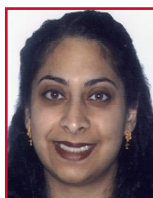


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Evaluating inhalation safety of fragrances

Research Institute for Fragrance Materials Respiratory Research Initiatives

ABSTRACT

Fragrances are intended to be enjoyed through the sense of smell. Therefore, one primary route of exposure to fragrance materials is inhalation. The Research Institute for Fragrance Materials (RIFM) has an active scientific program to verify the inhalation safety of fragrance materials. With a multi-faceted approach, RIFM has established projects to identify parameters of sensitization, estimate potential physiological fate and effects using a computational fluid dynamics model, and calculate air concentrations to determine potential inhalation exposure based on human ventilation. Within this comprehensive program, RIFM has also taken the initiative to develop alternative research methods. The Respiratory Sciences Program at RIFM is designed to further support the safe use of fragrances.

INTRODUCTION

In an era where consumers are increasingly concerned about asthma and inhalation allergies, it is important to understand the significant differences between natural fragrance materials and synthetic, or "nature-identical", fragrance materials used in personal care products and substantiate their lack of effects on sensitization and allergy development. Many studies over the last two decades have suggested various effects, both physiological and psychological, from inhalation exposure to fragrances and/or fragranced products (1-9). With specific attention to inhaled effects, the focus of this article is to present the research activities of the Respiratory Sciences Program at the Research Institute for Fragrance Materials, (RIFM), relating to inhalation sensitization, allergy, and asthma. The RIFM Respiratory Sciences Program is a rich research program that provides detailed information regarding the safe use of fragrances in diverse air care products.

ALLERGIES AND THE LUNG

Allergic reactions are first initiated by an exposure period with the allergen or antigen where the immune

system is triggered to respond to a subsequent exposure to the antigen. This is called the sensitization, or induction, phase and the subsequent challenge exposure is called the elicitation phase. Following sensitization, there are two main types of allergic reactions (Figure 1) involving the respiratory tract (10-12): Type I reactions and Type IV reactions. Type I reactions are characterized by a soluble antigen causing mast-cell activation. The primary immune reactant of Type I reactions is immunoglobulin E (IgE) and is associated with allergic rhinitis and acute asthmatic events. Type IV reactions are slightly more complex but are also characterized by the presence of a soluble antigen. Within the Type IV subgroup, two effector mechanisms may occur; Th1 or Th2. Th1 reactions are initiated by macrophage activation resulting in the release of Th1-specific chemokines, cytokines, and cytotoxins that lead to the development of contact dermatitis. Th2 type reactions are propagated by eosinophil activation that results in the release of Th2-specific inflammatory mediators and cytotoxins. It is the Th2 reaction that produces chronic asthma and/or chronic allergic rhinitis. Cytokine monitoring in *in vitro* and *in vivo* studies continue to illustrate the importance specific cytokines and chemokines exert over these reactions (10, 13-15). Although asthma and respiratory allergies are primarily governed by Th2 lymphocyte activity, recent data also suggest that Th1 lymphocytes may also be involved (12, 16).

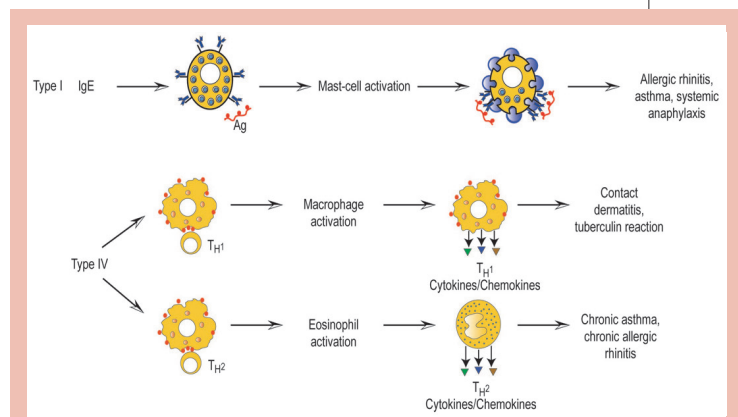


Figure 1. Type I and Type IV allergic responses. Adapted from Immunobiology by Charles Janeway, et al., 2001

The respiratory system presents a uniquely dynamic environment (Figure 2). The key regions of the airway are the upper airway, trachea, and alveoli. The upper airway is comprised of the oropharynx to the mainstem bronchi which bifurcate into the lower airways. The trachea contains ciliated columnar epithelium and, in conjunction with the production of mucus from the goblet cells, the cilia are part of a major defence mechanism of the lung known as the mucociliary escalator. In the trachea, the presence of intraepithelial lymphocytes is critical as a first response to antigens. The alveoli, and in particular the alveolar capillary-membrane, is integral to the gas exchange function of the lung and is the primary site of inflammatory activity due to its proximity to the systemic circulation. Noting the regions and the specific size of materials that can enter each region is important to our understanding of the fate and effects of environmental and occupational allergens and/or irritants (17, 18).

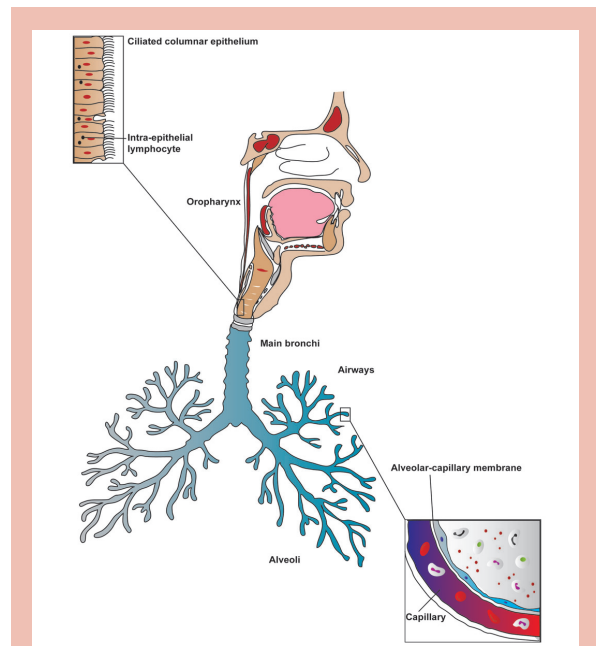


Figure 2. Schematic of the respiratory system. Adapted from Respiratory Medicine, An Illustrated Colour Text by Colin Selby, 2002

RIFM RESPIRATORY RESEARCH INITIATIVES

The fragrance industry has developed a multipath approach to verifying the inhalation safety of fragrance materials. Genetic variation plays a large role in the prevalence of allergy to fragrances, and other materials, in the population. Environmental factors may significantly affect certain genetic polymorphisms post-natally or modify genetics at conception; both of these

factors can contribute to the overwhelming inter-individual variability that is observed with regards to allergy prevalence (19). In order to address the issues and concerns described, RIFM initiated the Respiratory Sciences Program approximately 8 years ago. The objective of the program is to further elucidate the physiological effects of

inhalation exposure to fragrance materials in a consumer product, when that product is used as intended. The overall goal of this research program is to continue to ensure there are no adverse respiratory effects to fragrances in the consumer population and surrounding environments. There are various advisory committees that guide the development of the research programs at RIFM. For the Respiratory Sciences Program, the Respiratory Sciences Working Group (RSWG), comprised of toxicologists from the fragrance industry, consumer products industry, and members of RIFM, establishes research priorities based on consumer and industrial observations. The independent Expert Panel (REXPAN) provides scientific oversight for research conducted by RIFM; all the members of REXPAN are academic scientists and clinicians with no industry associations. For the Respiratory Sciences Program, an appointed adjunct of the Expert Panel, the Respiratory Clinical Advisory Panel (CAP), consists of three academic scientists representing the fields of pulmonology, physiology, and psychology. REXPAN is an important aspect in the development of the research programs at RIFM (20-22), and integral to the systematic review

of fragrance materials based on priorities established by structural groups; volume of use, potential exposure, and new safety information may also move the review of a particular material up the priority list and additional testing may be necessary to address specific concerns. While the RSWG provides internal review for the research conducted by the Respiratory Sciences Program, REXPAN, and its clinical adjunct advisory group, provide an independent external review.

Formal requests for proposals are posted to contract research organizations and universities currently working in the field of study. Proposals are received and competitive bids are extensively reviewed by REXPAN for expertise and research quality. All studies are monitored closely by RIFM staff scientists and data generated from all completed studies is submitted for publication in peer-reviewed journals; publishing in the open literature is a way to ensure a second external review process.

The first step in the Respiratory Sciences Program was to determine the fragrance product form that resulted in the greatest human exposure. Therefore, a series of studies was conducted to ascertain the maximum air concentration of selected fragrance raw materials in environmentally controlled chamber studies. 9 materials were selected based on volume of use and the ability to represent, not only a range of volatilities, but distinct chemical classes. These selected materials had also been previously characterized as either a skin or lung irritant or skin sensitizer.

Three different product forms were selected: a pressurized aerosol air freshener, a plug-in heated oil air freshener, and a fine fragrance (23). We now have data detailing total air concentrations of each material from each product type (24). Not surprisingly, the aerosol air freshener resulted in the greatest exposure. Subsequently, to verify the safety of fragrance exposure, the Respiratory Sciences Program embarked on a new type of study, the first of its kind in the fragrance industry: a clinical inhalation pilot study. Human subjects were exposed to the 9 fragrance materials used in the chamber studies. Subjects included non-

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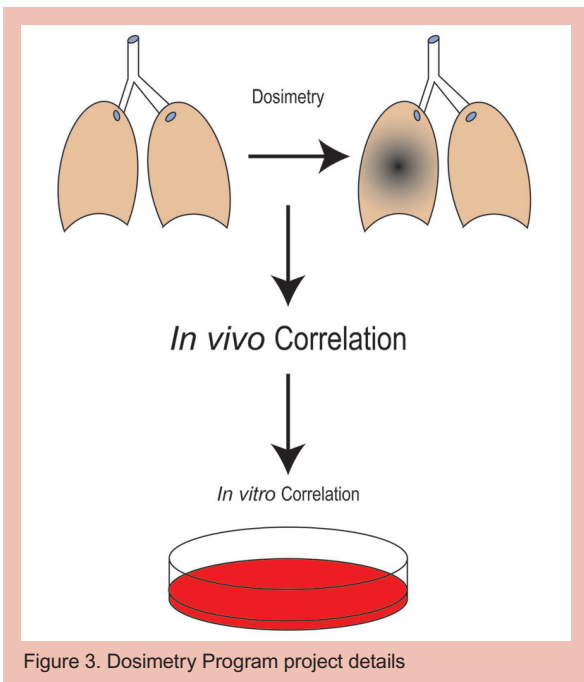


Figure 3. Dosimetry Program project details

asthmatic, mild asthmatic, and moderate asthmatic individuals. Each subject underwent 4 exposures with each subject serving as his/her own control. The specific endpoints studied were exhaled nitric oxide, forced expiratory volume (FEV₁), differential cell counts from induced sputum and nasal lavage samples, and a comprehensive questionnaire to gain insight to the perception of the fragrance mix and any subjective symptoms experienced by each subject. This study was completed in May 2008. Based on the data analysis (final report and publication pending) that has been done to date, no statistically significant differences were observed between non-asthmatics and mild asthmatics across all exposure scenarios. All FEV₁ (a gold standard measure of pulmonary function) (25, 26) values are within the expected range for adults and exhibit normal variability. Exhaled nitric oxide values (a marker of inflammation) all fall within published ranges (27). The conclusion of the study, thus far, is that no statistically significant changes have occurred in the mild asthmatic population that would suggest an effect due to the materials tested. Data from the moderate asthmatic population is currently being analyzed. The final report is expected to be published in a peer-reviewed journal later this year (28). Based on the completion of the chamber studies and the clinical pilot study, RIFM has established 5 main areas of research for the Respiratory Sciences Program. It should be noted that these areas of research are currently in the development process and will continually evolve.

1. **Data Mining:** RIFM is conducting a comprehensive review of all inhalation exposure literature in the RIFM database. Each study is assessed for type of study (chronic, subchronic, acute, developmental, metabolic, neurotoxicological, etc.), quality of data, and overall significance of the findings.
2. **Dosimetry Program:** This project is a three part program that will evaluate lung deposition and dosimetry profiles of selected materials, and assess potential physiological effects (Figure 3). The potential effects will be corroborated in a 2-week in vivo inhalation study, after which select parameters will also be evaluated in an in vitro system using cells representative of identified affected cell types. Histopathology, respiratory function, bronchoalveolar lavage fluid, differential cell counts, cytokine analysis and clinical chemistry will be evaluated in the in vivo

model. The *in vitro* phase will select primary cell types for study based on which cell type is located in the areas of greatest deposition (as determined by the deposition phase) and evaluate certain parameters that are determined by the *in vivo* phase; if a well-characterized cell line is available, these may be used in place of primary cells. Morphological changes in cell structure, cell viability, cytokine analysis, and certain neuropeptides (nasal epithelium only) will be studied. Calculation of cell numbers per surface area and known delivered concentrations of each material will provide dosimetry information that has been shown to correlate with *in vivo* data (29).

3. **Respiratory Sensitization Pilot Project:** There are no known fragrance materials that have been shown to be respiratory sensitizers. However, of particular relevance to today's topic, respiratory sensitization is an area of research under much debate. There are two main schools of thought: those who believe that primary dermal induction followed by subsequent respiratory challenge produces inhalation allergy and those who believe that primary respiratory sensitization is required prior to respiratory elicitation. The RSWG and CAP selected a proposal that will utilize a three-dimensional cellular co-culture in an air-liquid system known as a Cultex system (Figure 4) (30). Immortalized human Type II epithelial cells, primary human alveolar macrophages, and immature dendritic cells, derived from human monocytes, will be placed into culture as shown here. Materials can be delivered in aerosol, gas, or insoluble particulate forms at a set flow rate and concentration. Cross-talk between cell types can be studied without overt destruction of cellular structures (unlike the use of precision cut lung slices that would require tissue matrix destruction for the purposes of isolating specific cell types). From this system, cytokine profiles will be evaluated in the conditioned media collected, as well as from the cells themselves, using a specially designed protein array targeting distinction between irritation and sensitization profiles (31-33).
4. **Inhalation Exposure Assessment Modelling:** The purpose of this study is to determine, mathematically, potential air exposure, given specific values attributed to human breath rate (depth of

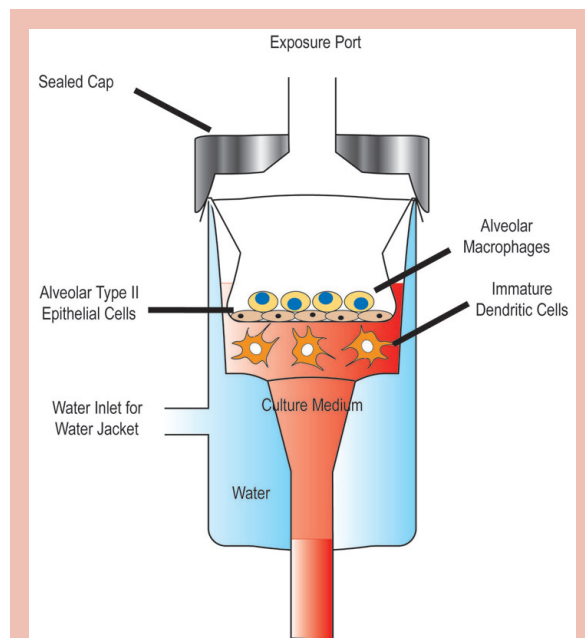


Figure 4. Respiratory sensitization triple co-culture design

ventilation, air exchange ratios, flow of material, product type, human activity during use, etc.) from a single material from multiple sources using a two box model system (Figure 5). This model is used extensively by the U.S. Environmental Protection Agency and other environmental and human health protection agencies in the United Kingdom and the Netherlands (1, 34). The goal of the systemic exposure quantitative risk assessment is to base accurate use limits for each material on potential cumulative consumer exposure by oral, dermal, and inhalation routes. The 3 product categories for the inhalation component are: aerosol air fresheners/fine fragrances, candles/incense, and heated oil plug-in air fresheners/reed diffusers.

5. *Occupational Exposure Scenarios*: The aim of this project is to determine the potential for occupational exposure, in industry factories, and develop a risk assessment strategy to address each scenario. This research will benefit the completion of REACH (Registration, Evaluation, Authorization, and Restriction of Chemical substances)- required information for chemical dossiers in the European Union.

CONCLUSION

Basic research in respiratory effects of fragranced products is necessary to support the continued safe use of fragrance materials. New advanced techniques and greater availability of resources are evident. Historical evidence has demonstrated safe use of fragrances in various types of products; however, we aim to provide strong scientific data with respect to any respiratory exposure. RIFM's Respiratory Sciences Program is poised to address fragrance safety for human health and the environment (Figure 6) and will actively pursue collaboration in the interest of sound science.

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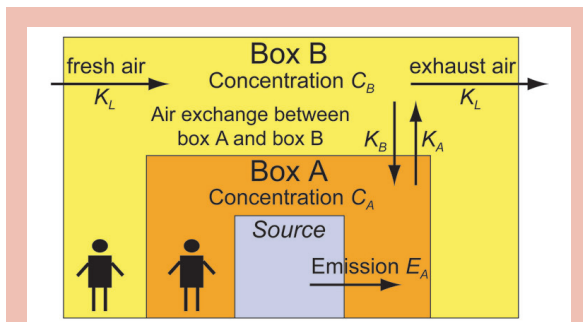


Figure 5. Two-box air exposure model diagram where K_L = air exchange rate of fresh air, K_A and K_B represent the air exchange rate between Box A and Box B, C_A and C_B = air concentration in Box A and Box B, and E_A represents the emission rate of the material of interest from its source

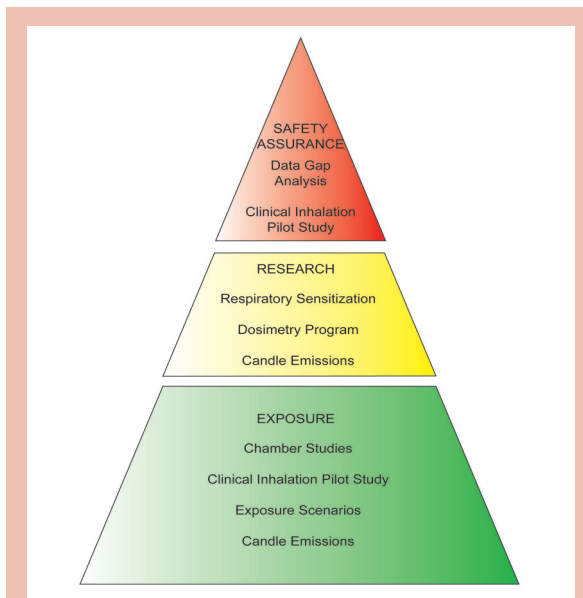


Figure 6. Current structure of the evolving RIFM Respiratory Sciences Program

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