COSMETIC INGREDIENT REVIEW

CIR Resource Document

Dermal Penetration, Absorption, and other Considerations for Babies and Infants in Safety Assessments

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1. Introduction

1.1. Preamble

The purpose of this report is to serve as a resource document to which the CIR Expert Panel will refer in safety assessment reports when appropriate. This report summarizes and evaluates information gathered from the scientific literature that compares the potential for the systemic absorption of ingredients of topically-applied cosmetic products in babies and infants, with the potential for systemic absorption in older children and adults. Among the topics addressed here are the development of the diffusion barrier of the skin, which is largely attributed to the *stratum corneum* (SC), and the development of biotransformation capacities of the skin, which can also influence systemic absorption through the skin. In addition, this report addresses some of the issues that the CIR Expert Panel considers when assessing the safety of cosmetic ingredients used on children.

The focus of this report is on the barrier function of the skin in normal, full-term babies and infants compared with that of adults. The primary issue addressed is whether normal, full-term babies and infants have unique susceptibilities to topical exposures to cosmetic ingredients, because of differences in the diffusion or functional barriers of the skin, compared with adults.

For example, the solubility, and thus the rate of percutaneous absorption, of weak acids and bases in aqueous solutions will generally depend on the pH of the solution, as described by the Henderson-Hasselbalch equation. However, the natural pH of the skin is a critical parameter that determines the permeability of the skin. The decrease in skin pH that occurs especially in babies from birth to about 6 months of age, which may continue up to 2 years of age, can help to explain, in large part, the corresponding decrease observed in the potential dermal absorption of many substances, including ingredients (not limited to weak acids and bases) of topically-applied cosmetic products. The CIR Expert Panel considers such factors in cosmetic ingredient safety assessments when appropriate.

It is important to emphasize, at the outset, that cosmetic products are intended to be used on healthy, intact skin. Accordingly, the purview of the CIR Expert Panel includes assessing the safety of cosmetic ingredients applied to normal skin, and not to damaged or diseased skin. For example, over-the counter (OTC) and prescription products used to treat atopic dermatitis or diaper rash are regulated as drugs by the U.S. FDA, and should be used under medical supervision; ingredients as used in such products are outside the purview of the CIR Expert Panel. The Panel acknowledges that some cosmetic products could be confused with OTC products for treating diaper dermatitis. However, the focus of the Panel is on assessing the safety of ingredients as used in cosmetic products, not in prescription or nonprescription drugs.

Further, this report does not address the potential for enhanced penetration or systemic absorption of ingredients of topically-applied products through the skin of pre-mature neonates. Cosmetic products are not intended for use on pre-term neonates, whether or not they are also pre-mature.

Finally, this document does not provide a detailed account of the Panel's approach to addressing the greater ratios of body-surface area / body mass of babies, infants, and young children compared with those of adults. The CIR

Expert Panel recognizes that babies, infants and children represent a distinct subpopulation for risk and safety assessments, and routinely considers the greater skin-surface area to body-mass ratio in children when performing cosmetic ingredient safety assessments.

1.2. Age Groupings

Children and adults can be grouped approximately as follows, based on anticipated differences in development, susceptibilities, and exposure characteristics, and a variety of similar groupings given in the literature.[1-6] The following groups are recognized for the purposes of this resource document:

- Neonate (newborn)
- Baby (birth up to 6 months)
- Infant (6 months up to 2 years)
- Preschool (2 years up to 6 years)
- Preadolescent (6 years up to 12 years)
- Adolescent (12 years up to 18 years)

Neonates can be pre-term (i.e., born at estimated gestational age, GA, < 37 weeks; 9¼ months) full-term (GA 37 to 42 weeks; 8½ to 10½ months), or post-mature (GA > 42 weeks; 10½ months).[7] From the biological (as opposed to biophysical) perspective, pediatric dermatologists refer to children from birth to 6 months of age as babies and from 6 months to 2 years of age as infants.[6]

1.3. Anatomy of the Skin

The skin, in cross-section, has three distinct, primary layers or regions, including the epidermis, the dermis and the subcutis or hypodermis (see illustrations: Integumentary System, Structure and Function of the Skin, or The Integumentary System). The epidermis is the outer layer of the skin, which can vary in thickness from ½ mm on the eyelids to 1½ mm on the palms and soles. The epidermis contains connective tissue, hair follicles, and sweat glands. The five layers of the epidermis include (from outermost to innermost) the *stratum corneum* (SC), *stratum lucidum*, *stratum granulosum*, *stratum spinosum*, and the *stratum basale*. The outermost layer, the SC, consists of corneocytes (i.e., fully mature keratinocytes that originate and migrate from the *stratum basale* to the SC), which are enucleated, packed with keratin, highly interlocked with one another, and embedded in an extracellular hydrophobic lipid matrix.

The dermis, which lies beneath the epidermis, contains collagen and elastic fibers, as well as the dermal vasculature, lymph vessels, and nerves. The dermis projects into the overlying epidermis in ridges called papillae in the outermost (papillary) layer of the dermis. The epidermal swellings that project downward between the papillae of the dermis are called rete ridges (see illustration: Structure and Function of the Skin). Sweat glands and sebaceous glands lie in the deeper (reticular) layer of the dermis.

The hypodermis, also called the subcutis or superficial fascia, is a layer of subcutaneous tissue beneath the dermis, consisting of a network of collagen and fat cells that provide a cushioning function, among other functions.

1.4. *Stratum Corneum* (SC) and Significance of Body-Surface / Body-Mass Ratio

The outermost layer of epidermis, the SC, is generally considered to be the primary rate-limiting barrier to loss of water from the body, the absorption site of most topically-applied substances, and a significant route of systemic exposure to microorganisms in full-term neonates, babies, infants and adults.[8-18] In comparison, the layers of the epidermis and dermis under the SC, although much thicker than the SC, offer little resistance to the evaporation of water or the systemic absorption of toxicants.[9]

The SC is a tough, cohesive layer composed of flat, enucleated cells lacking energy-dependent synthetic capacity, corneocytes packed with the fibrous protein keratin and held together by a lamellar matrix of an extracellular lipid mixture of ceramides, cholesterol, and free fatty acids.[10,18-20] The SC is often thought of as a passive, inert barrier to the diffusion of water and other substances through the skin.[9,10,12,17]

The importance of the barrier function of the SC is magnified in babies and infants.[8] Percutaneous absorption and systemic exposures in babies and infants are generally assumed to be greater than in older children and adults because of the immaturity of the skin as a barrier to absorption (higher pH of the skin yields decreased barrier function and increased risk of irritation) and the greater body-surface-area to body-mass ratio of babies and infants compared with older children and adults, among other reasons.[2,21-24] For example, neonates have approximately three times the body-surface-area to body-mass ratio of children about 13 years old.[24]

The default inter-individual uncertainty factor of 10 routinely used to evaluate margins of safety (MoS) estimates in safety assessments is generally considered to be adequate to address potential differences between children and adults in relative absorption through intact skin.[23,25-28] However, an additional safety factor may be considered if there is data indicating that the inter-individual variability for a specific ingredient may exceed a factor of ten.[24,29]

2. Development of the Skin

The development of the skin is not fully complete at birth. Parturition and early postnatal life involves a rapid adaptation of the skin to life outside the uterus, including maturation of the structure and function of the skin, which begins during the third trimester (28-36 weeks; 7-9 months) of pregnancy.[7,10,30]

2.1. Skin Function and Physiology

The physical barrier properties of the skin are generally considered to be located almost entirely in the SC, and dependent on its thickness and integrity.[31] The viable epidermis below the SC continually replenishes the SC by cell division in the basal layer.[31] When the mitotic rate of the basal layer increases, because the skin is damaged for example, there are two histological changes: (1) the basal layer increases in area and heaps up to form undulations (i.e., the rete ridges) of the dermal-epidermal junction, and (2) the epidermis becomes thicker as the number of keratinocytes increases, especially in the *stratum spinosum*, and the keratinocytes differentiate and migrate into the SC.

The complex protective barrier of the SC is made up of lipids synthesized by the epidermis and natural moisturizing factors (NMFs), known as the hydro-lipid system.[31] NMFs are hygroscopic substances in the SC involved in water binding within corneocytes.[32]

2.2. Development of Epidermis and Dermis

The coordinated development of the dermis, epidermis, and associated tissues begins during the first trimester (around the 2nd month of gestation).[9,32-34] The following subsections provide additional detail.

2.2.1. Stratum Corneum Development

The development of the SC begins during the third trimester, around week 24 (month 6) of gestation, and continues through weeks 32 to 40 (months 8 to 10).[9,12,18,35-39]

2.2.2. Gestational Age (GA) 24 Weeks (6 months) to Full-Term Birth

The cellularity and thickness of the epidermis steadily increase from gestational week 24 (month 6) to term. Before GA 30 weeks (7½ months), the epidermis is thin and is characterized by few cell layers, barely perceptible rete ridges (i.e., the undulations of the basal layer of the dermal-epidermal junction), and a poorly formed SC.[31] Well-defined rete ridges and well-developed SC appear around the 34th week (8½ month) of gestation.[9,18,31,32] The barrier function of the skin at 34th weeks (8½ months) of gestation is comparable to neonates.

The vernix caseosa develops at the end of the third trimester, coincident with terminal differentiation of the epidermal keratinocytes of the SC.[18,23,32,40-43] The vernix caseosa is a protective hydrophobic biofilm (i.e., hydrophobic mantle) containing fatty acids, squalene, wax esters, triglycerides, cholesterol and ceramides.[7,18] The vernix caseosa does not directly contribute to the barrier function of the skin, although it contains some lipids that help maintain hydration levels of the skin, and may have some antimicrobial properties as well.

2.2.3. Full-Term Birth to Two Years of Age

The full-term neonate has all of the skin structures of an adult, and these structures do not undergo substantial changes after birth.[4] The epidermis continues to thicken for about four months after birth, attributable primarily to the proliferation of cells in the basal layer, which causes a mounding or heaping of that layer and a corresponding deepening of the rete ridges. However, the SC of the full-term neonate is remarkably capable of fulfilling its key functions soon after birth, especially that of providing an effective semipermeable diffusion barrier between the inside and outside of the body under basal conditions.[23]

On the other hand, the skin of babies and infants is continually adapting during the postnatal period, in a manner that optimizes the balance among growth, thermoregulation, and the water-barrier and protective functions of the skin, in contrast to the relatively steady-state of adult skin.[4,12,18,32,43-46 35,43]

As noted above, full-term neonates are born after 37 weeks GA. Premature neonates born after 34 weeks GA generally have dermal barrier functions similar to full-term neonates and babies up to 6 months of age.[6] The skin of infants is relatively mature, compared to the skin of babies, but does not yet function as a fully-mature permeability

barrier. As explained below, the immaturity of the barrier in babies and infants can be largely attributed to the elevated pH of the skin, as well as to the super-moisturization of the skin in the diaper area and body folds. The normalization of the pH of the surface of the skin, and thus the maturity of the barrier function of the skin, is largely complete by 6 months of age, although in some individuals there may be a further decline in surface pH and improvement in barrier function between 6 months and 2 years of age. This factor helps to explain why babies and infants continue to have increased risk of dermatitis and infections and why they recover more slowly from damage by exposures to irritants.

There are exogenous and endogenous dermal acidifying mechanisms in the skin, which are responsible for the development and maintenance of the skin's protective "acid mantle." The major acidifying mechanism that is immature in neonates is the endogenous secretory phospholipase A2 (sPLA2) mechanism, which breaks down phospholipids to release free fatty acids (FFAs) in the skin.[6] Delayed maturation of the barrier function of neonatal skin is attributable to the low expression and activity sPLA2). As the expression and activity of these enzymes increase in the skin after birth, they yield free fatty acids that acidify the skin and contribute to the barrier function of the skin.

When the barrier is compromised in babies, the pH of the skin increases, and this increase activates serine proteases (SPs) that release pro-inflammatory cytokines, which helps to explain the increased tendency for dermatitis and other types of inflammatory reactions.[6]

The barrier function of the skin at about 34 weeks GA and thereafter is sufficient for life after birth. However, barrier repair is slower in babies than in adults, and can continue to be delayed for up to about 2 years of age.[6]

The immature barrier of babies and infants will be manifested by increased potential for evaporative water loss from the skin, increased potential for dermal penetration and percutaneous absorption of ingredients of topically-applied products, increased susceptibility to infections, inflammation, and blistering, and delayed repair. These signs of immaturity are notable especially in the skin of pre-term neonates until postnatal age (PNA) 2 to 3 weeks and in full-term neonates during the first 3 to 5 days of life.[6,9,12,22,47,48]

For example, the blanching response to topical phenylephrine increases in pre-term neonates (GA < 37 weeks; 9¼ months) with decreasing GA at birth, and disappears 2 to 3 weeks later, demonstrating that the dermal penetration of the drug in pre-term neonates is attributable to the poor epidermal barrier.[8,9,12,12,47] The blanching effect, which is attributable to the phenylephrine-induced local contraction of blood vessels in the skin, was minimal or absent in full-term neonates (GA > 37 weeks; 9¼ months), indicating low skin penetration and absorption of the drug.[8,12,47] There is no clear consensus about the relative effectiveness of the SC barrier in babies after about the first postnatal month.[18,32] SC barrier function involves a complex interplay of factors such as corneocyte maturity/hydrophilicity, lipid amount and phase, density of appendages, surface micro-relief, and diffusion-path length, all of which could help explain some of the differences between the skin of babies and infants and adult skin.[32]

2.2.4. Histological and Other Changes

The following subsections describe some of the histological and other changes of the skin during development.

2.2.4.1. Basement Membrane and Dermis

The cohesion structures in the basement membrane of neonatal skin are similar in type and density to adult skin.[4,49,50] There are also many fibroblasts producing elastic and collagen fibers in the newborn dermis, although fewer than in adult skin.[4] The elastic fibers develop further after birth, and are completely mature at about 3 years of age. The water, glycogen, and hyaluronic acid contents of the dermal extracellular matrix decrease during development after full-term birth, while dermatan-sulfate content increases.[4,51]

2.2.4.2. Epidermis

Histologically and ultrastructurally, the epidermis of full-term neonates (GA > 37 weeks; 9¼ months) is well developed, and does not show much difference compared to the epidermis of adults.[4,4,34,52,53] The dermis is somewhat thinner, the rete ridges shallower, and the appendages denser, but the epidermis and SC are nearly identical to their adult counterparts.[8-10,12,18,23,31,53,54]

The full thickness of infant skin (epidermis and dermis) is about 40–60% that of adult skin.[18,55] The cohesion and adhesion of epidermal cells in newborn skin are not fully developed and the connection at the epidermal/dermal junction is weaker than in adult skin.[23] Thus, infant skin blisters more readily than adult skin, for example. The rete ridges progressively deepen in the skin of infants during GA 36 to 40 weeks (9 to 10 months), yielding a thicker and more cellular epidermis up to PNA 16 weeks (4 months).[9,31]

2.2.4.3. Stratum Corneum

Histologically, the SC does not appear to be fully differentiated in newborn skin before GA about 34 weeks (8 ½ months), based on the fewer layers of cornified cells compared with adult skin, and SC maturation may not be complete until GA about 37 weeks (9¼ months).[7,31,32,43,56]

2.2.4.4. Microstructure of the SC/Epidermis

Stamatas et al. (2010)[57] investigated skin microstructure in babies and infants *in vivo* and compared it with that of adult skin using fluorescence spectroscopy, video microscopy, and confocal laser scanning microscopy. The SC of the babies and infants was 30% and the epidermis 20% thinner than in adults. The corneocytes and granular cells were 20% and 10% smaller, respectively, in the skin of the babies and infants compared to adults, suggesting more rapid cell turnover in babies and infants. The skin of the babies and infants also differed from adult skin in papillae density and size distribution. A direct relationship between SC morphology and the structure of dermal papillae was observed only in baby and infant skin. The transition from papillary to reticular dermis was observed only in adult skin. The authors indicated that the qualitative and quantitative differences in the microstructure may help explain some of the reported functional differences, especially the differences in water-handling properties, between adult skin and the skin of babies and infants.

2.2.4.5. Hydrolipid Film

The hydrolipid film of the skin is a protective water-in-oil (w/o) mixture composed mainly of sebum from sebaceous glands and water from eccrine glands. It is not fully developed in babies.[4]

Sebum consists mainly of squalene, wax esters, cholesterol esters and triglycerides, and possibly free cholesterol and free fatty acids.[7,18] Sebum secretion increases after birth, reaching rates during the first week of life comparable to adult rates.[7] Sebaceous gland activity in the neonate during this first week is thought to be stimulated by preceding transplacental exposure to maternal hormones, because sebum secretion is greater in the early neonatal period than at PNA 6 months.[7,18,58] Sebum secretion then remains relatively low and constant until pre-puberty, when an increase in sex-hormone production causes a new rise in secretion.[7]

Sebum lipids are major constituents of the Marchionini's protective cutaneous hydrolipid film, which has primarily an antimicrobial function. This hydrolipid layer is also thought to serve as a plasticizer, lubricant, and antioxidant.[7,18,59]

2.2.4.6. Triggers and Mechanisms of Structural Development

During the postnatal period there is development of the SC, so that even pre-term neonates born at GA greater than about 35 weeks (8 ¾ months) have barrier function, as determined by reduction in transepidermal water loss (TEWL) similar to that of full-term neonates and infants within 2 to 4 weeks after birth.[7-9,12,18,31,36,56,59-61] The stimulus for rapid epidermal maturation and skin-surface acidification, especially in the pre-term neonate, appears to be the change from the amniotic fluid environment to extra-uterine air, and the accompanying low external humidity that stimulates cell turnover in the outer layers of the skin.[7,9,12,31]

The molecular mechanisms of postnatal epidermal-barrier development in neonates are not fully understood, although there is evidence for a complex interplay of regulatory mechanisms involving skin-surface acidity, calcium-ion gradient, and nuclear-hormone receptors/ligands (i.e. topical peroxisome-proliferator-activated-receptor activators and liver X-receptor activators).[7,60,62]

2.2.5. Changes in Biophysical Measurements

The development of the functional transepidermal-barrier properties of the skin parallels the histological development of the dermis and epidermis before and after birth.[12,18,31,63] The morphology and lipid composition of the epidermis in full-term neonates closely resemble those of older children, and the basal transepidermal barrier is effective at birth, although recovery from external insults that affect the barrier function of the skin is slower in neonates than in older children. The parameters of skin physiology undergo dynamic changes during the first 3 months of life, especially during the neonatal period.[10,31,63,64] These rapid changes during the first months after birth are reflected in measurements of biophysical parameters, such as TEWL and skin-surface pH, as well as measures of the total water content of the SC, water gradient through the SC, and water absorption and desorption rates in the skin. This is discussed in greater detail in the following subsections. These biophysical measurements are used to assess skin function, but they are generally not good indicators of the ability of the skin to serve as a barrier to skin penetration and absorption.

2.2.5.1. Transepidermal Water Loss (TEWL)

Based on TEWL and percutaneous absorption studies, full-term neonates (GA > 37 weeks; 9¼ months) and even late pre-term neonates (GA > 30 weeks; 7½ months) appear to have SC with barrier properties comparable to those of adult skin.[4,7,12,18,32,32,56,61,63]

The high TEWL associated with the drying of the skin in the first 4 hours after birth is subsequently substantially reduced to rates as low as about 6 g⁄m^2⁄h within a week or two, depending on the measurement technology, which is consistent with TEWL measurements in older children (PNA > 1 month) and adults.[4,7,9,18,23,61,63,65-67]

However, this finding is not universal. For example, Nikoloski et al. (2003) found average TEWL ranging from 15 to 30 g/m^2/h in infants 3 to 12 months of age compared with around 6 to 8 g/m^2/h in adults, using a closed chamber method, suggesting that the water-barrier function of the skin continues to develop during the first year of life.[32,46,68]

2.2.5.2. Acidity (pH)

The skin-surface pH in neonates ranges from 6.2 to 7.5 at birth, which is responsible for the immaturity of the epidermis.[4,6,7,18,23,41,46,65,69]

Most studies indicate that the pH declines rapidly in the first week of life, and more gradually up to the fourth week, to pH about 4.5 to 5.5, which is within the dermal pH range of older children and adults (4.0 to 5.9).[4,7,10,23,43,45,46,53,65 18,70] A single study reported that pH measurements of the skin of the volar forearm and, especially, the buttocks of babies and infants up to 2 years of age were statistically-significantly higher than in adults.[65]

As noted above, acidification of the skin surface (i.e., "acid mantle" development) is essential for normal SC barrier maturation, homeostasis, and repair, because it enables pH-dependent extracellular lipid processing and turnover by β-glucocerebrosidase and acidic sphingomyelinase, formation of functional lipid lamellae, regulation of desquamation, and control of bacterial skin flora.[4,6,18,23,30,65,70-75] The "acid mantle" of the SC is thought to arise from the secretion of sebum (free fatty acids), sweat (lactic acid), free amino acids, *cis*-urocanic acid (from histidine), pyrrolidone carboxylic acid, filaggrin breakdown products, and the action of the Na+/H+ antiporter during exocytosis of lamellar bodies.[4,6,7,18,43,45,65] However, the hydrolysis of phospholipids and triglycerides of epidermal origin produces most of the free fatty acids in the SC. Sebum-derived fatty acids are probably less important, because the pH of the surface is low in areas of the skin where sebaceous glands are few compared to sebaceous-gland enriched areas.

However, the buffering capacity of the skin surface is much lower in babies and infants than in adults.[4] Occlusion or bathing with alkaline soaps can readily alkalinize the skin surface of babies and infants.[70,75]

2.2.5.3. Hydration

Following a period of evaporative drying, SC hydration in full-term neonates during the first days of postnatal life is lower on most sites of the body, compared with older children (PNA 3 to 48 months) and adults.[7,18,43,46,58,76] This is partly explained by the presence of the hydrophobic mantle (vernix caseosa), which protects the fetus from intrauterine maceration.[7,76,77] Subsequently, SC hydration increases substantially from 2 weeks to up to 1 to 3

months after birth, corresponding with a parallel increase in the permeability of hydrophilic compounds and decrease in the permeability of lipophilic compounds in the skin.[10,18,32,43,45,46,65,74]

However, some authors report measurements of hydration, pH, and other biophysical properties suggesting that, functionally, the SC continues to mature throughout the first or second year of life. For example, SC hydration (measured as capacitance) and pH were reported to be statistically-significantly greater throughout the second year of life, compared with adults.[65] Further, SC water absorption/desorption rates, total water content, and the steepness of the water gradient through the SC were greater than, and hygroscopic NMF content of the SC was less than, and more variable than in adults throughout at least the first year of life.[32]

The amount of water in the SC and the gradient of water across the thickness of the SC, in particular, reflect the maturation of the SC in the skin of babies and infants, and influence skin surface morphology, desquamation, and the expression of keratins and other epidermal proteins.[18,32]

2.3. Development of Dermal Microcirculation

Birth triggers a series of events in cutaneous vascularization, and the microcirculation of the skin continues to develop up to PNA 14 to 17 weeks (3½ to 4¼ months).[7] Immediately after birth, the microvasculature is a horizontal dense plexus with a disordered capillary network, and capillary loops are not detectable except in the nail beds, palms and soles. Capillary loops begin to appear in other areas in the second week postpartum, and are widespread 12 to 15 weeks (3 to 3¾ months) thereafter.[7] The capillary loops form lastly in the skin creases.

2.4. Summary

In summary, the SC provides an effective basal semi-permeable barrier soon after birth, if not at birth, although it continues to develop over the course of the first 6 months to 2 years of age.

3. Biotransformation in the Skin

This section discusses the effects of biotransformation enzymes on the absorption of substances topically applied to the skin.

The SC is generally thought of as a passive, inert barrier to diffusion. However, the skin can also serve as a metabolism barrier for some topically-applied substances. This is illustrated, for example, by the substantial reduction in the dermal absorption of topically-applied parabens, which is attributable to the biotransformation of parabens in the skin. The skin has all of the major biotransformation enzymes of the liver except, almost invariably, at lower levels, and at much lower levels for some enzyme systems. The biotransformation capacity of the skin is generally more saturable than that of the corresponding systems in the liver. Nevertheless, the skin has a substantial capacity to biotransform substances that enter and pass through the SC if the substances remain in the epidermis long enough.

Very little information was found to address the development of enzyme systems in human skin. Thus, the subsections below include information about the development of biotransformation capacities in the liver, which has a rich literature in comparison.

3.1. First Pass Effect

The absorption of chemicals through the skin is often thought to be the result of simple diffusion, with the SC serving as a passive, inert, rate-limiting barrier.[17] However, the skin expresses all of the major Phase 1 and Phase 2 enzymes and metabolic functions found in the liver and other tissues.[59,78] The specific activities of these enzymes in subcellular fractions of the skin are generally lower than their counterparts in the liver (0.1%–28% for Phase 1; 0.6%– 50% for Phase 2).[59] However, viable skin still has a substantial capacity to metabolize many xenobiotics.[17,59,79]

Esters, primary amines, alcohols, and acids are especially susceptible to metabolism in the skin.[59,80] Esterases and amidases are highly active in human skin, although these enzymes appear to be much more active in keratinocyte cultures than in excised whole skin or dermis-derived fibroblast cultures.[81] Esters can be extensively metabolized by non-specific esterases in the skin to yield the corresponding alcohols and acids.[59] Examples include benzyl acetate, dimethyl-, diethyl-, and dibutylphthalates, retinyl palmitate, herbicide esters, and methyl salicylate.[59] Primary amines are acetylated, alcohols and acids undergo oxidation/reduction reactions, and acids are conjugated with glycine in the skin.

In addition, conjugation with glutathione, sulfate, and glucuronic acid occurs in the skin.[59]

The capacity of the skin to metabolize xenobiotics can be an important factor determining the rates, extents, and forms (parent compounds and their metabolites) in which they exert local effects on the skin and systemic effects.[17,59] For example, *in vitro* studies clearly demonstrated extensive "first-pass" metabolism of benzo(a)pyrene (BaP) or testosterone topically applied to metabolically-viable, full-thickness skin samples from humans and other mammals, unlike previously frozen skin samples, yielding a complete spectrum of metabolites in the receptor fluid.[17] See diagram of static-type diffusion cell, for an image of the type of apparatus used in this kind of study (image is from: http://www.eurofins.com/agroscienceservices/chemistry/dermal-absorption.aspx). These studies showed that "first-pass" metabolism through mouse skin can be induced by topical BaP exposure to increase the permeation of BaP by 2- to 3-fold, and inhibited by potassium cyanide (KCN) to substantially reduce BaP permeation.

Thus, the intact, viable epidermis can function as a "metabolizing membrane," providing a metabolic as well as a diffusional barrier to percutaneous absorption, depending on the metabolic competency of the epidermal cells and the physicochemical and biological properties of the topically-applied substances and their metabolites.[17,78] The "first-pass effect" can serve as a rate-limiting barrier for compounds that bind to or otherwise remain for a sufficiently long time in the skin to produce inactive metabolites.[59]

However, the "metabolic barrier" will be negligible if the physicochemical barrier of the SC substantially limits the percutaneous penetration of a compound, or if the compound permeates the skin and enters the systemic circulation

too rapidly to enable significant "first-pass" metabolism of the compound in the skin. On the other hand, the "first-pass effect" may enhance permeation and systemic toxicity of polar metabolites formed from a lipophilic compound in the skin.[59]

It is also important to recognize the substantial differences between a "first-pass effect" in the skin after skin contact compared with the "first-pass effect" in the liver after oral exposure.[78] A compound will be susceptible to metabolism only in the relatively small area to which it is applied on the skin. In contrast, the compound will be dispersed throughout the entire liver before entering the systemic circulation after oral exposure. This factor, together with the generally lower enzymatic activities in the skin, indicates that metabolism will be more readily saturated, and the "first-pass effect" may be substantially less after skin contact than after ingestion. It is also important to note that assessing "first-pass" metabolism of a topically-applied compound, *in vivo*, is complicated by the potential for metabolites found in the skin to originate from the metabolism of the compound in the liver as well as in the skin.

3.2. Ontogeny of Biotransformation Enzymes

Unlike development of structural/anatomical and physiological/functional parameters, there is a dearth of research on the ontogeny of biotransformation-enzyme systems specifically in the skin of babies, infants and older children. Thus, little is known about differences in the metabolism of xenobiotics in the epidermis of full-term babies and infants compared with adults, although the differences could be responsible for important dissimilarities in the relative rates of metabolism, the nature of the metabolites produced and, thereby, the potential for these substances to cause local and systemic effects.[82]

In comparison, there is much more information in the scientific literature about the development and maturation of biotransformation enzymes in the liver. The ontogeny of biotransformation enzymes in the skin might be similar enough to that of liver enzymes to provide some insights by analogy. However, it should be emphasized that extrapolating information on the development of liver-enzyme systems to make assumptions about the development of dermal-enzyme systems is speculative, and must be approached with caution. This is because there is little scientific evidence to support the reliability or validity of such extrapolations. Note, for example, the substantial differences observed in the developmental timelines of the biological functions of several organ systems that can play critical roles determining the susceptibility of children to exposures (Figure 1).[2]

3.2.1. Neonatal Biotransformation Capacity in the Liver

Neonates display both Phase I and Phase II metabolic capacity for most substances in the liver, although it may be immature and can be quite low for some substances.[5] Generally, the biotransformation capacity in neonates and infants is difficult to predict based solely on PNA, because the maturation rates of Phase 1 and Phase 2 pathways vary widely by metabolic pathway and inter-individually, and the metabolic pathways can be induced by *in utero* or *post-partum* exposures to inducing agents.[83] In non-induced neonates (pre-term or full-term), the overall capacity for biotransformation is low, especially during the first two weeks of life, compared with older children and adults.[4,83,84]

Ginsberg et al. (2002) demonstrated the immaturity of metabolic and clearance systems in the first weeks to months of life, based on the evaluation of a pharmacokinetics database of 45 drugs covering a wide range of chemical structures, mechanisms of action, and metabolism and clearance pathways, including Phase I, Phase II and renal excretion pathways.[85] On average, half-lives were 3 to 5 times longer in premature neonates, and 2 to 3 times longer in full-term neonates, compared with the corresponding half-lives in adults, although the half-life for CYP1A2 substrates were 9 times longer in full-term neonates than in adults. However, these differences disappeared by 2-6 months of age, after which the half-lives were comparable to, or shorter than, those of adults for specific drugs and pathways.[4,10,83,85-87]

The biotransformation rates of several drugs in the neonate can increase precipitously from about 1/3 to 1/5 of adult rates to 2 to 6 times greater than adult rates by 2 to 3 months up to 2 to 3 years after birth, followed by a gradual decline to adult rates after puberty.[88]

3.2.2. Development of Biotransformation Capacity in the Liver

Figure 1.d, below, illustrates general trends in the timelines for the development of several major Phase I and II hepatic biotransformation pathways.[2] The specific enzymes involved in xenobiotic metabolism, as well as intermediary metabolism, typically develop at different ontogenic stages. Some enzymes increase rapidly days before birth, some increase soon after birth, and others develop around the time of weaning.[23,86] Hines (2008) suggested three categories of individual hepatic drug-metabolizing enzymes (DMEs), based on current knowledge of their ontogenies, including the following:[89]

(1) Enzymes with concentrations that peak during the first trimester (GA < 12 weeks; 3 months), remain high or decrease during gestation, but are absent or greatly diminished within 1 to 2 years after birth (e.g., CYP3A7, FMO1, SULT1A3/4, SULT1E1, and perhaps ADH1A)
(2) Enzymes with relatively constant concentrations throughout gestation and postnatal life (e.g., CYP3A5, CYP2C19, and SULT1A1)
(3) Enzymes with undetectable or very low concentrations during the second or third trimester (GA 16 to 37 weeks; 4 to 9¼ months), but which increase substantially at birth or within 1 to 2 years after birth (e.g., ADH1C, ADH1B, CYP1A2, CYP2C9, CYP2D6, CYP2E1, CYP3A4, FMO3, and SULT2A1)

The enzymes in category (3) that have onset or significantly increasing expression during the perinatal period (e.g., CYP3A7, CYP2C, CYP2D6 and CYP2E1) typically also exhibit substantially greater degrees of inter-individual variability during this period, compared with later postnatal ages.[89-92] Based on limited data, the polymorphic enzymes CYP2D6 (category 3) and CYP2C19 (category 2) appeared to exhibit especially high inter-individual variability, leading Dorne et al. (2005) to suggest that some neonates could be particularly susceptible to compounds that are deactivated by these enzymes.[92]

Generally, the variability in elimination half-lives for many chemicals tends to be greatest for full-term neonates between 1 week and 1 month of age, presumably because of the developmental changes taking place during that period.[2,93]

3.2.2.1. Glucuronidation

Glucuronidation reactions are greatly reduced in neonates, compared with older children and adults, corresponding to the substantially lower activities of uridine 5'-diphosphate (UDP)-glucuronosyltransferases (UGTs) in the neonates.

Reduced glucuronidation capacity in neonates is responsible for a number of well-characterized medical conditions, including neonatal jaundice caused by the accumulation of bilirubin in the serum and typically lasting for about 10 days in full-term neonates. Subsequently, the serum bilirubin concentrations decline steadily for about two weeks, and reach adult levels by about 3 to 6 months of age, corresponding to increases in UGT activity toward bilirubin.[22,94,95]

Other examples of medical conditions attributable to the immaturity of conjugation enzyme systems include "gray baby" syndrome from the systemic accumulation of the antibiotic chloramphenicol administered *i.v.* in neonates, and ''gasping baby'' syndrome from the systemic accumulation of benzoic acid after exposure to the preservative benzyl alcohol in umbilical catheter flushing solutions or *i.v.* injectable products.[10,84,95-99]

Strassburg et al. (2002) detected no transcripts of 9 UGT genes (i.e., UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B10, UGT2B15, representing the "typical repertoire" in humans) in the livers of two fetuses (GA ~ 20 weeks), but found transcripts of all of the UGT genes in all liver samples from children (PNA 7 to 14 months) and adults.[100] The expression levels of these genes were constant in the livers of all subjects more than 6 months old, except for UGT1A9 and UGT2B4, which appeared to be up-regulated in an age-dependent manner from 6 months up to 18 months (UGT1A9) or 24 months (UGT2B4) after birth.

However, the catalytic activities of hepatic microsomal preparations tested *in vitro*, using 18 substrates, appeared to be 3- to 40-fold less in children 6 to 12 months, 13 to 18 months and 19 to 24 months of age, compared with adults, including ibuprofen (24-fold), amitriptyline (16-fold), 4-tert-butylphenol (40-fold), estrone (15-fold), and buprenorphine (12-fold) in 13- to 24-month old children.[100] The authors concluded that hepatic glucuronidation does not correlate well with the expression of UGT genes, and may not approach adult rates until after 2 years of age for numerous substrates, including steroid hormones, phenolic substances, and opioids.

3.2.2.2. Sulfation

Sulfation appears to provide a major, broad-spectrum detoxification mechanism during human development, as well as playing a central role in steroid hormone biosynthesis, catecholamine metabolism, and thyroid hormone homeostasis, among other processes.[101,102] In contrast to glucuronidation, sulfation reactions display considerable capacities in the fetus, at birth, and thereafter.[22,84,92,95,102,103]

For example, acetaminophen sulfate is the major metabolite of acetaminophen from birth to about 9 years of age, after which acetaminophen glucuronide is the major metabolite.[84,95,104-106] Thus, the active sulfation of acetaminophen appears to compensate, at least to some extent, for the reduced glucuronidation of acetaminophen in neonates and older children up to around adolescence.[10,95,101,102,107] The weight-normalized clearance of

acetaminophen in infants 6 to 16 months of age is the same as the clearance observed in adults, and exceeds adult clearance for a period thereafter.[105,108,109]

As for acetaminophen, the sulfate conjugation of salicylamide and morphine has been reported to be similar in neonates and adults, in contrast to glucuronidation.[10,95,104,105,110] In particular, morphine is sulfated at rates comparable to adults by 2 to 6 months of age, although it is only poorly sulfated in neonates.

3.2.2.3. Glutathione-, Acetyl-, and Thiopurine-S-methyl- Transferases

Like the UGTs, glutathione-transferase activity is low during the first 6 months of life, and acetyltransferase activity remains low until about 2 years of age.[22,27] On the other hand, thiopurine *S*-methyltransferase (TPMT) activity is reported to be about 50% greater in neonates than in race-matched adults, although exhibiting polymorphism consistent with that observed for this enzyme in adults.[10,111]

3.2.2.4. Carboxylesterases

Carboxylesterases (CEs) play integral roles in the metabolism and detoxification of xenobiotics by hydrolyzing chemicals containing carboxylic acid ester, amide, and thioester functional groups.[112] CEs can also catalyze transesterification reactions.

The liver has the highest carboxylesterase (CE) activity of all of the organs, and expresses two major isozymes, including hCE1 and hCE2, although hCE2 is predominately expressed in the gastrointestinal tract.[112] Additionally, hCE1 and hCE2 differ markedly in the ability to hydrolyze some substances. For example, oseltamivir and deltamethrin are rapidly hydrolyzed only by hCE1, but aspirin and irinotecan are hydrolyzed predominantly by hCE2.

Pope et al. (2005) found no statistically significant difference between mean baby/infant (PNA 2 to 24 months) and adult (20 to 36 years old) hepatic CE activities in an *in vitro* system, using p-nitrophenyl acetate as the substrate, and the IC_{50}s of the CE inhibitor chlorpyrifos oxon in this system were comparable across the liver samples from all subjects ≥3 months of age. The amounts of carboxylases measured in the microsomal preparations were lower, and the sensitivity of the *in vitro* system to chlorpyrifos was statistically-significantly greater, for liver samples from the single 2-month-old subject, compared with the older subjects (3 months to 36 years of age).[113] The authors noted the limited scope of their study, which evaluated only 5 liver samples from individuals ≤ 2 years old, some of whom were on medications (e.g., corticosteroids) that could have influenced CE expression.

In comparison, Yang et al. (2009) found that hepatic hCE1 and hCE2 gene expression was about 70% in a group of 34 children (0 days to 10 years old), compared with 22 adults (≥ 18 years of age).[112] In addition, the hydrolysis rates of aspirin, osetamivir, deltamethrin and permethrin by the liver microsomes obtained from the group of children were only about 25% of the rates using microsomes prepared from the adults. Further, the authors noted substantially greater within-group inter-individual variability of the expression of both hCE1 (218-fold) and hCE2 (21-fold) among the children, compared to those of the adults (12-fold for hCE1; 4-fold for hCE2), which corresponded well to the elevated inter-individual variability in the hCE1 and hCE2 protein content (100-fold) and hydrolytic activity (127-fold) of liver extracts from the children.

Except for hCE1 among the children ≤ 1 year of age ($p = 0.004$), these researchers found no statistically significant linear correlation between age and either hCE1 or hCE2 expression, which they speculated is attributable, at least in part, to diseases and exposure to therapeutic agents in the older children and adults studied.[112]

3.2.2.5. Summary

The metabolic capacity of many, if not most, hepatic enzyme systems mature rapidly in the neonates, exhibiting, and even exceeding, adult capacities within about 6 months to 1 year after birth.[5,10] The capacities of these systems to detoxify or potentiate xenobiotics can reasonably be assumed to be lower in neonates and infants ≤ 6 months than in older children and adults.

However, for some substrates, the metabolizing capacity may more gradually approach adult levels only after 1 to 3 or more years of age, particularly for substrates for which metabolism may depend exclusively or almost exclusively on the activity of UGTs or glutathione- or acetyl-transferases.[10,114] Thus, with the possible exception of the latter substrates, children older than about 6 months probably have hepatic enzyme capacities similar to those of adults.

The information currently available in the scientific literature is not sufficient to establish that generalities based almost entirely on studies on the development of hepatic enzyme systems in humans can be extended to the development of such systems in the skin. However, extrapolating the hepatic developmental data to infer the likely corresponding development of cutaneous metabolic activities appears to be the best we can do, given the substantial gaps in current knowledge and the uncertainties about the ontogeny of these systems in the skin. Thus, as for the liver, the enzymatic activities in the skin of neonates and infants ≤ 6 months of age may be assumed to be lower, and more variable, than in the skin of older children and adults.

4. Susceptibility to Carcinogens and Tumor Promoters

The potential for greater susceptibility of neonates and infants to carcinogenicity has been explored in numerous animal studies since around the mid-1940s.[115-123] The neonatal mouse, in particular, is highly sensitive to direct-acting genotoxic carcinogens, which cause mutations by covalently binding to DNA to form exogenous DNA adducts.[119,120,120,124] Direct-acting carcinogens include electrophiles that bind to DNA without requiring metabolic activation, as well as chemicals (procarcinogens) that require biotransformation to produce metabolites (proximate carcinogens) that bind covalently to DNA.

Most direct-acting chemical carcinogens are procarcinogens, which require biotransformation to produce reactive proximate carcinogens.[119,120,124-126] For example, neonatal mice are extraordinarily susceptible to direct-acting hepatic carcinogens, in part because they possess biotransformation capacities sufficient to produce reactive metabolites.[120,124] However, the greater susceptibility of neonatal mice is often largely attributable to substantially greater DNA-replication rates in the liver, compared with those of adult mice. Observations such as these have led to the development of neonatal rodent tumorigenicity bioassays as animal models for testing chemicals for the potential to cause cancer.[124-126]

However, neonatal mice have proven to be insensitive to indirect-acting carcinogens that can yield tumors through secondary mechanisms.[124,125] Secondary mechanisms, which do not involve direct reaction of the carcinogen with DNA, include tumor promotion through the stimulation of cellular proliferation or the inhibition of programmed cell death (i.e., apoptosis).

In sum, the animal studies noted above, which were performed mostly on particularly sensitive strains of mice, suggest that neonates may be more susceptible than adults to exposures to direct-acting genotoxic carcinogens, but not to exposures to indirect-acting nongenotoxic carcinogens, such as tumor promoters.

5. Discussion and Conclusions

The potential dermal penetration and systemic absorption of ingredients of topically-applied cosmetic products in normal, full-term babies and infants is governed by two major factors, including the development of the (1) SC as a diffusion (physical and chemical) barrier, and (2) biotransformation-enzyme systems and capacities in the skin.

The CIR Expert Panel concluded that the SC provides an effective basal diffusion barrier at birth or within a few days of birth. The Panel considered evidence that the SC continues to develop over the first 6 months to two years of life. However, the Panel was confident that the SC in normal, full-term neonates is an efficient barrier that continues to develop incrementally throughout childhood.

The Panel noted the absence of capillary loops in the dermal papillae of neonates until about 2 weeks after birth, which continue to develop gradually until about 3 months of age. The absence of these loops probably reduces the rate of systemic absorption of lipophilic and other ingredients that can penetrate the SC, compared to the absorption rates in older children and adults.

However, the biotransformation capacities of the skin represent an important consideration for topically-applied ingredients that penetrate the SC and remain in the dermis for durations sufficient for the production or deactivation or potentially toxic metabolites. Unfortunately there is very little information available on the development of biotransformation capacities in the skin. To the extent that development in the skin parallels development in the liver, many enzyme systems in the skin will be fairly mature by about six months of age. Liver-enzyme systems, in general, tend to mature very rapidly in newborns. The major exceptions are the enzymes that catalyze glucuronidation reactions. Enzymes that catalyze reactions other than glucuronidation can increase substantially by about six months of age, and even exceed biotransformation rates of adults during childhood (e.g., sulfotransferases).

The Panel noted that toxicokinetic information in ingredient safety assessment reports include metabolism data when appropriate. Thus, for, example, if an ingredient can be expected to penetrate the SC and its metabolism in the liver depends primarily on glucuronidation pathways, then topical exposures to babies and infants may warrant greater scrutiny and caution from the Panel. This is because glucuronidation activity is generally an effective detoxification and elimination mechanism that is lower in the liver, and probably in the skin as well, in babies and infants than in adults. On the other hand, the Panel will be less concerned if an ingredient that penetrates the SC is metabolized by sulfotransferase pathways to produce nontoxic metabolites, because sulfotransferase activity is

typically much greater in babies and infants than in adults.

The Panel concluded that the information currently available in the scientific literature reveals that the skin of babies and infants is capable of fulfilling its key functions, especially that of providing an effective semipermeable diffusion barrier under basal conditions. However, susceptibility to external insults that affect the barrier function of the skin is greater, and recovery from such insults is slower, in babies than in older children. As the expression and activity of sPLA2 increase in the skin after birth, they yield free fatty acids that acidify the skin, which is a critical factor in the complete maturation of the barrier function of the skin. The normalization of the pH of the surface of the skin, and thus the maturity of the barrier function of the skin, is largely complete by 6 months of age, although in some individuals there may be a further decline in surface pH and improvement in barrier function between 6 months and 2 years of age.

The Panel recognized the substantial knowledge gaps in the scientific literature about the development of biotransformation capacities specifically in the skin, and the significant uncertainties of extrapolating from the ontogenetic information about the liver to the skin. The greatest uncertainties appear to be associated with the reported differences in biotransformation capacities between adults and babies/infants up to about 6 months of age. Some of these differences, for example differences in caboxylesterase activities, are not clearly attributable specifically to differences between adults and babies/infants, rather than simply to inter-individual differences. Further, some compounds may inhibit or induce biotransformation enzymes in the skin of babies and infants, as well as adults.

However, the nature and significance of these factors has been ill-explored in the scientific literature. The Panel encouraged basic-science investigators to explore these gaps and develop information likely to help refine safety assessments in the future, and resolved to monitor the pertinent scientific literature.

Further, the CIR Expert Panel recognizes that, in exposure assessments, babies and infants represent a distinct subpopulation because they have a greater skin-surface area to body-mass ratio, as well as greater dermal permeability for up to 6 months to 2 years of age, compared with adults. Thus, the Panel routinely considers the greater skin-surface area to body-mass ratio in children when performing cosmetic ingredient safety assessments. The Panel also emphasized that they will continue to interpret MoS estimates, as appropriate, to ensure the safety of cosmetic ingredients for babies, infants and other children.

In addition, the CIR Expert Panel is keenly aware of the potentially greater susceptibility of neonates to carcinogens, as well as to other potential toxicological effects. The postulated reasons for greater susceptibility of neonates are many, including the effect of the rate of cell division on the fixation of mutations before repair can occur, the immaturity of biotransformation systems that can deactivate direct-acting carcinogens, the proliferation of mutated cells together with normal cells in target organs as part of normal ontogeny, and the hormonal status of the neonate, to name a few.[115,116,124] The Panel routinely evaluates the physicochemical properties of cosmetic ingredients and data from carcinogenicity, genotoxicity, and other relevant toxicological studies to ensure that cosmetic ingredients do not have the potential to cause cancer in babies, infants, or any other members of the human population.

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7. Figure