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Investigation on the use of expired make-up and microbiological contamination of mascaras

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Synopsis.

OBJECTIVES: This study investigated the habits of female students regarding make-up use, and quantifies the microbiological contamination of mascaras worn by this population.

METHODS: To this end, 44 students answered a structured questionnaire to evaluate the use of expired make-up, shared usage and reports of adverse effects. Subsequently, make-up samples were collected to check the manufacturing registration and the expiration date and its visibility on the label. The total counts of microorganisms and identification of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in mascara samples collected were performed as described in the Brazilian Pharmacopea (4th edition)

RESULTS: According to the results obtained, 97.9 % (43/44) of participants reported that they use or have previously used makeup after the expiration date, with mascara being the most frequently mentioned product. It was observed that on the sample collection date, 70.5% (31/44) of the students had some type of expired make-up. The microbiological analysis of 40 mascara samples revealed $2.54 \pm 1.76 \ 10^4 \ \text{UFC mL}^{-1}$ bacteria and $2.55 \pm 1.54 \ 10^4 \ \text{UFC mL}^{-1}$ fungi. Analysis revealed the presence of *S. aureus* in 79% of samples and of *P. aeruainosa* in 13%.

CONCLUSION: The results are interesting because they show that women tend to continue to use make-up beyond the expiry date. Frequently, these products have a high level of contamination with pathogenic microorganisms.

Résumé.

OBJECTIFS: Cette étude a examiné les habitudes des étudiantes concernant l'utilisation de maquillage, et de quantifier la contamination microbiologique des mascaras portés par cette population.

METHODES: À cette fin, 44 étudiants ont répondu à un questionnaire structuré pour évaluer l'utilisation de maquillage expiré, utilisation partagée et des rapports d'effets indésirables. Par la suite, des échantillons de maquillage ont été recueillis pour vérifier l'inscription de fabrication et la date d'expiration et sa visibilité sur l'étiquette.

RESULTATS: Les chiffres totaux des micro-organismes et l'identification de *Pseudomonas aeruginosa* et de *Staphylococcus aureus* dans

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des échantillons de mascara recueillis ont été réalisés comme décrit dans la pharmacopée brésilienne (4e édition). Selon les résultats obtenus, 97,7% des participants ont déclaré qu'ils utilisent ou ont déjà utilisé du maquillage après la date d'expiration, avec le mascara en tant que produit le plus fréquemment mentionné. Il a été observé qu'à la date du prélèvement des échantillons, 70,5% des élèves possédaient des produits de maquillage expirés. L'analyse microbiologique des 40 échantillons de mascara révélait 2,54 \pm 1,76 10^4 UFC mL $^{-1}$ de bactéries et 2,55 \pm 1,54 10^4 UFC mL $^{-1}$ champignons. L'analyse a révélé aussi la présence de S. aureus dans 79% des échantillons et de P. aeruginosa dans 13%.

CONCLUSION: Les résultats sont intéressants car ils montrent que les femmes ont tendance à utiliser du maquillage expiré. Souvent, ces maquillages ont un niveau élevé de contamination par des micro-organismes nocifs.

Introduction

Currently, Brazil is the third largest market for the sale and consumption of personal hygiene/perfume/cosmetics products, with revenues of U\$ 43 028.5 million in 2011. Of this value, 7.3% corresponds to make-up products [1]. In the eye make-up product lines, which reached the global sum of \$11.89 billion, Brazil remains in sixth place with a market share of 4.6% (representing \$550 million) after the United States (24.5%), Japan (10.1%), United Kingdom (6.4%), Germany (5.9%) and France (4.9%) [2]. Besides beautifying, facial cosmetics have fundamental importance in stressing the harmonious features of the face, disguising imperfections and enhancing the personality of a woman [3].

Beauty products should be easy to use, effective and safe. Make-up products, especially those used in the eye area may trigger a number of allergic and infectious reactions. The main adverse effects include contact dermatitis by irritation, allergic contact dermatitis and contact dermatitis by photosensitivity (phototoxicity), which may start an inflammatory reaction [4–10]. A study conducted between 1977 and 1983 detected 713 cases of cosmetic dermatitis out of an estimated total of 13 216 patients with contact dermatitis. Skin care products, hair preparations and facial make-up were responsible for the majority of these reactions [11].

The adverse effects tend to occur not only due to individual susceptibility, but also the improper handling of these products, poor

preservation and the use of products after the expiration date [12]. Additionally, when several people share the same product, an intense contamination may take place, because each individual has a characteristic flora on their skin and this can be harmful to another individual. Improper storage is also a problem widely observed in homes, because users store their make-up in the bathroom, which is a damp place that favours the growth and development of potentially pathological organisms, like fungi and bacteria [6, 13]. Another possible way make-up can become contaminated is through poor handling procedures during manufacturing, which can negatively affect the conservation of make-up properties.

Of the different types of make-up products, those for the eye area merit special attention because of the proximity and contact with this region, and thus, the higher probability of causing irritation or, if the product is contaminated, ophthalmic infections. There is a wide variety of cosmetics for the eye area which have many different functions and contain diverse ingredients that may cause adverse effects [14]. The cosmetics used on the ocular region are the main cause of eyelid dermatitis, due to their pigments, resins, preservatives and vehicles [5]. Mascara is one of the most popular cosmetic products, used to lengthen evelashes and make them thicker, highlighting the feminine face [15]. However, this product is at a greater risk of contamination, being an aqueous-based formulation [4]. Also, the way it is handled may play a role in contamination, because of the greater chance of bacterial deposits originating from the environment and from the surface of the eyelashes, making the product more susceptible to infections.

Staphylococcus epidermidis and Staphylococcus aureus proliferate in contaminated mascaras. The most common infections caused by these microorganisms occur especially when the surface of the eyeball is damaged, in other words, traumatized [4]. Pseudomonas aeruginosa is the main agent of eye infections like conjunctivitis, keratitis and ophthalmitis, which may threaten the integrity of the eye, destroying tissues and damaging visual acuity [16]. Infections by P. aeruginosa have been reported to occur due to contaminated mascara, trauma to the eye or bad hygiene [17]. Fungi can also be found in contaminated mascaras, although less frequently than bacteria, being related to immune-compromised people or those who wear contact lenses [4, 16].

The microbial limit in medicines, cosmetics and related products may be defined as the complete absence of viable forms (in the case of sterile drugs) or as a set parameter, restricted or not to certain microbial strains (non-sterile). There are some microbiological quality parameters that must be respected, where the cosmetic products for the eye area should have no more than 10^2 UFC per g mL, besides the absence (in 1 g or 1 mL) of *P. aeruginosa, S. aureus* and total or faecal coliforms [18, 19].

The quality and performance requirements for mascara are as follows: They should (i) be non-irritating as they are applied so close to the eyes, (ii) go on evenly and not harden the eyelashes or form blobs, (iii) make the eyelashes look thick and long, (iv) make the eyelashes curl effectively, (v) have an appropriate lustre, (vi) have an appropriate drying time, (vii) not go on to the lower eyelids when dry and their appearance must not be spoiled by sweat, tears or rain, (viii) be easy to remove, (ix) be easy to use throughout their period of use, (x) not be contaminated by microorganisms [3]. Finally, it is important to ascertain the make-up usage habits of women, in order to avoid misuse, as well as the use of products with expired quality that may lead to adverse health issues. In this context, in this study, the use of make-up after its expiration date by college students from a university. Also, the number of viable

microorganisms presents in mascaras and the specific presence of the bacteria *P. aeruginosa* and *S. aureus* in these products was determined, considering that these pathogens are associated with a high risk of ocular infections.

Material and methods

This study was divided into three stages. The first stages refer to the data collection, applying a transversal study. The population consisted of college students of the pharmacy course at an university, over 18 years old, who agreed to participate in the research after signing an informed consent term. The third stage consisted of the microbiological evaluation of the mascara samples collected in the second part.

Stage I: application of the questionnaire to the make-up users

A structured questionnaire was administered individually to the participants after reading the consent term. The aim of this stage was to evaluate the use of make-up beyond the expiry date, shared usage and reports of adverse effects. The questionnaire applied had open and closed questions. Some questions were 'Do you use expired make-up or have you ever used?', 'If you do use, which ones?', 'Have you ever observed any adverse reaction while using expired make-up?', 'Which reactions?', 'Do you share your make-up with other people?', 'Do you sleep with the make-up?', 'From how often you discard the make-up, even if not expired?'. The study protocol was approved by the Ethics Committee for Research of URI University (177/TCH/08). The variables of interest at this stage were the use of expired make-up, shared usage, adverse effects after use and registration of expiration date on make-up.

Stage II: make-up collection to evaluate the expiration date

In this second stage, the conditions under which the cosmetics of the participants which were currently being used were kept in their homes were evaluated. The make-up products were collected from the participants for analysis and to register the information on a form. The make-up samples were kept in plastic bags, identified with a tag and sealed to preserve the integrity of the product. At this stage, no chemical or microbiological testing was carried out, only the verification of the presence of the manufacturing and expiration dates on the label of these products. The visibility of this information on the label of the cosmetics was also evaluated. The questions evaluated were 'Was there any expired make-up in the kit?','Which ones?', 'How many make-ups were expired?', 'For how long the make-ups were expired?', 'Did all make-ups in the package have visible validity dates?', 'Which make-ups did not have an expiration date?'. After the data collection, the cosmetics were returned to the participants within twenty-four hours, with the exception of the mascaras, which were used in stage III.

The data were analysed using the software SPSS (Statistical Package of the Science Social), v. 16.0 for Windows, which provides distribution parameters (average and frequency).

Stage III: microbiological evaluation of the mascara samples

During Stage II, 40 mascaras were collected. After the collection, the samples were stored until the moment of analysis in individual plastic bags. As controls, we used four samples of new products, purchased at local pharmacies. The procedure for total microbial

count in plaques was performed as described in the Brazilian Pharmacopeia V [20].

Pseudomonas aeruginosa evaluation

A nutrient broth was prepared for the bacteria, to which 1 mL of the product under examination (mascara) was added followed by incubation at a temperature of 37°C for 2 days. After this period, the samples were inoculated by the pour plate method into Cetrimide agar. The dishes were incubated at 35°C for 24 h. For the P. aeruginosa identification, three confirmatory tests were performed. The first involved growth at 41°C, because all P. aeruginosa species grow at this temperature. In the second test, one colony was incubated at 35°C for 24 h after which time the yellow fluorescence was observed at a wavelength of 210 mm. The last test performed was the cytochrome oxidase test in which a drop of N-N-dimethyl-p-phenylenediamine solution was added to each colony and the change from an intense pink to black colour was indicative of a positive result. The presence of P. aeruginosa in the mascara was considered when positivity was observed in two or three confirmatory tests [20].

Staphylococcus aureus evaluation

Approximately, 1 mL of nutrient broth for bacteria and fungi was transferred to a petri dish containing phenol red mannitol agar. This plaque was incubated at 35°C for 48 h. Four identification tests were performed. The first test was gram staining, looking for the presence of gram-positive cocci. The catalase test was also performed in which hydrogen peroxide was added to a colony. The test was considered positive after the rapid appearance of sustained gas bubbles. The coagulase test procedure consisted of the addition of a colony from each mascara sample to a tube with $500 \mu L$ of rabbit plasma for the positive control and water was used instead of the plasma for the negative control. The tubes were maintained in a water bath at 36°C for 24 h and the subsequent observation of clotting characterized a positive result. Finally, we determined the deoxyribonuclease activity. The colonies were subcultured in test tubes containing test agar for deoxyribonuclease. These tubes were incubated at 37°C for 18 h, and the formation of colourless area around the bacterial growth indicated a positive result. The presence of S. aureus in the samples was only considered when positivity was observed in two or more confirmatory tests [20].

Results and discussion

The correct use of medicines is a determinant factor of therapeutic efficacy, that is, the expected result. Likewise, the appropriate application of cosmetics also ensures the desired aesthetic benefit from these products. Therefore, knowledge of how cosmetics are used by the consumer might aid an understanding of the side effects often associated with these products, such as allergies and eye irritation. Important parameters for evaluating make-up usage behaviour were evaluated in this study, including records of allergic reactions due to the use of cosmetics, make-up sharing and the use of make-up beyond the expiry date products.

This study included 44 students from different semesters of the course. The age ranged between 18 and 28 years (21.52 ± 2.28) . Of the total participants interviewed, 97.7% (43/44) reported that they use or have previously used make-up beyond the expiration date. Table I describes the make-up most used after the expiration date as reported by the participants. The products frequently used

Table I Frequency of make-up use by students after the expiration date

Make-up	N	% *
Mascara	40/44	86.3
Eye pencil	35/44	79.5
Lipstick/Lip Gloss	32/44	72.7
Eye shadow	28/44	63.6
Face powder	21/44	47.7
Foundation	20/44	45.5
Concealer	13/44	29.5
Others	10/44	22.7

^{*}Percentage in relation to total number of participants.

Table II Adverse effects following the use of make-up after the expiration data

Adverse reactions	Number of citations	% *	% [†]
Tearing	8/44	18.2	36.4
Erythema	6/44	13.6	27.3
Itchiness	4/44	9.0	18.2
Irritation	3/44	6.8	13.6
Allergy	1/44	2.2	4.5

^{*}Percentage in relation to the total number of participants.

were mascara, eye pencil, lipstick/lip gloss and eye shadow, with 86.3, 79.5, 72.7 and 63.6 of use frequency, respectively (Table I).

In 70.5% (31/44) of the responses, it was observed that there were no reports of adverse effects after wearing expired make-up. In the case of students who had some adverse effects after the use of expired make-up (13/44), the most common manifestations were tearing (36.4%), redness (27.3%) and itchiness (18.2%; Table II). It should be noted that in some cases, more than one reaction was reported by a single participant. The adverse reactions highlighted are mainly related to make-ups used on the eyes area, because the shedding of tears was reported by mascara, eyeshadow and pencil usage, allergy to eyeshadow and powder, redness caused by eyeshadow and foundation, and the itching by the eyeshadow and powder usage.

Guin [7] showed that 29% of 203 users had some adverse reaction to make-up. This result is similar to that reported by the participants of this study (29.5%). Mascara, being the make-up most frequently used beyond the expiration date may have contributed to the high incidence of adverse effects. Mascaras can elicit dermatitis along the eyelid due to the components used in its formulation (colourants and preservatives) [4, 8] and also the presence of microorganisms (*P. aeruginosa* and *S. aureus*) [16]. An allergic reaction in the eyes was the less frequent, 2.2% (Table II), in contrast to Guin [7] results, who reported 13.3% of participants with allergies to make-up. This reaction is an allergic dermatitis caused by an allergen, which can trigger a series of hypersensitive reactions, one of the responses that more affect women who wear make-up [8]. Common allergens can be the constituents of the cosmetic formulation, mainly preservatives and fragrances [9].

[†]Percentage of occurrence of adverse effects in relation to total citations.

Watery eyes (tearing) was reported by 18.2% of the participants and this reaction can be caused by many types of make-up used near the eye region, such as eye pencil and mascara, which were identified as being expired but reported as being used in this situation. However, when these reactions may occur they are not necessarily associated with the use of cosmetics. According to the questionnaire responses, make-up sharing with other individuals was reported by 61.4% (27/44) of the students and 68.2% (30/44) do not remove their make-up before sleeping. Make-up sharing is very common among young women, such as the students who participated in this study. However, it may increase the risk of problems resulting from bacterial contamination [13, 21].

Most of the students interviewed (79.5%; 35/44) said that they discard make-up products after 1 year of use. In the literature, it is described that the ideal period of use before discarding eye cosmetics products is 3 months after purchase or before if the product has dried or the colour has changed [4, 13]. Mascaras, particularly water-based products, are easily contaminated with bacteria, which readily grow in water. Preservative systems, such as parabens, mercurials and formalin derivates, are usually added to mascara to prevent microbial proliferation. However, the effectiveness of the preservatives is dependent on the several factors, including time of use, sharing and form of use [22]. Reports have described that 92% of women admit to keeping their mascara for longer than 6 months, nearly two-thirds use eye make-up that is over 2 years old and a quarter of those surveyed share eye make-up with friends, family and colleagues [23].

The second part of this study consisted of evaluating the packaging of the cosmetic products that were collected from students. There was a high percentage of students (70.5%) that had some kind of make-up product which had already expired on the day of the sample collection. The make-up samples analysed which were beyond the expiration data had expired for more than 1 year (34.1%), 6 months (13.6%) or 3 months (13.6%). Considering all of the make-up products which had expired, 86% were products applied to the eye region (mascara, eye pencil and eye shadow), 22.7% were lipstick/lipgloss and 6.8% were foundation (Fig. 1).

The information contained on the packaging serves as guide for correct use by the consumer, for instance, the expiration date. Of the 44 participants, it was observed that $42\ (95.5\%)$ had some type of make-up product for which it was not possible to identify

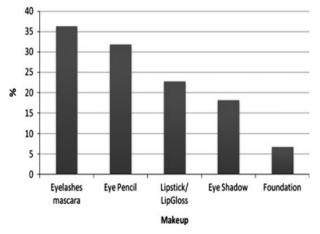


Figure 1 Make-up products that had past their expiration date.

the expiration date, that is, the printing was no longer visible. Most of such products collected were cosmetics for the eye region and lips (Table III).

In the case of around 70% of the students interviewed, expired make-up was collected (Table III). These results suggest that these students use the cosmetics under this condition. We verified that 95.5% of the participants had a make-up product on which no expiration date could be found on the packaging. According to specific Brazilian legislation [24], information on the product need only appear on the secondary packaging of the make-up and it is not mandatory to provide the expiration date on the primary packaging. However, in most cases, the secondary packaging is discarded after the purchase. Subsequently, the consumer may use the product for a long period of time and frequently beyond the expiration data, enabling product contamination and the development of ocular diseases and allergy.

In the microbiological evaluation, we analysed 40 mascaras collected from the volunteer students. The bacterial growth was $2.54 \pm 1.76 \, 10^4 \, \mathrm{UFC} \, \mathrm{mL}^{-1}$ and fungi $2.55 \pm 1.54 \, 10^4 \, \mathrm{UFC} \, \mathrm{mL}^{-1}$. As controls, four new samples were used. None of the controls showed *S. aureus* or *P. aeruginosa* contamination and the colony counts for bacteria and fungi were extremely low, that is, less than $100 \, \mathrm{UFC} \, \mathrm{mL}^{-1}$, not exceeding the levels recommended by legislation [18]. In the research on pathogens, it was verified that in 79% of the samples analysed, *S. aureus* was present and 13% were contaminated by *P. aeruginosa*. The skin harbours *S. aureus*, which lives symbiotically with it, and this may be correlated with the result obtained in this study [19]. This relationship is only of concern when the presence of *S. aureus* causes skin disease or eye disease such as conjunctivitis [5].

In 1981, Dawson and Reinhardt [25] performed a study on 1345 samples of cosmetics for the eye area. Of these, 67% were contaminated with one or more species of microorganisms and the genera most frequently found were *Staphylococcus*, *Micrococcus*, *Corynebacterium* and *Moraxella*. Because these are present in our regular flora, the majority of the contaminants found in these products belonged to the regular microbiota [25]. Maidana et al. [26] conducted a study in patients with infectious keratitis and observed the presence of several microorganisms in the lesions. The main species found were *S. aureus* (in 19.2% of the samples) and *P. aeruginosa* (in 12.8% of cases) [26]. The bacteria *S. aureus* and *S. epidermids* were also isolated from eyeliner samples [27]. Therefore, it can be concluded that these microorganisms, when present, may become highly pathogenic, compromising the ocular surface.

An important factor is the shared use of eye make-up products because each person has a particular microbiota and at the

Table III Frequency of students who had make-up with no visible printed date on the packaging

Make-up	Frequency	%
Mascara	34/44	77.3
Eye pencil	28/44	63.6
Lipstick/ Lipgloss	25/44	56.8
Eye shadow	22/44	50.0
Face powder	10/44	22.7
Foundation	6/44	13.6
Concealer	4/44	9.0
Others	5/44	11.3

Table IV Recommendations to avoid contamination of eye make-up products

Do not moisten cosmetic products.
Do not share or swap products.
Avoid using eye cosmetics if you have an eye infection.
Do not use old eye cosmetics.
Do not store cosmetics at temperatures above 85° F or in moist places.
Avoid using make-up with Kohl (contains heavy metal salts such as lead)
Insert contact lenses before applying mascara
Replace make-up after bacterial infections
Discard make-up after colour, odour and texture alterations
Do not use old applicators in new products
See an optometrist if you experience eye problems

Adapted from ref. [13].

moment a make-up product is shared the contamination is instantaneous. The presence of fungi and bacteria at the time of product manufacturing is very low, and thus, in such cases, because the manufacturing standards are very strict, it can be assumed that this contamination did not originate in the factory but instead is attributable to the user [19]. In a study performed by Pack *et al.* [12], after 3 months of using new mascaras a contamination level higher than that recommended by the governmental organs [18] was observed. Therefore, the authors suggest that the maximum period of mascara usage should be 3 months.

Another extremely important aspect to be considered is the contamination occurring during the use or storage of the product, which is difficult to predict. Thus, adequate care regarding the correct use of topical products should be encouraged. For example, the use of spatulas for the removal of the product and packaging from tubes can provide a significant reduction in contamination by *P. aeruginosa* and *S. aureus* [19]. The packaging should protect the product from the environment, thereby minimizing the loss of the constituents and maintaining the physico-chemical stability and efficacy of the constituents. The product should be stored under appropriate conditions so that degradation of its components do not occur, that is, away from moisture, which is the main cause of microbial proliferation [28].

In addition, the correct production of a cosmetic is extremely important in order to reduce the final level of microbiological contamination of the product. The packaging of the products requires important precautions and procedures that are in agreement with good manufacturing practices [29]. A study performed by Fiorentino *et al.* [29] revealed that glass and plastic packaging allow mould and yeast colonies to grow, and 7 of the 12 samples analysed contained *Aspergillus fumegatus*, a potentially contaminating mould found in hospital environments, and this compromises the microbiological quality of the cosmetic products. Therefore, package is a source of contamination when it does not obey the criteria for microbial quality.

It is not known if the consumers are aware that their products are contaminated or if they throw them away due to changes in the coloration and/or odour caused by microorganisms. Most consumers realize that contamination has occurred following the use of a make-up product only when organoleptic changes are noted [28]. The contamination arises mainly because the consumers do not take proper care of the product or there is the lack of adherence to the requirements on the part of the industries that manufacture the packaging [12]. In the packaging of the control samples, instructions for use were not observed and only the composition was verified. Table IV shows some guidelines that should be observed regarding the use of make-up products.

The correct use of a product ensures efficiency for the consumer and less risk to health. The results of this study highlight that microbiological contamination is a serious problem. Considering the risks generated by both the inappropriate usage and the possibility of inadvertently using products beyond the expiration date, public health campaigns warning of the need for the correct usage of these products are of great interest, as well as the mobilization of the Brazilian health authorities in order to require the printing of the expiration date on the primary packaging of the cosmetics products.

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References

- ABIHPEC, Brazilian Association of Cosmetic, Toiletry and Perfumery Industry. Sector Overview 2011 (2011) Available at: http:// www.abihpec.org.br/wp-content/uploads/ 2012/04/Panorama-do-setor-2011-2012-INGL%C3%8AS-04-FEV-2013.pdf, accessed 30 April 2013.
- ABIHPEC, Brazilian Association of Cosmetic, Toiletry and Perfumery Industry. Abiphec yearbook 2009-2010. Available at: http:// www.abihpec.org.br/en/2011/08/anuarioabihpec-20092010, accessed 30 April 2013.
- Mitsui, T., ed. New Cosmetic Science. 1st edition. Elsevier Science, Amsterdam (1998).
- Draelos, Z.D. Special considerations in eye cosmetics. Clin. Dermatol. 19, 424–430 (2001).

- Draelos, Z.D., ed. Procedures in Cosmetic Dermatology Series: Cosmeceuticals. Saunders Elsevier, China (2009).
- Saxena, M., Warshaw, E. and Ahmed, D.D. Eyelid allergic contact dermatitis to black iron oxide. Am. J. Contact Dermat. 12, 38– 39 (2001).
- Guin, J.D. Eyelid dermatitis: experience in 203 cases. J. Am. Acad. Dermatol. 47, 755– 765 (2002).
- Loden, M. and Wessman, C. Mascaras may cause irritant contact dermatitis. Int. J. Cosmet. Sci. 24, 281–285 (2002).
- Biebl, K.A. and Warshaw, E.M. Allergic contact dermatitis to cosmetics. *Dermatol. Clin.* 24, 215–232, vii (2006).
- Castanedo-Tardan, M.P. and Zug, K.A. Patterns of cosmetic contact allergy. *Dermatol. Clin.* 27, 265–280, vi (2009).

- Adams, R.M. and Maibach, H.I. A five-year study of cosmetic reactions. J. Am. Acad. Dermatol. 13, 1062–1069 (1985).
- Pack, L.D., Wickham, M.G., Enloe, R.A. and Hill, D.N. Microbial contamination associated with mascara use. *Optometry.* 79, 587–593 (2008).
- FDA, Food and Drug Administration. Use Eye Cosmetics Safely. Available at: http:// www.fda.gov/ForConsumers/ConsumerUp dates/ucm048943.htm, accessed 30 April 2013.
- Barata, E.A.F., ed. A Cosmetologia: Princípios Básicos. Tecnopress, São Paulo (1995).
- 15. Rieger, M.M., ed. *Harry's Cosmeticology*, 8th edition. Chemical Publishing, Gloucester (2000).
- Esteva, E. Infecciones Oculares, tipos tratamiento y consejo farmacéutico. Offarm: Farm. y Sociedad. 25, 58–62 (2006).

- 17. O'Donoghue, M.N. Eye cosmetics. *Dermatol. Clin.* **18**, 633–639 (2000).
- Brazil. Resolution RE n. 481(09/27/1999).
 Ministry of Health, Diario Oficial da União, Brazilia (1999).
- Pinto, T.J.A., Kaneko, T.M. and Ohara, M.T. ed. Controle Biológico De Qualidade De Produtos Farmacêuticos, Correlatos E Cosméticos, 3rd edition. Atheneu, Sao Paulo (2010).
- Brazilian Pharmacophea, v.1, 5th edn, Available at: http://www.anvisa.gov.br/hotsite/ cd_farmacopeia/index.htm, accessed 16 June 2010
- Wilson, L.A., Sawant, A.D., Simmons, R.B. and Ahearn, D.G. Microbial contamination of contact lens storage cases and solutions. Am. J. Ophthalmol. 110, 193– 198 (1990).

- Bhadauria, R. and Ahearn, D.G. Loss of effectiveness of preservative systems of mascaras with age. *Appl. Environ. Microbiol.* 39, 665–667 (1980).
- Optometrists, T.C.o. Foresight, The Annual Student Newsletter 2007 to 2008. The College of Optometrists, London (2008).
- Brazil. Resolution nº 211 (05/14/2005).
 Ministry of Health, Diario Oficial da Uniao, Brazilia (2005).
- Dawson, N.L. and Reinhardt, D.J. Microbial flora of in-use, display eye shadow testers and bacterial challenges of unused eye shadows. *Appl. Environ. Microbiol.* 42, 297–302 (1981).
- 26. Maidana, E., Gonzáles, R., Melo Soares, L.A.J. and Souza, L.B. Ceratite infecciosa em crianças: estudo microbiológico e epidemiológico em um hospital universitário de As-

- sunção Paraguai. *Arq. Bras. Oftamol.* **68**, 828–832 (2005).
- Flores, M., Moríllo, M. and Crespo, M.L. Deterioration of Raw Materials and Cosmetic Products by Preservative Resistant Microorganisms. *Int. Biodeterior. Biodegradation* 40, 157–160 (1997).
- Lundov, M.D., Moesby, L., Zachariae, C. and Johansen, J.D. Contamination versus preservation of cosmetics: a review on legislation, usage, infections, and contact allergy. Contact Derm. 60, 70–78 (2009).
- Fiorentino, F.A.M., Ricarte, P.C., Correa, M.A., Gianinni, M.J.S.M., Issac, V.L.B. and Salgado, H.R.N. Análise microbiológica de Embalagens para o Acondicionamento de Medicamentos e Cosméticos. *Lat. Am. J. Pharm.* 27, 757–761 (2008).