Risk analysis tools for toxicological profile of cosmetics
S Kapoor, S Saraf

INTRODUCTION
Skin is the effective barrier to the environmental. Disturbances to this barrier lead to the development of various skin problems. Cosmetic products are widely used by every socio class of human being to cleanse, perfume, protect and change the appearance of skin. Most cosmetic products contain a combination of ingredients such as emulsifiers, preservatives, thickener, colour, fragrance, UV filters (sunscreens), humectants, occlusive agents and pH stabilizers. Sometimes the ingredients in cosmetics can have unintended side effects. Most serious side effects have been noticed from various studies for certain cosmetic ingredients. Thus, it is necessary to have the best knowledge possible about each ingredient used, both in relation to its characteristics as well as to its toxicological data, taking into account the many potential risks related to its cosmetic use. The safety of a cosmetic product is based on the safety of its ingredients, the latter being evaluated by toxicological testing (Pauwels and Rogiers, 2004). Toxicity testing has been concentrated on ingredients, and particularly on those that are intended to react with biological matrices and therefore are of most concern for human health. The Threshold of Toxicological Concern (TTC) is a concept that refers to the establishment of a level of exposure for all chemicals, whether or not there are chemical specific toxicity data, below which there would be no appreciable risk to human health (Kroes, 2005).

Some activist groups have targeted cosmetics as possible human health threats, claiming that cosmetic ingredients are not adequately tested for safety and may pose risks to consumers. The groups allege that industry practices related to safety testing are flawed, that there is little government oversight, and that cosmetics contain cancer-causing chemicals and other toxicants (Ross, 2006).

When a formulation is being considered for safety a risk assessment must consider the general toxicological profile of the ingredients and their level of exposure. More recently the toxicity and efficacy of cosmetics and their ingredients has been under the spotlight. So cosmetic which are used mainly for beautification purpose is not free from side effects that myth should be cleared in front of consumers. There are some ingredients that found to be toxic and banned by all legislations but highly used by cosmetic industry, about which common consumers even academicians, pharmacist, cosmetician and chemist are also not aware. Various regulatory bodies like Food and drug administration (FDA), European union (EU) e.t.c. has identified the cosmetic ingredients that are either approved or prohibited on the basis of the toxic effects produced by them (Table 1, 2 and 3). Results of toxicology and safety testing are not always publicly disclosed, cosmetic manufacturers have internal processes, programs, and testing protocols that are designed to ensure the safety of the products they produce.

The present paper describe about the key principle of risk assessment used for evaluation of toxicological profile of cosmetics with special reference to the methodologies and
techniques used for managing risk at optimum level. Objective of this review to motivate all academicians, chemists, beauticians, pharmacist, industrialist and researchers to perform risk assessment study of cosmetics by adopting proper legislation and publish. This would be helpful to upgrade the knowledge, increased the trust and disclosed the myths of common consumers regarding cosmetic products.

**Figure 1**
Table 1: List of ingredients with approved limits in sunscreens

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredient</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-Aminobenzoic acid</td>
<td>15 %</td>
</tr>
<tr>
<td>2</td>
<td>Aurochome</td>
<td>5 %</td>
</tr>
<tr>
<td>3</td>
<td>Citrate</td>
<td>2 %</td>
</tr>
<tr>
<td>4</td>
<td>Hexamethylenetetramine</td>
<td>2 %</td>
</tr>
<tr>
<td>5</td>
<td>Hydroquinone</td>
<td>2 %</td>
</tr>
<tr>
<td>6</td>
<td>Menthol</td>
<td>2 %</td>
</tr>
<tr>
<td>7</td>
<td>Octinoxide</td>
<td>18%</td>
</tr>
<tr>
<td>8</td>
<td>Octyl dimethylsalicylate</td>
<td>7.5%</td>
</tr>
<tr>
<td>9</td>
<td>Octyl trisalicylate</td>
<td>5%</td>
</tr>
<tr>
<td>10</td>
<td>Oleic acid</td>
<td>6%</td>
</tr>
<tr>
<td>11</td>
<td>PEG 80</td>
<td>10%</td>
</tr>
<tr>
<td>12</td>
<td>Phenylbenzimidazole sulfone acid (Epinridole)</td>
<td>4%</td>
</tr>
<tr>
<td>13</td>
<td>Sodium benzoate</td>
<td>18%</td>
</tr>
<tr>
<td>14</td>
<td>Titanium dioxide</td>
<td>25%</td>
</tr>
<tr>
<td>15</td>
<td>Terephthalic acid</td>
<td>12%</td>
</tr>
<tr>
<td>16</td>
<td>Zinc oxide</td>
<td>25%</td>
</tr>
</tbody>
</table>

**Figure 2**
Table 2: List of Prohibited and Restricted Ingredients

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredient</th>
<th>Side effect</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nickel</td>
<td>Photo-contact sensitisation</td>
<td>Cereals, fats, soaps, shampoos, creams, adhesives</td>
</tr>
<tr>
<td>2</td>
<td>Mercury</td>
<td>Potential allergy, skin irritation</td>
<td>Cereals, fats, soaps, shampoos, creams, adhesives</td>
</tr>
<tr>
<td>3</td>
<td>Halogenated surfactants</td>
<td>Photocatalysis</td>
<td>Cereals, fats, soaps, shampoos, creams, adhesives</td>
</tr>
<tr>
<td>4</td>
<td>Vinyl chloride</td>
<td>Dizziness, headache, dizziness, and mucous accumulation</td>
<td>Hair sprays, cosmetic aerosol products</td>
</tr>
<tr>
<td>5</td>
<td>Manganese complexes</td>
<td>Skin granulomas</td>
<td>Antiperspirants</td>
</tr>
<tr>
<td>6</td>
<td>Chlorides</td>
<td>Hepatosplenic congestion</td>
<td>Cosmetic products</td>
</tr>
<tr>
<td>7</td>
<td>Methylene chloride</td>
<td>Burns and malignant tumors of the lung</td>
<td>Hair sprays, cosmetic aerosol products</td>
</tr>
<tr>
<td>8</td>
<td>Chlorinated paraffins</td>
<td>Skin irritation</td>
<td>Cosmetic aerosol products</td>
</tr>
<tr>
<td>9</td>
<td>Hexachlorophene</td>
<td>Toxic effect</td>
<td>Antiperspirants</td>
</tr>
</tbody>
</table>

Note: Mercury HMT 0.0005% in eye cosmetics and HMT 0.001% in other Cosmetics, Hexachlorophene HMT 0.1%.

**Figure 3**
Table 3: List of banned Ingredients

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredient</th>
<th>Side effects</th>
<th>Used in</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paraben</td>
<td>Allergic reactions and skin rashes</td>
<td>Antimicrobial agents</td>
</tr>
<tr>
<td>2</td>
<td>Ethylhexylmethylsiloxane (EEMA), 2-octylcyclopentene (TEA)</td>
<td>Allergic reactions, eye irritation, freckles of hair and skin</td>
<td>Edemizers, foaming agents</td>
</tr>
<tr>
<td>3</td>
<td>Isopropyl Urea, Imidazolidinyl Urea</td>
<td>Contact dermatitis</td>
<td>Preservatives</td>
</tr>
<tr>
<td>4</td>
<td>Sodium Laurylethyl Sulfate</td>
<td>Eye irritation, skin rashes, allergic reactions</td>
<td>Detergents in shampoo</td>
</tr>
<tr>
<td>5</td>
<td>Petroleum</td>
<td>Dryness and Cracking</td>
<td>Emollients</td>
</tr>
<tr>
<td>6</td>
<td>Propylene Glycol</td>
<td>Allergic reactions, liver and eyes</td>
<td>Inhalants</td>
</tr>
<tr>
<td>7</td>
<td>PVP Copolymer</td>
<td>Vomiting and damage of the lung</td>
<td>Hair sprays</td>
</tr>
<tr>
<td>8</td>
<td>Streptomycin Chloride</td>
<td>Allergic reactions</td>
<td>Hair conditioners and cures</td>
</tr>
<tr>
<td>9</td>
<td>Synthetic Colors</td>
<td>Hair dyes</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Synthetic Fragrances</td>
<td>Headaches, dizziness, rash, hypersensitivity, unusual constipation</td>
<td>Creams and deodorants</td>
</tr>
</tbody>
</table>

Note: Do not buy a cosmetic that has the word "fragrance" on the ingredients label.

**RISK ASSESSMENT AND RISK MANAGEMENT**

Each cosmetic finished product is an individual and unique combination of ingredients. No ingredients, whether naturally occurring or manufactured, is absolutely free from potential toxic effects. To allow a better safety evaluation risk associated with cosmetic ingredients should be assessed and properly managed [WHO, 2001; EC, 2000]. General principles and practice adopted for risk assessment process, is represented in Fig 1.

**Figure 4**
Figure 1: Principles and practice of the risk assessment process

Risk assessment is the process which identifies and quantifies the risk resulting from a specific use or occurrence of chemical or physical agents (ingredients). This risk can be assessed by 4 ways.

Hazard identification: It is based on the results of in vivo tests, in vitro tests, clinical studies, accidents, human epidemiological studies. In this the intrinsic physical, chemical and toxicological properties of the product/ingredient under consideration are studied to identify whether the substance has the potential to damage human health or not.

Dose-response assessment: In this the relationship between the toxic response and the exposure of ingredient is studied. In the case of an effect with a threshold, the dosage at which no adverse effects are observed (NOAEL) is determined. If the NOAEL is not available, the LO(A)EL is used. In the
case of non-threshold carcinogens, a dose-descriptor (e.g., T$_{25}$) is determined [Dybing et al., 1997].

Exposure assessment: Study in which the amount and the frequency of human exposure to the product is determined.

Risk characterization: It is used to examine the probability of the ingredient/molecule to cause the damage to human health and its potency to produce damage.

Risk Management is a decision-making process in which the optimal steps are selected for reducing a risk produced by ingredients to an acceptable level. Risk management (Fig 2) procedure is often controlled by legislation, which lays down allowable limits of exposure and puts specific duties on management and workers [Beck et al., 1994; Dayan, 1999; Loprieno, 1999; Rogiers, 2002a; Masson, 1999; Sanner 2001].

**Figure 5**

Figure 2: Legislation control over risk management system.

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**RISK ANALYSIS**

Risk analysis of cosmetic ingredients should be carried out by performing physicochemical analysis, toxicological analysis (SCCNFP/0690/03, 2003) and microbial contamination examination.

**PHYSICOCHEMICAL ANALYSIS**

A small molecular weight hydrophobic compound is more likely to penetrate through the skin than a high molecular weight hydrophilic compound and a highly volatile compound could cause significant inhalation exposure when present in a product applied to the skin. Hence, physical and chemical properties of cosmetic ingredients should be seen as they may be able to predict certain toxicological properties. The basic and minimal specifications such as chemical identity, physical form, molecular weight (M.W), purity of the chemical, characterization of the impurities or accompanying contaminants, solubility, partition coefficient (Log Po/w) of any ingredient should be considered and the information obtained from study of above points must be included in each toxicological dossier.

**TOXICOLOGICAL PROFILE ANALYSIS**

The assessment of the toxicological potential is the most important step in the hazard evaluation of a cosmetic ingredient and consists in a series of distinct toxicity studies. This study carried out in terms of evaluations of distinct toxicological parameters which are as follows.

1) **ACUTE TOXICITY**

It is evaluated to describe the adverse effects on health, which may result from a single exposure to a substance via the oral, dermal or inhalation route [ECB, 2003]. The in vivo acute oral toxicity test was originally developed to determine the LD50-value (the dose at which 50% of the animal dies, Table 4) of the compound under investigation. It is now replaced by the alternative methods that are fixed dose method [EC B.1 bis, 1992; OECD 1992], acute toxic class method [EC B.1 tris, 1996; OECD, 1996], up-and-down procedure [OECD, 1998].

2) **IRRITATION AND CORROSIVITY**

This is evaluated in terms of skin irritation, skin corrosivity and mucous membrane irritation produced by cosmetic ingredients.

A) **SKIN IRRITATION AND SKIN CORROSIVITY**

Skin irritation tests have been developed to assess the potential of a certain substance to cause redness and edema after a single topical application and skin corrosion tests assess the potential of a substance to cause irreversible necrosis through the epidermis and into the dermis, following the application of a test substance for the duration period of 3 minutes up to 4 hours. Corrosive reactions are notified by formation of ulcers, bleeding, and scabs. At the end 14 days, discoloration of the skin, alopecia, and scars will be observed to assess the extent of skin corrosion reactions [OECD, 1981]. Validated in vivo [Draize, 1965] and in vitro [Archer and Liebisch, 1998] method that is used for evaluating skin irritation and skin corrosion is mentioned in table 4 and table 5 respectively.

B) **MUCOUS MEMBRANE IRRITATION**

Mucus membrane irritation will be accessed by determining ocular irritation produced by cosmetic ingredients after a single application. Ocular irritation tests have been developed to assess the potential of a certain substance to
cause chemosis, discharge, redness to the conjunctiva, swelling of the iris and opacity to the cornea. Classical in vivo ocular irritation test [Kay and Calandra, 1962, Table 5] and the HET-CAM test [Gilleron et al., 1996] is a valid in vitro alternative method for evaluating mucous membrane irritation (Table 5).

3) SKIN SENSITIZATION

A skin sensitizer is an agent that is able to cause an allergic response in susceptible individuals. Three common in vivo laboratory animal test methods (Table 4) widely in being used to evaluate the potential of a substance to cause skin sensitization are Local Lymph Node Assay (LLNA), Guinea Pig Maximisation Test (GPMT) and Buehler test [OECD, 2002; Magnusson and Kligmann, 1969; Buehler, 1965]. Till date there is no validated in vitro test method accepted for skin sensitization.

4) DERMAL / PERCUTANEOUS ABSORPTION

Human exposure to cosmetic ingredients occurs mainly via the skin. In order to reach the circulation cosmetic ingredients must cross a number of cell layers of the skin. The in vivo and in vitro dermal / percutaneous absorption studies have been described by several international bodies [ECETOC, 1993; US EPA, 1996]. Dermal / percutaneous absorption is the amount of dermally applied substance remaining in the residual skin (excluding the stratum corneum) plus the amount of dermally applied substance which has transpassed the skin and is detected in the receptor fluid. The sum is considered to be systemically available [Bronaugh, 2005].

5) REPEATED DOSE TOXICITY

Repeated dose toxicity comprises the adverse general toxicological effects occurring as a result of repeated daily dosing with exposure to, a substance for a specific part of the expected lifespan of the test species [ECB, 2003]. The 28-day and 90-day oral toxicity tests in rodents are the most commonly used repeated dose toxicity tests and often give a clear indication on target organs and type of systemic toxicity. Currently no validated or generally accepted alternative methods are available for replacing animal testing.

6) MUTAGENICITY/GENOTOXICITY

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. Genotoxicity is a broader term and refers to potentially harmful effects on genetic material that are not necessarily associated with mutagenicity. Several in vitro gene mutation test (Table5) and in vivo tests are available and have been described for evaluating mutagenicity and genotoxicity of cosmetic ingredients [OECD, 1984; ECB, 17, 2000], but for most cosmetic ingredients, in vivo tests are not considered imperative. For testing the potential genotoxicity/mutagenicity/carcinogenicity of oxidative hair dye ingredients a new strategy was adopted by the SCCNFP in June 2003 [SCCNFP/0720/03, 2003].

7) CARCINOGENICITY

Substances are defined as carcinogenic if they induce tumours, increase their incidence, malignancy when they are inhaled, ingested, dermally applied or injected [ECB, 2003]. In vivo carcinogenicity tests [Amur et al., 1991, Table 4] and in vitro Syrian Hamster Embryo (SHE, Table 5) Transformation Test [OECD, 1996b] is the most commonly performed methodologies to evaluate carcinogenic action of cosmetic ingredients.

8) REPRODUCTIVE TOXICITY

Reproductive toxicity is used to describe the adverse effects induced by a substance on any aspect of mammalian reproduction. The most commonly performed in vivo reproduction toxicity studies are done by teratogenicity test [Amur et al., 1991, Table 4]. Three alternative in vitro methods (Table 5), Whole Embryo Culture test (WEC) Micro Mass test (MM) and Embryotoxic Stem cell Test (EST) are used to evaluate reproductive toxicity of cosmetic ingredients [ESAC, 2001].

9) TOXICOXIKINETIC STUDIES

Toxicokinetic studies explain the time-dependent fate of a substance within the body. This includes absorption, distribution, biotransformation and its excretion [ECB, 2003]. In specific cases, in vivo or in vitro biotransformation studies are required to prove and to exclude certain adverse effects associated with cosmetic ingredients. Several in vitro models (hepatocytes in suspension or culture) are suitable for biotransformation studies (Table 5), however, none of these models has been validated [Blaauboer et al., 1994; Coecke et al., 1999] since so far for evaluation purpose. Finally, toxicokinetic studies are of importance in extrapolating both in vitro and in vivo animal data to man.

10) PHOTO-INDUCED TOXICITY

It is measured in terms of photoinirritation and photomutagenicity of cosmetic ingredients.
a) Photoirritation/photosensitization – in vitro method for the determination of the phototoxicological profile of all UV light absorbing chemicals and especially for those cosmetic ingredients to be used as UV filters [SCCNFP/0069/98]. The reliability and relevance of the in vitro 3T3 NRU phototoxicity test (Table 4) is already being evaluated for a number of substances with a chemically different structure [Spielmann et al, 1998] and various in vivo methods used for assessment (Table 4).

b) Photomutagenicity/photogenotoxicity – photomutagenicity/photogenotoxicity test is desirable for UV radiation absorbing cosmetic ingredients is assessed by SCC guidelines in 1990 [Dean et al., 1992].

11) HUMAN DATA VS DATA
Tests in animals and alternative methods carried out for toxicity evaluation of ingredients are of limited predictive value with respect to human exposure. Therefore, a skin compatibility test with human volunteers is needed scientifically and ethically, to confirm that there are no harmful effects when applying a cosmetic product for the first time to human skin or mucous membranes.

In general, acute toxicity, irritation and corrosivity, skin sensitization, dermal/percutaneous absorption, repeated dose toxicity, mutagenicity/genotoxicity studies are considered the minimal base set requirements for toxicological evaluation of all cosmetics ingredients. However, when considerable oral intake is expected or when the data on dermal/percutaneous absorption indicate a considerable penetration of the ingredients through the skin then only carcinogenicity, reproductive toxicity and toxicokinetics studies may become necessary. Photo-induced toxicity data are specifically required when the cosmetic product is expected or intended to being used on sunlight-exposed skin. Human data are extremely useful and should be included whenever available.

**Figure 6**
Table 4: methodologies, test and animal models

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Animal model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Comet assay</td>
<td>Albino rats</td>
<td>Palme et al., 1984</td>
</tr>
<tr>
<td>2</td>
<td>Primary Dermal Irritability</td>
<td>Rabbits</td>
<td>Davis, 1965</td>
</tr>
<tr>
<td>3</td>
<td>Ocular irritability</td>
<td>Rabbits</td>
<td>Keay et al., 1965</td>
</tr>
<tr>
<td>4</td>
<td>Accumulated Dermal Irritability</td>
<td>Rabbits</td>
<td>Davis, 1965</td>
</tr>
<tr>
<td>5</td>
<td>Primary Dermal Photo-Irritiation</td>
<td>Albino guinea pigs</td>
<td>Gollet et al., 1985</td>
</tr>
<tr>
<td>6</td>
<td>OPMT</td>
<td>Albino guinea pigs</td>
<td>Morgenstern, 1969</td>
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<tr>
<td>7</td>
<td>Phototoxicity</td>
<td>Albino guinea pigs</td>
<td>Gollet et al., 1985</td>
</tr>
<tr>
<td>8</td>
<td>Photomutagenicity</td>
<td>Albino guinea pigs</td>
<td>Mailbach et al., 1996</td>
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<tr>
<td>9</td>
<td>Photoreactivity</td>
<td>Albino guinea pigs</td>
<td>Wegmann, 1979</td>
</tr>
<tr>
<td>10</td>
<td>Non-maximum Dermal Phototoxicity</td>
<td>Albino guinea pigs</td>
<td>Wegmann, 1979</td>
</tr>
<tr>
<td>11</td>
<td>Phototoxicity</td>
<td>Albino guinea pigs</td>
<td>Mailbach et al., 1996</td>
</tr>
<tr>
<td>12</td>
<td>Oral LD 50</td>
<td>More rats</td>
<td>OECD, 1992</td>
</tr>
<tr>
<td>13</td>
<td>Oral Macous Irritability</td>
<td>Albino rabbits</td>
<td>Monksy et al., 1962</td>
</tr>
<tr>
<td>14</td>
<td>Oral Macous Irritability</td>
<td>Rats</td>
<td>Denz et al., 1994</td>
</tr>
<tr>
<td>15</td>
<td>Carcinogenicity</td>
<td>More rodents</td>
<td>Amdur et al., 1991</td>
</tr>
<tr>
<td>16</td>
<td>Teratogenicity</td>
<td>More rodents</td>
<td>Amdur et al., 1991</td>
</tr>
<tr>
<td>17</td>
<td>LLENA</td>
<td>More</td>
<td>OECD, 2002b</td>
</tr>
</tbody>
</table>

**Figure 7**
Table 5: methodologies with evaluation parameters

<table>
<thead>
<tr>
<th>S.No</th>
<th>Technique</th>
<th>Evaluation parameters</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HET-CAM</td>
<td>Ocular irritation</td>
<td>Spielmann et al., 1997</td>
</tr>
<tr>
<td>2</td>
<td>BCSP</td>
<td>Ocular irritation</td>
<td>Giller et al., 1996</td>
</tr>
<tr>
<td>3</td>
<td>Cytotoxicity (Aqueous gel dilution test)</td>
<td>Ocular irritation</td>
<td>Wallin et al., 1987</td>
</tr>
<tr>
<td>4</td>
<td>Cytotoxicity (Neutral Red method)</td>
<td>Ocular irritation</td>
<td>Hassel et al., 1997</td>
</tr>
<tr>
<td>5</td>
<td>Cytotoxicity (MTT Method)</td>
<td>Ocuar irritation</td>
<td>Hassel et al., 1997</td>
</tr>
<tr>
<td>6</td>
<td>Cytotoxicity (MTT Method)</td>
<td>Ocuar irritation</td>
<td>Berenbaum et al., 1998</td>
</tr>
<tr>
<td>7</td>
<td>Phototoxicity Test</td>
<td>Phototoxicity</td>
<td>Spielmann et al., 1994</td>
</tr>
<tr>
<td>8</td>
<td>Ames test</td>
<td>Photoreactivation and sensitization</td>
<td>OECD, 1985, EEC, 13/14, 2006</td>
</tr>
<tr>
<td>9</td>
<td>Alteration test</td>
<td>Photoreactivation and sensitization</td>
<td>OECD, 1990, EEC, 10, 2006</td>
</tr>
<tr>
<td>10</td>
<td>Gene mutation test</td>
<td>Photoreactivation and sensitization</td>
<td>OECD, 1994, EEC, 17, 2004</td>
</tr>
<tr>
<td>11</td>
<td>ZF-test</td>
<td>Carcinogenicity</td>
<td>OECD, 1990</td>
</tr>
<tr>
<td>12</td>
<td>WEC test</td>
<td>Reproductive toxicity</td>
<td>ESAC, 2001</td>
</tr>
<tr>
<td>13</td>
<td>MM test</td>
<td>Reproductive toxicity</td>
<td>ESAC, 2001</td>
</tr>
<tr>
<td>14</td>
<td>EEC test</td>
<td>Reproductive toxicity</td>
<td>ESAC, 2001</td>
</tr>
<tr>
<td>15</td>
<td>Hepatotoxicity test</td>
<td>Toxicokinetic study</td>
<td>Amdur, 1998</td>
</tr>
</tbody>
</table>

**III) MICROBIAL EXAMINATION**

Preservatives are added as ingredients in cosmetic products to make the products free from microbial side effect. To evaluate the potency of preservative efficacy challenge test is widely used which determines that preservative are in sufficient quantity to keep the product free from toxic effect [BIS, 2006]. COLIPA has proposed the microbial limit for the purity of cosmetics (Table 6). Membrane filtration and
plate count method is used for qualitative and quantitative specifications of microbes [Behravan et al, 2004].

**Figure 8**  
Table 6: Limit for microbial purity of Cosmetics

<table>
<thead>
<tr>
<th>Gam</th>
<th>Category</th>
<th>TVC (CFU per gm or per ml)</th>
<th>Aerobic Bacteria</th>
<th>Yeasts &amp; Moulds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baby/Eye Products</td>
<td>&gt;10^7</td>
<td>&gt; 10^4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Oral Products</td>
<td>&gt; 10^5</td>
<td>&gt; 10^5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Products containing Natural Raw Material</td>
<td>&gt; 10^7</td>
<td>&gt; 10^5</td>
<td></td>
</tr>
</tbody>
</table>

**METHODOLOGIES AND TECHNIQUES**

Some validated in vitro methodologies are already reported in the literature for monitoring specific toxicological parameters, but presently no validated in vitro alternative methods are available for the repeated dose animal toxicity studies nor there relevant proposals are ready for prevalidation/validation [Worth and Balls, 2002; Rogiers, 2002b]. The toxicological evaluation of a cosmetic product should be certainly carried out with full knowledge of the pharmacotechnical, toxicological, pharmacokinetic, regulatory, clinical phase’s areas and Scientific Committee of Cosmetics and Non-Food Products Intended (SCCNFP), Organization for Economic Cooperation and Development (OECD), European Cosmetic, Toiletry and Perfumery Association (COLIPA), Cosmetics, Toiletries and Fragrances Association (CTFA), Bureau of Indian standard (BIS) and world health organization (WHO) guidelines. There are various in vitro (Table 1), in vivo (Table 4) and clinical methodologies (Table 7) for evaluating the toxicological potential of chemical substances.

**Figure 9**  
Table 7: Clinical methodologies for evaluation of ingredients toxicity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test/ Compatibility Assays</th>
<th>Number of Human Volunteers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Primary &amp; accumulated cutaneous irritation</td>
<td>50</td>
<td>Mailbach et al, 1995; Baseltter et al, 1997</td>
</tr>
<tr>
<td>3</td>
<td>Soap Chamber Test</td>
<td>15</td>
<td>Aggar 1984; Herman et al, 1972; Frenck et al, 1979</td>
</tr>
<tr>
<td>4</td>
<td>Conspicuity</td>
<td>5</td>
<td>Zehnder et al, 1985</td>
</tr>
<tr>
<td>5</td>
<td>Dermal Sensitization</td>
<td>50</td>
<td>Mailbach et al, 1960; Klignan et al, 1967</td>
</tr>
</tbody>
</table>

**CALCULATION OF RISK FACTORS**

Risk factors for a cosmetic ingredient are calculated in terms of Margin of Safety (MoS), Systemic Exposure Dosage (SED) and Life time cancer risk.

1) **MOS**

![Figure 10](image)

**NO(A)EL**: The No Observed (Adverse) Effect Level is the outcome of long-term toxicity studies, such as 28-day, 90-day tests.

**SED**: The Systemic Exposure Dosage of a cosmetic ingredient is the amount expected to enter the blood stream (and therefore be systemically available) per kg body weight and per day. It is expressed in mg/kg body weight/day.

This MoS value [Renwick, 1998] is used to extrapolate from a group of test animals to an average human being, and subsequently from average humans to sensitive subpopulations (Fig.3). These factors can be further subdivided as indicated in Fig.4 [WHO, 1994]. It is generally accepted that the MoS should at least be 100 to declare a substance safe for use.

**Figure 11**  
Figure 3: Schematic representation of the extrapolation from animal to man

![Figure 11](image)

**Figure 12**  
Figure 4: Subdivision of the Margin of Safety

2) **SED**

For testing skin absorption of product that mimics human
exposure, normally 1-5 mg/cm² for a solid and up to 10 µl/cm² for liquids should be used in vitro tests. Exceptions may exist with oxidative hair dyes, where 20 mg/cm² usually are applied for 30 minutes. There are two ways of calculating the SED

A) DERMAL ABSORPTION IN µG/CM

Calculations of the SED should preferably be based on the absolute amount bioavailable (µg/cm²) after a certain time period, based on the highest anticipated concentration.

Figure 13

\[
\text{SED (mg/kg bw/day)} = \frac{\text{DA}_\text{a} (\mu g/cm^2) \times 10^{-3} \cdot \mu g \times \text{SSA (cm}^2) \times F \times \text{(day}^{-1})}{60 \text{ kg}}
\]

SED (mg/kg bw/day) = Systemic Exposure Dosage

\( \text{DA}_\text{a} (\mu g/cm^2) \) = Dermal Absorption reported as amount/cm²

\( \text{SSA (cm}^2) \) = Skin Surface Area expected to be treated with the finished cosmetic product

\( F \times \text{(day}^{-1}) \) = Frequency of application of the finished product

60 kg = Default human body weight

B) DERMAL ABSORPTION IN PERCENTAGE

Calculations of the SED may also be based on the percentage dermally absorbed, lowest concentration anticipated.

Figure 14

\[
\text{SED = } \frac{A (g/day) \times 1000 mg/g \times C(\%)/100 \times \text{DA}_\text{p}(\%)/100}{60 \text{ kg}}
\]

SED (mg/kg bw/day) = Systemic Exposure Dosage

\( A (g/day) \) = Amount of the cosmetic product applied daily

\( C(\%) \) = Concentration of the ingredient under study in the finished cosmetic product on the application site

\( \text{DA}_\text{p}(\%) \) = Dermal Absorption expressed as a percentage

60 kg = Default human body weight

3) LIFETIME CANCER RISK

Determination of the lifetime cancer risk is carried out in several distinct steps. The dose-descriptor \( T_{25} \) is defined as the chronic dose rate that will give 25% of the animal’s tumours at a specific tissue site after correction for spontaneous incidence, within the standard life time of that species. The determination of \( T_{25} \) is described in details by Dybing et al. [1997]. From the experimental data an animal dose descriptor (\( T_{25} \)) is determined and converted to a human dose descriptor (\( \text{HT}_{25} \)) by using the following formula [Sanner et al. 2001]. Subsequently, the lifetime cancer risk is determined by linear extrapolation to the actual exposure dose. Finally, a commentary statement is generated stating whether an overall evaluation of all data available indicates that the actual risk may be higher or lower than the calculated risk.

Figure 15

\[
\text{HT}_{25} = \frac{T_{25}}{(\text{body weight human}/\text{body weight animal})^{0.25}}
\]

\[
\text{Lifetime cancer risk} = \frac{\text{SED}}{\text{HT}_{25}^{0.25}}
\]

SED represents the lifetime daily dose expressed in mg/kg bw/day.

CONCLUSION

As time progressed, through invasions and migrations and cultures merged there has been profound effect on the value of cosmetics. Cosmetic products are developed to be applied to human skin and external mucosa and to be used by the general public. Skin and mucous membrane irritation are the most frequently observed reactions. Cosmetic products should be free from side effects as their use is concern to human health. To access the risk and the safety of cosmetic products it is necessary to determine the characteristics and toxicological data of cosmetic ingredients. With a view to checking the chemical nature of the ingredient and its degree of purity, methods should be devised for identification, qualitative and quantitative control. The maximum admitted concentration of ingredients in formula of cosmetic product must be based on toxicological values, as it is a dose that makes the poison. Toxicological data can be obtained by adopting standard methodologies and techniques. Study of risk assessment reveals specific toxicity associated with the particular ingredients that can be useful to avoid its use in cosmetics. Calculations of risk factors determine the margin of safety of ingredients that can be helpful in risk management. Paper upgrades the knowledge of common consumers, academicians, chemist and beautician about cosmetics. At last study conclude that there is a need of integrated research by adopting proper guidelines at scientific level to eliminate the risk at zero level to produce safe cosmetics.
ACKNOWLEDGEMENT

Authors are thankful for the time to time guidance provided by professionals of Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, C.G.

References


r-29. ESAC. (2001). Statement on the scientific validity of the Embryonic Stem Cell Test (EST), the Micromass Test and the Post implantation Rat Whole-Embryo Culture Assay.
Risk analysis tools for toxicological profile of cosmetics

- in vitro tests for embryo toxicity. ECVAM Scientific Advisory Committee (ESAC), 17th Meeting, 16-17 October, Ispra, Italy.


r-64. SCCNFP/0069/98, Final. (1998). Opinion on in vitro methods to assess phototoxicity in the safety evaluation of cosmetic ingredients or mixtures of ingredients, adopted by the SCCNFP during the plenary session of 25 November.


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