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Levels of soya aeroallergens during dockside unloading as measured by personal and static sampling

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Soya is an important worldwide agricultural product widely shipped and imported in bulk. It contains a number of recognised allergens and the use of soya products and its dockside unloading have been associated with occupational asthma and community episodes of asthma. Two recognised inhalation soya allergens, soya trypsin inhibitor (STI) and hydrophobic soya protein (HSP), were measured in personal and static air samples collected at a United Kingdom (UK) dock during 3 days of unloading three bulks of processed soya beans and soya pelletised husk. Static samples included task-related and those taken at the workplace perimeter and neighbouring sites. Soluble total protein (STP) and gravimetric dust analyses were also undertaken. While gravimetric dust results in personal air samples were below half of the current UK exposure limit of 10 mg m^{-3} for grain dust, and generally less than 0.5 mg m^{-3} for the static samples, airborne concentrations for STI and HSP ranged between 0-3,071 and 11-12,629 ng m^{-3}, respectively, while the correlation between the two specific allergen measurements was generally good (Rank Spearman coefficient 0.74). The data from this investigation suggest that HSP is a more sensitive indicator of soya exposure than STI, but only for soya husk, while STI may be equipotent in detecting exposure to both hull and bean derived soya products. Both assays appear sensitive techniques for investigating the control of exposure to allergenic soy material. The endotoxin level in the husk bulk was 15-60-fold that found in the two chipped bean bulks.

KEY WORDS: *allergens; air samples; atmospheric monitoring; hydrophobic soya protein (HSP); soya trypsin inhibitor (STI)*

Soya has become one of the most important agricultural products worldwide, being a widely used source of protein, oil, and biofuel. The United Kingdom (UK) imported some 3.3 million tonnes of soya in 2010, largely as processed beans and husks (hulls) or whole beans. Soya oil alone accounted for about 0.11 million tonnes. The principal uses are in poultry feed, dairy cattle, and pig rations. Other major uses of soya products, including soya flour and oil, are in the food and bakery industries. Soya products are imported at a number of UK ports equipped to handle bulk grain, agrochemicals, and foodstuffs.

While constituents of soya are one of the top eight causes of food allergies, proteins within the soybean and its husk are also occupational and environmental allergens by inhalation. Outbreaks of epidemic asthma related to soya dust inhalation have been reported in a number of harbour cities such as Verona (1), St Nazaire (2), New Orleans (3), Naples (4), Cartagena (5), Tarragona (6), Coruña (7), Valencia (7), and Barcelona (8). Exposure to soya dust and soya flour has been reported to cause occupational asthma (OA) in persons working in a variety of occupations, such as farmers, mill workers, soybean handlers, and bakers (9-12).

Allergens causing OA in bakers seem to differ from those related to asthma outbreaks in the community (13). Serum from asthma epidemic patients reacted to an acidic low molecular mass glycoprotein (<14 kDa) located principally in the hulls and dust of soybeans unloaded in the harbour (14, 15). Subsequent studies described two isoallergens, Gly m 1a and Gly m 1b, with molecular weights of 7.0 and 7.5 kDa, respectively, as the main allergens responsible (16). These isoallergens were highly homologous with the hydrophobic soybean protein (HSP) described by Odani (17). Individual allergic response to inhaled soybean flour components differed in bakers (13), but mainly involved higher molecular weight allergens (10, 13, 18). A study of bakers with work-related respiratory symptoms and sensitised to soya flour suggested that Kunitz soybean trypsin inhibitor (STI) (21 kDa, pI 4.5) and lipoxidase were the major allergens (10). Subsequently, bronchial reactivity to Kunitz STI was confirmed in two bakers with work-related asthma symptoms exposed to soya flour (19).

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A number of immunoassays for monitoring soya aeroallergens had been investigated as part of the MOCALEX project (20) funded by the European Union (EU). The results suggested that the optimal immunoassay for soya aeroallergen exposure assessment may depend on the type of work and the local soy dust composition. One of the methods developed was a sensitive and specific polyclonal-based amplified immunoassay for HSP (21) that has subsequently been used to monitor exposure from soya plant activities in Barcelona.

STI is a stable protein and allergen found in soya beans, flour, and the husk (22). Therefore, its measurement could act as a useful surrogate of inhalation exposure to soya-associated allergens in general, as well as reflect exposure to a specific major soya allergen. The Health and Safety Laboratory (HSL) had developed a polyclonal antibody based immunoassay for STI in order to monitor atmospheric levels of soya dust in bakeries.

Organic dusts, such as that derived from soya products, are complex mixtures containing not only allergenic proteins derived from soya itself, but also moulds and bacteria including pyrogenic material, such as endotoxins, that can cause a range of respiratory symptoms.

An occupational hygiene investigation of soya handling at a UK dock was initiated by the Health and Safety Executive (HSE) subsequent to reported respiratory symptoms in some dockyard workers and a neighbouring workplace from the previous soya unloading. This report compares the two immunoassays for HSP and STI, soluble total protein (STP), and gravimetric dust analysis measured in air samples during this investigation. Extractable levels of both allergens, STP, and endotoxin were also measured in the off-loaded bulks. It had originally been planned to measure endotoxin in the air samples, but the background level of endotoxin found in the batch of buffer used to extract the air filter samples precluded this.

MATERIALS AND METHODS

Immunoassays to STI and HSP were developed at HSL and the Vall d'Hebron Hospital, Barcelona, respectively. The rabbit polyclonal sandwich immunoassay for HSP has been described previously (21). The STI immunoassay was based on a polyclonal sandwich assay using commercially available antisera (Chemicon Ab1239, Temecula, USA) for capture antibody and as detection antisera after conjugation with long chain N-hydroxysulfosuccinimidobiotin (Vector labs, Peterborough, UK). Avidin-peroxidase (Vector labs) and 3,3′,5,5′-Tetramethylbenzidine (Insight Biotechnology, Wembley, UK) were used for colour development after stopping with 0.5 mol L^{-1} sulphuric acid and reading the absorbance at 450 nm. The standard range of the STI assay covered 0-25 ng mL^{-1} with a lower limit of quantitation of around 0.1 ng mL^{-1} calculated using ProQuant software (Qivx Inc, Fort Collins, USA).

Soluble total protein (STP) was measured using a bicinchoninic acid dye-binding method (Sigma-Aldrich, Poole, UK) and standardised against 1 mg mL^{-1} bovine serum albumin standard (Sigma-Aldrich, Poole, UK). Gravimetric analyses were carried out according to Methods for the Determination of Hazardous Substances Guidance No. 14 (23). Endotoxin was measured using a commercial Limulus Amebocyte Lysate assay (Lonza, Slough, UK) and employing a standardized spiking technique to check for any interference in the extracts. Results for soluble protein, allergens, and endotoxin readily extractable from the bulk samples were expressed per unit weight of the bulk material.

Workplace and unloading procedure

The dock where the soya was unloaded and the samples collected is situated to the west of the centre of a UK city with 250,000 inhabitants. Container ships are emptied after mooring using a track-guided grab crane operating from the dockside. This deposits the loose granular soya material from the ship's hold into one of two hoppers from where it is guided directly into a tipper lorry or onto a conveyor for transport into one of several warehouses. Once partially empty, a slewer excavator is lifted into the hold to move the remaining cargo, allowing continued use of the grab crane. Final emptying of the ship's hold may involve a skid-steer loader and manual labour to clean the hold. Within the warehouses, vehicles push the cargo into large piles, while other loaders load tipper lorries to transport the soya offsite. Figure 1 shows a diagram of the dock soya operation and the neighbouring area. There is no processing of soya on-site. Twelve workers work twelve-hour shifts and rotate between jobs.

Air sampling

Personal and static air samples (n=37) were collected over 3 days of unloading of three soya bulks. Personal samples were collected from those undertaking dockside supervision, the operator of the slewer excavator in the ship's hold, individuals who monitored operations from both the ship's hatch above the hold and the dock-side hopper during a shift, and individuals who spent most or all of their time working in the soya warehouses during loading or moving of bulk material by shovel loader or pusher vehicles.

Static samples included those related to specific work tasks as well as those positioned to gauge potential exposure in the wider environment. Static samples related to specific work tasks included the crane cab, by the open hatch of the ship, beneath the conveyor hopper, and in the vehicle cabins of both pusher and shovel loader. Other static samples identified in Figure 1 were collected to monitor the perimeter and neighbouring areas; these included the eastern end of the conveyor, beneath the conveyor between railway line and road, just in front of the East soya warehouse, by the road entrance to the west of warehouse 107, trolley

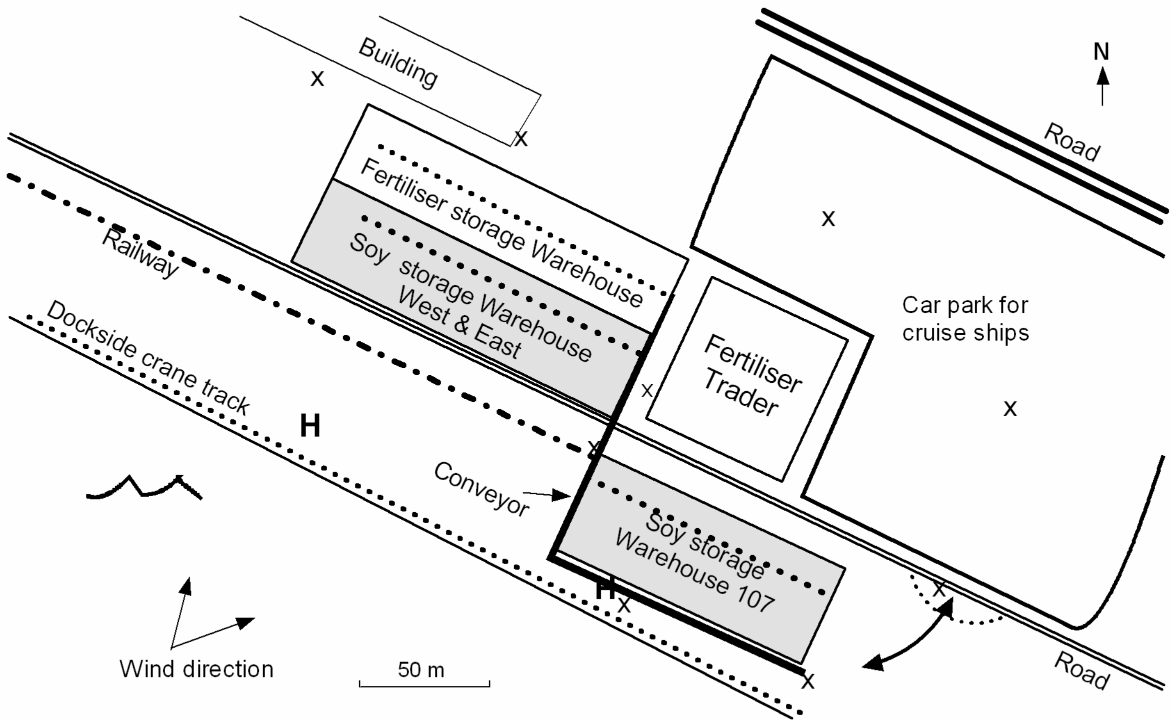

Figure 1 *Diagram of dock and surrounding area where soya was unloaded. The two warehouses coloured grey were where soya was stored. The position of the two hoppers used during unloading are identified by 'H'. The conveyor system takes bulk along two sides of warehouse 107 over the railway and road and to the soya/fertiliser warehouse. Branches of the main conveyor (shown as dotted lines) deposit the bulks into the warehouses, where they are moved by pusher vehicles. 'X' identifies the sampling sites at the site perimeter (e.g., at the road entrance to the east of warehouse 107, in front of east soya storage warehouse) or neighbouring areas (e.g., within car park used for passengers on cruise ships or end of building to north of fertiliser storage warehouse). Wind direction during the 3 days of sampling are shown with average daily winds speeds between 1.70-3.35 m s^{-1}*

shelter and lamp-post within the car park used by cruise line customers, at the end of a building P north of a fertiliser storage warehouse and by steps of the boundary wall of building P.

All sampling employed glass microfiber (GFA) filters in Institute of Occupational Medicine (IOM, Edinburgh, UK) sampling heads applying an airflow of 2 L min^{-1}. The median (range) durations of air sampling in personal and static samples were 412 (158-561) and 476 (158-580) minutes, respectively. On all of the three days, five static air samples were collected at the same positions within the dock site, its perimeter, and neighbouring areas. On the first day of sampling, pelletised soya husk was unloaded and transported by lorry to an off-site warehouse for storage, while on days 2 and 3 two different ground soya meals were handled and transported to warehouses using the conveyor system.

After gravimetric analyses, the filters were extracted in 2 mL of extraction buffer (0.1 % Tween-20 in PBS; Sigma-Aldrich, Poole, UK), for 2 h on roller mixers. Samples were analysed for STI and STP at HSL, and then sent frozen to the aforementioned collaborating laboratory in Spain for HSP measurement. Bulk soya samples (2 chipped soya beans and 1 pelletised soya husk) were extracted at 10 % w/v with extraction buffer and analysed as above, but with endotoxin measurement.

Non-parametric statistical analyses, including Rank Spearman correlation and Freidman's test, were carried out using MedCalc statistical software V13 (MedCalc Software, Ostend, Belgium). Actual *p*-values are quoted, with $p<0.05$ considered as statistically significant.

RESULTS

Workplace observations during sampling included that, while the vehicles (not lorries) used in the ship's hold and warehouses were fitted with HEPA filters, their doors were opened at intervals either to clear accumulated dust off the windscreens or for other reasons, allowing dust into the cabs. The crane grabber frequently spilled soya above the hold and hopper leading to peaks of airborne dust. The enclosure around the conveyor was to keep the weather out rather than contain the bulk in. Significant airborne dust was visible in warehouses where soya was being piled up or loaded onto tipper lorries for transportation.

The amount of STI found in soybean and pelletised husk (hull) bulk samples was relatively constant (233-798 µg g^{-1}); while as expected the HSP was a far greater component of the husk bulk sample compared to the ground soya bean samples (2824 *vs.* 178 µg g^{-1}) (Table 1). The amount of extractable endotoxin was considerably higher in the husk

Table 1 *Amounts of two specific allergen measurements, total protein, and endotoxin extracted from the bulk samples*

Bulk material	STI ($\mu g\ g^{-1}$)	HSP ($\mu g\ g^{-1}$)	STP ($mg\ g^{-1}$)	Endotoxin ($EU\ g^{-1}$)
Pelletised soya husk (hull)	798	2824	21	80364
Fine ground soya	270	196	44	4630
GM-free ground soya	233	178	42	1309

STI-soya trypsin inhibitor
HSP-hydrophobic soya protein
STP-soluble total protein

bulk compared to the chipped bean bulks. The pelletised husk bulk showed some evidence of breakdown, presumably as compaction during transportation.

Inhalable dust exposures for personal samples (gravimetric), expressed as eight-hour time weighted average (TWA) ranged from 1.2-4.5 mg m^{-3}; the current UK workplace exposure limit (WEL) for both flour dust and grain dust is 10 mg m^{-3} (24). Static samples gave dust levels in the range 0.1-3.9 mg m^{-3}.

In all air samples, STP concentrations ranged from 6 to 183 µg m^{-3}; the median value being 51 µg m^{-3}; the median values (range) for the allergens STI and HSP were 47 (ND-3,071) and 266 (11-12,629) ng m^{-3}, respectively. Medians and ranges for the allergens in air samples characterised as personal, job-related static and environmental static are shown in Table 2, as well as data for individual tasks and sites. HSP results were generally higher than STI but well correlated (Rank Spearman correlation coefficient r=0.74, Table 2, Figure 2), except for the three samples collected at the same peripheral static monitoring site (end of building P), where significant levels of HSP were detected but STI levels were at or below the assay's detection limit .

Rank correlation coefficients between the air measurements are shown in Table 3. The correlation between the two specific allergen measurements was significantly better than the correlations between the allergen measurements and total soluble protein (STP). The Passing & Bablock regression equation between the two specific protein assays was HSP=(-16.4)+6.04(STI) with no significant deviation from linearity. Airborne dust levels were significantly better correlated with STI allergen than with total soluble protein (STP), and better, albeit not significantly, than between dust and HSP levels.

Statistical analysis (Friedman test), comparing results for the five static sampling positions sampled on the three consecutive days, suggested that HSP results on day 1 were higher than on days 2 and 3 (medians 898, 42, and 111 ng m^{-3}, respectively; p=0.04). No significant differences were found over the days for STI and STP. These air sampling results are consistent with unloading of the pelletised husk material on day 1.

DISCUSSION

While all the personal air samples were less than half of the current UK WEL for grain dust; such exposures,

Table 2 *Measured levels of allergens in air samples by group (personal, job-related static and environmental static) and for each specific job, site. Median and (range) shown where possible*

Sample	Days sampled	STI ($ng\ m^{-3}$)	HSP ($ng\ m^{-3}$)
All personal samples	**1-3**	**130 (33-3071)**	**583 (170-12629)**
Dockside supervision*	1-3	55 (51-1045)	582 (170-12629)
Slewer operator in hold	1-2	(63-3071)	(585-6528)
Combined hopper and hatch man	2-3	(197-223)	(266-885)
Most/all time moving soya in warehouse	3	(42-601)	(255-2215)
All job-related static samples	**1-3**	**216 (11-845)**	**1970 (40-7438)**
Crane cab	1-2	(11-19)	(64-2925**)
Open hatch of ship	2	26	40
Beneath conveyor hopper	3	845	2757
Pusher/shovel cabs in warehouses	1-2	269 (216-699)	1970 (1150-7438**)
All environmental static samples	**1-3**	**21 (ND-494)**	**86 (11-1684)**
Eastern end of conveyor	1-3	16 (7-56)	97 (42-118)
Beneath conveyor between railway & road	1-3	389 (71-494)	630 (353-1684**)
In front of East soya warehouse	3	27	24
Road entrance, west of warehouse 107	1-3	19 (2-24)	54 (26-85)
Two sites in cruise ship car park	2-3	13 (ND-40)	26 (11-125)
End of P building	1-3	ND (ND-4)	87 (30-1300**)
Steps at boundary wall for P building	1-3	56 (7-80)	339 (27-898**)

STI-soya trypsin inhibitor; HSP-hydrophobic soya protein; ND-not detected
**high value where dockside supervisor cleaned the conveyor*
***high value associated with day 1 when husk bulk was unloaded*

including soya, are acknowledged to contain allergenic proteins that may sensitise workers or precipitate respiratory symptoms in those already sensitised. Therefore in reducing the possibility of poor health outcomes, it is particularly important to control exposure to such biological material to as low as practically possible, rather than meeting the dust WEL.

This study made it possible to measure significant amounts of two proven soya inhalation allergens, namely HSP and STI, in both personal and static air samples. Three air samples were below the detection limit for STI, but all samples had measurable amounts of HSP. There is currently limited published air monitoring data for the immunoassay measurements of the two specific allergens. Using the same HSP assay, data collected as part of the MOCALEX study (20) showed air values ranging from 582 to 19,171 ng m^{-3} on different days in monitoring between a hull processing and flour machines in one plant located in Barcelona docks that unloaded and processed soya (flour and oil). In another soy plant that only unloaded soya beans from ships, sampling near the scales in the grain weighing room gave HSP results that ranged between 765-4,441 ng m^{-3} (personal communication Dr Susana Olles-Gomez). The HSP results in our study involving personal samples (range 170-12629 ng m^{-3}) and job-related static samples (range 40-7437 ng m^{-3}) are of the same order as the Barcelona results. However, as might be expected, our study has lower static results where static sampling was undertaken at the perimeter, or outside, of the dockside area.

A report on monitoring soya exposure in Verona (1) using a monoclonal antibody immunoassay for Gly 1 m showed a peak level of 171 ng m^{-3} close to a ship during unloading. If the results from the Gly 1 m and HSP assays are comparable, then our study would suggest the possibility of significantly larger peaks of exposure in the open environment of the dock during unloading. In a study of two soy processing plants in South Africa (25), where STI measurements were undertaken on personal air samples, measured STI values ranged between 24-32,990 ng m^{-3} ; higher values were apparent in the late process stages where the processes of de-hulling, sifting, milling, and bagging of the final product were undertaken. In comparison, the STI values at the dockside from this study appear somewhat lower.

The level of endotoxin extractable from the husk bulk was 15-60-fold greater than that found in the two chipped bean bulks. Endotoxin exposure is associated with lung inflammation and organic dust toxic syndrome. Interestingly, high levels of endotoxin in soya husk have also been

Table 3 *Rank Spearman correlation coefficients (95 % confidence intervals) and their significance between the four air measurements*

	Dust	HSP	STI
HSP	0.71 (0.49-0.84) *p*<0.0001	-	
STI	0.84 (0.70-0.92) *p*<0.0001	0.74 (0.54-0.86) *p*<0.0001	-
STP	0.62 (0.36-0.79) *p*=0.0001	0.52 (0.23-0.72) *p*<0.001	0.41 (0.1-0.65) *p*=0.012

STI - soya trypsin inhibitor
HSP - hydrophobic soya protein
STP - soluble total protein

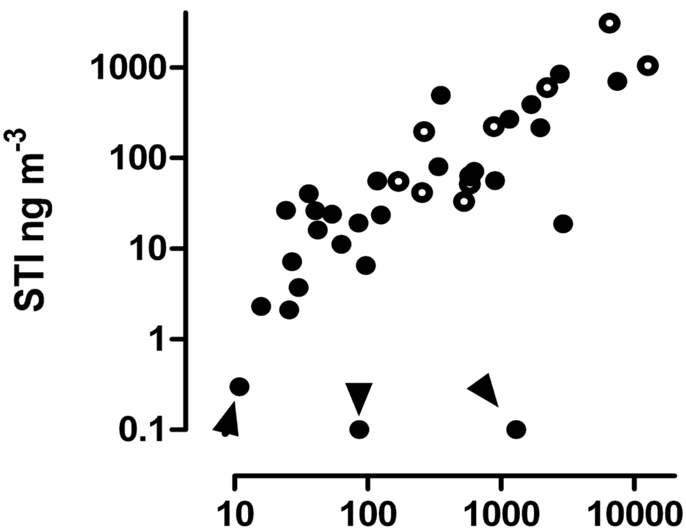

Figure 2 *Correlation between soya trypsin inhibitor (STI) and hydrophobic soya protein (HSP) results for air monitoring data. Filled circles represent static samples; unfilled circles represent personal samples. The three points identified by an arrow were from the same peripheral static monitoring site and the STI levels were at, or below, the detection limit*

reported by Harris-Roberts (26). Unfortunately we could not measure the levels of endotoxin in the air samples, so the actual inhalation risk from endotoxin and soya remains unclear.

We can find no published data that relates airborne levels of HSP or STI to the risk of developing sensitisation, or provoking symptoms in those already sensitised. However, studies on other occupational allergens have reported that averting or decreasing allergen exposure in the workplace is the most effective approach for preventing asthma (27). Thus, this current lack of data does not obviate the uses of either the HSP and STI assays as a means of monitoring the effectiveness of workplace control measures in reducing exposure to inhalable soya allergens to as low as is reasonably practical. Both specific protein immunoassays (STI and HSP) can measure allergens in soya beans and soya hull with adequate sensitivity. In discussing a number differing of aeroallergen measurements for soya, it had been highlighted that airborne samples from different work environments with soy exposure may contain soya dust with quite different compositions (20). The HSP assay appears relatively more sensitive than the STI assay, but from the bulk sample results the latter assay appears to be equipotent in detecting exposure to soya products from the bean and the husk.

The measurement of soluble total protein (STP) in air samples has been suggested as a relatively inexpensive means of identifying general inhalation exposure to biological material rather than the more expensive measurement of specific allergenic proteins. Data from this study suggest that the ability to readily measure proven inhalation allergens that relate to the specific commodity being handled seems to offer significant advantages over the non-specific measurement of total protein.

This study also indicates significant potential personal exposure to soya allergens for those working in enclosed spaces such as the ship's hold and storage warehouses, even though they were operating vehicles with HEPA-filtered cabs. Workers carrying out such activities wore respiratory protective equipment. The high allergen levels measured within the cabs of vehicles operating in the warehouse confirmed the need for protective equipment. Personal and static samples showed that proximity to the hoppers during unloading was associated with significant airborne allergen levels. Interestingly, the static sample in the crane cab showed a high level of HSP when the husk material was being unloaded, while STI levels were low. As expected, the airborne levels of the allergens at dock-site perimeter or in neighbouring areas, which were largely in the prevailing wind direction, were lower than those close to the activities generating airborne soya dust, but were still readily measureable. However, as with some of the job-related static samples (e.g., crane cab), the unloading of bulk soya husk was associated with peak HSP airborne levels in these sampling sites away from the point sources of dust. It is unfortunate that we did not monitor the car-park for cruise ship customers on the day of husk unloading (day 1), as it would give additional insight into the environmental spread of the husk associated HSP allergen.

The reason for the possibly anomalous STI and HSP results (Figure 2) for the same individual environmental static site (end of building P) over the three days is unclear. It could reflect the different physical properties of hull and bean dusts in terms of their dispersion under prevailing atmospheric conditions. Further analysis of particle size of dusts from beans and their hulls may be informative in terms of environmental health risk from handling soya products.

We hope that a body of airborne results for the HSP and STI from various occupational and environmental exposures to soya products can be developed. This would in turn further help the interpretation of such measurements in terms of monitoring control of exposure to soya allergens.

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Razine aeroalergena soje za vrijeme iskrcavanja na lučkom doku

Soja je gotovo u cijelome svijetu važan poljoprivredni proizvod, pa se prevozi često i u velikim količinama. Sadrži mnoštvo poznatih alergena, a korištenje proizvoda od soje i rukovanje njima prilikom iskrcavanja robe povezivani su s pojavama profesionalne astme. Razine dvaju inhalacijskih alergena, soja tripsin inhibitora (STI) i hidrofobnog proteina sojina zrna (HSP), mjerene su u uzorcima zraka prikupljenim osobnim uzorkovačem i stacionarnim uzorkovačima u jednom britanskom lučkom doku tijekom 3 dana iskrcavanja triju pošiljaka prerađena sojina zrna i kuglica sojine mahune. Također je provedena analiza ukupnih topivih proteina i gravimetrijska analiza prašine. Rezultati gravimetrijske analize uzoraka prikupljenih osobnim uzorkovačem bili su dobrano ispod polovice trenutačne granice izloženosti u Velikoj Britaniji (10 mg m^{-3} za prašinu sjemena) i općenito ispod 0,5 mg m^{-3} kod statičnih uzoraka, a razine STI-a i HSP-a u zraku prikupljenom stacionarnim uzorkovačem bile su unutar raspona 0-3.071, odnosno 11-12.629 ng m^{-3}. Korelacija između ta dva alergena bila je dobra (Rank Spearmanov koeficijent 0,74). Rezultati ovoga istraživanja pokazali su da je HSP precizniji pokazatelj izloženosti soji od STI-a, no samo za sojine mahune; STI bi mogao biti precizniji pokazatelj u pogledu proizvoda od sojine ljuske ili zrna. Obje su se vrijednosti pokazale preciznim tehnikama za praćenje izloženosti sojinim alergenima. Razina endotoksina u pošiljci sojinih mahuna bila je 15 do 60 puta veća od one u dvjema pošiljkama usitnjenog zrna.

KLJUČNE RIJEČI: *alergeni; uzorci zraka; praćenje okolišnih parametara; hidrofobni protein sojina zrna (HSP); soja tripsin inhibitor (STI)*