Safety/Testing of Fragrances

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Fragrance materials have been a part of the skin's environment since before recorded history. The materials themselves change somewhat with the times, but many of the currently employed fragrance materials date from the third millenium B.C. and Biblical references abound.

Fragrances are ubiquitous. From the baby products to the embalming fluids and cosmetics used at death, humans are exposed to fragrance materials. In addition to cosmetics, fragrances are found in household and garden sprays, room fresheners, floor waxes and furniture polishes, insect repellents, bathroom cleansers, scouring powders, oven cleaners, shoe polish, incense, sachets, pomanders, and tobacco.

When one considers the long history of the use of fragrances, their broad distribution, and

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our length of exposure to them, one is impressed with the very few examples of injury to humans that can be attributed to these materials. The only problems reported have been occasional rashes on the skin and even more specifically, light-induced rashes.

Yet there is a persistent myth in the cosmetic industry that any problem encountered in the safety testing of a new cosmetic must be attributed to the fragrance component. Only a systematic screening of all the materials used in fragrances by an independent scientific body and the systematic and voluntary zealous response by the industry to eliminate potential offenders can dispel this myth.

The Research Institute for Fragrance Materials, Inc., an international nonprofit organization, was established in 1966 by the industry to carry out research on the many ingredients employed in perfumery. The sole purpose of RIFM is to assure the safety of perfumery raw materials.

The Board of Directors is composed of the chief executive officers of the member companies, elected at an annual meeting. In order to ensure an independent scientific status for the Institute, it is structured so that the only link between the administrative branches and the scientific arms is the president, who performs a dual role as scientist and administrator. The president has available the advice of a Scientific Committee composed of perfumers, research scientists, and analytical chemists drawn from the fragrance industry. Judgments in matters pertaining to the evaluation of safety are made completely independently by a Panel of Experts, who are toxicologists, pharmacologists, or dermatologists drawn from the academic world and have no connection whatever with the fragrance industry.

I. MATERIALS

Fragrance raw materials are derived from many geographical, natural, and chemical sources. Any program of safety evaluation has to consider the origin of the materials, types of uses, and the concentrations used.

First of all, some definitions are necessary because some of the terminology as used in the fragrance industry is different from that used elsewhere. Every industry has its own jargon.

Fragrance Material

1. An *essential oil* is produced by steam or water distillation of a plant or flower—or of an animal part or product. Example: anise oil.
2. A *concrete* is a solvent extracted product with the solvent subsequently removed—a waxy solid. Example: thyme concrete
3. An *absolute* is made by dispersing the concrete in alcohol followed by filtration and distillation so that only the alcohol-soluble portions of the concrete remain. Then the alcohol is driven off. Example: jasmine absolute
4. A *gum* is a natural exudation of a plant—usually acidic solids. Example: olibanum or frankincense
5. Something made in an organic chemistry plant is an *aroma chemical.* This constitutes the bulk of the raw materials used in perfumery. Example: cyclohexyl acetaldehyde

Fragrance Specialty

A combination by a perfumer, an artist, is a basic working tool. This may consist of 80 to 150 raw materials and is characterized as a specific company's woodsy, mossy, or floral, or oriental, or rose, or muguet, tabac, or leather fragrance specialty. Usually, the composition of these compounds is a closely guarded secret—arrived at only after years of creative expertise. They are rarely sold.

Fragrance Oil

A fragrance oil is a finished fragrance that might be made from various admixtures of these specialties to produce a desired fragrance. This would later be diluted with alcohol to produce a perfume, diluted more for a cologne or toilet water, and especially adapted for use in soaps, aerosols, etc. It would not be the same formulation for all these applications—to smell the same in each use, it has to be adapted for the particular vehicle.

An elegant perfume that one might purchase at a boutique is a very complex mixture of natural and synthetic materials and may easily contain as many as 600 raw materials, many of which are also components of flavors.

The Research Institute for Fragrance Materials is conducting its program only on the starting raw materials. These are carefully selected by the Scientific Committee.

The raw materials are selected on the basis of the following criteria:

- they must be representative of the material in actual use by the industry
- they must conform to the specifications and standards, if any, of the Fragrance Materials Association of the United States (FMA), the International Fragrance Association of Geneva (IFRA), or of the Prodarom Scientific Committee of Grasse, France

II. METHODS

The chief parameters selected for toxicological screening of all these materials include tests for allergenicity and photoirritation on human skin, tests for general toxicity in animals by the oral and dermal route, and other procedures where required by the Panel of Experts.

> "Facing up to the facts of real life, one has to establish priorities for safety evaluation in regard to the fragrance components being tested and the tests that are most necessary."[1]

When each raw material arrives at the Institute, a retain sample is taken and the rest is sent out to various commerical laboratories for testing. A sample in petrolatum is prepared by the Institute for repeated insult patch testing,[2] or maximization testing on human skin using, where feasible, a tenfold exaggeration of the maximum use level to which human skin could be exposed.[3,4]

Acute oral and acute dermal LD_{50}s are determined as a general measure of toxicity and, wherever pertinent, a test for photoirritation of human skin is included. The results of these preliminary data are reviewed by the Panel of Experts, who decide whether additional work is indicated.

In the course of testing these materials, it was found that the Kligman Maximization Test gave more uniformly reproducible and consistent results in the hands of two testing facilities than the repeated insult patch procedure. Consequently, this was chosen as the preferred test for potential allergenicity. In this procedure four materials are tested on each subject. It was learned that each of these materials had to be completely unrelated; that is, one cannot test two essential oils, two acetate esters, etc., in the same group.

Failure to recognize that cinnamic alcohol and hydroxycitronellal are both alcohols has frequently produced confusing results by people doing skin tests when both are tested simultaneously. Cinnamic alcohol contains impurities which are sensitizers; these cross react with the hydroxycitronellal and give a false positive reaction to the hydroxycitronellal often causing the investigator to classify each as a sensitizer quite incorrectly.

Table I

Group I	Group II
Citronella oil (Java)	Citronella oil (Ceylon)
Aldehyde C-11 undecylic	Aldehyde C-11 undecylenic
Allyl caproate	Allyl cyclohexyl propionate
Musk xylol	Musk tibetene

Table II. Concentration in Final Product (%)

	Soap	Detergent	Creams, Lotions	Perfume
Usual	0.01	0.002	0.005	0.09
Maximum	0.15	0.024	0.030	0.20

Typical test groupings might be those shown in Table I. All of these materials would be tested at a ten times highest use level. For example, if a material has the percent concentrations in final products as shown in Table II that can come into contact with human skin, it will be the highest one multiplied by ten, where feasible, that determines the level for maximization testing. In this case, 20%. This gives a considerable exaggeration of exposure. When tested in this fashion, the test becomes a pass or fail test, any positive result is taken to disqualify a material from further use.

Tests are also carried out for potential photoirritation to human skin by testing undiluted materials on the skin of hairless mice, swine, and humans by the procedures used by Forbes and Urbach, using natural sunlight and the solar ultraviolet simulator. [5]

Tests for photoallergy have been carried out by Dr. Kays Kaidbey using the photomaximization test.[6]

90-Day percutaneous toxicity tests have been carried out on selected materials of different chemical types to explore the possibility of systemic effects.

III. OBSERVATIONS

Among the potential allergens shown by the maximization or repeated insult patch test methods are the following:

Acetyl Isovaleryl
Alantroot Oil
Anisylidene Acetone
Benzylidene Acetone
p-t-Butyl Phenol
Carvone Oxide
Cassia Oil
Cinnamic Aldehyde
Cinnamic Aldehyde Methyl Anthranilate
Cinnamon Bark Oil Ceylon
Citral
Costus Oil
Cyclamen Alcohol
Diethyl Maleate
2,4-Dihydroxy-3-Methyl Benzaldehyde
Dihydrocoumarin
Dimethyl Citraconate
Ethyl Heptine Carbonate
Fig Leaf Absolute
Hexahydrocoumarin
Hydroabietyl Alcohol
Isoeugenol
6-Isopropyl Decalol
alpha-Methyl Anisalacetone
Methyl Crotonate
Methyl Heptine Carbonate
3-Methyl-2(3) Nonenenitrile
Pentylidene Cyclohexanone
Perilla Aldehyde
Peru Balsam
Phenylacetaldehyde
Propylidene Phthalide
Pseudo lonones
Verbena Oil

Among those items identified as potential photoirritants are the following:

Angelica Root Oil
Bergamot Expressed
Cumin Oil
Dimethyl Anthranilate
Fig Leaf Absolute
Lemon Oil CP
Limes Expressed
Orange Oil Bitter
Phantolid
Rue Oil
Verbena Oil

Among those items identified as photoallergens by the photomaximization test of Kaidbey & Kligman are the following:

4,6-Dimethyl-8-T-Butyl Coumarin
7 Methoxy Coumarin
6 Methyl Courmarin
7 Methyl Courmarin
4-Methyl-7-Ethoxycoumarin

One neurotoxic item has been discovered[7,8,9] which is Acetyl Ethyl Tetramethyl Tetralin (AETT).

In addition, one depigmenting allergen has been demonstrated: p-t-Butyl Phenol

One can readily see by reviewing these items that they occur among natural ingredients as well as chemical ones. The fact that an item occurs in nature is no guarantee of safety.

Monographs on the individual fragrance raw materials are being published as a regular feature of *Food and Cosmetics Toxicology.*[10]

IV. DISCUSSION

Testing for allergens is not a simple proposition. While animal methods have proved satisfactory in the hands of Klecak of Givaudan, Basle by the Open Epicutaneous Test (OET)[11] and Barbara James of Colworth House, Unilever, in general, these are far more sensitive than tests done on humans and based on them alone, many items would be disqualified from use by the industry. One would be reluctant on ethical grounds to use only the animal test and test only demonstrated sensitizers on humans.

Maximization testing is not without its difficulties, one of which we call the "spillover effect." While others have referred to this before[4,12] it becomes striking in its effect in the maximization procedure. In this test, four unrelated materials are tested on each of 25 human subjects. In the event one of the four test materials turns out to be an overwhelming sensitizer, false

Table III

Material and Concentration Tested	Subjects Sensitized
Costus oil, 4%	25/25
Jasmine absolute, 3%	2/25
Hydroxycitronellol, 10%	0/25
Amyris oil, high gravity, 10%	0/25

Table IV

Material and Concentration Tested	Subjects Sensitized
Phenylacetaldehyde, 2%	11/25
Phenylacetaldehyde dimethyl acetal, 2%	2/25
Butyl methyl hydrocinnamic aldehyde, 5%	9/25
Lemon oil, 10%	0/25

weak positive results may occur with the other three materials. When these three materials are subsequently retested out of the context of the serious allergen and in the same or different group of subjects, they prove to be negative. Table III lists one result. When the jasmine sample was retested out of the potent sensitizer (costus) context it was found to be negative.

In another instance, the four items listed in Table IV were tested together. When the phenylacetaldehyde dimethyl acetal and butyl methyl hydrocinnamic aldehyde were retested separately,they were unequivocally negative.

In another instance, one of the investigators doing maximization testing relocated his laboratories, going from a prison population of predominately male blacks to a university student population of mixed male and female—white, black, and Oriental individuals. The result was an increased susceptibility on the part of the new test subjects to the irritating effects of sodium lauryl sulfate (SLS) used as a pretreatment. Some false positive results were obtained which proved to be negative in repeated tests using lower concentrations of the SLS. Although considered insignificant, in the interest of completeness, these results are included in the pertinent monographs. This observation is explained in detail in a recent publication.[4]

V. UNEXPLAINED OBSERVATIONS (QUENCHING)

In the course of maximization testing, three instances have arisen in which a pure aldehyde, isolated from a natural source, has proved to be a strong sensitizer. Upon examining the essential oil from which it was isolated, the oil did *not* induce sensitization even though the aldehyde was present in concentrations as high as 85%. It appeared that something in the natural oil was inhibiting (quenching) the induction of sensitization. As a test of this hypothesis, several terpenes and alcohols found along with the aldehyde in the natural composition were combined with the aldehydes in question. It appears now to be a consistent finding that these three aldehydes, although potent sensitizers per se, do not sensitize in selective 1:1 mixtures.

There is no suggestion at this point that this is anything more than an unexplained observation. The implications are that there may be materials that abort the induction of the allergic condition in the human. Do these key materials combine with aldehydes to form a new compound? Do they work with *all* aldehydes? Do they inhibit the penetration of the allergen through human skin? All allergens? Do they compete for binding sites on the cells responsible for the sensitization manifestation? Do they prevent the formation of haptens?

Efforts have been made to explain these findings, which cannot, incidentally, be reproduced in the guinea pig.[13,14]

Certainly the fact that a material has been proved to have sensitization potential in any given system, human or guinea pig, should be followed up by carefully planned epidemiological studies using human subjects with contact dermatitis and paired cohorts with normal skin. The International Contact Dermatitis Research Group is a group of professional dermatologists doing such work on frequently encountered allergens in the environment.

It is of interest to note that both "natural" and "synthetic" materials are found among the allergens detected thus far.

Elimination of any single material from fragrances already made and sold, most of which have good safety records, is a considerable hardship to an industry. Certainly, these items can be eliminated from the palette of the artist in new creations. However, with an industry that has close to 5000 materials in its armamentarium, eliminating the few potential troublemakers is only good insurance.

The fact that the fragrance industry has been so relatively trouble free may in part be attributable to the fact that many fragrance materials are also used in flavors. In sensitizing guinea pigs to dinitrochlorobenzene (DNCB), it has been observed that the development of cutaneous reaction may be effectively aborted by the prior administration of the DNCB as a component of the animal's diet.

This has also been observed in the human. This phenomenon of immunosuppression has been extensively reviewed by Lowney.[15,16] Perhaps there would be more fragrance allergies if these same materials had not been used in flavors over the years. It is an interesting point for conjecture.

Also there is probably more quenching in the complex mixtures than is understood.

It is also of interest to note that several potent sensitizers, citral, cinnamic aldehyde, etc., as demonstrated by the human maximization tests have been shown to be quite harmless by the Soap and Detergent Association retrospective studies or when used in household products at quite low concentrations.[17-19]

In the testing of photoallergens by the photomaximization procedure several structure-activity relationships have been demonstrated in the substituted coumarins, that is, the coumarin molecule is in itself non-sensitizing and non-allergenic when tested as described above. However the substitution of a methyl group in the 6 position makes a potent photoallergen. Substituting in the 7 position makes a less potent one. Substituting a methoxy group makes a more potent photoallergen and also makes the material a sensitizer without ultraviolet light. Substitution of additional methyl or other groups around the molecule attenuate the photoallergenicity as does partial saturation of the molecule; for example, dihydrocoumarin and hexahydrocoumarin are not photoallergens but are potent allergens. Completing the saturation to form octahydrocoumarin produces a molecule as harmless as is the original coumarin.[20]

In the area of photoirritation there arises much confusion and controversy in view of the recent appearance of sun products intentionally containing components of bergamot oil, the removal of which from perfumes was so vigorously urged by Marzulli and Maibach eleven years ago.[21] This is further complicated by the recent observations that 5 MOP and 8 MOP may be photocarcinogenic.[22-24]

VI. CONCLUSION

The Research Institute for Fragrance Materials, Inc., using predictive methods, continues its ongoing program to identify potential sources of toxicity among the raw materials used by the industry. It continues to publish its reports in an impartial fashion in the well established, refereed, international journal, *Food and Cosmetics Toxicology*.

The North American Contact Dermatitis Group and the related International Contact Dermatitis Group will continue to do their diagnostic, epidemiological studies on humans suffering from contact dermatitis to determine the causes of the illness and to establish the frequency worldwide of reactions to various environmental factors.

Other groups such as the Soap & Detergent Association will continue to publish their findings of actual usage data.

The International Fragrance Association in Geneva will continue to publish in its Code of Practice what that group considers guidelines for safe usage of fragrance materials based on RIFM and other data.

The interrelationship of these various organizations is described in another publication.[25]

The fragrance industry enjoys a good reputation as a remarkably safe industry: the incidence of reported human injury problems is very low. With all of the groups mentioned above continuing as guardians of fragrance safety, albeit from differing points of view, the record can only improve in the future.

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