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Assessment of soy aeroallergen levels in different work environments

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Summary

Background Airborne soybean hull proteins are known causes of asthma epidemics around harbours and soy processing plants. Soy flour dust proteins may cause occupational allergy in food and feed industries.**Objective** To compare enzyme immunoassays (EIAs) for soy hull and soy flour aeroallergens, exposure assessment in various work environments.**Methods** Airborne dust samples ($n = 324$) from soy unloading and/or processing plants, the animal feed industry and pig stables were analysed by two soy flour assays: one assay for measuring complete soy hull proteins and two assays for measuring the purified low-molecular-weight (LMW) soy hull allergens.**Results** Immunoblotting confirmed strong differences between antibody specificities and soy preparations. The results of the two soy flour assays and the assay for measuring complete soy hull proteins were highly correlated ($r > 0.85$). The two LMW soy hull assays also showed a strong mutual correlation ($r = 0.91$), but much less correlation with assays for measuring soy flour and complete soy hull. The levels of LMW soy hull proteins were the highest at sites of soybean unloading or processing, while soy flour levels were particularly high in the soy and animal feed industry.**Conclusions** The optimal EIA procedure for soy aeroallergen exposure assessment depends on the type of work environment and the local soy dust composition. Thus, the type of work environment should always be taken into account in future soy allergy studies in order to prevent a possible underestimation of the workers' actual risk of developing soy allergy.**Keywords** epidemic asthma, immunoassays, occupational asthma, soy flour, soy hull

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Introduction

Soy is one of the major allergenic foods and contains a high diversity of allergenic proteins [1]. Allergic reactions can also occur when soy flour or soybean dust is inhaled. In 1934, Duke [2] described several cases of asthma triggered by dust from a nearby soybean mill, and Bush and Cohen [3] reported in 1977 a case of asthma due to soybean flour inhalation. The most intensively investigated examples of soy-induced airway disease are the asthma outbreaks in the city of Barcelona caused by unloading of soybeans in the harbour [4]. Work-related allergy to soy dust has only incidentally been reported as an occupational hazard, in bakeries, in animal feed factories and in food processing plants where soy flour is

handled [3, 5]. The allergens involved in occupational asthma may, however, differ from the causative agents in the Barcelona epidemics. Allergens causing epidemic asthma would be mainly low-molecular-weight (LMW) proteins concentrated in the soybean hull [6, 7]. Specifically, the major allergens responsible for the soy asthma epidemics are two isoallergens (Gly m 1A and Gly m 1B) with molecular weights (MW) of 7.0 and 7.5 kDa [8]. In contrast, the major allergens from soy flour dust would predominantly be high-molecular-weight (HMW) proteins [9, 10]. On the other hand, soy-induced occupational asthma is probably not exclusively caused by soy flour [11]. Several immunoassays have been described for quantifying airborne levels of the LMW soy allergens [7, 12, 13]. A human IgE enzyme immunoassay

(EIA)-inhibition method is currently used for daily monitoring of the soy hull LMW (SHLMW) aeroallergens in the city of Barcelona [12]. Because the widespread use of human sera in these assays is complicated by their limited availability and a relatively low sensitivity, other assays have been developed, such as the monoclonal antibody-based method to quantify Gly m 1 [13]. However, the high specificity of a monoclonal antibody-based EIA – depending on the recognition of a single epitope – could also be a disadvantage if in some soy dust mixtures the epitope is masked and the actual allergen exposure level is thus underestimated [14]. We therefore recently also developed polyclonal rabbit IgG-based assays for soy hull antigens, of which one, an ‘amplified EIA’, combines a high sensitivity with reasonably high specificity while avoiding the risk of false-negatives associated with the use of monoclonal antibodies [15].

To our knowledge, no published study has described immunoassays for airborne soy allergens in the occupational environment.

Hence, the aims of this study were (1) to develop several new immunoassays for the measurement of airborne soy allergens: the human IgG4 soy flour protein (SFP) inhibition EIA, the rabbit SFP sandwich EIA, the rabbit SHLMWE (soy hull LMW allergenic protein) inhibition EIA and the rabbit complete soy hull extract (CSHE) inhibition EIA; and (2) to assess their suitability and that of the amplified SHLMWE sandwich EIA [15] for monitoring of soy aeroallergens at different workplaces. The study was part of the six-laboratory European Research Project MOCALEX (Measurement of Occupational Allergen Exposure), whose purpose was to compare existing methods and develop new techniques for the measurement of important occupational bio-allergens [16]

Materials and methods

Airborne dust samples

Airborne soy allergen-containing dust was sampled with a parallel sampling device [17, 18], in three countries:

- In Spain, samples were taken in two soy plants: one that unloads and processes soy (flour and oil) (company 1) and another that only unloads soy beans from ships (company 2). The parallel sampling unit was placed between the hull processing machine and the flour machine in company 1 and near one of the soy grain scales in the weighing room of company 2.
- In the Netherlands, samples were taken in the animal feed industry (company 3) and a soy-processing plant (flour and oil) (company 4). In the animal feed industry, the sampler was placed in the department where crude ingredients are weighed and mixed. In the soy plant, the sampler was placed in the area where

crushing of soybeans, separation of hulls and grinding of soy flakes were performed.

- In Germany, sampling took place in two pig stables, where soy-containing animal feed was used regularly (companies 5 and 6).

The sampling time varied from 30 min to 6 h to provide a wide range of soy aeroallergen concentrations. A total of 324 filters were used to perform this study and to investigate the effects of different extraction procedures on soy allergen yields [18]. Filter eluates were stored at -20°C until analysis.

Preparation of extracts

Three different extracts were prepared from soybeans. The preparation of HMW SFPs was carried out originally as part of a food allergy study, essentially as described by Burks et al. [19]. Briefly, soybeans from a local grocery were crushed and extracted in phosphate-buffered saline (PBS), followed by defatting, filtration and dialysis of the extract. The other two, crude CSHE and its purified LMW fraction (SHLMWE) were obtained as described previously [15]. The protein concentrations of CSHE and SHLMWE were determined by a bicinchoninic acid assay (Pierce Chemical Co., Rockford, IL, USA), and of SFP by the modified Lowry assay [20, 21].

Characterization of the extracts

The protein composition of the extracts was studied by SDS-PAGE under reducing conditions on a Novex Xcell II mini-cell electrophoresis unit (Invitrogen, Carlsbad, CA, USA). Proteins were incubated for 10 min at 70°C in sample buffer and separated on a precast 10% NuPAGE gel (Novex, Invitrogen, Carlsbad, CA, USA).

Antibodies

Because IgG4 antibodies to food proteins can be found in many non-allergic individuals [22, 23], IgG4 anti-SFP-positive human sera could be selected by screening a large set of sera, and a pool was made of eight sera from the bakery industry with the highest titres. Rabbit antisera were obtained by immunization with SFP, SHLMWE and CSHE, essentially as described previously [15, 24]. The Ig fraction from the anti-SFP antiserum was isolated by ammonium sulphate precipitation, and IgG of anti-SHLMWE and CSHE was isolated with a protein A-agarose column (Pierce). Part of the rabbit anti-SFP and anti-SHLMWE IgG fractions was biotinylated and used as a detection antibody in their respective sandwich EIAs.

Specificity tests with a series of other plant-derived extracts, including wheat, rye, barley and maize, and

various other potentially relevant common airborne or food allergens, were performed to assess the specificity of rabbit and human anti-SFP. Only the SFP preparation showed the capacity to inhibit the binding of the antibodies to the coated SFP proteins (not shown).

Immunoblotting

The reaction profile of the antibodies was analysed by immunoblotting. Nitrocellulose membranes with proteins blotted from the SDS-PAGE gels were blocked with 5% non-fat dry milk blotting grade blocker (Bio-Rad, Richmond, CA, USA) for 1 h, and then incubated for 90 min with appropriate dilutions of pooled human IgG4-positive serum (1/10–1/60), rabbit IgG anti-SFP (1/3000–1/20 000), rabbit IgG anti-SHLMWE (1/5000–1/10 000) or rabbit IgG anti-CSHE (1/1000–1/8000). After washing, the membranes were incubated with horseradish peroxidase (HRP)-labelled mouse anti-human IgG4 for IgG4 blots (1/10 000) and with HRP-labelled goat anti-rabbit IgG (H+L) at 1/20 000 for rabbit IgG anti-SFP, anti-SHLMWE and anti-CSHE blots (both from Southern Biotechnology Associates, Birmingham, Ala, USA). The binding of HRP was detected by incubation for 10 min with the Super-Signal West Pico Chemiluminescent Substrate (Pierce).

Immunoassays

SFP human IgG4 inhibition EIA (SF-h). The SF-h assay for SFP was performed essentially as described previously for the wheat allergen IgG4 inhibition EIA [24]. Microtitre plates (Microton, Greiner – Bio One, Alphen a/d Rijn, the Netherlands) were coated overnight at 4 °C with SFP at 5 µg/mL (200 µL/well), washed with PBS-0.05% Tween-20 (PBT) and blocked for 60 min at 37 °C with PBT-0.2% gelatine (PBTG), which was also used as a diluent in the subsequent steps. Test samples diluted 1/5, 1/10 and 1/20, and SFP standard (eight serial dilutions from 4 µg/mL to 31 ng/mL) were added, both at 100 µL/well, after which 100 µL of the human anti-SFP IgG4-positive pooled serum diluted 1/200 was added. On each plate, there were three wells of the 0% inhibition control (no antigen, only the IgG4 serum pool) and three blanks (no antigen, no serum). After incubation for 120 min at 37 °C, IgG4 binding was measured by incubation (1 h) with HRP-labelled mouse monoclonal anti-human IgG4 (1/1000; Sanquin, Amsterdam, the Netherlands), and finally, o-phenylenediamine (OPD) (Sigma-Aldrich, St Louis, MO, USA) with 0.015% H₂O₂. After stopping the reaction with 50 µL of 2 M HCl, the OD at 492 nm was read. SFP concentrations in test samples were calculated from the SFP standard calibration line made by semi-log curve fitting with the Softmax program (Molecular Devices Corporation; Menlo Park, CA, USA). The lower and upper limits of detection (LOD) of the

assay were defined as the standard concentrations, giving 15% and 85% inhibition, respectively [24].

Rabbit SFP sandwich EIA (SF-r). The protocol for SF-r was derived from the analogous sandwich EIA for wheat proteins [24]. Microtitre plates (Microton) were coated overnight with rabbit IgG anti-SFP at 10 µg/mL in PBS (100 µL/well), and blocked with PBTG. The SFP standard was added at eight serial dilutions from 2 µg/mL to 16 ng/mL, and test samples at 1/5, 1/10 and 1/20 (100 µL/well). After a 1 h incubation for 60 min, captured soy proteins were quantified by 1 h incubations with biotinylated rabbit anti-SFP (1/200), and 1/2000 avidin-alkaline phosphatase (AP; Calbiochem, Darmstadt, Germany), and 60 min with 1 mg/mL paranitrophenylphosphate (Sigma-Aldrich) in 10 mM diethanolamine containing 2 mM MgCl₂ (pH 10.6). The reaction was stopped with 50 µL of 2 M NaOH, and the OD was read at 405 nm. Assay results were calculated with the four-parameter curve fitting option (Softmax). Lower and upper LODs were defined as the SFP concentrations giving OD₄₀₅ values of 0.05 and 3.0 above the mean OD of the blanks (PBTG instead of sample).

Rabbit SHLMWE and CSHE inhibition EIAs (SH-inh and CSH-inh). Wells of high-binding microtitre plates (Costar, Cambridge, MA, USA) were coated with 100 µL/well of SHLMWE (1.25 µg protein/mL) in 0.2 M Na₂CO₃/NaHCO₃ buffer (pH 9.2) overnight at room temperature. After three washes with PBST (0.1 M PBS with 0.1% Tween 20, pH 7.4), the wells were blocked with PBS-1% bovine serum albumin (BSA) (1 h, 37 °C), and incubated overnight at room temperature with 50 µL of either test samples or the SHLMWE reference preparation plus 50 µL/well of rabbit IgG anti-SHLMWE at 1/25 000. After three washes with PBST, plates were incubated (1 h 37 °C) with 100 µL/well of HRP-labelled goat anti-rabbit IgG (γ heavy chain) (Southern Biotechnology Associates) at 1/7000, and washed again. The reaction was developed with 0.3% H₂O₂, 3,3',5,5'-tetramethyl-benzidine in acetate buffer, pH 5.5, at room temperature in the dark. After 20 min, the reaction was stopped with 2 M H₂SO₄ and the OD was read at 450 nm Titertek Multiskan Plus MKII (Labsystems, Helsinki, Finland). The standard curve was made from six data points using a four-parameter logistic curve fit, and the percentage inhibition was calculated by comparison with the OD₄₅₀ in wells with non-inhibited antibody. Genesis software release 2.16 Life Sciences Ltd, (Basingstoke, UK) was used to calculate the results. The same procedure was used for CSH-inh, except that the microwells were coated with 100 µL of CSHE (4.9 µg protein/mL), and that rabbit IgG anti-CSHE (1/10 000 dilution) was used as the primary antibody, and HRP-labelled goat anti-rabbit IgG was used at 1/1000.

The specificity of the assays was studied by testing extracts from several common legumes (green bean, green pea, chickpea and peanut), other legumes and cereals

unloaded in the Barcelona harbour, such as corn, rye, field pea and wheat fodder (prepared as described by Gómez-Ollés et al. [15]) and the outdoor allergens *Aspergillus fumigatus*, *Penicillium notatum*, *Platanus hybrida* and *Parietaria judaica* (Laboratorios Leti, Madrid, Spain).

Rabbit SHLMWE amplified sandwich EIA (SH-amp). SH-amp was performed as described previously [15]. Briefly, the rabbit IgG anti-SHLMWE was used both as the coated capturing antibody and – in biotinylated form – as the detecting antibody, and biotin binding was detected with poly-HRP80-streptavidin (1/15 000; Research Diagnostics, Flanders, NJ, USA). The sensitivity of the assay was 40 pg/mL. The calibration standard series of SHLMWE ranged from 0.039 to 5 ng/mL, and results were read from a standard curve constructed from eight data points using a four-parameter logistic curve Genesis software release 2.16 (Life Sciences Ltd, Basingstoke, UK).

Statistical analysis

Statistical procedures for comparison of EIAs were essentially as described in the previous study comparing wheat allergen EIAs [24]. The percentage of positive samples, the geometric mean and range of soy aeroallergen levels were calculated. Samples with undetectable levels were assigned a value of two-third of the lower limit of detection. Because data showed in general log-normal distributions, quantitative comparisons between EIAs were made by calculating Pearson's correlation coefficients for ln-transformed allergen concentrations, but only for samples with detectable levels in both assays. Agreement between methods that measure the same allergens was determined by calculating the geometric mean of the ratio among soy aeroallergen levels detected by two methods, both soy flour or both LMW soy hull assays, each time in only double-positive samples. SPSS release 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

Results

Characterization of soy protein standard preparations

The SFP and the two soy hull extract preparations (SHLMWE and CSHE) had a strongly different composition (Fig. 1a). Coomassie staining after SDS-PAGE of SFP revealed 13 protein bands between 5 and 133 kDa (lane 2), whereas the SHLMWE showed two heavily stained bands at 5–6 kDa and weak bands at 10–12 kDa (lane 3), and the CSHE showed nine protein bands between 5 and 101 kDa (lane 4). The lowest MW band of the soy hull extracts was identified by peptide mass fingerprinting as a hydrophobic seed protein, described by Odani et al. [25]. This protein is highly homologous with the main allergen of

the Barcelona asthma epidemics, Gly m 1, and a low amount of it – or a protein with very similar MW – could also be seen in the SFP preparation.

Comparison of antibody-binding profiles

The antibodies used in the various EIAs differed markedly in their anti-soy reaction patterns (Fig. 1b). Human IgG4 recognized in SFP predominantly larger proteins with MWs from 40 to 102 kDa (lane 2) and showed in the CSHE blot weak reactions (lane 10), but failed to recognize any band of SHLMWE (lane 6). Rabbit IgG anti-SFP reacted with more SFP proteins, not only in the 40–102 kDa range (lane 3). Rabbit IgG anti-SFP also reacted strongly with LMW proteins of SHLMWE (lane 7), and with both HMW and LMW bands of CSHE (lane 11). Rabbit IgG anti-SHLMWE reacted with four bands from 5 to 12 kDa of the SHLMWE (lane 8), and – with much less intensity – also with some HMW proteins of SFP (lane 4) and CSHE (lane 12). The rabbit IgG anti-CSHE reacted with four bands of CSHE with MWs close to 5, 7, 18 and 54 kDa (lane 13), in SFP with various proteins of 30–60 kDa (lane 5), and in the SHLMWE with LMW proteins (lane 9).

Assay characteristics

The two rabbit LMW soy hull EIAs differed strongly in sensitivity: the LOD of SH-inh was 200 ng/mL, while SH-amp had a greater sensitivity with a LOD of only 0.04 ng/mL. The two flour EIAs, which used the same SFP preparation, but different antibodies, had a similar LOD (10–40 ng/mL), and the LOD of CSH-inh was approximately 40 ng/mL. In specificity studies, SH-inh was found to be highly specific, with no cross-reactivity with any of the tested non-soy extracts. Field pea and corn extracts showed some reactivity in CSH-inh, but only up to 50% inhibition at the highest protein concentrations tested (data not shown). The SH-amp showed some cross-reactivity with soy seed and field pea, and with some cereal extracts (corn, rye and wheat fodder) at very high protein concentrations [15], while all other allergen preparations were completely negative.

Intra-day coefficients of variation (CV) in the various EIAs varied from 5% to 19%, and inter-day/inter-assay CVs (for samples tested twice on different days) varied from 16% to 20%.

Airborne soy protein measurements with the different immunoassays

A total of 324 airborne dust samples, from six different workplaces, were analysed by the five EIAs. For some assays, the numbers of valid results were slightly lower due to technical failures. As shown in Table 1, SH-inh resulted in the lowest numbers of positive samples (23%),

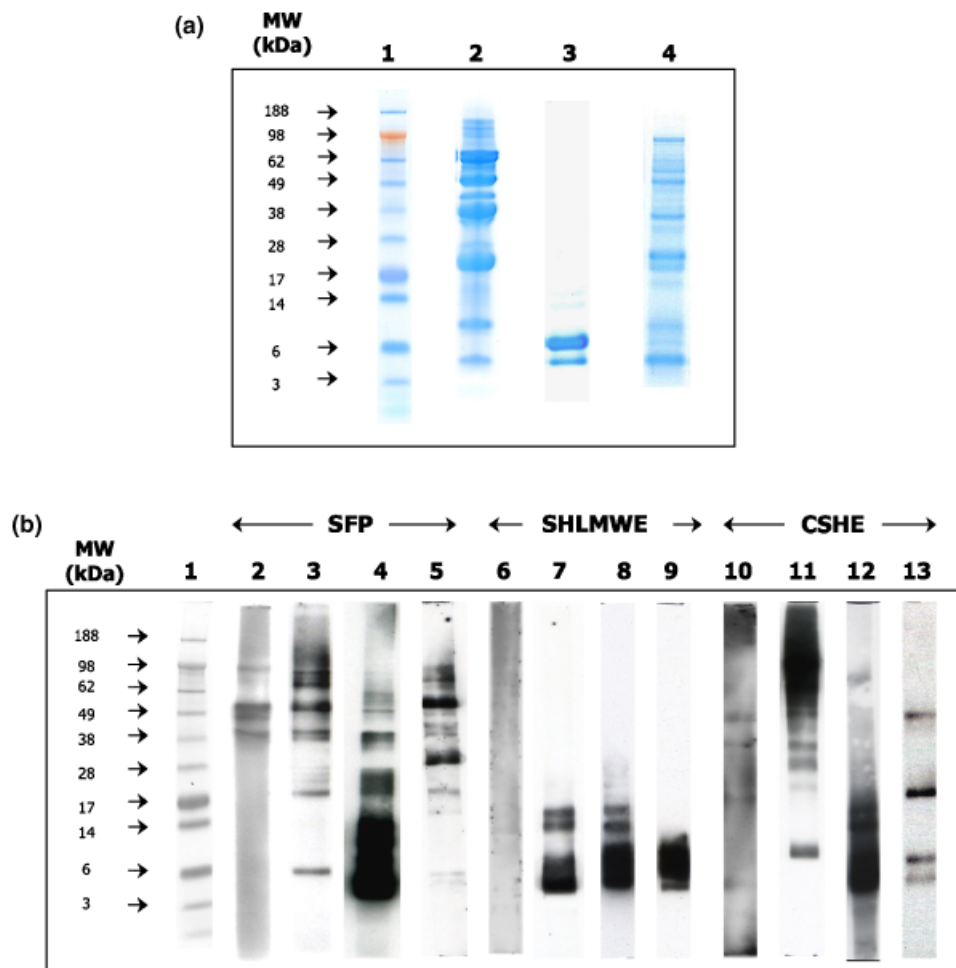


Fig. 1. Electrophoresis and immunoblotting analysis. (a) SDS-PAGE of soy standards Coomassie stained. Lane 1, molecular weight markers (MW); lane 2, soy flour protein (SFP); lane 3, soy hull low molecular weight allergenic protein SHLMWE and lane 4, complete soy hull extract (CSHE). (b) Western blot of the anti-soy antibodies against the three soy standards. Lane 1: MW markers; lanes 2, 6 and 10: immunoblots with human IgG4; lanes 3, 7 and 11: immunoblots with rabbit IgG anti-SFP; lanes 4, 8 and 12: immunoblots with rabbit IgG anti-SHLMWE and lanes 5, 9 and 13: immunoblots with rabbit IgG anti-CSHE.

in accordance with its low sensitivity, whereas SH-amp yielded the highest percentage of positive results (87%).

Figure 2 shows the results for pair-wise EIA comparisons. Pearson's correlation coefficients calculated for samples with levels $>LOD$ in both assays revealed high correlations between the EIAs using human or rabbit anti-SFP antibodies ($r=0.87$; Fig. 2a), and between the two EIAs designed to measure the LMW soy hull antigen ($r=0.91$; Fig. 2b). In contrast, comparison of the LMW soy hull assays with soy flour assays showed only a moderate level of correlation for the SH-amp ($r=0.53$ and 0.61 ; Figs 2c and d), and a weak or hardly any correlation for the SH-inh ($r=0.34$ and 0.06 ; Figs 2e and f).

Correlation analyses for CSH-inh showed a somewhat surprising pattern: its results correlated only moderately ($r=0.54$ and 0.69 ; Figs 2g and h) with the values measured by LMW soy hull assays, while on the other hand strong

correlations ($r=0.86$ – 0.92 ; Figs 2i and j) were found with the results of both soy flour assays.

Differences due to sampling location

Correlations between assay results were examined in more detail by taking into account the source of airborne dust samples, which clearly also determined the frequency of positive results (Table 1). The assay with the lowest sensitivity, the SH-inh, was positive for approximately 50% of the samples from facilities where soy was unloaded (companies 1 and 2), but for only 17% of the samples from the animal feed industry (company 3) and 22% of the samples from the soy processing plant (company 4), and no LMW soy hull protein at all was detected in samples from pig stables (companies 5 and 6). In contrast, both the two soy flour assays and the CSH-inh

Table 1. The percentage of positivity, geometric mean and range of soy aeroallergens in 324 dust sample eluates analysed by five different immunoassays for each sampling site

Worksite	Parameter	Assays				
		SF-h	SF-r	SH-inh	CSH-inh	SH-amp
Company 1. Soybean unloading and processing	% > LOD	64.8	66.7	55.6	69.8	88.9
	GM (all)	169.1	93.3	529.5	291.7	82.6
	GM (> LOD)	353.9	271.2	1681.8	816.4	202
	Range	35–1037.2	6.3–773.1	120–4000	27–2160	0.05–2231.6
Company 2. Soybean unloading	% > LOD	9.3	40.7	44	69.2	83.3
	GM (all)	55.7	9.3	217.8	99	39
	GM (> LOD)	165.4	28.3	504.7	176.3	137.5
	Range	31.4–220.4	0.9–85.3	90–1220	27–580	0.05–669
Company 3. Animal feed industry	% > LOD	72.2	83.3	22.2	81.6	90.7
	GM (all)	173.9	56.9	166.4	219.2	19.1
	GM (> LOD)	289	83.7	475.8	351.2	33.6
	Range	44.6–1584.7	5.1–472.5	120–1900	27–1960	0.08–414.3
Company 4. Soy processing	% > LOD	77.8	83.3	17.3	88.2	83.3
	GM (all)	461.8	130.5	150.8	396.7	14.8
	GM (> LOD)	834.7	258.9	370.5	567.7	44.2
	Range	44.9–4329.7	3.2–3550.2	90–710	27–3810	0.05–216.7
Company 5. Pig stable 1	% > LOD	50	46.3	0	55.6	83.3
	GM (all)	80.8	10.7	142.1	63.3	0.7
	GM (> LOD)	140.7	19.3	–	125.3	1.1
	Range	44.9–273.2	5.4–63.2	130–160	27–230	0.05–6.2
Company 6. Pig stable 2	% > LOD	20.4	24.1	0	33.3	90.7
	GM (all)	62.9	9.8	124.9	37.5	0.9
	GM (> LOD)	85.1	25.9	–	72.4	1.2
	Range	43.5–139.9	6.1–51.9	110–160	27–130	0.05–21.2
All	% > LOD	49.1	57.4	23	66.4	86.7
	Range	31.4–4329.7	0.9–3550.2	90–4000	27–3810	0.05–2231.6

LOD, limits of detection; CSH, complete soy hull; SF-h, SFP human IgG4 inhibition EIA; SF-r, Rabbit SFP sandwich EIA; SH-amp, Rabbit SHLMWE amplified sandwich EIA.

found the highest numbers of positive samples in the animal feed industry (company 3) and the soy processing plant (company 4), followed closely by the soy unloading and processing plant (company 1). In fact, the prevalence of positive samples found with these three assays was similar for most locations, but not for the soybean unloading plant (company 2) where SF-h detected soy antigens in only 9% as compared with a relatively high 69% for CSH-inh and 41% for SF-r.

The type of sampling location also had an impact on the correlations between EIAs results, as illustrated by the use in Fig. 2 of separate symbols for each sampling site. It appeared that not only levels of soy aeroallergen measured in the various EIAs differed depending on the location where the sampling was performed, but that their relative ranking also depended on the applied EIA. Accordingly, in all analyses showing a low moderate correlation (Figs 2c and h), the results for each sampling site tended to be clustered in a specific area of the scatter plot. Thus, Table 1 and Fig. 2a show that the soy flour assays measured the highest levels in the Dutch animal feed and

soy factories (companies 3 and 4) and the Spanish soy unloading a processing plant (company 1), whereas samples collected in companies 2, 5 and 6 – soy unloading only, and pig stables – had on average much lower values. The agreement analyses corroborate those findings, showing that the magnitude of difference between both methods was not related to the antigen load but to the sampling sites with the best agreement between SF-h and SF-r assays in samples from company 1 (Fig. 3a). On the other hand, the two assays for LMW soy hull proteins showed the highest levels in companies 1 and 2, thus at the two worksites where soybeans were unloaded, followed by the animal feed and soy factories in the Netherlands, which on average had approximately threefold lower levels than company 1 (Fig. 2b). With both LMW soy hull assays similar outcomes were observed, with the best agreement between SH-inh and SH-amp assays in samples from company 1, followed closely by samples from company 2 (Fig. 3b). The CSH-inh again appeared to take an intermediate position: the highest values were found at the worksites where soybeans are processed, while the levels

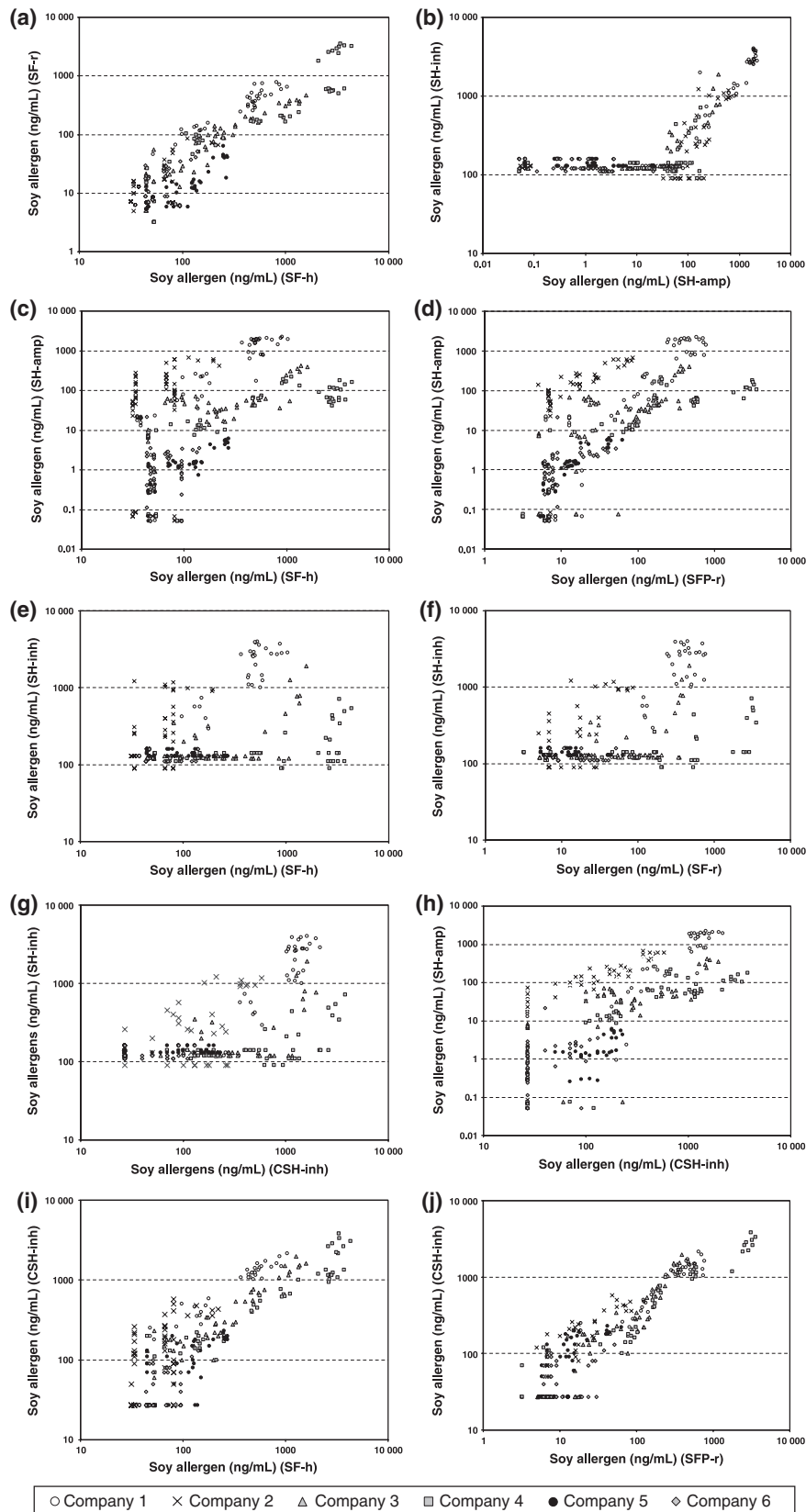


Fig. 2. (a-j) Scatter plots showing correlation between soy allergen concentrations measured by the five immunoassays in 324 airborne dust samples obtained at six different companies.

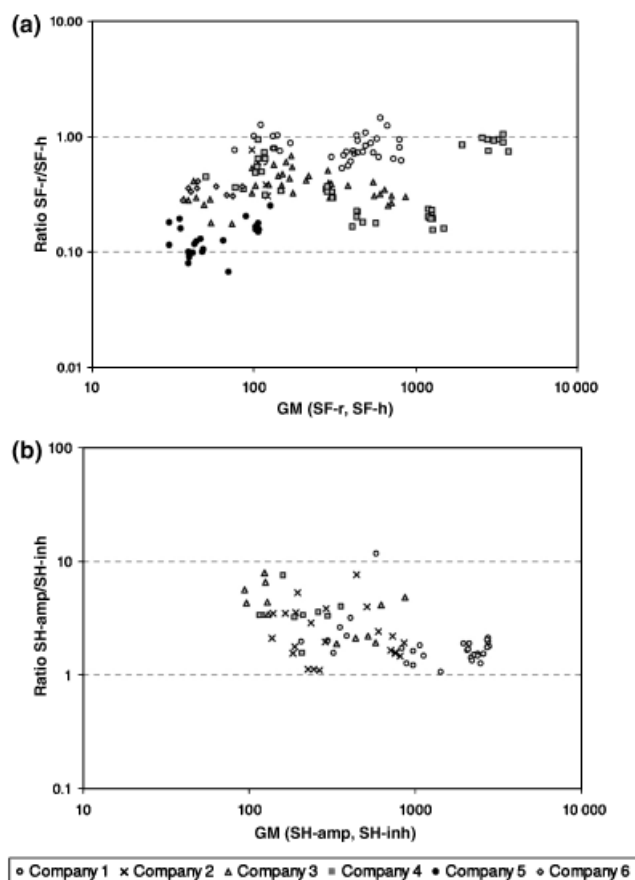


Fig. 3. Relative difference (ratio) plotted against the geometric mean of soy aeroallergen levels measured by two methods designed to measure the same antigen. Only double-positive samples are included. (a) Agreement study between soy flour assays. (b) Agreement study between low molecular weight soy hull assays.

in company 2 – only soybean unloading – were nearly three to fourfold lower, and approximately twofold lower than in the Dutch animal feed industry, which showed intermediate levels. The levels in the pig stables were in general low for all the immunoassays.

Discussion

It has been well established that the allergens responsible for the adverse reactions following oral ingestion of soy are not the same as the causative agents of soy dust-induced asthma epidemics, and the latter may differ from the proteins involved in cases of occupational asthma in bakers exposed to soy flour [10]. LMW proteins from the soybean hull [6, 7], Gly m 1 [8, 26] and Gly m 2 [27] have been identified as the main allergens that cause the asthma outbreaks, while allergens responsible for soybean flour-induced occupational asthma would be mainly HMW proteins [9, 28]. Soy hull components may, however, also have been involved in occupational asthma reported in animal feed manufacturers [11].

Thus, 'soy aeroallergen exposure' might vary markedly between worksites where soy is handled, not only quantitatively – in the levels of exposure – but also qualitatively – in the relative presence of different soy allergenic components in the dust, and this could make soy exposure assessment a complex issue.

In this study, airborne soy allergen levels were measured with five EIAs with different specificity and/or sensitivity. The SF-h and SF-r EIAs for SFP were both developed and performed by IRAS in the Netherlands, for application in the animal feed industry, while the three other assays were developed by HUVH in Spain, primarily for the monitoring of outdoor air soybean dust and were thus focused on soy hull extracts and isolated soy hull allergens. A large series of airborne samples were collected in three European countries in soy plants, animal feed industries and in pig farms. The general design of the MOCALEX study implied the use of an identical airborne parallel sampling procedure at all worksites, with filters that had been pre-weighed in a central laboratory (IRAS), and to which they also were returned after sampling for post-weighing and extraction [18]. Replicate aliquots of the same filter eluates were either analysed at IRAS or at HUVH. Thus, the use of the same eluates in the five immunoassays precluded any discrepancies in soy allergen yields due to the application of different storage or extraction procedures, and differences in the reported concentrations should be completely due to differences in EIA characteristics. Analysis of the standard soy preparations by SDS-PAGE showed that the CSHE and the SFP contained proteins with a wide range of MWs, while the purified soy hull allergen preparation (SHLMWE) contained practically only proteins with LMW. The immunoblotting experiments reflected the three different binding profiles of the antibodies used in the EIAs. The largest difference was seen between the IgG4 in the pool of selected human sera, which reacted mainly with HMW proteins, and the rabbit anti-SHLMWE antibody that predominantly bound to the LMW protein identified by mass spectrometry as a hydrophobic seed protein, which is highly homologous to Gly m 1, the major allergen responsible for the asthma outbreaks in Barcelona [8]. The rabbit anti-SFP and anti-CSHE antibodies recognized both LMW and HMW proteins.

Comparison of allergen levels measured by the different EIAs showed a very strong correlation between several of the methods, with the highest correlation coefficient (0.92) between the results of SF-r and CSH-inh. Poor correlations were, however, also found, with, e.g. practically no correlation ($r=0.06$) between SF-h and SH-inh. The percentage of positive samples also differed in the various assays, with values ranging from 23% to 87%. The SH-amp yielded the highest percentage of positive results, and the sensitivity of the assay (0.04 ng/mL) was much better than that of inhibition assays (10–200 ng/mL). These differences between the EIAs can be attributed in

part to differences in the specificities of antibodies and allergen standards, as found in other studies where differences in antibody specificities accounted for up to 800-fold differences [29].

Reported soy aeroallergen levels, even agreement between assays for measuring either soy flour or LMW soy hull proteins, also depended strongly on the sampling site. Notably, the relative ranking of exposure levels in various work environments depended on the EIA applied, and this could also be ascribed to the different specificities of the EIAs. Thus, the data strongly suggest that airborne samples from different work environments with 'soy exposure' may contain 'soy dust' with quite different compositions. For example, soy hull antigens are more commonly found at sites where whole soybeans are unloaded and stored, such as at the harbour silos in Barcelona [6, 8], whereas in a work environment where processed soybean and flours are used – like the feed and food industries – the airborne soy proteins might be mainly soy flour derived. Differences in soy allergen composition of airborne samples and differences in EIA antibody specificity might also explain the very low correlations between some of the assays, notably the SH-inh, which detects only soy hull antigens, and the SF-h, which exclusively detects HMW allergens, assays. On the other hand, at several sampling locations both types of assays were positive, which suggests that there may be quite a few worksites where both types of soy allergens occur simultaneously and their levels even correlate.

Thus, no unambiguous answer can be given to the question regarding the optimal procedure for airborne soy allergen exposure assessment. In addition to the sensitivity, specificity and reproducibility of the assays and the availability of reagents, a common difficulty especially when working with human sera, the work environment that is going to be evaluated should be considered. Gly m 1, the previously identified prominent allergen involved in soy asthma epidemics [8], was in this study indeed found relatively often at the harbour sites where soy is unloaded. Thus, CSH-inh and SH-amp EIAs can be recommended for soybean dust measurements in this work environment. Because of its high sensitivity and specificity, SH-amp might be the first test of choice, but for budget reasons CSH-inh could be a worthwhile alternative.

For work environments where soy flour predominates, such as bakeries or the animal feed industry, SF-h, SF-r and CSH-inh might be suitable EIAs. These assays had similar absolute sensitivities, and the antibodies used had similar allergen-binding properties, and the EIAs showed high mutual correlations and a percentage of samples with detectable values that was also similar for most locations. SF-h and SF-r gave the highest levels in animal feed industries and soy-processing plants. Nevertheless, CSH-inh also yielded higher concentrations at the soy processing plant, but the levels of soy allergen at animal feed industries were twofold lower. Given the much higher titre

of the rabbit antisera, and the better long-term availability of the reagents, SF-r and CSH-inh would be the first choices for use in the animal feed and soy-processing industry. The allergenicity of SFP is, however, less well documented than that of soy hull proteins, and for this reason it could be of value to include as yet each of SF-h, SF-r and CSH-inh EIAs in future research studies of assessment of soy aeroallergen levels in facilities where soy flour is present, to improve the understanding and advancement of this issue.

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