Retrospective study of the allergens analysis in cosmetic products following the nfen16274 standard and the eu 2017/1410 regulation

Abstract
The presence of allergens and dangerous compounds in cosmetic products is a current social problem. Indeed, it can cause various cutaneous and allergic reactions. Thus, the European standard NF EN 16274, a regulation on allergens in cosmetics, request from manufacturers to write the name of the 24 allergens on their labels when they exceed a certain threshold. However, currently, only 23 of these allergens should be labelled because of a new regulation published in August 2017, the EU 2017/1410, banned the use of atranol, chloroatranol and one allergen included in the previous standard, the lyral, in cosmetic products. Therefore, a retrospective study was carried out by the EXPERTOX laboratory on cosmetic products studied by gas chromatography and mass spectrometry between January 2015 and July 2017. Products in the study are fragrances, oils, soaps, creams and raw materials used in the manufacture of cosmetics. The results show the clear predominance of allergens in perfumes and soaps while no raw material contained allergens. Linalool and limonene are two of the most common allergens in products, while four allergens in the standard are completely absent from the products analyzed. Differences according to the origin of the product are also visualized: the fragrances coming from France have more allergens than those coming from Switzerland.

Keywords: cosmetics, retrospective study, allergens, nfen16274, standard, 2017/1410 regulation

Abbreviations: GC-MS, gas chromatography mass spectrometry; LOD, limit of detection; PCS, product containing the substance

Introduction
Cosmetics are believed to cause skin reactions in 15 to 20% of the population. These reactions can be either an irritant contact or an allergic contact that can lead to the appearance of eczema. The irritant contact can be caused by deodorants, aftershave or shampoos. In addition, the European Scientific Committee on Consumer Safety has indicated in a report that 1 to 3% of the European population suffers from allergic reactions resulting from the exposure of the skin to certain ingredients contained in cosmetic products, especially perfumes. Thus, since 2005, 26 substances known to be allergens must be compulsorily included on the labels of cosmetic products in order to inform consumers when those substances exceed a certain threshold in the final product. This threshold is 0.001% in a product without rinsing such as a cream and 0.01% in a product with rinsing such as a shampoo. Among these substances, 2 are natural extracts, 8 are exclusively of synthetic origins and finally, 16 can have either a natural or a synthetic origin. 56 other substances can also be characterized as being capable of causing an allergic reaction but are not necessarily indicated on the label by the producers.

In 2013, the European ban on animal testing in the cosmetics industry led to the introduction of alternative methods for the detection and the analysis of allergens. The “European Union Reference Laboratory for Alternatives to Animal Testing” has developed three skin sensitization methods that can reveal allergens. There are two in vitro tests: the Keratinosens and the human Cell Line Activation Test and one chemical test: the Direct Peptide Reactive Assay. Self-testers are also available and recommended by many manufacturers to highlight the presence of allergens. However, they have negative points such as a lack of harmony in the conditions of use and instructions between the different sellers. In August 2017, the European Commission introduced a new regulation: the regulation number 2017/1410. Lyral, atranol and chloroatranol have been banned from use in cosmetic products. Indeed, these are the substances that cause the most important number of allergic reactions. The measure will apply from August 23, 2019 and will be fully applicable from August 23, 2021.

The EXPERTOX laboratory uses the gas chromatography and mass spectrometry (GC-MS) for a full validation of the presence of allergens. Therefore, it is intended to apply the European and French standard NF EN 16274 and the EU 2017/1410 regulation. The first one has implemented a method of quantification and analysis of allergenic fragrances in cosmetic finished products or in the raw materials used to create them. Thus, a retrospective study, based on cosmetic products, was carried out between January 2015 and July 2017 in order to study the 24 different allergenic substances indicated in this standard and atranol and chloroatranol. The aim was to detect the presence and the distribution of allergens according to the different types of cosmetics’ products and to the geographical origin of the products.

Materials and methods
Standards, reagents and solvents
The selected compounds used in the present work are: Anise Alcohol, Amyl Cinnamal, Amyl cinnamyl Alcohol, α-Isomethylionone, Benzyl Alcohol, Benzyl Benzoate, Benzyl Cinnamate, Benzyl Salicylate,
Citrone, Cinamyl Alcohol, Cinamal, Citral, Coumarin, Eugenol, Farnesol, Geraniol, Hexylcinnamal, Hydroxycitronellal, Isoeugenol, Lyral®, Lilial®, Limonene, Linalool, Methyl-2-octynoate were acquired from Sigma-Aldrich. All fragrance standards have a known purity. The internal standard used is 1,4-dibromobenzene and was supplied by Sigma Aldrich. The solvent, supplied by Servilab, is Methanol. All reagents are of analytical grade. The solvent is of GC-MS analysis grade. For the quantification of atranol and chloroatranol, the samples were diluted to one-tenth in ethanol provided by servilab and analyzed by GC-MS.

The retrospective study of allergen analysis was carried out on 55 cosmetic products studied between January 2015 and July 2017 by the EXPERTOX laboratory. Among them, there are 25 fragrances (eau de toilette and eau de parfum), 13 soaps, 6 creams, 4 oils, 4 raw materials extracted from plants and used to create cosmetic products and 3 lotions. The 24 allergens indicated in the standard NF EN 16274 were analyzed for each product. Their concentrations were studied according to different parameters such as the type of product and their country of manufacture. Moreover, atranol and chloroatranol were studied in 18 raw materials in 2017 but were never detected. During the analysis of the cosmetic products, it was detected that 5 allergens (limonene, linalool, citral, geraniol and citronellol) were present in more than half of the products (Table 1). More than 80% of the studied products contained the first two allergens (81% of the products contained limonene and 89% contained linalool). It should be known that many essentials oils contain linalool and that linalool is present in many plants. Both are used in cosmetics especially for fragrances and soaps. The absorption of limonene by the respiratory route is more than 50% and may cause irritation of the upper aerodigestive tract. In contrast, dermal toxicity is low and is only caused by limonene metabolites when the compounds are oxidized. Citronellol is non-mutagenic and non-carcinogenic. For linalool, when it is applied to the skin as a fragrance ingredient, it is also non-carcinogenic. For humans, after a cutaneous application with massage, the linalool is present in the blood after 5 minutes. Nevertheless, the toxicity of this allergen is not severe. Thus, the linalool has a sensitizing power although the number of allergic reactions due to this allergen is low and it occurs only when the linalool is oxidized in contact with oxygen.

The other three allergens (citral, geraniol and citronellol) detected in more than half of the cosmetic products studied by EXPERTOX are mainly used for soaps and perfumes. These allergens are neither mutagenic nor carcinogenic. Essential oils, plants (for geraniol and citronellol) and some fruits (for geraniol) contain these allergens. Citral and citronellol can also be produced by chemical synthesis. The most common symptom caused by citral is a skin irritation and the inhalation can cause coughing. Nevertheless, citral is a weak skin sensitizer. For geraniol and citronellol, some studies have been carried out. A 5-year US study showed that geraniol is involved in 1.1% (8 out of 713 cases) of patients with contact dermatitis caused by cosmetics. This allergen is known to have a sensitizing effect and to cause contact allergies sometimes accompanied by a hyperpigmentation. According to another study conducted in vitro on human skin, it was shown that citronellol was rapidly absorbed in significant amounts by all layers of the skin. Thus, if the application to the skin is too high, it would cause moderate to severe skin irritation. Nevertheless, inhalation toxicity would be low. Citronellol would cause skin sensitization in 0.4% of individuals using cosmetics. In addition, 8 substances were detected in less than 10% of the products (Tables 1 & 2): cinamyl alcohol was detected in 9% of the studied products, isoeugenol in 2% of the products, farnesol in 7% and methyl 2-octynoate in 4% of the products. The 4 other substances (benzyl cinnamate, anise alcohol, amylcinnamyl alcohol and amyl cinamal) were never quantified in the carried out tests. These 4 substances totally absent from the cosmetics studied in the EXPERTOX laboratory have only a few studies concerning their toxicity. Nevertheless, they are neither mutagenic nor carcinogenic. Amylcinnamyl alcohol and amyl cinnamal do not exist in natural form and they are obtained by chemical synthesis. In very large quantities, amylcinnamyl alcohol can cause hives but remains a weak skin sensitizer. A study published in 1999 revealed that 7 out of 179 people suspected to be allergic to allergens had a positive reaction when 20% alpha amylcinnamyl alcohol was applied to them (even if it is a very high concentration).

Table 1 Table of the 55 allergens’ analysis in cosmetic products carried out between January 2015 and July 2017

| Allergens | Number of products containing the substance (PCS) | Average % of the substance for PCS | Lower value (in %) | Upper value (in %) | Cream (n=6) | Number of PCS | Average % substance for PCS | Upper value (in %) | Number of PCS | Average % of the substance for PCS | Upper value (in %) |
|-----------|-----------------------------------------------|----------------------------------|--------------------|--------------------|-------------|-------------|--------------------------|-------------------|-------------|----------------------------------|-------------------|
| Amyl Cinnamal | 0 | Nothing | <LOD | <LOD | 0 | Nothing | <LOD | 0 | Nothing | <LOD | 0 | Nothing | <LOD |
| Benzy1 Alcohol | 17 | 0.0813 | <LOD | 0.4438 | 2 | 0.0615 | 0.0672 | 9 | 0.1311 | 0.4438 |
| Cinamyl Alcohol | 5 | 0.0055 | <LOD | 0.1538 | 0 | Nothing | <LOD | 5 | 0.0065 | 0.0138 |
| Citral | 33 | 0.1555 | <LOD | 15.915 | 0 | Nothing | <LOD | 19 | 12.383 | 15.915 |
| Hydroxycitronellal | 12 | 0.2978 | <LOD | 14.278 | 0 | Nothing | <LOD | 8 | 0.432 | 14.278 |

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### Table continued...

| Allergens                        | Number of products containing the substance (PCS) | Average % of the substance for PCS | Lower value (in %) | Upper value (in %) | Cream (n=6) | Number of PCS | Average % of the substance for PCS | Upper value (in %) | Fragrance (fau de toilette & eau de parfumi) (n=25) | Number of PCS | Average % of the substance for PCS | Upper value (in %) |
|----------------------------------|-----------------------------------------------|-----------------------------------|--------------------|--------------------|--------------|----------------|-----------------------------------|--------------------|--------------------------------------|--------------|-----------------------------------|--------------------|
| Isoeugenol                      | 1                                             | 0.0014                            | <LOD               | 0.0014             |              |                |                                   |                    |                                      |              |                                   |                    |
| Annylemnarryl Alcohol           | 0                                             | Nothing                           | <LOD               | <LOD               |              |                |                                   |                    |                                      |              |                                   |                    |
| Benzyl Salicylate               | 13                                            | 0.3724                            | <LOD               | 40.671             | 3            | 0.0102         | 0.027               | 6                  | 0.7969         | 40.671         | 19                            | <LOD               |
| Cinnamal                        | 7                                             | 0.0583                            | <LOD               | 41.583             | 1            | 0.11           | 0.11                | 4                  | 44.634         | 0.1583         | 5                              | <LOD               |
| Coumarin                        | 25                                            | 0.1324                            | <LOD               | 2.49               | 1            | 0.073          | 0.073               | 11                 | 0.2766         | 2.49           | 5                              | <LOD               |
| Geraniol                        | 29                                            | 0.3115                            | <LOD               | 29.625             | 0            | Nothing        | <LOD               | 19                 | 0.4323         | 29.625         | 5                              | <LCD              |
| Lyra®                           | 10                                            | 0.4945                            | <LOD               | 29.498             | 0            | Nothing        | <LOD               | 5                  | 0.8119         | 29.498         | 4                              | <LOD               |
| Anise Alcohol                   | 0                                             | Nothing                           | <LOD               | <LOD               | 0            | Nothing        | <LOD               | 0                  | Nothing         | <LOD           | 0                              | <LOD               |
| Benzyl Cinnaminate              | 0                                             | Nothing                           | <LOD               | <LOD               | 0            | Nothing        | <LOD               | 0                  | Nothing         | <LOD           | 0                              | <LOD               |
| Fornesol                        | 4                                             | 0.0072                            | <LOD               | 126                | 0            | Nothing        | <LOD               | 4                  | 72             | 126            | 0                              |                    |
| Lilale                           | 19                                            | 0.7901                            | <LOD               | 81.667             | 2            | 0.0205         | 0.0316              | 6                  | 24.259         | 81.667         | 25                | <LOD               |
| Linalool                        | 49                                            | 0.6911                            | <LOD               | 60.874             | 5            | 0.0274         | 0.0649              | 25                 | 12.416         | 60.874         | 5                 | <LOD               |
| Benzyl Benzoate                 | 13                                            | 0.1                               | <LOD               | 0.9707             | 4            | 0.0089         | 0.026               | 5                  | 0.2127         | 0.9707         | 4                 | <LOD               |
| Citronellol                     | 29                                            | 0.1187                            | <LOD               | 10.853             | 1            | 0.0125         | 0.0125              | 16                 | 0.2016         | 10.853         | 2                 | <LOD               |
| Hexyl Cinnamal                  | 7                                             | 0.7533                            | <LOD               | 40.710             | 0            | Nothing        | <LOD               | 4                  | 11.918         | 40.710         | 1                 | <LOD               |
| Limonene                        | 45                                            | 10.828                           | <LOD               | 56.772             | 4            | 40.059         | 0.0169              | 23                 | 20.406         | 56.772         | 12                | <LOD               |
| Methyl-2-ocmoate                | 2                                             | 0.0766                            | <LOD               | 9.65               | 0            | Nothing        | <LOD               | 2                  | 0.0766         | 0.0965         | 12                | <LOD               |
| α-Isomethyliodonone             | 27                                            | 0.2246                            | <LOD               | 24.737             | 2            | 0.0653         | 0.0781              | 12                 | 0.4578         | 24.737         | 0                 |                    |

| Allergens                        | Oil (n=4)                         | Soap (n=13)                      | Lotion (n=3)       | Raw material - plant extract (n=4) |
|----------------------------------|----------------------------------|---------------------------------|--------------------|-----------------------------------|
|                                  | Number of PCS | Average % of the substance for PCS | Upper value in % | Number of PCS | Average % of the substance for PCS | Upper value in % | Number of PCS | Average % of the substance for PCS | Upper value in % | Number of PCS |
| Amyl Cinnamal                    | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               |                   |
| Benzyl Alcohol                   | 1               | 0.0006      | 0.0056          | 5               | 0.0176      | 0.0341          | 0               | Nothing     | < LOD           | 0               |                   |
| Cinnamyl Alcohol                 | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               |                   |
| Citral                           | 2               | 0.0242      | 0.0302          | 12              | 0.0458      | 0.1247          | 0               | Nothing     | < LOD           | 0               |                   |
| Hydroxycitronellial              | 0               | Nothing     | < LOD           | 4               | 0.0295      | 0.0643          | 0               | Nothing     | < LOD           | 0               |                   |
| Eugenol                          | 0               | Nothing     | < LOD           | 5               | 0.0075      | 0.036           | 0               | Nothing     | < LOD           | 0               |                   |
| Isoeugenol                       | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               |                   |
| Amylnylemnarryl Alcohol          | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               | Nothing     | < LOD           | 0               |                   |

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Retrospective study of the allergens analysis in cosmetic products following the nfen16274 standard and the eu 2017/1410 regulation

Allergens

| Allergens          | Oil (n=4) | Soap (n=13) | Lotion (n=3) | Raw material - plant extract (n=4) |
|--------------------|-----------|-------------|--------------|-----------------------------------|
|                    | Number of PCS | Average % of the substance for PCS | Upper value in % | Number of PCS | Average % of the substance for PCS | Upper value in % | Number of PCS | Average % of the substance for PCS | Upper value in % | Number of PCS |
| Benzy1 Salicylate   | 1         | 0,0023      | 0,0023       | 3       | 0,009       | 0,024       | 0     | Nothing | < LOD 0         | 0       |
| Cinnamal           | 0         | Nothing     | < LOD 0      | 2       | 0,0221      | 0,0344      | 0     | Nothing | < LOD 0         | 0       |
| Coumarin           | 1         | 0,0012      | 0,0012       | 12      | 0,0161      | 0,1466      | 0     | Nothing | < LOD 0         | 0       |
| Geraniol           | 0         | Nothing     | < LOD 0      | 10      | 0,0819      | 0,2471      | 0     | Nothing | < LOD 0         | 0       |
| Lyral®             | 1         | 0,0461      | 0,0461       | 4       | 0,2097      | 0,4712      | 0     | Nothing | < LOD 0         | 0       |
| Anise Alcohol      | 0         | Nothing     | < LOD 0      | 0       | Nothing     | < LOD 0     | 0     | Nothing | < LOD 0         | 0       |
| Benzy1 Cinnamate   | 0         | Nothing     | < LOD 0      | 0       | Nothing     | < LOD 0     | 0     | Nothing | < LOD 0         | 0       |
| Farnesol           | 0         | Nothing     | < LOD 0      | 0       | Nothing     | < LOD 0     | 0     | Nothing | < LOD 0         | 0       |
| Lilal 0            | 1         | 0,003       | 0,003        | 7       | 0,023       | 0,0849      | 3     | 0,0835 | 0,0975         | 0       |
| Linalool           | 3         | 0,0793      | 0,118        | 13      | 0,1862      | 0,3943      | 3     | 0,0998 | 0,012          | 0       |
| Benzy1 Benzoate    | 0         | Nothing     | < LOD 0      | 1       | 0,0004      | 0,0004      | 3     | 0,0667 | 0,0751         | 0       |
| Citronella         | 1         | 0,0011      | 0,0011       | 11      | 0,0185      | 0,0642      | 0     | Nothing | < LOD 0         | 0       |
| H exyl Cinnamil    | 1         | 0,0049      | 0,0049       | 2       | 0,2504      | 0,4943      | 0     | Nothing | < LOD 0         | 0       |
| Limonene           | 2         | 0,6097      | 0,7343       | 13      | 0,1195      | 0,4178      | 3     | 0,0233 | 0,0303         | 0       |
| Meth 1-2-oe noate  | 0         | Nothing     | < LOD 0      | 0       | Nothing     | < LOD 0     | 0     | Nothing | < LOD 0         | 0       |
| α-Isomethylionone  | 1         | 0,0229      | 0,0229       | 12      | 0,0348      | 0,1396      | 0     | Nothing | < LOD 0         | 0       |

Bold: The 5 allergens present in more than half of the products; Italic: The 8 allergens present in less than 10% of the products; Underlined: Lyral®

Table 2 Summary table of the 55 analysis performed

| Product         | Number of products studied (n=) | Number of standard allergens detected in products (1/24 allergens) | Total number of allergens detected | Number of allergens per product | Total % of allergens per product |
|-----------------|---------------------------------|---------------------------------------------------------------|-----------------------------------|--------------------------------|---------------------------------|
| Cream           | 6                               | 27                                                            | 4,5                               | 0,12                           |                                 |
| Fragrance       | 25                              | 198                                                           | 7,92                              | 5,52                           |                                 |
| Oil             | 4                               | 11                                                            | 15                                | 575                            | 0,4                             |
| Soap            | 13                              | 116                                                           | 8,92                              | 0,61                           |                                 |
| Lotion          | 3                               | 12                                                            | 4                                 | 0,18                           |                                 |
| Raw Material    | 4                               | 0                                                             | 0                                 | 0                              |                                 |

Lyral® was detected in 10 products during the analysis (Table 1). However, since 2017, the European Regulation has banned this substance from cosmetics products because it is considered as an important sensitizer and can cause a high number of allergic reactions. Indeed, a study, published in 2004 and conducted in 20 dermatological departments in Germany, showed that 62 out of 3245 patients presented a positive allergic reaction following contact with a perfume with a lyral® concentration of 5%. Thus, manufacturers should remove the products containing this substance. It is important to notice that during the analysis, linalool was detected in all the perfumes (Table 1), all the soaps and all the lotions. Limonene has also been quantified in all soaps and lotions but in a lower proportion than linalool. Finally, all the lotions contained lilial and benzy1 benzoate as well. These two allergens can be obtained by a chemical synthesis and benzy1 benzoate also exists in natural form. They have perfuming properties and are neither mutagenic nor carcinogenic. Both are weak skin sensitizers.

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Nevertheless, only three lotions were studied, so these results are not representative. Among the 55 analysis, we can notice the clear predominance of the presence of different types of allergens in eau de parfum and eau de toilette, and in soaps compared to other products with respectively 20 out of 24 allergens for all fragrances and 16 out of 24 for all the soaps (Table 1). In contrast, no allergen was detected in the raw materials. However, when data are reported for one product in each category, soaps have the highest number of different allergens per product with an average of 8.9 substances for one product, one more than the fragrances (7.9 per product) and far ahead of creams (4.5), lotions (4) and oils (3.75). Moreover, the proportion of allergens present in the fragrances is higher than their proportion in the other products (Table 2). By adding the percentage of each allergenic substance (Table 1) by type of product, we can notice that the total proportion of allergen is 5.52% in the fragrances. That is 46 times more than in creams (where they represent 0.12% of the product), 30 times more than in lotions (0.18% of the product), 14 times more than in oils (0.40% of the product) and 9 times more than in the soaps (0.61% of the final product) per product (Figure 1). Moreover, the proportion of allergens in these final products is considerably higher in France with an average of 6.64% of allergens substances in the product, whereas this proportion is less than 1% in final products created in Switzerland (Figure 2).

**Conclusion**

Finally, a comparative analysis of eau de parfum and eau de toilette according to their geographical origin could be carried out. Thus, the study of allergens in fragrances produced in France (20 samples) compared to those produced in Switzerland (5 samples) indicated that the number of allergens per product was higher in France than in Switzerland with respectively 8.9 and 7 allergens. The results of this retrospective study of the analysis carried out between January 2015 and July 2017 have revealed a difference in the proportion of allergens in cosmetics according to product type. It has also been shown that 5 allergens were present in most of the products (citral, geraniol, linalool, citronellol, limonene) while others were completely absent from the products analysed. Moreover, lyral® was quantified in 10 of 55 products although it has been banned by a recent legislation. These allergens are skin sensitizers, at different levels depending on the allergen. They can cause cutaneous or allergic reactions, eczema, hives etc. when the consumers are using cosmetics products. This is why, as indicated by the European standard NF EN 16274, the 24 volatile allergens must be written on the labels when their concentration exceeds a certain threshold. In addition, following regulation number 2017/1410, it will be necessary to remove products containing lyral®, atranol and chloroatranol because it is banned from cosmetic products.

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None.

**Conflict of interest**

The author declares no conflict of interest.

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