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ORIGINAL PAPER

Post-consumer use efficacies of preservatives in personal care and topical drug products: relationship to preservative category

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Abstract Ninety-six used personal care and topical OTC drug items collected from consumers in the USA were examined for the presence of microbial contaminants. Of the eye and face product type containing global preservative chemistries (i.e., acceptable for use in Japan without major restrictions), 55% yielded numbers of microorganisms in excess of 500 CFU/g ($P < 0.1814$). For the mascara products with global preservative chemistries, 79% yielded numbers of microorganisms in excess of 500 CFU/g ($P < 0.024$). Products containing global preservative chemistries accounted for 88% ($n = 14$) of the products that had microbial contents above 10^4 CFU/g ($P < 0.001$). Prominent contaminants were species of *Staphylococcus*, *Pseudomonas*, *Klebsiella, Streptococcus*, *Lactobacillus*, *Bacillus*, *Corynebacterium*, and yeast. In general, under the stress of consumer use, products preserved with global preservative chemistries did not maintain as adequate preservation as products with non-global preservatives.

Keywords Preservatives · Microbial contamination · Drugs · Cosmetics · Mascara

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Introduction

Cosmetic and over-the-counter (OTC) drug products that are applied to the facial area may be considered "high consequence" products because, with repeated use, they are subject to microbial contamination that may result in a potential adverse health effect for the user [16]. Water is a common ingredient of cosmetic and topical drug formulations and water often enters anhydrous products during consumer use [5]. The presence of water provides a condition for the growth of bacteria and fungi [2]. To prevent microbial spoilage during production and the intended use period for the product by the consumer, manufacturers add chemical preservatives. Preservatives need to target not only Gram positive and Gram negative bacteria but the eukaryotic fungi as well. The choice of preservatives is a concern because compounds that target eukaryotic fungi are anticipated to have a higher probability of invoking toxic or hypersensitivity reactions in humans than those that target prokaryotic bacteria [11]. The preservatives typically are used at very low levels, i.e., less than 1% of the formulation, and are targeted at species such as *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Serratia* spp., and *Aspergillus niger* [3].

Cosmetics, as manufactured, are not sterile and are released for marketing according to internal release criteria based on guidelines from the Cosmetic, Toiletry and Fragrance Association (CTFA; i.e., no more than 500 cfu/g or ml for eye area products and no more than 1,000/g for other products) [6]. The CTFA recommended a microbial limit of <500 cfu/g for Gram positive bacteria as acceptable for the release of eye area type cosmetics into market, however many manufacturers have lower more stringent release criteria. Gram negative bacteria, at any level, are to be avoided according to the CTFA guidelines. The CTFA has

no microbiology guidelines for used products. The FDA cosmetic compliance program uses the 500 cfu/g mark as a flag to initiate identification of Gram positive contaminants in cosmetics collected during investigations. All Gram negative contaminants are selected for identification [10, 12]. The FDA has no set density levels for Gram negatives relative to product release and no microbial limit values for used products. The Agency does require that formulations pass a valid Preservative Efficacy Test (PET) prior to marketing, and that marketed products comply with the FD&C Act in that they are safe for the consumer during reasonable use [9, 17]. The PET not only is a FDA requirement but a scientific means of approximating the microbial insults from a consumer and validating the preservative system [4, 8].

The United States, the European Union (EU), and most countries, some with restrictive labeling, allow the use of most of the common preservatives [7, 14]. The EU, Japan, and the Unites States have codified laws regarding the allowance/disallowance of cosmetic products formulated with specific preservative chemistries in addition to specific labeling requirements. The laws regarding cosmetics in the United States include a negative list of what preservatives are disallowed, and the laws regarding cosmetics in the EU and Japan include positive lists of what preservatives are permitted. The EU has an additional labeling requirement stating the PAO (period after opening) in number of months the cosmetic can be used [15]. Whereas Japan, which has major concerns for possible toxicity reactions, has in general a more restrictive regulatory program. Preservative chemistries permitted in all countries are referred to as global preservatives, and those without universal acceptance are nonglobal preservatives. Thus the term "global preservatives" generally has become defined as a preservative that is accepted in Japan. For example, the Japanese government prohibited the import of all cosmetic products containing formalin-donor preservatives into Japan until 2001. In 2001 the use of imidazolidinyl urea and dimethyldimethylolhydantoin (DMDM) formalin donors were approved for "rinse-off" cosmetics only, with restrictions not to exceed a low use level of 0.3% [14]. This regulatory action improved the preservation options for rinse-off products in the hair care industry because these formalin donors can be used in lower concentrations in some surfactant based hair care formulations. However, high consequence "leave-on" products (i.e., not rinsed off after application) in the skin care industry generally required higher levels of these formalin donors to achieve efficacious preservative levels. Table 1 lists some examples of common preservatives and their permitted uses. This study compares the densities of culturable aerobic microorganisms of used cosmetic products containing global (GPC) and non-global (NPC) preservative chemistries in high consequence "leave-on" products.

Materials and methods

Ninety-six in-use high consequence personal care and topical over-the-counter (OTC) drug items were donated from consumers. No demographic information on the consumer or use of the product was obtained. Only items in visually good physical condition were studied. The global or non-global regulatory status of the products, i.e., their preservatives, was determined by the preservative chemistry as disclosed on their label panels. All high consequence products in the study were leave-on products. Of the 96 products obtained, 53 were NPC and 43 were GPC. Sixty-eight were categorized as eye and face type products, 24 as mascara type products, 15 were OTC drug products, and four were hand type products. Samples originated from Georgia, Oklahoma, California, New York, North Carolina, Colorado, Florida, and Maryland. Product name, product type, size, manufacturer, preservatives, and drug status information were documented. The preservative chemistries were obtained from the ingredient disclosure on the packaging, or, if that was not available, the ingredients were researched in retail stores and the ingredients identified from on the shelf products. Samples (collected during the period 2005–

Table 1 Frequently used preservatives sorted by their regulatory status

Globally (Japan) (GPC) approved w/o application restrictions (leave-on products)	Not globally (Japan) (NPC) approved w/o application restrictions
Esters of p-hydroxybenzoic acid (parabens)	DMDM hydantoin
Sorbic acid	Imidazolidinyl urea
Benzoic acid	Diazolidinyl urea
Benzyl alcohol	Sodium hydroxymethylglycinate
Phenoxyethanol	Methylchloroisothiazolinone/Methylisothiazolinone
Benzalkonium chloride	Quaternium 15
Triclosan	Iodopropynylbutylcarbamate
Chlorphenesine	
Dehydroacetic acid	
Chlorhexidine digluconate	

Individual countries may differ in their labeling requirement for certain preservatives

Table 2 Incidence and densities of recoverable aerobic cultures of bacteria and fungi from in-use cosmetics and over-the-counter drugs (OTC) with GPC and NPC

CFU/g	GPC	NPC	OTC drugs[a]
$<5 \times 10^2$	16/43 (37%)	34/53 (64%)	8/15 (53%)
5×10^2–10^3	11/43 (26%)	17/53 (32%)	7/15 (47%)
$>10^4$	16/43 (37%)	2/53 (4%)	0
Total	43/43 (100%)	53/53 (100%)	15/15 (100%)

[a] All OTC drugs had GPC

2006) were kept under refrigeration until testing, and were tested within 1 week after receipt.

Samples were diluted 1/10 (either 1 g, or the preferred 10 g of product, depending on remaining sample contents) in a filter-sterilized sodium pyrophosphate buffer (0.1%, pH = 7.0) containing 0.1% sodium thioglycollate and 0.5% polysorbate 80. Products whose packaging contained application tools were sampled using the tool to obtain material, e.g., mascara wands, blush applicators. For enumeration and recovery of microorganisms we used the 5-point Most Probable Number (MPN) method. From the 1/10 diluted samples, 1 mL of each diluted sample was used in the first set of MPN tubes with 1/2 strength Tryptic Soy Broth (Becton, Dickinson and Co., Sparks, MD). A volume of 0.1 ml from the last MPN tube, which most likely contained the dominant culturable microorganism, was spread-plated onto Tryptic Soy Agar. The plates were incubated at 30°C for at least five days and cfu recorded. Basic identifications from isolated colonies were performed with Biolog™. Additional screening of the samples was conducted as required on selective and differential media including: Mannitol Salt Agar, Pseudomonas Isolation Agar and Malt Agar (Becton, Dickinson and Co., Sparks, MD), and CHROMagar ECC (CHROMagar, Paris, France). Data were analyzed using a one-sided Fisher's exact test for statistical independence [1].

Results

The overall incidences and density ranges of microorganisms in the in-use products are presented in Table 2. Sixty-three percent of the cosmetics with GPC yielded cfu in excess of 500/g, whereas only 36% of NPC yielded these levels ($P < 0.0075$). Of the products in excess of 10^4 cfu/g, the greater susceptibility to contamination of GPC (88%) compared to NPC (12%) was statistically evident ($P < 0.001$).

Subcategory analyses of eye and face products and mascaras among in-use cosmetics are given in Table 3. Eye and face products constituted 72% (13 of 18) of all the combined samples that yielded cfu above 10^4/g with samples with GPC (12 of 18) at a significantly higher incidence than NPC ($P < 0.001$). Mascaras with GPC showed a significantly higher incidence of samples with CFU between 10^2–10^3 cfu/g compared to NPC ($P = 0.0244$). A subcategory for the four hand products was not created because of the small sample size.

The incidence of the most common microorganisms among the in-use products is indicated in Table 4. In general, *Staphylococcus* spp., mainly *S. aureus,* were the most common contaminants identified from all samples, but densities in most positive samples, particularly NGC, were less than 5×10^2 cfu/g.

There were 28 samples that did not yield culturable microorganisms. In the subset of these 28 samples, there was no significant difference between GPC samples and the NPC samples ($P = 0.18$) regarding the detection of organisms.

Table 4 Incidence of more common genera of microorganisms from in-use cosmetics

	GPC	NGC
Staphylococcus	5	17
Pseudomonas	4 (4)	1
Bacillus	6 (3)	2
Total samples	15	19

No. samples positive (no. samples with densities $>10^6$). Identifications were not undertaken with most samples with less than 2.5×10^2/g, unless Gram-negatives species were present

Discussion

Preserving cosmetic and topical drug products is a complicated process where formulators must account for the sum of microbiological adversities from raw materials, manufacturing, packaging, and consumers [11]. Cosmetic products must be safe for use by the consumer while produced

Table 3 Incidence and densities of aerobic bacteria and fungi in in-use eye and face products and mascaras

CFU/g	Face and eye area		Mascara	
	GPC[a]	NPC[a]	GPC[a]	NPC[a]
$<5 \times 10^2$	13/29 (45%)	23/39 (59%)	3/14 (21%)	7/10 (70%)
5×10^2–10^3	4/29 (14%)	15/39 (38%)	7/14 (50%)	2/10 (20%)
$>10^4$	12/29 (41%)	1/39 (3%)	4/14 (29%)	1/10 (10%)
Total	29	39	14	10

[a] Global preservative chemistries and non-global preservative chemistries, respectively

economically by their manufacturers. One aspect of safety consists of ensuring that products do not pose an undo risk of microbial contamination while in-use by the consumer [13]. There are no legal requirements from the FDA for the release of finished cosmetics into commerce other than the products be safe.

Our acceptance of products for this study was based essentially on that they were in current use and they lacked obvious visual evidence of adulteration. The limited number of samples, necessary administrative constraints on collection of demographic data, and lack of specific information on the various formulations preclude our recommendations for use of specific preservatives in any given product.

Our data suggest that NPC, in regard to the risk of an adverse event from microbial contamination, provide a greater degree of safety than GPC, particularly with eye area and facial area in-use cosmetics. In in-use cosmetics using GPC, 63% had microbial contents >500 cfu/g whereas 36% of the NPC had microbial densities >500 cfu/g. The results of the comparison of the GPC group and the NPC group found that there was a significant difference between their microbial contents ($P < 0.0075$). Products using GPC accounted for 88% of the products that had microbial contents >10 E4 cfu/g ($P < 0.001$). Although 28 samples containing GPC and NPC did not yield culturable microorganisms, this study compared the densities of culturable aerobic microorganisms of used cosmetic products containing global (GPC) and non-global (NPC) preservative chemistries in high consequence "leave-on" products. We do not interpret our data on the relatively high contaminant incidence of used OTC drugs that they are inadequately preserved compared to cosmetics. The degree of contamination of OTC products such as skin care creams may be related more to mode of use and packaging.

Perceived (but still mostly unsubstantiated) health hazards from preservative exposures have stimulated recent pursuit by consumers and marketers for "natural" or preservative-free personal care products. Our data suggest that the less intensive GPC may not be as efficacious as NPC in preventing post marketing contamination events. Still, formulations with either preservative system may become contaminated with potentially harmful microorganisms during the stress of consumer use. Both consumers and manufacturers should be cautious in their adaptation to environmentally friendly personal care products (particularly those used in the eye area) that risks for infections are not increased.

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References

1. Berger RL, Boos DD (1994) *P* values maximized over a confidence set for the nuisance parameter. J Am Stat Assoc 89:1012–1016. doi:10.2307/2290928
2. Brannan DK (1992) Cosmetic microbiology. Encyclopedia of microbiology. Academic Press, Boca Raton, vol 1, pp 593–603
3. Brannan DK (1995) Cosmetic preservation. J Soc Cosmet Chem 46:199–220
4. Brannan DK, Dille JC, Kaufman DJ (1987) Correlation of in vitro challenge testing with consumer-use. Appl Environ Microbiol 53:1827
5. Campana R, Scesa C, Patrone V, Vittoria E, Baffone W (2006) Microbiological study of cosmetic products during their use by consumers: health risk and efficacy of preservative. Lett Appl Microbiol 43:301–306. doi:10.1111/j.1472-765X.2006.01952.x
6. Cosmetic T, Fragrance Association (1983) Microbiological limit guidelines for cosmetics and toiletries. CTFA technical guidelines. Cosmetic, toiletry, and fragrance association, Inc., Washington
7. European Economic Community (EU) (1999) Cosmetics legislation. Directive 76/768/EEC, Annex VI, http://www.leffingwell.com/cosmetics/vol_1en.pdf
8. Farrington JK, Martz EL, Wells SJ, Ennis CC, Holder J, Levchuk JW et al (1994) Ability of laboratory methods to predict in-use efficacy of antimicrobial preservatives in an experimental cosmetic. Appl Environ Microbiol 60:4553–4558
9. Federal Register (1977) Notice of intent to propose regulations. 45 FR, pp 54837–54838
10. Food and Drug Administration (FDA) (2000) Center for food safety and applied nutrition. Domestic cosmetics program, cosmetic and color technology, chap 29, http://www.cfsan.fda.gov/~comm/cp29001.html
11. Geis PA (2006) Preservation strategies. In: Geis PA (ed) Cosmetic microbiology, 2nd edn. Taylor & Francis, New York
12. Hitchins AD, Tran TT, McCarron JE (1998) Microbiological methods for cosmetics. In: Tomlinson LA (ed) Bacteriological analytical manual, chap 23, http://www.cfsan.fda.gov/~ebam/bam-toc.html
13. Hugbo PG, Onyekweli AO, Igwe I (2003) Microbial contamination and preservative capacity of some brands of cosmetic creams. Trop J Pharm Res 2:229–234
14. Ministry of Health Labour and Welfare (2000) Communication No. 331. Standards for cosmetics. Article 42, No. 331. http:///.mhlw.go.jp/English/topics/cosmetics
15. Orus P, Leranoz S (2005) Current trends in cosmetic microbiology. Int Microbiol 8:77–79
16. Parker MT (1972) The clinical significance of the presence of micro-organisms in pharmaceutical and cosmetic preparations. J Soc Cosmet Chem 23:415–426
17. Rope B, Jimenez I (1999) Validation of test methods to ensure reliable results in the lab. Glob Cos Ind 165:24