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Study of the composition of 140 shampoos: similarities and differences depending on the sales channel used

Background: Shampoo is a hair care product designed to clean the skin and hair of the scalp. Among the ingredients that go into the making of a shampoo are detergents, conditioners, thickeners, sequestering agents, pH adjusters, preservatives, and active ingredients such as anti-dandruff agents. **Objectives:** The purpose of this study was to identify the composition of 140 shampoos available in pharmacies, in stores of a mass-market chain, or from mail-order retailers. Forty-one shampoos were advertised as “gentle”, 12 as specially formulated for infants, 35 as anti-dandruff, and 52 without any particular claim. **Materials and Methods:** We analysed the cleansing base, preservatives, and anti-dandruff agents when relevant and identified the allergens regardless of whether or not they are listed under Regulation (EC) No. 1223/2009 as one of the 26 regulated substances. **Results:** We discovered that unlike shampoos sold in stores of a mass-market chain and those available from mail-order retailers, those sold in pharmacies expose users to some of the 26 substances listed under Regulation (EC) No. 1223/2009. We also determined that baby shampoos sold in pharmacies are allergen-free. Regarding anti-dandruff formulations, the largest variety of active ingredients was found in shampoos sold in pharmacies. Overall, the most common active ingredients were olamines, zinc pyrithione, azoles, selenium disulphide, and plant extracts. **Conclusion:** Shampoos sold in pharmacies appear to contain fewer allergens listed under Regulation (EC) No. 1223/2009 compared to those sold elsewhere.

Key words: shampoo, pharmacy, supermarket, cleansing base, allergen, preservative, anti-dandruff agent

The word “shampoo” (derived from the Hindi word meaning “to massage”) is a hygiene product intended to clean hair and scalp. <http://fr.wiktionary.org/w/index.php?title=shampoo&action=edit&redlink=1> In the monograph entitled “Liquids for cutaneous application” (*Liquida ad usum dermicum*), the seventh edition of the European Pharmacopoeia provides the following definition: “*Shampoos are liquid or sometimes semi-liquid preparations to be applied on the scalp and then rinsed and eliminated with water. They generally form foam when mixed with water. Shampoos are emulsions, suspensions or solutions. They usually contain surfactants*”.

In practice, the vast majority of shampoos are solutions, although there are some cream shampoos. The following schematic definition can be given:

A shampoo = a cleansing base (water + surfactants) + additives ± active ingredients [1, 2].

The cleansing base is an aqueous solution of surfactants and its effectiveness is based on three or four of the following properties of surfactants; the ability to:

- wet: tendency to spread on a surface;
- emulsify: to emulsify sebum and lipophilic substances;

- clean: to remove and dissolve dirt from a solid surface;
- foam: to generate foam which plays an important psychological role for this type of product.

All these properties contribute to the cleaning of the solid surface, *i.e.* hair in this case [2, 3].

We were interested in the composition of approximately 100 shampoos (“gentle”, baby, anti-dandruff, and without any particular claim), available through different sales channels (pharmacies, stores of a mass-market chain, and mail-order retailers), in order to shed light on possible differences in the nature of the raw materials chosen, *i.e.* the surfactants used in the cleansing base, preservatives, or active ingredients.

Materials and methods

Materials

In total, 140 shampoos were studied. Forty-one (27 sold in pharmacies and 14 in stores of a mass-market chain) were marketed as “gentle”, 12 (six sold in pharmacies and six in stores of a mass-market chain) were presented as formulated

for infants, 35 (15 sold in pharmacies, 17 in stores of a mass-market chain, and three through mail-order retailers) were marketed as anti-dandruff formulations, and 52 (29 in stores of a mass-market chain and 23 through mail-order retailers) were sold without any particular claim.

Methods

For each shampoo, the number and nature of surfactants constituting the cleansing base, the number of allergens as defined under Regulation (EC) No. 1223/2009, and the number and nature of preservatives have previously been determined [4]. In the case of anti-dandruff shampoos, we also looked at the number and nature of active ingredients.

Statistical analysis

Boxplots were used as a standard graphical method to describe groups of values in terms of quartiles. Using this method, it is possible to visualise five statistical features: the segment in the middle as the median; the two segments delimiting the box as the first and third quartile, and two whiskers (corresponding to 1.5 interquartile range). This tool is used to provide quick insight into the dispersion of data. Moreover, it allows an easy comparison of the dispersion of different groups. The preliminary conclusion provided by graphical tools must then be confirmed by statistical hypothesis testing. The Mann Whitney test was used to investigate whether two shampoos were different or not according to p values; for $p < 0.05$, the null hypothesis was rejected, therefore indicating an impact on the measurements.

Results

Comparison of the cleansing base

The 58 surfactants we were able to identify in the 140 shampoos studied are presented in *table 1*. The cleansing base was always composed of several surfactants. The number of surfactants found in shampoos marketed as “gentle”, for babies, and as anti-dandruff is presented *figure 1*.

Approximately 44% of the surfactants identified were found in the “gentle” shampoos sold in pharmacies. Of these, cocamidopropyl betaine (9.2%), coco glucoside (9.2%), sodium laureth sulphate (9.2%), sodium cocoyl glutamate (8.5%), and glyceryl oleate (7.7%) were the most prevalent. “Gentle” shampoos sold in pharmacies were most likely to contain cocamidopropyl betaine (12/27; 44%), coco glucoside (44%) or glyceryl oleate (37%) in the cleansing base. In approximately 19.7% of the cases, the cleansing base contained a combination of cocamidopropyl betaine and coco glucoside (*figure 2*).

Of the “gentle” shampoos sold in retail stores, only 14% of the surfactants identified were present in the cleansing bases. These were mostly sodium laureth sulphate (24%), cocamidopropyl betaine (20%), and glycol distearate (16%). “Gentle” shampoos sold at stores of a mass-market chain were most likely to contain a combination of sodium laureth sulphate (12/14; 86%) and cocamidopropyl betaine (71%). The likelihood of a formulation with cocamidopropyl betaine and sodium laureth sulphate was 61% (*figure 3*).

There was no significant difference in the number of surfactants contained in the cleansing base of shampoos designated as “gentle”, sold through the three sales channels studied (pharmacies, stores of a mass-market chain, and mail-order retailers). An average of three surfactants

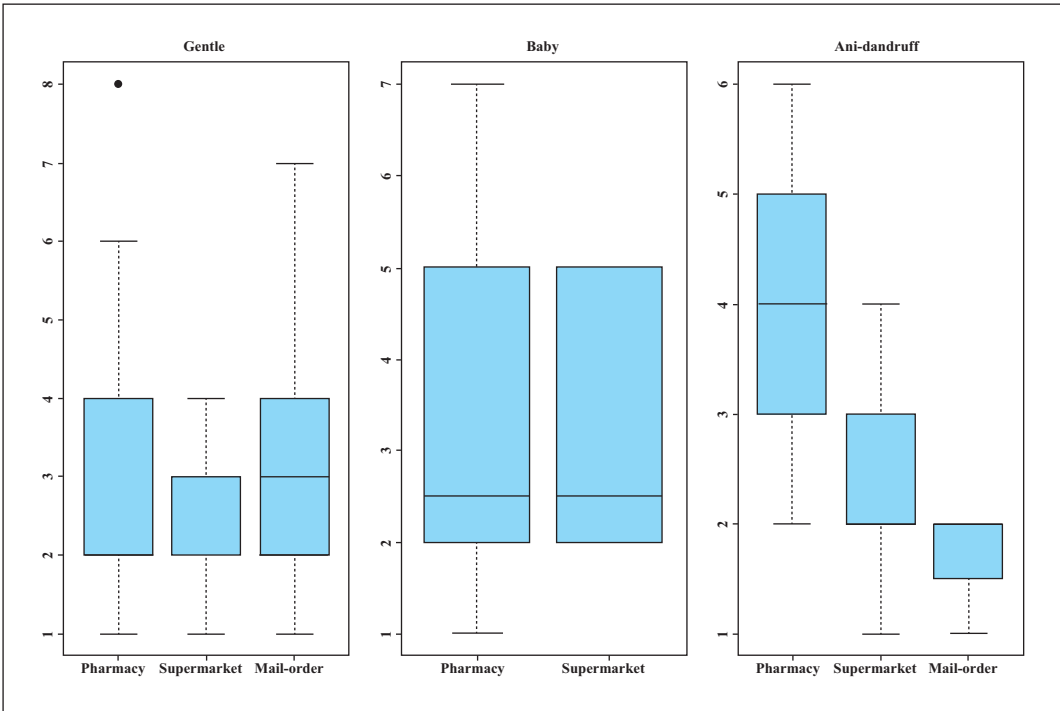


Figure 1. Box plots showing the number of surfactants in cleansing bases, according to shampoo category and sales channel.

Table 1. Surfactants found in shampoos studied.

Surfactants	“Gentle” shampoos (pharmacy)	“Gentle” shampoos (mass- market)	Shampoos for babies (pharmacy)	Shampoos for babies (mass- market)	Shampoos without any claim (mass- market)	Shampoos (mail-order retailers)	Anti- dandruff shampoos (pharmacy)	Anti- dandruff shampoos (mass- market)	Anti- dandruff shampoos (mail-order retailers)
Acetamide MEA	0	0	1	0	0	0	1	0	0
Ammonium laureth sulphate	0	0	0	0	1	1	0	0	0
Ammonium lauryl sulphate	2	2	0	0	2	5	2	1	1
Babassuamidopropylbetaine (<i>antistatique, synergiste de mousse</i>)	1	0	0	0	0	1	0	0	0
Capryloyl glycine	0	0	1	0	0	0	3	0	0
Caprylyl/capryl glucoside	2	0	2	0	0	3	0	0	0
Ceteareth-60 myristyl glycol	1	0	2	0	0	0	2	0	0
Cetearyl glucoside	1	0	0	0	0	0	0	0	0
Cocamide DEA	0	1	0	0	0	0	1	0	0
Cocamide MEA	3	1	0	0	6	3	0	3	0
Cocamide MIPA	1	1	0	0	5	1	4	0	0
Cocamidopropyl betaine	12	10	2	2	21	12	4	7	2
Cocamidopropyl hydroxysultaine	0	0	0	0	1	0	0	0	0
Coco-betaine	3	5	0	1	6	2	6	5	0
Coco-glucoside	12	0	4	1	3	4	2	3	0
Decyl glucoside	5	0	0	1	5	8	4	0	1
Dimethicone propyl PG-betaine	0	0	0	0	0	2	0	0	0
Disodium coco-glucoside citrate	1	0	0	0	0	0	0	0	0
Disodium cocoamphodiacetate	5	3	0	1	4	0	1	3	0
Disodium cocoyl glutamate	0	0	0	0	0	4	1	0	0
Disodium laureth sulfosuccinate	2	0	0	1	1	1	0	1	0
Disodium lauryl sulfosuccinate	0	0	0	0	0	0	1	0	0
Disodium lauroamphodiacetate	0	0	1	0	0	0	0	0	0
Disodium ricinoleamido MEA-sulfosuccinate	1	0	0	0	0	0	2	0	0
Distearyl phthallic acid amide	0	0	0	0	0	0	2	0	0
Glyceryl caprylate	0	0	1	0	0	0	0	0	0
Glyceryl oleate	10	0	0	1	1	2	4	3	0
Glyceryl laurate	1	0	0	0	0	0	2	0	0
Glyceryl linoleate	0	0	0	0	0	0	1	0	0
Glyceryl palmitate	0	0	0	0	0	0	1	0	0

Table 1. (Continued)

Surfactants	“Gentle” shampoos (pharmacy)	“Gentle” shampoos (mass- market)	Shampoos for babies (pharmacy)	Shampoos for babies (mass- market)	Shampoos without any claim (mass- market)	Shampoos (mail-order retailers)	Anti- dandruff shampoos (pharmacy)	Anti- dandruff shampoos (mass- market)	Anti- dandruff shampoos (mail-order retailers)
Glyceryl stearate	1	0	0	0	1	0	1	0	0
Glycol distearate	6	80	0	0	17	4	3	10	0
Lauramidopropyl betaine	0	0	0	0	0	1	0	0	0
Laureth-2	0	0	0	0	0	0	2	4	0
Laureth-3	0	0	0	0	2	0	0	1	0
Laureth-4	0	0	0	0	11	0	0	4	0
Laureth-7	0	0	0	0	0	0	1	0	0
Laureth-23	0	0	0	0	7	0	0	0	0
Laureth-5 carboxylic acid	0	0	0	0	1	0	1	1	0
Lauryl betaine	2	0	0	0	0	0	3	0	0
Lauryl glucoside	2	0	0	2	3	3	0	0	1
Lauryl hydroxyethyl imidazoline	0	0	1	0	0	0	0	0	0
Magnesium laureth sulphate	0	0	0	0	0	0	1	0	0
Methyl glucose sesquistearate	0	0	0	0	0	1	0	0	0
Olive oil glycereth-8 esters	0	0	1	0	0	0	0	0	0
PEG-4 dilaurate	0	0	0	0	0	0	3	0	0
PEG-4 laurate	0	0	0	0	0	0	3	0	0
PPG-5-ceteth-20	0	0	0	0	0	0	0	3	0
PEG-7 glyceryl cocoate	4	3	0	2	6	3	1	2	0
PEG-30 glyceryl cocoate	0	0	0	1	0	0	0	0	0
PEG-15 cocopolyamine	1	0	1	0	0	0	0	0	0
PEG-40 glyceryl cocoate	0	0	0	0	0	10	0	0	0
PEG-40 hydrogenated castor oil	0	0	1	0	5	0	0	2	0
PEG-55 propylene glycol oleate	1	0	0	0	0	3	1	0	0
PEG-60 almond glycerides	0	1	0	0	1	0	1	0	0
PEG-80 glyceryl oleate	0	0	0	0	0	0	1	0	0
PEG-90 glyceryl isostearate	0	0	0	0	0	0	1	0	0
PEG-120 methyl glucose dioleate	0	0	0	0	1	0	1	2	1
PEG-120 methyl glucose trioleate	0	0	0	0	0	0	0	1	0
PEG-150 distearate	0	0	1	0	0	0	0	0	0
PEG-200 hydrogenated glyceryl palmitate	3	0	0	2	3	0	0	1	0
Polysorbate 20	2	0	2	2	0	0	2	0	0

Table 1. (Continued)

Surfactants	“Gentle” shampoos (pharmacy)	“Gentle” shampoos (mass- market)	Shampoos for babies (pharmacy)	Shampoos for babies (mass- market)	Shampoos without any claim (mass- market)	Shampoos (mail-order retailers)	Anti- dandruff shampoos (pharmacy)	Anti- dandruff shampoos (mass- market)	Anti- dandruff shampoos (mail-order retailers)
Poly sorbate 80	0	0	0	0	0	0	1	0	0
PPG-2 hydroxyethyl coco/isostearamide	0	0	0	0	1	0	0	0	0
PPG-5-cetheth-20	0	0	0	0	0	0	0	2	0
Saponaria officinalis root extract	0	0	0	1	0	0	0	0	0
Sodium C12-13 pareth sulphate	0	0	0	0	1	0	0	0	0
Sodium C14-16 olefin sulfonate	1	0	0	0	1	0	1	0	0
Sodium coceth sulphate	0	0	0	0	0	1	0	0	0
Sodium cocoamphoacetate	4	0	2	1	0	1	4	1	0
Sodium cocoate	0	1	0	0	0	0	0	0	0
Sodium coco-glucoside tartrate	1	0	0	0	0	0	0	0	0
Sodium coco-sulphate	0	0	0	0	1	1	0	0	0
Sodium cocoyl apple amino acids	1	0	0	0	0	0	0	0	0
Sodium cocoyl glutamate	11	0	2	0	2	1	0	0	0
Sodium cocoyl isethionate	1	0	0	0	0	1	0	0	0
Sodium laureth sulphate	12	12	1	4	21	9	12	16	1
Sodium lauroamphoacetate	0	0	1	0	0	0	0	0	0
Sodium lauroyl oat amino acids	2	0	0	0	0	1	0	0	0
Sodium lauroyl glutamate	0	0	0	1	0	1	0	1	0
Sodium lauroyl sarcosinate	2	0	0	0	0	1	2	0	0
Sodium lauryl glucose carboxylate	1	0	0	0	0	2	0	0	0
Sodium lauryl sulphate	4	2	0	0	2	5	0	2	1
Sodium lauryl sulphoacetate	2	0	0	0	0	1	0	0	0
Sodium methyl cocoyl taurate	0	0	0	0	0	1	1	0	0
Sodium myristoyl glutamate	2	0	0	0	0	0	0	0	0
Sodium polynaphthalenesulphonate	0	0	0	0	0	0	0	7	0
Sodium xylensulphonate	0	0	0	0	1	3	0	2	0
Sorbitan caprylate	1	0	0	0	2	1	0	1	0
Sorbitan sesquicaprylate	0	0	0	0	1	0	0	0	0
Undecylenamidopropyl betaine	0	0	0	0	0	0	3	0	0
Zinc coceth sulphate	0	0	1	0	0	0	0	0	0

was found in the cleansing base; mostly sodium lauryl ether sulphate, cocamidopropyl betaine, and glycol distearate. The difference in the number of preservatives used in the formulations was not statistically significant between shampoos available in pharmacies and stores of a mass-market chain ($p=0.72$), but was significantly lower in shampoos sold through mail-order retailers ($p=0.038$). The real difference between shampoos sold in pharmacies and those sold in stores of a mass-market chain was the number of allergens from the list of 26 regulated substances under Regulation (EC) No. 1223/2009 ($p=0.038$). No difference was identified when shampoos sold in stores of a mass-market chain were compared with those available from mail-order retailers ($p=0.34$).

Only two sales channels (pharmacies and stores of a mass-market chain) were studied in the case of baby shampoo; the difference in number of surfactants between the different shampoos was not significant ($p=1$) (figure 4).

A comparison between “gentle” and baby shampoo sold in pharmacies showed that the formulations were similar with regards to the number of surfactants that comprise the cleansing base ($p=0.86$) (figure 5).

Analysis of the cleansing base of anti-dandruff shampoos sold in pharmacies showed that nearly 45% of the surfactants identified (table 1) were found in these formulations. The most common surfactants found were sodium laureth sulphate (12.9%) and cocamidopropyl betaine (6.5%). The most common cleansing base of anti-dandruff shampoo sold in pharmacies was a combination of sodium laureth sulphate (12/15; 80%) and coco-betaine (40%) (figure 6).

Analysis of the cleansing base of anti-dandruff shampoos sold in stores of a mass-market chain showed that nearly 30% of the surfactants identified (table 1) were found in these formulations. Of these, the most prevalent were: sodium laureth sulphate (17.7%), glycol distearate (11%), cocamidopropyl betaine (7.7%), and sodium polynaphthalene sulfonate (7.7%). All anti-dandruff shampoos sold in stores of a mass-market chain, except for one, contained sodium laureth sulphate. Glycol distearate (59%),

cocamidopropyl betaine (41%), and sodium polynaphthalene sulfonate (41%) were also fairly prevalent (figure 7). Only 8% of the identified surfactants (table 1) were found in the cleansing base of shampoos sold through mail-order retailers. Of these, cocamidopropyl betaine was the most prevalent (25%). Two out of three shampoos studied contained this surfactant (figure 8).

The cleansing base was also compared between shampoos with no particular claim and “gentle” and baby shampoos, sold in stores of a mass-market chain (figure 9); no statistically significant difference was identified between baby shampoos and shampoos with no specific claim ($p=0.37$).

Allergens

The allergens that have to be listed on the packaging pursuant to Regulation (EC) No. 1223/2009 and their nature are presented in figure 10 and table 2. It is important to note that there are also allergenic substances that are not on the list of 26 allergens which, by law, must also be listed on the packaging. Those which we have been able to identify are presented in table 3.

The number of allergens found in shampoos from retail stores was compared between shampoos without any particular claim and “gentle” and baby shampoos (figure 11); more allergens were identified in shampoos sold without any specific claim compared to baby shampoos and this difference was statistically significant ($p=0.065$). The nature of all allergenic substances found in shampoos with no specific claim sold in retail stores, regardless of whether they are on the list of 26 regulated substances, is presented in table 4.

The most significant difference between shampoos sold in pharmacies and those sold in retail stores was the number of allergens from the list of 26 regulated substances under Regulation (EC) No. 1223/2009, ($p=0.038$). Fewer allergens were found in baby shampoos compared to “gentle” shampoos ($p=0.016$). However, there was no difference in the number of allergens between shampoos sold in retail

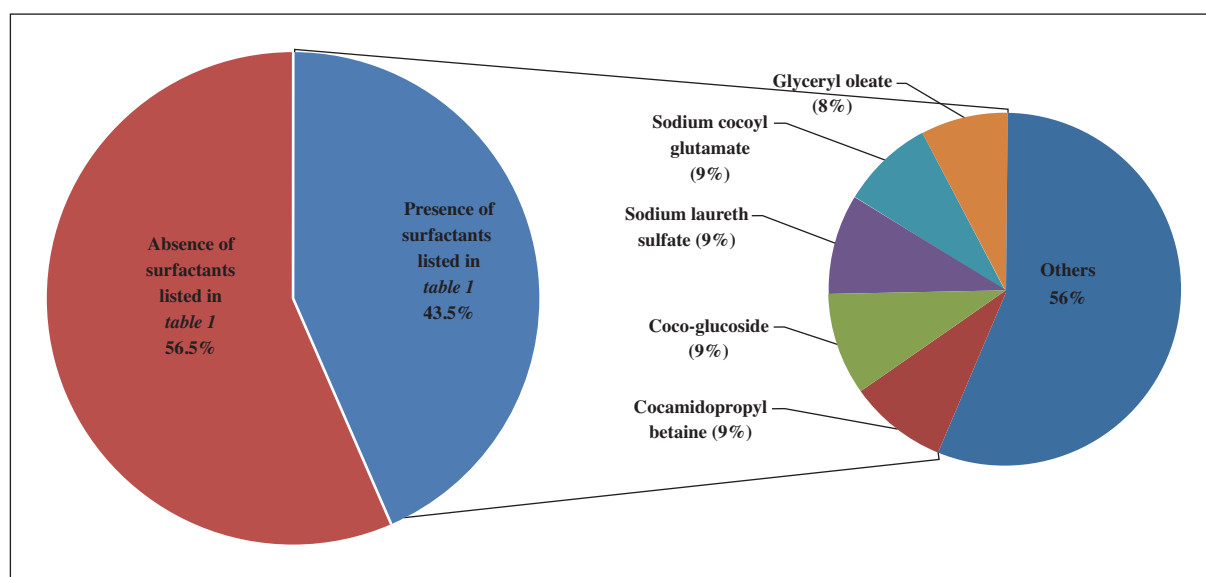


Figure 2. Analysis of the cleansing base in “gentle” shampoos sold in pharmacies.

stores and those available online ($p=0.34$), between “gentle” shampoos and baby shampoos sold in retail stores ($p=0.12$), or between baby shampoos and shampoos with no specific claim sold in stores of a mass-market chain.

Preservatives

The number of preservatives identified according to where the shampoo was available is presented in *figure 12*. The nature of the preservatives identified is presented in *table 5*. Shampoos with no specific claim sold in retail stores contained the same number of preservatives as other types of shampoo (*table 6*).

Different preservatives are used in order to cover a broad antimicrobial spectrum. The number of preservatives in shampoos from retail stores was compared, and no difference was identified between “gentle” shampoos, baby shampoos, and shampoos with no specific claim (*figure 13*).

Anti-dandruff agents

The number and nature of anti-dandruff agents identified in the shampoos studied are presented in *table 7*, and a comparison between the different sales channels is presented in *figure 14*.

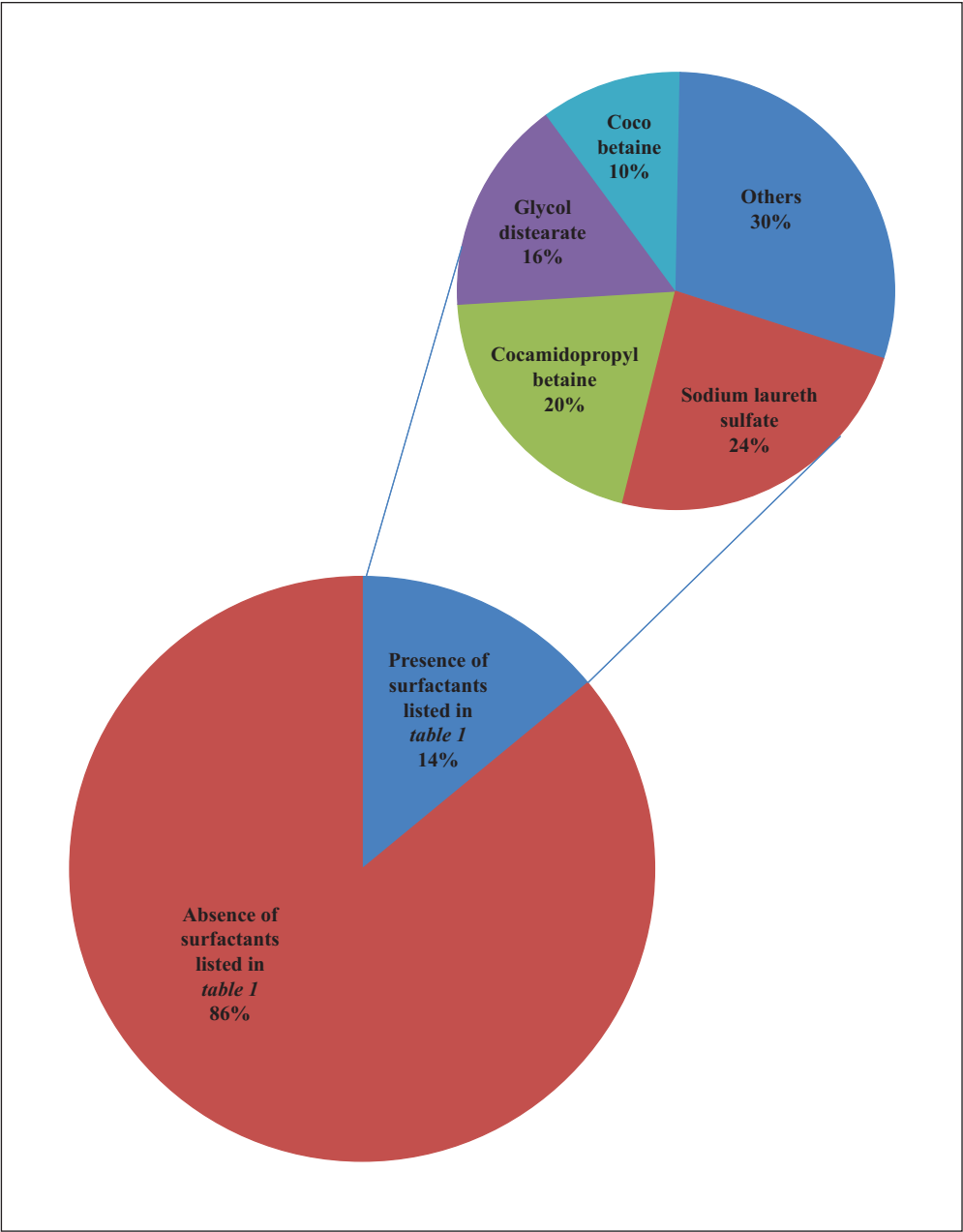


Figure 3. Analysis of the cleansing base in “gentle” shampoos sold in a mass-market chain.

Table 2. Allergens found in shampoos studied according to Annex III of EU Cosmetic Regulation 1223/2009.

Allergens	“Gentle” shampoos (pharmacy)	“Gentle” shampoos (mass-market)	Shampoo for babies (pharmacy)	Shampoos for babies (mass- market)	Anti-dandruff shampoos (pharmacy)	Anti-dandruff shampoos (mass-market)
Alpha-isomethylionone	0	4	-	-		2
Amyl cinnamal	1	-	-	-	-	5
Benzyl alcohol	6	3	-	-	-	
Benzyl salicylate	1	4	-	-	1	5
Butylphenylmethyl propional	0	1	-	-	1	3
Citral	4	1	-	-	-	-
Citronellol	1	2	-	-	1	3
Coumarin	1	4	-	-	-	2
Eugenol	1	-	-	-	-	-
Farnesol	1	-	-	-	-	-
Geraniol	2	2	-	-	-	3
Hexyl cinnamal	0	6	-	-	-	6
Hydroxycitronellal	1	-	-	-	-	-
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	1	-	-	-	-	-
Limonene	10	7	-		2	9
Linalool	6	7	-	1	2	12

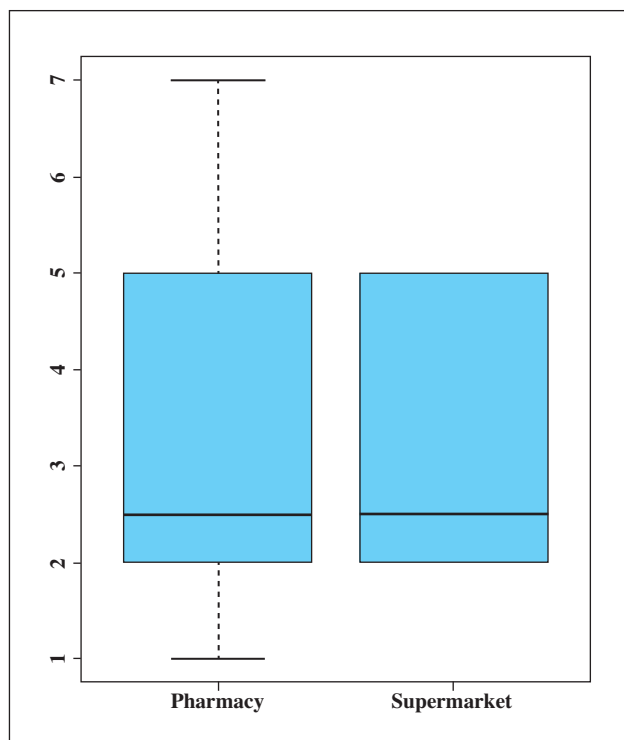


Figure 4. Box plot showing the number of surfactants in cleansing bases in shampoos for babies, according to sales channel.

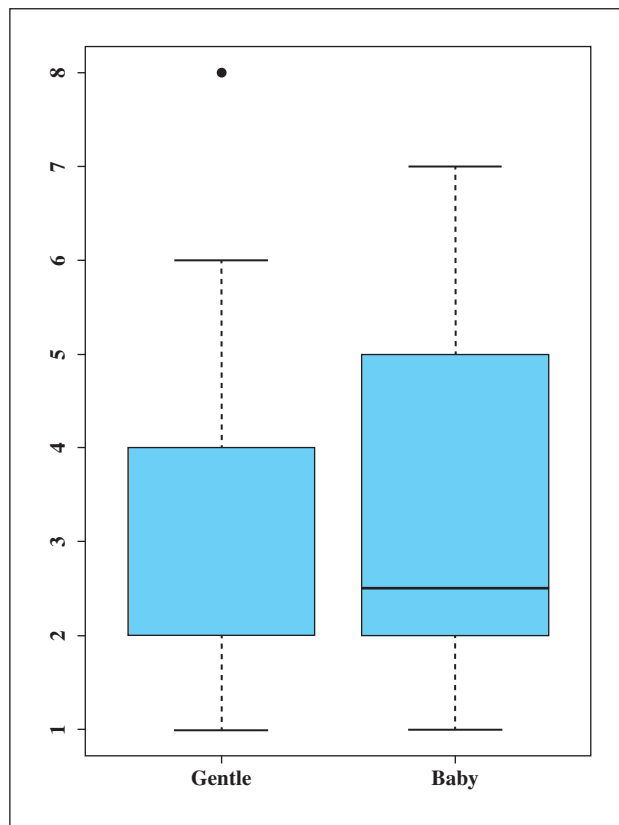


Figure 5. Box plot showing the number of surfactants in cleansing bases in “gentle” and baby shampoos.

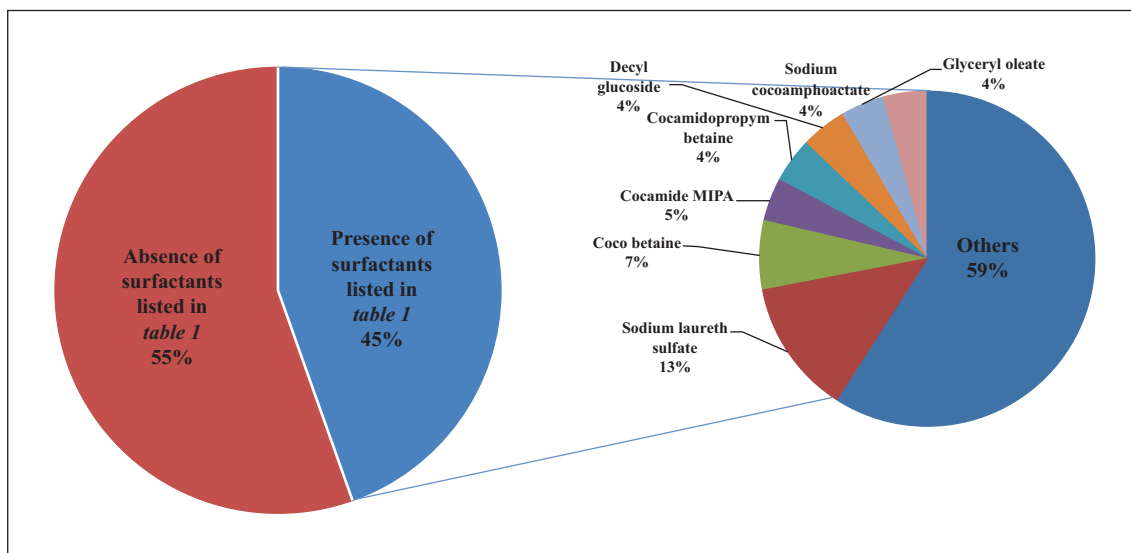


Figure 6. Analysis of the cleansing base in antidandruff shampoos sold in pharmacies.

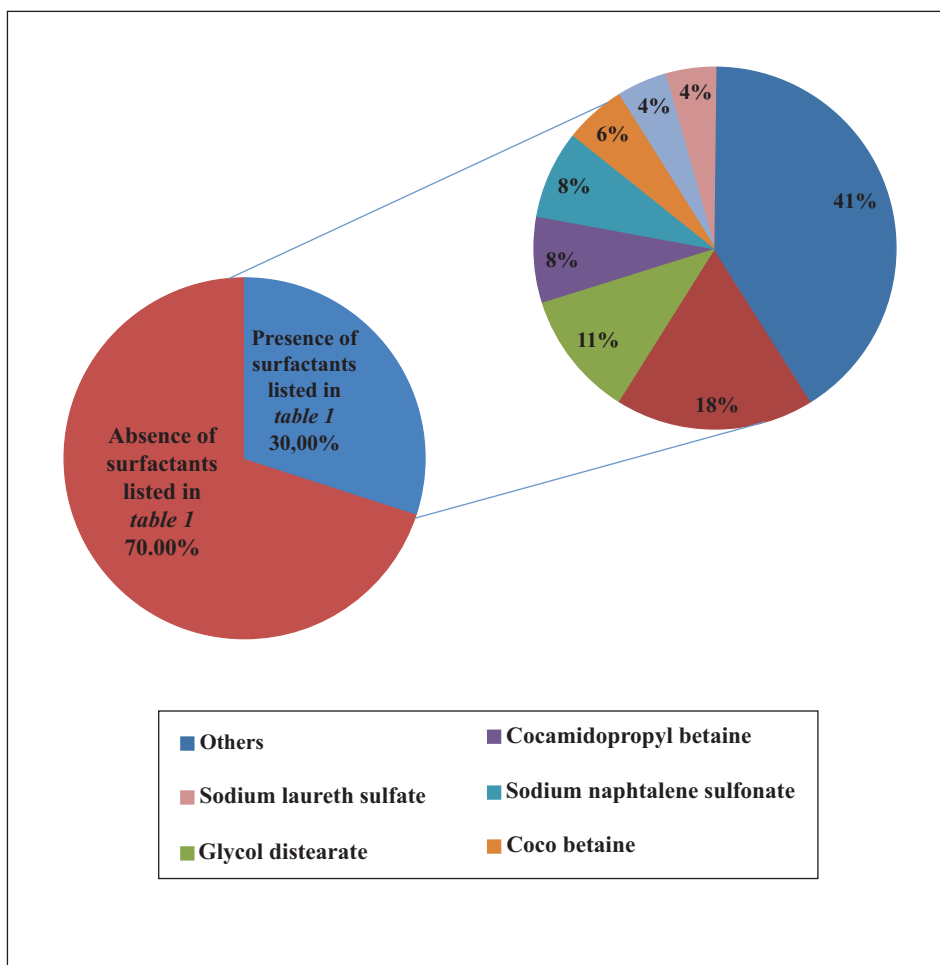


Figure 7. Analysis of the cleansing base in antidandruff shampoos sold in supermarkets.

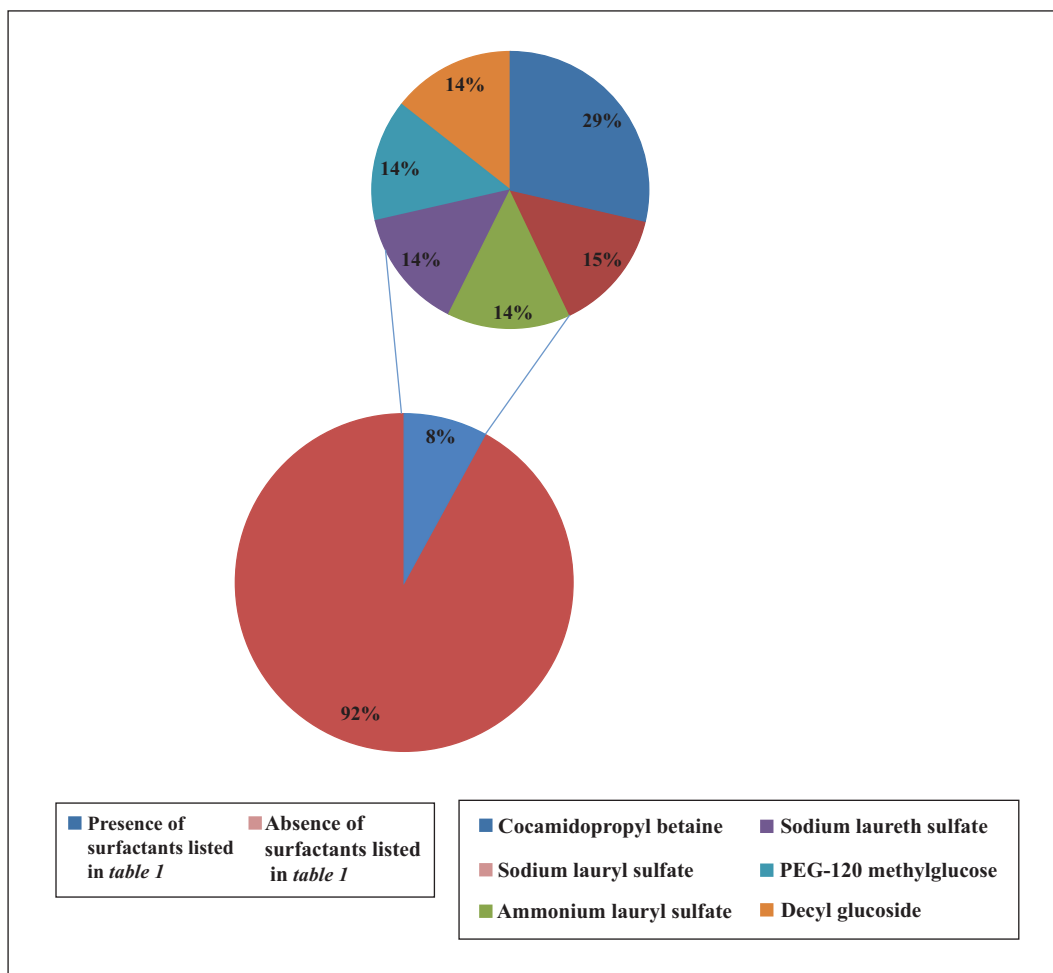


Figure 8. Analysis of the cleansing base in antidandruff shampoos obtained from mail-order retailers.

Table 3. Other allergens than those listed under EU Cosmetic Regulation 1223/2009.

Allergens	“Gentle” shampoos (pharmacy)	“Gentle” shampoos (mass-market)	Shampoos for babies (mass-market)	Anti-dandruff shampoos (pharmacy)	Anti-dandruff shampoos (mass-market)
Hydrolysed wheat or rice protein	3	-	1	-	2
DMDM hydantoin	-	-	-	2	2
Iodopropynyl butylcarbamate	3	-	-	-	-
Methylchloroisothiazolinone	1	1	1	-	1
Methylisothiazolinone	6	2	1	2	7

Discussion

With very few exceptions, shampoos are considered as cosmetics, moreover, from a regulatory point of view, the laws in force have only ever recognized shampoos as cosmetics. The concept of dermo-cosmetics was developed in both Europe and the United States for marketing purposes in the 1970s and has no regulatory basis [5]. There are only small differences in the cleansing base of the shampoos available through the different sales channels.

The main surfactants found are anionic surfactants including an alkyl ether sulphate: sodium lauryl ether sulphate. Although it was thought that this substance had completely disappeared, the amount of sodium or ammonium lauryl sulphate found was not negligible. In fact, four shampoos with no specific claim sold in retail stores contained these substances. More surprisingly, six “gentle” shampoos sold in pharmacies also contained these substances, as did four “gentle” shampoos of the 14 sold in retail stores. In contrast, lauryl sulphate is not present in baby shampoos sold in pharmacies or retail stores. This is worth noting insofar

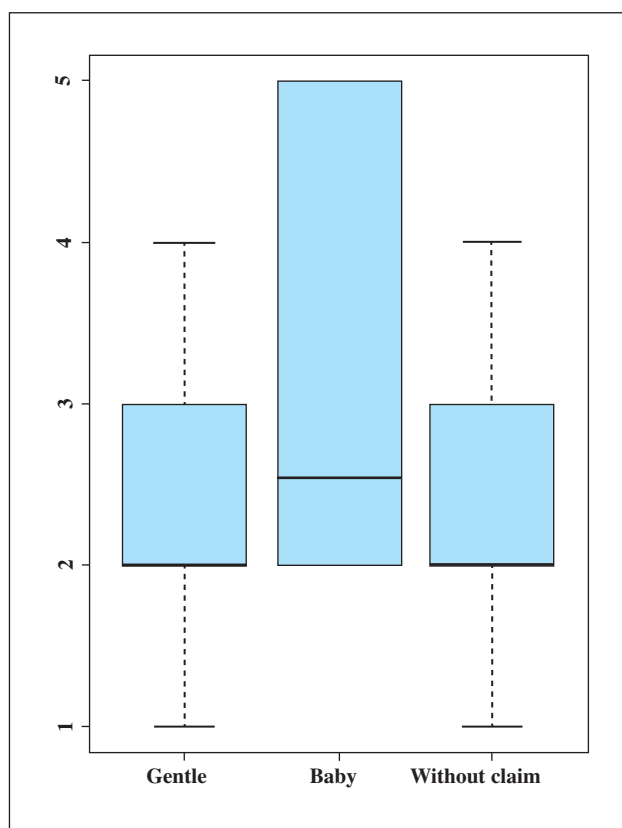


Figure 9. Box plot showing cleansing base composition in “gentle” shampoos, shampoos for babies, and shampoos without any claim, sold in supermarkets.

as the particularly irritating effect of this surfactant is known [6-12]. As is common knowledge, the ethoxylation of this type of molecule leads to less irritation and therefore better tolerance by the scalp [8-13]. To overcome the irritating effect of these surfactants and formulate “gentle” shampoos, amphoteric and non-ionic surfactants are combined. Of the amphoteric surfactants, we found mostly betaine derivatives, such as coco-betaine, but especially cocamidopropyl betaine, a synthetic surfactant belonging to this family obtained from coconut oil and dimethylaminopropylamine. This is well tolerated, even though for a time it was suspected to be allergenic [14-16].

The true cause of the undesirable effects observed is due to the synthetic intermediate, dimethylaminopropylamine [17, 18].

The advantage of non-ionic surfactants is that they can be formulated within a wide range of pH values and do not cause much irritation, while still having a cleansing effect. The disadvantage is that they create only a small amount of foam. Alkanolamides were identified in the shampoos studied. These are surfactants, good foaming agents, and foam stabilizers. They also make it possible to control the viscosity of the shampoo [19]; cocamide MEA (*figure 15*) is an example.

When using any of the members of this family of surfactants, one should pay attention to the way in which nitrosamines are produced [20], with care during formulation, in this case avoiding the use of a *formaldehyde-releaser* as a preservative [21, 22].

Table 4. Allergens found in shampoos without any particular claim.

Allergens	Number of shampoos with identified allergen
From the list of 26 regulated substances of Regulation (EC) No 1223/2009	
Alpha-isomethyl ionone	3
Benzyl alcohol	13
Benzyl benzoate	1
Benzyl salicylate	8
Butylphenylmethylpropional	5
Citronellol	9
Coumarin	4
Geraniol	5
Hexylcinnamal	7
Limonene	15
Linalool	17
Other allergens	
Hydrolysed protein	2
Benzophenone-3	1
Benzophenone-4	2
Diazodinyl urea	1
Ethylhexyl methoxycinnamate	1
Iodopropynyl butylcarbamate	1
Methylchloroisothiazolinone	4
Methylisothiazolinone	5
2-bromo-2-nitropropane-1,3-diol	1

Among the non-ionic surfactants, we also identified sugar esters that are perfectly biodegradable. Decyl glucosides are derivatives of glucose and fatty alcohols with between eight and 16 carbon atoms (lauryl glucoside, coco glucoside, decyl glucoside, and caprylyl/capryl glucoside). We found decyl glucoside particularly in “gentle” shampoos sold in pharmacies (in five products). This surfactant is currently considered a contact allergen [23-26]. The real allergen could be an impurity as in the case of cocamidopropyl betaine.

Although the cleansing bases all appeared similar, we highlight a statistically significant difference in allergen content between shampoos sold in pharmacies and elsewhere. In any case, ingredients with sensitizing potential, other than the substances listed in Annex X, should be taken into account. In this regard, protein hydrolysates were found in so-called “gentle” shampoos sold in pharmacies and a baby shampoo sold in retail stores (*table 3*). The latter, used as conditioners, are known for their sensitizing properties and hence their sensitizing effect [27-31]. The same goes for certain preservatives such as DMDM hydantoin or isothiazolinones [32-34]. DMDM hydantoin is a formaldehyde-releaser, hence its sensitizing effect [32, 35, 36]. Isothiazolinones (*figure 16*) are a family of biocides known for their sensitizing properties since the late 1980s. Kathon® CG, for example, is a mixture of methylchloroisothiazolinone and methylisothiazolinone in a mass ratio of 3/1. Of the two molecules, methylchloroisothiazolinone is the most prominent sensitizer. It can be found in many products, especially cosmetics and detergents, but also in paints, glues, *etc.* [32, 35, 37-43]. After the virtual disappearance of methylisothiazolinone and methylchloroisothiazolinone in the 1990s, we

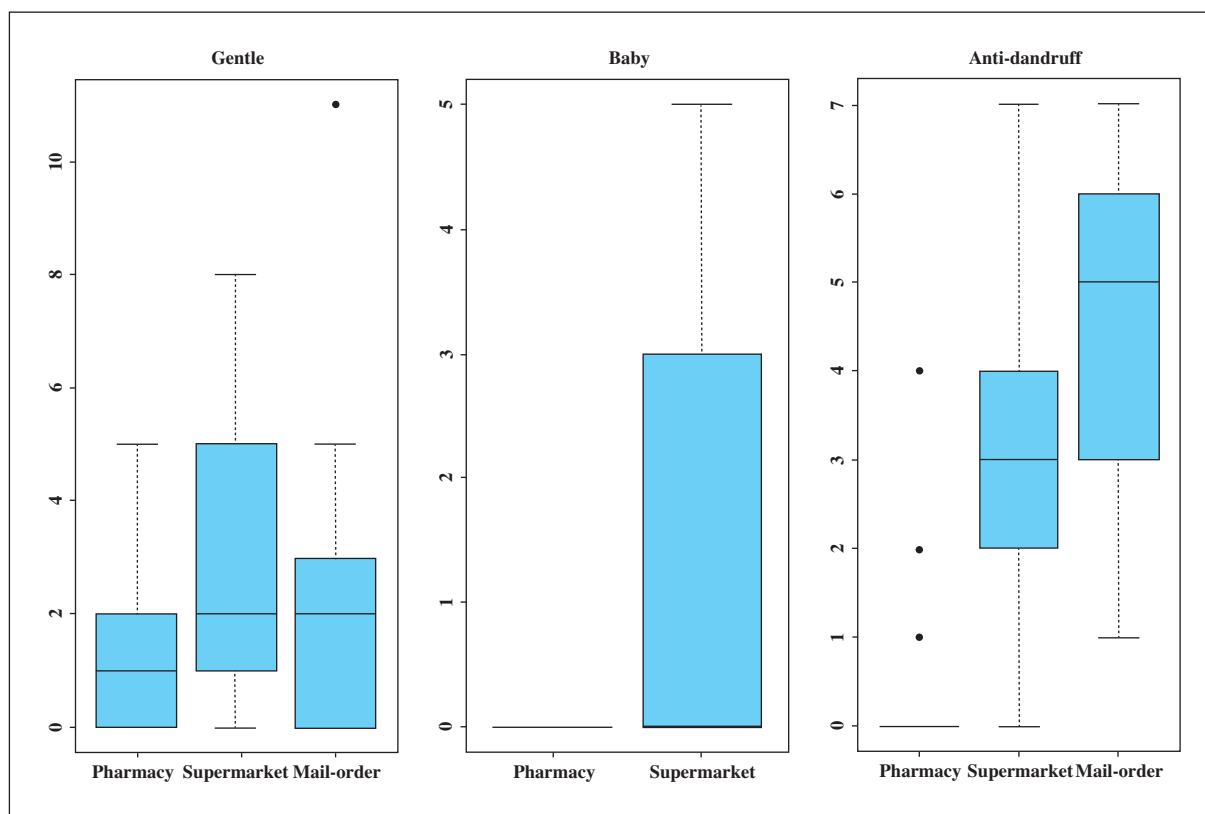


Figure 10. Box plots showing allergens found in “gentle” shampoos, shampoos for babies, and shampoos without any claim, according to sales channel.

Table 5. Preservatives found in shampoos for babies and anti-dandruff shampoos.

Preservatives	Shampoos for babies (pharmacy)	Shampoos for babies (mass-market)	Anti-dandruff shampoos (pharmacy)	Anti-dandruff shampoos (mass-market)
Benzalkonium chloride	1	-	-	-
Benzoic acid	10	2	3	2
Cetrimonium chloride	2	2	-	1
Dehydroacetic acid	4	-	-	1
Diazolidinyl urea	1	-	-	-
DMDM hydantoin	-	-	2	2
Ethylparaben	6	-	8	-
Formic acid	-	2	-	-
Iodopropynyl carbamate	-	-	3	-
Phenoxyethanol	10	2	3	4
Potassium sorbate	7	5	2	2
Methylchloro isothiazolinone	-	1	-	1
Methyl isothiazolinone	6	2	2	7
Methylparaben	3	-	4	1
Piroctone olamine	1	-	6	8
Propylparaben	2	-	4	1
Salicylic Acid	4	7	14	8
Sodium benzoate	18	13	8	13
Sodium salicylate	1	-	-	-
Sorbic acid	1	4	-	-
Zinc pyrithione	-	-	3	-

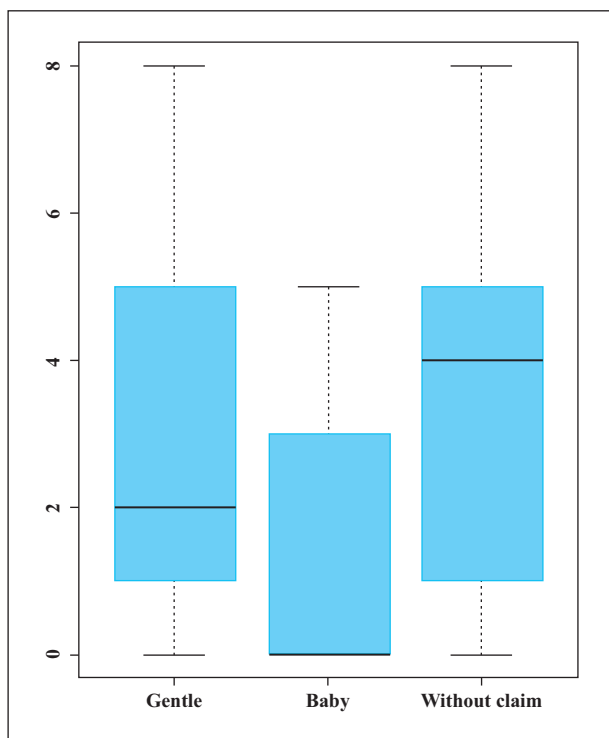


Figure 11. Box plot showing allergens found in “gentle” shampoos, shampoos for babies, and shampoos without any claim, sold in supermarkets.

discovered that they were again being used in cosmetics following the controversy around parabens and the media pressure that led to their removal from formulations [44].

Kathon® CG could even be described as an emerging allergen [45]. We found that methylisothiazolinone is present in many of the shampoos studied, even in those sold in pharmacies. The regulation of this preservative listed in Annex V has recently changed. Indeed, Annex V has been amended in accordance with Regulation (EU) No. 2016/1198 of 22 July 2016; methylisothiazolinone is now no longer permitted in leave-on products; it can, however, be used in rinse-off products, at a concentration of up to 0.01% [46]. This situation is regrettable to the extent that it is known that allergic reactions can occur when rinse-off products are used. Lastly, iodopropynyl butylcarbamate (*figure 17*) was found in three shampoos sold exclusively in pharmacies. Due to its fungicidal and bactericidal properties, it is used as a preservative in cosmetics and other industrial products, such as wood varnish [47, 48]. It was identified as a contact allergen about 30 years ago but is still used and found in cosmetics [47, 49-51].

Phenoxyethanol, sodium benzoate, and potassium sorbate were very common preservatives found in the shampoos studied (*tables 5, 6*) and are common in cosmetics in general, as reported by other authors [52]. Unlike those mentioned above, they do not pose any particular hazard. The real difference that we noticed between shampoos sold in pharmacies and those sold in retail stores is the number of allergens from the list of 26 under Regulation (EC) No. 1223/2009. However, there are substances not on that list that can cause allergies, as we have seen with preservatives. There are other ingredients that may be involved in causing eczema or contact urticaria. We also identified protein hydrolysates and UV filters, such as benzophenones (*tables 3, 4*).

The protein hydrolysates are mostly from wheat proteins. Of “gentle” shampoos, 28.5% sold in retail stores,

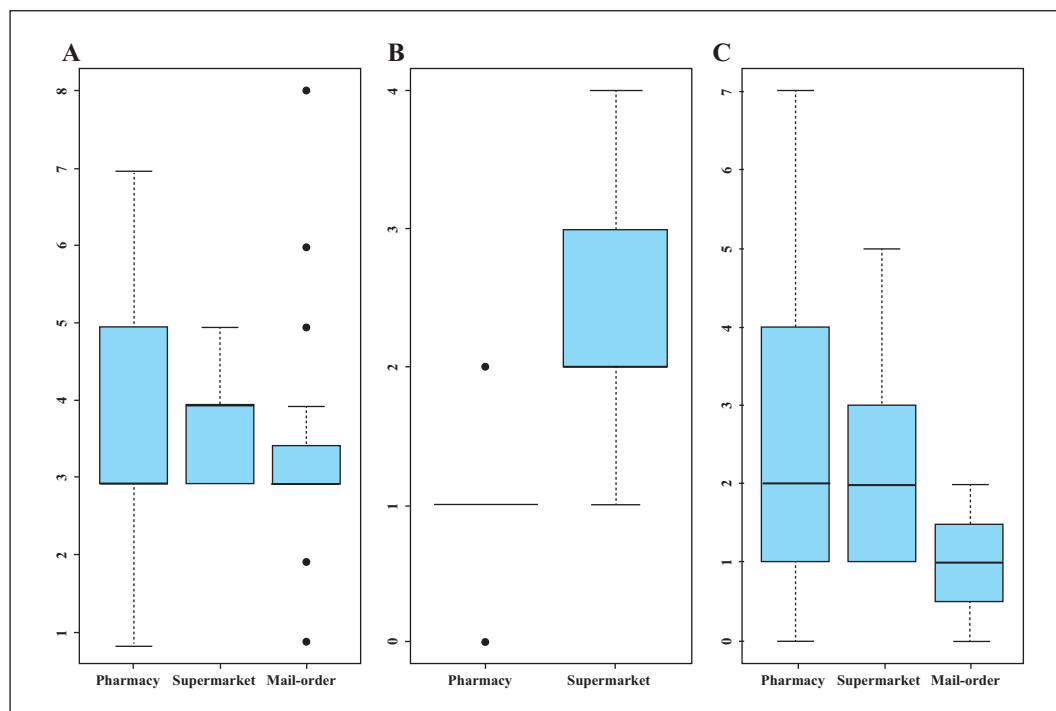


Figure 12. Box plots showing preservatives found in “gentle” shampoos (A), shampoos for babies (B), and anti-dandruff shampoos (C), according to sales channel.

compared to 18.5% of those sold in pharmacies, contained a protein hydrolysate. This goes to show that the term “gentle” does not refer to anything significant, neither in terms of the cleansing base nor allergenic properties. Indeed, the sensitizing potential of protein hydrolysates used as conditioners in shampoos has long been known [53-56]. Annex III of Regulation (EC) No. 1223/2009 has been amended in accordance with Regulation (EU) No. 2017/2228 of 4th December 2017, published in the Official Journal of the European Union on 5th December, to limit the average molecular weight of peptides in hydrolysed wheat protein in cosmetic products [57]. The benzophenones found in the products studied were benzophenone-4 and benzophenone-3 (oxybenzone). These molecules are clearly identified as causes of contact dermatitis, photocontact dermatitis, photocontact urticaria, and anaphylaxis [58-61]. Allergies resulting from the use of shampoos can therefore be due to various ingredients, even if they are primarily preservatives such as methylisothiazolinone alone or mixed with methylchloroisothiazolinone, formaldehyde releasers or iodopropynyl butylcarbamate which have been identified as the main culprits [62]. Moving on to anti-dandruff shampoos, we found that there were many more different anti-dandruff agents in shampoos sold in pharmacies. When dandruff is caused by the proliferation of yeasts of the *Malassezia* genus, it makes sense to use shampoos with fungicidal agents as part of the formulation [63-67]. Regardless of whether products were sold in pharmacies or retail stores, the most common active ingredients

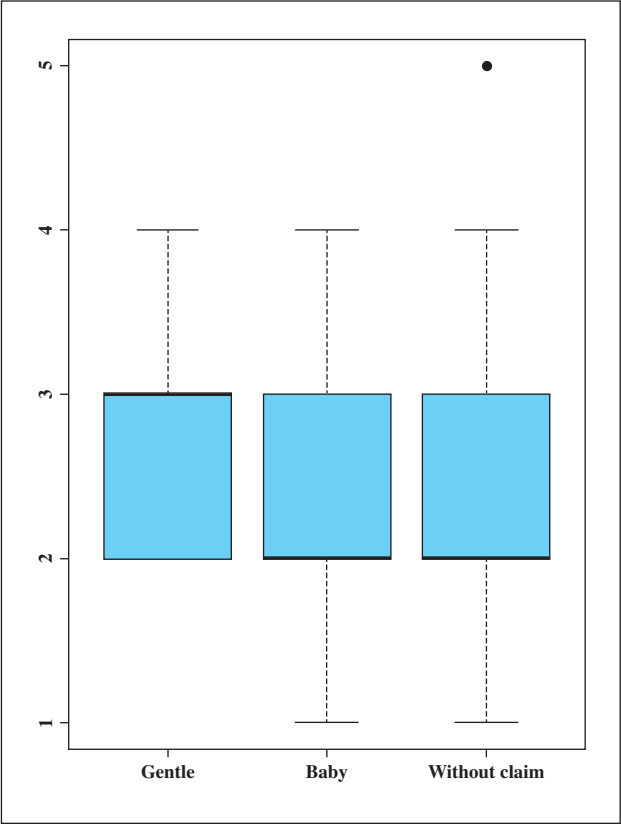


Figure 13. Box plots showing the number of preservatives in “gentle” shampoos, shampoos for babies, and shampoos without any claim sold in supermarkets.

Table 6. Preservatives found in shampoos without any particular claim.

Preservatives	Number of shampoos with identified preservative
Benzoic acid	2
2-Bromo-2-nitropropane-1,3-diol	1
Cetrimonium chloride	5
Dehydroacetic acid	3
DMDM hydantoin	-
Formic acid	4
Iodopropynyl carbamate	1
Phenoxyethanol	5
Potassium sorbate	10
Methylchloroisothiazolinone	4
Methylisothiazolinone	5
Methylparaben	1
Salicylic Acid	9
Sodium benzoate	25
Sodium salicylate	1
Sodium sulphite	1

were piroctone olamine, zinc pyrithione, and salicylic acid (table 7).

The greatest diversity of anti-dandruff active ingredients was found in shampoos sold in pharmacies. However, there were differences in terms of cutaneous tolerance. We will first focus on piroctone olamine and zinc pyrithione

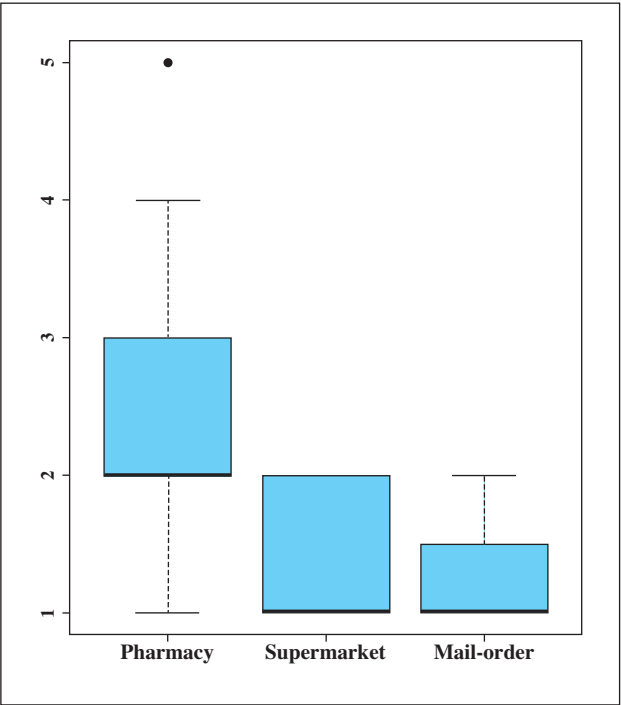
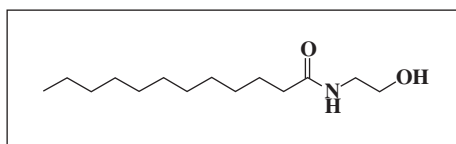
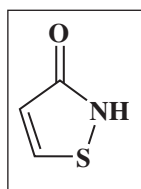
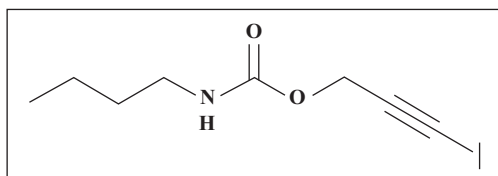


Figure 14. Box plots showing the number of active agents in anti-dandruff shampoos, according to sales channel.

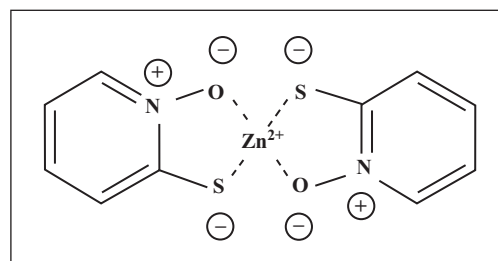
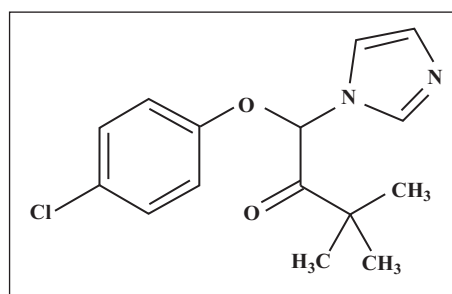
Table 7. Anti-dandruff agents found in shampoos.

Active ingredient	Anti-dandruff shampoos (pharmacy)	Anti-dandruff shampoos (mass-market)	Anti-dandruff shampoos (mail-order retailers)
Climbazole	2	1	-
Ciclopirox olamine	1	-	-
Juniperus oxycedrus extract	3	-	1
Miconazole nitrate	1	-	-
Myrtus communis extract	1	-	-
Piroctone olamine	6	8	2
Salicylic acid	14	8	1
Selenium sulphide	2	-	-
Serenoa serrulata extract	1	-	-
Sodium shale oil sulfonate	2	-	-
Tropaeolum majus extract	1	-	-
Undecylenic acid	1	-	-
Zinc pyrithione	3	8	-

**Figure 15.** Chemical formula of cocamide MEA.**Figure 16.** Chemical formula of isothiazolinone.**Figure 17.** Chemical formula of iodopropynyl butylcarbamate.

(figure 18), two molecules with antifungal properties widely used as anti-dandruff agents.

Zinc pyrithione, acting on the hair infundibulum, has a dual mode of action. It interferes with the proton pump of microorganism membranes and also exhibits its own cytotoxic effect as cell turnover is known to be greatly accelerated in the presence of dandruff [68-72]. A number of studies have reported increased efficacy when combined with climbazole, an antifungal azole also used to treat dandruff [73-76]. Its irritating effect and sensitizing potential [77-82] should encourage the use of other molecules, however, the alternative is not necessarily climbazole as this is an azole derivative (figure 19).

**Figure 18.** Chemical formula of zinc pyrithione.**Figure 19.** Chemical formula of climbazole.

At concentrations of up to 2% in shampoos, climbazole is able to significantly reduce *Malassezia* on the scalp of a person suffering from dandruff by inhibiting C-14 demethylase [83-85]. Its mode of action is similar to that of other azole antifungals. While it is neither mutagenic nor carcinogenic, it should be used frugally in cosmetics in order to prevent the development of resistance [86, 87]. Of the shampoos studied, only two sold in pharmacies and one sold in retail stores contained this product. We found very limited use of miconazole nitrate (one shampoo sold in pharmacies). At a concentration of 2%, it seems to be as effective as ketoconazole, used at the same concentration [88]. This is the active ingredient in Daktarin® and is not regulated at all for use in cosmetics, and one can only recommend the same caution as with climbazole.

On the other hand, piroctone olamine appears to be the most interesting molecule as it has been proven superior in terms of effectiveness and user safety [89-91].

Ichthyol, cade oil, and various plant extracts were also found in the shampoos studied that were sold in pharmacies. In the past, tars were used to treat squamous conditions, but coal tars were banned by Directive 2004/93/EC as their use in shampoos was found to be carcinogenic [90-97]. However, tars obtained from bituminous rock such as ichthyol and vegetable tars such as cade oil (that we rarely found in shampoos; see *table 8*) can still be used. Ichthyol or ichthyolammonium (INCI sodium shale oil sulfonate) is a sedimentary tar derived from the dry distillation of bituminous or shale rock, resulting from the decomposition of animal matter such as fish. The European Pharmacopoeia defines it as “*a tar of viscous consistency, of black-brown colour, with a characteristic pungent odour*”. There are very few studies on its composition. About 100 aromatic compounds including alkylbenzene, alkyl-naphthalene, and alkylanthracene have been identified [98]. The lack of information accounts for the sporadic use of this substance. The situation is fairly similar for cade oil and the limited data available are insufficient to determine its safety in cosmetics [99].

In contrast, the effectiveness of plant extracts has been more or less documented. The antimicrobial effect of nasturtium leaf extract is most likely attributable to the high levels of glucosinolate glucotropaeolin found [100]. There are no data on the possible adverse effects of shampoos containing this substance. Hence, our knowledge of the effect of nasturtium in shampoos for oily hair and in anti-dandruff shampoos is mainly due to practical experience rather than scientific experimentation. This is not the case for myrtle extract. There is a fair amount of documentation on the bioactive molecules found in this plant. 1,8-cineole (eucalyptol), α -pinene, myrtenyl acetate, limonene, linalool, and α -terpinolene are considered to be the compounds responsible for the biological activity observed, particularly antimicrobial activity [101-104]. One can easily assume that the risk of allergy must be considered [105]. The third most common extract found in one shampoo sold in pharmacies was *Sabal serrulata*. The only documented use of this extract is in cases of benign prostatic hyperplasia with hypothetical activity [105-108]. Selenium disulphide (SeS_2) should be studied separately. From a regulatory point of view, selenium is prohibited in cosmetic products, except for one of its salts, disulphide, which can be used at a concentration of up to 1% in anti-dandruff shampoos in accordance with Regulation (EC) No. 1223/2009. Higher concentrations can be found in shampoos which are considered medicinal. This is the case for the product called “Selsun” with a 2.5% concentration of selenium disulphide. We only found this in two shampoos sold in pharmacies under the brand name “Dercos”. Regarding effectiveness, a dose of 1% appears to be sufficient. However, we were encouraged by the fact that few products were shown to contain selenium disulphide in our study, given its toxic effect. This ingredient is likely to cause telogen effluvium and hair discolouration when used in shampoo. Moreover, it is probably carcinogenic [109-114].

To continue our study of active ingredients found in shampoos, we intend to investigate salicylic acid. Dandruff can be caused by two very different clinical conditions. In one, dandruff spontaneously falls from the scalp and this is referred

to as “pytirisias capitis simplex”; in the other, dandruff adheres to the scalp and this is referred to as “pytirisias capitis steatoides” [115]. Salicylic acid is the active ingredient of choice in these two cases due to its keratolytic, bacteriostatic, and fungicidal properties [116]. Indeed, we found salicylic acid in 14 shampoos sold in pharmacies and eight sold in retail stores. In Europe, this ingredient is regulated; it is one of the authorised preservatives at a maximum concentration of 0.5% and can also be used for other purposes in rinse-off hair products at a concentration of up to 3%. When concentrations of 20 to 30% are required for a peeling effect [117], its use at a dose of 3% and in combination with another active ingredient, such as ciclopirox olamine, has been shown to be of interest for the treatment of oily dandruff [118]. ■

References

1. Trüeb RM. Shampoos: ingredients, efficacy and adverse effects. *J Dtsch Dermatol Ges* 2007; 5: 356-65.
2. Couteau C, Coiffard L. *La formulation cosmétique à l'usage des professionnels et des amateurs*. Le Moniteur Ed. Pro-Officina coll, 2014.
3. Larpent C. *Tensioactifs*. Techniques de l'ingénieur, ref. K342 V1. 1995.
4. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.
5. Couteau C, Coiffard L. Les “dermocosmétiques” et les produits de soins et d'hygiène pour animaux, deux types de produits absents de la réglementation. *Médecine & Droit* 2017; 146-7: 131-5.
6. Agner T, Serup J. Sodium lauryl sulfate for irritant patch testing-a dose-response study using bioengineering methods for determination of skin irritation. *J Invest Dermatol* 1990; 95: 543-7.
7. Wilhelm KP, Surber C, Maibach HI. Effect of sodium lauryl sulfate-induced skin irritation on *in vitro* percutaneous absorption of four drugs. *J Invest Dermatol* 1990; 96: 963-7.
8. Wilhelm KP, Surber C, Maibach HI. Effect of sodium lauryl sulfate-induced skin irritation on *in vivo* percutaneous penetration of four drugs. *J Invest Dermatol* 1991; 97: 927-32.
9. Patil SM, Singh P, Maibach HI. Cumulative irritancy in man to sodium lauryl sulfate: the overlap phenomenon. *Int J Pharm* 1994; 110: 147-54.
10. Patterson SE, Williams JV, Marks JG. Prevention of sodium lauryl sulfate irritant contact dermatitis by Pro-Q aerosol foam skin protectant. *J Am Acad Dermatol* 1999; 40: 783-5.
11. Charbonnier V, Morrison BM, Paye M, Maibach HI. Subclinical, non-erythematous irritation with an open assay model (washing): sodium lauryl sulfate (SLS) versus sodium laureth sulfate (SLES). *Food Chem Toxicol* 2001; 39: 279-86.
12. Horita K, Horita D, Tomita H, Yasoshima M, Matsunaga K. Effects of different base agents on prediction of skin irritation by sodium lauryl sulfate using patch testing and repeated application test. *Toxicology* 2017; 382: 10-5.
13. Effendy I, Maibach HI. Detergent and skin irritation. *Clinics Dermatol* 1996; 14: 15-21.
14. Brand R, Delaney TA. Allergic contact dermatitis to cocamidopropylbetaine in hair shampoo. *Aus J Dermatol* 1998; 39: 121-2.
15. Jackson E. Cocamidopropyl betaine: new allergen or tempest in a tea pot? *Am J Contact Derm* 1999; 10: 109.
16. Oei HD. Eyelid dermatitis due to contact allergy to cocamidopropyl betaine. *J Allergy Clin Immunol* 2007; 119: S203.
17. McFadden JP, Ross JS, White IR, Basketter DA. Clinical allergy to cocamidopropyl betaine: reactivity to cocamidopropylamine and lack of reactivity to 3-dimethylaminopropylamine. *Contact Dermatitis* 2001; 45: 72-4.

18. Suuronen K, Pesonen M, Aalto-Korte K. Occupational contact allergy to cocamidopropyl betaine and its impurities. *Contact Dermatitis* 2012; 66: 286-92.
19. Klein K, Palefsky I. C.2: Shampoo Formulation. *Handbook for Cleaning/Decontamination of Surfaces 1*. 2007: 277-304.
20. Fan TY, Goff U, Song L, Fine DH, Arsenault GP, Biemann K. N-nitrosodiethanolamine in cosmetics, lotions and shampoos. *Food Cosmet Toxicol* 1977; 15: 423-30.
21. Dunnett PC, Telling GM. Study of the fate of Bronopol and the effects of antioxidants on N-nitrosamine formation in shampoos and skin creams. *Int J Cosmet Sci* 1984; 6: 241-7.
22. Fournier P. Biotransformation of dimethylnitrosamine. *J Toxicol Clin Exp* 1990; 10: 283-96.
23. Le Coz CJ, Meyer MT. Contact allergy to decyl glucoside in anti-septic after body piercing. *Contact dermatitis* 2003; 48: 279-80.
24. Gijbels D, Timmermans A, Serrano P, Verreycken E, Goossens A. Allergic contact dermatitis caused by alkyl glucosides. *Contact Dermatitis* 2014; 70: 175-82.
25. Grey KR, Hanson JL, Hagen SL, Hylwa SA, Warshaw EM. Epidemiology and co-reactivity of novel surfactant allergens: a double-blind randomized controlled study. *Dermatitis* 2016; 27: 348-54.
26. Loranger C, Alfalah M, Ferrier Le Bouedec MC, Sasseville D. Alkyl glucosides in contact dermatitis. *Dermatitis* 2017; 28: 5-13.
27. Varjonen E, Petman L, Makinen-Kiljunen S. Immediate contact allergy from hydrolyzed wheat in a cosmetic cream. *Allergy* 2000; 55: 294-6.
28. Pecquet C, Bayrou O, Vigan M, Raison N, Lauriere M. Hydrolysed wheat protein: a new allergen in cosmetics and food. *Contact Dermatitis* 2004; 50: 182-3.
29. Laurière M, Pecquet C, Bouchez-Mahiou I, et al. Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. *Contact Dermatitis* 2006; 54: 283-9.
30. Livideanu C, Giordano-Labadie F, Paul C. Contact dermatitis to hydrolyzed wheat protein. *Contact Dermatitis* 2007; 57: 283-4.
31. Airaksinen L, Pallasaho P, Voutilainen R, Pesonen M. Occupational rhinitis, asthma, and contact urticaria caused by hydrolyzed wheat protein in hairdressers. *Ann Allergy Asthma Immunol* 2013; 111: 577-9.
32. de Groot AC, Bruynzeel DP, Bos JD, et al. The allergens in cosmetics. *Arch Dermatol* 1988; 124: 1525-9.
33. Travassos AR, Claes L, Boey L, Drieghe J, Goossens A. Non-fragrance allergens in specific cosmetic products. *Contact Dermatitis* 2011; 65: 276-85.
34. Kręcis B, Chomiczewska-Skóra D, Kieć-Świerczyńska M. Preservatives as important etiologic factors of allergic contact dermatitis. *Med Pr* 2015; 66: 327-32.
35. Uter W, Frosch PJ, & IVDK Study Group, the German Contact Dermatitis Research Group, DKG. Contact allergy from DMDM hydantoin, 1994-2000. *Contact Dermatitis* 2002; 47: 57-8.
36. Kireche M, Gimenez-Arnau E, Lepoittevin JP. Preservatives in cosmetics: reactivity of allergenic formaldehyde-releasers towards amino acids through breakdown products other than formaldehyde. *Contact Dermatitis* 2010; 63: 192-202.
37. Larsen WG. Methylchloroisothiazolinone-methylisothiazolinone (Kathon CG). *J Am Acad Dermatol* 1989; 20: 703.
38. Gonzalo-Garijo MA, Caballero ML, Gil-Micharet MS, Moneo I, Pérez-Calderón R, García-Borrue L. Hypersensitivity reactions due to nitrile gloves. *J Allergy Clin Immunol* 2012; 129: 562-4.
39. Latheef F, Wilkinson SM. Methylisothiazolinone outbreak in the European Union. *Curr Opin Allergy Clin Immunol* 2015; 15: 461-6.
40. Gonçalves M. Methylisothiazolinone in rinse-off products: additional fuel to the world epidemics of allergic contact dermatitis to isothiazolinones. *Br J Dermatol* 2015; 173: 11.
41. Leiva-Salinas M, Francés L, Silvestre JF. Update on allergic contact dermatitis due to methylchloroisothiazolinone/methylisothiazolinone and methylisothiazolinone. *Actas Dermo-Sifiliográficas* 2014; 105: 840-6.
42. Pirmez R, Fernandes AL, Melo MG. Photoaggravated contact dermatitis to Kathon CG (methylchloroisothiazolinone/methylisothiazolinone): a novel pattern of involvement in a growing epidemic? *Br J Dermatol* 2015; 173: 1343-4.
43. Puangpet P, Chawarung A, McFadden JP. Methylchloroisothiazolinone/methylisothiazolinone and methylisothiazolinone allergy. *Dermatitis* 2015; 26: 99-102.
44. Golden R, Gandy J. Comment on the publication by Darbre et al. *J Appl Toxicol* 2004; 24: 297-9.
45. Mowad CM. Methylchloroisothiazolinone revisited. *Am J Contact Dermat* 2000; 11: 115-8.
46. Commission Regulation (EU) 2016/1198 of 22 July 2016 amending Annex V to Regulation (EC) No. 1223/2009 of the European Parliament and of the Council on cosmetic products.
47. Bryld L, Agner T, Rastogi S, Menné T. Iodopropynyl butylcarbamate: a new contact allergen. *Contact Dermatitis* 1997; 36: 156-8.
48. Johnson W Jr.. Iodopropynyl butylcarbamate. *Int J Toxicol* 2017; 36: 37S-8S.
49. Aschenbeck KA, Warshaw EM. Allergenic ingredients in personal hygiene wet wipes. *Dermatitis* 2017; 28: 317-22.
50. Deza G, Giménez-Arnau AM. Allergic contact dermatitis in preservatives: current standing and future options. *Curr Opin Allergy Clin Immunol* 2017; 17: 263-8.
51. Kwon HJ, Kim JM, Oh WJ, Park KY, Kim MN, Seo SJ. Allergic contact dermatitis caused by a moisturizer containing iodopropynyl butylcarbamate. *Clin Exp Dermatol* 2018; 43: 62-3.
52. Pastor-Nieto MA, Alcántara-Nicolás F, Melgar-Molero V, Pérez-Mesonero R, de Eusebio-Murillo E. Preservatives in personal hygiene and cosmetic products, topical medications, and household cleaners in Spain. *Actas Dermo-Sifiliográficas* 2017; 108: 758-70.
53. Niinimäki A, Niinimäki M, Mäkinen-Kiljunen S, Hannuksela M. Contact urticaria from protein hydrolysates in hair conditioners. *Allergy* 1998; 53: 1078-82.
54. Olaiwan A, Pecquet C, Mathelier-Fusade P, Francès C. Contact urticaria induced by hydrolyzed wheat proteins in cosmetics. *Ann Dermatol Venereol* 2010; 137: 281-4.
55. Barrientos N, Vázquez S, Domínguez JD. Contact urticaria induced by hydrolyzed wheat protein in cosmetic cream. *Actas Dermosifiliogr* 2012; 103: 750-2.
56. Kobayashi T, Ito T, Kawakami H, et al. Eighteen cases of wheat allergy and wheat-dependent exercise-induced urticaria/anaphylaxis sensitized by hydrolyzed wheat protein in soap. *Int J Dermatol* 2015; 54: e302-5.
57. Regulation (EU) 2017/2228 of 4 December 2017 amending Annex III to Regulation (EC) No. 1223/2009 of the European Parliament and of the Council on cosmetic products.
58. Peluso AM, Bardazzi F, Tosti A. Photocontact dermatitis due to Eusolex 4360. *Contact Dermatitis* 1991; 25: 65-6.
59. Alanko K, Jolanki R, Estlander T, Kanerva L. Occupational allergic contact dermatitis from benzophenone-4 in hair-care products. *Contact Dermatitis* 2001; 44: 188.
60. Sasseville D, Nantel-Battista M, Molinari R. Multiple contact allergies to benzophenones. *Contact Dermatitis* 2011; 65: 179-81.
61. Heurung A, Raju SI, Warshaw EM. Benzophenones. *Dermatitis* 2014; 25: 3-10.
62. Zirwas M, Moennich J. Shampoos. *Dermatitis* 2009; 20: 106-10.
63. Schmidt A. *Malassezia furfur*: a fungus belonging to the physiological skin flora and its relevance in skin disorders. *Cutis* 1997; 59: 21-4.
64. Borda LJ, Wikramanayake TC. Seborrheic dermatitis and dandruff: a comprehensive review. *J Clin Invest Dermatol* 2015; 3: 2.
65. Sommer B, Overby DP, Kerr RG. Identification and characterization of lipases from *Malassezia restricta*, a causative agent of dandruff. *FEMS Yeast Res* 2015; 15: 7.
66. Gaitanis G, Velegriaki A, Mayser P, Bassukas ID. Skin diseases associated with *Malassezia* yeasts: facts and controversies. *Clin Dermatol* 2013; 31: 455-63.
67. Garrett NL, Singh B, Jones A, Moger J. Imaging microscopic distribution of antifungal agents in dandruff treatments with stimulated Raman scattering microscopy. *J Biomed Opt* 2017; 22: 66003.
68. Gibson WT, Chamberlain M, Parsons JF. The effect and mode of action of zinc pyrithione on cell growth. I. In vitro studies. *Food Chem Toxicol* 1985; 23: 93-102.
69. Priestley GC, Brown JC. Acute toxicity of zinc pyrithione to human skin cells in vitro. *Acta Derm Venereol* 1980; 60: 145-8.

70. Yasokawa D, Murata S, Iwahashi Y, *et al.* DNA microarray analysis suggests that zinc pyrithione causes iron starvation to the yeast *Saccharomyces cerevisiae*. *J Biosci Bioeng* 2010; 109: 479-86.
71. Schwartz JR, Rocchetta H, Asawanonda P, Luo F, Thomas JH. Does tachyphylaxis occur in long-term management of scalp seborrheic dermatitis with pyrithione zinc-based treatments? *Int J Dermatol* 2009; 48: 79-85.
72. Schwartz JR, Shah R, Krigbaum H, Sacha J, Vogt A, Blume-Peytavi U. New insights on dandruff/seborrheic dermatitis: the role of the scalp follicular infundibulum in effective treatment strategies. *Br J Dermatol* 2011; 165: 18-23.
73. Mills KJ, Hu P, Henry J, Tamura M, Tiesman JP, Xu J. Dandruff/seborrheic dermatitis is characterized by an inflammatory genomic signature and possible immune dysfunction: transcriptional analysis of the condition and treatment effects of zinc pyrithione. *Br J Dermatol* 2012; 166: 33-40.
74. Schwartz JR, Bacon RA, Shah R, Mizoguchi H, Tosti A. Therapeutic efficacy of anti-dandruff shampoos: a randomized clinical trial comparing products based on potentiated zinc pyrithione and zinc pyrithione/climbazole. *Int J Cosmet Sci* 2013; 35: 381.
75. Schwartz JR. Zinc pyrithione: a topical antimicrobial with complex pharmaceuticals. *J Drugs Dermatol* 2016; 15: 140-4.
76. Chen G, Ji C, Miao M, *et al.* Ex-vivo measurement of scalp follicular infundibulum delivery of zinc pyrithione and climbazole from an anti-dandruff shampoo. *J Pharm Biomed Anal* 2017; 143: 26-31.
77. Muston HL, Messenger AG, Byrne JP. Contact dermatitis from zinc pyrithione, an antidandruff agent. *Contact Dermatitis* 1979; 5: 276-7.
78. Yates VM, Finn OA. Contact allergic sensitivity to zinc pyrithione followed by the photosensitivity dermatitis and actinic reticuloid syndrome. *Contact Dermatitis* 1980; 6: 349-50.
79. Goh CL, Lim KB. Allergic contact dermatitis to zinc pyrithione. *Contact Dermatitis* 1984; 11: 120.
80. Nigam PK, Tyagi S, Saxena AK, Misra RS. Dermatitis from zinc pyrithione. *Contact Dermatitis* 1988; 19: 219.
81. Pereira F, Fernandes C, Dias M, Lacerda MH. Allergic contact dermatitis from zinc pyrithione. *Contact Dermatitis* 1995; 33: 131.
82. Nielsen NH, Menne T. Allergic contact dermatitis caused by zinc pyrithione associated with pustular psoriasis. *Am J Contact Dermat* 1997; 8: 170-1.
83. Richter E, Wick A, Ternes TA, Coors A. Ecotoxicity of climbazole, a fungicide contained in antidandruff shampoo. *Environ Toxicol Chem* 2013; 32: 2816-25.
84. Pople JE, Moore AE, Talbot DC, Barrett KE, Jones DA, Lim FL. Climbazole increases expression of cornified envelope proteins in primary keratinocytes. *Int J Cosmet Sci* 2014; 36: 419-26.
85. Cavana P, Petit JY, Perrot S, *et al.* Efficacy of a 2% climbazole shampoo for reducing *Malassezia* population sizes on the skin of naturally infected dogs. *J Mycol Med* 2015; 25: 268-73.
86. Pérez-Rivera AA, Hu T, Aardema MJ, Nash JF. Evaluation of the genotoxicity of the imidazole antifungal climbazole: comparison to published results for other azole compounds. *Mutat Res* 2009; 672: 27-39.
87. Parker JE, Warrilow AGS, Price CL, Mullins JGL, Kelly DE, Kelly SL. Resistance to antifungals that target CYP51. *J Chem Biol* 2014; 7: 143-61.
88. Buechner SA. Multicenter, double-blind, parallel group study investigating the non-inferiority of efficacy and safety of a 2% miconazole nitrate shampoo in comparison with a 2% ketoconazole shampoo in the treatment of seborrheic dermatitis of the scalp. *J Dermatolog Treat* 2014; 25: 226-31.
89. Rivalland P, Coiffard L, Lelaure M, Roeck-Holtzhauer YD. Evaluation de l'activité antifongique de deux dérivés et test d'innocuité in vivo de shampooings à visée antipelliculaire ainsi formulés. *Int J Cosmet Sci* 1994; 16: 77-83.
90. Lodén M, Wessman C. The antidandruff efficacy of a shampoo containing piroctone olamine and salicylic acid in comparison to that of a zinc pyrithione shampoo. *Int J Cosmet Sci* 2000; 22: 285-9.
91. Schmidt-Rose T, Braren S, Fölster H, *et al.* Efficacy of a piroctone olamine/climbazole shampoo in comparison with a zinc pyrithione shampoo in subjects with moderate to severe dandruff. *Int J Cosmet Sci* 2011; 33: 276-82.
92. Olansky S. Whole coal tar shampoo: a therapeutic hair repair system. *Cutis* 1980; 25: 99-104.
93. Lowe NJ, Breeding JH, Wortzman MS. New coal tar extract and coal tar shampoos. Evaluation by epidermal cell DNA synthesis suppression assay. *Arch Dermatol* 1982; 118: 487-9.
94. Nenoff P, Hausteil UF, Fiedler A. The antifungal activity of a coal tar gel on *Malassezia furfur* in vitro. *Dermatology* 1995; 191: 311-4.
95. Faure P, Antognanelli C. Traitement du psoriasis par les goudrons: passé et présent. *Rev Hist Pharm* 1996; 84: 352-5.
96. Paghdal KV, Schwartz RA. Topical tar: back to the future. *J Am Acad Dermatol* 2009; 61: 294-302.
97. van Schooten FJ, Moonen EJ, Rhijnsburger E, van Agen B, Thijssen HH, Kleinjans JC. Dermal uptake of polycyclic aromatic hydrocarbons after hairwash with coal-tar shampoo. *Lancet* 1994; 344: 1505-6.
98. Koch J, Moser R, Demel J. Analyse des ammoniumbituminosulfonate ichthyol. *Arch pharm* 1985; 10: 198-206.
99. Johnson W Jr.. Final report on the safety assessment of *Juniperus communis* extract, *Juniperus oxycedrus* extract, *Juniperus oxycedrus* tar, *Juniperus phoenicea* extract, and *Juniperus virginiana* extract. *Int J Toxicol* 2001; 20: 41-56.
100. Kleinwächter M, Schnug E, Selmar D. The glucosinolate-myrosinase system in nasturtium (*Tropaeolum majus* L.): variability of biochemical parameters and screening for clones feasible for pharmaceutical utilization. *J Agric Food Chem* 2008; 56: 11165-70.
101. Gardeli C, Papageorgiou V, Mallouchos A, Theodosios K, Komaitis M. Essential oil composition of *Pistacia lentiscus* L and *Myrtus communis* L: evaluation of antioxidant capacity of methanolic extracts. *Food Chemistry* 2008; 107: 1120-30.
102. Wannes WA, Mhamdi B, Sriti J, Bettaieb I, Tounsi MS, Marzouk B. Fatty acid and glycerolipid changes during Tunisian myrtle (*Myrtus communis* var. *italiq*) fruit ripening. *J Food Biochem* 2011; 35: 177-94.
103. Cannas S, Molicotti P, Ruggeri M, *et al.* Antimycotic activity of *Myrtus communis* L. towards *Candida* spp. from isolates. *J Infect Dev Ctries* 2013; 7: 295-8.
104. Alipour G, Dashti S, Hosseinzadeh H. Review of pharmacological effects of *Myrtus communis* L. and its active constituents. *Phytother Res* 2014; 28: 1125-36.
105. Vilaplana J, Romaguera C. Allergic contact dermatitis due to eucalyptol in an anti-inflammatory cream. *Contact Dermatitis* 2000; 43: 118.
106. Plosker GL, Brogden RN. *Serenoa repens* (Permixon). A review of its pharmacology and therapeutic efficacy in benign prostatic hyperplasia. *Drugs Aging* 1996; 9: 379-95.
107. Koch E. Extracts from fruits of saw palmetto (*Sabal serrulata*) and roots of stinging nettle (*Urtica dioica*): viable alternatives in the medical treatment of benign prostatic hyperplasia and associated lower urinary tracts symptoms. *Planta Med* 2001; 67: 489-500.
108. Novara G, Giannarini G, Alcaraz A, *et al.* Efficacy and safety of hexanic lipidosterolic extract of *serenoa repens* (permixon) in the treatment of lower urinary tract symptoms due to benign prostatic hyperplasia: systematic review and meta-analysis of randomized controlled trials. *Eur Urol Focus* 2016; 2: 553-61.
109. Grover RW. Diffuse hair loss associated with selenium (Selsun) sulfide shampoo. *J Am Med Assoc* 1956; 160: 1397-8.
110. Cummins LM, Kimura ET. Safety evaluation of selenium sulfide antidandruff shampoos. *Toxicol Appl Pharmacol* 1971; 20: 89-96.
111. Danby FW, Maddin WS, Margesson LJ, Rosenthal D. A randomized, double-blind, placebo-controlled trial of ketoconazole 2% shampoo versus selenium sulfide 2.5% shampoo in the treatment of moderate to severe dandruff. *J Am Acad Dermatol* 1993; 29: 1008-12.
112. Moore FR, Urda GA, Krishna G, Theiss JC. Genotoxicity evaluation of selenium sulfide in vivo and in vivo/in vitro micronucleus and chromosome aberration assays. *Mutat Res* 1996; 367: 33-41.
113. National Toxicology Program. Selenium sulfide. *Rep Carcinog* 2011; 12: 376-7.
114. Gilbertson K, Jarrett R, Bayliss SJ, Berk DR. Scalp discoloration from selenium sulfide shampoo: a case series and review of the literature. *Pediatr Dermatol* 2012; 29: 84-8.
115. DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR, Dawson TL Jr.. Three etiologic facets of dandruff and

seborrheic dermatitis: *Malassezia* fungi, sebaceous lipids, and individual sensitivity. *J Invest Dermatol Symp Proc* 2005; 10: 295-7.

116. Madan RK, Levitt J. A review of toxicity from topical salicylic acid preparations. *J Am Acad Dermatol* 2014; 70: 788-92.

117. Arif T. Salicylic acid as a peeling agent: a comprehensive review. *Clin Cosmet Invest Dermatol* 2015; 8: 455-61.

118. Squire RA, Goode K. A randomised, single-blind, single-centre clinical trial to evaluate comparative clinical efficacy of shampoos containing ciclopirox olamine (1.5%) and salicylic acid (3%), or ketoconazole (2%, Nizoral) for the treatment of dandruff/seborrheic dermatitis. *J Dermatol Treat* 2002; 13: 51-60.