Toxicological Aspects of Chemical Penetration Enhancers

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25.1 Introduction

Dermal delivery is perhaps one of the oldest methods of drug administration. In ancient times cutaneous wounds and other skin problems were treated with animal fats and resins. The dermal as well as the transdermal drug delivery concept is based on the ability of certain substances to increase the cutaneous permeability. The skin is the largest organ of the body in mammals, and it is also the first line of defense against foreign materials. For this reason not all substances can reach the body system through intact skin. The skin is composed of three layers, epidermis being the outermost layer, which is supported by a nourishing layer of fibroelastic tissue called the dermis, and a variable deep layer, composed mainly of adipose tissue called the hypodermis or subcutis. The epidermis is composed of several layers (called strata) of epithelial cells. The *stratum corneum* is the most external stratum of the epidermis. This is composed of a thick lamella of keratin and keratinized cells embedded in lipid matrix,

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which function as a practically impermeable barrier that makes it difficult for dermal and transdermal delivery of drugs. The goal of a penetration enhancer is to increase the permeability of the *stratum corneum* without altering the physical integrity of this layer. In order to meet this goal, chemical penetration enhancers have been used, which must have specific characteristics, such as being pharmacologically inert, nonirritant, nonimmunogenic (non-allergenic or non-sensitizing), and nontoxic to the skin or to the body system as a whole. Although all the mentioned aspects are important, the main objective of this chapter is to describe some of the reported toxicological features for a few of the currently most utilized skin penetration enhancers. Among the most used penetration enhancers are the following chemical classes: terpenes, fatty acids, fatty alcohols, alcohols, glycols, laurocapram (Azone®), sulfoxides, pyrrolidones, and surfactants.

25.2 Terpenes

Terpenes are natural substances contained in plant essential oils. These compounds are designated by the general formula $(C_5H_8)_n$. However, these nonaromatic compounds may also include oxygen atoms. This class of substances may provide the right balance between safety and penetration-enhancing ability (Ahad et al. 2009 and Narishetty and Panchagnula 2004a). The mechanism of action of terpenes is based either on their ability to increase the *stratum corneum* lipid fluidity or on the perturbation of the barrier integrity of the *stratum corneum* (Gao and Singh 1998). Some potential mechanisms by which terpenes operate is by disrupting the bilayer lipids in the cellular membrane of keratinocytes or by their interaction with intracellular proteins (Williams and Barry 2012). The terpenes are widely used in the pharmaceutical and food industry, mainly as medicines, flavoring, and fragrance agents. Based on its penetration-enhancing ability, eucalyptol (1,8-cineole) has been reported to be one of the most potent terpenes (Ahad et al. 2009 and Narishetty and Panchagnula 2004a). Cineole's major use in the pharmaceutical industry is as a flavoring in expectorants and cough syrups. It is used in the cosmetic industry as a flavoring in toothpastes and mouthwashes, as well as in perfumery. It is also used in the food industry (e.g., chewing gum and ice cream) (Medi et al. 2006). This substance was the most promising chemical penetration enhancer among terpenes when tested in vitro across rat skin for propanolol, indomethacin, imipramine, urea, and zidovudine (Amnuaikit et al. 2005; Ogiso et al. 1995; Narishetty and Panchagnula 2004b; Jain et al. 2002). Cineole may induce several systemic reactions in humans when ingestion or exposure to vapors occurs. Among the reactions that have been reported in humans is transient coma with symptoms including epigastric burning, nausea, vomiting, vertigo, ataxia, muscle weakness, occasional cyanosis, respiratory edema, miosis, and convulsions (Gosselin et al. 1976). Other effects include fever, albuminuria, painful urination, hematuria, transient renal lesions, and respiratory failure with death as final outcome (Gosselin et al. 1976). Fatalities have occurred with oral doses as low as 3.5 mL given neat. However, recovery has occurred after an oral dose as high as 30 mL (Gosselin et al. 1976). The probable lethal oral dose in humans is 50–500 mg/kg (between 1 teaspoon and one ounce for a 70 kg person). Exposure to vapor causes irritation to the human eye, which is perceptible at 175 ppm in air, and is reported to be unpleasant at 720–1,100 ppm. Slight transient damage to corneal epithelium may be induced by direct contact with cineole (Grant 1986). Apparently nonlethal doses of this terpene induce hepatic microsomal enzymes (Gosselin et al. 1976). In general, 1,8-cineole was of low to moderate acute oral toxicity in rodents, causing central nervous system and respiratory effects and inducing increased hepatic enzyme activity and bile secretion. According to De Vincenzi et al. (2002), the mice are less susceptible than rats to the toxicity of 1,8-cineole. This author found that toxicity may be induced in rats dosed orally with this terpene at 600 mg/kg, while a similar toxic profile may be induced in mice when dosed orally at 1200 mg/kg (De Vincenzi et al. 2002). The design of this study did not allow the establishment of a no observable effect level (NOEL) or no observable adverse effect level

(NOAEL) for cineole in mice or rats. The oral LD_{50} of eucalyptol in rats is 2480 mg/kg. In mice the lowest published lethal dose (LD_{50}) was established at 50 mg/kg when dosed subcutaneously, and LD_{50s} of 100 and 2500 mg/kg were observed when dosed by intramuscular or oral routes, respectively (NTP-PAFA 2006). In dogs the LD_{50} was established at 1500 mg/kg when administered subcutaneously (NTP 1991). In rats, eucalyptol induces increased levels of glucuronyl transferase with no changes in histopathology such as no observed effects on the size of liver cell endoplasmic reticulum. The activity of other liver enzymes, including P450 cytochromes CYP3A1, CYP3A2, CYP2B1, CYP2B2, methoxyresorufin O-demethylase (MROD), benzyloxyresorufin O-debenzylase (BROD), and pentoxyresorufin O-depenylase (PROD), was increased in male rats when dosed orally at 400 mg/kg of eucalyptol (NTP-PAFA 2006). In a different study, performed also in rats, 1,8-cineole increased the production of β-2 microglobulin in the kidney of male rats when administered orally for 28 days at a dose of 500 mg/kg (Kristiansen and Madsen 1995). The genotoxic potential of 1,8-cineole was assessed in AMES (bacterial reverse mutation test or *Salmonella*/microsome test) (Haworth et al. 1983), chromosomal aberration, and sister chromatid exchange tests (Galloway et al. 1987), with negative genotoxicity for the first two and positive results for the last test. Cineole was not carcinogenic when dosed at 12 g/kg for 8 weeks in a mouse primary lung tumor model (Stoner et al. 1973.). Cineole showed teratogenic effects in rats when dosed at 2 g/kg during gestation days 19–22 (NTP-PAFA 2006).

Regardless of the potential systemic toxic effects of eucalyptol (1,8-cineole), this terpene was not irritant when applied in solution to the skin of human volunteers or when applied to rabbit skin. However, nasal irritation and rapid heartbeat occurred in some children after its direct instillation into the nostrils. Cineole also induced allergic skin reactions in several turpentine-sensitive subjects (BIBRA 1991). These reactions may be explained by the association of 1,8-cineole-induced itching in mice with the mast cell degranulation, which possibly involves opioidergic and adenosinergic mechanisms (Santos and Rao 2002). In summary, 1,8-cineole is well tolerated when applied onto the skin, but it may cause low to moderate systemic effects when ingested or administered via subcutaneous or intramuscular injection.

L-Menthol is another terpene that is widely used as a flavoring, disinfectant, and cooling compound in confectionary products, liquors, chewing gums, toothpastes, cosmetics, and common cold ointments for human purposes (OECD-SIDS 2003). In countries such as Canada, l-menthol is registered for control of mites in apiculture (OECD-SIDS 2003).

L-menthol is a food ingredient generally recognized as safe (GRAS), and not much monitoring of its toxicological potential is performed. The most common routes of consumer exposure to l-menthol are the oral and dermal; mainly when mentholated products are used. Other routes of exposure are inhalation from mentholated cigarettes or mentholated cleaning products. Maximum usage levels in food, cosmetic, and pharmaceutical products have been established. The recommended levels for the addition of l-menthol to products are up to 2 % for oral care products, up to 4 % for pharmaceuticals, up to 0.45 % to cigarettes, up to 0.3 % to tobacco, and up to 1 % to perfumed products (OECD-SIDS 2003).

In humans the l-menthol is well absorbed by the oral route. The absorption through skin is slower than oral absorption. It appears that absorption by inhalation is also very efficient (OECD-SIDS 2003). The toxicological potential of l-menthol has been studied in humans and in laboratory species. In rats, skin irritation, such as slight subepidermal edema and swelling in collagen fibers, was observed at 5 % l-menthol formulations (Narishetty and Panchagnula 2004b). In humans, the ingestion of high doses of l-menthol may cause abdominal pain, convulsions, nausea, vomiting, vertigo, ataxia, drowsiness, and coma (OECD-SIDS 2003). Some of these symptoms were observed in children ingesting about 200–250 mg/kg. The symptoms were fully reversible by 4 days post exposure (OECD-SIDS 2003). The ingestion of

8000–9000 mg of l-menthol by three volunteers (corresponding dose of approximately 120 mg/kg) caused a cold burning sensation in the mouth, throat, and esophagus; a cold sensation in mucous membranes of the nose and on the skin of the hand and feet; and fatigue (OECD-SIDS 2003). Mild abdominal discomfort was reported when l-menthol was dosed at 20 mg/kg (OECD-SIDS 2003). Adverse neurological effects (central nervous system or CNS) were observed in a 13-year-old boy exposed by to approximately 200 mg of menthol by inhalation (O'Mullane et al. 1982). Similar effects were reported in a woman after smoking 80 mentholated cigarettes for 3 months. This woman showed insomnia, unsteady gait, mental confusion, depression, vomiting, and cramp in the legs (Luke 1962). Reflex apnea was induced in children after administration of l-menthol in their nostrils. The clinical manifestations were laryngospasm, spasm of the glottis or instant collapse, dyspnea, apnea, unconsciousness, cyanosis, and hyperextended extremities (OECD-SIDS 2003). It is possible that these symptoms are not a result of a direct poisoning effect, but a reflex reaction of the trigeminal nerve (OECD-SIDS 2003).

In laboratory animals, the liver weights of rats dosed orally with menthol at a dose of 200 mg/kg for 28 days were increased, and a non-dose-related vacuolization of hepatocytes was also reported. No toxicological effects were observed at doses lower than 200 mg/kg. However, it was not possible to establish a NOEL in this study. Therefore, the 200 mg/kg should be the established NOAEL in this study, based on the described hepatic effects (OECD-SIDS 2003). Rats exposed for 71–79 days to l-menthol vapors presented pulmonary and tracheal irritation, but no toxicological systemic effects (OECD-SIDS 2003). In subchronic studies (lasting for 13 weeks) performed in rats and mice, this compound did not induce any alterations in organ weights when dosed up to 937/998 mg/kg in male/female rats or up to 3913/4773 mg/kg in male/female mice. A slight increase in severity of spontaneous interstitial nephritis was reported after microscopic examination of kidneys from the male rats dosed at the highest dose level. Reduction in body weight gain was the only effect observed in mice when dosed at the highest dose. The NOAELs derived from these studies were 937 and 998 mg/kg for the male and female rats, respectively. The NOAELs in mice were 1956 and 2386 mg/kg in males and females, respectively (OECD-SIDS 2003).

A chronic study (103 weeks) in rats dosed orally with l-menthol (about 188 and 375 mg/kg/day) reported minimal test article-related effects that included a slight increase in spontaneous chronic nephritis in male rats at both dose levels and a slightly reduced body weight in females. The NOAELs in this study were 375 mg/kg in the males and 188 mg/kg in the females (OECD-SIDS 2003). A NOAEL of 667 mg/kg was established in a chronic study performed in mice dosed orally for 103 weeks with d-/l-menthol at dose levels of 334 and 667 mg/kg (OECD-SIDS 2003).

Menthol has been reported to be non-genotoxic and noncarcinogenic based on results from standard genotoxicity and carcinogenicity tests. No information exists regarding the potential of l-menthol to induce reproductive toxicity. However, this compound proved to be non-teratogenic when administered orally to species such as rat, rabbit, mouse, and hamster at doses ranging from 185 to 425 mg/kg, which are not maternally toxic doses (OECD-SIDS 2003). All menthol isomers present a similar safety-toxicology profile.

In summary, terpenes are penetration enhancers with an acceptable safety profile when administered onto the skin or when administered at low-dose levels orally or by inhalation in adults and individuals provided they are not allergic to terpenes or to the delivered compounds. Based on results of some studies reported above, special care is needed when children or susceptible individuals are exposed to these compounds by inhalation or by ingestion of pure compound.

25.3 Fatty Acids

Fatty acids consist of long hydrocarbon chains with a terminal carboxyl group. Lauric acid, myristic acid, palmitic acid, linolenic acid, and oleic acid are some of the fatty acids that have

been used to increase the percutaneous absorption of hydrophilic and lipophilic drugs. Oleic acid is perhaps the most popular of these long-chain fatty acids. Oleic acid is an 18-carbon *cis*-mono unsaturated fatty acid. This review will be focused on the toxicological potential of some of the fatty acids used as penetration enhancers.

Increased cutaneous penetration of a wide variety of drugs has been demonstrated when oleic acid is used as an enhancer. Among those drugs are 5-fluorouracil, salicylic acid, tamoxifen, estradiol, and progesterone (Gao and Singh 1998). Although extensive efforts to define the mechanism of action of this penetration enhancer have been devoted, it has not been possible to fully describe how oleic acid can increase the permeability of the *stratum corneum*. However, based on existing reports, it is clear that the penetration-enhancing effect of this enhancer on human skin is in part due to the interaction with and the modification of lipid domains in the *stratum corneum*, as would be expected for a long-chain fatty acid with a *cis* configuration (Barry 1991). This interaction and modification of lipids in the *stratum corneum* consequently increases the drug flux in the epidermis (Barry 1991). Touitou and collaborators (2002) demonstrated by scanning electron microscopy that skin treated with 10 % oleic acid in ethanolic solution induced the generation of pores on the surface of epidermal keratinocytes (Touitou et al. 2002).

The potential toxicity of oleic acid and other fatty acids has also been studied. Erythema and edema are the most common effects reported after cutaneous administration of lauric acid, palmitic acid, myristic acid, stearic acid, or oleic acid in 5 % (w/v) alcohol solutions (Liebert 1987). Boelsma and collaborators (1996) reported that oleic acid induced skin irritation and inflammatory infiltrate as consequence of cytokine activation (Boelsma et al. 1996). Other effects in the skin are follicular epidermal hyperplasia produced after daily (6 times per week for 1 month) topical application of undiluted commercial grade oleic acid in mice (Liebert 1987). Myristic acid induced thinning of collagen fibers and dermal lymphocytic-histiocytic infiltration after topical administration in depilated skin of rabbits (Liebert 1987). Local cutaneous edema was induced by stearic acid in rats after topical application of a cosmetic containing 2 % of this acid. The same effect was observed in rabbits receiving 20 applications of 2 mL/kg of 2 % stearic acid (Liebert 1987). The topical application of lauric acid in intact and abraded skin of rabbits induced erythema at 72 h posttreatment and also induced blanching and coriaceous tissue in the abraded areas.

Studies to assess photosensitization have been conducted using variable concentrations (from 25 to 100 %) of stearic acid topically applied to guinea pigs. No photosensitizing effect was found at any of the dose levels tested (Liebert 1987).

The potential of oleic acid to induce skin comedones was evaluated. Very large comedones were induced by UVA- irradiated and nonirradiated oleic acid in New Zealand white rabbits. In this study, the lipid peroxide concentration was positively correlated with the degree of comedones formation (Liebert 1987).

Studies have been conducted to assess the potential of fatty acids to induce ocular irritation. No irritation or minimal ocular irritation was produced in rabbits after ophthalmic administration of 0.1 mL of oleic acid. The ocular instillation of commercial grade lauric acid caused corneal opacity, mild conjunctivitis, and iritis during the first 72 h post dosing. However, this effect was not observed when the same acid was administered as an 8.7 % aqueous dilution in rabbits or as 1 % aqueous preparation of a soap containing 1.95 % of the acid. No ocular irritation was induced by administration of commercial grade palmitic or stearic acid in rabbits, while slight conjunctival irritation was induced by administration of commercial grade myristic acid in the same animal species (Liebert 1987).

In studies administering oleic acid by ways different than topical administration, other effects associated to the test article were reported. The subcutaneous administration of oleic acid at volumes from 0.25 to 0.5 mL for 400 days had no adverse effects in the growth of treated albino mice. However, the life duration of both males and females was lower than the life duration in control animals. Nonclinical toxicity values have been established for oleic acid in different

laboratory species. An oral LD_{50} of 74 g/kg was established in rats. The LD_{50} is reduced to 2.4 mg/kg when dosed intravenously (IV) in the same species. The IV LD_{50} in mice is 230 mg/kg, while the dermal LD_{50} in guinea pig is higher than 3000 mg/kg (HSDB 2008). Acute exposure to doses as high as 21.5 mL/kg of oleic acid and up to 10 g/kg of commercial grade lauric, palmitic, and myristic acids by oral gavage to rats resulted in no deaths and no significant findings at necropsy (HSDB 2008). Severe pulmonary damage (mainly pulmonary edema) and death were induced by IV injection of oleic acid at 42 mg/kg in rats (HSDB 2008). In dogs, the repeated injection of oleic acid at 0.09 g/kg over a period of 1–3 months induced significant pulmonary changes, including thrombosis, and cellular necrosis. In a different study using dogs, severe pulmonary edema was induced following an IV injection of 60 mg/kg of oleic acid (HSDB 2008).

The subchronic and perchronic oral exposure to oleic acid caused deterioration on health and subsequent death after 17 weeks of continuous oral treatment of rabbits at a dose of 4.5 g/kg/day, while normal growth and health was reported in rats orally dosed with 7.5 g/kg/day for up to 24 weeks (HSDB 2008).

The genotoxic and carcinogenic potential of oleic acid has also been assessed by several authors. All the published results report a negative genotoxic potential of oleic acid when tested in bacterial and mammal systems. No carcinogenic or potentiating carcinogenic effect was identified when mice or rats were dosed orally with oleic acid or with a combination of this acid with a tumorogenic substance such as benzo(a)pyrene. Therefore, it was concluded by different authors that oleic acid is not tumorogenic (HSDB 2008).

No evidence of maternal or fetal toxicity (teratogenicity) was identified in a reproductive toxicity assessment of oleic acid dosed topically in rats. Although the oral administration of this enhancer did not affect the fertility of male rats, apparently it impaired the reproductive capacity in females by interfering with parturition and mammary gland development when dosed at 7.5 g/kg/day in diet for 16 weeks in Sprague-Dawley rats (HSDB 2008).

In summary fatty acids have a good safety profile when administered topically onto the skin or even into the eye, causing only minimal or slight transient irritation. No severe systemic effects have been reported when oleic acid is administered orally; however, severe pulmonary effects can be induced by intravenous administration of these compounds.

25.4 Fatty Alcohols, Ethanol, and Glycols

25.4.1 Fatty Alcohols

Fatty alcohols are substances used as penetration enhancers for several drugs. Studies using different fatty alcohols have been performed in order to assess their penetration potential using melatonin as a permeant through porcine and human skin in vitro (Williams and Barry 2012). Fatty alcohols from octanol to myristyl alcohol have been evaluated. However, the skin irritation induced by saturated fatty alcohols has not been studied widely (Kanikkannan and Singh 2002). In one of the few published reports, Kanikkannan and collaborators assessed the skin irritation potential of several fatty alcohols in rat hairless skin. Using erythema as a skin irritation indicator, lauryl alcohol proved to be the most irritant of the tested fatty alcohols, which included myristyl alcohol, tridecanol, decanol, undecanol, nonanol, and octanol (Kanikkannan and Singh 2002). However, lauryl alcohol induced a lower transepidermal water loss (TEWL) than other fatty alcohols such as myristyl alcohol and undecanol. The authors concluded that octanol and nonanol were less irritant than the other fatty alcohols tested when used to increase the permeation of melatonin in hairless rat skin in vivo (Kanikkannan and Singh 2002).

25.4.2 Ethanol

Ethanol is commonly used in many transdermal formulations and is often the solvent of choice for use in patches and other products with direct exposure to skin. Ethanol can act as a cutaneous

penetration enhancer through several potential mechanisms of action. Among those mechanisms is the volatile capacity of the ethanol, which may remove some of the lipid fraction from the *stratum corneum* when used at high concentrations, and for prolonged times, this mechanism may improve drug flux through skin. The solvent features of ethanol can increase the solubility of the drug in vehicle, increasing penetration of the drug. Moreover, it is also possible that the rapid permeation of ethanol or its evaporative loss from the donor phase modifies the thermodynamic activity of the drug in the formulation (Williams and Barry 2012). Ethanol has been used to enhance the penetration of several compounds such as levonorgestrel, estradiol, hydrocortisone, imipramine hydrochloride, naloxone, zidovudine, and 5-fluoroacil in rat skin and of estradiol in human skin in vivo (Panchagnula et al. 2001; Jain et al. 2002; Williams and Barry 2012). Although several reports exist in the scientific literature regarding the safety of ethanol, there is no up-to-date risk assessment of ethanol application on the skin. The main concern of topical ethanol applications for human health is its potential carcinogenic effect because there is ambiguous evidence for the carcinogenicity of ethanol when orally consumed in alcoholic beverages. However, there is no evidence which associates the topical administration of ethanol with an increased risk of cutaneous cancer (Lachenmeier 2008). Even though there is no evidence which links the cutaneous administration of ethanol with increased risk for cancer in skin, other factors such as synergy with other chemicals should be taken into consideration. Therefore, each formulation containing ethanol should be evaluated for its carcinogenic potential.

The use of ethanol is associated with skin irritation or contact dermatitis, especially in individuals with an aldehyde dehydrogenase (ALDH) deficiency. It has been demonstrated that relatively low blood concentrations of ethanol and its metabolite acetaldehyde may be measured after regular cutaneous application of ethanol. However, those measurable levels are below the toxicological threshold. Percutaneous toxicity after application of ethanol through lacerated skin has been reported to be possible, especially in children (Lachenmeier 2008). Some side effects of the transdermal patches using ethanol as permeation enhancer have been reported. Among those side effects are cutaneous intolerance (manifested by signs such as erythema) and allergic contact dermatitis, which in some regard have been associated with ethanol as one of the causal agents. However, in some of the reported cases, the combination of effects between the different components of the dosed preparations cannot be ruled out. Therefore, it is inconclusive whether ethanol or other agents in the products that come in contact with skin were the real causes for the side effects observed (Heard et al. 2006; Heard and Screen 2008). In other studies, it was not possible to detect changes in the TEWL after the topical administration of ethanol (Loffler et al. 2007; Kramer et al. 2002).

In an exceptional review published by Lachenmeier and collaborators in 2008, the authors draft a list of conclusions regarding the safety of topical administration of ethanol. Among those conclusions the authors mention the following: (A) Topically applied ethanol on un-lacerated human skin will not cause acute or systemic toxic effects, which can only occur if applied on damaged skin; especially in children. (B) Adverse effects of topically applied ethanol may include skin irritations to allergic contact dermatitis. (C) Ethanol and its metabolite acetaldehyde are potentially carcinogenic for humans; however, only limited evidence supports the carcinogenicity of mouthwashes and a complete lack of data about the carcinogenicity of all other groups of products. (D) Further concerns include the permeation-enhancing capabilities of ethanol, which could lead to an increased absorption of other components of topically applied formulations. (E) Safety assessments of ethanol in any form of application must include the carcinogenic and genotoxic properties of ethanol and its metabolite acetaldehyde.

In summary, ethanol is a compound that is widely used as penetration enhancer for several drugs. Although there are some concerns regarding the potential carcinogenic effect of topical administration of ethanol, no evidence which

could demonstrate this effect has been found. Therefore, the main proved concerns regarding potential toxicity of topical administration of ethanol are those related to local skin or eye irritation.

25.4.3 Glycols (Propylene Glycol (PG) and Polypropylene Glycol (PPG))

The most known compound among the glycols is propylene glycol (PG), whose activity is thought to result from solvation of α-keratin within the *stratum corneum* (Ahad et al. 2009). PG has been used as a stand-alone penetration enhancer and also widely used as a vehicle for penetration enhancers and shows synergistic action when used with, for example, oleic acid (Williams and Barry 2012). However, even though several pharmaceutical formulations contain unsaturated fatty acids such as oleic acid and PG, the general use of this combination is limited due to the high prevalence of dermal side effects including *stratum corneum* lipid extraction and damage to viable epidermal cells, which presumably occurs because of the acidic nature of the fatty acids (Sintov et al. 1999; Touitou et al. 2002).

The toxicology of PGs and PPGs has been assessed in several studies. Sax and collaborators in 1979 (CIR 1994) reported an acute oral LD_{50} of 21 g/kg for PG in five female Fischer strain rats, while in 1976 Bartsch reported an acute oral LD_{50} of 25 mL/kg in 10 male and female Sprague-Dawley rats (CIR 1994). In the Sprague-Dawley rat, the LD_{50} of PG is reduced to 13 g/kg or to 6.2 mL/kg after intraperitoneal or intravenous administration, respectively (CIR 1994). In specific pathogen-free NMRI (SPF-NMRI) mice, the LD_{50} reported by Bartsch and collaborators is 6.4 mL/kg (CIR 1994).

Significant decreases in the levels of fibrinogen, albumin, and globulin in plasma were detected in Wistar strain rats after the acute oral administration of PG at 9.66, 19.32, or 38.64 mmol/kg. These results suggest that PG may affect the hepatic function in either the synthesis or the secretion of proteins in the treated rats (CIR 1994). Lethality has also been reported in horses. After an accidental oral administration of 3.8 L of PG to a 400–500 kg male horse, signs of ataxia, pain, salivation, and excessive sweating were observed within 10–15 min post dosing. All signs, with exception of ataxia, resolved within 5 min. However, the next day, the animal became increasingly ataxic and died of apparent respiratory arrest. Death occurred approximately 28 h after exposure. No macroscopic changes were observed at necropsy. There was a PG concentration of 9,000 mg/L in serum and 7,500 mg/L in renal fluids (combined blood and urine). Histological changes included moderate myocardial perivascular edema with dilation of lymphatics and moderate pulmonary edema characterized by proteinaceous material in alveoli and in some of the bronchioles. Hepatic lesions consisted of scattered single-cell hepatocytic necrosis and minimal acute suppurative pericholangitis. Peracute renal infarcts characterized by multiple linear areas of coagulative tubular necrosis were also observed (CIR 1994).

For polypropylene glycols (PPGs), the acute toxicity of PPGs of various molecular weights was evaluated in Sherman strain rats. The mean LD_{50} values were 2.91 g/kg for polypropylene glycol 425 (PPG 425), 2.15 g/kg for polypropylene glycol 1025 (PPG 1025), and 9.76 g/kg for polypropylene glycol 2025 (PPG 2025), respectively. The animals treated with any of the mentioned PPGs showed sluggishness, prostration, tremors, convulsions, and rapid death. At necropsy, the following macroscopic changes were observed: minor pulmonary hemorrhage, congestion in the liver and spleen, and renal ischemia (CIR 1994). In a different study, male rats received different oral doses of 10 % aqueous PPG 1200 and the resulting LD_{50} was 640 mg/kg. Additional studies assessing the oral toxicity on PPGs of various molecular weights have reported LD_{50} values in rats ranging from 0.5 to more than 40 g/kg.

The acute oral toxicity of PPG 1200 was tested in dogs. A subconvulsant effect was observed after administration of 50 mg/kg (CIR 1994). In guinea pigs the PPG 1200 LD_{50} value for males and females was 1,320 mg/kg.

Additional acute oral toxicity studies in guinea pig have reported LD_{50} values ranging from 1.5 to 17 g/kg (CIR 1994).

The acute toxicity for PPGs using parenteral administration has also been calculated. Shaffer and collaborators in 1951 reported LD_{50} values of 0.46 g/kg for PPG 425, 0.23 g/kg for PPG 1025, and 4.47 g/kg for PPG 2025 when dosed via intraperitoneal route to Sherman rats. The same authors reported LD_{50} values of 0.41 g/kg for PPG 425, 0.12 g/kg for PPG 1025, and 0.71 g/kg for PPG 2025 when the compounds were administered via intravenous into Sherman rats (CIR 1994). Death was observed in all treated groups regardless of the route of administration. Death was preceded by tremors, prostration, frothing at mouth, and audible rales (CIR 1994).

Studies in mice involving intraperitoneal dosing of various PPGs reported LD_{50} values of 700 mg/kg, 195 mg/kg, 12 mg/kg, and 3,600 mg/kg for PPG 400, PP G750, PPG 1200, and PPG 2000, respectively (CIR 1994).

In dogs, the intravenous administration of an aqueous solution of PPG 400 at 10–20 mg/kg resulted in tremors with convulsions. Convulsions were also observed in dogs dosed IV with PPG 750 at 8–15 mg/kg. Convulsions and death were observed in dogs treated IV with 20 mg/kg of PPG 750. The IV administration of PPG 1200 elicits tremors or convulsions when dosed at 7 or 15 mg/kg, respectively, while convulsions and death were observed in dogs dosed at 20 mg/kg with the same compound. No adverse effects were observed in dogs dosed IV with PPG 2000 at 100 mg/kg (CIR 1994).

The assessment of the acute toxicity of PPG 1200 when injected IM into dogs resulted in subconvulsant effects or mild convulsions in dogs treated with 45 or 60 mg/kg, respectively (CIR 1994).

The subchronic toxicity of PG was assessed in rats dosed IV for three consecutive days with 100 % PG at 0.75, 1.5, or 3 mL/kg. Slight hyperemia of the intestinal mucosa was the most prominent finding in animals dosed with the lowest or the highest dose. Similar changes were reported in dogs dosed with 100 % PG at 1.5 mL/kg (CIR 1994). Cats dosed with 41 % PG in dry feed developed moderate polyuria and polydipsia. These changes are consistent with renal excretion of PG, which acts as an osmotic diuretic. Other signs in these cats were decreased activity, mental depression, and slight to moderate ataxia, which may be related to the metabolism of PG to D-lactate (CIR 1994).

No significant adverse effects were reported in subchronic oral studies using rats dosed with PG. The most significant effects observed were related to the urinary system and were characterized by hematuria (CIR 1994).

The acute dermal toxicity of PG in rabbits was 20,800 mg/kg. PG is essentially nonirritating to the skin and mildly irritating to the eyes. Several studies support that PG is not a skin sensitizer (CIR 1994; OECD-SIDS 2001). Repeated exposures of rats to PG in drinking water or feed did not result in adverse effects at levels up to 10 % in water (estimated at about 10 g/kg/day) or 5 % in feed (dosage reported as 2.5 g/kg/day) for periods up to 2 years. In cats, two studies of at least 90 days duration show that a species-specific effect of increased Heinz bodies was observed (NOAEL = 80 mg/kg/day; LOAEL = 443 mg/kg/day), with other hematological effects (decrease in number of erythrocytes and erythrocyte survival) reported at higher doses (6–12 % in diet, or 3.7–10.1 g/cat/day). PG did not cause fetal or developmental toxicity in rats, mice, rabbits, or hamsters (NOAELs range from 1.2 to 1.6 g/kg/day in four species). No reproductive effects were found when PG was administered at up to 5 % in the drinking water (reported as 10.1 g/kg/day) of mice. PG was not genotoxic as demonstrated by a battery of in vivo (micronucleus, dominant lethal, chromosome aberration) and in vitro (bacterial and mammalian cells and cultures) studies. No increase in tumors was found in all tissues examined when PG was administered in the diet of rats (2.5 g/kg/day for 2 years) or applied to the skin of female rats (100 % PG; total dose not reported; 14 months) or mice (mouse dose estimated at about 2 g/kg/week; lifetime). These data support lack of carcinogenicity for PG (OECD-SIDS 2001).

In summary, after an analysis of a series of laboratory studies (some of those described above), the Cosmetic Ingredient Review (CIR)

expert panel issued several conclusions regarding the safety of PG. Among the studies performed were acute, subchronic and short-term animal studies, which suggested little toxicity beyond slight growth and body weight decreases. Little ocular or skin irritation and no sensitization were observed in experimental animals. Small increases in fetal malformations were seen in mice injected subcutaneously with PG, but a continuous breeding reproduction study in mice showed no reproductive toxicity following oral administration. The compound was also negative in a wide range of mutagenesis studies, and no carcinogenicity potential was detected in mice and rat studies. Clinical data in humans showed skin irritation and sensitization reactions to PG in normal subjects at concentrations as low as 10 % under occlusive conditions and dermatitis patients at concentrations as low as 2 %. In these studies, test concentrations ranged from 2 to 100 %. Reactions were observed at concentrations as low as 10 % in predictive tests and as low as 2 % in provocative tests. Based on these results the general conclusion of the experts was, "A concentration limit for propylene glycol is considered necessary." It was decided that the 50 % concentration is safe for the use in cosmetics. Moreover, a more recent review by another panel agreed with what had been concluded earlier. This review panel stated: "True allergic reactions to propylene glycol are uncommon and the clinical significance has probably been overestimated". Additional studies have been performed after these reviews. In two repeated insult patch tests, no indication of allergy was noted upon challenge. The incidence of irritation responses was 1 in 104 tested individuals. These findings support the conclusion issued by the FDA's experts, in regard to the safety of the use of PG in cosmetics up to concentrations of 50 % (CIR 1994).

25.5 Azone® (Laurocapram)

Azone® (1-dodecylazacycloheptan-2-one, laurocapram), along with its derivates, is probably the most investigated penetration enhancers and is effective at low concentrations for both lipophilic and hydrophilic drugs but suffers from toxicity problems. The enhancing effect of laurocapram (Azone®) is attributed to different mechanisms, such as insertion of its dodecyl group into the intercellular lipidic bilayer, increase of the motion of the alkylic chains of lipids, and fluidization of the hydrophobic regions of the lamellate structure (Lopez-Cervantes et al. 2006). It is thought that the penetration-enhancing effects of Azone® are exerted by interacting with the intercellular lipids of the *stratum corneum* causing an increase in their fluidity. The effects on the lipid bilayer are considered to be partially due to its C12 alkyl chain, which helps it insert among the acyl chain of lipids in the bilayers of the intercorneocyte space (Lopez-Cervantes et al. 2006). The enhanced fluidity of intercellular lipids is thought to facilitate the diffusion of the drug molecules through the hydrocarbon chains of the lipid bilayer in the *stratum corneum* (Lopez-Cervantes et al. 2006).

In humans, pure Azone® is poorly absorbed through skin, and the limited amount of Azone® absorbed is cleared rapidly by the kidneys (Wiechers et al. 1987). There have been several studies to assess the irritant potential of Azone®. The results of those studies are contrasting, some reporting that Azone® is a moderate irritant and others reporting that is not irritating (Barry and Bennett 1987; Lopez-Cervantes et al. 2006). In rats, it has been demonstrated that Azone® causes a minimal skin irritation and enhances the TEWL values (Fang et al. 2003a). It is possible that the skin irritation caused by the Azone® is the main reason for not using it as a penetration enhancer in transdermal systems.

Toxicological studies reveal a low toxicity for laurocapram, and for some derivates, a relationship exists between toxicity and the number of carbons in the alkyl chain. Laurocapram is minimally absorbed through the skin and rapidly eliminated from circulation in humans (Lopez-Cervantes et al. 2006). In cutaneous studies in rats, it was demonstrated that morphological microscopic changes in the skin after treatment with Azone® were located in the epidermis. Proliferation was observed in the superficial layer of the skin. The epidermis had increased numbers

of neutrophils and lymphocytes, indicating the inflammation of keratinocytes (Fang et al. 2003b). Laurocapram proved to be safe after cutaneous administration to humans in combination with methotrexate (Sutton et al. 2001; Demierre et al. 2003).

In summary, the safety of laurocapram administered by ways other than cutaneous administration has not been completely assessed. The assessment of the toxic potential of cutaneous administration of Azone® has generated contrasting results, reporting Azone® as a nonirritant in some reports and other results reporting this compound as a moderate skin irritant.

25.6 Sulfoxides

25.6.1 Dimethyl Sulfoxide (DMSO)

DMSO has been widely investigated as a percutaneous penetration enhancer for a wide range of drugs (Medi et al. 2006). Although DMSO was shown to be an effective percutaneous penetration enhancer, the toxicity problems associated with its use prevented the widespread use of DMSO in transdermal systems.

Several studies to assess the toxicity of DMSO have been reported. The acute LD_{50} levels have been calculated in mice and rats. The intravenous LD_{50} level at 24 h is 11 g/kg in mice, while the levels after intraperitoneal administration are 20.1 and 13.7 g/kg for mice and rat, respectively. LD_{50} levels after subcutaneous administration are 16.0 g/kg in the mouse and 13.7 g/kg in the Wistar rat. It was not possible to establish an acute oral LD_{50} in mice and rat. The maximum oral dose never fatal in mice was $\geq$14 g/kg and in rat was $\geq$15.0 g/kg, while the minimum dose always fatal was 14.0 g/kg in mice and 15 g/kg in Wistar rat (Caujolle et al. 1966). The most characteristic symptoms observed after administration of DMSO were catatonia of the tail (Straub's reaction) and hypothermia in the mouse. In the rat the main symptom was profuse lacrimal secretion. However, the toxic effects were observed only in doses close to the lethal dose when parenterally administered.

Smith and collaborators reported the assessment of the toxic potential of DMSO after dermal administration to rats (Smith et al. 1967). The authors immersed rats up to their necks in various concentrations of DMSO, not observing immediate effects. However, within 24 h, most of the animals treated with undiluted DMSO were dead, as were some animals dipped in 80 % DMSO-water. The single-dose dermal LD_{50} in mice and rats was estimated to be equal to, or less than, 50 and 40 g/kg, respectively (Smith et al. 1967).

Brown and collaborators painted undiluted DMSO onto five male hairless mice twice a week for 30 weeks with no discernible effect. Moreover, the same authors applied undiluted DMSO daily for 28 days to the clipped backs of guinea pigs and found no gross or microscopic evidence of damage (Brown et al. 1963).

In a series of experiments, DMSO was applied to shaved skin in the back of dogs and monkeys at doses of 3.3–33 g/kg/week for 6 months. The compound was used at concentrations from 60 to 100 %. The skin became transiently reddened and warm from the application, particularly with the undiluted DMSO. Furfuraceous and membranous desquamation of the epidermis began within 3 weeks and persisted throughout to the end of dosing period. There were no changes in behavior, body weight, hematology, blood chemistry, or urinalysis. No obvious changes were observed in organ weights, and some test article-related effects were seen at gross and microscopic examination at the site of administration in the skin. Other microscopic effects observed in the skin after dermal administration of DMSO to rats were hyperkeratosis, parakeratosis, and focal ulcerations, whereas to dogs and monkeys, it produced only desquamation (Smith et al. 1967). Changes observed by other authors when 90 % DMSO was applied to the trunk of 20 human patients were erythema in some patients and mild scaling and diffuse erythematous dermatitis in others. When DMSO was administered at doses double the ones described above, some individuals showed irritation. Skin biopsies revealed the presence of mild perivascular lymphocytic infiltration, moderate acanthosis, absence of the granular layer, and a parakeratotic, thickened cornified

layer. These changes partially resemble some changes observed in rats that were dipped into DMSO (Kligman 1965).

Regarding the ocular administration of DMSO, Brown and coworkers administered undiluted DMSO into the conjunctival sac of rabbits and noted no adverse effects (Brown et al. 1963). Similarly, using the same species, Smith and collaborators dosed undiluted and diluted 25 and 60 % DMSO (Smith et al. 1967). These authors found that DMSO caused a dose-related edema and erythema of the orbital tissues when administered at 60 and 100 % solutions. While the undiluted compound also produced lacrimation and drooping of the lower eyelid (Smith et al. 1967).

The assessment of the teratogenic potential of DMSO was assessed in rats, rabbits, and chickens. The results of those assessments revealed that the toxicity of DMSO is discreet, showing considerable tolerance in the species tested in single- or repeated-dose studies with duration of up to 30 days of administration of DMSO. The teratogenic effects of DMSO were demonstrated in chick embryo and in mammals (mice, rabbit, and rat). The main teratogenic effects in both mammals and chicken are effects on nervous system (anencephalia and microphalia), skeletal malformations, and celosomia (Smith et al. 1967).

The single- and repeated-dose experiments that were performed to assess the toxic potential of DMSO revealed some important properties of DMSO. First, DMSO is well absorbed following administration by all routes. This compound possesses a high local toxicity, characterized by local tissue irritation and destruction after dosing by most of the routes of administration. Since the LD_{50}s are all in the terms of grams per kilogram of body weight, DMSO possesses low systemic toxicity. Finally, the toxicological effects induced by DMSO are similar in all tested species.

In summary, although some adverse effects have been observed after a single- or repeated-dose administration of DMSO, those effects have not prevented the use of DMSO in the formulation compositions used in clinical development and veterinary products.

25.7 Pyrrolidones

A range of pyrrolidones and other structurally related compounds have been investigated as potential penetration enhancers in human skin. The most studied analogues of naturally occurring pyrrolidone carboxylic acid are 2-pyrrolidone (2P) and N-methyl-pyrrolidone (NMP). It has been demonstrated that 2P increases the skin permeability by enhancing the diffusivity of the drugs through polar routes of the skin (Southwell and Barry 1983). Consequently, as is the case for other enhancers, such as Azone®, pyrrolidones apparently are more active with hydrophilic rather than with lipophilic molecules (Williams and Barry 2012).

Regarding mechanism of action, pyrrolidones act well upon the *stratum corneum*. Apparently they act by altering the solvent nature of the membrane, and pyrrolidones have been used to generate "drug reservoirs" within the skin membranes. Those reservoirs allow the slow or sustained release of the permeant from the *stratum corneum*. However, as with other penetration enhancers, the clinical use of pyrrolidones is often prevented due to reported adverse reactions, which are mainly referred to as skin irritation.

Pyrrolidones have been used as permeation promoters for numerous molecules including hydrophilic and lipophilic permeants. Among those molecules are: nalone, 5-fluoroacil, hydrocortisone, betamethasone, indomethacin, luteinizing hormone-releasing hormone, mannitol, metronidazole, naloxone, progesterone, nitroglycerine, and sulfaguanidine (Williams and Barry 2012; Ahad et al. 2009; Medi et al. 2006).

Regarding safety, it has been reported that in humans, NMP and 2P cause skin irritation while increasing the bioavailability of betamethasone-17-benzoate (Ahad et al. 2009). In an in vivo vasoconstrictor bioavailability study, pyrrolidones caused erythema in some patients, although this effect had a relatively short duration. Also a toxic hygroscopic contact to N-methyl-2-pyrrolidone has been reported (Williams and Barry 2012).

In a toxicological evaluation of eprinomectin (Longrange Merial®), an injectable parasiticide approved for its use in cattle in the United States

and other countries, the toxic potential of NMP was assessed in several repeated dose studies. NMP was included in this product as an excipient (FDA-CVM 2001). The literature reports the carcinogenic potential of NMP in mice, but not in rats as described in the freedom of information document for NADA 141–3227, and was conducted to investigate the human food safety issues of the excipient. The NOEL identified in a 1 week oral gavage repeat-dose study in mice was 1000 mg/kg/day based on proliferative effects at 3000 g/kg/day (FDA-CVM 2001).

In subchronic studies the NOEL identified in a 90-day repeat oral dose study in mice was 167 g/kg/day based on the increased liver weight and increased incidence of centrilobular hepatocellular hypertrophy observed at 417 mg/kg/day. A similar study using Wistar rats dosed with the compound in the diet showed a NOEL of 40 mg/kg/day, based on a decrease of body weights, an increase in the thyroid weight, and changes in the chemical properties of urine (FDA-CVM 2001).

The NOEL obtained from a 90-day repeat oral dose study in dogs was 79 mg/kg/day based on the lack of body weight gain of the highest dose group (250 mg/kg/day) (FDA-CVM 2001).

After the analysis of the results from the subchronic and chronic studies performed to assess the toxic potential of NMP, the most appropriate toxicity study for determining the human health protective value of NMP residues in edible tissues was the 90-day oral toxicity study in mice. The NOEL of NMP for this study was 167 mg/kg/day (FDA-CVM 2001).

No teratogenic effects were observed in Sprague-Dawley rats dosed with up to 237 mg/kg/day. The NOEL identified in a developmental toxicity study in New Zealand white rabbits was 55 mg/kg/day with respect to maternal toxicity and 175 mg/kg/day with respect to developmental toxicity (FDA-CVM 2001).

In a multigeneration rat reproduction study with N-methyl-2-pyrrolidone, the NOEL for reproductive and developmental effects was established as 160 mg/kg/day of the compound. The 160 mg/kg/day dose level was established as the parental, reproductive, and developmental NOEL in this study (FDA-CVM 2001).

In summary, pyrrolidones and specifically NMP may cause skin erythema and irritation after cutaneous administration. When orally administered, this compound may be teratogenic at doses higher than 160 mg/kg/day and may have adverse effects on the body weight, thyroid weight, and liver, in which microscopic changes were observed in the hepatocellular areas, having hepatocellular hypertrophy.

25.8 Surfactants

As happens with other classes of skin penetration enhancers, surfactants are found in many existing therapeutic, cosmetic, and agrochemical preparations. Surfactants are usually added to formulations in order to facilitate the solubilization of lipophilic active ingredients. The surfactants have the ability of solubilize lipids within the *stratum corneum*. Surfactants are typically composed of a lipophilic alkyl or aryl fatty chain, together with a hydrophilic head group. These enhancers can be anionic or cationic. Anionic surfactants include sodium lauryl sulfate (SLS), while cationic surfactants include cetyltrimethyl ammonium bromide (Williams and Barry 2012). There is a wide use of nonionic surfactants in topical formulations as solubilizing agents. Among those, the polysorbates (ethoxylated sorbitan esters) are very common. Tween® 80 was reported to accelerate hydrocortisone and lidocaine penetration, and Tween® 20 improved the permeation of 5-fluoroacyl across hairless mouse skin (Kogan and Garti 2006).

Surfactants generally have low toxicity, and most have been shown to enhance the flux of materials permeating through biological membranes. The cationic surfactants are claimed to be more potent than anionic surfactants. Both types of surfactants have potential for damaging human skin; sodium lauryl sulfate (an anionic surfactant) is a powerful irritant for human skin (Ahad et al. 2009). Nonionic surfactants tend to be widely recognized as safe (Kogan and Garti 2006).

Turkoglu and Sakr investigated the irritation potential of sodium laureth sulfate alone and in

combination with lauryl glucoside, polysorbate 20, and cocamidopropyl betaine in 13 human subjects (Turkoglu and Sakr 2001). The highest irritation potential was observed with the composition containing sodium laureth sulfate, lauryl glucoside, and cocamidopropyl betaine together. Among the subformulations, cocamidopropyl betaine showed the highest irritation grade. It was concluded that the irritation potential of surfactants was related to the total surfactant concentration as well as to the chemical structure of the surfactant molecules (Ahad et al. 2009). Recently, new low-irritant surfactants based on caprylocaproyl macroglycerides for microemulsions as drug delivery vehicles for topical application were studied (Kogan and Garti 2006).

In general surfactants may be irritant for skin; however, regardless of the minimal adverse effects reported, surfactants should be considered of low toxicity or generally safe.

Conclusion

In the drug development process, it is important to perform a thorough assessment of the toxic potential of each of the components of a drug formulation. This review of the toxicology of several penetration enhancers provides a summary of the reported safety assessment for those enhancers. However, it is our responsibility as toxicologists to evaluate the potential toxicological effect that certain enhancers may have as components of formulations intended to be used in humans or in animals. The main toxicological effects, LD_{50}s, and NOELs/NOAELs described in this review are summarized in Table 25.1.

Table 25.1 Summary of Toxicological Effects of Chemical Penetration Enhancers

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/species tested and way of administration	Reference
Terpenes		
1,8-Cineole (eucalyptol)	In humans: transient coma, epigastric burning, nausea, vertigo, ataxia, muscle weakness, occasional cyanosis, respiratory edema, miosis, convulsions, fever, albuminuria, hematuria, painful urination, respiratory failure It also induced allergic skin reactions and tachycardia	Gosselin et al. (1976), BIBRA (1991), Santos and Rao (2002), NTP, PAFA (2006), NTP Repository (1991), Galloway et al. (1987), Stoner et al. (1973), De Vincenzi et al. (2002)
	Possible lethal dose in human: 50–500 mg/kg	
	LD_{50} 2480 mg/kg rat, oral	
	LD_{50} 2500 mg/kg mouse, oral	
	LD_{50} 100 mg/kg mouse, IM	
	LD_{LO} 1500 mg/kg dog, SC	
	Genotoxicity negative and carcinogenicity negative (at 12 g/kg for 8 weeks in mouse primary lung tumor model)	
	Teratogenic at 2 g/kg rat, oral	
	Central nervous system and respiratory effects and increased hepatic enzyme activity with bile secretion in rodents	

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
L-Menthol	In human: high oral doses may cause abdominal pain, convulsions, nausea, vomiting, vertigo, ataxia, drowsiness, coma	OECD-SIDS (2001, 2003), O'Mullane et al. (1982)
	250–250 mg/kg caused some of the signs above in children	
	120 mg/kg caused cold burning sensation in the mouth, esophagus, nasal mucosa, skin in humans	
	20 mg/kg caused mild abdominal discomfort	
	NOAEL 200 mg/kg increased hepatic weight, rat, 28 days, oral	
	NOAEL 937 mg/kg reduction body weight, male rat, 13 weeks, oral	
	NOAEL, 1956 mg/kg; male mice, 13 weeks, oral	
	NOAEL 188 mg/kg, rat, 103 weeks, oral	
	NOAEL 667 mg/kg, mice, 103 weeks, oral	
	Genotoxicity negative and carcinogenicity negative	
	Non-teratogenic at up to 425 mg/kg rat, mouse, rabbit, oral	
Fatty acids		
Oleic acid	Several species: erythema and edema, skin irritation, inflammatory infiltrate, follicular epidermal hyperplasia after cutaneous administration in 5 % (w/v)	Liebert (1987), Boelsma et al. (1996), HSDB (2008)
	Induction of comedones in rabbit skin	
	No ocular irritation in rabbits after ophthalmic administration of 0.1 mL oleic acid	
	LD_{50} 74 mg/kg, rat, SC	
	LD_{50} 2.4 mg/kg, rat, IV	
	LD_{50} higher than 3000 mg/kg, guinea pig, dermal	
	Rat: severe pulmonary damage and death, IV injection at 42 mg/kg	
	Dog: significant pulmonary changes, thrombosis, cellular necrosis, at 0.09 g/kg IV for 1–3 months	
	Severe pulmonary edema at 60 mg/kg IV in dogs	
	Genotoxicity negative, carcinogenicity negative (rat and mouse)	
	Reproductive effects in females (affect parturition and mammary gland development) at 7.5 g/kg/day in diet for 16 weeks, Sprague-Dawley rats	
Myristic acid	Rabbit: thinning collagen fibers and lymphocytic-histiocytic infiltration	Liebert (1987), HSDB (2008)
	Rabbit: slight conjunctival irritation after administration of commercial grade myristic acid	
	No acute toxicity in rats with up to 10 g/kg oral dose in rat	

(continued)

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
Stearic acid	Rats: local cutaneous edema after topical application 2 % solution	Liebert (1987)
	Rabbits: cutaneous edema after 20 applications 2 mL/kg 2 % solution	
	No photosensitizing, 100 % stearic acid, guinea pigs, topical	
	No ocular irritation in rabbits after administration of commercial stearic acid	
Lauric acid	Rabbits: erythema 72 h posttopical treatment	Liebert (1987), HSDB (2008)
	Rabbit: corneal opacity, mild conjunctivitis, and iritis after ophthalmic administration of commercial grade lauric acid. No effects when administered at a 8.7 % solution	
	No acute toxicity in rats with up to 10 g/kg oral dose in rat	
Fatty alcohols		
Octanol/nonanol	Octanol and nonanol are less irritant than decanol and undecanol in hairless rat skin in vivo	Kanikkannan and Singh (2002)
Ethanol	Associated to skin irritation or contact dermatitis in individuals with aldehyde dehydrogenase deficiency	Lachenmeier (2008)
	Application of ethanol on intact human skin does not cause acute systemic toxic effects	
	Ethanol and its metabolites are potentially carcinogenic for humans addicted to alcoholic beverages	
Glycols		
PG	LD_{50} 21 g/kg, Fischer rat, oral	CIR (1994)
	LD_{50} 13 g/kg, Sprague-Dawley rat, IP	
	LD_{50} 6.2 mL/kg, Sprague-Dawley rat, IV	
	LD_{50} 6.4 mL/kg SPF-NMRI mouse	
	PG may affect hepatic function (decreased fibrinogen, albumin, and globulin in plasma at 9.66, 19.32, or 38.64 mmol/kg in Wistar rat)	
	Horses: lethal when dosed at 3.8 L into a 400–500 kg horse. Ataxia, pain, salivation, excessive sweating, respiratory arrest, and death. Histology: myocardial perivascular edema, pulmonary edema, lymphangiectasia, suppurative pericholangitis, and renal infarcts	
	LD_{50} dermal 20.8 g/kg. No irritant to skin or eyes	
	No adverse effects in rats dosed 10 g/kg/day in water or 2.5 g/kg/day in feed for 2 years in rats	
	NOEL 80 mg/kg/day and LOAEL 443 mg/kg/day for 90 days in cats. Increased Heinz body formation	CIR (1994)
	Developmental NOELs from 1.2 to 1.6 g/kg/day in rat, rabbit, hamster, or mice	
	No reproductive effects at 10.1 g/kg/day in mice	
	Genotoxicity negative/carcinogenicity negative at up to 2.5 g/kg/day for 2 years in rats (oral drug in diet)	
	Carcinogenicity negative at up to 2 g/kg/week dermal application for lifetime (mice) or for 14 weeks (rat)	

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
PPGs	LD_{50} 2.91 g/kg (PPG425), 2.15 g/kg (PPG1025), 9.76 g/kg (PPG2025), Sherman rat, oral. Signs: sluggishness, prostration, tremors, convulsions, sudden death	CIR (1994)
	LD_{50} 640 mg/kg (PPG1200), rat, oral	
	Guinea pig: LD_{50} (PPG1200) 1.320 g/kg, oral	
	LD_{50} 0.46 g/kg (PPG425), 0.23 g/kg (PPG1025), 4.47 g/kg (PPG2025), Sherman rat, IP	
	LD_{50} 0.41 g/kg (PPG425), 0.12 g/kg (PPG1025), 0.71 g/kg (PPG2025), Sherman rat, IV	
	LD_{50} 700 mg/kg (PPG400), 195 mg/kg (PPG750), 12 mg/kg (PPG1200), 3600 mg/kg (PPG2000), mouse, IP	
	Dog: acute oral toxicity PPG1200 at 50 mg/kg with subconvulsant effect	
	Dog: tremors and/or convulsions with PPG400 at 10–20 mg/kg IV, PPG750 at 8–15 mg/kg IV, PPG1200 at 7–15 mg/kg IV. Death with PPG750 at 20 mg/kg IV, PPG1200 at 20 mg/kg IV	
	No adverse effects with 100 mg/kg PPG2000 in dogs dosed IV	
Azone (laurocapram)		
	Minimal skin irritation in rats	Fang et al. (2003a, b)
	Proliferation of epidermis and neutrophilic-lymphocytic infiltration of epidermis after cutaneous administration in rats	
Dimethyl sulfoxide (DMSO)		
	LD_{50} 11 g/kg in mice	Kligman (1965), Brown et al. (1963)
	LD_{50} 20.1 g/kg mice, IP; LD_{50} 16 g/kg, mice, SC	
	Maximum oral never fatal dose 14.0 g/kg, mice	
	Dermal application to rats induced: hyperkeratosis, parakeratosis, focal ulcerations	
	LD_{50} 13.7 g/kg rat, IP and SC	
	Maximum oral never fatal dose 15 g/kg, rat	
	Dog and monkey: 3.3–33 g/kg in shaved skin caused, reddened skin	
	Rabbits: no adverse effects after ocular application of undiluted DMSO	
	Teratogenic in chick embryo, mice, rabbit, rat after 30 days of dosing	

(continued)

Table 25.1 (continued)

Penetration enhancer	Toxic syndrome/lowest LD_{50}/lowest NOEL reported/ species tested and way of administration	Reference
Pyrrolidones		
NPM	Cause skin irritation	Ahad et al. (2009), Williams and Barry (2012), FOI NADA 141-327
	Cause skin erythema in some patients	
	NOEL 1000 mg/kg/day for 1 week, mice, oral. Based on proliferative effects at 3000 mg/kg/day.	
	NOEL 167 mg/kg/day for 90 days, mice and rat, oral. Based on increased liver weight and increased incidence of centrilobular hepatocellular hypertrophy at 417 mg/kg/day	
	NOEL 40 mg/kg/day for 90 days, drug in diet, Wistar rat	
	NOEL 79 mg/kg/day for 90 days, oral, dog. Based on lack of body weight gain at 250 mg/kg/day	
	NOEL 55 mg/kg/day, oral, rabbit. Based on maternal toxicity, and NOEL 175 mg/kg/day based on developmental toxicity	
	No teratogenic in Sprague-Dawley rat at up to 237 mg/day/kg	
	NOEL 160 mg/kg/day in a multigeneration reproductive toxicology in rat. This was a NOEL for parental and fetal toxicity	
Surfactants		
	Generally have low toxicity	Kogan and Garti (2006)
	The highest irritation potential was observed with combination of laureth sulfate, lauryl glucoside, and cocamidopropyl betaine	

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