Measuring Dust on Skin with a Small Vacuuming Sampler—A Comparison with Other Sampling Techniques

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Airborne skin exposure to allergens and irritants may cause dermatitis. There are few methods for assessing skin exposure to airborne particles. We have modified and tested a vacuuming sampler for removing particles from the skin. The sampler was compared with two other skin and surface exposure sampling techniques. These were based on surrogate skin (a patch sampler—adhesive tape on an optical cover glass) and a tape stripping removal procedure. All three samplers measure the mass of dust on skin. Dust containing starch was deposited onto the skin in a whole-body exposure chamber. Samples were taken from forearms and shoulders and analysed using optical microscopy. With the different sampling techniques small differences in the results were obtained. Agreement between the vacuuming sampler and the tape stripping technique was good. The comparison between patch and tape stripping procedure indicated a slight overestimation for the patch. The three techniques are applicable for assessing skin exposure to particles and for dose–effect studies. The vacuuming method will be further developed and applied in workplace studies. The technique allows for dust sampling from large areas of skin.

Keywords: aerosol; airborne; dermal; dust; exposure chamber; patch sampling; skin exposure; tape stripping; vacuuming sampler

INTRODUCTION

Exposure to hazardous compounds may occur by inhalation, dermal contact or ingestion. In occupational hygiene, inhalation has traditionally been considered the most important route. Recently however, the dermal exposure pathway has received more and closer attention, for instance in the EC Dermal Exposure Network (DEN) 1997–2000 and in the RISKOFDERM project (RISKOFDERM, 1999).

Contact dermatitis may be caused by skin exposure to airborne particles. Glass fibres and cement and wood dusts cause irritant contact dermatitis (Lachapelle, 1986; Derave et al., 1998). The dust of tropical woods, pine, some plants and medicaments may cause allergic contact dermatitis (Lachapelle, 1986; Estlander et al., 2001). Skin may be affected by dust containing nickel and some other metals (Schubert, 2000; Tinkle et al., 2003; Mäkinen and Linnainmaa, 2004).

Skin exposure measurement follows different principles (Fenske, 1993). Direct methods involve removal procedures, use of a surrogate skin and fluorescence tracer techniques. Most of these techniques were developed, and are primarily used, for agents other than particles. Removal procedures most frequently used include wiping, rinsing and cleaning (Brouwer et al., 2000). Removal by tape stripping of the outermost skin layer, the stratum corneum, has been popular in recent years (Surakka et al., 2000; Nylander-French, 2000). Vacuuming techniques have been employed mostly for sampling from surfaces other than skin. They have been regarded as poor collectors due to their low removal efficiency.

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There are few validated and standardized methods for measuring skin exposure. For developing methods, an approach using a basic concept (a model) for the different factors influencing skin exposure has been suggested (Schneider et al., 1999). The terminology is based on a conceptual model of the different steps leading to exposure—the transport of a substance from the source to the surface of the skin. Methods for measuring not only skin exposure but also that of other surfaces, e.g. clothes that contribute to exposure by contact and transport between such surfaces are of interest.

The present aim was to develop a small vacuuming sampler for measuring dust on skin and to compare it with other techniques such as a surrogate skin (patch) and a tape stripping procedure for use in workplaces as well as in controlled laboratory experiments. They should all measure the mass of dust particles deposited on skin. We sought to use simple, practical and inexpensive equipment already available in an occupational hygiene laboratory. Also, since dust and particles were the agents of interest, we stressed the possibility of performing microscopic studies of morphological and other particle characteristics.

**MATERIALS AND METHODS**

**Experimental design**

A small and handy vacuuming sampler for removing particles from the skin was constructed and its use was compared with two other skin and surface exposure sampling techniques. These involved a surrogate skin sampler (a patch sampler with adhesive tape on an optical cover glass) and a tape stripping procedure. The samples were analysed with optical microscopy. All three samplers measure the mass of dust on skin.

The dust particles used in the study were chosen either because they are known to cause occupational skin and respiratory tract symptoms (wheat flour) or because they may serve as model substances (cornstarch).

Healthy subjects were exposed to the dusts in a whole-body exposure chamber. Areas on forearms and on shoulders were exposed and sampled. The exposure lasted either 30 min (cornstarch) or 60 min (wheat flour) for all subjects. The exposure studies were approved by the Regional Ethics Committee at Karolinska Hospital, Solna, Sweden.

**Equipment for exposure setting**

The whole-body exposure chamber has been used in studies of respiratory effects from bakery dust (Gripenbäck et al., 2003; Grünewald et al., 2003) and pinewood dust (Gripenbäck et al., 2005), and it has been described previously (Lidén et al., 1998). It consists of a chamber sufficient for one subject and a sluice. The floor area in the chamber is 1.8 x 1.5 m and height is 2.1 m (volume 5.7 m³). Dust is generated into the chamber using a solid particle disperser, an RBG-1000 particle generator (Palas GmbH, Germany) and transported through a tube containing a krypton 85 source for neutralisation of electrical charges on the particles. The chamber includes facilities for measuring airborne dust concentration with conventional sampling methods or on-line with direct-reading instruments. Temperature and humidity can be recorded, but not controlled, during exposure. All challenges with subjects were performed normally at air exchange rates of 12–18 changes per hour. The airborne dust concentration of cornstarch was targeted at ~8 mg/m³, and for wheat flour the airborne concentrations were between 4.2 and 8.6 mg/m³ as measured in 37 mm open-face Millipore cassettes with AAWP membrane filters (pore size 0.8 μm, Millipore Corp., USA), at 2 l/min.

Inhalable airborne particle size distribution curves from measurements with PIDS impactors (personal inhalable dust spectrometer) (Gibson et al., 1987; Ramachandran et al., 1996) inside the whole-body exposure chamber are shown in Fig. 1.

**Equipment for measuring skin exposure**

A small and handy vacuuming sampler, a patch sampler (surrogate skin) and a tape stripping procedure were used.

A small filter sampler for removing deposited particles from the skin by vacuuming was constructed. It consists of a three-piece, 25 mm filter cassette (Nuclepore Corp., USA) with a specially designed metallic (copper) nozzle with an opening of 10 mm in length and about 1 mm in width (Fig. 2). The nozzle has small openings on each side to prevent it from adhering to the skin during vacuuming. A sampling pump is connected to the cassette—a portable pump or a stationary pump can be used. Before sampling, the airflow through the cassette was adjusted to a fixed value with an external rotameter. A working flow of 10 l/min was used. This corresponds to an air velocity in the nozzle ~14 m/s. Such a high air velocity results in particle deposition not only on the membrane filter but inside the sampler as well. Dust deposited in all three parts of the sampler (headpiece with nozzle, tube and filter) was combined and evaluated as one sample. The sampler can be loaded with different types of membrane filters depending on the requirements of forthcoming analysis. In our studies, we used a smooth-surface filter made of polycarbonate with pore size 0.4 μm (Nuclepore Corp., USA). The actual vacuuming was done by moving the

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*Byrne, 2000; Glass et al., 2003.* Surrogate skin collectors (patches or pads) are used to measure the mass of material deposited on the surface of the patch (Soutar et al., 2000; Eriksson et al., 2004).
vacuuming sampler repeatedly over the exposed area 5–6 times with the nozzle in contact with the skin. Normally, each sampler vacuumed a skin area of 12 cm².

**Tape stripping.** This is a technique that removes deposited particles and also some of the stratum corneum cells, using adhesive tape. Tape stripping was done with 1.9 cm Scotch Magic tape (3M, USA). Prior to stripping, the tape was statically neutralized with a static charger (Staticmaster no. 2U500, NRD Inc., USA) to eliminate the risk of losses due to static charges. The tape was placed with the sticky surface on the skin. Slight pressure was applied with a silicon-surfaced roller (width 2 cm), which was moved 10 times over the tape. The tape was then carefully removed from the skin and placed with the sticky side up on an objective glass for analysis. Normally, one tape strip covered a skin area of 5.7 cm². Each area was always stripped twice.

**The patch sampler.** The patch sampler with a sampling area of 4.2 cm² consists of a microscopy cover glass covered with adhesive tape placed in a specially designed two-piece plastic holder (Fig. 3). The tape used (Scotch Pressure Sensitive Tape Klebeband, 3M, USA) is sticky on both sides and has good transparency to visual light. Prior to sampling, the patch

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**Fig. 1.** Particle size distribution curve for inhalable airborne cornstarch and wheat flour in the exposure chamber as measured with PIDS impactors.

**Fig. 2.** The vacuuming sampler. The opening of the inlet is 10 mm in length and 1 mm in width.

**Fig. 3.** Sampling areas and view of patch samplers mounted on shoulder and on forearm. The picture to the right shows the whole investigated area on the forearm (sampling with tape strips or vacuuming was performed within the dotted area).
sampler was conditioned overnight in an oven at 35°C to eliminate excess volatile compounds in the adhesive material.

Skin exposure measurements

Sampling. For the exposure challenges with cornstarch in the whole-body chamber, all three different sampling techniques were used. The sampling was done using patches mounted on the forearms before exposure and sampling with vacuuming and tape stripping after exposure (for patch position and the area evaluated after exposure, see Fig. 3). For the challenges with wheat flour, only patches and tape stripping were used since at that time the vacuuming sampler had not been constructed.

To reduce the effect of possible non-homogeneous particle deposition, two areas not in direct contact were evaluated as one sample and compared with two other non-contiguous areas.

The number of sampling sets from forearms or shoulders varied. From each exposure challenge, either one or two sampling sets were performed depending on whether two forearms or two shoulders were evaluated.

Sample preparation and analysis. All samples were stained and immersed in a suitable immersion liquid before the microscopical evaluation. The cornstarch was stained black with Lugol’s iodine solution (BHD Laboratory Supplies, UK), and wheat flour starch was stained red with Schiff’s reagent (BHD Laboratory Supplies, UK).

The Nuclepore filter inside the vacuuming sampler is not suitable for light microscopy because it is not easily made transparent. The cornstarch particles on the surface filter and from inside all parts of the vacuuming sampler were collected by rinsing the sampler’s interior and stained prior redeposition by liquid filtration onto a new 37 mm cellulose ester filter with pore size 0.8 μm (Millipore, USA). The Nuclepore filter was also checked after redeposition under a stereo microscope for residual (black) starch. The new cellulose ester filter with the stained cornstarch particles was collapsed with acetone after drying and glycerol was used as immersion liquid. Patches and tape strips were prepared by staining and immersing in glycerol without the redeposition procedure.

Cellulose ester filters were not used during vacuuming since the subsequent staining procedure resulted in a difficult background for microscopical analysis. These filters are also unsuitable for redeposition since particles remain stuck inside the filter.

The analysis was done with different techniques of light microscopy as described below.

Cornstarch. The cornstarch particles were counted at 10x objective magnification on a Leitz DM microscope (Leica, Germany) using a screen computer system (Lundgren et al., 1995). Polarizing filters were also used in order to examine the typical crystalline starch cross. In Fig. 4, a typical microscopical viewing field of stained cornstarch can be seen. Only spherical particles (stained and/or crystalline) were counted. Mass was calculated after comparison to a calibration curve based on filter samples spiked with known amounts of cornstarch particles. The counting criteria used were counts of >100 starch particles or at least 30 viewing fields.

The spiked calibration samples were produced by liquid filtration onto 37 mm cellulose ester filters, 0.8 μm (Millipore, USA) with known amounts of cornstarch direct from laboratory beakers (not stained with iodine). Ten more calibration samples were produced by pouring known amounts of cornstarch into the vacuuming samplers (with Nuclepore filters) and staining and preparing them by redeposition in the same way as a real sample. The purpose of this was to check the efficiency of the preparation step involving redeposition of particles onto cellulose ester filters. The calibration curve can be seen in Fig. 5. As there was no difference between the two spiking techniques, any loss of starch particles due to the redeposition procedure seems to be negligible.

The detection limit, defined as three times the variation (sigma) of background counting, was ≈0.2 μg/cm² (calculated from 31 different blank samples—patches, tape strips and membrane filters).

Two experienced microscopists evaluated the samples. For 40 samples counted by both, no statistical difference was found (average ratio 0.98, confidence interval (CI) of 0.95–1.01).

Wheat flour dust. The starch amount in wheat flour was estimated using image analysis in an optical microscope at different magnifications. The starch was stained selectively and the area of the coloured and strongly red fluorescing starch, the main component of wheat flour, was determined in a
stereomicroscope at ×10 magnification and in an EPI-fluorescence Leitz DM microscope at ×10 and ×40 objective magnification (Leica, Germany). Computerized image analysis (Optilab, Graftek, France) was used. The volume of each starch particle observed in the microscope was measured by using different shape factors depending on the size of the single starch particle or of a cluster of starch and proteins. A density of 1.5 g/cm³ was used for converting volume to mass. The final result of the starch content in each sample represented all calculations at different microscopic magnifications. The technique was calibrated and tested against a CRM 382 reference wheat flour (BCR, EUR 14553 EN) with known starch content. On three spiked filter samples, an average of 97% (CI 83–111%) of all starch was recovered. One spiked sample with ~630 μg wheat flour (CRM 382) was recalculated twice, yielding a coefficient of variation (CV) of as little as 1.5% [2.5% at the lower (×10) and 0.8% at the higher objective magnification (×40)]. The estimated detection limit was ≤0.2 μg/cm². Only one microscopist evaluated the samples.

**Validation of sampling methods**

The efficiency of a sampler in removing dust particles is an important factor when using vacuuming and tape stripping. Another important factor when studying and comparing skin exposure methods is the homogeneity of the dust on the areas evaluated. Data were needed for this skin deposition homogeneity as estimated by side-by-side sampling with the same method in the whole-body exposure chamber.

**Removal efficiency.** The removal efficiency of tape stripping was tested on the shoulder with wheat flour and on the forearms with cornstarch. An "overall" removal efficiency of tape stripping was performed for one subject by stripping the same area 10 times on the shoulder after exposure to wheat flour (a cumulative mass loading of 300 μg/cm² was measured on all strips). No starch was found on the strip 4–10. We found 96.4% of the mass on strip 1 and 99.8% when the mass on strip 2 was included. No difference in starch particle size was found between these two strips. For all other subjects exposed to wheat flour on the shoulders, the fraction found on the first strip was 95.4% (CI 93.2–97.5%, n = 11) when two successive strips were studied. On the forearm with cornstarch, the fraction found on the first strip compared with the cumulative amount for strips 1 and 2 was 89.8% (CI 88.8–90.8, n = 24).

How well the vacuuming sampler removed particles from the skin at airflow 10 l/min was examined by tape stripping of remaining particles on the area after vacuuming. The fraction found in the vacuuming sampler compared to the cumulative amount found in the sampler and on the tape strip was 95.7%, with a CI of 94.8–96.5% (n = 24). This vacuuming ability at a lower airflow (5 l/min) gave almost the same result (93.7% with a CI of 90.2–97.3%, n = 4).

**Homogeneity of the dust on the exposed skin areas.** To estimate how evenly and homogeneously particles were deposited on the forearms in the exposure chamber, 2 to 7 samples were collected with the same sampling method for a different number of

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**Fig. 5.** Calibration curve showing the number of starch particles found at different levels of spiked filters after counting in a light microscope.
repeated sampling sets. The variation of cornstarch particles found on the evaluated areas on forearms for each sampling method was calculated (Table 1). The amount of cornstarch removed from the skin varied between 7 and 17 \( \mu g/cm^2 \) for the different sets. The variation of wheat flour on shoulders as measured with patches was also evaluated. The dust loading of wheat flour starch found on the patches varied between 13 and 66 \( \mu g/cm^2 \).

Only when studying wheat flour on subjects’ shoulders did the variation occasionally exceed 10%. The variation on the forearms was low, on average \( \leq 6\% \). Such a low spatial deposition after exposure in the whole-body exposure chamber on a horizontal skin area indicated that the chosen area was suitable for comparing sampling techniques.

Calculations and statistics

Calculations and statistics consisted of testing the ratios between the result of the vacuuming sampler or the patch against the result of tape stripping (Student \( t \)-test, \( H_0: \mu_1/\mu_2 = 1, \alpha = 5\% \)). The 95% CI of the ratio was also calculated.

Other simple statistical calculations as means, ratios and CV were also used.

**RESULTS**

**Comparison between vacuuming and tape stripping**

The results obtained with the vacuuming sampler and with the tape stripping procedure were compared after exposure to cornstarch. Four subjects were exposed separately. Areas on both forearms were sampled, giving a total of eight sampling sets to be evaluated. Tape strips were also sampled on areas already vacuumed. The result for vacuuming, tape strip after vacuuming and the two results from strips 1 and 2 is presented graphically in Fig. 6. The relevant data is also shown in Table 2.

In Table 3, the comparison is presented as the average value (with confidence intervals) of the ratios of starch found with the vacuuming sampler to that found with tape strip 1 and cumulatively with strips 1 and 2.

Statistical testing (Student \( t \)-test) indicated no difference in result when comparing sampling by vacuuming against using the first strip. Adding tape strip 2 (i.e. tape strips 1 and 2) gave a small statistical difference between the two techniques—average values with vacuuming were 9% lower.

### Table 1. Variation in particle loading between areas on forearms and shoulders

<table>
<thead>
<tr>
<th>Particles</th>
<th>Sampling methods</th>
<th>Areas exposed</th>
<th>No. of sampling sets</th>
<th>No. of samples per set</th>
<th>CV (%)</th>
<th>CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>Vacuuming</td>
<td>Forearm</td>
<td>8</td>
<td>2–4</td>
<td>5.3</td>
<td>2.6–8.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>Tape stripping</td>
<td>Forearm</td>
<td>16</td>
<td>2–7</td>
<td>6.4</td>
<td>4.5–8.2</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>Patch</td>
<td>Forearm</td>
<td>8</td>
<td>2–4</td>
<td>4.3</td>
<td>2.6–6.0</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>Patch</td>
<td>Shoulder</td>
<td>10</td>
<td>2–3</td>
<td>9.4</td>
<td>4.7–14</td>
</tr>
</tbody>
</table>

Measurements taken on subjects during repeated sampling sets.

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**Fig. 6.** Mass of cornstarch measured on forearms by vacuuming, tape stripping after vacuuming and tape strips 1 and 2 for eight sampling sets.
Comparison between patch sampling and tape stripping

Patch sampling and tape stripping were compared after exposure to both cornstarch and wheat flour. Samples were evaluated on forearms (cornstarch) and on shoulders (wheat flour). Seven subjects were exposed to cornstarch (12 sampling sets) and nine to wheat flour (11 sampling sets). The result for the patches and the cumulative result from the two tape strips (1 and 2), are presented in Table 4.

Table 5 presents the comparison as the average value (and CI) of the ratios of starch found with the patches to that found by tape strips 1 and 2.

DISCUSSION

A vacuuming sampler for measuring dust particles deposited on human skin was constructed and evaluated. The sampler construction is simple, and sampling is non-invasive. The sampler measures the mass of dust found on the skin after a period of exposure expressed as µg per cm².

Using a small and handy vacuuming sampler for removing particles from the skin is a tempting idea. Some small vacuuming samplers have been constructed for experimental sampling from surfaces other than the skin (Wheeler and Stancliffe, 1998). However, their removal efficiency was low, which has limited their use and interest (Byrne, 2000). Our vacuuming sampler consists of parts from a standard sampling filter holder but with a specially designed metal nozzle. All the parts of the samplers were regarded as potential dust collectors and accordingly evaluated together.

The analytical technique used in this study was counting and measuring starch particles with light microscopy. It was both a simple and a reliable procedure with good agreement between two microscopists. A slightly tedious procedure is the redeposition of sampled dust from the vacuuming sampler onto a filter for microscopical analysis. It would be more convenient if the sampling filter, after a thorough rinsing of the walls and nozzle, could be analysed directly. This was not possible in the present study because of the high microscopical counting background when staining with iodine.

Rinsing, as well as redeposition procedures, increases the risk of analytical losses as any analytical preparatory step may do. Even when ultrasonic treatment is used, not all vacuumed particles might be rinsed off from the inner parts of the sampler onto the filter as easily as large cornstarch particles. Redeposition, which worked well for cornstarch in the present study, may cause analytical losses of greater magnitude.
importance at lower concentrations. However, redeposition was needed because of the microscopical analysis used. If we had vacuumed some other type of insoluble particles and chosen another analytical procedure, no redeposition procedure might have been needed. These particles would have been rinsed off from all inner parts of the sampler down to the connecting membrane filter, which would then be directly analysed with an appropriate technique. Further development of the vacuuming device should address enhancement of collection of particles onto a collecting media so as to avoid rinsing and redeposition procedures.

Two other techniques for measuring particles deposited on human skin were also evaluated—tape stripping and sampling with patches. They are both quite simple and almost non-invasive, and they also measure the mass of dust found on the skin. Removing the outermost skin layer, the stratum corneum, by stripping with adhesive tape is an established technique (Nylander-French, 2000; Surakka et al., 2000). However, we know of no study using the same procedure for removing particles deposited on the skin and at the same time evaluating the content of particles found on the tape. Sampling with patches is an established technique for assessing volatile compounds on skin (Soutar et al., 2000) but rarely used for particle exposure (Vermeulen et al., 2000). Our patch sampler was specially constructed for use with light microscopy—an important analytical tool when studying airborne particles. Morphology and particle size determination combined with chemical analysis with EPI-fluorescence microscopy can give valuable characterization data of deposited particles. An advantage is that the patches are quite easy to handle and can be analysed afterwards with different techniques depending on the particles studied.

How efficient a sampler is in removing dust particles is an important variable when testing vacuuming and tape stripping. The vacuuming of cornstarch removed ~95% of the dust deposited on forearms. Tape stripping with only one strip removed ~90% of the cornstarch and 95% of the wheat flour. An ‘overall’ tape stripping test with wheat flour (10 successive tape strips evaluated on one subject) indicated that >99% was found on the two first strips. The small difference between cornstarch and wheat flour in tape stripping efficiency was not evaluated. Possible explanations could be particle size effects, other different material behaviour or differences in the adhesive strength of the tape due to different batches of tape.

An important factor when studying and comparing skin exposure methods is the homogeneity of the areas evaluated, since the same area could not be analysed repeatedly with the same method or with the comparing technique. Ideally, we need a large homogeneous area or at least we need to know the variation of the deposited material on the area evaluated. In our whole-body exposure chamber, the spatial homogeneity of deposition on the areas evaluated was very good. The variation on the forearms was low, 6% and below. Such low variation over a horizontal skin area within the whole-body exposure chamber makes the present exposure chamber suitable for skin sampling tests.

When comparing skin sampling methods, we found good agreement between the vacuuming sampler and tape stripping. They both remove >90% of the deposited dust. A small underestimation (an average of 9% lower values) could be found with the vacuuming sampler when compared with a twice-off stripping procedure. When only one tape strip was evaluated, an almost identical result was obtained.

The agreement between tape stripping and patch sampling differed slightly, with a small overestimation (average 21%) for the patch for cornstarch collected on the forearms and for wheat flour on the shoulder (on average 15%) when the tape stripping data were based on a twice-off stripping procedure. A slightly higher value on a patch might be explained by particles remaining stuck to the glue, while particles deposited on skin might fall off as the subject moved during exposure.

It is usually considered infeasible to compare different skin measuring techniques because of different sampling characteristics and hence differing results (Fenske et al., 1999). However, one comparison between a fluorescence tracer method and a rinsing procedure for spray painting (Roff et al., 2001) showed good agreement. Our study shows that, given control of some vital influencing parameters in an exposure scenario, good agreement between different sampling techniques may be achieved when studying the deposition of airborne particles.

The present vacuuming sampler was a prototype to test the feasibility of vacuuming particles from the skin. It worked as well as the tape stripping procedure for removing particles deposited on human skin, and had a high removal efficiency (~95% of the particles were found in the sampler). Based on the promising result, we also tested the vacuuming sampler during an exposure trial with pinewood dust and ran some minor laboratory tests with silica particles of different sizes in order to further develop and evaluate this user-friendly sampler. These data and other improvements of the sampler will be presented separately.

Vacuuming dust from skin is a tempting and attractive procedure. It is non-invasive and handling is safe and easy. Sampling simplicity in the field is an advantage compared to using a tape or patches. The greatest advantage may be the possibility of sampling from large areas than are practical with tape stripping and patches. With a vacuuming sampler, the sampling area may be increased as desired.
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