FINAL REPORT ON THE SAFETY ASSESSMENT OF PEG-30, -33, -35, -36, AND -40 CASTOR OIL AND PEG-30 AND -40 HYDROGENATED CASTOR OIL[1]

PEG Castor Oils and PEG Hydrogenated Castor Oils are a family of polyethylene glycol derivatives of castor oil and hydrogenated castor oil that are used in over 500 formulations representing a wide variety of cosmetic products. They are used as skin conditioning agents and as surfactants (emulsifying and/or solubilizing agents). The PEG Castor Oils and PEG Hydrogenated Castor Oils include various chain lengths, depending on the quantity of ethylene oxide used in synthesis. Although not all polymer lengths have been studied, it is considered acceptable to extrapolate the results of the few that have been studied to all ingredients in the family. Because a principal noncosmetic use of PEG Castor Oils is as solvents for intravenous drugs, clinical data are available that indicate intravenous exposure can result in cardiovascular changes. Results from animal studies indicate very high LD_{50} values, with some evidence of acute nephrotoxicity in rats but not in rabbits. Short-term studies with intravenous exposure produced some evidence of toxicity in dogs but not in rabbits. Intramusuclar injection produced no toxicity in several species, including dogs. Subchronic oral studies also were negative. No dermal or ocular irritation was observed in studies in rabbits. Irritation was seen during induction, but no sensitization was found on challenge in guinea-pig studies using up to 50% PEG-35 Castor Oil; however, this ingredient was found to be a potent adjuvant in guinea pigs and mice. No evidence of developmental toxicity was seen in mice and rat feeding studies. These ingredients, tested as vehicle controls, produced no mutagenic or carcinogenic effect. Clinical data are generally negative for irritation and sensitization, although some anaphylactoid reactions have been seen in studies of intravenous drugs in which PEG-35 Castor Oil was used as the vehicle. Because the maximum concentration used in animal sensitization studies was 50% for PEG Castor Oils and 100% for PEG Hydrogenated Castor Oils, it was concluded that PEG Castor Oils are safe for use in cosmetic formulations up to a concentration of 50% and that PEG Hydrogenated Castor Oils are safe as used in cosmetic formulations.

PEG-30, -33, -35, -36, and -40 Castor Oil and PEG-30 and -40 Hydrogenated Castor Oil are polyethylene glycol derivatives of castor

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel.

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oil (q.v.) and hydrogenated castor oil (q.v.) that are used in a variety of cosmetic products. This report reviews the safety data on these ingredients.

CHEMISTRY

Definition and Structure

PEG-30, -33, -35, -36, and -40 Castor Oil (generic CAS No. 61791-12-6) and PEG-30 and -40 Hydrogenated Castor Oil (generic CAS No. 61788-85-0) are polyethylene glycol derivatives of castor oil (q.v.) and hydrogenated castor oil (q.v.). The number associated with the name of the compound represents the average number of moles of ethylene oxide consumed in the reaction to form the compound. Other respective chemical names for the castor oil and hydrogenated castor oil compounds include (X = the number of moles of ethylene oxide) Polyethylene Glycol X Castor Oil; Polyethylene Glycol X Hydrogenated Castor Oil; Polyethylene Glycol X; and Polyethylene Glycol X Hydrogenated Castor Oil (Wenninger and McEwen, 1995a).

Chemical and Physical Properties

Because castor oil is a triglyceride containing approximately 87% ricinoleic acid, 7% oleic acid, 3% linoleic acid, 2% palmitic acid, 1% stearic acid, and a trace of dihydroxysteric acid (Budavari, 1989), PEG Castor Oils are predominantly glyceryl triricinoleyl polyethylene glycol and PEG Hydrogenated Castor Oils are predominantly tri-12-hydroxylstearyl polyethylene glycol.

PEG-36 Castor Oil is a light yellow and slightly viscous liquid with a mild fatty odor. It has a specific gravity of 1.05 to 1.06 at 25°/25°C and is soluble in water. A 1% aqueous (aq) solution of this ingredient has a pH range of 7.0 to 8.0 (Nikitakis and McEwen, 1990). PEG-40 Castor Oil is a nonionic amber colored liquid that is miscible in water and aqueous buffer solution. It has a density of 1057.0 kg^{-3}, a viscosity of 450 cps at 20°C, and a melting point of approximately 10°C. Over a concentration range of 0.0005% to 1.0% (w/v), the pH of PEG-40 Castor Oil aq is 5.0 to 6.0 (Yalabik-Kas et al., 1982).

Spectral Data

The ultraviolet spectra of PEG-40 Castor Oil has a maximum peak at 234 to 236 nm (Yalabik-Kas et al., 1982).

Method of Manufacture

In general, PEG Castor Oils are made by reacting ethylene oxide with castor oil. The number (X) in the name of the compound refers to the molar ratio (X:1) of ethylene oxide to castor oil (Au et al., 1991).

USE

Cosmetic

The PEG Castor Oils and PEG Hydrogenated Castor Oils are used as skin-conditioning agents and as surfactants that function as emulsifying or solubilizing agents in cosmetic formulations (Wenninger and McEwen, 1995b). The product formulation data submitted to the Food and Drug Administration (FDA) in 1995 are listed in Tables 1 and 2 (FDA, 1995). Collectively, these ingredients are used in more than 500 cosmetic products. Concentration of use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). Product formulation data submitted to the FDA in 1984 stated, however, that PEG-30 Castor Oil was used at concentrations of up to 50% and more and that PEG-40 Castor Oil was used at concentrations of up to 10%. PEG-30 Hydrogenated Castor Oil was used at concentrations of up to 0.1%, and PEG-40 Castor Oil was used at concentrations of up to 5% (FDA, 1984).

Noncosmetic

In the pharmaceutical industry, PEG-35 Castor Oil is used as an emulsifier and solubilizer in pharmaceuticals containing volatile oils, fat-soluble vitamins, diazepam, propanidid, alfaxolone/alfadolone acetate, miconazole, methotrimeprazine, thiopental, and glycerin suppositories (Smolinske, 1992).

BIOLOGY

Cell Function

Nässberger (1990) reported that PEG-35 Castor Oil (diluted 1:1) decreased the spontaneous production of adenosine triphosphate (ATP) in isolated rat kidney mitochondria by 4%. At greater dilutions no such effect was observed. During oxidative phosphorylation, PEG-35 Castor Oil inhibited ATP production by 9.0% to 36.9% at dilutions of 1:160 to 1:1. A dilution of 1:640 caused only minimal inhibition.

PEG-35 Castor Oil selectively inhibited the activation of protein kinase C (PKC) at submicromolar concentrations in vitro. In a study

with PKC isolated from human leukemia ML-1 cells, PEG-35 Castor Oil interacted with the main enzyme activator of PKC, diacylglycerol, and prevented the enzyme from binding to and activating PKC. These inhibitory effects were not observed with studies of other polyoxyethylated nonionic solvents, which the investigators believed ruled out the possibility that PEG-35 Castor Oil acted by virtue of a detergent effect (Zhao et al., 1989).

The inhibitory effects of PEG-35 Castor Oil on PKC activity were tested further using 12-*o*-tetradecanolyphorbol-13-acetate (TPA), which mimicked the effects of intracellular diacylglycerol in activating PKC and inducing phosphorylation of cellular proteins. When added

Table 1. Cosmetic product formulation data on the PEG Castor Oil Family

Castor Oil	Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
PEG-30 Castor Oil	Hair dyes and colors (all types requiring caution statements and patch tests)	1437	72
	Cleansing	771	1
	Listing under		4
	1995 Total		***77***
PEG-33 Castor Oil	Hair conditioners	624	1
	Shampoos (noncoloring)	916	2
	Tonics, dressings, and other hair-grooming aids	624	1
	Other hair preparations	382	1
	Other skin-care preparations	782	8
	1995 Total		***13***
PEG-35 Castor Oil	Tonics, dressings, and other hair-grooming aids	624	1
	Face and neck (excluding shaving preparations	261	1
	Skin fresheners	228	1
	Indoor tanning preparations	62	1
	1995 Total		***4***
PEG-36 Castor Oil	Body and hand (excluding shaving)	987	1
	Other skin-care preparations	782	2
	1995 Total		***3***

Table 1. Cosmetic product formulation data on the PEG Castor Oil Family (*continued*)

Castor Oil	Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
PEG-40 Castor Oil	Other bath preparations	144	1
	Eye makeup remover	89	1
	Other fragrance preparations	158	1
	Hair conditioners	693	8
	Hair sprays (aerosol fixatives)	348	2
	Permanent waves	423	10
	Tonics, dressings, and other hair-grooming aids	624	6
	Wave sets	104	3
	Other hair preparations	382	4
	Bath soaps and detergents	339	1
	Cleansing	771	15
	Face and neck (excluding shaving preparations)	261	3
	Body and hand (excluding shaving preparations	987	10
	Moisturizing	873	8
	Night	220	2
	Paste masks (mud packs)	276	8
	Skin fresheners	228	2
	Other skin-care preparations	782	18
	Suntan gels, creams, and liquids	196	2
	Indoor tanning preparations	62	2
	Listing under trade name of mixtures		63
	1995 Total		***170***

Source. FDA (1995).

extracellularly to human myeloblast ML-1 cells, PEG-35 Castor Oil greatly reduced the phosphorylation of proteins induced by TPA. It also inhibited the growth of ML-1 cells dose dependently but had no effect on TPA-induced cell differentiation. Because PKC is involved with cellular regulation, the researchers suggested that inhibition of PKC by PEG-35 Castor Oil may alter cellular functions (Chuang et al., 1991).

PEG-35 Castor Oil also affects the physiologic functioning of renal proximal tubule cells. Primary proximal tubule cells from the kidneys of New Zealand White rabbits were cultured to retain the functional polarity of the proximal tubule, and the effects of 156, 391, and 782

Table 2. Cosmetic product formulation data on the PEG Hydrogenated Castor Oils

Castor oil	Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
PEG-30 Hydrogenated Castor Oil	Deodarants (underarm)	293	1
	Shaving cream	152	1
	Cleansing	771	2
	Moisturizing	873	1
	1995 Total		***5***
PEG-40 Hydrogenated Castor Oils	Other baby products	30	1
	Bubble baths	204	2
	Other bath preparations	144	18
	Eye shadow	597	3
	Eye makeup remover	89	1
	Mascara	211	3
	Other eye makeup preparations	130	2
	Colognes and toilet waters	776	18
	Other fragarance preparations	158	2
	Hair conditioners	693	1
	Hair sprays (aerosol fixatives)	348	3
	Shampoos (noncoloring)	916	2
	Tonics, dressings, and other hair-grooming aaids	624	31
	Wave sets	104	6
	Other hair preparations	382	11
	Hair bleaches	112	2
	Blushers (all types)	283	1
	Face powders	305	3
	Foundations	333	1
	Makeup bases	159	1
	Bath soaps and detergents	339	3
	Deodorants (underarm)	293	2
	Other personal cleanliness products	317	7
	Aftershave lotion	236	22
	Other shaving preparation products	60	4
	Cleansing	771	19
	Face and neck (excluding shaving preparations)	261	3

Table 2. Cosmetic product formulation data on the PEG Hydrogenated Castor Oils (*continued*)

Castor oil	Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
PEG-40 Hydrogenated Castor oils	Body and hand (excluding shaving preparations)	987	32
	Moisturizing	873	9
	Paste masks (mud packs)	276	5
	Skin fresheners	228	11
	Other skin-care preparations	782	21
	Suntan gels, creams, and liquids	196	2
	Indoor tanning preparations	62	3
	Other suntan preparations	62	1
	Listing under trade name		11
	1995 Total		***268***

Source. FDA (1995).

μg/mL PEG-35 Castor Oil on the capacity of these cells to generate a pH gradient was determined. PEG-35 Castor Oil significantly inhibited the development of a pH gradient after 24 h and essentially eliminated it by 72 h. This effect was reversible. A decrease of 15% also occurred in cell viability following 72 h of exposure to 782 μg/mL PEG-35 Castor Oil. The investigators concluded that PEG-35 Castor Oil affected the capacity of rabbit primary proximal tubular cell cultures to carry out a characteristic physiologic function and proposed that the toxicity of the compound itself may contribute to nephrotoxicity observed with cyclosporine A, the drug for which it is a vehicle (Sokol et al., 1990).

PEG-35 Castor Oil also has been reported to affect neurites in vitro. In cultures of differentiating N1E.115 neuroblastoma cells treated with 0.005% PEG-35 Castor Oil, neurite outgrowth was inhibited 50% in serum-free medium. Surviving neurites were shorter in length and were disfigured. Deficits in rapid axonal transport also were detected. The investigators concluded that PEG-35 Castor Oil "impairs neurite outgrowth and disrupts organellar motility in a fashion that might account for neuropathologic effects *in vivo*" (Brat et al., 1992).

The effect of PEG-40 Hydrogenated Castor Oil on epithelial integrity was investigated using monolayers of human intestinal epithelial cells. Specifically, intracellular enzyme activity and morphology were studied, and cell monolayer permeability was determined by measuring the transport of marker molecules and by measuring transepithelial electrical resistance. At concentrations of 0.010

to 7.1 mM, PEG-40 Hydrogenated Castor Oil had no effect on permeability; however, it caused a dose-dependent decrease in dehydrogenase activity, and, at a concentration of 7.1 mM, it caused alterations in a monolayer morphology (Anderberg et al., 1992).

Cytotoxicity

The toxicity of PEG-30 Castor Oil to hepatocytes was investigated by O'Hara et al. (1989). Hepatocytes isolated from male CD rats were treated with 0.063% to 1.0% PEG-30 Castor Oil for 5 h. Cell injury was measured by intracellular K^+, and cell death was measured by lactate dehydrogenase leakage. Cell injury and leakage were observed at concentrations between 0.25% and 1.0% after 5 h. The maximum no-effect concentration was 0.125%.

PEG-35 Castor Oil was cytotoxic to the porcine renal epithelial cell line LLC-PK_1. Using electron and fluorescence microscopy, a reduction in cell adherence at concentrations between 0.01% and 0.1% and alterations in intracellular morphology at lower concentrations were observed. The investigators stated, "This finding supports suggestions based on clinical experience that [PEG-35 Castor Oil] may be responsible for a part of the nephrotoxic effects associated with cyclosporin A treatment" (Nässberger et al., 1991).

Hemodynamic Effects

In Vitro

In a study by Board (1993), PEG-35 Castor Oil inhibited the transport of 2,4-dinitrophenyl glutathione (GSDNP) out of intact human erythrocytes. Approximately 20% of the total transport activity was not inhibited, which the investigators suggested was caused by the inhibitory effect differentiating between glutathione transporters. No hemolysis occurred at concentrations of up to 10% (v/v), the maximum concentration tested. Inhibition was not caused by a depletion of intracellular ATP because studies using a 1:1000 dilution of PEG-35 Castor Oil did not change the intracellular ATP concentrations.

Kongshaug et al. (1991) studied the interaction of PEG-35 Castor Oil with human plasma lipoproteins and nonlipoproteins using ultracentrifugation. They found that at concentrations of 1 to 3 mg/mL PEG-35 Castor Oil associates substantially with low-density lipoproteins; however, such an association was not observed at concentrations between 12 and 116 mg/mL. PEG-35 Castor Oil has a destructive effect on high-density lipoproteins, which was observable at all concentrations tested but was particularly severe at concentrations of 3.6 mg/mL or more PEG-35 Castor Oil. PEG-35 Castor Oil had no observable effect on human serum albumin or other heavy proteins.

The effect of PEG-35 Castor Oil on endothelial function and vascular smooth muscle was investigated using isolated rat hearts. Hearts from AO/Olac rats were perfused using a modified Langendorf preparation (Langendorf, 1935). Groups of eight hearts were perfused with 50, 100, 500, and 1000 ng/mL PEG-35 Castor Oil, and basal coronary flow during perfusion was determined. Each heart was perfused separately with 5-hydroxytryptamine (5-HT) and nitroglycerine (GTN), and coronary flow was monitored to determine the effects of PEG-35 Castor Oil on changes induced by these vasodilators. A control group of hearts was perfused with buffer only, followed by perfusion with the two vasodilators.

At a dose of 50 ng/mL, PEG-35 Castor Oil caused a slight decrease in coronary flow, which was similar to that observed with the control experiment. At greater concentrations, however, PEG-35 Castor Oil caused dose-dependent coronary vasodilation. The greatest increase observed was a 24.8% increase in flow with 1000 ng/mL PEG-35 Castor Oil. No change occurred in the vasodilatory response to 5-HT and GTN in the control experiment; however, in the experiments with 500 and 1000 ng/mL PEG-35 Castor Oil, a significant reduction occurred in the 5-HT response, but no appreciable change occurred in the GTN response. The researchers concluded that PEG-35 Castor Oil caused endothelial dysfunction (Mankad et al., 1992).

In Vivo

The effect of PEG-35 Castor Oil on blood flow to various organs was studied using dogs. A group of five dogs was anesthetized and intravenously injected with 2 mL of a solution containing 1 g/mL PEG-35 Castor Oil over a 90-min period. Cardiac output; mean arterial pressure; and renal, hepatic, and pancreatic blood flow were monitored. A control group of five dogs also was anesthetized but received no infusions. Statistically significant changes were observed in the cardiac output, mean arterial pressure, and hepatic blood flow of the treated group compared with the control group. Curves for cardiac output and mean arterial blood pressure over the 90-min infusion period indicated a marked decrease immediately following the infusion of a few milliliters of PEG-35 Castor Oil, followed by a recovery toward baseline and then another rapid fall with time. A precipitous decline in hepatic blood flow occurred, which returned to baseline rate after approximately 60 min. In the control group, cardiac output, mean arterial blood pressure, and hepatic blood flow were relatively stable. The curve for pancreatic blood flow was similar to the bimodal pattern seen with cardiac output and mean arterial blood flow. The difference between this curve and that of the control group approached, but did not reach, statistical significance. A rapid linear decline occurred in renal blood flow over time in the PEG-35 Castor Oil–treated group, but this decline was not sta-

tistically different from that observed with the control group (Bowers et al., 1991).

In a study with different types of species, PEG-35 Castor Oil depressed the blood pressure of dogs and cats, but not of rabbits, following intravenous administration at concentrations of 30 to 100 mg/kg and 100 mg/kg, respectively. The depressant effects observed with the dogs and cats was thought to be of allergic nature (BASF, no date, a).

Histamine Release

In in vitro studies with rat peritoneal mast cells, PEG-35 Castor Oil alone did not stimulate histamine release (Ennis et al., 1985). When administered in conjunction with histamine-releasing agents, however, PEG-35 Castor Oil potentiated the release of histamine with some agents but inhibited the release with others (Ennis et al., 1986).

PEG-30 Castor Oil (Constantine and Lebel, 1979) and PEG-35 Castor Oil (Lorenz et al., 1977; Lorenz et al., 1982; Ennis et al., 1985) caused histamine release and severe hypotension when administered intravenously to dogs. Oxethylated oleic acid was the most effective constituent of PEG-35 Castor Oil (Lorenz et al., 1977). In studies with miniature pigs, PEG-35 Castor Oil was also a histamine releaser, but only after a second exposure to the compound. In addition, hypertension, as opposed to hypotension, was observed (Glen et al., 1979). Although PEG-35 Castor Oil alone did not cause histamine release in studies with humans (Doenicke et al., 1973; Lorenz, 1975), this compound is believed to cause histamine release when administered in combination with certain anaesthetic drugs (Lorenz, 1975). This effect is thought to play a role in clinical anaphylactoid reactions caused by drugs dissolved in PEG-35 Castor Oil (Lorenz et al., 1982).

Pharmacologic Effects

In Vitro

Multidrug resistance was reversed in vitro by PEG-35 Castor Oil in studies with human myeloma cells (Schuurhuis et al., 1990), Ehrlich ascites tumor cells (Friche et al., 1990), and the R100 and K562 cell lines (Woodcock et al., 1990). PEG-35 Castor Oil is thought to modulate resistance by binding to the plasma membrane P-glycoproteins and preventing the efflux of drugs from cells (Friche et al., 1990; Woodcock et al., 1992).

In Vivo

Antidiuretic effects were observed when Sprague-Dawley rats were orally administered 2.5 mL/kg PEG-35 Castor Oil. The researchers

attributed this observation to the laxative action induced by this compound (Coppi et al., 1971).

ANIMAL TOXICITY

Acute Toxicity

Oral

LD_{50} values of formulations containing 2.0% PEG-25 Hydrogenated Castor Oil and 0.25% PEG-40 Hydrogenated Castor Oil were reported to be more than 15.0 g/kg for rats (CTFA, 1982a; CTFA, 1982b). For a formulation containing 3.0% PEG-60 Hydrogenated Castor Oil, the LD_{50} for rats was more than 5.0 g/kg (CTFA, 1976a).

Intravenous

PEG-35 Castor Oil impaired renal function following intravenous administration in male Wistar rats. Five rats were injected with 0.7 mg/kg/min PEG-35 Castor Oil for 2 h, and a control group of five rats was injected with the same volume of 0.9% NaCl. Control measurements of blood pressure, renal blood flow, creatinine clearance, and urine output were determined prior to infusion. Then blood pressure and renal blood flow were determined five times during infusion and urine was collected at 30-min intervals for clearance determination. A slight decrease occurred in renal blood flow in some of the rats during the first 30 min of infusion, but no changes in arterial blood pressure or creatinine clearance were detected. Renal blood flow and creatinine clearance decreased at 45 min and decreased to 50% of their initial values at 90 min. Arterial blood pressure was only modestly reduced. Urine volume initially increased during the first 30 min of infusion but began to decrease after 45 min. It decreased to less than 50% of initial control values at 105 min, whereas in the control group urine flow doubled. The investigators concluded that PEG-35 Castor Oil caused vasoconstriction of renal arteries, which induced a more than 50% decrease in renal blood flow and glomerular filtration rate without affecting blood pressure (Thiel et al., 1986).

Short-Term Toxicity

Intravenous

Transient cholestasis occurred when PEG-35 Castor Oil was administered intravenously to rats. This condition was both dependent and independent of bile acid secretion and was accompanied by a marked

reduction in bilirubin excretion with no significant changes in serum bilirubin concentrations (Roman et al., 1989).

PEG-30 and PEG-35 Castor Oil (0.5 mL/kg) each were administered intravenously to one male and one female beagle dog. A pair of control dogs was injected with 0.9% NaCl. Injections were performed daily for 30 days. The dogs were observed daily for signs of toxicity, and blood samples were taken after 9, 16, 23, and 31 days. All of the dogs were killed on day 31 for necropsy. Clinical signs of toxicity were observed in both treatment groups and included edematous wrinkling of the skin above the eyes, flushing of the skin of the external ears, and shaking or rubbing of the head. These signs were more pronounced in the dogs treated with PEG-35 Castor Oil. Salivation and rhinorrhea also were observed in both treatment groups but only for the first 10 days. Thrombocytopenia occurred in the dogs treated with PEG-30 Castor Oil and increased platelet counts were observed in the dogs administered PEG-35 Castor Oil. Clinical chemistry changes included increases in serum concentrations of total cholesterol, triglycerides, total lipids, and percentage of chylomicrons. Electrophoretic patterns indicated a decrease in the percentage of α lipoproteins and demonstrated the appearance of a new peak near the origin. Changes in the lipid and lipoprotein values were more marked in the PEG-35 Castor Oil–treated dogs. Excessive amounts of lipid were present in the spleen, lymph nodes, liver, and kidneys at histopathologic examination (Hacker et al., 1981).

Six male and six female rabbits were intravenously injected in the ear vein with 4.0 mL/kg of 25% aq PEG-35 Castor Oil (= 1.0 g/kg) for 5 consecutive days. A control group of two male and two female rabbits was injected with the same volume of saline solution following the same protocol. The animals were weighed daily, and hematologic evaluations were performed prior to the study and on day 5. Two rabbits, one male and one female, were killed on day 5, three male and three female rabbits were killed on day 8, and the remaining rabbits were killed on day 12. There were no clinical signs of toxicity in any of the rabbits. The only significant changes was a decrease in hemoglobin content compared with both initial values and that of the controls. At necropsy, no evidence of lipid accumulation or any other adverse macroscopic changes was present (BASF, no date, b).

A different batch of PEG-35 Castor Oil was tested using similar procedures. A group of two male and two female rabbits was injected in the ear vein with 4.0 mL/kg of 25% PEG-35 Castor Oil (= 1.0 g/kg) on 5 consecutive days. The animals were observed for signs of toxicity during the study and were killed on day 7 for necropsy. No clinical signs of

toxicity were observed. At necropsy, one rabbit had an enlarged spleen, but no significant pathologic changes were observed during macroscopic or microscopic examination (BASF, no date, b).

PEG-40 Hydrogenated Castor Oil was also tested for short-term toxicity. Groups of 60 Sprague-Dawley rats were administered daily injections of 300, 900, and 2700 mg/kg PEG-40 Hydrogenated Castor Oil via the tail vein for 4 wk. A control group of 60 rats was administered saline. At the end of 4 wk, all except 10 animals from each group were killed for necropsy. The remaining animals were maintained for an additional 6 wk without treatment. At doses of 300 and 900 mg/kg PEG-40 Hydrogenated Castor Oil, no systemic toxicity was observed; however, at a dose of 2700 mg/kg, slight ataxia was observed and body weight was reduced significantly in the males and slightly in the females. Feed intake also was reduced accordingly. At the end of 4 wk, the number of reticulocytes was increased but was not significantly different from control values. Microscopic evaluation produced evidence of a storage process in the splenic reticulum, but these effects apparently did not cause functional disturbance. At necropsy, heart weight was increased and ovary weight was reduced in the females. Hemorrhages and thrombosis were observed at the injection sites of both experimental and control animals, and thrombophlebitis was found during microscopic evaluation. These effects were found to be reversible in the animals that were maintained for an additional 6 wk without treatment. Body weights, feed intake, and reticulocyte numbers normalized, and damage at the injection sites healed. Microscopically, the injection sites had no changes, and only slight accumulation in the splenic reticulum was observed (BASF, 1976).

Intramuscular

Dogs (number not specified) were alternately injected intramuscularly in the right and left flank with 1.0 mL of 50% PEG-35 Castor Oil. Each dog was given a total of 11 injections. Spotty reddening of the skin was observed at the injection sites, but no resorptive toxicity or macroscopic lesions were observed (BASF, no date, c).

In another study, rabbits and guinea pigs (numbers not specified) were alternately injected intramuscularly in the right and left flank with 0.5 mL and 0.1 mL PEG-35 Castor Oil, respectively. Both species were given a total of 10 injections. No irritation of the skin or resorptive poisoning was observed. At microscopic examination of the muscle tissue, nonspecific foreign body reaction of resorptive character was found at the injection sites; however, this lesion was transient (BASF, no date, c).

Subchronic Toxicity

Oral

In a 90-day feeding study, groups of 15 Sherman-Wistar rats were fed diets containing 0.01%, 0.04%, 0.16%, 0.64%, 2.5%, and 5.0% (initially 10.0%) PEG-40 Castor Oil. A control group of 30 rats was fed untreated feed. The animals were weighed at weekly intervals and feed intake was measured. Blood samples were taken from two male and two female rats prior to the study and then periodically during the study. After 8 wk on the diet, the lightest two male and two female rats were killed for necropsy and tissues were removed for microscopic examination. At the end of the study, the lightest two male and two female rats also were killed for necropsy. After 1 wk on the diet, the animals of the 10.0% treatment group stopped eating the feed, so the concentration was reduced to 5.0% PEG-40 Castor Oil. Weight gain, feed intake, and hematology results were comparable between the experimental groups and the control group. No significant gross or microscopic lesions were found at either 8 wk or 90 days (Industrial Biology Research and Testing Laboratories, no date, a).

Dogs also were used in a 90-day feeding study with PEG-40 Castor Oil. One beagle each was fed a diet containing 0.04%, 0.64%, or 5.0% PEG-40 Castor Oil. A control group of three dogs was fed untreated feed. The animals were weighed at weekly intervals and feed intake was measured. Blood samples were taken prior to the study and then periodically during the study. At the end of the 90 days, the dogs were killed and necropsy was performed. No significant difference was observed in weight gain, feed intake, or hematologic values between the dogs fed the PEG-40 Castor Oil diet and the untreated control dogs. At necropsy, perilobular cellular infiltration and parasitic granulomas were observed, but these conditions also were present in the control animals and were attributed to parasitic infection and migration rather than a treatment-related effect (Industrial Biology Research and Testing Laboratories, no date, b).

The subchronic toxicity of PEG-40 Hydrogenated Castor Oil also was investigated. Groups of 20 male and 20 female Sprague-Dawley rats were given feed containing 32,000-ppm or 64,000-ppm PEG-40 Hydrogenated Castor Oil. A group of 25 male and 25 female rats was fed a diet containing 100,000-ppm PEG-40 Hydrogenated Castor Oil and a control group of 20 male and 20 female rats was fed untreated feed. All of the animals survived the study period. During the study, no significant changes in feed intake, body weight gain, or hematologic evaluations were observed. When 20 male and 20 female rats from each group were killed for necropsy after 6 mo, body and organ weights were within the parameters of those of the control group and no significant

changes in gross or microscopic lesions were found. The remaining five male and five female rats fed the 100,000-ppm diet were fed untreated feed for an additional 21 days. No signs of toxicity were observed clinically and no lesions were observed at necropsy in these rats (BASF, no date, d).

A summary of a 6-mo feeding study with dogs reported that groups of three male and three female beagle were fed diets containing 1.0%, 2.5%, and 5.0% PEG-40 Hydrogenated Castor Oil. A control group of dogs was given untreated feed. During the study, no significant changes in behavior, feed intake, or body weight gain were observed. Hematologic and biochemical parameters and urine analyses were similar to those of the control group. One male dog of the low-dose group died before termination of the study, but its death was considered unrelated to treatment. When the remaining animals were killed for necropsy at the end of the study, body and organ weights were within normal limits and no gross changes were observed. No lesions were observed in tissues examined microscopically (BASF, no date, e).

A PEG Castor Oil (number of moles of ethylene oxide was not specified) was tested as a vehicle control in a study with CD-1 mice. Forty mice (20 of each sex) were given 10% PEG Castor Oil in their drinking water for 90 days. A nontreated control group was given deionized water. The animals were observed for signs of toxicity twice a day and body weights were taken at weekly intervals. Necropsy was performed on the animals either when they died during the study or when they were killed at the end of the study. Hematology and clinical chemistry determinations also were performed. All of the mice survived until the end of the study. PEG Castor Oil seemed to make the drinking water less palatable, because the mean fluid consumption was significantly less for the treated animals compared with the untreated control mice. Terminal body weights of the PEG Castor Oil–treated group did not differ significantly from the those of the untreated group. The absolute and relative weights of the kidneys and liver were significantly greater, and the weight of the brain was significantly less in the treated group than in the control group. Significant differences in hematology included greater hematocrit and lower polymorphonuclear values in the male mice. Among the clinical chemistry parameters studied, the percentage of calcium and creatinine was greater and the BUN–creatinine ratio was lower in experimental mice of both sexes. Female mice also had greater cholesterol and albumin values, and male mice had greater plasma alkaline phosphatase values. No significant differences between the PEG Castor Oil group and the groups administered a drug dissolved in PEG Castor Oil were observed (Borzelleca et al., 1985).

In a similar study in which PEG Castor Oil (number of moles of ethylene oxide was not specified) was tested as a vehicle control, 10 male and 10 female Sprague-Dawley rats were administered 0.5% PEG Castor Oil in drinking water for 13 wk. All of the rats survived the study. The PEG Castor Oil–treated rats had slightly lower water consumption than did untreated control animals, but no significant differences in body weight gain were observed. The only change in organ weights occurred with the brain weight, expressed as a percentage of body weight, which was lower than that of the untreated group. No significant changes were observed in the biochemical, hematologic, and histologic parameters investigated (Villeneuve et al., 1985).

Dermal

A formulation containing 0.25% PEG-40 Hydrogenated Castor Oil was applied by gentle inunction to the shaved skin on the backs of 10 male and 10 female Sprague-Dawley rats. Applications were made once daily for 5 days-wk over a 13-wk period. The daily dose, 1640 mg/kg/day of the formulation, was considered to be 100 times the average daily use level of the product by consumers. Behavioral observations were made daily, body weight was measured weekly, and blood and urine samples were evaluated during weeks 7 and 13. Clinical chemistry parameters also were monitored. At the end of the study, all of the rats were killed and necropsy performed. No deaths occurred during the study and no treatment-related changes in body weight gain, behavior, hematology, urinalysis, or clinical chemistry parameters were observed. After five doses, minimal irritation and desquamation were observed and persisted until the end of the study. The mean relative hepatic weight for male rats was significantly greater compared with the untreated controls; however, this finding was not considered toxicologically significant because no significant lesions were observed at microscopic examination (CTFA, 1984).

In a similar study, two groups of 10 female ChR-CD rats were given topical applications of 284 or 2840 mg/kg of a formulation containing 3.0% PEG-60 Hydrogenated Castor Oil. Applications were made five times a week for 13 consecutive weeks. As seen in the previous study, the only treatment-related lesions occurred on the skin. Slight erythema and drying of the skin was observed; however, control animals also exhibited these effects. At necropsy, no gross lesions were observed. The hepatic weights of the rats treated with 2840 mg/kg of the formulation and the renal-to-body weight ratio of the rats treated with 284 mg/kg of the formulation were significant. It was noted, however, that these changes were within the normal accepted range for the laboratory and that no significant lesions were observed during histopathologic examination (CTFA, 1977a).

Nephrotoxicity

The isolated perfused rat kidney model was used to assess the acute nephrotoxic effects of cyclosporine and its vehicle, PEG-35 Castor Oil. In this model, the right kidney from male Sprague-Dawley rats was perfused with 100 mL of a perfusate solution at normothermic temperature. After 50 min of perfusion, cyclosporine dissolved in PEG-35 Castor Oil or 200 μL PEG-35 Castor Oil was added to the perfusate, and perfusion was continued for an additional 130 min. Control experiments were conducted with the perfusate alone. Serial determination of renal hemodynamics and tubular function was made over the 3-h period. Marked vasoconstriction occurred following perfusion with PEG-35 Castor Oil, and renal blood flow and glomerular filtration rate were reduced by 45% and 28%, respectively, after 3 h. A statistically significant increase occurred in renal vascular resistance. The investigators concluded that PEG-35 Castor Oil had a direct toxic effect on the tubular cells. Because similar results were observed in tests with cyclosporine dissolved in PEG-35 Castor Oil, the investigators suggested that PEG-35 Castor Oil is a contributing factor in severe acute nephrotoxicity sometimes observed in patients following prolonged treatment with intravenous cyclosporine (Hirsch et al., 1987; Besarab et al., 1987).

Cyclosporine in PEG-35 Castor Oil, diluted in NaCl, was evaluated for nephrotoxicity in a study using male Fisher rats. Three control rats were administered intravenous PEG-35 Castor Oil in NaCl, and six control rats were administered NaCl alone for 15 days (volume of intravenous administration not reported). The rats were killed on day 15, and functional studies (inulin clearance) were performed and microscopic examination of the kidneys conducted. No evidence showed that PEG-35 Castor Oil caused alterations in the glomerular filtration rate. Numerous cytoplasmic dark crystals were observed in the proximal tubules of both groups receiving cyclosporine in PEG-35 Castor Oil and the control group receiving PEG-35 Castor Oil and NaCl. Unlike the rats treated with cyclosporine, the rats administered PEG-35 Castor Oil and NaCl had no vacuolization of the proximal tubules. Because no crystals were observed in the tubules of the rats treated with NaCl alone, the investigators concluded that PEG-35 Castor Oil caused the crystal structures in the proximal tubules (Verani, 1986).

No evidence of nephrotoxic effects were observed with PEG-35 Castor Oil in a study using New Zealand White rabbits. In this study, groups of five rabbits were given daily intravenous injections (1.0 mL) of cyclosporine in PEG-35 Castor Oil diluted in saline for 30 days. One control group of rabbits was administered saline, and another received only PEG-35 Castor Oil. The rabbits were placed in metabolic cages for

24-h urine collections. Serum and whole blood also were collected and analyzed regularly during the study. At the end of the study, cardiac puncture was performed to obtain both heparinized whole blood and serum for creatinine and cyclosporine determinations. Tissue samples were taken from different regions of each kidney and microscopic and ultrastructural evaluations were performed. Reductions in creatinine clearance and the development of leukocyte infiltrates, tubular atrophy, and interstitial fibrosis of the kidneys were observed in rabbits treated with cyclosporine. Structural changes also were observed in specimens examined by light and electron microscopy; however no morphologic or functional changes were observed in the rabbits treated with PEG-35 Castor Oil alone. The investigators concluded that PEG-35 Castor Oil did not contribute to the compromise in renal structure and function observed with cyclosporine administration (Thliveris et al., 1991).

Anaphylactoid Reactions

Twenty-percent PEG-35 Castor Oil was administered intravenously to dogs at a constant 30 mL/h; the infusion was stopped when the systolic arterial pressure decreased by more than 50% of the control. This treatment induced a significant decrease in blood pressure and cardiac output associated with massive increases in plasma histamine and catecholamine. The investigators suggested that the large increase in histamine release was indicative of acute mast cell degranulation. Thoracopulmonary compliance decreased rapidly and was reduced markedly by the end of infusion. A significant reduction in blood volume, which was caused by a decrease in plasma volume, was observed. Hematocrit increased significantly by the end of infusion, whereas platelet and leukocyte counts sharply decreased. It was also noted that 6 of 13 dogs had cutaneous erythema and edema of their paws and muzzle. The investigators concluded that 20% PEG-35 Castor Oil induced cardiovascular collapse, and that PEG-35 Castor Oil–induced shock "is of the anaphylactoid type, and includes cutaneous erythema and edema, hypotension, venous pooling, plasma extravasation, histamine and catecholamine release, and decreases in dynamic thoracopulmonary compliance and leucocyte and platelet counts" (Gaudy et al., 1987).

Dermal Irritation

When undiluted PEG-35 Castor Oil was applied to the shaved backs or to the external ears of albino rabbits for more than 20 h (experimental details not provided), slight transient irritation was reported (BASF, no date, c).

Undiluted PEG-40 Hydrogenated Castor Oil reportedly caused reddening and scaling of the skin when applied to the backs of albino rabbits for 20 h (experimental details not provided). Only slight transient reddening was reported when applications were made to the external ears of rabbits for 20 h (BASF, no date, c).

The primary skin irritation potential of a formulation containing 2.0% PEG-25 Hydrogenated Castor Oil was minimal. Only one of nine rabbits had evidence of erythema 24 h after a single application of the formulation under an occlusive patch (amount not stated). The overall primary irritation index (PII) was 0.11/8 (CTFA, 1982c). For formulations containing either 0.25% PEG-40 Hydrogenated Castor Oil or 3.0% PEG-60 Hydrogenated Castor Oil, the PIIs were 0.22/8 and 0.67/8, respectively (CTFA, 1982d; CTFA, 1976b).

Dermal Sensitization

The flanks of 10 guinea pigs were shaved, degreased with ether, and 50% PEG-35 Castor Oil in acetone was applied to the left flank of each animal (painted three times sequentially with a saturated cotton swab) for 10 consecutive days. The right flanks were left untreated. After a 12-day nontreatment period, 5% PEG-35 Castor Oil in acetone was applied to the right flank (as above, after degreasing with ether). The skin was observed for signs of irritation after 12 h. A control group of three guinea pigs was untreated during the induction phase of the experiment but was given a single application of 5% PEG-35 Castor Oil. Slight reddening of the skin was observed during induction with PEG-35 Castor Oil, but no signs of irritation were observed after the challenge application. When this study was duplicated, the same results were obtained (BASF, no date, f).

In a subcutaneous sensitization study, 10 daily injections with 0.1% PEG-35 Castor Oil (1 × 0.05 mL; 9 × 0.1 mL) were administered to guinea pigs (number not specified) in their backs. After a 13-day nontreatment period, a challenge injection of 0.1% PEG-35 Castor Oil (1 × 0.05 mL) was administered into the neck. Reddening occurred around the injection sites during induction, but no sign of sensitization was present (BASF, no date, f).

Tachon et al. (1983) also assessed the allergenic potential of PEG-35 Castor Oil. Ten consecutive intradermal injections of 0.5 mL PEG-35 Castor Oil were performed to the backs of 10 male and 10 female Dunkin-Hartley guinea pigs, and an occlusive patch was applied for 48 h. After a 12-day nontreatment period, the animals were challenged with 0.5 mL PEG-35 Castor Oil on their abdomens. When macroscopic evaluations were performed 48 h following challenge, 60% of the male and 50% of the female guinea pigs had doubtful reactions; however, no

evidence of sensitization was observed at microscopic examination. The investigators concluded that PEG-35 Castor Oil was not a sensitizing agent.

Adjuvancy

Descotes et al. (1983) conducted three studies to determine the adjuvancy of PEG-35 Castor Oil. In the first study, groups of six female Dunkin-Harley guinea pigs were injected with 100 μg bovine serum albumin (BSA) in 0.05 mL PEG-35 Castor Oil. A positive control groups of animals was injected with BSA in Freund's complete adjuvant (FCA), and a negative control group of animals was treated with BSA in saline. Three weeks later the animals were intradermally injected in the right flank with BSA in saline. Skin evaluations were made at 2, 24, and 48 h. PEG-35 Castor Oil caused erythema and skin induration that was of the same severity as seen with FCA.

In the second study, groups of 10 Swiss mice were injected subcutaneously in the back with 10^8 sheep erythrocytes in either 0.005 or 0.01 mL PEG-35 Castor Oil in saline. A control group of mice was injected with sheep erythrocytes in saline. Five days later, the mice were administered an eliciting dose of 10^8 sheep erythrocytes in the hind footpad. The percent increase in footpad swelling was taken as a measure of delayed hypersensitivity. Both doses of PEG-35 Castor Oil caused significant increases in footpad thickness.

In the third study, 10^9 sheep erythrocytes in 0.01 and 0.05 mL PEG-35 Castor Oil in saline was administered intraperitoneally to 10 Swiss mice. A control group of mice was treated with erythrocytes in saline only. Eight days later, blood samples were taken from the mice to measure hemagglutinin concentrations. PEG-35 Castor Oil had no effect on antibody concentrations. Based on the three studies, the investigators concluded that PEG-35 Castor Oil is "a potent adjuvant of cellular immune response."

Ocular Irritation

A 5% active solution (pH = 6–8) of PEG-5 Hydrogenated Castor Oil (w/w) (0.1 mL) was instilled into the left conjunctival sac of six New Zealand white rabbits. Three of the eyes were rinsed after 30 sec. The rights eyes left untreated served as controls. Scoring according to the method of Draize (1944) was made at 24, 48, and 72 h postinstillation. Slight to mild corneal opacity was observed in two unrinsed eyes at 24 h and persisted until 72 h. None of the other treated eyes had evidence of corneal damage. Slight iridial damage was observed in one unrinsed eye at 24 and 48 h, and in another unrinsed eye at 48 and 72 h. Iridial

damage also was observed in one rinsed eye at 24 h, but it cleared by 72 h. At 24 h, all of the treated eyes had mild to moderate conjunctival irritation manifest as hyperemia, chemosis, and discharge. One rinsed eye and one unrinsed eye cleared by 72 h, whereas the remaining eyes were irritated through 72 h. The 24-h maximum mean total score was 36.3/110 for the unrinsed eyes and 13.7/110 for the rinsed eyes. The investigators concluded that PEG-5 Hydrogenated Castor Oil was severely irritating to unrinsed eyes and mildly irritating to rinsed eyes (Product Safety Labs, 1988).

When 50 mm^3 of 50% aq PEG-35 Castor Oil in acetone was instilled into the conjunctival sac of rabbits (number not specified), lacrimation and mild irritation of the conjunctiva were observed. A 30% aq solution did not cause any signs of irritation (BASF, no date, c).

Undiluted and 50% aq PEG-40 Hydrogenated Castor Oil was instilled (0.05 mL) into the conjunctival sacs of rabbits (number not specified), and observations were made at 24 and 48 h. Slight transient reddening of the conjunctiva was observed with both concentrations (BASF, no date, c).

No irritation was observed when the eyes of six rabbits were instilled (volume not given) with a formulation containing 2.0% PEG-25 Hydrogenated Castor Oil (CTFA, 1982e). In a similar study, a formulation containing 0.25% PEG-40 Hydrogenated Castor Oil caused mild transient irritation. The total irritation scores on days 1 to 3 postinstillation were 1/110. All eyes were clear by day 4 (CTFA, 1982f).

A formulation containing 3.0% PEG-60 Hydrogenated Castor Oil (volume not given) caused minimal irritation to the eyes of 2 of 6 rabbits. The irritation score for both rabbits was 2/110 24 h after instillation. All signs of irritation disappeared by 48 h (CTFA, 1976c).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

PEG-40 Hydrogenated Castor Oil was tested for teratogenic effects in a feeding study with Sprague-Dawley rats. Two groups of pregnant rats, 30 in one group and 27 in the other, were fed diets containing 50,000 ppm or 100,000 ppm PEG-40 Hydrogenated Castor Oil, respectively, on days 0 to 20 of gestation. Two control groups of 26 and 29 rats were fed untreated feed. All of the animals were observed for signs of toxicity during gestation and were killed on day 20 for evaluation of the uteri. No evidence of either maternal or fetal toxicity was present. A slight but not statistically significant increase occurred in the number of resorptions in the group treated with 100,000 ppm. The type and number of malformations and anomalies found in the fetuses of the experimental groups were similar to those found among the fetuses from the control groups. The investigators concluded

that PEG-40 Hydrogenated Castor Oil was not teratogenic (BASF, no date, g).

Negative results were also obtained in a teratogenicity study with NMRI mice. Two groups of pregnant mice, 25 in one group and 31 in the other, were fed diets containing either 5000 ppm or 10,000 ppm PEG-40 Hydrogenated Castor Oil on days 6 to 15 of gestation. Two groups of 26 and 28 mice were given untreated feed. There was no statistically significant evidence of either maternal or fetal toxicity. The few malformations observed among the fetuses of the treated dams were similar to type and number to those found in the control groups (BASF, no date, h).

Lane et al. (1982) tested PEG-30 Castor Oil as a vehicle control in a multigeneration study that was modified to include a screening for dominant lethal and teratogenic effects. Ten male and 30 female ICR Swiss mice (F/0) were administered 1% PEG-30 Castor Oil in their drinking water continuously throughout the study. After 35 days on the test solution, the mice were randomly mated to produced F/1A litters. Two weeks following the weaning of the F/1A litters, the F/0 mice were rerandomized and mated to produce F/1B litters. Then the F/0 mice were mated randomly again 2 weeks after the weaning of the F/1B litters to produce F/1C litters.

Ten male and 30 female weanling mice from the F/1B litters also were administered 1% solutions of PEG-30 Castor Oil in their drinking water and were mated in nonsibling matches to produce F/2A litters. Body weight and fluid consumption, as well as fertility and gestational indices, were determined regularly for the parental mice. Twenty-one day survival studies were conducted on the litters from the F/1A, F/1B, and F/2A matings.

F/1C and F/2B matings also were produced to screen for dominant lethal and teratology effects. In these studies, female mice were killed during gestation and the number of fetal implants, early and late resorptions, viable fetuses, and dominant lethal factors were determined. Fetuses were removed and individually evaluated for gross defects, and one third of them were examined for skeletal and visceral malformations.

No significant changes in reproductive performance were observed in any of the matings. Mean litter size, postnatal body weights, and survival indices also were unaffected. The only significant change observed in both the dominant lethal and teratology screenings was an increase in the ratio of dead fetuses to live fetuses.

PEG-35 Castor Oil also was tested as a solvent control in a teratogenicity study. Groups of pregnant ICR and C57B1/10Dg mice (numbers not specified) were orally given 0.05 mL/10 g body weight 8% PEG-35 Castor Oil and 10% propylene glycol in water on either day 9, 10, or

11 of gestation. An untreated control group of pregnant mice was used for comparison. All of the mice were killed on day 18 of gestation, and the fetuses and placentas were removed for examination. PEG-35 Castor Oil did not have any significant effects on growth or development in any of the treatment groups (Cusic and Dagg, 1984, 1985).

In the following studies, PEG-30 Castor Oil and PEG-35 Castor Oil were tested as negative vehicle control substances. No untreated control animals were used for comparison.

Burkhalter and Balster (1979) used PEG-30 Castor Oil as a vehicle control in a behavioral development evaluation. Male and female albino ICR mice were given daily oral doses of 10 mL/kg of a solution containing one part PEG-30 Castor Oil and eight parts saline. The mice were mated after 3 wk of treatment with PEG-30 Castor Oil, and the dams continued to receive daily doses of PEG-30 Castor Oil through gestation and lactation. A total of five litters was used. Each of these litters was randomly reduced to eight pups, and oral administration of PEG-30 Castor Oil to the pups was initiated 7 d following birth and continued for the remainder of the study. On days 7 to 21, the pups were weighed, and a battery of tests to determine neurobehavioral development was conducted. These tests included measurement of righting reflex, forepaw grasp, rooting reflex, cliff-drop aversion, auditory startle response, bar-holding ability, eye opening, motor performance and learning measures, and placing and grasping responses. In general, the pups experienced gradual weight gain and progressive neurobehavioral development.

PEG-35 Castor Oil was used as the vehicle control in a study using embryo cultures. Embryos from pregnant Swiss Webster mice were removed on day 8.5 of gestation and grown in culture containing 130 μg/mL PEG-35 Castor Oil for 24 h. At the end of culture, the embryos were evaluated for viability and only viable embryos were examined for malformations. Of the 44 embryos examined, only three had abnormalities, including defect of the neural tube, facial arch, and cranial rotation. The mean somite number was 24.2, mean crown-rump length was 2.25, and mean protein content was 86.5 μg per embryo (Uhing et al., 1993).

MUTAGENICITY

Au et al. (1991) investigated the clastogenic and coclastogenic activity of PEG-35 Castor Oil using male ICR mice. Cytogenetic and metabolite analyses were conducted with groups of five mice given 0.1 mL/g of 0.03%, 0.3%, or 3.0% PEG-35 Castor Oil orally. Other groups of mice were treated with benzene in olive oil or benzene in combination with the various doses of PEG-35 Castor Oil. An untreated control group and

a vehicle control (olive oil) group also were used. The mice in the single-treatment groups were killed at 30 h, and the mice receiving the combined treatment were killed after the first treatment. Bone-marrow samples were harvested at the end of the study and evaluated for micronuclei (MN) frequencies in polychromatic erythrocytes (PCE).

The investigators also tested the effect of PEG-35 Castor Oil in hepatic cytochrome P450 isoenzyme expression in liver. Male Swiss albino CD1 mice were given 0.01 mL/g body weight 3% PEG-35 Castor Oil in water orally. Another group of mice was given benzene followed by 3% PEG-35 Castor Oil 1, 3, and 5 h later. The mice given PEG-35 Castor Oil only were killed 1, 3, 5, 15, or 30 h after treatment, whereas the mice treated with both PEG-35 Castor Oil and benzene were killed after 30 h. The livers were removed from all of the mice for evaluation.

PEG-35 Castor Oil did not cause any significant or dose-dependent increses in MN but did enhance significantly the clastogenicity of benzene. When PEG-35 Castor Oil was administered 1, 3, and 5 h after benzene treatment, an inverse time-dependent change occurred in MN frequencies. The enhancement effects of PEG-35 Castor Oil were attributed to its ability to induce the cytochrome P450I family when it was administered 1 h after benzene treatment. No positive synergistic effect was observed when PEG-35 Castor Oil was administered at later intervals. The investigators also noted an increase in *trans,trans* muconic acid (a genotoxic metabolite of benzene) in the urine following combined treatment.

PEG-30 Castor Oil and PEG-35 Castor Oil also were used as negative vehicle controls in several studies. PEG-30 Castor Oil was used in a chromosomal aberration assay using Chinese hamster ovary (CHO) cells and micronucleus and spermhead abnormality assays with mice (Blazak et al., 1988). Machemer and Lorke (1978) used PEG-35 Castor Oil in dominant lethal tests on male and female mice, micronucleus tests on male and female mice, and a spermatogonial test using Chinese hamsters. PEG-35 Castor Oil also was used in a study to detect sex-linked recessive lethals in *Drosophila* spermatozoa (Kortselius, 1978). In all of these studies, known mutagens were used as positive controls and had significant evidence of mutagenicity compared with the PEG-30 Castor Oil and PEG-35 Castor Oil vehicle controls.

CARCINOGENICITY

In the following studies, the PEG Castor Oils were used as vehicle control substances, and no untreated control groups were used.

In an oral carcinogenicity study of several agents, PEG-30 Castor Oil was used as a vehicle control. Male Sprague-Dawley rats were given 1

mL of 10% PEG-30 Castor Oil by gavage three times a week for 16 wk and then once per week for an additional 10 wk. All of the rats were killed during week 77 and necropsy was performed. No untreated control group of animals was used. Of the 29 rats examined, the following neoplasms (and number of neoplasms) were found: benign liver tumor (1), keratoacanthoma (1), pituitary adenomas (4), prostate carcinoma in situ (1), Leydig cell tumor of the testis (1), ear spindle cell sarcoma (1), pancreatic islet cell adenoma (1), spleen lymphomas (2), mammary fibroma (1), subcutaneous myxolipoma (1), subcutaneous fibromas (2), and adrenal adenoma (1). No comment was made by the investigators regarding the normal range of occurrence of these tumors in their historical data base (Fiala et al., 1987).

In another study, a PEG Castor Oil (number of moles of ethylene oxide was unspecified) was used as a vehicle control in a lung adenoma assay using A/J mice. Two groups of 20 female mice were given either 0.2 mL of 2% PEG Castor Oil three times a week for 8 wk or 0.2 mL of 2% PEG Castor Oil twice each dosing day following the same dosing schedule. A positive control group of 40 mice was given benzo[*a*]pyrene (BaP) in PEG Castor Oil following the first dosing schedule. No untreated control group was used. All of the animals were killed 8 mo after the first dose and were examined for neoplasms. The neoplastic response was not significantly different between the two PEG Castor Oil groups, so the data were pooled. Only two of the mice died during the study. Twenty-nine percent of the mice had lung neoplasms, and the average number of neoplasms per mouse was 0.32. None of the mice had squamous cell papillomas or carcinomas of the nonglandular stomach. Of the BaP-treated group, four mice died, 61% of the mice had lung neoplasms, the average number of neoplasms per mouse was 1.42, and 92% of the mice had squamous cell neoplasms of the nonglandular stomach (Robinson et al., 1987).

In a tumor-promotion study, a 2% solution of PEG Castor Oil (unspecified number of moles of ethylene oxide) was used as a vehicle control. PEG Castor Oil (0.2 mL) was orally administered to 110 female SENCAR mice three times a week for 2 wk. After a nontreatment period of 2 wk, 1.0 μg of TPA in acetone was topically applied to the mice three times per week for 20 wk. No untreated control group of animals was used. At the end of the study, 15% of the mice had neoplasms. A total of 20 neoplasms was present, and the neoplasms–animal ratio was 0.18. The investigators also reported the result of 90 mice treated with dimethylsulfoxide followed by TPA treatment: 6% of the mice had neoplasms, there were a total of 5 neoplasms, and the neoplasm–animal ratio was 0.06 (Robinson et al., 1989).

CLINICAL STUDIES

Hemodynamics

Eight men who were previously tested intravenously with a drug dissolved in 20% PEG-35 Castor Oil were given 0.15 mL/kg PEG-35 Castor Oil intravenously over a 10-sec infusion period. Blood samples were taken 1, 5, 10, 20, and 30 min postinjection for histamine analysis, and blood pressure and heart rate was monitored. No increase in plasma histamine concentrations or effects on blood pressure and heart rate were detected (Doenicke et al., 1973).

Dermal Irritation

Twenty subjects were patch tested with 30% PEG-35 Castor Oil in water and 100% PEG-40 Hydrogenated Castor Oil on the skin of their backs. Observations were made after 24 and 48 h. No signs of irritation were observed (University Clinic Eppendorf, 1951–1954).

A 24-h single insult patch test of a formulation containing 2% PEG-25 Hydrogenated Castor Oil was conducted using 20 subjects. One subject developed mild erythema and two subjects had barely perceptible reactions (CTFA, 1982g). In a similar study, a formulation containing 0.25% PEG-40 Hydrogenated Castor Oil caused 1 of 20 subjects to develop a mild reaction to the formulation (CTFA, 1981).

The cumulative irritation potential of a formulation containing 3% PEG-60 Hydrogenated Castor Oil was conducted using 12 volunteers. Occlusive patches of 0.2 mL of the formulation were applied to the backs of each subject for 23 h for 21 consecutive days. Test sites were scored 24 h after each application. The composite total score was 22/756. The investigators concluded that this formulation was essentially nonirritating (Hill Top Research, 1976).

Dermal Irritation and Sensitization

A formulation containing 0.05% PEG-40 Hydrogenated Castor Oil was tested in a repeated insult patch test using 120 volunteers. The formulation (0.10 mL) was applied under occlusive patches to the backs of each subject for 24 h on Mondays, Wednesdays, and Fridays for 3 wk. After a 2-wk nontreatment period, challenge patches of the formulation were applied to previously untreated sites.

Five subjects had one incidence each of barely perceptible erythema during the induction phase of the study. One of these subjects also had a mild reaction to the challenge application at both the 24-h and 48-h readings. One subject, who showed no reaction during the induction phase, had a barely perceptible reaction at the 48-h challenge reading.

Follow-up testing of these two subjects was conducted using the formulation "as is" and at a 1:3 dilution in water. Reactivity was not confirmed in one subject, but the other subject had a very weak reaction to the "as-is" formulation at the 24-h grading period. The investigators noted that this reactivity was much less than at challenge and was of questionable clinical significance. They concluded that this formulation was not an allergic sensitizer (CTFA, 1977b).

Using the same procedures, a formulation containing 0.25% PEG-40 Hydrogenated Castor Oil was tested for allergic contact sensitization potential on 86 subjects. Two subjects had minimal irritation during the induction phase of the study, but neither reacted to the challenge patch. One subject, who had no signs of irritation during induction, had faint erythema at the 24-h grading period only. The investigators concluded that this formulation was not a sensitizer (CTFA, 1982h).

A formulation containing 3.0% PEG-60 Hydrogenated Castor Oil also was tested using the same repeated insult patch procedures with 102 subjects. No signs of irritation were observed in any of the subjects durng induction. Only one doubtful reaction was observed at the 48-h reading after challenge. Follow-up testing of this subject with the formulation and a 1:3 dilution of the formulation was negative (CTFA, 1976d).

Jones and Kennedy (1988) reported two cases of eczema from a topical medicament used to treat leg ulcers. Patch tests with the constituents of this cream implicated PEG-40 Castor Oil (0.1% and 1% pet.) as the sensitizing agent. This ingredient induced severe grade-3 reactions at 48 and 96 h in both patients. The allergenicity of these reactions was supported by tests with 10 control patients, who had negative reactions to 0.1% and 1% PEG-40 Castor Oil.

Anaphylactoid Reactions

Several case studies of anaphylactoid reactions associated with intravenous administration of drugs dissolved in PEG-35 Castor Oil have been reported. In all of these cases, PEG-35 Castor Oil could not be diretly implicated as the cause for the adverse reaction; however, such factors as tolerance of the drugs alone when administered orally, treatment with drugs from the same family not dissolved in PEG-35 Castor Oil, and similar in several types of drugs in which PEG-35 Castor Oil was the solvent strongly suggest that PEG-35 Castor Oil was the cause of the anaphylaxis.

Most of the case reports are of intravenous treatment with cyclosporine dissolved in PEG-35 Castor Oil (Van Hooff et al., 1987; Magalini et al., 1986; Ptachcinski et al., 1985; Howrie et al., 1985; Chapuis et al., 1985; Leunissen et al., 1985; Friedman et al., 1985;

Kahan et al., 1984). In general, subjects experienced flushing, bronchospasm, dyspnea, chest pains, pruritus, urticaria, and hypotension within minutes of injection. Reactions were documented occurring both after the first dose and after multiple doses. In some instances, the researchers were able to determine that the subjects had previous exposure to other drugs dissolved in PEG-35 Castor Oil.

Intravenous treatment with vitamin K_1 (phytonadione) also has been linked with anaphylactoid reactions (de la Rubia et al., 1989; Lefrere and Girot, 1987; Rich and Drage, 1982; Barash et al., 1976). Within minutes of a bolus injection, patients developed facial flushing, hypotension, chest pain, dyspnea, and abdominal pain. In the five cases reported, four subjects were administered the drug an undiluted and one was administered a diluted form. In one case, anaphylactoid reactions were prevented in a subsequent injection by using a diluted form of the drug and administering it at a slower infusion rate. Three of the cases occurred on the first injection, and two occurred after a second injection, which resulted in one death.

The anesthetic drug, Althesin, which was a combination of alphaxalone and alphadolone, was withdrawn from the market in 1983 because of the high incidence of anaphylactic reactions to the solvent, PEG-35 Castor Oil (Smolinske, 1992).

Anaphylactic reaction also have been associated with intravenous treatments with diazepam (Hüttel et al., 1980), teniposide (Siddal et al., 1989), and disoprofol (Briggs et al., 1982).

The mechanism behind these types of reactions is not clear. Doenicke et al. (1973) reported that although histamine release occurs in patients treated with drugs dissolved in PEG-35 Castor Oil, this vehicle alone did not induce histamine release. It is believed that this compound causes histamine release when administered in combination with certain anaesthetic drugs (Lorenz, 1975). Watkins et al. (1976) also proposed that the surfactant properties of PEG-35 Castor Oil enhance the immunogenicity of concurrently administered drugs.

In a study by Radford et al. (1982), activation of the alternative complement pathway was associated with subjects reacting to first exposure to Althesin, whereas the activation of the classic complement pathway was associated with reactions to a repeat exposure to the drug. In general, patients reacting to a repeat exposure to the drug have more severe clinical reactions, which are immunologically related.

In a 10-year survey of 118 patients with Althesin-related hypersensitivity, 1% of the cases were classified as immunoglobin E–mediated type I hypersensitivity, 36% to immune complement-mediated reactions involving other antibodies, 40% to alternate pathway complement C3 activation, and 23% to mixed reactions (Watkins, 1986).

SUMMARY

PEG-30, -33, -35, -36, and -40 Castor Oil and PEG-30 and -40 Hydrogenated Castor Oil are polyethylene glycol derivatives of castor oil and hydrogenated castor oil that are used in a variety of cosmetic products as emulsifiers or solubilizing agents. Formulation data submitted to the FDA in 1995 reported a total of 540 cosmetic formulations containing these ingredients.

At the cellular level, PEG-35 Castor Oil affects a variety of cellular functions, including ATP production, PKC activation, renal proximal tubule cell function, and neurite outgrowth. PEG-40 Hydrogenated Castor Oil affected the integrity of human epithelial cells.

In the pharmaceutical industry, PEG-35 Castor Oil and PEG-40 Castor Oil are commonly used as solvents for intravenous drugs. Therefore, much of the toxicity data available on this family of ingredients are specifically on intravenous use.

Hemodynamic studies indicate that intravenous exposure to PEG-35 Castor Oil causes alterations in cardiac output, blood pressure, blood flow to various organs, and histamine release. Endothelial dysfunction of isolated rat hearts also was altered by exposure to PEG-35 Castor Oil.

The oral LD_{50} of two cosmetic formulations containing either 2.0% PEG-25 Hydrogenated Castor Oil or 0.25% PEG-40 Hydrogenated Castor Oil was reported to be more than 15.0 g/kg for rats. For a formulation containing 3.0% PEG-60 Hydrogenated Castor Oil, the LD_{50} for rats was more than 5.0 g/kg.

PEG-35 Castor Oil causes acute nephrotoxicity in rats. In the isolated perfused rat kidney model, this ingredient induced vasoconstriction and reduced renal blood flow and glomerular filtration rate in rats. Another study reported that PEG-35 Castor Oil caused the development of crystals in the proximal tubules of rats; however, no nephrotoxic effects were observed in a study using rabbits.

Impairment of renal function also was observed in acute intravenous studies of PEG-35 Castor Oil using rats.

In short-term studies, the toxicity of PEG-35 Castor Oil to dogs following intravenous exposure was greater than that of PEG-30 Castor Oil. Changes in lipid and lipoprotein values and accumulation of lipid in the spleen, lymph nodes, liver, and kidneys were observed; however, no toxicity or any adverse macroscopic or microscopic changes were observed in two studies using rabbits. A study of PEG-40 Hydrogenated Castor Oil produced some evidence of toxicity as well as a storage process in the splenic reticulum, but no functional disturbance occurred.

Following repeated intramuscular injections with 50% PEG-35 Castor Oil, no significant signs of toxicity were observed in dogs. Negative results also were obtained in studies with rabbits and guinea pigs.

No significant signs of toxicity were observed in subchronic oral studies of 5% PEG-40 Castor Oil using rats and dogs, 100,000-ppm PEG-40 Hydrogenated Castor Oil using rats, and 5% PEG-40 Hydrogenated Castor Oil using dogs. Results were also negative in subchronic dermal studies using rats for formulations containing 0.25% PEG-40 Hydrogenated Castor Oil or 3.0% PEG-60 Hydrogenated Castor Oil.

Undiluted PEG-35 Castor Oil and PEG-40 Hydrogenated Castor Oil caused mild transient dermal irritation when applied to the skin of rabbits. Primary irritation studies of formulations containing either 2.0% PEG-25 Hydrogenated Castor Oil or 0.25% PEG-40 Hydrogenated Castor Oil produced minimal signs of irritation.

In a sensitization study with guinea pigs, 50% PEG-35 Castor Oil caused irritation during the induction phase of the experiment, but no sensitization was observed following a challenge application of 5% PEG-35 Castor Oil. Similar results were obtained in intradermal studies.

Some evidence showed that PEG-35 Castor Oil was a potent adjuvant of cellular immune response in studies using guinea pigs and mice.

No ocular irritation was observed in rabbits with either 30% aq PEG-35 Castor Oil or a formulation containing 2.0% PEG-25 Hydrogenated Castor Oil. Slight, transient ocular irritation was observed with undiluted and 50% aq PEG-40 Hydrogenated Castor Oil and with formulations containing either 0.25% PEG-40 Hydrogenated Castor Oil or 3% PEG-60 Hydrogenated Castor Oil.

A diet of 100,000-ppm PEG-40 Hydrogenated Castor Oil fed to pregnant rats through 20 days of gestation did not cause teratogenic effects to the fetuses. Similarly, no teratogenic effects were observed when pregnant mice were fed 10,000-ppm PEG-40 Hydrogenated Castor Oil.

In reproductive and developmental toxicity studies in which 1% PEG-30 and 8% PEG-35 Castor Oil were used as vehicle controls, adverse effects on fertility or development following oral administration were not observed.

PEG-35 Castor Oil did not cause any significant clastogenic effects in mice but did produce coclastogenic effects when administered in combination with benzene. PEG-30 and PEG-35 Castor Oil were used as negative vehicle controls in a variety of mutagenicity assays. In these studies, known mutagens had significant evidence of mutagenicity compared with these vehicles.

The only available data on the carcinogenic potential of the PEG Castor Oils were from studies in which these ingredients were used as vehicle controls. In clinical studies, PEG-35 Castor Oil had no effect on the plasma histamine concentration of men following intravenous administration.

A 30% solution of PEG-35 Castor Oil and 100% PEG-40 Hydrogenated Castor Oil were not irritating to the skin of 20 subjects. Negative results also were obtained in single 24-h insult patch tests with formulations containing either 2.0% PEG-25 Hydrogenated Castor Oil or 0.25% PEG-40 Hydrogenated Castor Oil. In a cumulative irritation study, a formulation containing 3% PEG-60 Hydrogenated Castor Oil was nonirritating.

No evidence of sensitization was shown in clinical studies with formulations containing either 0.05% or 0.25% PEG-40 Hydrogenated Castor Oil, or 3.0% PEG-60 Hydrogenated Castor Oil.

PEG-35 Castor Oil has been associated with case reports of anaphylactoid reactions following intravenous administration of drugs dissolved in this vehicle. Although PEG-35 Castor Oil has not been directly implicated as the cause for these types of reactions, factors such as tolerance of the drugs alone when administered orally, treatment with drugs from the same family not dissolved in PEG-35 Castor Oil, and similar reactions observed in several types of drugs in which PEG-35 Castor Oil was the solvent strongly suggest that PEG-35 Castor Oil was the cause of the anaphylactic reactions.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel reviewed the available safety data on the PEG Castor Oils and the PEG Hydrogenated Castor Oils and agreed that these ingredients seem to have little toxicity. The only adverse reactions observed were anaphylactoid reactions in intravenous studies. Because this route of exposure does not occur from cosmetic use, the Panel was not concerned about this type of risk. The Panel did express concern over the lack of current concentration of use data. It was agreed that concentration limits should be based on the available test data on irritation and sensitization. The highest concentration tested yielding negative results for the PEG Castor Oil family was 50% PEG-35 Castor Oil in a sensitization study with guinea pigs. For the PEG Hydrogenated Castor Oil family, undiluted PEG-40 Hydrogenated Castor Oil was negative in animal and clinical irritation studies. The Panel agreed that the chemical similarity of these two ingredients to the other ingredients in their respective families allows for extrapolation of the concentration limits to these other ingredients.

CONCLUSION

Based on the irritation and sensitization data presented in this report, the CIR Expert Panel concludes that PEG-30, -33, -35, -36, and -40 Castor Oil are safe for use in cosmetics at concentrations up to 50% and that PEG-30 and -40 Hydrogenated Castor Oil are safe for use at concentrations of up to 100%.

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