

## ABSTRACT

Products containing fragrance materials, such as air fresheners, are often used by consumers to enhance their surroundings or to remove unpleasant odours. There are concerns expressed by consumers regarding the potential for fragrance materials to either cause or exacerbate asthma-like symptoms. We hypothesized that fragrance has negligible adverse health effects on asthmatic and non-asthmatic subjects. We present preliminary clinical data from a pilot study designed to study the possible effects of a surrogate air freshener on healthy/non-smoking non-asthmatic and mild asthmatic subjects. The aim was to measure pulmonary function, subjective symptoms, and cellular and sub-cellular changes occurring 15 and 30-minutes post-exposure to a non-fragranced Control Substance (CS) and a fragranced Test Substance (TS). Exposure to CS or TS was arranged in a stratified double-blind crossover design. A minimum two-week washout period was allowed between consecutive exposures. End-points included subjective symptoms, forced expiratory volume (FEV<sub>1</sub>), exhaled nitric oxide (eNO), and sputum cell counts as well as cytokine/chemokine analysis in induced sputum and nasal lavage. Time-course subjective symptom scores were recorded to measure symptom changes. The subjects were required to record symptom scores pre-exposure, every five minutes during exposure, and 1, 2, and 3 hours post-exposure. We have already tested twenty-four subjects - 12 non-asthmatics and 12 mild asthmatics. Analysis of our current data suggests little difference between CS and TS exposures for the 15 minute or 30 minute exposure times in both non-asthmatic and mild asthmatic subjects. This study is ongoing with further recruitment of 12 moderate asthmatics for the final phase of the study. All data will be compiled and analyzed for statistical significance upon collection of all data points. Data generated from this study will assist in the design of future studies to aimed at gaining better understanding of the specific physiological and potential psychological effects of inhaled fragrance materials.

## INTRODUCTION

Many consumer products used in homes contain fragrance materials, including cosmetics, toiletries, and household air care and laundry products. Although considerable information exists on the dermal effects of exposure to fragrance materials (De Groot *et al.*, 1997; Buckley *et al.*, 2000; Johansen, 2003; Bickers *et al.*, 2003), relatively little information exists on inhalation exposure to these substances and what does exist suggests that inhalation is a minor route of systemic exposure (Cadby *et al.*, 2002).

There is limited evidence that exposure to certain fragrance materials may induce allergic and asthma-like symptoms in sensitive individuals. It is noteworthy that in comparison to dermal effect data for fragrance materials, inhalation data are relatively sparse.

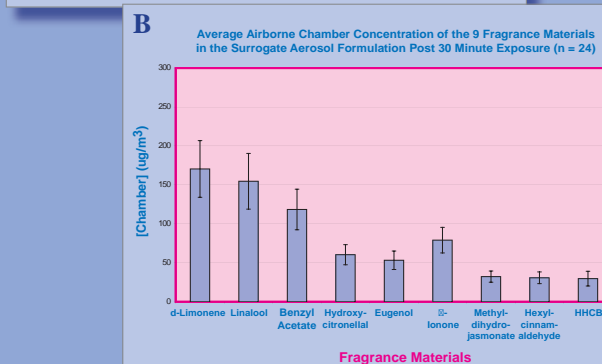
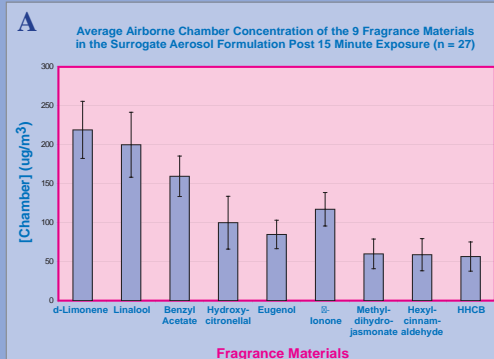
This study was designed: 1) to examine the effects of selected fragrance materials on healthy, non-smoking, non-asthmatic, mild and moderate asthmatic subjects with respect to the elicitation of cellular and sub-cellular changes following inhalation exposure; 2) to examine potential trends in changes of selected objective and subjective endpoints for each subgroup as defined by gender, asthma, and allergy status.

## MATERIAL and METHODS

### STUDY DESIGN

- N=12 each of non-asthmatics, mild and moderate asthmatics (total=36).
- Randomized, double blind cross-over; each person as own control.
- Each person received 15' and 30' exposures to Test and Control Substances (total = 4 exposures).

**FIGURE 1:** Airborne chamber concentrations from 15 min (A) and 30 min (B) of exposure of both non-asthmatic and mild asthmatic individuals; chamber concentration is independent of subject inside chamber



## VOLUNTEER SUBJECTS

- Healthy, non-smoking, non-asthmatic and asthmatic (mild/moderate) subjects in the age range of 18-70 yrs
- Eligibility criteria included current medical condition will be determined through evaluation of Toxcon's baseline, asthma, dermal and eye health questionnaires, pulmonary function testing, skin prick testing (SPT), dermal patch testing (see below), nasal exam, and odor discrimination/identification. All non-asthmatic subjects had a negative methacholine challenge while all mild asthmatic subjects had a positive methacholine challenge. Asthma definitions follow the guide lines set forth by the Global Initiative for Asthma (GINA) ([www.ginasthma.com](http://www.ginasthma.com)).
- Patch testing protocol, briefly, fragrance materials were mixed in a soft petrolatum, using the concentrations listed below, and applied to 8 mm Finn Chambers. The chambers were affixed to the right and left side of the back of each subject for 30-60 minutes (left) or 48 hours (right). Following removal of the chambers, the test area was rinsed with distilled water. Urticaria scoring was based on the International Contact Dermatitis Research Group (ICDRG) scoring system.

## OBJECTIVE MEASUREMENTS

- FEV<sub>1</sub> and eNO (pre- and immediate post-exposure).
- Induced sputum and nasal lavage at 3-hours post-exposure; differential cell counts, and cytokine profile pending analysis.

## SUBJECTIVE MEASUREMENTS

- Self-reporting and ranking of clinical symptoms and odor perception.

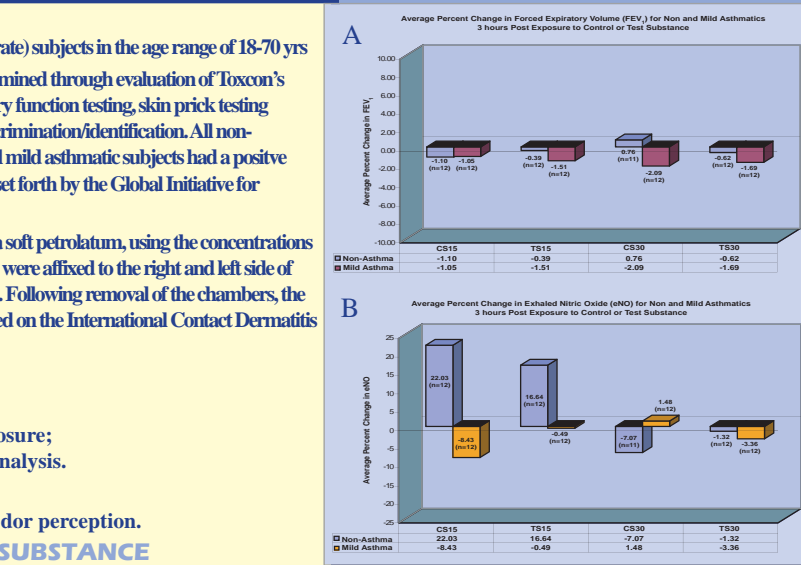
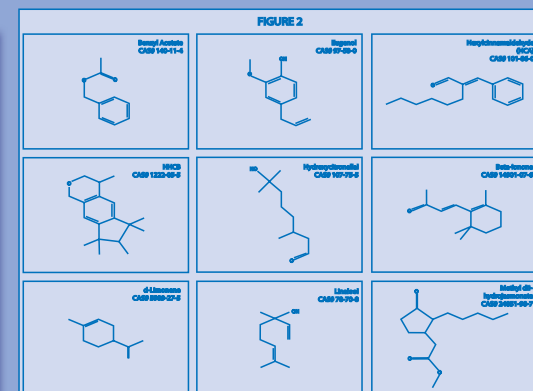
## PRESSURIZED AEROSOL AIR FRESHENER SUBSTANCE

- Preparation: Takasago International Corporation, NJ.
- Fragrance Sources: Coordinated by International Flavors and Fragrances, NJ.
- Formulation concentrations:

- ♦ Actives (Figure 2):
  - o 0.06% benzyl acetate, eugenol, HCA, HHCB, hydroxycitronellal, β-ionone, d-limonene, linalool, MDJ
  - o Purities of actives: 97.5 – 99.8%
- ♦ Other ingredients:
  - o 0.20% butylated hydroxyanisole (BHA) CAS # 25013-16-5)
  - o 0.50% food grade emulsifier, polyglycerol ester of oleic acid
  - o 29.00% hydrocarbon propellant (20% propane, 80% isobutene)
  - o 69.76% water
  - o Purities of other ingredients: >95%

## EXPOSURE CHAMBER

- Approximately 9.1 m<sup>3</sup>
- Temperature: 22 ± 2°C
- Relative humidity: 50 ± 10%
- Air changes per hour: 0.6 ± 0.1 ACH
- Air Change Rate was measured using SF6 Tracer Decay Method

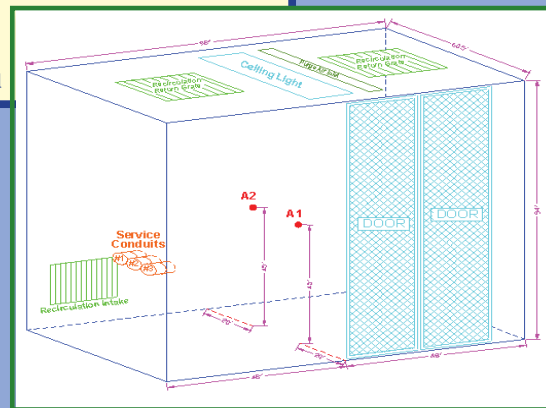


**TABLE 1:** Symptoms experienced by subjects upon exposure to either control or test substance, include, but are not limited to: Cough, Shortness of breath, Itching eyes, Watery eyes, Runny nose, Sneezing, Dry Throat, Skin rash, Headache, Dizziness, and Nausea.

|                       | Baseline |       | 5-Min |       | 10-Min |       | 15-Min |       | 1-hr  |       | 2-hr  |       | 3-hr  |       |
|-----------------------|----------|-------|-------|-------|--------|-------|--------|-------|-------|-------|-------|-------|-------|-------|
|                       | CS-15    | TS-15 | CS-15 | TS-15 | CS-15  | TS-15 | CS-15  | TS-15 | CS-15 | TS-15 | CS-15 | TS-15 | CS-15 | TS-15 |
| Non-Asthmatic (n=12)  | 4        | 4     | 4     | 5     | 4      | 4     | 4      | 4     | 4     | 3     | 2     | 2     | 3     | 2     |
| Mild Asthmatic (n=12) | 5        | 6     | 6     | 7     | 5      | 7     | 6      | 6     | 5     | 8     | 6     | 6     | 3     | 6     |

|                                   | Baseline |       | 5-Min |       | 10-Min |       | 15-Min |       | 20-Min |       | 25-Min |       | 30-Min |       | 1-hr  |       | 2-hr  |       | 3-hr  |       |   |
|-----------------------------------|----------|-------|-------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|-------|-------|-------|-------|-------|-------|---|
|                                   | CS-30    | TS-30 | CS-30 | TS-30 | CS-30  | TS-30 | CS-30  | TS-30 | CS-30  | TS-30 | CS-30  | TS-30 | CS-30  | TS-30 | CS-30 | TS-30 | CS-30 | TS-30 | CS-30 | TS-30 |   |
| Non-Asthmatic (n=12) <sup>a</sup> | 1        | 1     | 3     | 5     | 3      | 5     | 2      | 2     | 3      | 2     | 3      | 2     | 3      | 2     | 0     | 1     | 1     | 1     | 0     | 0     | 0 |
| Mild Asthmatic (n=12)             | 5        | 4     | 5     | 4     | 7      | 5     | 7      | 4     | 9      | 6     | 8      | 6     | 7      | 6     | 4     | 5     | 3     | 2     | 2     | 2     | 2 |

<sup>a</sup>n=11 for the Non-Asthmatic CS-30 category



**FIGURE 3:** Exposure Paradigm  
All equipment is pre-washed and wiped with isopropyl alcohol. The chamber is purged with HEPA/charcoal-filtered clean air. Once the door is closed, no mechanical ventilation occurs during the experiment.

## RESULTS

- Smell perception testing showed various levels of microsmia.
- Figure 1 shows average airborne concentrations (15' and 30') for non-asthmatic and mild asthmatic exposures and are comparable to those previously reported.
- % eNO (total) changes (Figure 4A) were variable, regardless of post exposure time, control substance and test substance.
- % FEV<sub>1</sub> changes (Figure 4B) did not show any differences between any of the groups for both post exposure time periods.
- Preliminary data shows that subjects in all groups showed various subjective symptoms (Table 1) and fragrance perceptions (Table 2).

## CONCLUSIONS

The preliminary results of the study are inconclusive with respect to selected respiratory endpoints (FEV<sub>1</sub> and eNO), pending completion of the moderate asthmatic population. Few subjects showed a decrease in FEV<sub>1</sub> post exposure to TS, that did not always occur in conjunction with an increase in eNO. Though the results show inconsistent changes in eNO, a more important analysis of cytokine/chemokine production in the induced sputum and nasal lavage samples is expected to reveal an allergy/asthma specific inflammatory profile. It is interesting to note that, while not statistically significant, more mild asthmatic individuals reported experiencing a variety of symptoms related to hyperresponsiveness upon exposure to the fragrance TS. However, the symptoms described appear to be unrelated to the subjects' perception of the fragrance test substance (pleasant vs. unpleasant). No significant adverse events were noted in either the non-asthmatic or mild asthmatic population.

## REFERENCES

- DeGroot, A.C. and Frosch, P.J. "Adverse Reaction to Fragrances: A Clinical Review." *Contact Dermatitis*, Vol. 36, No. 2, 1997, pp. 57-86.
- Buckley, D.A., Wakelin, S.H., Seed, P.T., Holloway, D., Rycroft, R.J.G., White, I.R., and McFadden, J.P. "The Frequency of Fragrance Allergy in a Patch-Test Population Over a 17-Year Period." *British Journal of Dermatology*, Vol. 142, 2000, pp. 279-83.
- Johansen, J.D. "Fragrance Contact Allergy: A Clinical Review." *American Journal of Dermatology*, Vol 4(11), 2003, pp. 789-98.
- Bickers, D., Calow, P., Greim, H., Hanifin, J.M., Rogers, A.E., Saurat, J.H., Sipes, I.G., Smith, R.L., Tagami, H. "A Toxicologic and Dermatologic Assessment of Linalool and Related Esters When Used as Fragrance Ingredients." *Food and Chemical Toxicology*, Vol. 41, 2003, pp. 919-42.
- Cadby, P.A., Troy, W.R., Middleton, J.D., and Vey, M.G.H. "Fragrances: Are They Safe?" *Flavour and Fragrance Journal*, Vol. 17, 2002, pp. 472-77.

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**TABLE 2:** Scent perception of either control or test substance by all subjects to date. Subjects were requested to respond to questions as described in Fragrance Perception Legend

| Fragrance Perception Legend   |  |   |        |
|---|--|---|--------|
| Participants were asked to answer the following questions by placing a mark on a 10cm line:               |  |   |        |
| P   | S  | C   |        |
| How pleasant is the fragrance in the room?  | How strong is the fragrance in the room? | How would you rate your level of comfort in the room? |        |
| Their score is recorded, from left to right, as a number in centimetres. The scores translate roughly as: |  |   |        |
| 0   | Extremely unpleasant/weak/uncomfortable  |   |        |
| 2.45  | Somewhat unpleasant/weak/uncomfortable   |   |        |
| 4.9   | Neither                                  |   |        |
| 7.65  | Somewhat pleasant/strong/comfortable     |   |        |
| 10.00   | Extremely pleasant/strong/comfortable    |   |        |
|   |  | Non-Asthmatic (n=12)                                  |        |
|   |  | Pleasant  | Strong |
| CS-15   | 5.14                                     | 4.09  | 6.16   |
| TS-15   | 5.29                                     | 5.29  | 5.88   |
| CS-30 <sup>a</sup>  | 4.98                                     | 3.91  | 5.63   |
| TS-30   | 5.40                                     | 4.79  | 5.98   |
| a n=11 only for the non-asthmatic CS-30 group   |  |   |        |
|   |  | Mild Asthmatic (n=12)                                 |        |
|   |  | Pleasant  | Strong |
| CS-15   | 5.17                                     | 4.08  | 6.58   |
| TS-15   | 6.04                                     | 4.92  | 6.84   |
| CS-30   | 5.51                                     | 4.73  | 6.30   |
| TS-30   | 6.29                                     | 5.40  | 6.27   |