A new approach to characterize dermal systemic exposure by use of chemicals' permeability coefficient (Kp) in finite dose – Application to some ingredients of nail polish by skin and nail exposure routes

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Abstract— *To evaluate systemic chemical exposure, the permeability coefficient can be used to estimate absorption. This parameter characterizes the transfer rate of a substance in a vehicle across a membrane. The biological membrane thickness is a factor of resistance to permeability. Thus, it seems interesting to take this parameter into consideration in the calculation of the absorption.*

In this study, a calculation model of systemic exposure through the skin and the nail has been developed for finite dose conditions. It represents a new approach to systemic exposure assessment and is based on the following assumption: systemic exposure to a molecule is achieved when this substance has completely crossed the biological membranes. We used skin and nail thickness to integrate the permeability coefficient in the formula. The permeability coefficient, the membrane thicknesses and the contact time represent what is called the systemic absorption factor which can be incorporated in external exposure formulas. Presented in an exponential form, it can be used to determine the amount absorbed versus time and to assess the systemic exposure to an applied amount which is finite.

Keywords— Safety testing, Skin barrier, Nail physiology, Systemic/Internal exposure, Permeability coefficient, Thickness

I. INTRODUCTION

Chemical risk assessment is defined as a process to calculate or estimate the probability of an adverse health effect which occurs after humans are exposed to a substance. This process consists of three important steps: (i) hazard assessment (identification and characterization), (ii) exposure assessment (external or systemic) and (iii) risk characterization [1-3].

A cosmetic product is currently defined as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odors" [4].

Some reference studies assess external exposure to cosmetic products [5-10]. However, a risk assessment to cosmetic products is currently not possible. It is necessary to know the ingredients and their concentrations in the finished product. Because regulations do not oblige industries to supply ingredient concentrations in the finished cosmetic product [4], and because there is limited data available in the literature [11, 12], it was necessary to be able to assess ingredient concentrations. A method was therefore developed to estimate ingredient concentrations from a standard composition of cosmetic products [13]. In this study, an exposure assessment to nail polish composition was performed by inhalation, oral and dermal (skin and nail) routes and was based on external nail polish exposure data carried out by Ficheux *et al.* [14]. According to the literature, these two studies have shown that the dermal route could not be considered as negligible in cosmetics exposure. However, the exposure assessment was performed by considering an absorption rate of 100% as recommended by the security agencies [15-17]. The skin and nails are very effective barriers against external substances and studies on chemical permeability often show a low passage of chemicals across these membranes. Justified as overly protective for the dermal route, such an external exposure assessment cannot be considered realistic in the risk assessment for the consumer; a systemic exposure assessment would be more appropriate.

It is necessary to determine the potency of percutaneous absorption of chemical substances in a systemic dermal exposure assessment. This can be characterized by the absorption percentage of the applied dose, the flux (J) and the permeability

coefficient (Kp) [18]. J corresponds to the passage of a substance amount per unit area of a membrane as a function of time ($mg.(cm^2.s)^{-1}$), whereas Kp is a rate of transfer of a molecule across a membrane ($cm.s^{-1}$).

The absorption percentage is the percutaneous absorption factor most often reported in the literature for reasons of measurement simplicity (*in vitro* and *in vivo*) and use in the exposure assessment. However, the absorption percentage depends entirely on the experimental conditions in which it is measured including the period of exposure and the dose applied. It can vary considerably from one study to another for the same chemical and therefore can just be applied to a defined exposure scenario [19].

The flux and the permeability coefficient allow an exposure assessment over time in steady state condition. When Fick's first law of diffusion is applicable (i.e. homogeneous membrane, no change in physicochemical membrane properties…), Kp is constant over the range of concentrations. The permeability coefficient is a measure of the substances' penetration speed through a membrane. Kp provides a much more consistent basis to characterize the dermal absorption potential of chemical substances in a solvent (usually water) [18-21].

To characterize the quantity absorbed (QA), some risk assessors have integrated the Kp by multiplying the chemical concentration in the vehicle (C) (i.e. the flux: J = Kp x C), the exposed surface (S) and the exposure time (t):

$$\mathbf{QA = J \times S \times t = Kp \times C \times S \times t} \quad (1)$$

The skin is a barrier in constant contact with the external environment; it is essential for the protection and the homeostatic maintenance of body [22-24]. It can histologically be divided into three main layers. (i) The epidermis, which is often divided into two separate layers in percutaneous absorption models: the *stratum corneum* (outermost layer) and the viable epidermis (other epidermal layers), (ii) the dermis and (iii) the hypodermis.

The *stratum corneum* (SC) is the outermost layer of the epidermis and consists of several layers of completely keratinized, dead cells that are constantly desquamated (i.e. corneocytes). These closely packed interdigitated corneocytes are embedded in a highly organized, dense lipid matrix. It is recognized that chemical transport through the SC is essentially by passive diffusion via the intercellular lipid pathway (i.e. lipid matrix) [23, 24].

The viable epidermis (VE) consists of three layers (*stratum granulosum*, *stratum spinosum* and *stratum basale* representing the outermost layer to the innermost, respectively) defined as a predominantly hydrophilic avascular environment (≈ 40% protein, 40% water and 15-20% lipids) and essentially composed of keratinocytes. If chemical transport in the VE is considered to be by diffusion, this process occurs through the aqueous medium [23-24].

The dermis (D) and hypodermis (HD) are more complex structures composed of various cells such as fibroblasts (which produce connective tissue), mast cells, macrophages and melanocytes, which are the most predominant cells. These hydrophilic layers are characterized as a dense irregular connective tissue with a felt work of collagen (essentially D), elastic, and reticular fibers embedded in an amorphous ground substance of mucopolysaccharides. They are also made up of a network of blood and lymphatic vessels. This characteristic can affect the diffusion and transport of chemical molecules through the skin by an increase in clearance [22-24].

The SC is often seen as the main barrier to chemical molecules in cutaneous absorption models and many models are based on the Kp of molecules for this membrane only [19]. It was demonstrated that the VE could have significant role in the absorption of chemicals. If, like the SC, the VE is considered as a homogeneous membrane, both these layers can be treated as a "diffusion resistance series". The steady state resistance barrier (1/Kp) of the total epidermis would then be equal to the sum of the resistances of diffusion of the SC and VE [24]. In their percutaneous absorption model, Cleek and Bunge integrated the contribution of the VE in the resistance to the absorption of molecules by defining a correction parameter B as the ratio of Kp_{SC} and Kp_{VE} [25]. Thus, it is possible to generate corrections to SC permeability (most commonly measured or estimated parameter) taking into account epidermal resistance in a two-compartment absorption model [24, 25]. The permeability of chemicals through D and HD can also be integrated in an absorption model if the same assumptions, necessary to the validity of Fick's law, are applied. However, studies have shown that the dermis does not act as a significant barrier to penetration [19]. Consequently to the structural complexity of these layers, the assumption of transport by simple diffusion in a homogeneous membrane is not as relevant as for the epidermis. Vascularization reinforces this fact and in order to ensure a protective risk assessment, we consider that systemic exposure is achieved when the molecules are passed through the epidermis and not the skin in whole. Thus, D and HD are not often taken into account in skin absorption models.

Like the skin, the nail is a barrier in permanent contact with the outside environment.

The nail plate, conventionally named nail, is schematically characterized by a roughly rectangular and relatively dense flat surface covering vascular tissues: nail bed and matrix [26]. The nail plate is structurally divided into three main layers: (i) the dorsal nail plate composed of few compact and keratinized epithelial cells; (ii) the intermediate nail plate contains softer keratin and represents three quarters of the nail thickness; (iii) the ventral nail plate is comprised of soft hyponychial keratin with one or two cell layers [27]. Cysteine-rich, keratin fibers are linked by disulfide bonds that confer physical stability to proteins. The nail plate forms a dense and a relatively rigid structure [28].

In a per-ungueal absorption assessment, the nail plate can be seen as the real barrier against exogenous elements [27, 29]. Like the skin, if the nail plate is considered as a homogeneous membrane, substance absorption can be characterized by using Kp. The nail consists of 10% to 30% water and of 0.1% to 1% lipids, so it can be defined as a hydrophilic membrane [30-34].

The skin and nails are membranes with variable thickness (all layers combined). These variabilities can be intra-individual (i.e. anatomical site), inter-individual, age-related etc. [26, 35-38].

Systemic exposure is characterized as a passage from an exogenous entity into the blood. To reach the systemic circulation, a molecule must pass through the membranes separating the external environment than internal environment. Molecule absorption according to time is inversely proportional to the thickness of these membranes [19].

In percutaneous absorption models, the absorbed dose is characterized *via* a flux by multiplying the Kp by the chemical concentration in the solvent (Equation 1). The absorbed dose quantity per time unit is related to an exposure surface. The membrane thickness resistance is implicitly integrated in the calculation by use of Kp, but it is defined in advance and is fixed (i.e. standardized by measuring or estimating the Kp). Incorporating membrane thickness variabilities in the systemic exposure calculation model could be interesting in exposure assessment. Kp and J are estimated as an "infinite-constant-dose" in steady-state conditions. Their use in the exposure assessment is subject to the same rules. However, in most real exposure scenarios, these conditions are rare. For example in cosmetics, even if the frequency of use is important, the product is not applied continuously. The exposure assessment should be carried out for finite doses [15].

In this study, we propose an algebraic model to calculate systemic dermal exposure in finite dose conditions. Currently, the exposure assessment by skin route is too often performed using a percentage of absorption (by default of experimental data: using 10% or 100%). This unit of percutaneous transfer is easy to use but it has too much bias to be used in different exposure scenarios. The calculation method of systemic exposure that we propose allows improving the accuracy of the exposure assessment to be more close to the reality. Furthermore, it can be used on different exposure scenarios. It is simple to use in risk assessment even if this model can be considered a rough estimate only from a scientific point of view.

This model is an alternative to the existing ones with a different approach to the utilization of permeability coefficient. It is based on the use of substance Kp with respect to the diffusion resistance induced by membrane thickness according to time. Kp is directly used as transfer speed and not as a flux. This model is applicable considering the main assumptions: diffusion is the main way for substance transfer through the skin and nails, this diffusion is constant during the exposure (i.e. no interferences in diffusion), the membranes are homogeneous, the epidermis and the nail plate are the main barriers to dermal absorption and finally, systemic exposure to a molecule is reached when this substance has completely crossed the biological membrane which separates the internal environment from the external environment. The systemic exposure calculation through the skin and nails was applied to some ingredients of a standard nail polish. Exposure results were compared to results obtained with US-EPA and RIVM methods in order to validate this new model. This study complements two previous publications on the nail polish exposure assessment: (i) exposure assessment to nail polish product [14] and (ii) exposure assessment to nail polish composition [13].

II. MATERIALS AND METHODS

2.1 Permeability coefficient

2.1.1 Theoretical overview

In the systemic exposure assessment, the substance passage across biological membranes is difficult to model realistically because there are many influential factors and variables (i.e. related to the physicochemical complexity of membranes). Assuming that the transport of molecules through the skin can be described by Fick's first law, absorption by passive diffusion models can be used as a significant approach by evaluators. The permeability coefficient and the flux are key

parameters to characterize the substance absorption over time, and their estimation is based on this main assumption. They meet specific validity criteria [19]:

- The *Stratum corneum* is considered to be the rate-limiting membrane of the skin absorption;
- The full thickness of the SC contributes to the diffusion barrier;
- The SC is a homogeneous medium;
- Penetrant and vehicle molecules diffuse across the SC as individual entities (i.e. there is no carrier effect);
- There are no size-limiting pores to affect absorption;
- The SC is not changed progressively by the vehicle or penetrant;
- Penetrant concentration changes do not alter SC or vehicle properties.

The permeability coefficient characterizes the transfer rate of a substance in a vehicle (commonly water) across a membrane. When Fick's first law prevails, Kp can be defined as a steady-state flux of chemical across the skin normalized for concentration [19]. Experimentally, it is often evaluated from the measured steady-state flux (J_{ss}) and the differential concentration (ΔC) between the donor and receiver phase (i.e. the constant concentration across the membrane) (Equation 2).

$$\mathbf{Kp} = {}^{\mathbf{J_{ss}}}/_{\mathbf{\Delta C}} \tag{2}$$

Where Kp is the permeability coefficient of chemical ($cm.h^{-1}$); J_{ss} is the steady-state flux ($mg.(cm^2.h)^{-1}$); and ΔC is the chemical concentration through the membrane($mg.cm^{-3}$).

If Kp is not estimated experimentally from J_{ss}, it can be obtained by the theoretical expression of Kp *via* the diffusion coefficient [19, 39, 40] (Equation 3).

$$\mathbf{Kp_{SC}} = \frac{\mathbf{K_{SC/W}} \times \mathbf{D_{SC}}}{\mathbf{l_{SC}}} \tag{3}$$

Where Kp_{SC} is the permeability coefficient of substance through the *stratum corneum* ($cm.h^{-1}$); $K_{SC/W}$ is the partition coefficient between *stratum corneum* and water (unitless); D_{SC} is the diffusion coefficient of substance through the *stratum corneum*($cm^2.h^{-1}$); and l_{SC} is the *stratum corneum* thickness (cm) (Default value = 10^{-3} cm).

Ideally, the Kp should be calculated from Equation 3. This requires knowledge of all the parameters needed ($K_{SC/W}$, D_{SC} and L_{SC}). However, $K_{SC/W}$ and D_{SC} are difficult to characterize. Empirically correlation algorithms have been developed to approximately estimate Log Kp with parameters commonly found in the literature (Equation 4) [19, 20, 39, 41, 42].

$$\mathbf{LogKp} = \mathbf{b} + \mathbf{a} \times \mathbf{LogK_{OW}} - \mathbf{c} \times \mathbf{MW} \tag{4}$$

Where $K_{SC/W}$ is the partition coefficient between *stratum corneum* and water (unitless); K_{OW} is the octanol/water partition coefficient (unitless); MW is the molecular weight ($g.mol^{-1}$); and a, b, c are correlation coefficients empirically defined (unitless).

This algorithm (Equation 4) is used as a base in many Quantitative Structure-Permeation Relationship (QSPeRs) models for skin permeation (20, 43-50]. These models were mainly developed by linear regressions from databases of substances whose Kp_s were measured experimentally according to their physicochemical properties. QSPeRs (or QSARs) models are relatively simple tools to estimate Kp or J when experimental measurements are lacking [15, 17, 40]. Although these models have limitations on their use, they have been widely studied and developed to assess skin absorption of molecules in an aqueous vehicle as accurately as possible. Guidance and criteria for validation of QSPeRs have been developed by the Organization for Economic Cooperation and Development to estimate and characterize these models [51, 52].

Currently, one of the best known and most commonly used models in the evaluation of percutaneous transfer is that of Potts and Guy [20]. This is the one used in this study. Conversely, due to the small contribution of nail in the exposure assessment

and the low permeability of this membrane, few studies relating to this substances transfer are available [53, 54]. Moreover, these studies mainly involve drug substances for the topical treatment of nail diseases. In this study we propose a model based on a simple linear regression of experimentally measured Kp and MW values of 37 molecules.

2.1.2 Method applied

2.1.2.1 Skin permeability coefficient (Kps)

The model developed by Potts and Guy is based on the structure of the algebraic Equation 4 [20]. They used a large compilation of published skin permeability coefficients (Kp_s) from aqueous vehicles (reflecting the substance permeability coefficient through the *stratum corneum*) [55]. By multiple regression of these experimental values with MW (18 to >750) and K_{OW} (-3 to +6) molecules, they generated Equation 5 with a reasonable correlation ($r^2 = 0.67$ for 93 compounds) [20, 42].

$$\mathbf{LogKp_S = -6.3 + 0.71 \times LogK_{OW} - 0.0061 \times MW} \quad (5)$$

Expressed in centimeters per second in the Potts and Guy model, the estimated Kp_s was re-expressed in centimeters per week ($cm.week^{-1}$) for the exposure assessment.

2.1.2.2 Ungual permeability coefficient (Kp_u)

Base on the same assumptions regarding Kp_s estimation (homogeneous membrane, transfer by diffusion etc.), we developed a simple QSPeRs model to predict permeability coefficients of molecules across fingernails (Kp_u).

The correlation between Kp_u of 37 molecules experimentally measured in an aqueous solvent and their molecular weight was carried out [32, 56-60]. By linear regression of Kp_u with MW, the regression Equation 6 was obtained ($r^2 = 0.67$) (Figure I).

$$\mathbf{LogKp_U = -0.0087 \times MW - 5.6006} \quad (6)$$

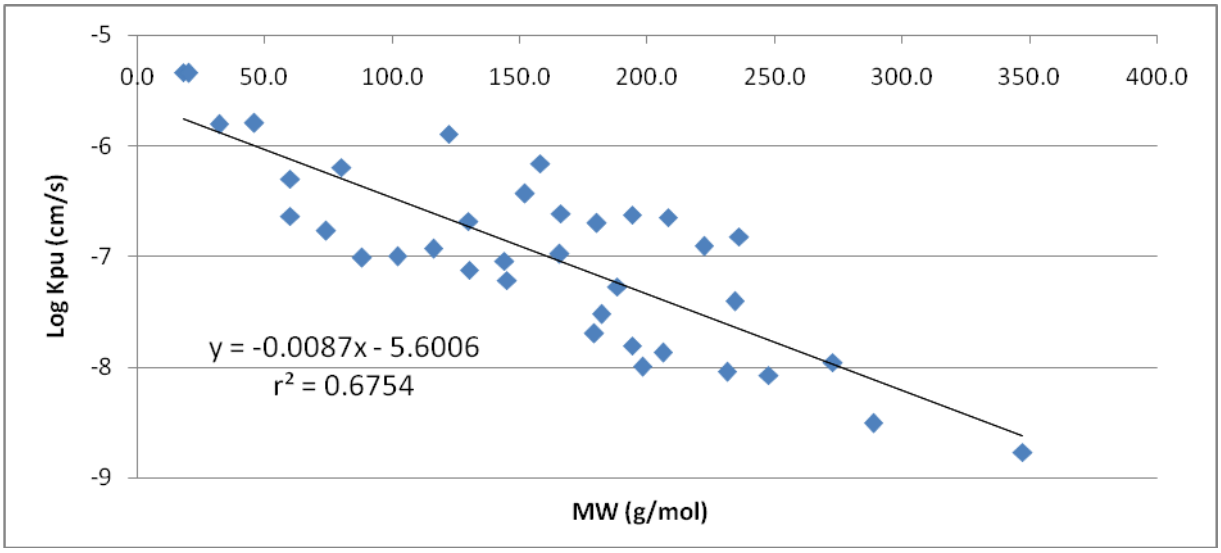

FIGURE I: A PLOT OF EXPERIMENTALLY MEASURED KPU VERSUS MW OF MOLECULES. THE SOLID LINE REPRESENTS THE LINEAR REGRESSION BETWEEN THESE TWO PARAMETERS WHICH GAVE US EQUATION 6.

Expressed in centimeters per second in this model, the estimated Kp_u was re-expressed in centimeters per week ($cm.week^{-1}$) for the exposure assessment.

2.2 Systemic exposure model

2.2.1 Theoretical overview

Systemic exposure to chemical substances is estimated by the absorbed amount per kilogram of body weight per time unit (SED). In dermal exposure, this assessment is often obtained by the exposed and absorbed quantity per unit area (AD), based on body surface exposed (S), the exposure frequency (F) and the body weight of the individual (BW) (Equation 7) [19].

$$SED = \frac{AD \times S \times F}{BW} = \frac{QA \times F}{BW} \quad (7)$$

Where SED is the systemic exposure dose (mg.(kg bw.day)$^{-1}$); AD is the substance absorbed dose (mg.cm^{-2}); S is the body surface exposed (cm^2); F is the exposure frequency (day^{-1}); QA is the quantity of substance absorbed (mg); and BW is the body weight (kg bw).

When dermal absorption is expressed as an absorption percentage, AD can then be simply estimated (Equation 8) (Adapted from ECHA [61]; SCCS [16]). It is considered that the substance of interest is totally absorbed (100 %) in the worst-case scenario. The absorbed quantity corresponds to the exposed quantity per surface unit. If an absorption percentage value is available, it is necessary to assume that the experimental conditions in which the substance absorption quantity is measured are equivalent (or similar) to the exposure conditions. If the substance is contained in a liquid, AD is not based on the quantity of substance applied to a certain surface area of body, but on the substance concentration in the mixture that is in contact with the skin (Equation 9) (Adapted from ECHA [61]).

$$AD = \frac{Q \times WF \times A}{S} \quad (8)$$

Or

$$AD = C \times l_P \times A \quad (9)$$

Where AD is the substance absorbed dose (mg.cm^{-2}); Q is the product quantity (mg); WF is the weight fraction of the substance in the final product (%); A is the total absorption (i.e. 100%) or absorption percentage under defined conditions (with a correction factor if it is necessary); S is the body surface exposed (cm^2); C is the substance concentration in a product (mg.cm^{-3}); and l_P is the thickness of product layer on membrane (cm).

When dermal absorption is expressed by a transfer factor across a membrane such as Kp or J, AD it is more complex to estimate and interpret (i.e. many validity criteria to consider).

Kp represents a substance transfer rate in a solvent (generally water) through a membrane. The amount of exposed substance is therefore expressed as a concentration. The result of the multiplication of a concentration and a Kp gives a flux through a membrane (Equation 2). J represents an AD per time unit. To simply obtain AD, J can be multiplied by the contact time (t) (Equation 10).

$$AD = Kp \times C \times t \quad (10)$$

Where AD is the substance absorbed dose (mg.cm^{-2}); Kp is the permeability coefficient (cm.day^{-1}); C is the substance concentration (mg.cm^{-3}); and t is the contact time (day).

This approach is used to characterize AD for inorganic water contaminants [19, 39].

Cleek and Bunge established two equations for calculating DA taking into account the SC absorption in non-steady state conditions and the contribution of VE in the percutaneous absorption according to exposure time [25, 62, 63]. These equations were used as a base and are recommended by the US-EPA for the calculation of DA [19]. The equations were updated in new report of US-EPA in 2007 [39].

The US-EPA (2007) recommends using this model for calculating DA to estimate systemic exposure by the dermal route. Equation 11 is used to characterize the substance absorption process by the SC (i.e. generally low exposure time, non-steady state). Equation 12 is used to characterize the substance absorption process taking into account the VE contribution (i.e. generally long exposure time, steady state reached).

$$\text{If } t \leq t^*: \quad AD = 2 \times FA \times Kp \times C \times \sqrt{\frac{6 \times t_{lag} \times t}{\pi}} \tag{11}$$

$$\text{If } t > t^*: \quad AD = FA \times Kp \times C \times \left[\frac{t}{1+B} + 2 \times t_{lag} \times \left(\frac{1 + 3 \times B + 3 \times B^2}{(1+B)^2}\right)\right] \tag{12}$$

Where AD is the substance absorbed dose ($mg.cm^{-2}$); FA is the fraction absorbed (i.e. relative to desquamation of SC - unitless); Kp is the permeability coefficient ($cm.day^{-1}$); C is the substance concentration ($mg.cm^{-3}$); t is the contact time (day); t_{lag} is the lag time (day); t* is the time to reach steady state (day); B is the ratio of Kp_{SC} relative to Kp_{VE} (unitless).

In these formulas, time is the limiting factor of the absorbed dose (i.e. concentration in donor phase is constant). Under conditions of exposure to certain products (such as cosmetics), the quantity may be a limiting factor to be considered (i.e. topical application of a finite dose remains in contact for a varying length of time). When the exposure conditions are considered to be simplistic (diffusion transport, no washing off, no evaporation etc.), the amount absorbed in a finite dose can be obtained by expressing the simple diffusion equation in the Laplace domain [42, 64, 65]. Anissimov proposed equations (not shown) based on this principle whose use is governed by several conditions (volume capacity of the donor phase and membrane, exposure time etc.).

To calculate AD by incorporating the concept of finite dose, the RIVM provides an equation in which the amount of the applied substance is multiplied by its absorption coefficient (unitless) in an exponential form (Equation 13) (Adapted from RIVM [66]).

$$AD = \frac{Q \times \left(1 - e^{-Kp \times s \times t / V}\right)}{S} \tag{13}$$

Where AD is the substance absorbed dose ($mg.cm^{-2}$); Q is the substance amount on the skin (mg); Kp is the permeability coefficient ($cm.day^{-1}$); S is the body surface exposed (cm^2); t is the contact time (day); and V is the donor volume (cm^3) (V = Q/C).

2.2.2 Method applied

2.2.2.1 Background

In a previous study, Chevillotte *et al.* proposed a method to assess external exposure to cosmetics product composition [13]. They used Equation 14 to estimate exposure by dermal route (skin and nail) for nail polish ingredients.

$$WED_{s\&u} = \left(\frac{F \times Q \times WF \times (S/(NS + NWS))}{BW}\right) \times (1 + Binom.) \tag{14}$$

Where $WED_{s\&u}$ is the Weekly Exposure Dose for dermal exposure route ($\mu g.(kg\ bw.week)^{-1}$); F is the frequency of use ($use.week^{-1}$); Q is the quantity of product applied on nail per coat ($\mu g.use^{-1}$); WF is the ingredient weight fraction in the final product (%); S is the surface of nail or nail wall (cm^2); NS is the nail surface (cm^2); NWS is the nail wall surface (cm^2); BW is the body weight (kg bw) and (1 + binom.) is probability that people apply a second coat of nail polish (unitless).

From this equation, AD can be expressed by Equation 15 which is similar to Equation 11. QA represents the amount of substance absorbed by skin or ungual route for the applied product quantity (i.e. external exposure).

$$AD = \frac{QA}{S} = \frac{Q \times WF \times ({}^{S}/_{(NS + NWS)}) \times A}{S} \quad (15)$$

Where AD is the substance absorbed dose (µg.cm^{-2}); QA is the substance quantity absorbed (µg); Q is the quantity of product applied on nail per coat (µg.use^{-1}); WF is the ingredient weight fraction in the final product (%); S is the surface of nail or nail wall (cm²); NS is the nail surface (cm²); NWS is the nail wall surface (cm²); A is the total absorption (i.e. 100%) or absorption percentage under defined conditions (with a correction factor if it is necessary).

2.2.2.2 Exposure model

Conventionally, AD is estimated by a flux through the membrane by multiplying Kp and C (Equation 10). C represents the substance amount available for absorption relative to the vehicle volume in which it is dissolved (usually water). Assuming that the quantity of the exposure substance is fully available for absorption, we chose to measure percutaneous absorption directly using Kp as an absorption factor (not using a flux). The quantity subject to absorption is not dependent on the exposed surface area (µg.(cm^2.week)$^{-1}$) but on resistance induced by the thickness of the membranes versus time (mg.(cm.week)$^{-1}$). QA is then estimated by Equation 16 where Kp, t and l represent a set named systemic absorption factor (SAF - unitless) in this study.

In Equation 16, the quantity absorbed is directly dependent on Kp. In a relatively long exposure time scenario and when the Kp of the substance is high, the cumulative quantity absorbed over time could be greater than the quantity originally applied. This case corresponds to conditions in which the quantity of substance available is infinite (i.e. the initial quantity in the donor phase is still available). Integrating the systemic absorption factor in an exponential form as in Equation 13 ensures the initial dose is never exceeded (Equation 17).

$$QA = Q \times WF \times \left({}^{S}/_{(NS + NWS)}\right) \times \left({}^{Kp \times t}/_{l}\right) \quad (16)$$

$$QA = Q \times WF \times \left({}^{S}/_{(NS + NWS)}\right) \times \left(1 - e^{-{}^{Kp \times t}/_{l}}\right) \quad (17)$$

Where QA is the substance quantity absorbed (mg); Q is the quantity of product applied on nail per coat (µg.use^{-1}); WF is the ingredient weight fraction in the final product (%); S is the surface of nail or nail wall (cm²); NS is the nail surface (cm²); NWS is the nail wall surface (cm²); Kp is the permeability coefficient (cm.week^{-1}); t is the contact time (week); l is the membrane thickness (cm).

Based on the following assumptions, namely that:

- substance transfer through the skin and nails is mainly by diffusion;
- thickness is an important factor in the transfer resistance of the molecules through a membrane, and should be considered in the exposure calculations;
- the SC possesses physicochemical characteristics that are the main barrier to percutaneous absorption of most molecules;
- Ep is a homogeneous structure similar to SC, and represents the real layer thickness to be crossed to reach the systemic circulation;
- the nail plate represents the real barrier against exogenous elements and the three layers that compose it form a homogenous membrane;

Equation 18 provides a new approach to characterize the systemic exposure dose by the dermal route (skin or nail) using Kp.

$$SED = \frac{F \times AD \times S}{BW} = \frac{F \times QA}{BW} = \frac{F \times Q \times WF \times \left(S/(NS + NWS)\right) \times \left(1 - e^{-Kp \times t/l}\right)}{BW} \quad (18)$$

Where SED is the Systemic Exposure Dose for dermal exposure route (µg.(kg bw.week)$^{-1}$); F is the frequency of use (use.week^{-1}); AD is the substance absorbed dose per use (µg.(cm^2.use)$^{-1}$); QA is the substance quantity absorbed (µg); S is the surface of membrane exposed (cm^{-2}); NS is the nail surface (cm^{-2}); NWS is the nail wall surface (cm^{-2}); Q is the quantity of product applied on nail per coat (µg.use^{-1}); WF is the ingredient weight fraction in the final product (%); Kp is the permeability coefficient of the substance through the membrane (cm.week^{-1}); t is the contact time (week); l_s is the membrane thickness (cm).

Equation 19 is applied to the systemic exposure assessment to nail polish ingredients, and that used as an example in this study.

$$SED_{s\&u} = \left(\frac{F \times Q \times WF \times \left(S/(NS + NWS)\right) \times \left(1 - e^{-Kp_{s\&u} \times t/l_{s\&u}}\right)}{BW} \right) \times (1 + Binom.) \quad (19)$$

Where $SED_{s\&u}$ is the Systemic Exposure Dose for skin and ungual exposure route (µg.(kg bw.week)$^{-1}$); F is the frequency of use (use.week^{-1}); Q is the quantity of product applied on nail per coat (µg.use^{-1}); WF is the ingredient weight fraction in the final product (%); S is the surface of membrane exposed (cm^{-2}); NS is the nail surface (cm²); NWS is the nail wall surface (cm²); $Kp_{s\&u}$ is the permeability coefficient of the substance through the skin (= Kp_{SC}) or nail plate (cm.week^{-1}); t is the contact time (week); $l_{s\&u}$ is the skin (= l_{EP}) and nail plate thickness (cm); BW is the body weight (kg bw); and (1 + binom.) is probability that people apply a second coat of nail polish (unitless).

In order to compare our results with those obtained from conventional formulas, we have adapted Equation11 (proposed by the US-EPA [19, 39]) and Equation 13 (proposed by the RIVM [66]) to our input data to assess exposure with the same scenarios (Equation 20 and 21, respectively). In these systemic exposure models, the substance concentration is present in the input data. For cosmetic products we do not have this parameter data (not supplied by industry in relation to current legislation). We possess a weight fractions distribution provided by the proposed method of Chevillotte *et al.* [13]. The product density of 0.999 g.cm^{-3} is equivalent to a water density of 0.998 g.cm^{-3} at 20-22 °C. Thus, we assume that all substance densities are equivalent to water and that C is equal to WF (µg/cm^3).

$$\text{If } t \leq t^*: \quad SED_s = \left(\frac{2 \times F \times Kp \times C \times NWS \times \sqrt{\frac{6 \times t_{lag} \times t}{\pi}}}{BW} \right) \times (1 + Binom.) \quad (20)$$

$$SED_{s\&u} = \left(\frac{F \times Q \times WF \times \left(S/(NS + NWS)\right) \times \left(1 - e^{-\frac{Kp_{s\&u} \times S \times t}{q/C}}\right)}{BW} \right) \times (1 + Binom.) \quad (21)$$

Where SED_s is the Systemic Exposure Dose for skin exposure route (µg.(kg bw.week)$^{-1}$); t_{lag} is the lag time (week); t* is the time to reach steady state (week); t is the contact time (week); $SED_{s\&u}$ is the Systemic Exposure Dose for skin or ungual exposure route (µg.(kg bw.week)$^{-1}$); F is the frequency of use (use.week^{-1}); Q is the quantity of product applied per coat (µg.use^{-1}); WF is the ingredient weight fraction in the final product (%); S is the surface of membrane exposed (cm^{-2}); NS is the nail surface (cm²); NWS is the nail wall surface (cm²); $Kp_{s\&u}$ is the permeability coefficient of the substance through the skin (= Kp_{SC}) or nail (cm.week^{-1}); q is the quantity of substance applied per coat [= Q x WF x (S/ (NS x NWS))] (µg); C is the substance concentration in the vehicle (≈WF) (µg/cm^3); BW is the body weight (kg bw); and (1 + binom.) is probability that people apply a second coat of nail polish (unitless).

To determine systemic exposure by the skin route with the US-EPA's formula, it was necessary to know (i) B because Equation 20 is usable only if $B \leq 0.6$, (ii) t_{lag} to use it in the equation and to calculate t*, and (iii) t* to verify that the conditions of application of the formula are fulfilled (i.e. $t \leq t^*$) [19].

2.3 Exposure assessment

2.3.1 Data used

Within the framework of an application of the proposed method, an example of systemic exposure to some ingredients of nail polish was estimated. The data used came from these sources: (i) global data of exposure to nail polish taken from the study of Ficheux *et al.* [14]; (ii) ingredient concentrations (i.e. weight fractions) were obtained using the method described in the first study of Chevillotte *et al.* [13]; and (iii) specific data to assess systemic exposure by the dermal route as defined in this document.

2.3.1.1 Data from the study of Ficheux et al.

Some of the data relating to the French population's consumption habits was obtained *via* a web enquiry conducted in partnership with a survey institute. In accordance with quota sampling, this survey was conducted on 1512 women (18-85 years) and 301 children (0-17 years). The main parameters obtained were: percentage of users, frequency of use, contact time (i.e. wearing time), number of coatsapplied and body weight. Data such as quantities applied, nail area, nail width and nail wall perimeter were obtained from laboratory tests on 110 volunteers (18-62 years). Other data used in this study were obtained from the literature (e.g. nail wall area).

2.3.1.2 Data from the study of Chevillotte et al.

The amounts of ingredient available for absorption were obtained by multiplying the quantity of product applied by the weight fractions of ingredients in the final product. Using the order of composition of marketed products (nail polish here) and much of the data available on the web (patent publication), it was possible to propose a qualitative (i.e. a standard composition) and quantitative (i.e. weight fraction per ingredient or ingredient families) composition of nail polish.

In our study, we chose as an example 6 ingredients from 3 different families: (i) Butyl acetate (BA) and isopropyl alcohol (IA) derived from the family of "organic solvents", (ii) acetyl tributyl citrate (ATC) and ethyl tosylamide (ET) from the family of "plasticizers", and (iii) benzophenone (Be) and citric acid (CA) from the family of "agents of physicochemical properties control".

2.3.1.3 Specific parameters for the systemic exposure assessment

To switch from an external exposure to an internal exposure, an absorption factor (SAF) has been proposed in our model. It is composed of a set of specific factors necessary for the absorption evaluation: the permeability coefficient, the contact time of the substance on the membrane and the membrane thickness.

The substances' Kp_s was obtained via the model of Potts and Guy (see section 2.1.2.1) and the Kp_u was obtained using the model presented in section 2.1.2.2.

t represents the nail polish contact time with membranes. Originally, this contact time was estimated as the exposure time to nail polish according to the results of Ficheux *et al.* with a median value of 1 week [14]. However, tests carried out on the product showed that it was completely dry in approximately 30 minutes. Solvent evaporation leads to hardening of product. We chose to estimate the percutaneous absorption over a period of 30 minutes for reasons that are developed in section 4.2.1.2 of the discussion.

The epidermal and nail plate thicknesses were obtained by data adjustment from a few publications based on average distribution form independently of age, sex or body sites [67-70].

2.3.2 Monte Carlo probabilistic method

Calculations of exposure were realized according to the probabilistic Monte Carlo method on the basis of 10,000 iterations [71]. Realized by age class, exposure calculations were performed using the input data described previously and which had earlier been adjusted to theoretical distributions with the Chi2 goodness of fit test with @Risk 6 software (Palisade Corp.). The distributions obtained from the simulations were used to characterize the P50 and the P95 of exposures. "Life-long"

exposures were calculated by weighting exposures obtained by age based on the number of years of each of the respective classes over a lifetime of use estimated at 85 years.

III. RESULTS

In this study, we chose to assess systemic exposure to 6 ingredients of a standard nail polish to illustrate the proposed calculation model. Life-long exposure was estimated by age group based on consumption data described by Ficheux *et al.* [14]. External exposure was performed for the same ingredients with the same input data in order to perform a comparison with systemic exposure data. The results obtained were represented by the median (P50) and the ninety-fifth percentile (P95) of distributions.

3.1 Permeability coefficients and quantitative data

The weight fractions of substances and their concentrations in the finished product were obtained with the method proposed by Chevillotte *et al.* [13]. The Kp of substances through skin and nails were estimated in centimeters per week from the results calculated by Equations 5 and 6, respectively. B, t_{lag} and t* were calculated from equations given by the US-EPA [39]. All of these results are shown in Table 1. B is lower than 0.6 for all substances and t* is always greater than t. Thus, the formula 20 is applicable [19, 39].

TABLE 1
NECESSARY DATA TO CALCULATE THE EXPOSURE OF THE 6 INGREDIENTS COMMONLY FOUND IN NAIL POLISH COMPOSITION

Substances	No CAS	Quantitatives data (fromChevillotte*et al.*[13])				Physicochemicalproperties		Skin		Nail		Skin				
		WF (unitless)		C (µg/cm3)		MW	LogKow	LogKps (cm/s)	Kps (cm/week)	LogKpu (cm/s)	Kpu (cm/week)	B (unitless)	tlag		t*	
		P50	P95	P50	P95								hour	week	hour	week
Butylacetate	123-86-4	28.0	46.6	280291	465515	116.16	1.25	-6.12	0.4577	-6.61	0.1481	0.0113	0.4696	0.0028	1.1269	0.0067
Isopropylalcohol	67-63-0	4.8	13.1	47675	131499	60.10	0.05	-6.63	0.1414	-6.12	0.4551	0.0025	0.2279	0.0014	0.5470	0.0033
Acetyltributyl citrate	77-90-7	4.6	7.9	45793	79180	402.48	4.29	-5.71	1.1814	-9.10	0.0005	0.0543	18.8398	0.1121	45.2156	0.2691
Ethyltosylamide	1077-56-1	3.7	8.4	37376	84218	199.27	1.87	-6.19	0.3924	-7.33	0.0280	0.0127	1.3712	0.0082	3.2909	0.0196
Benzophenone	119-61-9	0.2	0.5	2479	4592	182.22	3.18	-5.15	4.2449	-7.19	0.0394	0.1312	1.1006	0.0066	2.6414	0.0157
Citricacid	77-92-9	0.1	0.3	1089	3007	192.12	-1.64	-8.64	0.0014	-7.27	0.0323	0.0000	1.2504	0.0074	3.0010	0.0179

3.2 Exposure assessment

3.2.1 External exposure

Table 2 presents results obtained in the context of as external exposure assessment by exposure route (skin and nail), by ingredient and by age group. The quantity absorbed for one coat was obtained by Equation 15 where A = 100 %; it was expressed in micrograms (µg). External exposure was calculated for one coat and for all coats (Equation 14). It was expressed in micrograms per kilogram body weight per week (µg.(kg bw.week)$^{-1}$).

3.2.1.1 By exposure route

The results of QA and exposure show that the nail is the main exposure route for all ingredients (≈ 3 – 4 times greater).

3.2.1.2 By ingredient

The results of QA and exposure show that BA presents the highest values; IA, ATC and ET have approximately similar values; and Be and CA provide the smallest values. Generally, the values have a tendency to decrease with the estimated weight fractions for skin or ungual routes (Table 1 and Table 2).

TABLE 2
RESULTS OF GLOBAL QA AND WED BY INGREDIENT, AGE GROUP AND EXPOSURE ROUTE

	Age (years)	Quantity absorbed for 1 coat (µg)					Exposure for 1 coat (µg.(kg bw.week)$^{-1}$)					Exposure for all coats (µg.(kg bw.week)$^{-1}$)				
Substances		0-12	13-17	18-34	35-85	Life-long	0-12	13-17	18-34	35-85	Life-long	0-12	13-17	18-34	35-85	Life-long
		Skin														
Butyl acetate	P50	1.05E+04	1.06E+04	1.05E+04	1.06E+04	1.09E+04	3.17E+02	1.83E+02	1.53E+02	1.26E+02	2.19E+02	3.17E+02	2.92E+02	2.42E+02	1.91E+02	3.08E+02
	P95	2.57E+04	2.52E+04	2.48E+04	2.50E+04	2.47E+04	2.75E+03	1.16E+03	7.94E+02	6.72E+02	8.23E+02	2.75E+03	1.96E+03	1.36E+03	1.12E+03	1.16E+03
Isopropyl alcohol	P50	1.85E+03	1.86E+03	1.85E+03	1.86E+03	1.90E+03	5.82E+01	3.33E+01	2.75E+01	2.32E+01	4.04E+01	5.82E+01	5.28E+01	4.39E+01	3.47E+01	5.65E+01
	P95	6.22E+03	6.28E+03	6.19E+03	6.13E+03	6.18E+03	5.34E+02	2.45E+02	1.72E+02	1.39E+02	1.82E+02	5.34E+02	4.05E+02	2.87E+02	2.29E+02	2.53E+02
Acetyl tributyl citrate	P50	1.65E+03	1.66E+03	1.66E+03	1.66E+03	1.71E+03	4.76E+01	2.75E+01	2.22E+01	1.81E+01	3.34E+01	4.76E+01	4.26E+01	3.52E+01	2.77E+01	4.64E+01
	P95	4.43E+03	4.38E+03	4.41E+03	4.41E+03	4.34E+03	4.24E+02	1.99E+02	1.40E+02	1.12E+02	1.41E+02	4.24E+02	3.26E+02	2.40E+02	1.88E+02	2.01E+02
Ethyltosylamide	P50	1.44E+03	1.44E+03	1.44E+03	1.44E+03	1.49E+03	4.47E+01	2.58E+01	2.15E+01	1.78E+01	3.07E+01	4.47E+01	4.03E+01	3.36E+01	2.71E+01	4.33E+01
	P95	4.15E+03	4.12E+03	4.13E+03	4.16E+03	4.08E+03	3.98E+02	1.80E+02	1.21E+02	9.93E+01	1.30E+02	3.98E+02	2.95E+02	2.07E+02	1.62E+02	1.79E+02
Benzophenone	P50	9.19E+01	9.23E+01	9.17E+01	9.27E+01	9.52E+01	2.77E+00	1.59E+00	1.28E+00	1.07E+00	1.91E+00	2.77E+00	2.50E+00	2.02E+00	1.59E+00	2.66E+00
	P95	2.43E+02	2.41E+02	2.43E+02	2.40E+02	2.38E+02	2.38E+01	1.07E+01	7.45E+00	6.11E+00	7.80E+00	2.38E+01	1.80E+01	1.30E+01	1.02E+01	1.11E+01
Citric acid	P50	4.21E+01	4.22E+01	4.23E+01	4.23E+01	4.37E+01	1.29E+00	7.41E-01	6.10E-01	5.08E-01	8.88E-01	1.29E+00	1.17E+00	9.63E-01	7.67E-01	1.24E+00
	P95	1.42E+02	1.41E+02	1.41E+02	1.42E+02	1.41E+02	1.30E+01	5.71E+00	4.09E+00	3.22E+00	4.24E+00	1.30E+01	9.35E+00	7.01E+00	5.30E+00	5.98E+00
		Nail														
Butyl acetate	P50	4.01E+04	4.03E+04	4.02E+04	4.03E+04	4.07E+04	1.19E+03	6.93E+02	5.76E+02	4.74E+02	8.18E+02	1.19E+03	1.08E+03	8.98E+02	7.22E+02	1.15E+03
	P95	8.80E+04	8.78E+04	8.81E+04	8.74E+04	8.82E+04	1.01E+04	4.28E+03	2.96E+03	2.44E+03	3.04E+03	1.01E+04	7.28E+03	5.07E+03	4.07E+03	4.25E+03
Isopropyl alcohol	P50	7.01E+03	7.01E+03	7.00E+03	6.98E+03	7.06E+03	2.21E+02	1.26E+02	1.05E+02	8.87E+01	1.50E+02	2.21E+02	2.00E+02	1.63E+02	1.31E+02	2.12E+02
	P95	2.22E+04	2.21E+04	2.22E+04	2.21E+04	2.25E+04	1.96E+03	8.75E+02	6.38E+02	5.03E+02	6.66E+02	1.96E+03	1.50E+03	1.07E+03	8.37E+02	9.20E+02
Acetyl tributyl citrate	P50	6.35E+03	6.31E+03	6.33E+03	6.31E+03	6.40E+03	1.81E+02	1.03E+02	8.50E+01	7.00E+01	1.25E+02	1.81E+02	1.61E+02	1.33E+02	1.05E+02	1.74E+02
	P95	1.54E+04	1.55E+04	1.55E+04	1.55E+04	1.57E+04	1.58E+03	7.34E+02	5.06E+02	4.12E+02	5.14E+02	1.58E+03	1.19E+03	8.78E+02	6.87E+02	7.32E+02
Ethyltosylamide	P50	5.45E+03	5.48E+03	5.48E+03	5.45E+03	5.53E+03	1.69E+02	9.71E+01	8.10E+01	6.84E+01	1.16E+02	1.69E+02	1.52E+02	1.27E+02	1.02E+02	1.63E+02
	P95	1.48E+04	1.48E+04	1.49E+04	1.48E+04	1.50E+04	1.46E+03	6.46E+02	4.46E+02	3.59E+02	4.73E+02	1.46E+03	1.08E+03	7.66E+02	5.85E+02	6.53E+02
Benzophenone	P50	3.52E+02	3.51E+02	3.52E+02	3.50E+02	3.55E+02	1.03E+01	5.89E+00	4.90E+00	4.05E+00	7.08E+00	1.03E+01	9.47E+00	7.61E+00	6.10E+00	9.88E+00
	P95	8.53E+02	8.57E+02	8.52E+02	8.53E+02	8.62E+02	8.81E+01	3.93E+01	2.75E+01	2.19E+01	2.84E+01	8.81E+01	6.64E+01	4.77E+01	3.67E+01	3.96E+01
Citric acid	P50	1.60E+02	1.60E+02	1.60E+02	1.60E+02	1.62E+02	4.90E+00	2.77E+00	2.30E+00	1.92E+00	3.30E+00	4.90E+00	4.40E+00	3.62E+00	2.87E+00	4.62E+00
	P95	5.13E+02	5.12E+02	5.11E+02	5.17E+02	5.16E+02	4.55E+01	2.10E+01	1.48E+01	1.17E+01	1.57E+01	4.55E+01	3.47E+01	2.52E+01	1.96E+01	2.17E+01

3.2.1.3 By age group

QA is roughly similar for all ingredients for the same exposure route but the exposure results are different. Individuals between 0 and 12 years old are the most exposed regardless of the exposure route or the number of layers of varnish applied. Conversely, persons aged 35-85 years are less exposed.

3.2.2 Systemic exposure

Table 3 presents the results obtained for the systemic exposure assessment by exposure route (skin and nail), by ingredient and by age group with the proposed calculation model which is the subject of this study. The quantities absorbed for one coat were obtained using Equation 20 and were expressed in micrograms (µg). Systemic exposure was calculated for one coat and for all coats using Equations 19 and 20-21 respectively (SED). It was expressed in micrograms per kilogram body weight per week (µg.(kg bw.week)$^{-1}$).

TABLE 3
RESULTS OF SYSTEMIC QA AND SED BY INGREDIENT, AGE GROUP AND EXPOSURE ROUTE

Substances	Age (years)	Quantity absorbed for 1 coat (µg)					Exposure for 1 coat (µg.(kg bw.week)$^{-1}$)					Exposure for all coats (µg.(kg bw.week)$^{-1}$)				
		0-12	13-17	18-34	35-85	Life-long	0-12	13-17	18-34	35-85	Life-long	0-12	13-17	18-34	35-85	Life-long
		Skin														
Butyl acetate	P50	1.75E+03	1.76E+03	1.75E+03	1.76E+03	1.81E+03	5.26E+01	3.05E+01	2.53E+01	2.10E+01	3.64E+01	5.26E+01	4.87E+01	4.03E+01	3.17E+01	5.14E+01
	P95	4.29E+03	4.21E+03	4.14E+03	4.19E+03	4.11E+03	4.56E+02	1.93E+02	1.31E+02	1.13E+02	1.37E+02	4.56E+02	3.24E+02	2.25E+02	1.86E+02	1.94E+02
Isopropyl alcohol	P50	1.01E+02	1.02E+02	1.02E+02	1.02E+02	1.04E+02	3.20E+00	1.82E+00	1.51E+00	1.27E+00	2.20E+00	3.20E+00	2.90E+00	2.40E+00	1.90E+00	3.11E+00
	P95	3.40E+02	3.46E+02	3.43E+02	3.39E+02	3.40E+02	2.94E+01	1.35E+01	9.40E+00	7.57E+00	9.96E+00	2.94E+01	2.22E+01	1.57E+01	1.25E+01	1.38E+01
Acetyltributyl citrate	P50	6.19E+02	6.18E+02	6.20E+02	6.20E+02	6.39E+02	1.78E+01	1.03E+01	8.33E+00	6.78E+00	1.25E+01	1.78E+01	1.60E+01	1.32E+01	1.03E+01	1.74E+01
	P95	1.66E+03	1.64E+03	1.67E+03	1.66E+03	1.62E+03	1.60E+02	7.41E+01	5.20E+01	4.23E+01	5.28E+01	1.60E+02	1.22E+02	8.95E+01	7.07E+01	7.49E+01
Ethyl tosylamide	P50	2.08E+02	2.08E+02	2.08E+02	2.09E+02	2.15E+02	6.44E+00	3.72E+00	3.12E+00	2.57E+00	4.43E+00	6.44E+00	5.86E+00	4.86E+00	3.91E+00	6.28E+00
	P95	6.03E+02	6.02E+02	6.00E+02	6.01E+02	5.90E+02	5.75E+01	2.59E+01	1.75E+01	1.45E+01	1.87E+01	5.75E+01	4.30E+01	2.98E+01	2.35E+01	2.57E+01
Benzophenone	P50	7.48E+01	7.52E+01	7.47E+01	7.54E+01	7.76E+01	2.26E+00	1.29E+00	1.05E+00	8.75E-01	1.55E+00	2.26E+00	2.04E+00	1.65E+00	1.30E+00	2.16E+00
	P95	1.98E+02	1.96E+02	1.98E+02	1.95E+02	1.94E+02	1.95E+01	8.72E+00	6.09E+00	5.00E+00	6.31E+00	1.95E+01	1.46E+01	1.05E+01	8.32E+00	9.04E+00
Citric acid	P50	2.33E-02	2.35E-02	2.34E-02	2.35E-02	2.38E-02	7.14E-04	4.15E-04	3.39E-04	2.82E-04	4.92E-04	7.14E-04	6.51E-04	5.34E-04	4.28E-04	6.89E-04
	P95	7.96E-02	7.92E-02	7.99E-02	7.93E-02	7.94E-02	7.20E-03	3.15E-03	2.28E-03	1.81E-03	2.36E-03	7.20E-03	5.19E-03	3.87E-03	2.97E-03	3.34E-03
		Nail														
Butyla cetate	P50	3.54E+02	3.55E+02	3.54E+02	3.54E+02	3.59E+02	1.05E+01	6.12E+00	5.08E+00	4.20E+00	7.21E+00	1.05E+01	9.60E+00	7.90E+00	6.33E+00	1.01E+01
	P95	7.74E+02	7.76E+02	7.78E+02	7.70E+02	7.78E+02	8.97E+01	3.77E+01	2.63E+01	2.15E+01	2.68E+01	8.97E+01	6.44E+01	4.52E+01	3.58E+01	3.75E+01
Isopropyl alcohol	P50	1.89E+02	1.89E+02	1.89E+02	1.88E+02	1.91E+02	5.94E+00	3.36E+00	2.81E+00	2.38E+00	4.02E+00	5.94E+00	5.36E+00	4.41E+00	3.51E+00	5.69E+00
	P95	6.00E+02	6.01E+02	5.99E+02	5.96E+02	6.02E+02	5.31E+01	2.37E+01	1.71E+01	1.36E+01	1.78E+01	5.31E+01	4.03E+01	2.86E+01	2.23E+01	2.48E+01
Acetyl tributyl citrate	P50	1.81E-01	1.80E-01	1.81E-01	1.80E-01	1.82E-01	5.16E-03	2.95E-03	2.43E-03	1.99E-03	3.57E-03	5.16E-03	4.63E-03	3.79E-03	2.98E-03	4.97E-03
	P95	4.43E-01	4.44E-01	4.45E-01	4.45E-01	4.47E-01	4.53E-02	2.09E-02	1.44E-02	1.18E-02	1.48E-02	4.53E-02	3.45E-02	2.53E-02	1.96E-02	2.08E-02
Ethyltosylamide	P50	9.10E+00	9.18E+00	9.15E+00	9.10E+00	9.24E+00	2.83E-01	1.62E-01	1.36E-01	1.14E-01	1.93E-01	2.83E-01	2.55E-01	2.15E-01	1.71E-01	2.74E-01
	P95	2.49E+01	2.47E+01	2.49E+01	2.49E+01	2.50E+01	2.45E+00	1.09E+00	7.44E-01	6.07E-01	7.82E-01	2.45E+00	1.80E+00	1.28E+00	9.92E-01	1.10E+00
Benzophenone	P50	8.24E-01	8.26E-01	8.29E-01	8.25E-01	8.35E-01	2.42E-02	1.39E-02	1.15E-02	9.55E-03	1.66E-02	2.42E-02	2.23E-02	1.79E-02	1.44E-02	2.32E-02
	P95	2.01E+00	2.03E+00	2.01E+00	2.02E+00	2.03E+00	2.09E-01	9.21E-02	6.49E-02	5.20E-02	6.61E-02	2.09E-01	1.56E-01	1.12E-01	8.56E-02	9.37E-02
Citric acid	P50	3.08E-01	3.09E-01	3.09E-01	3.07E-01	3.12E-01	9.45E-03	5.34E-03	4.43E-03	3.73E-03	6.39E-03	9.45E-03	8.55E-03	6.97E-03	5.56E-03	8.94E-03
	P95	9.97E-01	9.97E-01	9.90E-01	9.96E-01	1.01E+00	8.84E-02	4.05E-02	2.87E-02	2.25E-02	3.06E-02	8.84E-02	6.69E-02	4.89E-02	3.79E-02	4.21E-02

3.2.2.1 By exposure route

QA and SED values of BA, ATC, ET and Be are higher by the skin route than by the ungual route. Conversely, IA and CA show higher values for the ungual route.

3.2.2.2 By ingredient

Independently of age groups, BA is the substance that has the highest rate of absorption and SED by skin and ungual routes. For the skin route, BA is followed by ATC, ET, IA and Be in this order respectively and CA provides the smallest values. For the nail route, BA is followed by IA and ET and by Be, CA and ATC.

3.2.2.3 By age group

QA and SED values roughly follow the same trends as those observed for external exposure.

3.2.3 Comparisons of external versus systemic exposure

To compare data calculated for external exposure and systemic exposure, a ratio between results was calculated (Table 4).

The difference between the values obtained (QA and exposure) in the external and a systemic evaluation during a contact time of 30 min varies according to ingredients and exposure routes. In all cases, the values are higher for the external evaluation.

TABLE 4

COMPARATIVES RESULTS OF EXPOSURE FOR ONE COAT BY INGREDIENT, AGE GROUP AND EXPOSURE ROUTE

Ratio between exposure results	Comparison of exposure results for 1 coat (unitless)										
	Exposure route		Skin					Nail				
	Substances \ Age (years)		0-12	13-17	18-34	35-85	Life-long	0-12	13-17	18-34	35-85	Life-long
Global Systemic (new model)	Butyl acetate	P50	6.03	6.00	6.04	6.02	6.02	113.27	113.30	113.32	112.81	113.41
		P95	6.04	6.01	6.06	5.95	6.02	112.74	113.65	112.65	113.63	113.44
	Isopropyl alcohol	P50	18.19	18.28	18.23	18.20	18.34	37.14	37.38	37.39	37.25	37.29
		P95	18.14	18.16	18.35	18.34	18.24	36.93	36.96	37.27	36.93	37.35
	Acetyl tributyl citrate	P50	2.68	2.67	2.67	2.67	2.67	35086.83	35045.39	35084.39	35156.34	34933.34
		P95	2.64	2.68	2.69	2.65	2.67	34991.71	35161.91	35124.97	34891.20	34813.67
	Ethyltosylamide	P50	6.94	6.94	6.90	6.92	6.93	598.12	598.81	596.54	600.59	601.57
		P95	6.92	6.93	6.88	6.84	6.94	597.38	592.64	599.35	591.01	605.06
	Benzophenone	P50	1.23	1.23	1.22	1.23	1.23	425.94	423.57	425.87	424.14	426.41
		P95	1.22	1.23	1.22	1.22	1.24	422.08	426.10	423.28	422.08	429.21
	Citric acid	P50	1805.59	1784.40	1797.39	1799.82	1804.41	518.33	518.63	520.19	514.78	516.55
		P95	1801.42	1812.07	1797.60	1780.41	1800.28	515.25	518.79	517.41	518.83	514.47
Systemic (new model) Systemic (RIVM model)	Butyl acetate	P50	5.27	1.84	1.86	1.86	2.34	0.85	0.30	0.30	0.29	0.37
		P95	5.96	2.02	2.04	2.11	2.86	0.94	0.30	0.31	0.30	0.43
	Isopropyl alcohol	P50	5.68	1.90	1.92	1.93	2.48	0.88	0.30	0.30	0.31	0.38
		P95	6.17	2.17	2.18	2.05	2.86	0.93	0.31	0.33	0.30	0.43
	Acetyl tributyl citrate	P50	4.85	1.74	1.77	1.68	2.17	0.87	0.29	0.29	0.29	0.37
		P95	5.26	1.86	2.00	1.97	2.69	0.92	0.31	0.31	0.30	0.42
	Ethyltosylamide	P50	5.36	1.87	1.89	1.86	2.37	0.85	0.29	0.29	0.29	0.37
		P95	6.08	2.11	2.05	2.13	2.92	0.96	0.31	0.31	0.31	0.42
	Benzophenone	P50	3.35	1.46	1.44	1.41	1.73	0.87	0.29	0.30	0.29	0.36
		P95	3.66	1.50	1.59	1.56	2.09	0.94	0.30	0.31	0.30	0.43
	Citric acid	P50	5.76	1.94	1.96	1.94	2.48	0.87	0.29	0.29	0.30	0.37
		P95	6.60	2.13	2.17	2.06	2.84	0.90	0.31	0.31	0.30	0.42
Systemic (new model) Systemic (EPA model)	Butyl acetate	P50	1.93	0.67	0.66	0.66	0.84	Not possible (calculs of B.tlag, and t* are specific of skin)				
		P95	2.29	0.73	0.73	0.75	1.03					
	Isopropyl alcohol	P50	3.01	1.02	1.01	1.02	1.33					
		P95	3.31	1.13	1.17	1.09	1.50					
	Acetyl tributyl citrate	P50	0.27	0.09	0.09	0.09	0.12					
		P95	0.30	0.10	0.10	0.10	0.14					
	Ethyltosylamide	P50	1.16	0.39	0.40	0.39	0.50					
		P95	1.35	0.44	0.42	0.43	0.60					
	Benzophenone	P50	0.68	0.23	0.23	0.23	0.29					
		P95	0.74	0.25	0.26	0.26	0.36					
	Citric acid	P50	1.32	0.45	0.44	0.45	0.57					
		P95	1.57	0.49	0.51	0.48	0.66					

3.2.3.1 Skin route

For this exposure route, the ingredient with the greatest difference between external and systemic evaluation is CA with an approximate mean ratio of 1800. IA, ET and BA present ratios of 18, 7 and 6 respectively. The lowest differences are for ATC and Be with ratios of 3 and 1.25.

3.2.3.2 Nail route

Generally, differences between external and systemic evaluations are greater for the ungual route than the skin route. ATC presents the biggest ratio approximated at 35000. ET, CA and Be results show ratios of 600, 500 and 400 respectively. BA results provide a ratio of 100 and IA a ratio of 40.

IV. DISCUSSION

4.1 Permeability coefficients

The model proposed to assess systemic exposure through the skin and nail is based on the use of permeability coefficients (Kp). Generally, it is recommended to use the Kp of substances which have been characterized experimentally and listed in a database [17, 19, 49]. In this study, the decision to use estimation methods was mainly due to the lack of available ingredient data in the database (especially for the ungula route). We do not discuss in this paper the general limits of Kp the in absorption estimation according to the real biological characteristics of membranes (heterogeneity, metabolism etc.). We only consider its influence in the systemic exposure model.

4.1.1 Kp_s

The model of Potts and Guy is a simple model to estimate Kp [20]. It is based on a large compilation of published Kp from aqueous solutions, and thus a wide range of physicochemical properties [MW (18 to >750) and K_{OW} (-3 to +6)] [55]. Estimated by a multiple regression, the r^2 of 0.67 suggests that approximately two thirds of the variability in the data was explained by the model. It is therefore interesting to estimate the Kp of a wide range of chemicals such as those composing cosmetics and can be considered as a relatively good prediction model. For these reasons, because it is one of the most commonly used model in the evaluation of percutaneous transfer and is recognized by the US-EPA, we used it as a first approach to estimate the Kp of nail polish ingredients.

The transfer rate of chemical entities through the skin depends on the resistance induced by the physicochemical structure of the membrane compared to the substance's properties. In this Kp_s prediction model, the limiting transfer rate of entities through the skin is implicitly correlated with the lipophilic nature of SC [20, 42]. Thus, the Kp_s for highly lipophilic substances (i.e. Log K_{ow} of 3-4) is greatly overestimated [25, 42]. This explains the high permeability coefficient of ATC and particularly the Kp of Be for which the MW is lowest.

The used QSPeRs model was established with molecules whose permeability coefficients were measured from an aqueous solvent [55]. However, in many cosmetics (perfumes, deodorants, nail products etc.), the main solvent is not water but a set of organic compounds. It has been shown that, depending on the type of solvent used during the percutaneous absorption measurement, the vehicle could have an influence on the permeability [72-82].This difference between aqueous vehicles and organic solvent vehicles can induce errors in Kp_s predictions. Indeed, according to the physicochemical characteristics of the solvent, this one penetrates the skin more or less easily. Depending on the affinity (i.e. solubility) that certain molecules present, solvents can maximize or minimize absorption [45, 75]. In addition, organic solvents have the ability to alter the biochemical structure of the skin and in particular the *stratum corneum* composed largely of lipids [72, 75-77, 79, 80]. Structurally disorganized, the skin is more permeable to exogenous substances. Van der Merwe and Riviere also showed that the correlation coefficient between octanol/water and the *stratum corneum*, valid in an aqueous solvent, is not valid when ethanol or an ethanol/water mixture is used. Thus, it is generally not recommended to use Kp for substances present in a non-aqueous solvent. However, because there is no predictive model of Kp for substances in these solvents or correction factor of Kp, we assume that all products are composed of aqueous solvent.

4.1.2 Kp_u

In contrast to the skin, nails possess a slightly lipid constitution (<1%) concentrated mainly in the ventral and dorsal layers [34, 56]. Thus, the nail plate is often characterized as a hydrophilic gel membrane rather than a lipophilic partition membrane. The influence of Kow on the substance transfer across the nail is discussed, but recent studies suggest that the

permeability of the matrix is independent of this physicochemical parameter [29, 53, 57]. All authors agree that the MW is the critical physicochemical property in ungual permeation of substances (i.e. the main physico-chemical parameter that would be likely to influence nail absorption). Thus we chose to use the MW as a discriminating factor in the Kp_u prediction model (section 2.1.2.2).

In their study, Kobayashi *et al.* showed that Kp_u of ionized substances were lower than non-ionic molecule drugs, but it was caused by a small increase in the apparent molecular weight due to ion hydration [57]. They proposed two prediction models based on a simple linear regression between the MW and Kp_u ($cm.s^{-1}$) of ionic and non-ionic drugs.

In view of these conclusions, we chose in our model not to take into account ionization of substances and to pool all listed data to cover the most widest range of substances in an aqueous vehicle (i.e. 18 <MW<347.2 $g.mol^{-1}$; -8.77<experimental $LogKp_u$<-5.34). With this simplistic model, the MW represents 67% of the correlation found. This model can be considered as a relatively good Kp_u predictor for substances located in this weight range for lack of more complete and accurate prediction models. Although ATC does not fit into this range of MW, its Kpu was nevertheless used in this study. Like the other substances, it is an application example of the method of exposure calculation and no other Kp_u data is available in the literature.

As for the establishment of the skin permeability coefficient model, the Kp_u of substances used to define the model were experimentally measured in an aqueous solvent. However even for 30 minutes, organic solvents could have a significant impact on the estimation of the permeability of molecules through the nails. A study conducted by Walter *et al.* [56] showed that the permeability coefficient was about 5-fold lower using a pure alcohol relative to a diluted alcohol [27]. The authors concluded that water facilitated the transport of the substances through the nail [33, 56]. As described above, the nail plate is characterized as a hydrogel which seems more permeable to hydrophilic molecules. Capable of absorbing between 30% and 50% of its weight in water, the nail plate's hydration can have an impact on its permeability [83-85]. The aqueous solvent used would lead to nail swelling. This would result in an increase in the space between keratin fibers with the consequent formation of larger pores through which molecules can diffuse more easily [86]. Conversely, organic solvents cause a decrease in permeability. They would dehydrate the nail, tighten pores and increase the resistance which would result in strengthening their barrier property [53, 60]. Organic solvents could also cause a decrease in permeability by reducing the solution's conductivity (i.e. organic solvents affect the mobility of ions that affect the diffusion coefficient) [60]. In this study, Kp_u is probably overestimated. Like the Kp_s model, many factors could affect the ungual transfer estimation and are not considered. However it is also possible to use experimentally measured Kp_u or future Kp_u prediction models more suitable for the exposure calculation model proposed.

4.1.3 Kp_s vs Kp_u

Table 1 shows that the transfer rate of substances by the ungual route is generally lower than by the cutaneous route (mean factor of 10 between the two). This result is consistent with what is generally observed in the literature concerning transfer rate through the nail (i.e. low permeability). Both the predictive models are based on the MW used. Smaller substances tend to cross membranes faster than large molecules. This is observable by a comparison of the Kp of ATC and Be or BA and ET which have a relatively close LogKow (Log Kow affects the prediction Kp_s). Due to the lipophilic nature of the SC, substances with a large LogKow (i.e. a significant lipophilicity) will tend to cross the barrier more readily than hydrophilic molecules. This fact explains why the Kp_s of Be is higher than the Kp_s of ET for a MW in the same range.

Like other exposure models, the parameter Kp is the major contributor to uncertainties in the assessment of dermal systemic exposure [39]. In this exposure model, Kp experimentally measured or estimated with more precision can be used. For the cutaneous route, there are studies of the comparative QSPeRs models to select the most appropriate model according to the characteristics of the substances and the membrane complexity [18, 42, 87-89].

The results of these two prediction models of substance transfer rates seem appropriate for use in the proposed exposure model. The influence of Kp in the exposure assessment will be consistent with the theory.

4.2 Model to estimate systemic exposure

4.2.1 Discussion of results

4.2.1.1 External exposure assessment

In the external exposure assessment, the absorption of substances through the membranes is considered as a whole. The only variable parameter is the surface ratio between the exposed skin (i.e. nail wall) and the nail plate. The amount of product applied is directly correlated to the surface; QA and WED are proportional to this factor. This obvious fact explains the difference observed in QA and the external exposure dose between cutaneous and ungual routes. In a comparison by ingredient, the difference is explained by the weight fractions estimated. Indeed, as described above, in external exposure there is no resistance to absorption. The amount absorbed is directly proportional to the amount of ingredient applied. By age group, differences in exposures to ingredients were consistent with what has been described in the publication of Ficheux *et al.* on exposure to nail polish [14]. Frequencies of use and body weight of individuals are significant factors in exposure assessment results.

4.2.1.2 Systemic exposure assessment

In the systemic exposure assessment, the permeability resistance involvement was included in the formula. It represented by the systemic absorption factor (SAF) including the permeability coefficient (i.e. transfer rate of substances through a membrane), the membrane thickness (i.e. thickness that the substances have to cross before reaching the systemic circulation) and the contact time (exposure time).

As an example of exposure, we worked on nail polish. The product contact time evaluated by Ficheux *et al.* had a median value of about 1 week. In this study, the contact time was reassessed for a period of 30 minutes. This decision is justified considering the impact of solvent evaporation and solidification of the product on the substance transfer. It was found that the evaporation of organic solvents contained in the product was complete after 30 minutes [14]. In terms of evaporation time, the nail polish viscosity increases until it becomes solid. These 30 minutes correspond to the time required for drying and complete setting of nail polish. Thus, two elements may be demonstrated:

(i) After 30 minutes, organic solvents are completely evaporated and are no longer in contact with the skin. In the performed exposure assessment, the evaporation kinetics of the solvent are not included. We assume that the total amount of each ingredient of this family is available for absorption during this exposure period. This phenomenon could be integrated by applying a correction factor exp(-at), but in order to not complicate the method, we abstained to applying it.

(ii) After 30 minutes, all remaining ingredients are aggregated forming a dense and compact layer and are no longer available for absorption. Even with this short contact time (compared to the real contact time of 1 week), we assume that the exposure assessment is protective. Indeed, it has been demonstrated that increasing the viscosity of a solution has a negative impact on the penetration of constitutive substances by decreasing its conductivity and ion mobility [60]. Nail varnish has a relatively high viscosity originally and this viscosity increases significantly for the first 30 minutes.

By comparing the results of QA and systemic exposure of substances by exposure route, it is interesting to note that these are correlated with the permeability coefficients. Ingredients that have the highest Kp by one route have a greater QA and SED than by the other exposure route (BA, ATC, ET and Be for cutaneous route; IA and CA for ungual route). For the exposure time and the membrane thickness used in this study, Kp appears to be the discriminating factor in the substances absorption. Despite these results, the impact of membrane thickness on the absorption factor, and therefore on the estimation of QA and SED, can be observed by comparing BA and IA. These two ingredients present relatively similar Kp by cutaneous and ungual routes of exposure, respectively. When the contact time is the same, the ratio between the values obtained in the external and the systemic exposure assessment corresponds to the involvement of the thickness (regardless of the amount applied in the comparison: the higher the ratio, the lower the substance transfers through the membrane because the thickness is greater. BA (Kp_s = 0.4577 cm.week^{-1}) presents a ratio of 6. IA (Kp_u = 0.4551 cm.week^{-1}) presents a ratio of 37. The ratio between both (6.2) is substantially the same as the ratio between the median of membrane thicknesses (6.6).

Comparison of the results of QA and SED by ingredient shows the impact of Kp on exposure, but also that the impact of the amount of substance is consequent. For example, BA has a lower Kp_u than IA but the substance remains the most exposed through the nail because the amount applied is great (6 times higher).

The exposure difference between age groups is explained in the same way as for external exposure (i.e. influence of the frequency and of body weight).

Described in section 2.2.2.2, the SAF used in exponential form to calculate QA allows to obtained exposure in finite dose conditions. A constraint on the use of this exponential is that the absorption kinetic of substances across membranes is governed by this function. Although it is an approximation, the kinetics of cumulative QA vs. time obtained with SAF in our model are close to what is presented in the literature [64, 65, 90, 91]. Using SAF in exponential form enables us to simply and relatively justly characterize exposure in finite dose conditions from Kp. This approach is used by the RIVM to assess exposure [66].

4.2.1.3 External vs. systemic exposure

As described previously, a systemic exposure assessment takes into account the membrane resistance in the substance transfer (integrated in the absorption factor in our model). The values obtained for external exposure are higher than those obtained for systemic exposure for a short contact time. This is consistent relative to the contribution of substances Kp and the membrane thickness in systemic exposure. We tested the impact of time on QA and SED for a contact time of 1 week found by Ficheux *et al.* [14]. The results showed that the SED was then very close to external exposure (data not shown). Whether through the dermal or ungual route, QA and SED follow the substance transfer rates (i.e. the external and systemic ratio for substances with a high Kp is smaller than the ratio of molecules with a low Kp).

The results are conclusive and consistent with theory. The absorption factor presented seems usable to assess systemic exposure. It can be used to determine QA according to time considering the substances transfer rate and the membrane thickness. It also retains the relative importance of other factors (frequency and amount applied) in the exposure assessment.

4.2.2 Comparison with systemic exposure models presented in the literature

To compare the new exposure model with calculation formulas already established in the literature models, we adapted formulas proposed by (i) the RIVM (Equation 21) [66] and (ii) the US-EPA [19, 39] based on Cleek and Bunge's model (Equation 20) [25]. The results are expressed as a ratio between SED for one coat by age and exposure route (Table 4). There were no differences compared to ratios of QA and SED for all coats (data not shown). The aim is to validate our model by a comparison of results obtained with this new model and models currently used in systemic exposure assessment.

4.2.2.1 RIVM model

The RIVM model was developed to estimate exposure to a finite dose (i.e. by incorporating Kp in exponential form).

The ratios show that the results are generally relatively close in the conditions of this study (Table 4). For skin, the ratio obtained per ingredient is around 2 with the highest ratios (between 4 and 6) for 0-12 year olds. For the ungual route it is about 0.3 for age groups ranging from 13 to 85 years old and 0.9 for 0-12 year olds.

The difference in ratio between the group of 0-12 year olds and the other age groups is explained by the surface parameter used in the RIVM formula (Equation 21) which is not suitable for this age group in the conditions of this study. Indeed, the estimated skin and nail surface present a factor of 3 between the 0-12 year olds and the other age groups [14]. This factor 3 was found in the exposure results from the RIVM formula. However, the distribution of quantities used is the same for all age groups (data unavailable for 0-12 year olds). In realistic conditions, the amount of product applied is proportional to the application surface area. A readjustment of the amount distribution to the exposed surface should be performed.

It is also interesting to note that the model developed in this study gives a higher SED than the RIVM by cutaneous exposure (ratio > 1) and conversely by the ungual route (ratio < 1). Both models are similarly algebraically structured. To integrate Kp, our model is based on the membrane thickness (section 2.3.1.3). The RIVM model is based on the solution thickness on the membrane [$l_{sol} = 1/(S/(q/C))$] (cm).

The median thickness of the epidermis and the nail plate are estimated at 0.0075 cm and 0.0499 cm, respectively. The product layer thickness on the membrane under the study conditions was estimated at 0.0151 cm (0.0448 cm with the membrane surface of 0-12 year olds). Because the Kp is a transfer rate, the greater the thickness, the lower the exposure. The skin thickness is less than the thickness of the solution on the skin, exposure is therefore greater in our model and conversely for ungual exposure.

Kp is a substance transfer rate across a membrane. It is based on the substance diffusion coefficient and the membrane thickness (Equations 3 and 4). The thickness is therefore specific to the membrane and not to the solution in which the substance of interest is found. With the assumption that systemic exposure is achieved when the substance has crossed the

membrane, the use of membrane thickness appears to be most appropriate to integrate Kp vs. time in the exposure calculation.

4.2.2.2 EPA model

The EPA model is not applicable to the ungual route due to the estimation of parameters B, t_{lag} and t* that are specific to the skin. This model is different from the RIVM model and the one that we propose. The absorbed dose is characterized as a flow multiplied by the contact surface (Equation 20). Thus expressed, absorption is not limited by the amount (C constant) but only by exposure time. Unlike the model proposed in this study, the EPA exposure model is based on the assumption that the absorption continues sometime after the exposure to the substance has ceased (i.e. everything that penetrates into the SC is available to systemic exposure) [39]. A time correction factor described by Cleek and Bunge is used to include the reserve effect of the SC in non-steady state conditions ($t \leq t^*$) (section 2.2.1 – Equation 16) [25, 62]. As this model is different from what we propose, it is difficult to compare them.

What is described above and in section 2.2.1 explains that the exposure results of the EPA model are slightly higher than those obtained by our model (Table 4). Like the RIVM model, the exposure results for 0-12 year olds are three times higher. This can be explained by a distribution of amounts which is not adapted to the exposed surface area (section 4.2.2.1). Although there are slight differences in results (except IA) they are relatively close and consistent.

4.2.3 The new model in the exposure assessment

As previously reported the aim of this study is to propose a simple calculation model to assess systemic exposure and can be used as an alternative to existing models. Like these other models, it can only be used under certain assumptions that simplify a complex reality and an accurate exposure assessment in finite dose (end of introduction and section 2.2.2.2).

Indeed, we assume that the total amount applied (Q) is fully available to the absorption and the absorbed dose (AD) is governed by the absorption factor (SAF). However, the volatility of certain substances such as solvents suggests that there is a decrease of Q versus time. The importance of this concept in the calculation of the exposure is presented in the study of Frasch *et al.* [92]. Include this variable in the calculation would decrease drastically the exposure to some substance. We deliberately chose not to take into account the evaporation of volatile compounds in order not complicate the model and stay protective in exposure assessment. AD and SED are therefore overestimated but we are protective for the consumer.

Another major assumption of the proposed model is that the molecules are in an aqueous solvent. This assumption is required to use Kp directly into the formula. This model is suitable for assessing exposure to molecules in an aqueous solvent, but in the case of nail polish, solvents are organic type and have a significant influence on the transfer rate (Section 4.1). The US-EPA suggests using the "maximum flux" (Jmax) as tools for assessment of percutaneous transfer that is independent of solvents [19]. However this flux is usable only when the interaction of the solvent with the membrane is negligible; which is not the case of organic solvents (section 4.1). Furthermore, in this model is not a flux that is used but the Kp. The choice to consider aqueous solvents for the use of Kp is necessary for this simple model of systemic exposure assessment. This allows for a reasonable first estimate and protective given the complexity of the transfer across membranes (desquamation, Lagtime, etc.).

It is also considered that, in this model, the transport of molecules across the membrane occurs essentially by diffusion. This assumption is very simplistic compared to reality and the plurality of pathways of transport, especially through the skin [89, 93-95]. However, this assumption is a fundamental point that is used in all transfer prediction models through membranes using the Kp (section 2.1.1) and in the most common systemic exposure models. For a simple model as proposed in this study, this assumption is necessary.

Thus there are proved limits in this model that is not strictly representative of a real systemic exposure. However with a new approach to the use of Kp, it allows to obtain a simple and protectress systemic exposure assessment to molecules through the skin and nails. Additional variables can be easily added to the basis formulation to enhance the estimation (evaporation kinetics, correction coefficients of the transfer speed depending on the solvent used, etc.).

V. CONCLUSION

In this study, a new calculation model of systemic exposure through the skin and the nail has been developed for finite dose conditions. It is based on a different approach to the models currently available in the literature: systemic exposure to a molecule is achieved when this substance has completely crossed the biological membrane which separates the internal

environment from the external environment. Kp is directly used as transfer speed and not as a flux. We used skin and nail thickness to integrate Kp in the formula. The permeability coefficient, the membrane thicknesses and the contact time represent what is called the systemic absorption factor. Presented in an exponential form, it can be used to determine the amount absorbed versus time and to assess the systemic exposure to an applied amount which is finite.

In this study, six ingredients commonly found in the nail polish composition have been used as examples in the exposure assessment. The comparison of the results obtained with this model with results obtained from models of the RIVM and the US-EPA have shown that they are relatively close; moreover they are consistent with the theory in the application conditions. Like other models, assumptions and application criteria limit the accuracy of exposure estimation. However, the new equation structure reduces the number of parameters needed to assess systemic exposure and reduces uncertainties. Except for Kp, all of the "input data" can be integrated under distribution form. The use of probabilistic the Monte Carlo method enables us to consider all of these parameter variability's, including the parameters that compose the absorption factor (l and t). Among other things, this method enables us to assess exposure by integrating inter-and intra-individual variability's existing in the thickness of biological barriers and whose impact on the systemic exposure may be significant depending on the exposure time (contact time) and the transfer rate of the molecules (Kp).

As with the other models, the most significant uncertainties of the proposed model are associated with the use of Kp. Indeed, it involves many theoretical constraints that must be considered (i.e. transfer only by diffusion). However, this model can be easily adapted according to exposure conditions (more accurate and suitable Kp for substances and types of biological membranes etc.) and the desired accuracy (integration of additional parameters such as B, FA or evaporation).

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