancer in rats and mice. A total of 158 4-week-old F344 rats and OF1 mice were included in the studies. Rats were given a single i.p. injection of 20 mg/kg azoxymethane (a known carcinogen) in saline, whereas mice were given 4 weekly injections of 5 mg/kg azoxymethane. All animals were divided into dietary groups. Thirty days after the start of each experiment, the animals were killed, their colons evaluated, and aberrant crypt foci (ACF) were counted.

For the first study, 28 male rats were divided into five groups. The control group (12 rats) were fed the standard AIN 76 diet. Four groups of four rats were fed diet supplemented with 5% (*w*/*w*) of either polyethylene glycol 800 (PEG), Poloxamer 188, polyoxyethylene 100 stearate ester, or polyoxyetheylene 100 stearyl ether. Poloxamer 188 suppressed ACF, with only 0.7 ± 1.0 ACF/colon, compared to 53 ± 14 ACF/colon in the control group. Poloxamer 188 caused 71 times fewer ACF than in the control group.

The second study involved dividing animals into three groups of 10 rats fed AIN diet and pure drinking water or supplemented with 5% (*w*/*v*) PEG or Poloxamer 188. In the third study, 60 female mice were grouped into three equal groups and fed AIN diet and pure drinking water or supplemented with 5% (w/v) PEG or Poloxamer 188. Both PEG and Poloxamer 188 decreased the number of ACF in rats and mice, as compared to controls, with Poloxamer causing a greater decrease in ACF than PEG in treated mice. The number of ACF/colon in control rats and those administered Poloxamer 188 was 81 ± 13 and 12 ± 07, respectively, and in mice was 47 ± 20 and 11 ± 06, respectively.

In the fourth study, female rats were divided into seven groups. Two groups of 10 rats were fed AIN diet with or without 5% Poloxamer 188. Five groups of four rats were fed diets with 5% Poloxamer 188, Poloxamer 181, Poloxamer 407 or L64, or NP30 (a PEG nonylphenyl ether). Rats given Poloxamer 188 had 56 times fewer ACF than in control rats and 6 rats out of 10 had no ACF at all. Poloxamer 407 halved the number of ACF but Pluronic L64 and NP30 had little effect. Poloxamer 181 also had little effect on the ACF number in the rats, as compared to controls (Parnaud et al. 2001).

Multidrug-Resistant Cancer

As noted earlier in the section on Multidrug Resistance–Associated Protein, drug efflux transport proteins (ATP-binding cassette proteins) function to pump selected agents out of mammalian cells, including therapeutic drugs. The activity of these drug efflux transport proteins is inhibited by Poloxamers. Poloxamer concentrations below those needed to form micelle structures are effective in increasing the activity of anticancer cytotoxic agents. The mechanism of action appears to be a decrease in ATP levels selectively in multidrug-resistant (MDR) cells associated with exposure to Poloxamers. It has been suggested that a successful strategy for treating MDR cancers could be based on selective energy depletion in MDR cancer cells, including the use of Poloxamers.

CLINICAL ASSESSMENT OF SAFETY

Clinical Testing

Poloxamer 188

Connaugton and McCarthy (1982) reported a double-blind crossover study with 10 male subjects, age 20 to 25 years, which examined the effect of a combination of ispaghula and Poloxamer 188 on gastrointestinal transit time. Ispaghula is the dried husks of the ripe seeds of Plantago Ovata used as a bulk laxative treatment in chronic constipation. The hypothesis was that Poloxamer 188 would increase the hydration of feces to allow for easier passage during defecation. During two 5-day periods, with 2 days in between, the volunteers received either a placebo or the active agent. The active agent was a sachet of granules made up of 3 g of isphaghula and 0.2 g of Poloxamer 188. Both the active agent and placebo were taken dispersed in water.

The 10 subjects were divided into two groups and one group took the isphaghula/Poloxamer 188 combination 1 week and placebo the next, whereas the other group took the placebo first and the active agent second. Throughout the study, volunteers took 20 radio-opaque markers on the second morning of each 5-day period to assess the efficacy of the agents. Stools passed after the markers were taken were collected and the number of markers in each was determined.

More markers were excreted on days 2 and 3 when volunteers were taking the ispaghula/Poloxamer 188 mixture. Every volunteer excreted a greater number of markers during the week of taking the active agent. The authors concluded that the ispaghula/Poloxamer 188 combination shortened intestinal transit time while causing a small degree of loose bowel action (Connaugton and McCarthy 1982).

Dire and Welsh (1990) evaluated the efficacy of Poloxamer 188 as a wound cleanser over 3 consecutive months on patients with minor, uncomplicated soft-tissue lacerations requiring suture. During the first month, normal saline was used to irrigate wounds, whereas 1% povidone-iodine (PI) and Poloxamer 188 were used during the second and third months, respectively. All wounds were irrigated with 200 ml or more of each solution and grossly contaminated wounds were scrubbed with a porous sponge saturated with the same solution. Wounds were draped and sutured and when patients returned for suture removal, wounds were examined for symptoms of infection and graded on a scale from 0 (no infection) to 4 (systemic symptoms). Data were collected on 531 patients; 189, 184, and 158 patients were treated with saline, PI, and Poloxamer, respectively.

The overall wound infection rate for all 531 patients was 5.6%. In the saline, PI, and Poloxamer groups, the infection rates were 6.9%, 4.3%, and 5.6%, respectively. Of the 13 infected wounds in the saline group, 8 were grade 1 and 5 grade 2. In the

PI group, 5 were grade 1 and 3 were grade 2, whereas the 9 in the Poloxamer group were grade 1. No grade 3 or 4 infections were noted in any group. The authors report that there was no statistically significant difference between the three wound irrigants studied (Dire and Welsh 1990).

O'Keefe et al. (1996) investigated the safety and efficacy of Poloxamer 188 after reperfusion with primary angioplasty. In a randomized double-blind placebo trial, 150 patients (23 did not complete the trial) were randomized into a group of 98 to receive Poloxamer 188 and 52 who were administered saline only and served as a control group. Patients in the treatment group received 300 mg/kg/h of Poloxamer 188 for 1 h, then 30 mg/kg/h for an additional 47 h. The placebo group received saline for 48 h.

Poloxamer was well tolerated except for an elevated serum creatinine level (>2.0 g/dl) in 12 of the 98 patients treated with Poloxamer, compared to 2% of the 52 control patients. However, all patients with elevated serum creatinine levels and azotemia had preexisting renal dysfunction. Patients in the treatment and control group had similar data concerning reinfarction, cardiogenic shock, and mortality. Also, infarct size, left ventricular ejection fractions, and myocardial salvage were statistically similar between groups (O'Keefe et al. 1996).

Schaer et al. (1996) investigated the effects of Poloxamer 188 (150 mg/ml buffered saline) on patients receiving thrombolytic therapy for acute myocardial infarction. All 114 patients had ongoing chest pain and evidence of anterior infarction or nonanterior infarction. Patients received either placebo or Poloxamer 188 intravenously beginning immediately after the initiation of thrombolytic therapy and injection of 20 to 30 mCi ^{99m}Tc sestamibi. Initially, 45 patients received placebo or a low dose of Poloxamer 188 (150 mg/kg/h for 1 h and then 15 mg/kg/h for 47 min). After this dose was determined to be safe, the remaining 69 patients received placebo or high-dose Poloxamer (300 mg/kg/h for 1 h and then 30 mg/kg/h for 47 h). A total of 75 patients received Poloxamer 188 and 39 were given vehicle placebo. Tomographic imaging with ^{99m}Tc sestamibi and radionuclide angiography at 5 to 7 days after infarction were used to determine myocardium at risk of infarction, infarct size, myocardial salvage, and left ventricular ejection fraction.

Myocardial infarct size was reduced by 38%, median myocardial salvage was reduced by 13% (compared to 4% with placebo), and median ejection fraction was improved by 13% in patients treated with Poloxamer 188. There was also a 1% incidence of reinfarction in those treated with Poloxamer, as opposed to the 13% in patients treated with vehicle only. No organ toxicity or adverse hemodynamic effects were noted in patients treated with Poloxamer 188 (Schaer et al. 1996).

Adams-Graves et al. (1997) tested the beneficial effects of Poloxamer 188 in patients suffering from sickle cell disease (SCD). Twenty-five male and 25 female patients suffering from SCD were enrolled in the randomized, double-blind, placebo-controlled trial. Twenty-eight volunteers received a continuous two-stage, intravenous infusion over 48 h consisting of a 60-min loading dose of 300 mg/kg followed by a maintenance infusion of 30 mg/kg/h of Poloxamer 188 in buffered saline. The other 22 patients received the vehicle placebo. Three patients withdrew from the study before completion. Efficacy variables that were measured included duration of painful episodes, duration of hospitalization, average pain intensity, and analgesic use.

Compared to the placebo group, the treated patients had a median duration of painful episodes that were reduced by 13 to 36 h. Duration of hospitalization was statistically insignificantly decreased by 1 to 2 days. There was also a 3- to 5-fold reduction in the use of analgesics in the treated group. The average pain intensity in the treated group was significantly decreased from baseline to 72 h ($p = 0.034$) and from baseline to 168 h ($p = 0.049$). Of the 24 Poloxamer-treated patients, 24 (42%) had a recurrent painful episode 1 to 3 weeks post-infusion, as did 8 of 18 (44%) of the control group. The authors stated that Poloxamer 188 may be beneficial for SCD patients who experience acute painful episodes (Adams-Graves et al. 1997).

The Collaborative Organization for RheothRx Evaluation (CORE) investigated the effects of Poloxamer 188 (RheothRx) on patients with acute myocardial infarction (CORE 1997). Initially, 963 patients were randomized into control or treatment groups. Treatment consisted of a 1-h loading infusion of 150 mg/ml Poloxamer 188 at a rate of 1.5 ml/kg/h; patients were then divided into regimens for subsequent maintenance infusions.

The regimens were as follows: A, no maintenance infusion; B, 23-h infusion at the lower rate; C, 47-h infusion at the low rate; D, 23-h infusion at the high rate; and E, 47-h maintenance infusion at the high rate. Infusion rates were determined according to age and baseline serum creatinine (Cr) levels and were designed to achieve a steady-state blood concentration of 0.5 and 1.0 mg/ml for the low- and high-dose regimens, respectively.

A few months after the study began, acute renal dysfunction (ARD) caused the termination of high-dose regimens D and E and the 47-h low-dose regimen. A month later, regimen B was also suspended until further evaluation of the renal toxicity of Poloxamer 188 could be determined. It was determined that renal toxicity occurred more often in the older patients or those with elevated Cr levels prior to study initiation. The eligibility criteria were modified to exclude patients older than 74, those with a history of renal dysfunction, or with serum Cr levels 135 μmol/L.

The study was resumed but all patients received 0.15 ml/kg/h of Poloxamer 188 (150 mg/ml). Regimen B (23-h low rate) was reinstated and a new regimen (Y) of a 1-h loading, as before, plus an 11-h infusion at low dose to create a regimen between A (loading dose only) and B. Renal dysfunction was still observed in patients in regimens A, Y, and B (3.1%, 2.7%, and 4.1%, respectively) compared to those in the control group (1.0%).

No significant difference in mortality, cardiogenic shock, or reinfarction at 35 days was observed between groups. Poloxamer 188 caused a higher incidence of sinus tachycardia (24.7% versus 21.6%, $p = 0.02$), atrial flutter (3.0% versus 1.3%, $p =$

0.019), atrial fibrillation (10.2% versus 7.3%, $p = 0.082$), pericarditis (6.6% versus 4.7%, $p = 0.055$), and clinical (21.9% versus 17.9%, $p = 0.005$) as well as radiological (15.3% versus 12.3%, $p = 0.12$) evidence of heart failure, as compared to the control group. Those in the treatment groups also had lower left ventricular ejection fractions and little difference in infarct size, as compared to control patients. Overall, Poloxamer 188 caused high levels of ARD and though adverse effects were less prevalent at lower doses, there was no favorable effect on infarct size, left ventricular function, or mortality (CORE 1997).

Maynard et al. (1998) included patients suspected as having had an acute myocardial infarction (AMI) at the time of hospitalization in a Poloxamer 188 study. Patients were randomly assigned to the placebo ($n = 99$) or Poloxamer 188 ($n = 97$) group and received a 48-h infusion of each. An initial 31 patients received 300 mg/kg/h Poloxamer 188 or placebo for the first hour and 30 mg/kg/h for the subsequent 47 h. A second group of patients aged 65 and older received placebo or Poloxamer 188 at a rate of 150 mg/kg/h for the first hour and 15 mg/kg/h for the next 47 h. Infarct size, left ventricular ejection fraction, mortality rate, and acute renal dysfunction were compared between the test and placebo groups.

Poloxamer 188 did not reduce infarct size compared to placebo but did cause a lower left ventricular ejection fraction. The mortality rates of the groups were comparable. There was a higher occurrence (12% versus 2%) of acute renal dysfunction in the treated group (Maynard et al. 1998).

Dermal Irritation and Sensitization

Leaf (1967) tested a paste of Poloxamer 188 on 10 volunteers. The paste was applied to the forearm every other day for a total of 10 applications. No skin irritation was observed. After 14 days rest, a challenge paste was applied and there was no evidence of skin sensitization in any of the volunteers. No further details were provided.

CTFA (2004d) reported on the evaluation of a moisturizing cleanser containing 5% Poloxamer 184 to determine its capacity to induce skin irritation and/or allergic sensitization following a repeat insult patch test. One hundred subjects (93 female, 5 male; age range from 16 to 70) were enrolled in this study. Only 98 subjects completed the test.

Ten test patches with ~0.1 ml of the test materials were applied to the upper backs of the subjects, five on the right and five on the left. The patches were applied for 24 h every Monday, Wednesday, and Friday for 3 consecutive weeks. Twenty-four hours following application, the patches were removed by the subjects at home. The patches were applied to the same sites repeatedly. Test site reactions were graded just before applications two through nine and the next test date following application nine.

In the sixth week of the study, the challenge phase was conducted. A single patch of each material was applied to virgin sites. Twenty-four hours later, the patches were removed. Reactions were scored 24 and 48 h after removal.

There were no reactions observed from the test material during either the induction phase or challenge phase. It was concluded that the moisturizing cleanser containing 5% Poloxamer 184 did not exhibit any potential for inducing allergic sensitization in this test (CTFA 2004d).

Ivy Laboratories (1993) reported a study which evaluated the contact-sensitization potential of eye lotion containing 8.0% Poloxamer 185. Healthy, adult volunteers over 18 years of age were used in this investigation. Patches were applied to the upper outer arm, volar forearm or back of each subject. The test material was tested on the subjects in order to determine whether it was irritating and whether sodium lauryl sulfate (SLS) was required. About 0.1 g of the test material was applied to the skin site under a 15-mm disc of nonabsorbing Webril cotton cloth. The patch was sealed with occlusive tape to ensure close contact with the skin. The patch remained in place for 48 h. It was then removed and the site examined for evidence of irritation. There was no evidence of irritation (erythema, scaling, etc.) in the subjects with the test sample during the pre-test phase. Induction was therefore conducted with SLS pretreatment.

Approximately 0.1 ml of aqueous SLS (0.5%) was applied to an assigned site under a 15-mm disc of Webril cotton and the patch was fastened to the skin with occlusive tape for 24 h. Twenty-four hours later, the SLS patch was removed and 0.1 g of the test material was applied to the same site before the sites were again covered with the induction patch. The patch remained in place for 48 h (or 72 h when placed over a weekend), then removed and the site examined for irritation. If there was no irritation present, a 0.5% aqueous SLS patch was again reapplied to the same site for 24 h, followed by reapplication of a fresh induction patch with the test material to the same site. This 24-h SLS pretreatment followed by 48 h of test material sequence was continued for a total of five induction exposures. If irritation developed at any point during the induction phase, the 24-h SLS pretreatment patch was eliminated and only the test material was reapplied to the same site after a 24-h rest period during which no patch was applied.

After a 10-day rest period following the last induction patch application, the subjects were challenged with a single application of the test material to a new skin site on the opposite arm, volar forearm, or back in order to determine if sensitization had developed. Pretreatment with SLS was performed prior to challenge since the test material was found to be non-irritating in the pretest phase. About 0.1 ml of a 10% aqueous solution (SLS) was applied to a fresh skin site under a 15-mm disc of Webril cotton and covered with occlusive tape. The SLS patch remained in place for 1 h, then removed and the test material was applied to the same site. The challenge patch was then covered by occlusive tape and left in place for 48 h. After that period, the patch was removed and the site scored 1 h later and again 24 h later for any reaction.

Based on the absence of any significant response, it was concluded that the test sample did not possess a detectable

contact-sensitization potential and hence is not likely to cause contact sensitivity reactions under normal use conditions (Ivy Laboratories 1993).

Ivy Laboratories (2003) also investigated the contact-sensitization potential of a topical product in human skin using the maximization assay method. Twenty-six healthy, adult volunteers (18 to 60 years of age) were used in this study; 12 males and 14 females. One subject was eliminated from the study for unknown reasons, leaving the remaining 25 to complete the investigation.

Patches were applied to the upper arm, volar forearm or back of each subject. During the induction phase, about 0.05 ml of aqueous SLS (0.25%) was applied to a designated site under a 15-mm disc of Webril cotton cloth and the patch was fastened to the skin with occlusive tape for 24 h. Twenty-four hours later, the SLS patch was removed and 0.05 ml of the test material (face cleanser: 3.0% Poloxamer and 0.5% aqueous) was applied to the same site before the site was covered again with the induction patch. The patch remained in place for 48 h (or for 72 h when placed over the weekend), following which it was removed and the site again examined for irritation. If there was no irritation present, a 0.25% aqueous SLS patch was reapplied to the same site for 24 h, followed by reapplication of a fresh induction patch with the test material to the same site. This sequence was continued for a total of five induction exposures.

After a 10-day rest period following the last induction patch application, the subjects were challenged with single application of the test material to a new skin site on the opposite arm, forearm or side of back in order to determine if sensitization had developed. Pretreatment with SLS was performed prior to challenge. Approximately 0.05 ml of a 5.0% aqueous solution was applied to a fresh skin site under a 15-mm disc of Webril cotton and covered with occlusive tape. The SLS patch remained in place for 1 h. It was then removed and the test material was applied to the same site. The challenge patch was then covered by occlusive tape and left in place for 48 h. After that period, the patch was removed and the site scored 1 h later and again 24 h later for any reaction.

There were no instances of contact allergy recorded at either 48 or 72 h after the application of the challenge patches. Therefore, the sample face cleanser (0.5% aqueous) did not possess a detectable contact-sensitizing potential and is not likely to cause contact sensitivity reactions under normal conditions of use (Ivy Laboratories 2003).

CTFA (2004f) reported on an allergic contact sensitization test for a peel-off mask containing 5% Poloxamer 185. The purpose of the study was to determine the capacity of 10 different materials to induce skin irritation and/or allergic sensitization via a predictive patch test procedure. There were originally 119 adult males and females, 18 to 70 years old, chosen to participate in the study. However, three withdrew from the study for unrelated reasons.

The patch was applied to the upper backs of the subjects. Five patches were placed on the right side and five were placed on the left side adjacent to the midline. The test materials were added to the patches shortly before application to the subjects. All samples were applied to all the subjects for 24 h every Monday, Wednesday, and Friday for 3 consecutive weeks. The patches were removed by the subjects at home 24 h after application. The samples were applied to the same sites (upper right and left side of back) unless severity of reactions justified changing the application site. In such cases, patches were applied to nearby, previously unpatched sites. Skin reactions were scored just before applications two through nine and the next test date following application nine. In most cases, this was about 24 h after patch removal.

Challenge applications were done on Tuesday in week 7 of the study. A single patch of each test material was applied to previously unpatched virgin sites. They were removed 24 h following application. Reactions were scored 24 and 48 h after removal. Subjects showing challenge patch reactions indicative of possible induced sensitization participated in follow-up testing following a 2-week rest period. Based on the absence of any significant response, it was concluded that within the limits imposed by the sample size and the test procedure itself, the peel-off mask did not exhibit any potential for inducing allergic sensitization (CTFA 2004f).

SUMMARY

Poloxamers are polyoxyethlyene, polyoxypropylene block polymers. The benzoate esters are formed from a reaction of benzoic acid and the particular Poloxamer. The trade name Pluronic® block polymer is often used to describe these chemicals as used in medical and/or research applications.

Poloxamers are synthesized at high temperature and pressure from propylene glycol, to which is added propylene oxide followed by ethylene oxide. Synthesis is done in the presence of an alkaline catalyst such as sodium or potassium hydroxide, which is neutralized and incorporated into the final product. The impurities of commercial grade Poloxamer 188 include low-molecular-weight substances, including aldehydes and both formic and acetic acids as well as 1,4-dioxane and residual ethylene oxide and propylene oxide. Maximum concentrations have been established for ethylene oxide, propylene oxide, and 1,4-dioxane in Poloxamers at 1, 5, and 5 ppm, respectively.

In cosmetics, most Poloxamers function as surfactants, emulsifying agents, cleansing agents, and/or solubilizing agents, althought Poloxamer 188 functions as an antimicrobial agent and Poloxamer 182 Dibenzoate functions as a skin conditioning agent and emollient. Poloxamers have been reported to be used in 141 cosmetic products, at concentrations from 0.005% to 20%.

Noncosmetic uses include use as a food additive and in a wide variety of medical applications, including tissue engineering, gene transfer, drug delivery, and cancer therapy.

At sufficiently high concentrations (>20 mg/ml), Poloxamers can affect structure and function of fibrin in plasma. Polox-

amers can inhibit platelet aggregation at concentrations as low as 0.05 mg/ml.

Poloxamer 108 or Poloxamer 188 injected intravenously in several animal species is rapidly and virtually completely excreted in the urine, did not appear in serum at appreciable levels, but did appear in lung, liver, brain, and kidney tissue in detectable amounts. The pharmacokinetics of Poloxamer 188 given via intravenous perfusion in rats was characterized by a steady-state plasma level by 46 h after initiation of perfusion. Steady-state levels were dose dependent. A similar study in dogs found that a dose-dependent steady state was reached at 7 days, with a mean plasma clearance between 49.4 and 87.9 ml/h. A single unidentified metabolite (with a MW of 1600 daltons and a block copolymer structure), representing 10% to 12% of the Poloxamer 188, given was detected in plasma. Poloxamer 407 injected intravenously into rats was almost totally excreted in urine over the first 24 h. No Poloxamer 407 was detected in urine from 24 to 96 h after injection. Poloxamer 407 was detected in liver and kidney tissue.

In humans, the plasma concentration of Poloxamer 188 (given intravenously) reached a maximum at 1 h, then reached a steady state. At steady state, the same single metabolite seen in dogs was also seen, at around 40% of the injected Poloxamer.

Poloxamers have been shown to modify drug efflux transport protein activity, thereby enhancing the effectiveness of anticancer drugs in multidrug-resistant cancer cells. Poloxamers also increase the bioavailability of orally administered pharmaceutical drugs and increase the permeability of the blood brain barrier to pharmaceutical drugs. Poloxamers have antimicrobial activity against some bacterial strains, but not others. Intravenous injections of Poloxamer 188 was shown to stimulate phagocytic activity in spleen and liver macrophages in male lipopolysaccharide non-responder mice in one study.

Poloxamers generally were ineffective in wound healing, but were effective in reducing postsurgical adhesions in several test systems. Poloxamer 188 inhibited adhesive interactions among proteins and cells in rabbits following cerebral ischemia and improved survival following hemorrhagic shock. Poloxamer 188 reduced reperfusion-induced myocardial necrosis in dogs. Poloxamers did not affect neurological outcome after hypothermic circulatory arrest in dogs or thrombus formation in pigs. Poloxamers induced some learning deficits in rats, but did not impair performance in a memory task.

Poloxamers have been shown to inhibit the growth of mammalian cells in culture, but the effects appear to be cell line specific.

Poloxamer 407 causes hypercholesterolemia and hypertriglyceridemia in rats, possibly by inhibiting heparin-releasable lipoprotein lipase and/or by stimulating the release of free fatty acids from adipocytes. Mice that were chronically exposed to Poloxamer 407 in the diet resulted in elevated plasma cholesterol levels and development of atherogenic arterial lesions.

Poloxamers are relatively nontoxic to animals, with LD_{50} values reported from 5 to 34.6 g/kg. Poloxamers 335 and 403 injected intramuscularly in rabbits caused an increase in creatine phosphokinase levels, whereas Poloxamers 238 and 407 did not, suggesting that lipophilicity is related to the increases seen. Acute intravenous toxicity tests in rats and dogs of Poloxamers 181 and 407 as vehicles demonstrated that single doses of 12 ml/kg/min were lethal, with death caused by hypervolemia and pulmonary edema. Another acute intravenous toxicity test in dogs identified significant toxicity, but not death, at 9.6 ml/kg/min. Acute intravenous injection of purified and unpurified 4% Poloxamer 188 in rats produced an increase in spleen weights in the animals receiving the unpurified material only, but all other tissue and organ weights were comparable among test and control animals.

Short-term intravenous toxicity tests in rats at doses up to 4 g/kg demonstrated no change in body weights, diffuse hepatocellular vacuolization, and renal tubular dilation in kidneys, and dose-dependent vacuolization of epithelial cells in the proximal convoluted tubules, but overall the Poloxamer 108 appeared to be well tolerated. A short-term inhalation toxicity study of Poloxamer 101 at 97 mg/m^3 identified slight alveolitis after 2 weeks of exposure, which subsided in the 2-week postexposure observation period.

A short-term dermal toxicity study of Poloxamer 184 in rabbits at doses up to 1000 mg/kg produced slight erythema and slight intradermal inflammatory response in histological examination. No dose-dependent body weight, hematology, blood chemistry, or organ weight changes were seen, although some statistically significant reductions were seen at the 100 mg/kg level.

A 6-month feeding study in rats and dogs of Poloxamer 188 at exposures up to 5% in the diet produced no adverse effects. Likewise, Poloxamer 331 (tested up to 0.5 g/kg/day), Poloxamer 235 (tested up to 1.0 g/kg/day), and Poloxamer 338 (at 0.2 or 1.0 g/kg/day) produced no adverse effects. Poloxamer 338 (at 5.0 g/kg/day) produced slight transient diarrhea.

Poloxamer 188 at levels up to 7.5% in diet given to rats in a 2-year feeding study produced diarrhea at 5% and 7.5% levels, a small decrease in growth at the 7.5% level, but no change in survival. Another study reported that doses up to 0.5 mg/kg day^{-1} for 2 years produced yellow discoloration of the serum, high serum alkaline phosphatase activity, and elevated serum glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase activities.

Poloxamers 124, 182, and 235 produced minimal ocular irritation in the first day after instillation that disappeared in the second and subsequent days. Poloxamer 182 Dibenzoate and Poloxamer 105 Benzoate produced a similar pattern, except that the minimal irritation did not totally disappear until the third day.

Poloxamers are not dermal irritants or sensitizers in animals.

Data on reproductive and developmental toxicity of Poloxamers were not found.

An Ames test did not identify any mutagenic activity of Poloxamer 407, with or without metabolic activation.

No data were found suggesting that Poloxamers are carcinogenic. Several studies have investigated the anticarcinogenic effects of Poloxamers. Poloxamer 188 given to rats intravenously at 20 mg/kg body weight reduced the incidence of pulmonary metastasis from injected Walker 256 ascitic tumor cells from 85% in the controls to 16%. Rats injected with azoxymethane to produce colon tumors and given Poloxamer 188 (5%) in the diet had around 1 aberrant crypt foci/colon in a colon exam compared to around 53 aberrant crypt foci/colon in the group that did not receive Poloxamer 188. Use of Poloxamer 407 was only half as effective in the same test system. Pluronic L64 and Poloxamer 181 had little effect.

Poloxamers appear to increase the sensitivity to anticancer drugs of multidrug-resistant cancer cells.

In clinical testing, an Ispaghula/Poloxamer 188 combination shortened intestinal transit time while causing a small degree of loose bowel action. A clinical trial of 150 angioplasty patients received placebo or Poloxamer 188, but there was no difference in reinfarction, cardiogenic shock, and mortality between the groups. Patients (114) on thrombogenic therapy for acute myocardial infarction received placebo or Poloxamer 188. Compared to controls, those patients receiving Poloxamer 188 had a reduced myocardial infarct size and a reduced incidence of reinfarction, with no evidence of toxicity. Yet a third study of Poloxamer 188 on patients (963) with acute myocardial infarction failed to find an impact on infarct size, left ventricular function, or mortality, but did report a higher incidence of sinus tachycardia, atrial flutter, atrial fibrillation, pericarditis, and clinical signs of heart failure in the Poloxamer 188 group compared to controls. Poloxamer 188 given to patients suffering from sickle cell disease had decreased pain and decreased hospitilization, compared to controls.

Clinical tests of dermal irritation and sensitization were uniformly negative.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel expressed concern regarding the possible presence of 1,4-dioxane, ethylene oxide, and propylene oxide as impurities. Ethylene oxide is highly volatile and a known human carcinogen. They stressed that the cosmetic industry should continue to use the necessary purification procedures to keep the levels below the established limits for ethylene oxide, propylene oxide, and 1,4-dioxane in Poloxamers at 1, 5, and 5 ppm, respectively, as set by the USP.

The Panel noted that as far as limits are concerned for the ingredients or the final product of the ingredients, the chemicals will fall below the levels already given if diluted. Therefore, limits are not an issue since it would be an even safer product in this case.

The Panel recognized that certain ingredients in this group are reportedly used in a given product category, but the concentration of use is not available. For other ingredients in this group, information regarding use concentration for specific product categories is provided, but the number of such products is unknown. In still other cases, an ingredient is not in current use, but may be used in the future. Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used, and at what concentration, indicates a pattern of use.

Acute, short-term, subchronic, and chronic animal testing suggested a low order of toxicity. The Panel did note the absence of reproductive and developmental toxicity data, but the available data suggest that there would be little or no exposure of sex organs or any developing fetus. The smallest Poloxamer in current use is Poloxamer 105, which has a molecular weight of approximately 1848 Da. The smallest Poloxamer addressed in this safety assessment is Poloxamer 101 with an approximate molecular weight of 1122 Da. In either case, these molecules are large compared to molecules that are known to penetrate the stratum corneum. In addition, these molecules are water soluble, further suggesting that there should be little skin penetration and that their rates of penetration of the skin should be slow. Also, the available data demonstrate that Poloxamers that are introduced into the body via routes other than dermal exposure have a rapid clearance from the body.

The Panel considered the negative Ames test data, and noted an absence of mammalian genotoxicity data. Chronic exposure studies in rats and dogs failed to identify any carcinogenic effects. In addition, several studies demonstrate an anticarcinogenic effect of Poloxamers. Overall, the available data do not suggest any concern about carcinogenesis.

In both animal and clinical testing, Poloxamers were not irritating or sensitizing.

Based on these safety test data and the information that the manufacturing process can be controlled to limit unwanted impurities, the Panel concluded that these Poloxamers are safe as used.

CONCLUSION

The CIR Expert Panel concluded that Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, Poloxamer 105 Benzoate, and Poloxamer 182 Dibenzoate are safe as cosmetic ingredients in the practices of use and concentration as described in this safety assessment.

REFERENCES

Adams-Graves, P., A. Kedar, M. Koshy, et al. 1997. RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: A pilot study. *Blood* 90:2041–2046.

Alakhov, V., E. Klinski, S. Li, et al. 1999. Block copolymer-based formulation of doxorubicin. From cell screen to clinical trials. *Colloids Surf. B Biointerfaces* 16:113–134.

Anonymous. 1997. Featured excipient: Poloxamer. *Int. J. Pharm. Compound* 1:190–191.

Armstrong, J. K., H. J. Meiselman, and T. C. Fisher. 1995. Inhibition of red blood cell-induced platelet aggregation in whole blood by a nonionic surfactant, poloxamer 188 (RheothRx injection). *Thromb. Res.* 79:437–450.

Armstrong, J. K., H. J. Meiselman, R. B. Wenby, T. C. Fisher. 2001. Modulation of red blood cell aggregation and blood viscosity by the covalent attachment of pluronic copolymers. *Biorheology* 38:239–247.

BASF. 2002. Technical Bulletin: Pluronic Block Copolymer NF Grades (Poloxamer NF Grades).[2]

Batrakova, E. V., S. Lee, S. Li, A. Venne, V. Alakhov, and A. Kabanov. 1999. Fundamental relationships between the composition of pluronic block copolymers and their hypersensitization effect in MDR cancer cells. *Pharm. Res.* 16:1373–1379.

Batrakova, E. V., S. Li, V. Y. Alakhov, D. W. Miller, and A. V. Rabanov. 2003. Optimal structure requirements for pluronic block copolymers in modifying P-glycoprotein drug efflux transporter activity in bovine brain microvessel endothelial cells. *J. Pharmacol. Exp. Ther.* 304:845–854.

Batrakova, E. V., S. Li, S. V. Vinogradov, V. Y. Alakhov, D. W. Miller, and A. V. Rabanov. 2001a. Mechanism of pluronic effect on P-glycoprotein efflux system in blood-brain barrier: Contributions of energy depletion and membrane fluidization. *J. Pharmacol. Exp. Ther.* 299:483–493.

Batrakova, E. V., D. W. Miller, S. Li, V. Y. Alakhov, A. V. Rabanov, and W. F. Elmquist. 2001b. Pluronic P85 enhances the delivery of digoxin to the brain: *In vitro* and *in vivo* studies. *J. Pharmacol. Exp. Ther.* 296:551–557.

Batrakova, E. V., S. Li, W. F. Elmquist, D. W. Miller, V. Y. Alakhov, and A. V. Rabanov. 2001c. Mechanism of sensitization of MDR cancer cells by Pluronic block copolymers: Selective energy depletion. *Br. J. Cancer* 85:1987–1997.

Bentley, P. R., S. S. Davis, O. L. Johnson, R. C. Lowe, and C. Washington. 1989. Purification of pluronic F68 for perfluorochemical emulsfication. *J. Pharm. Pharmacol.* 41:661–663.

Blonder, J. M., L. Baird, J. C. Fulfs, and G. J. Rosenthal. 1999. Dose-dependent hyperlipidemia in rabbits following administration of Poloxamer 407 gel. *Life Sci.* 65:L261.

Cao, Y., X. Cai, L. Cui, Q. Shang, W. Liu, and W. Guan. 2002. Repair of porcine full-thickness skin defects with autologous tissue engineered skin. *Zhonghua. Wai. Re. Za. Zhi.* 40:24–26 [translated from Chinese].

Cao, Y., A. Rodriguez, M. Vacanti, C. Ibarra, C. Arevalo, and C. A. Vacanti. 1998. Comparative study of the use of poly(glycolic acid), calcium alginate and pluronics in the engineering of autologous porcine cartilage. *J. Biomater. Sci. Polym. Ed.* 9:475–487.

Carr, M. E., Jr., S. L. Carr, and A. A. High. 1996. Effects of poloxamer 407 on the assembly, structure and dissolution of fibrin clots. *Blood Coagul. Fibronolysis.* 7:109–113.

Carr, M. E., Jr., P. L. Powers, and M. R. Jones. 1991. Effects of poloxamer 188 on the assembly, structure and dissolution of fibrin clots. *Thromb. Haemost.* 66:565–568.

Chandler, D., M. R. Davey, K. C. Lowe, and B. J. Mulligan. 1987. Effects of emulsified perflurochemicals on growth and ultrastructure of microbial cells in culture. *Biotechnol. Letts.* 9:195–200.

Clarke, M. S., and P. L. McNeil. 1992. Syringe loading introduces macromolecules into living mammalian cell cytosol. *J. Cell Sci.* 102:533–541.

Clarke, M., M. Predergast, and A. Terry, Jr. 1999. Plasma membrane ordering agent pluronic F-68 reduces neurotransmitter uptake and release and produces learning and memory deficits in rats. *Learning Memory* 6:634–649.

Colbassani, H. J., D. L. Barrow, K. M. Sweeney, R. A. Bakay, I. J. Check, R. L. Hunter. 1989. Modification of acute focal ischemia in rabbits by poloxamer 188. *Stroke* 20:1241–1246.

Collaborative Organization for RheothRx Evaluation (CORE). 1997. Effects of RheothRx on mortality, morbidity, left ventricular function, and infarct size in patients with acute myocardial infarction. Collaborative Organization for RhethRx Evaluation (CORE). *Circulation* 96:192–201.

[2]Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

Comai, R., and A. C. Sullivan. 1980. Antiobesity activity of pluronic L-101. *Int. J. Obesity* 4:33–42.

Committee of Revision of the United States Pharmacopeial Convention. 1995. *The United States Pharmacopeia*, 18th ed., 2279–2281. Rockville, MD: United States Pharmacopeial Convention.

Connaughton, J., and C. F. McCarthy. 1982. Comparison of combination of ispaghula/poloxamer 188 and placebo on gastrointestinal transit time. *Ir. Med. J.* 75:93–94.

Consumer Product Testing Company. 1990. Primary dermal and ocular irritation in rabbits of FINSOLV PL-62 (Poloxamer 182 Dibenzoate). 16 pages.[2]

Consumer Product Testing Company. 1992. Primary dermal and ocular irritation in rabbits of FINSOLV PL-355 (Poloxamer 105 Benzoate). 16 pages.[2]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2004a. Current concentration of use of Poloxamers. Unpublished data submitted by CTFA. 2 pages.[2]

CTFA. 2004b. Single insult patch test of Poloxamer 184 in rabbits. Unpublished data submitted by CTFA. 1 page.[2]

CTFA. 2004c. Safety evaluation of Poloxamer 184: Four week subacute dermal toxicity study in rabbits. Unpublished data submitted by CTFA. 7 pages.[2]

CTFA. 2004d. Allergic contact sensitization test: Moisturizing cleanser containing 5% Poloxamer 184. Unpublished data submitted by CTFA. 8 pages.[2]

CTFA. 2004e. An evaluation of the allergy potential of Poloxamer 185: Guinea pig maximization procedure. Unpublished data submitted by CTFA. 6 pages.[2]

CTFA. 2004f. Allergic contact sensitization test: Peel-off mask containing 5% Poloxamer 185. Unpublished data submitted by CTFA. 8 pages.[2]

Davidorf, F. H., R. B. Chambers, O. W. Kwon, W. Doyle, P. Gresak, and F. Sylvan. 1990. Ocular toxicity of vitreal pluronic polyol F-127. *Retina* 10:297.

Dire, D. J., and A. P. Welsh. 1990. A comparison of wound irrigation solutions used in the emergency department. *Ann. Emerg. Med.* 19:704–708.

Edwards, C. M., G. P. Gambaretto, L. Conte, and K. C. Lowe. 1999. Evaluation of commercial and purified Pluronic F-68 in a human blood neutrophil bioassay. *Artif. Cells Blood Substit. Immobil. Biotechnol.* 27:171–177.

Edwards, C. M., J. A. May, S. Heptinstall, and K. C. Lowe. 1996. Effects of pluronic F-68 (poloxamer 188) on platelet aggregation in human whole blood. *Thromb. Res.* 81:511–512.

Edwards, C. M., S. Heptinstall, and K. C. Lowe. 1998. Pluronic F-68 inhibits agonist-induced platelet aggregation in human whole blood *in vitro*. *Artif. Cells Blood Substit. Immobil. Biotechnol.* 26:441–447.

Elibol, M., and F. Mavituna. Effect of perfluorodecalin as an oxygen carrier on actinorhodin production by Streptomyces coelicolor A3(2). *Appl. Microbiol. Biotechnol.* 43:206–210.

El-Kamel, A. H. 2002. *In vitro* and *in vivo* evaluation of Pluronic F127-based ocular delivery system for timolol maleate. *J. Pharm.* 241:47–55.

Follis, F., B. Jenson, K. Blisard, et al. 1996. Role of poloxamer 188 during recovery from ischemic spinal cord injury: A preliminary study. *J. Invest. Surg.* 9:149–156.

Food and Drug Administration (FDA). 2002. Frequency of use of cosmetic ingredients. *FDA Database*. Washington, DC: FDA.

Gosselin, A. M., and G. P. Biro. 1990. The cardiovascular effects of the surfactant pluronic F68 in anesthetized dogs. *Adv. Exp. Med. Biol.* 277:291–299.

Gottschalck, T. E., and G. N. McEwen, Jr. eds. 2004. *International Cosmetic Ingredient Dictionary and Handbook*, 10th ed. Washington, DC: CTFA.

Grindel, J. M., T. Jaworski, R. M. Emanuele, P. Culbreth. 2002. Pharmacokinetics of novel surface-active agent, purified poloxamer 188, in rat, rabbit, dog, and man. *Biopharm. Drug. Dispos.* 23:87–103.

Harkonene, P., S. Salo, T. Mattila-Sandholm, G. Wirtanen, D. G. Allison, and P. Gilbert. 1999. Development of a simple *in vitro* test system for the disinfection of bacterial biofilms. *Water Sci. Technol.* 39:219–225.

Howell, J. M., H. S. Dhindsa, T. O. Stair, and B. A. Edwards. 1993a. Effect of scrubbing and irrigation on staphylococcal and streptococcal counts in contaminated lacerations. *Anitmicrob. Agents Chemother.* 37:2754–2755.

Howell, J. M., T. O. Stair, A. W. Howell, D. J. Mudt, A. Falcone, S. R. Peters. 1993b. The effect of scrubbing and irrigation with normal saline, povidone iodine, and cefazolin on wound bacterial counts in a guinea pig model. *Am. J. Emerg. Med.* 11:134–138. Erratum in *Am. J. Emerg. Med.* 11:319.

Hunter, R. L., C. Ragannath, A. Tinkley, C. A. Behling, and F. Nolte. 1995. Enhancement of antibiotic susceptibility and suppression of Mycobacterium avium complex growth by poloxamer 331. *Antimicrob. Agents. Chemother.* 39:435–439.

Ivy Laboratories. 1993. Evaluation of the contact-sensitizing potential of a test agent, eye lotion containing 8.0% Poloxamer 185. Unpublished data submitted by CTFA. 10 pages.[2]

Ivy Laboratories. 2003. An evaluation of the contact-sensitization potential of a topical coded product (facial cleanser with 3% Poloxamer 184) in human skin by means of the maximization assay. Unpublished data submitted by CTFA. 10 pages.[2]

Jewell, R. C., S. P. Khor, D. F. Kisor, K. A. LaCroix, and W. A. Wargin. 1997. Pharmacokinetics of RheothRx injection in healthy male volunteers. *J. Pharm. Sci.* 86:808–812.

Johnston, T. P., H. Beris, Z. G. Wout, and J. L. Kennedy. 1993. Effects on splenic, hepatic, hematological, and growth parameters following high-dose poloxamer 407 administration to rats. *Int. J. Pharm.* 100:279–284.

Johnston, T. P., and S. C. Miller. 1985. Toxicological evaluation of poloxamer vehicles for intramuscular use. *J. Parenter. Sci. Technol.* 39:83–89.

Johnston, T. P., and W. K. Palmer. 1993. Mechanism of poloxamer 407-induced hypertriglyceridemia in the rat. *Biochem. Pharmacol.* 46:1037–1042.

Johnston, T. P., and W. K. Palmer. 1997. Effect of poloxamer 407 on the activity of microsomal 3-hydroxy-3-methylglutaryl CoA reductase in rats. *J. Cardiovasc. Pharmacol.* 29:580–585.

Justicz, A. G., W. V. Farnsworth, M. S. Soberman, et al. 1991. Reduction of myocardial infarct size by poloxamer 188 and mannitol in a canine model. *Am. Heart J.* 122(3 Pt 1):671–680.

Kabanov, A. V., and V. Y. Alakhov. 2002. Pluronic block copolymers in drug delivery: From micellar nanocontainers to biological response modifiers. *Crit. Rev. Ther. Drug Carrier Syst.* 19:1–72.

Kier, L. D., L. M. Wagner, T. V. Wilson, et al. 1995. Cytotoxicity of ethylene oxide/propylene oxide copolymers in cultured mammalian cells. *Drug. Chem. Toxicol.* 18:29–41.

Kim, J. P., R. L. Peiffer, and R. E. Holman. 1988. Pluronic polyol: A potential alloplastic keratorefractive material. *J. Cataract Refract. Surg.* 14:312–316.

Kuo, J. H. 2003. Effect of pluronic-block copolymers on the reduction of serum-mediated inhibition of gene transfer of polyethyleneimine-DNA complexes. *Biotechnol. Appl. Biochem.* 37(Pt 3): 267–271.

Lane, T. A., and V. Krukonis. 1988. Reduction in the toxicity of a component of an artifical blood substitute by superficial fluid fractionation. *Transfusion* 28:375–378.

Leach, R. E., and R. L. Henry. 1990. Reduction of postoperative adhesions in the rat uterine horn model with poloxamer 407. *Am. J. Obstet. Gynecol.* 162:1317–1319.

Leaf, C. W. 1967. Toxicology of some non-ionic surfactants. *Soap Chem. Spec.* 43:48.

Lechmann, T., and W. H. Reinhart. 1998. The non-ionic surfactant Poloxamer 188 (RheothRx) increases plasma and whole blood viscosity. *Clin. Hemorheol. Microcirc.* 18:31–36.

Li, C., W. K. Palmer, and T. P. Johnston. 1996. Disposition of poloxamer 407 in rats following a single intraperitoneal injection assessed using a simplified colorimetric assay. *J. Pharm. Biomed. Anal.* 14:659–665.

Li, B., Y. J. Wang, C. Q. Ling, and W. J. Wang. 1998. Acute toxicity test of a new pharmaceutical adjuvant: Poloxamer 407. *J. Chin. Pharm.* 9:111–112 [translated from Chinese].

Liu, M. W., J. A. Hearn, J. F. Luo, P. G. Anderson, G. S. Roubin, S. Iyer, and L. Bilodou. 1996. Reduction of thrombus formation without inhibiting coagulation factors does not inhibit intimal hyperplasia after balloon injury in pig coronary arteries. *Coron. Artery Dis.* 7:667–670.

Lowe, K. C., B. A. Furmidge, and S. Thomas. 1995. Haemolytic properties of pluronic surfactants and effects of purification. *Artif. Cells Blood Substit. Immobil. Biotechnol.* 23:135–139.

Magnusson, G., O. Thomas, and J.-A. Nyberg. 1986. Toxicity of Pluronic F-68. *Toxicol. Lett.* 30:203–207.

Marino, D. J. 1987. Evaluation of Pluronic Polyol F127 as a vehicle for petroleum hydrocarbons in the salmonella/microsomal assay. *Environ. Mutagen.* 9:307–316.

Mayer, D. C., S. J. Strada, C. Hoff, R. L. Hunter, and M. Artman. 1994. Effects of poloxamer 188 in a rabbit model of hemorrhagic shock. *Ann. Clin. Lab. Sci.* 24:302–311.

Maynard, C., R. Swenson, and J. A. Paris. 1998. Randomized, controlled trial of RheothRx (poloxamer 188) in patients with suspected acute myocardial infarction. RheothRx in Myocardial Infarction Study Group. *Am. Heart J.* 135(5 Pt 1):797–804.

Mezrow, C. K., M. Mazzoni, D. Wolfe, H. H. Shiang, R. S. Litwak, and R. B. Griepp. 1992. Poloxamer 188 improves neurologic outcome after hypothermic circulatory arrest. *J. Thorac. Cardiovasc. Surg.* 103:1143–1146.

Miller, D. W., E. V. Batrakova, and A. V. Kabanov. 1999. Inhibition of multidrug resistance-associated protein (MRP) functional activity with pluronic block copolymers. *Pharm. Res.* 16:396–401.

Mizrahi, A. 1975. Pluronic polyols in human lymphocyte cell line cultures. *J. Clin. Microbiol.* 2:11–13.

Moghimi, S. M., and J. C. Murray. 1996. Poloxamer-188 revisited: A potentially valuable immune modulator. *J. Natl. Cancer Inst.* 88:766–768.

Moghimi, S. M., and A. C. Hunter. 2000. Poloxamers and poloxamines in nanoparticle engineering and experimental medicine. *Trends Biotechnol.* 184:412.

Murhammer, D. W., and C. F. Goochee. 1990. Sparged animal cell bioreactors: Mechanism of cell damage and Pluronic F68 protection. *Biotechnol. Prog.* 6:391–397.

Naim, J. O., D. Pulley, K. Scanlan, J. R. Hinshaw, and R. J. Lanzafame. 1993. Reduction of postoperative adhesions to Marlex mesh using experimental adhesion barriers in rats. *J. Laparoendosc. Surg.* 3:187–190.

Nalbandian, R. M., R. L. Henry, K. W. Balko, D. V. Adams, and N. R. Neuman. 1987. Pluronic F-127 gel preparation as an artificial skin in the treatment of third-degree burns in pigs. *J. Biomed. Mater. Res.* 21:1135–1148.

Nash, V. J, T. P. Johnston, and W. K. Palmer. 1996. Effects of nicotinic acid on poloxamer 407 induced hyperlipidemia. *Pharmacotherapy* 16:10–15.

O'Keefe, J. H., C. L. Grines, M. A. DeWood, et al. 1996. Poloxamer-188 as an adjunct to primary percutaneous transluminal coronary angioplasty for acute mycardial infarction. *Am. J. Cardiol.* 78:747–750.

Palmer, W. K., E. E. Emeson, and T. P. Johnston. 1998. Poloxamer 407-induced atherogenesis in the C57BL/6 mouse. *Atherosclerosis* 136:115–123.

Parnaud, G., S. Tache, G. Peiffer, and D. E. Corpet. 2001. Pluronic F68 block polymer, a very potent suppressor of carcinogenesis in the colon of rats and mice. *Br. J. Cancer* 84:90–93.

Port, D. C., P. J. Garvin, and C. E. Ganote. 1978. The effects of Pluronic F-38 (Polyoxamer 108) administered intravenously to rats. *Toxicol. Appl. Pharmacol.* 44:401–411.

Portoles, M., M. F. Refojo, and F. L. Leong. 1994. Poloxamer 407 as a bacterial adhesive for hydrogel contact lenses. *J. Biomed. Mater. Res.* 28:303–309.

Reigel, D. H., B. Bazmi, S. R. Shih, and M. D. Marquardt. 1993. A pilot investigation of poloxamer 407 for the prevention of leptomeningeal adhesions in the rabbit. *Pediatr. Neurosurg.* 19:250–255.

Rice, V. M., A. Shanti, K. S. Moghissi, and R. E. Leach. 1993. A comparative evalutation of Poloxamer 407 and oxidized regenerated cellulose (Interceed [TC7]) to reduce postoperative adhesion formation in the rat uterine horn model. *Fertil. Steril.* 59:901–906.

Rodeheaver, G. T., L. Kurtz, B. J. Kircher, and R. F. Edlich. 1976. Pluronic F68: A promising new skin wound cleanser. *Am. J. Surg.* 132:67.

Rodeheaver, G. T., L. Kurtz, B. J. Kircher, and R. F. Edlich. 1980. Pluronic F68: A promising new skin wound cleanser. *Ann. Emerg. Med.* 9:572–576.

Schaer, G. L., T. L. Hursey, S. L. Abrahams, et al. 1994. Reduction in reperfusion-induced myocardial necrosis in dogs by RheothRx injection (poloxamer 188 N. F.), a hemorheological agent that alters neutrophil function. *Circulation* 90:2964–2975.

Schaer, G. L., L. J. Spaccavento, K. F. Browne, et al. 1996. Beneficial effects of RheothRx injection in patients receiving thrombolytic therapy for acute myocardial infarction. Results of a randomized, double-blind placebo-controlled trial. *Circulation* 94:298–307.

Schmolka, I. R. 1994. Physical basis for poloxamer interactions. *Ann. N Y Acad. Sci.* 720:92–97.

Silk, M., and E. Sigman. 1972. Effect of pluronic F68 on the development of tumor metastasis. *Cancer* 29:171–172.

Steinleitner, A., H. Lamber, C. Kazensky, and B. Cantor. 1991. Poloxamer 407 as an intraperitoneal barrier material for the prevention of postsurgical adhesion formation and reformation in rodent models for reproductive surgery. *Obstet. Gynecol.* 77:48–52.

Toth, K., R. B. Wenby, H. J. Meiselman. 2000. Inhibition of polymer-induced red blood cell aggregation by poloxamer 188. *Biorheology* 37:301–312.

Ulrich, C. E., R. G. Geil, T. R. Tyler, G. L. Kennedy, and H. A. Birnbaum. 1992. Two-week aerosol inhalation study in rats of ethylene oxide/propylene oxide copolymers. *Drug Chem. Toxicol.* 15:269–270.

United States Pharmacopeia The National Formulary (USP National Formulary) 2004. Poloxamer entry. pp. 2908–2909. Rockville, MD: US Pharmacopeial Convention, Inc.

Van Belle, E., L. Maillard, A. Rivard, et al. 1998. Effects of poloxamer 407 on transfection time and percutaneous adenovirus-mediated gene transfer in native and stented vessels. *Hum. Gene Ther.* 9:1013–1024.

Wahl, R., and W. C. Butterfield. 1976. The prevention of ulcers in the pylorus-ligated rat by intravenous pluronic F68. *J. Surg. Res.* 20:45–47.

Wang, Z. Y., and I. J. Stern. 1975. Disposition in rats of a polyoxypropylene-polyoxyethylene copolymer used in plasma fractionation. *Drug Metab. Dispos.* 3:536–542.

West, J. L., and J. A. Hubbell. 1995. Comparison of covalently and physically cross-linked polyethylene glycol-based hydrogels for the prevention of postoperative adhesions in a rat model. *Biomaterials* 16:1153–1156.

Willcox, M. L., M. M. Newman, and B. C. Paton. 1978. A study of labeled pluronic F68 after intravenous injection into the dog. *J. Surg. Res.* 25:349–356.

Wout, Z. G., E. A. Pec, J. A. Maggiore, R. H. Williams, P. Palicharla, and T. P. Johnston. 1992. Poloxamer 407-mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. *J. Parenter. Sci. Technol.* 46:192–200.

Xiao, N., X. C. Lu, H. S. Chen, Z. H. Yang, and K. L. Tian. 1989. Effect of fluorocarbon blood substitute on neutrophil phagocytic function. *Zhongguo. Yao. Li. Xue. Bao.* 10:537–539.