

# Inhaled latex allergen (Hev b 1)

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**Background:** IgE-mediated responses to natural rubber latex allergens have become a major health problem among recognized risk groups.

**Objective:** The purpose of this investigation was to measure the amounts of *Hevea brasiliensis* latex allergen (Hev b 1) inhaled and deposited on surfaces when latex or vinyl gloves were worn and compare the results with the conventional measures (breathing zone samplers) of occupational exposure.

**Methods:** Hev b 1 exposure was measured by nasal sampling and breathing zone sampling. Latex allergen exposure was generated by having each subject don a pair of powdered latex examination gloves and continuing his or her normal daily activity for 30 minutes. By means of adhesive tape, surface dust samples were collected from the surfaces of gloves, the subject's hands, and work areas. Sampling was performed with subjects wearing no gloves, subjects wearing powdered vinyl gloves, subjects wearing powdered latex gloves, and nearby colleagues wearing latex gloves. All samples were assayed through use of the HALOgen assay (Inhalix, Sydney, Australia) with a Hev b 1-specific mAb. Particles transporting latex allergen were identified by a surrounding immunostain halo, and these were quantified and reported as total numbers of particles inhaled, airborne, or found on surface areas evaluated.

**Results:** Study subjects inhaled 26 times more allergen when powdered latex gloves were worn than under the "no glove" and powdered vinyl glove conditions. During the same period, Hev b 1 particle levels measured in the ambient air through use of the breathing zone sampler increased by 24-fold. The median numbers of particles carrying Hev b 1 allergen per square centimeter on the surface of the hands after the wearing of latex and vinyl gloves were 1964 and 5, respectively. Latex allergen was physically associated both with cornstarch granules and with larger dust particles having a darker, more irregular appearance.

**Conclusion:** In a laboratory where gloves are worn for protection, the use of latex gloves resulted in a 26-fold increase in inhaled latex allergen over background levels measured while vinyl gloves were worn as controls. Low levels of latex exposure also occurred when vinyl gloves or no gloves were worn; the reasons for this are under investigation. (*J Allergy Clin Immunol* 2002;109:701-6.)

**Key words:** Hev b 1, latex, inhaled allergen, occupational exposure, HALOgen assay, nasal air sampler

IgE-mediated responses to natural rubber latex (NRL) allergen are an important health problem, primarily among health care workers (HCWs), rubber workers, and patients who have undergone multiple surgical procedures. Recent studies have estimated the prevalence of latex sensitivity to be 2% to 17% in HCWs without symptoms.<sup>1-3</sup> Antibodies specific for *Hevea brasiliensis* group 1 allergen (Hev b 1) have been reported in 13% to 52% of latex-sensitized HCWs<sup>4-7</sup>; moreover, of sensitized HCWs, 54% have latex-related asthma.<sup>8</sup>

Sensitization to latex occurs from skin, mucosal, or wound contact or from inhalation of airborne latex allergen adsorbed to glove powder. A variety of investigations have shown that allergenic latex proteins are associated with the cornstarch particles that are used to lubricate medical latex gloves.<sup>9,10</sup> The latex proteins absorbed by the glove powder become airborne when powdered gloves are donned or removed. The level of NRL exposure appears to be the most significant factor associated with the risk of latex allergy. Baur et al<sup>11</sup> have demonstrated that allergic symptoms observed in HCWs were significantly associated with the level of latex aeroallergen in the workplace. These findings imply that chronic inhalation of latex allergens could be an important risk factor for the development of hypersensitivity and the exacerbation of symptoms.

Systemic symptoms caused by latex aeroallergens might not be alleviated by simply avoiding direct personal contact with NRL products if coworkers continue to use powdered latex gloves. Although the risk of a worker's exposure is greatest when gloves are being directly donned or removed, aerosolized particles carrying allergenic NRL proteins might also be deposited throughout the environment and resuspended from surfaces, including those of surgical gowns or other clothing.

Classic methods of assessing latex exposure have involved the use of air samplers with high flow rates,<sup>12-14</sup> which are used over long periods.<sup>11,15,16</sup> Swanson et al,<sup>13</sup> for instance, measured NRL aeroallergen levels in their medical center using area samplers operating at 60 to 180 L/min and personal breathing zone samplers operating for 1 to 4 hours at 5 L/min.

Many studies have used RAST inhibition assays for measurement of airborne latex allergens.<sup>2,12,13,17</sup> This method of assessing allergen exposure has, however, been difficult to standardize among laboratories because it depends on the use of human sera containing latex-

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**Abbreviations used**

HCW: Health care worker  
 Hev b 1: *Hevea brasiliensis* group 1 allergen  
 IOM: Institute of Occupational Medicine  
 NRL: Natural rubber latex

specific IgE antibodies that vary in terms of specificity for different latex allergens as well as in terms of avidity and cross-reactivity.

We have developed very sensitive methods for the collection of inhaled starch granules, the measurement of Hev b 1, and the detection of allergen on skin or glove surfaces. Although our nasal samplers have been used effectively to assess exposure to domestic allergens,<sup>18-21</sup> this is the first report of their use in monitoring exposure to an occupational allergen such as latex. The measurement of allergen exposure in an occupational setting allows direct examination of the relationship between exposure and symptoms. It also permits the identification of activities that pose a high exposure risk and provides a method by which to verify exposure abatement. The attractive features of the nasal sampler/HALOgen assay combination are (1) its ability to reflect the amount of allergen that is inhaled and (b) its sensitivity, which allows the measurement of the low allergen levels that are associated with personal exposure.

The aim of this study was to measure personal inhalation and contact exposure to Hev b 1 that was associated with wearing medical latex gloves or alternatives.

**Methods****In vitro testing of the capture efficiency of the nasal samplers for Hev b 1 particulate**

Nasal air samplers (Inhalix, Sydney, Australia) and Institute of Occupational Medicine (IOM) air sampling devices (SKC, Dorset, United Kingdom) were mounted in series in such a way that all particles not captured by the adhesive in the nasal sampler would be collected on the IOM sampler membrane mounted immediately behind it. Three of these were connected to the same pump, and flow rates were adjusted to 2, 5, and 10 L/min, representing the range of normal airflow through a single nasal sampler during respiration. A 10-minute sample was collected, during which time a pair of latex powdered gloves was removed from a box and put on, each cuff being pulled 3 times every 2 minutes. Nasal sampler capture efficiency for particulate carrying Hev b 1 was reported as the number of Hev b 1 particles collected by the nasal sampler divided by the sum of the numbers of Hev b 1 particles collected on the combined nasal and IOM samplers placed in series. This procedure was repeated 5 times to assess sampling variation.

**Vinyl gloves and latex gloves: surface, nasal, and breathing zone samples**

Each of 8 laboratory workers provided informed consent to participate in the study. Nasal air samplers were used to collect inhaled particles while an IOM sampler operating at 2 L/min was attached to the subject's lapel. Latex exposure was generated by having the subject don a pair of gloves from a box and use them during normal daily activity for 30 minutes while breathing through the nose. Activities performed by the subject during the period of sampling included general laboratory work, data entry, and microscopy. Sur-

face dust samples were subsequently collected with adhesive tape (2 × 3 cm; Inhalix) from the gloves, hands, and work areas by pressing the adhesive against the surfaces at 4 different sites. This procedure was performed first with vinyl powdered gloves (Surgigloves, Medical Industries Australia, Sydney, Australia) and then with powdered NRL gloves (Microtouch, Johnson and Johnson Medical/Ethicon, Inc, Arlington, Tex).

**No gloves, vinyl gloves, latex gloves, neighbor wearing latex gloves: nasal and breathing zone samples**

In a second experiment conducted in the same laboratory, the protocol described above was used with 8 subjects, but this time the subjects initially wore no gloves and levels of Hev b 1 were monitored through use of nasal and IOM samplers. In a second and then a third sampling period, the subjects wore vinyl gloves and NRL gloves from previously unopened boxes. A fourth set of nasal and IOM samples were taken when a nearby colleague wore powdered latex gloves.

**HALOgen assay**

All samples were analyzed through use of the HALOgen assay (Inhalix) and a mAb directed against Hev b 1 (Johns Hopkins University, Baltimore, Md). Collection strips from the nasal sampler and adhesive tapes used for surface sampling were laminated with a porous protein binding membrane (pore size, 1 μm; Inhalix). The same membrane was used as the collection filter in the IOM air samplers and was later laminated with the same adhesive tape used for the nasal sampling and surface sampling. All membrane/adhesive sandwiches were briefly wetted in methanol and left in an elution buffer (Inhalix) overnight to allow elution of proteins from the particles. Samples were then incubated with the anti-Hev b 1 monoclonal (clone DACI#J9221-GH3, 10 μg/mL); this was followed by buffer washes, incubation with an antimouse IgG biotin conjugate (6.8 μg/mL; Inhalix), an additional buffer wash, and, finally, use of avidin alkaline phosphatase (3.7 μg/mL; Inhalix). Color was developed through use of NBT/BCIP (Inhalix). Particles transporting Hev b 1 were identified by the presence of a surrounding halo of immunostain, and these were counted and sized under 100× magnification by means of a light microscope.

For adhesive samples from the gloves and hands, the method of sample preparation was identical to that described for the nasal sampler membranes. Estimates of total Hev b 1-positive particle counts were made by evaluating small sections of the specimen and multiplying by a correction factor for the total area.

**Statistical analysis**

Differences in the Hev b 1-positive particle counts between the "no glove," "latex glove," and "vinyl glove" conditions for the same subjects were performed with Friedman tests (nonparametric) through use of Analyse-it for Excel (Analyse-It Software, Ltd, Leeds, United Kingdom). Positive particle data were log-transformed to normalize the data before regression analysis.

**RESULTS****Hev b 1 capture efficiency of the nasal samplers**

At 10 L/min, the nasal samplers captured an average of 71.3% (± 12.9% [SD]) of the particles that were positive for Hev b 1. The collection efficiency decreased at lower flow rates. Means of 65.4% (± 5.3%) and 54.5% (±

10.2%) of the Hev b 1–positive particles were captured at flow rates of 5 and 2 L/min, respectively (Fig 1).

### Vinyl gloves compared with latex gloves: surface, nasal, and breathing zone samples

When powdered latex gloves were donned, people inhaled 26 times more particulate Hev b 1 than when vinyl gloves were worn ( $P < .005$ , Friedman; Table I and Fig 2, *a* and *b*). Similarly, breathing zone levels (IOM sampler) of particulate Hev b 1 increased by 24-fold under the same conditions ( $P < .005$ , Friedman). The median number of particles carrying latex allergen found on the surface of latex gloves was 88 times higher than that for the surface of vinyl gloves. The surface of the hands carried 409 times more particles carrying Hev b 1 after the removal of latex powdered gloves than after the removal of vinyl gloves (Fig 2, *c* and *d*). Particles carrying Hev b 1 detected on the surface of the surrounding work area increased by approximately 13-fold when powdered vinyl gloves were replaced with powdered latex gloves.

On microscopy, most latex allergen was found to be associated with cornstarch granules, which appeared almost circular and translucent. Allergen was also associated with larger particles—possibly dust particles—which had a darker appearance and were more irregular in shape. For samples taken from gloves and hands after latex gloves were worn, cornstarch granules accounted for >95% of particles carrying Hev b 1 allergen.

### No gloves, vinyl gloves, latex gloves, neighbor wearing latex gloves: nasal and breathing zone samples

Figs 3 and 4 present the results of the second exposure study for the nasal and breathing zone samples, respectively. The increase in the level of inhaled and breathing zone particles carrying Hev b 1 when latex gloves were worn in comparison with when vinyl gloves were worn was approximately 20-fold (Figs 3 and 4), which is similar to the increase found in the first experiment (Table I). A 6-fold increase in the amount of inhaled particles carrying Hev b 1 was observed when a nearby colleague wore latex powdered gloves and the person sampling wore no gloves ( $P < .005$ , Friedman). For most subjects, the nearby colleague was approximately 3 meters away. Breathing zone levels were increased by 4-fold under the same circumstances.

There were no significant differences in the amount of particles carrying Hev b 1 inhaled (Fig 3) or collected by breathing zone samplers (Fig 4) while no gloves were worn and while vinyl gloves were worn ( $P = .4795$ , Friedman). When all of the other combinations were compared, each was significantly different ( $P < .005$ , Friedman). A significant positive correlation was observed for nasal samples versus breathing zone samples (Fig 5).

There was a moderate positive correlation between the nasal samples and the glove surface levels of particles carrying Hev b 1 with vinyl gloves ( $r^2 = 0.5569$ ) and latex gloves ( $r^2 = 0.2888$ ). The correlation observed

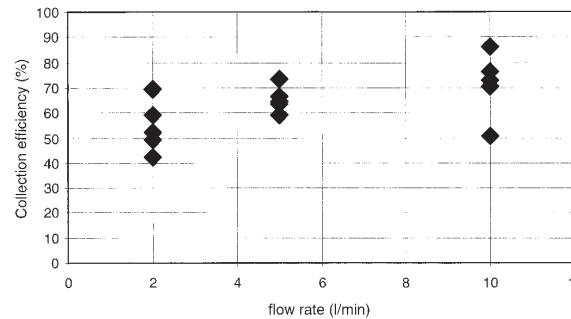


**FIG 1.** Collection efficiency of the nasal air sampler for Hev b 1–positive particles as a function of sampler air flow rate in liters per minute. Efficiency was computed as the number of Hev b 1 particles collected by the nasal sampler divided by the sum of the number of Hev b 1 particles collected by the nasal sampler and the number of Hev b 1 particles collected by the filter (IOM head) in series.

between breathing zone samplers and glove surfaces when vinyl gloves were worn ( $r^2 = 0.0184$ ) or when latex gloves were worn ( $r^2 = 0.2914$ ) was weaker.

The particles carrying latex allergen that were collected by the nasal sampler and the breathing zone sampler ranged in diameter from 5 to  $\geq 70 \mu\text{m}$ . For the nasal sampler, 1.6% of particles carrying Hev b 1 were  $\leq 5 \mu\text{m}$ , 38.1% were 6 to  $10 \mu\text{m}$ , 31.1% were 11 to  $20 \mu\text{m}$ , and 19.4% were 21 to  $30 \mu\text{m}$  in diameter. A similar collection pattern was observed for the breathing zone sampler, for which 0.95% of particles carrying Hev b 1 were  $\leq 5 \mu\text{m}$ , 34.3% were 6 to  $10 \mu\text{m}$ , 45.7% were 11 to  $20 \mu\text{m}$ , and 14.6% were 21 to  $30 \mu\text{m}$  in diameter.

## DISCUSSION

We have used nasal and breathing zone air samplers with a HALOgen assay because this enables the direct detection of airborne particles carrying Hev b 1 allergen during normal activities. It is widely accepted that a single allergen can be used as a marker for a source of allergen—eg, Fel d 1 and Der p 1 are used to estimate exposure to the array of allergens from cats and dust mites. There are presently 11 recognized latex allergens (Hev b 1 to Hev b 11), and in this study Hev b 1 was used as a representative allergen. We selected Hev b 1 as our marker latex allergen of choice because it is a protein that naturally resides on rubber particles, associates with cornstarch donning powder, and is thus readily inhaled. The sensitive HALOgen assay was able to detect airborne latex allergen in an undisturbed environment even when no gloves or vinyl gloves were worn. The Hev b 1 detected in the absence of latex gloves is assumed to be residual latex allergen either from surfaces or from the background of the system, being related to nonspecific binding of the mAb to particles; however, this seems unlikely, inasmuch as we have tested the anti-Hev b 1 monoclonal against dust mite, cat, dog, cockroach, and pollen allergens and found minimal nonspecific binding. This technique is sensitive enough to allow direct comparison of personal inhalation exposure with normal lab-

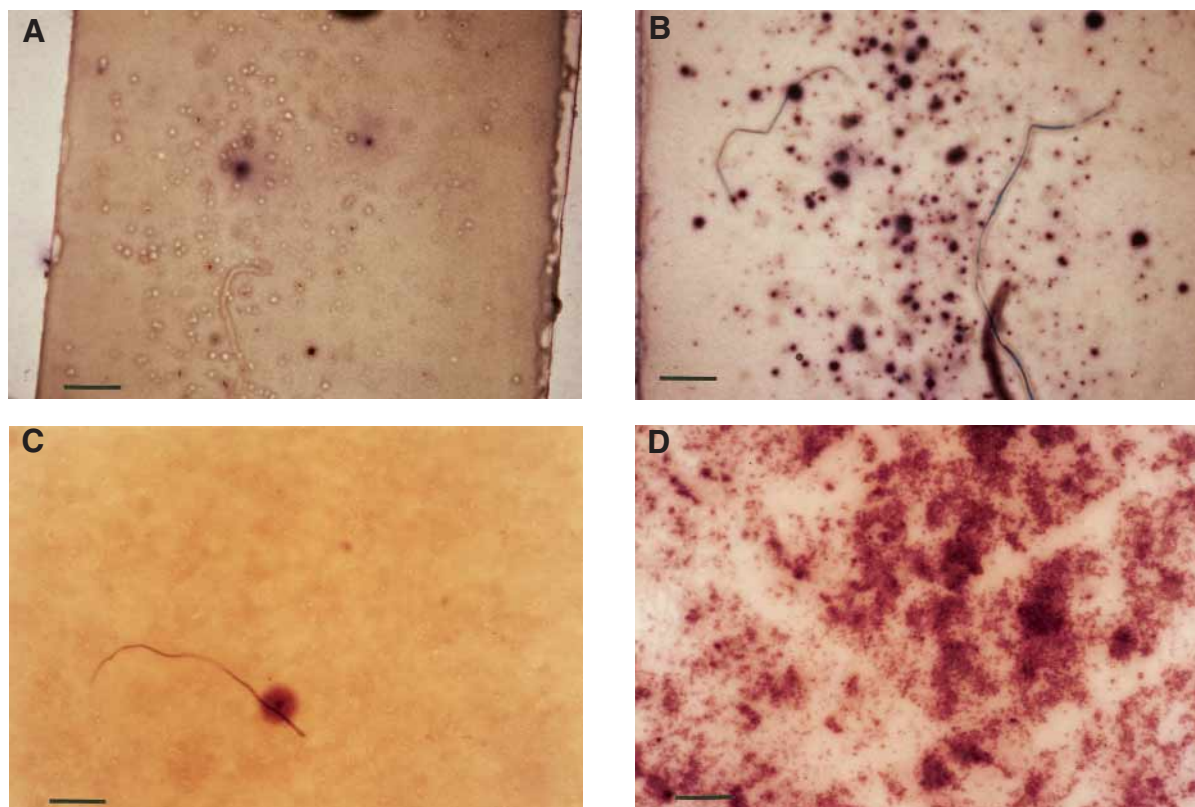


**FIG 2.** Particles HALOgen-stained for Hev b 1. **A**, Particles collected by nasal sampling while the subject wore vinyl gloves. **B**, Particles collected by nasal sampling while the subject wore latex gloves. **C**, Particles taken from the surface of the hands after vinyl gloves were removed. **D**, Particles taken from the surface of the hands after latex gloves were removed. Bars are 300  $\mu$ m.

**TABLE I.** Exposure to particles carrying Hev b 1 as a result of wearing latex or vinyl gloves

| Glove type           | Inhaled (nasal air sampler) | Breathing zone (IOM) | Surface samples   |                 |                 |
|----------------------|-----------------------------|----------------------|-------------------|-----------------|-----------------|
|                      |                             |                      | Desk              | Gloves          | Hands           |
| Vinyl control        | 7.5 (1-11)                  | 14.5 (6-78)          | 4.4 (2-14.8)      | 12.5 (1.8-45.2) | 4.8 (1-69.5)    |
| Natural rubber latex | 199 (56-351)                | 347.5 (116-1353)     | 56.8 (19.7-200.5) | 1102 (688-1416) | 1964 (916-2732) |

Each result is expressed as median (range). Inhaled and airborne samples were collected for 30 minutes while the 2 different gloves were worn. Surface samples were collected after the gloves were removed; results are expressed as numbers of particles per square centimeter.

IOM, Institute of Occupational Medicine air sampling device.

oratory levels of latex-carrying particles that are associated with the wearing of powdered latex or vinyl gloves.

We have developed a model for latex exposure that suggests that in a laboratory where latex gloves are used infrequently, there are low levels of airborne and surface latex allergens. The latex gloves used in these studies contained particles carrying Hev b 1, and when these gloves were donned, particles were transferred to the hands of the wearer, were carried to nearby surfaces, became airborne, and were inhaled by both the wearer and a nearby colleague. We have estimated the number of latex-carrying particles transferred from 1 pair of gloves to 1 pair of hands in 30 minutes of wearing to be approximately 850,000. When latex gloves were donned, latex contamination on nearby surfaces increased by 12.9-fold,

whereas airborne latex-carrying particles increased by 24-fold. This resulted in the wearer's experiencing a 26-fold increase in inhaled particles carrying Hev b 1, while a nearby colleague inhaled 6-fold more particles carrying Hev b 1. This latter finding of relatively high indirect exposure to latex might be significant for the development of occupational asthma in people with indirect exposure to latex materials.<sup>22</sup> Our results support the need to discontinue the use of latex powdered gloves in areas where sensitized individuals work. Sussman et al<sup>23</sup> and Charous et al<sup>12</sup> reported large reductions in the levels of latex protein when powdered latex gloves were replaced with powder-free gloves.

The observation that large numbers of Hev b 1-carrying particles are transferred to the hands after the

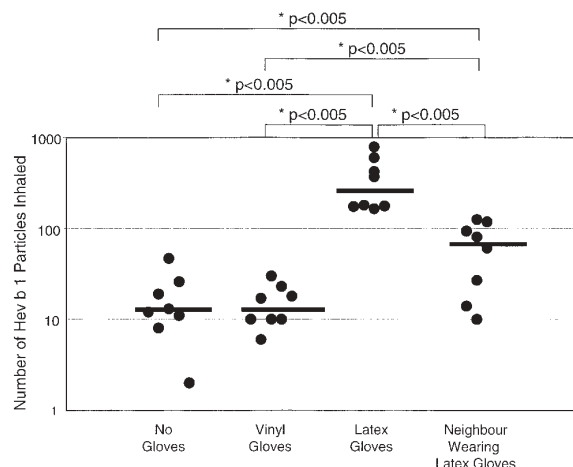


**FIG 3.** Numbers of Hev b 1-positive particles inhaled in the nasal sampler while the subjects wore no gloves, vinyl gloves, and powdered latex gloves and while a colleague was wearing powdered latex gloves in close proximity ( $\leq 3$  m away). The bar indicates the median for the group. Significantly elevated Hev b 1 particle levels were observed when the subjects wore powdered latex gloves or worked in an area where latex powdered gloves were being used by others.

wearing of latex powdered gloves provides some insight into the process of contact sensitization. When one wears a pair of such gloves, the hands begin to sweat and the powder, laden with latex allergen, becomes moistened. This can be likened to the elution step in the HALOgen assay, in which captured particles have the allergen leached out of them by soaking in an elution buffer. The allergen-rich solution in the glove-wearer's case is in direct contact with the hands and can be easily absorbed into the skin, especially in chapped and wounded areas; this might lead to sensitization.

There was a positive and significant relationship ( $r^2 = 0.7245$ ) between ambient airborne latex allergen, collected by a breathing zone sampler, and inhaled latex allergen, collected by the nasal sampler. This is consistent with a study conducted in Sweden in which allergen was detected in the air and on nasal samples during the cleaning of cages containing mice ( $r = 0.67$ ) and rats ( $r = 0.71$ ).<sup>24</sup> Allergen amounts reported in that study were 50 and 54 times higher for air sampling than for nasal sampling for mouse allergens and rat allergens, respectively. Our results for latex allergen collected from air and nasal samples show that the absolute amounts collected with breathing zone and nasal samplers were similar, the environmental area sampler collecting an average of 1.3 times more than the nasal samplers. Comparison of Figs 5 and 6 shows that the breathing zone and nasal samplers appear to collect similar numbers of particles when exposure is low (no gloves, vinyl gloves, or neighbor wearing latex gloves), but larger differences are observed at higher exposures.

The capture efficiency of the nasal sampler for collecting particles transporting Hev b 1 allergen was 71.3% at 10 L/min. Mean nasal inspiratory flow in normal

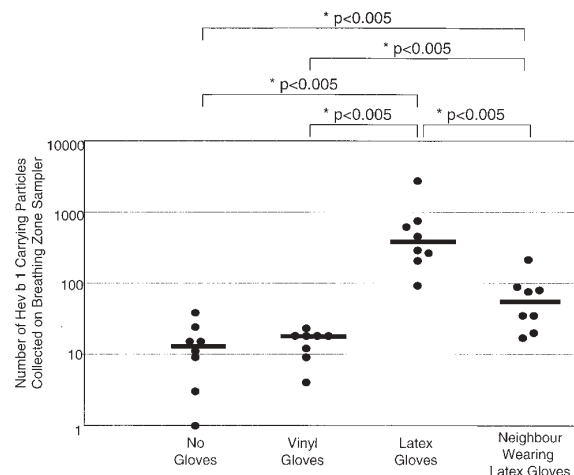


**FIG 4.** Numbers of Hev b 1-positive particles detected in the breathing zone sampler while the subjects wore no gloves, vinyl gloves, and powdered latex gloves and while a colleague was wearing powdered latex gloves in close proximity. The bar indicates the median for the group.

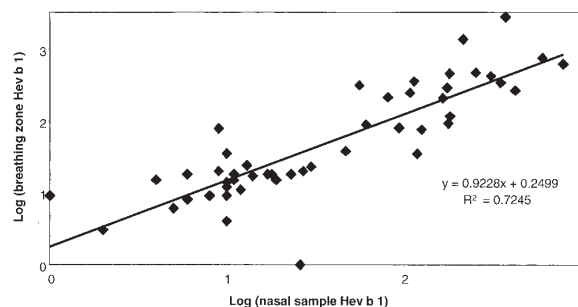


**FIG 5.** Correlation between the numbers of Hev b 1-positive particles as detected by the nasal sampler (x-axis) and the breathing zone area sampler (y-axis).

adults was measured at 18.8 L/min by Rodenstein et al,<sup>25</sup> which is within the range tested in our study if it is considered that the collection reported here for capture efficiency is through a single nasal sampler, which is normally worn in one nostril. If the collection efficiency of the nasal sampler is factored into the inhaled latex particle counts, the total number (median) of latex particles inhaled in 20 minutes while latex gloves are worn increases from 362 to 516.4. Similarly, the median number of particles carrying Hev b 1 inhaled while a nearby colleague wears latex gloves would be estimated at 100 as opposed to 71.5.

The nasal samplers have been shown to collect virtually 100% of particles with a diameter of 7.6 and 12  $\mu$ m, and approximately 50% of 5  $\mu$ m particles at flow rates associated with normal breathing.<sup>26</sup> The breathing zone sampler has been shown to capture 85% of particles  $\leq 25$   $\mu$ m in aerodynamic diameter and 70% of particles  $\geq 30$   $\mu$ m in aerodynamic diameter at a wind speed of 0.5 m/s.<sup>27</sup> Its performance is acceptably close to the

inspirability criterion published by Vincent and Armbruster.<sup>28,29</sup> Swanson et al<sup>13</sup> have reported that >80% of latex allergen is found on particles more than 7  $\mu$ m in diameter. This was confirmed in our study: we found most of the particles carrying Hev b 1 as collected by the nasal sampler and the breathing zone sampler to be 10 to 20  $\mu$ m in diameter.

HCWs, we believe, become sensitized to airborne allergen that is inhaled. It is widely recognized that cornstarch granules are a major carrier of latex allergens.<sup>9,10,30</sup> In this study, we were able to determine that more than 95% of the particles carrying Hev b 1 were cornstarch granules. Latex allergen was also found on larger particles, which had bigger and darker halos and therefore were carrying more Hev b 1 per particle. The origin of these larger particles is unknown.

In this article, we present and compare a number of methods for monitoring inhalation and contact exposure to environmental allergens. The results confirm the utility of these methods for assessing the magnitude of exposure to a major occupational allergen, NRL, in an occupational setting. The nasal sampler has enabled us to model methods and levels of exposure that result from wearing powdered latex gloves. Moreover, these techniques have permitted study of the effect of a latex substitute, vinyl, on exposure. Further studies will aim at examining latex allergen exposure in different occupational settings and investigate factors that influence the variation in the amount of latex allergens inhaled.

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