

Cosmetic Ingredient: Metabolism and Mechanism

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Abstract. As additives, scents, preservatives, stabilizers, surfactants, stains, and shine, a variety of chemical substances have been added into the creation of cosmetic, beauty, and personal care products. Many of these compounds, on the other hand, are bioactive, ecologically persistent, and have the potential to bioaccumulate, posing a major hazard to the environment and human health. To genuinely address this issue, new techniques and approaches are necessary. This review summarizes the use of preservatives, pigments, and UV filters in cosmetics. The mechanisms of toxicities of preservative, such as parabens, benzalkonium chloride, and chlorphenesin are illustrated. Parabens have non-cutaneous adverse health effects, including infertility, spermatogenesis, adipogenesis, and perinatal exposure effects. Benzalkonium chloride in preservative decreased cell viability and caused apoptotic cell death *in vitro*. Pigment also played an important role in cosmetics and, some pigmentations in cosmetics are made by heavy metal or have heavy metal as ingredients. Cadmium and Chromium are two commonly used heavy metal in cosmetics. UV filters absorb UV energy and transform it chemically, which can lead to breakdown and the formation of photo-unstable reactive intermediates. Future research may focus on the safety assessment of specific substance in the application of cosmetics.

Keywords: Cosmetics, Preservatives, Pigments, UV filter, Mechanim, Metabolism.

1. Introduction

In the United States, the cosmetics are defined as products that are intended for use on the human body for the purpose of cleansing, beautifying or altering appearance by the Federal Food, Drug, and Cosmetic Act (FD&C Act) [1]. Among these products are lipsticks, eye shadows, shampoos, hair colours, moisturizers for the skin, and perfumes. The history of cosmetics goes back at least 7000 years, and cosmetics have been distributed worldwide. The development of the cosmetics industry is growing faster and faster, from \$483 billion in 2020 to \$511 billion in 2021, the cosmetics market is expected to grow by 4.75% annually, to reach \$716 billion by 2025 and \$784.6 billion by 2027 [2].

However, the toxicity of cosmetics was not taken into consideration at the early times, which led to variety of negative adverse effects on those who used cosmetics, including deformities, blindness and even death. Harmful cosmetic products have been used throughout history, including ceruse (white lead) which was widely used during the Renaissance in the west and ancient Japan. Many ingredients of cosmetics are toxic and may cause side effects in our body, for example, some preservatives and pigments may cause damage to cells and some UV filter may even cause tumor formation. Even though there are limits of adding them, but these limits are not entirely effective. The cosmetics that being absorbed by our body may act as carcinogens, reproductive toxins, endocrine disruptors, mutagens and neuro-toxicities [3]. Thus, the metabolisms and mechanisms are critical for studying their toxicity.

This review summarizes the metabolisms and mechanisms as well as toxic effects of ingredients in cosmetics, including preservatives, pigment and UV filters.

2. Preservatives

As the most common ingredient present in cosmetics, preservatives are added to maintain the freshness and safety of the cosmetic, and prevent microorganisms from growing in different environments. Ideally, the preservative for cosmetics is colourless, water-soluble, odourless, non-toxic, not allergenic, not irritating, effective over a broad pH range, and have the ability to inhibit growth of a wide spectrum of bacteria and fungi [4]. However, the current preservatives are not perfect. Parabens, phenoxyethanol, formaldehyde, chlorphenesin, benzalkonium chloride are some of the commonly used preservatives for cosmetics in the past few years. It is vital to understand mechanisms and metabolisms of some preservatives to seek for approaches to preventing their toxic effects.

2.1. Parabens

Parabens are a group of parahydroxybenzoates or esters of parahydroxybenzoic acid (also called 4-hydroxybenzoic acid) chemically, common parabens include methylparaben, ethylparaben, propylparaben, butylparaben and heptylparaben. Although it is very common and affective as preservatives, it's very disputable in past years because many previous literatures have pointed that they may have potential relationship with adverse health effects such as endocrine activity, carcinogenesis, infertility, spermatogenesis [5]. Up to now, parabens is allowed to use in cosmetic products. However, no single paraben may have a concentration higher than 4 g/kg in consumer products, with the maximum adding limitation being 8 g/kg in consumer products [6].

2.1.1. Mechanism of action.

The mechanism of action of parabens while acting as antibacterial agent remains to be clarified. But some possible mechanisms that relates to it have been posted by previous literature. For example, it was proposed to be acted by inhibiting membrane transport or some key enzymes [7, 8]. One of the studies conducted pump-clamp experiments on reconstituted mechanosensitive channels of large conductance (MscL) liposomes. Researchers discovered that parabens interact with mechanosensitive channels, which opens the MscL channel, and osmolytes that act as osmoprotectants including potassium glutamate, trehalose, and glycine betaine are released from the bacteria. The osmotic gradients in bacteria will be upset as a result, even the channel just open for a very short time. Bacterial growth is slowed or impaired by this disruption of the MS channel function in vivo [9]. The other study treated *Escherichia coli* and *Bacillus subtilis* with methyl parabens, propyl parabens and butyl parabens. The result shows that all of three parabens have an effect on incorporation of 3H-dTTP in DNA of toluene-treated cells, and as the concentration of parabens increase, the percentage inhibition will increase. Furthermore, the larger the size of substituents, the greater the inhibition on DNA. Researchers investigated the effects of inhibition on RNA synthesis as well, the inhibition patterns were quite similar to those of DNA synthesis. But conversely, the larger the size of substituents, the smaller the inhibition is observed [10].

2.1.2. Metabolism.

The tests on rats showed that after dermal application, for methylparaben, propylparaben and butylparaben, the peak levels of blood are achieved about 1 hour after post application, then the levels dropped until the last quantifiable time point occurred about 24 hours later. All three parabens tested showed similar pharmacokinetic profiles in both males and females. Overall, over 50% of the parabens did not be absorbed, while 14–27% or <2% were excreted in the urine or feces. But by oral administration, the maximum of blood levels all occur only after 0.5-1 h after post-gavage. It will rapidly be absorbed from the digestion tract and blood, mostly of which (>70%) would excrete in the urine within 24 hours while 2% would retain in the tissues and carcasses and no more than 4% would exit the body through the feces [11]. The process of parabens' hydrolysis by nonspecific esterases occurs during oral and dermal administration. Esterases can be found in abundance at sites of entry, such as the skin, subcutaneous fat tissue, and digestive system [12]. In decreasing order, p-

hydroxybenzoic acid and its conjugates of glycine, glucuronic acid, and sulfuric acid were shown to be the major metabolites that excreted in urine [13]. The hydrolysis efficiency of parabens in organisms is highly depending on alkyl chain and tissues [14]. It's interesting to know from the vitro studies that, the hydrolysis rate of parabens is much faster in human or rat livers than in human skin [15].

2.1.3. Toxic effect.

Paraben may affect the Akt pathway activity in immortalized human meibomian gland epithelial cells, which can be indicated by P-Akt level. It will reduce cell survival and proliferation and also induce rounding, atrophy, poor adherence and detachment of those cells. The P-Akt level of IHMGECs when exposed to parabens, and other preservatives is significantly lower than those of controls, which means the preservatives reduced cell survival and proliferation [16]. Recently, it was also found that parabens in personal care products may affect pubertal onset in children by clinical Tanner Staging (a classification system used to document and track children's development and sequence of secondary sex characteristics during puberty) and accelerated failure time models. Propyl parabens would cause earlier genital development of boys and earlier menarche of girls while methyl paraben would cause pubic hair development and menarche of girls [17].

2.2. Benzalkonium chloride

Benzalkonium chloride (BAC) is aquaternary ammonium antiseptic and an antimicrobial agents and preservatives which frequently used in skin antiseptic, personal care products [18]. It's also the most common preservative in topical medication used to treat glaucoma.

2.2.1. Mechanism of action.

It was determined that BAC was a lytic biocide. As a cationic substance, it binds to oppositely charged cell walls electrostatically [19], which results in the effects on cells shown in figure 1. In short, BAC may lead to membrane damage, which could cause leakage of intracellular contents, such as ATP. Further, the loss of membrane integrity (lost its culturability) would prevent the cell from growing in culture media, and the cell respiration and metabolic activity would decrease [22].

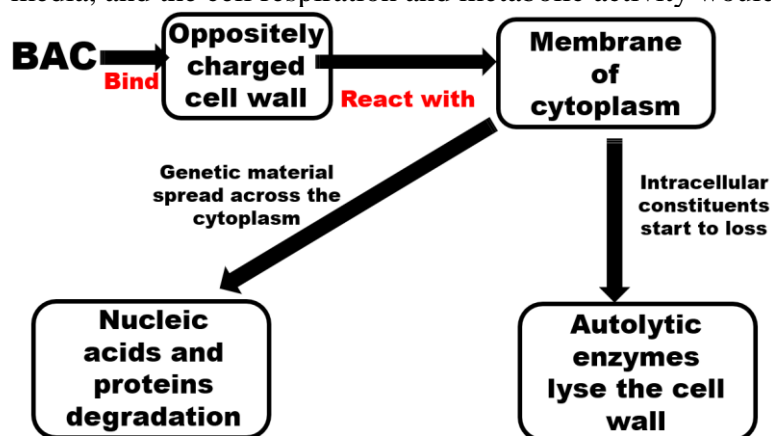


Figure 1. BAC effect on cells (modified from [20, 21])

2.2.2. Mechanism of toxic.

BAC was also found to have cytotoxic effect on human trabecular meshwork cells (HTMC), after exposed to BAC, cell viability of HTMC decreased with time and dose. After 24 hours of exposure, it also led to an increase in apoptosis in HTMC. BAK toxicity is caused by several mechanisms, including cell death signals (caspase-dependent apoptosis and apoptosis independent of caspase activity), cytokine induction, and oxidative stress mediated by the production of reactive oxygen species [23].

2.2.3. Metabolism.

In human liver microsomes (HLM), the metabolism of benzalkonium chloride was NADPH-dependent and it is reported that the half-lives as well as metabolic stability increase with the length of alkyl chain in BAC (C16 > C14 > C12 > C10). BACs with longer chains and more lipophilicity may have improved metabolic stability. By using liquid chromatography-mass spectrometry to compare with synthetic standards, the main metabolites of C10-BAC were identified as ω -hydroxy-, (ω -1)-hydroxy-, (ω , ω -1)-diol-, (ω -1)-ketone-, and ω -carboxylic acid. Different CYP enzyme will consume different amount of same BAC [24].

2.2.4. Toxic effect.

Benzalkonium chloride may cause neural toxicity in rat brains by inducing ROS, and causing apoptosis, decreasing cell viability and causing cell death [25]. Besides, although it is commonly used in eye drop as preservatives, benzalkonium chloride will damage the cornea. The result showed that the cell viability would decrease and the recovery of Transepithelial electrical resistance (TEER) would delay by benzalkonium chloride both depending on the dosage. And the TEER value after the treatment with 200 mg/mL BAK did not tend to recover within 120 min after treatment [26].

2.3. Chlorphenesin

Chlorphenesin (also called CHL) is a glycerol in which a 4-chlorophenyl group replaces one of the principal hydroxy groups. It is a synthesis preservative and has good antimicrobial, antifungal properties. Many products such as masks, foundation, moisturizer, concealer include this substance. However, it is restricted in cosmetic since it is classified as a weak eye irritant and will cause skin irritation and respiratory irritation.

2.3.1 Metabolism

The metabolism of chlorphenesin have been only studied in rats and dogs. After oral administration of radioactive drug to rats, gastrointestinal absorbance of CHL is very rapid, and peak blood radioactivity is measured after 30 minutes. Blood radioactivity has a half-life of approximately 140 minutes. The majority of the dose will be excreted in the urine after four hours, and the rest will primarily appear in the digestion tract and carcass. CO₂ was recovered as a small amount of radioactivity. Five end products of urine were found which include 3-p-chlorophenoxyacetic acid, p-chlorophenoxyacetic acid, unchanged drug, a conjugate of chlorophenol, and a conjugate of chlorphenesin [27].

2.3.2. Toxic effect.

The concern of CHL to health has reported by many literatures. Since CHL is known to degrade under the UV light, an experiment was done to study the toxicity of the transformation products after degradation. As a result of its degradation, chlorphenesin's overall ecotoxicity increased with increased irradiation time in both samples. [28]

Table 1. Summary of mentioned preservatives metabolism

Preservative type	Metabolism (oral)	Metabolism (dermal)	References
Parabens	>70% in urine, 2% in tissue, <4% in feces.	14%-27% in urine, <2% in feces.	[11]
BAC	Not defined	Not defined	[24]
CHL	Mainly in urine	Not defined	[27]

3. Pigment

Metals are ubiquitous in human daily life, whether in water, land, stone, or even in a large number of man-made objects [29]. Metals are also widely used in cosmetics as pigment; for example, eye shadow, lipstick, blush, and eyeliner. However, some metals, such as Cadmium and Chromium, are harmful to the human body and may even affect the human metabolism.

3.1. Cadmium

Cadmium is a very common heavy metal contaminant and is also used in small amounts as pigment in cosmetics because of its coloring ability. However, cadmium can cause a series of adverse reactions when it enters the human body. As mentioned above, cadmium decreases cell viability, enhances ROS and induces apoptosis. Although the amount of cadmium used in cosmetics is not high, long-term use can still have negative effects on the human body [29].

3.1.1. Metabolism.

When a group of human cells was exposed to various dose of cadmium for 24 hours, cell viability was substantially decreased (compared to the control group). It can be seen from table 1 that the reduce in cell viability was more pronounced after exposure to 5 and 10 μM cadmium, but the decrease in cell activity was very pronounced when exposed to 20 μM or higher cadmium concentrations [29].

The accumulation of Reactive oxygen species (ROS) may cause cellular damage [30]. In experiments, fluorescent probes can be used to detect ROS levels. After the cells were exposed under cadmium at concentrations of 20 μM , 40 μM , and 60 μM , the fluorescence intensity of the cadmium-treated group was 2.78, 4.20, and 4.68 times greater than that of the control group, respectively. The above set of data indicates that when the concentration of cadmium rises, the level of ROS also increases, which means that the cells are more prone to problems as the concentration of cadmium rises [29].

Bax and Bcl-2 are two proteins that can reflect apoptosis. As shown in Figure 3, the expression of Bax increased with enhancing cadmium concentration, while the expression of Bcl-2 decreased with enhancing cadmium concentration. This further suggests that cadmium may cause apoptosis.

3.2. Chromium.

Apart from cadmium, Chromium is another metal used as pigment in cosmetics. However, hexavalent chromium, like cadmium described above, can affect human metabolism and the normal functioning of individual organs [33].

Hexavalent Cr has been determined to be carcinogenic. In more detail, hexavalent Chromium may cause DNA damage, apoptosis, cell cycle regulation, cytoskeleton, morphological changes, energy metabolism, biosynthesis, oncogenes, bioenergetics, and can affect cells of the immune system associated with toxicity. Blood cells in the organism are also susceptible to hexavalent Chromium, and under the influence of Chromium, blood parameters can be altered, leading to a number of diseases [33].

3.2.1 Metabolism.

Tian and his research group [32] have done an experiment on how Chromium can affect chicken. They purchased forty chickens from regular sources, all of which were of similar weight. The experimenters kept them in a temperature of 36 °C and 60% humidity for a period of time and then divided them into two groups. Each group consisted of twenty chickens. Thirty-five days later, the experimentalists executed the forty chickens and found obvious differences between the two groups by autopsy. The first group of chickens did not find anything wrong with their livers because they were fed normally, while in the second group of chickens with Chromium poisoning. Their livers showed maceration, hemolysis, degeneration, nuclear scorching and central phagocytosis.

The International Agency for Research on Cancer (also known as the IRAC) classifies hexavalent chromium compounds as human carcinogens (Group 1) [34]. In different studies, among other things,

compounds containing chromium are also dangerous for humans. In addition to affecting metabolism, chromium compounds affect other things. For example, potassium dichromate has been shown to be one of the most potent sensitizing agents [35]. In Europe, 5.1% of chromium exposure allergy cases are reported every year and the incidence is higher in men than in women with increasing age [31].

4. UV Filter

Zinc oxide (ZnO) and titanium dioxide (TiO₂) are two of the most popular inorganic UV filters used in sunscreen products, which have long been utilized in sunscreens due to their capacity to effectively filter UV radiation. The bulk form of TiO₂ and ZnO results in thick, unattractive compositions that leave unsightly white stains on the skin after application. Sunscreen formulators utilize nanosized variants of these chemicals to avoid this annoyance. Furthermore, these shapes reflect UV light more effectively. The use of ZnO and TiO₂ nanoparticles allows for the creation of sunscreen compositions that are transparent, fluid, and simple to apply. Nanoparticles are particles with a diameter of fewer than 100 nanometers. Despite our lack of understanding of nanoparticles' biological impacts, we can find them in the majority of sunscreens [36].

Because of their tiny size, capacity to circumvent immunologic defense systems, complex with proteins, and stimulate reactive oxygen species (ROS) production, nanoparticles in sunscreens provide a potential toxicity risk. The surface reactivity of a particle determines its toxicity. Nanoparticles have a greater reactivity surface area than bigger particles due to their shape, and thus have a greater possibility to create ROS during a process catalysed by UV radiation.

Because these chemicals are primarily used as photocatalysts to produce energy in photovoltaic cells, concerns concerning ZnO and TiO₂ nanoparticle toxicity are centered on ROS production. When these chemicals are exposed to UV radiation, electrons are released, resulting in the formation of free radicals, peroxides, and other ROS [37]. The capacity of a particle to penetrate stratum corneum is determined by its molecular size. Stratum corneum cells have intercellular gaps of roughly 100 nm. Various topical medications or a stress such as UV ray exposure can alter and substantially expand these gaps [38,39].

4.1. Zinc oxide

Several studies have reported cytotoxic effects of ZnO nanoparticles on various mammalian cell lines. These particles can cause toxicity on the cells that make up the epidermal barrier, and later on organs reached by systemic circulation. Some investigations on the toxicity of ZnO nanoparticles on human dermal fibroblasts have been reported in recent years. Even at a relatively low concentration of 10 $\mu\text{g/mL}$, ZnO nanoparticles proved to be capable of inducing apoptosis in human dermal fibroblasts via the p53-p38 signaling pathway [40]. P38 MAP kinases are a kind of protein that responds to genotoxic stress by being active. They phosphorylate p53 when they are active, causing cell cycle arrest or death. The phototoxicity of bulk and nanoparticulate ZnO has also been investigated in the *Caenorhabditis elegans* worm [41].

4.2. Titanium dioxide

After using sunscreen, the initial degree of exposure to TiO₂ nanoparticle toxicity is through skin barriers. The concentrations of TiO₂ nanoparticles in genuine commercial sunscreens range from 3% to 15%. In vitro studies on human skin cells revealed cytotoxic and genotoxic effects of TiO₂ nanoparticles size [42]. Because of their photo-unstable nature, TiO₂ nanoparticles' ability to generate ROS generation resulted in DNA epidermal cell damage. Some researchers previously reported cytotoxicity of 450 nm TiO₂ particles after photoactivation with UVA on human skin fibroblasts [43]. Treatment of human skin fibroblasts with microcrystalline TiO₂ particles followed by UVA irradiation resulted in a considerable decrease in cytotoxicity. According to the findings, RNA retrieved from treated fibroblasts included substantial amounts of photo oxidation products. Researchers investigated the UVA-dependent cytotoxicity of several TiO₂ nanoparticles directly

isolated from commercial sunscreens [44]. The capacity of TiO₂ nanoparticles prevalent in marketed sunscreens to cause skin cell damage was convincingly proven in this investigation. This might result in DNA skin cell mutations that are passed down to daughter cells, resulting in tumor formation [45].

5. Conclusions

Cosmetics, as one of the most common daily necessities, are integrated into most people's lives. The commonness of cosmetics in people's lives is underscored by the cosmetic advertisements that can be seen everywhere. While the physical beauty and psychological pleasure of cosmetics are enjoyed, few people are aware of their side effects. Preservatives, UV filters, and pigment are extremely common and even indispensable ingredients in cosmetics, but they are actually toxic and can affect the human metabolism and internal organs at a certain level. Preservatives are used in a wide range of products, in fact, some skin care products also contain preservatives in addition to cosmetics. UV filters are often used in cosmetics as skin protection, and pigment is an indispensable part of eye shadow, lipstick, blush and other cosmetics that require color. Since pigment, UV filter, and preservatives are still difficult to be replaced in cosmetics, future research may focus on the use of chemicals that are harmless to humans in these three ingredients, as well as make effects on reducing the damage of the original chemicals to human metabolism and liver.

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