

Article

# Quantifying Dustiness, Specific Allergens, and Endotoxin in Bulk Soya Imports

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**Abstract:** Soya is an important bulk agricultural product often transported by sea as chipped beans and/or the bean husks after pelletisation. There are proven allergens in both forms. Bulk handling of soya imports can generate air pollution containing dust, allergens, and pyrogens, posing health risks to dockside workers and surrounding populations. Using an International Organization for Standardization (ISO) standardised rotating drum dustiness test in seven imported soya bulks, we compared the generated levels of dust and two major soya allergens in three particle sizes related to respiratory health. Extractable levels of allergen and endotoxin from the bulks showed 30–60 fold differences, with levels of one allergen (hydrophobic seed protein) and endotoxin higher in husk. The generated levels of dust and allergens in the three particle sizes also showed very wide variations between bulks, with aerolysed levels of allergen influenced by both the inherent dustiness and the extractable allergen in each bulk. Percentage allergen aerolysed from pelletized husk—often assumed to be of low dustiness—after transportation was not lower than that from chipped beans. Thus, not all soya bulks pose the same inhalation health risk and reinforces the importance of controlling dust generation from handling all soya bulk to as low as reasonably practicable.

**Keywords:** soya; biological dusts; organic dusts; aeroallergens; allergic asthma; atmospheric pollution

## 1. Introduction

Soya, commonly known as soy or soybean in North America, has become one of the most important worldwide agro-products. Large quantities are transported to Europe from South America. The UK imported about 3 million tonnes of soya in 2015, largely as coarsely milled or chipped beans, bean husks (hulls), and whole soya beans. The imported husk is of lower protein content and enters the animal feedstuff chain, largely used on farms as a poultry, pig, and dairy cattle feed. It is very often pelletised before shipping; one of the reasons is to reduce the potential for the generation of dusts. Uses of the beans are in the food and bakery industries. Chipped or milled beans may undergo further processing (e.g., production of flour or oil) for use in the food manufacturing industry, an industry that employs over 400,000 in the UK. Bulk soya products are imported at a number of UK ports specifically equipped to handle bulk grain, agrochemicals, and foodstuffs.

Soya is a well-recognised cause of food allergy, but proteins within the bean and the husk are also proven occupational and environmental allergens by inhalation [1–4]. Exposure to inhaled soya allergens has been linked with occupational asthma [4,5], rhinitis [6], hypersensitivity pneumonitis [7], and epidemics of asthma [8–14].

Asthma epidemics occurred in a number of harbour cities (best reported for Barcelona) importing bulk soya. Investigations demonstrated that exposure to soya allergens, particularly low molecular weight allergenic proteins present in soya husk, were associated with significant increases in immunological sensitisation by specific Immunoglobulin E (IgE) or skin prick tests and increases in the prevalence of respiratory symptoms such as wheeze, shortness of breath, allergic rhinitis, and even serious, life threatening asthma attacks [15]. Exposure to soya dust has also been associated with respiratory irritancy [16], and it is suggested that possible contaminating spoilage fungal allergens and endotoxins may also be implicated in both community asthma epidemics and affected workers [17,18]. Subsequent introduction of dust control measures in the storage silos at Barcelona reduced the occurrence of asthma epidemics. These epidemics represent a scenario where allergen-containing dust has migrated some considerable distance from the dockside, and highlights that managing these dusty materials that are moved in bulk represents an issue of concern for worker health and “bystander” effects in surrounding populations. Thus when assessing the risks from handling such bulk materials containing allergens, the properties of the dust may be important when considering the required control strategies.

Complaints of respiratory symptoms associated with one recent soya importation to the UK, together with the published literature about epidemic asthma in a number of harbour cities, had led to a atmospheric monitoring study in the UK port in question [19]. This raised our interest concerning possible differences between different soya bulk consignments in terms of their risk to health. Such differences may include intrinsic dustiness, particle size distribution, and other factors that might affect particle size and mass. In addition, the allergen content of the dust and the concentrations of allergen in different-sized particle fractions may influence the likelihood of exposure/deposition along the respiratory tract of dockside workers undertaking different tasks, as well as users of the soya material further down the animal feed supply chain. Also, smaller-sized particles are more buoyant and allow the possibility of dispersion of allergen, dependent on prevailing wind and climatic conditions, to communities proximal to dockside soya unloading activities.

A rotating drum testing method has been well established that can investigate the generated levels of a dust under standardised conditions that are associated with defined inhalable, thoracic, and respirable particle size fractions [20–22]. Inhalable particles are those of a size (aerodynamic diameter  $\leq 100 \mu\text{m}$ ) to enter the respiratory tract via the nose and mouth. Thoracic sized particles are defined as those small enough (aerodynamic diameter  $< 30 \mu\text{m}$ ) to penetrate past the larynx as far as the trachea and bronchial areas of the lung. Respirable particles (aerodynamic diameter  $< 10 \mu\text{m}$ ) are those small enough to enter the deepest part of the lungs. The rotating drum testing technique was used in this study to compare the intrinsic dustiness by gravimetric analysis in seven different bulk soya consignments recently imported into the UK and Ireland and extended to include measuring two major soya respiratory allergens in both the bulk samples and their generated dust fractions during dustiness testing. Five of these bulk samples were related with unloading where airborne monitoring using gravimetric and allergen measurement were undertaken. The two major soy allergens measured were the 20 kD Kunitz soya trypsin inhibitor (STI), given the allergen nomenclature Gly m T1 ([www.allergome.org](http://www.allergome.org)), and the hydrophobic seed protein allergen (HSP), which has the approved allergen designation Gly m 1 by the World Health Organisation and International Union of Immunological Societies WHO/IUIS ([www.allergen.org](http://www.allergen.org)). For both these allergens, sensitive and specific immunoassays have been developed and used to monitor atmospheric exposures [23]. HSP is highly homologous with the two low molecular weight allergens associated with asthma epidemics in harbour cities [24,25]. STI is one of the higher molecular weight allergens that has been implicated in bakery workers’ asthma [2,26,27]. While HSP protein is said to be largely associated with soya bean husk, STI is found in soya beans, flour and the bean husk [28].

The handling of bulk biological products, including those used as agrochemical feedstuffs, may pose a number of risks to respiratory health. Bulk soya imports are often considered a single hazard entity without consideration that differences between bulks may pose different relative health hazards

to both employees and communities in the vicinity of bulk unloading. An obvious difference is that a soy bulk may be of chipped beans or the bean husk, the latter being a cheap animal feed product but said to contain considerably more of the low molecular weight HSP allergen on a weight per weight basis than soya beans. Occupational and environmental exposure to bulk soya products that contain allergens, pyrogens, and other biological contaminants can also occur in the supply chain from dockside importation to end-users on farms.

Outside of those dock cities where asthma epidemics had occurred and among health specialists in allergy and asthma, the potential health risks from airborne soya proteins appear not to be universally recognised, even though large amounts of soya are energetically moved at dockyards and along the animal food chain. This study attempts to investigate whether all soya bulks may pose the same risk to respiratory health by examining the amounts of airborne dust and major soya allergens generated from different soya bulks during standardised rotating drum dustiness testing.

## 2. Materials and Methods

Seven bulk soya samples underwent dustiness testing. All seven bulk materials had been imported into the UK or Ireland within a 14 month period. Five of the bulks were soya meal (chipped beans), while the other two were soya husk products that had been pelletized before transportation. Each bulk sample was given a 2 letter code indicating where it was unloaded, followed by a digit to further uniquely identify the bulk. The seven bulks were associated with unloading from container ships at three different ports. Samples coded EN1, EN2, and EN3 were associated with unloading at a single dock in the south of the UK over a short period. Bulks coded as SC1 and SC2 were unloaded from a single container vessel in Scotland. Bulks coded as IR1 and IR2 were unloaded in Ireland. EN2 and SC2 were husk bulks. Bulk samples used in this study had been stored as received in airtight containers at  $-20\text{ }^{\circ}\text{C}$  until analysis.

Small duplicate samples (approximately 1 g) of each bulk sample were extracted with constant mixing for 2 h at room temperature at 10% *w/v* ratio using 0.1% Tween 20 (Sigma-Aldrich, Poole, UK) in pH 7.4 10 mM phosphate buffered saline. After centrifugation, the supernatants were then subsequently analysed for total soluble protein, the two intrinsic soya allergens (HSP and STI), the major allergen (WHO/IUIS designation Asp f 1) found in the common spoilage fungus *Aspergillus fumigatus*, and endotoxin. Soluble total protein was measured using a standard bicinchoninic acid methodology, the specific allergens (HSP and STI) by established sandwich immunoassays [23], and Asp f 1 by an enzyme linked immunosorbent assay (ELISA) using commercially available reagents (Indoor Biotechnology, Cardiff, UK). Endotoxin was measured using a commercial Limulus Amebocyte Lysate assay (Lonza, Verviers, Belgium), using the manufacturer's supplied methodology and employing a standardized spiking technique to check for any interferences 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.

Quality control samples were run at regular intervals for all assays in order to calculate the analytical imprecision of each assay.

Rotating drum dustiness testing was performed according to the standardized methodology using equipment supplied by JS Holdings (Stevenage, UK) [20,21]. Essentially, a fixed amount (35 mL) of bulk material is rotated at a set speed and time-period in a drum with vanes that lift and drop the bulk material during rotation. During the test, an air flow of 38 L/min through the drum is used to entrain any airborne dust that is then collected on an in-line series of two metal foams with different pore densities, 20 pores per inch (ppi) and 80 pores per inch, and finally a glass micro-fibre filter (Whatman GF/A grade, Sigma, Poole, UK). Each test of a bulk sample consisted of three replicate runs of the dustiness procedure with the gravimetric analysis of the foams and filter being used to calculate an average dustiness in the inhalable, thoracic, and respirable sized fractions.

The inhalable fraction is reflected on the amounts collected and weighed on the 20 ppi and 80 ppi foams and GF/A filter combined. The thoracic fraction is derived from the amounts on the 80 ppi foam and GF/A filter, while the respirable fraction equates to the amount found on the GF/A filter.

The moisture content of each bulk material was also measured using an automated Mettler Toledo HB43-S moisture analyser (Mettler-Toledo, Leicester, UK).

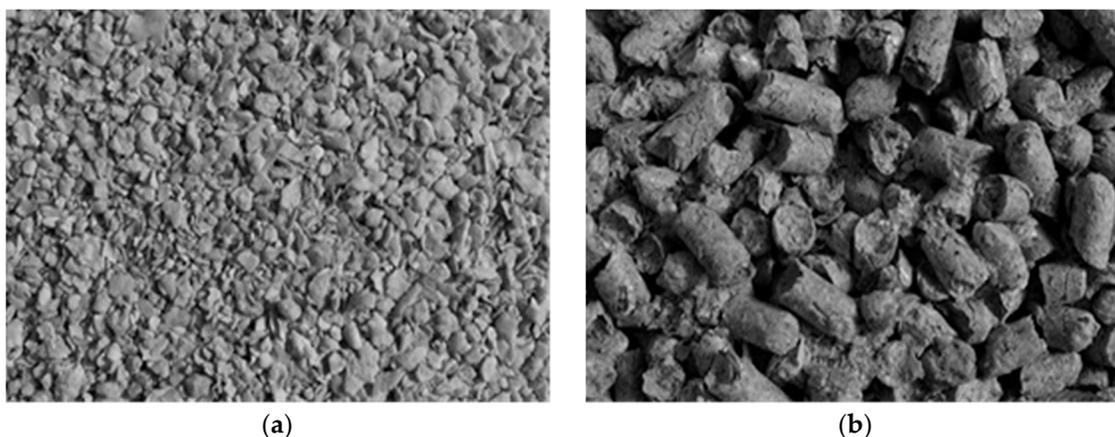
Extension of the standard drum dustiness methods for the intrinsic allergens (STI and HSP) and soluble proteins meant that after gravimetric analysis the foams (80 ppi and 20 ppi) and 37 mm GF/A filter were subjected to an extraction procedure using 0.1% Tween 20 in 10 mM phosphate buffered saline. Sixty millilitres (mLs) of extraction buffer was used for extraction of the 20 ppi and 80 ppi foams, and 15 mLs for the 37 mm GF/A filter. Foams and filters were extracted for two h using roller and orbital shakers to agitate the samples. Aliquots of the extraction buffer for each sample were removed and stored frozen until analysis for soluble total protein and the intrinsic allergens (STI and HSP) using the same immunoassay methods as employed for the extracts of the bulk materials.

Results from the drum dustiness testing for aerolysed dust and allergen measurements in the inhalable, thoracic, and respirable sized fractions were expressed either as per unit weight of bulk material or as a percentage of the amount of soluble protein or specific allergen that was extractable from the appropriate bulk.

Statistical analyses were performed using MedCalc v12.3 (MedCalc Software, Ostend, Belgium). Non-parametric tests or parametric tests after log transformation were applied. A significance level (alpha) of 0.05 was chosen.

### 3. Results

The five non-husk bulks looked visually similar as fairly coarse, irregularly sized chipped soya beans, generally in the size range 1–4 mm (Figure 1a). The two pelletized husk material showed evidence of being friable with varying degrees of breakdown of the original cylindrically shaped pellets of length 12–15 mm. (Figure 1b).



**Figure 1.** Photographs of representative bulk samples: (a) chipped soya beans; (b) cylindrical pelletised soya husk showing significant breakdown presumably from compaction during transportation.

The imprecision of the assays for protein, HSP, STI, Asp f 1 and endotoxin, calculated from quality control samples, were 8.3%, 11.1%, 12.3%, 13.7%, and 13.9% respectively.

The levels of readily extractable protein, the allergens (STI, HSP, and *Aspergillus f 1*), endotoxin, and moisture in the seven bulk samples are shown in Table 1. There was considerably more variation across the seven bulks in extractable specific allergen or endotoxin (STI coefficient of variation = 111%; lowest-highest showing 28-fold difference; HSP coefficient of variation = 148%; lowest-highest showing 54-fold difference and endotoxin coefficient of variation = 115%; lowest-highest showing 61-fold difference) than there was with extractable total protein (coefficient of variation = 44%; lowest-highest

showing 3-fold difference). The two husk products had clearly higher extractable levels of HSP (mean = 2862  $\mu\text{g g}^{-1}$ ) in comparison with the other soya meal products (mean = 122  $\mu\text{g g}^{-1}$ ). This distinction between husk and soya meal products is not apparent in terms of extractable STI levels. This is in agreement with HSP being more associated with husk, while STI is distributed both in husk and bean. The levels of extractable *Aspergillus f 1* allergen was relatively low across all the bulks and showed no correlation with the other allergens or soluble protein. Interestingly, the two highest levels of extractable endotoxin were associated with the two husk bulk samples. Technical issues with the extraction of endotoxin from the metal foams prevented analysis of endotoxin after drum dustiness testing.

**Table 1.** Mean concentrations of protein, allergens, and endotoxin readily extractable from duplicate bulk samples.

Code	Protein ( $\mu\text{g g}^{-1}$ )	STI ( $\mu\text{g g}^{-1}$ )	HSP ( $\mu\text{g g}^{-1}$ )	Asp f 1 ( $\mu\text{g g}^{-1}$ )	Endotoxin (EU $\text{g}^{-1}$ )	Moisture %
EN1	59,537	270	196	$18 \times 10^{-3}$	4630	5.73
EN2 *	20,609	798	2824	$19 \times 10^{-3}$	80,364	6.06
EN3	55,799	233	178	$33 \times 10^{-3}$	1309	13.89
SC1	28,070	65	68	$25 \times 10^{-3}$	1691	5.16
SC2 *	18,269	258	2900	$8 \times 10^{-3}$	52,769	8.71
IR1	36,600	28	116	$5 \times 10^{-3}$	51,455	8.26
IR2	37,745	37	54	$5 \times 10^{-3}$	5527	8.28

\* Husk bulk. EU is Endotoxin unit; 1 EU is equivalent to approximately 0.2 ng of endotoxin.

Results of the drum dustiness testing expressed per unit weight of bulk are shown in Table 2. Coefficients of variation for gravimetric dust results in triplicate runs were all less than 10%, a condition for validity of the rotating drum test [21]. Variability of generated levels of STI and HSP in the three fractions were widely different between bulks, with 50 to 580-fold differences between lowest and highest levels noted. The smallest respirable fraction showed the widest variability between bulks for both allergens.

**Table 2.** Mean outcomes from drum dustiness. Results are expressed per g of bulk material.

Code	Dust $\mu\text{g g}^{-1}$			Protein $\mu\text{g g}^{-1}$			STI $\text{ng g}^{-1}$			HSP $\text{ng g}^{-1}$		
	Inh	Thor	Resp	Inh	Thor	Resp	Inh	Thor	Resp	Inh	Thor	Resp
EN1	2467 (mod)	1007 (high)	81 (mod)	111 [9.8]	41 [7.3]	4 [20.1]	1309 [29.2]	505 [39.4]	70 [44.3]	1884 [16.9]	848 [13.6]	116 [39.4]
EN2 *	182 (vlow)	54 (low)	4 (vlow)	81 [28.4]	44 [47.4]	3 [54.8]	998 [7.2]	336 [29.1]	15 [50.4]	4626 [11.9]	1783 [15.8]	67 [35.5]
EN3	4337 (mod)	2500 (high)	710 (high)	262 [11.4]	137 [25.8]	18 [7.0]	177 [12.6]	78 [42.9]	15 [29.1]	782 [8.8]	187 [10.5]	29 [72.0]
SC1	616 (low)	242 (mod)	24 (low)	78 [7.0]	36 [13.0]	0.3 [23.4]	24 [17.0]	9 [26.2]	3 [27.1]	255 [16.4]	113 [9.3]	14 [27.3]
SC2 *	157 (vlow)	53 (low)	3 (vlow)	28 [8.9]	12 [28.7]	0.7 [27.0]	290 [36.7]	130 [34.1]	20 [25.7]	3481 [6.6]	1262 [20.9]	157 [31.3]
IR1	213 (low)	11 (vlow)	2 (vlow)	36 [9.2]	20 [24.2]	1 [15.7]	33 [12.1]	10 [23.9]	0.5 [34.3]	97 [18.3]	21 [37.2]	0.2 [12.4]
IR2	559 (low)	90 (low)	4 (vlow)	31 [6.6]	8 [6.0]	1 [26.8]	41 [13.4]	7 [41.2]	1 [25.6]	134 [7.1]	28 [12.9]	0.9 [18.4]

Classification of gravimetric dustiness as very low (vlow), low, moderate (mod) or high are as according to the European Committee for Standardization's document EN 15051 [21]. "Inh", "Thor", and "Resp" reflect inhalable-, thoracic-, and respirable-sized fractions. Numbers in square brackets indicate the coefficient of variation for triplicate measurements on each sample. \* represent husk products.

Analysis of variance of the type of bulk (husk or bean) on protein and specific allergen levels in the various sized fractions was made. After adjusting for the influence of fraction size, the type of bulk

material (husk or bean) was significant ( $p = 0.005$ ) for HSP but not STI or protein, with husk bulks associated with increased HSP levels. Albeit in a small cohort, Spearman rank correlation analyses showed no significant correlations ( $p > 0.05$ ) between the dust level and either allergen (STI or HSP) levels in the fractions. Soluble protein levels in the three fractions were correlated with  $p$  values around 0.05 with the dust fractions, but not allergen fractions. Thus, in these soya bulk samples, dust or soluble protein levels in the inhalable, thoracic, or respirable fractions were not strong indicators of the two major allergen levels generated in the same fractions.

Stepwise multiple regression analyses were performed on log-transformed variables to investigate whether the levels of allergens (STI and HSP) in the three health-related fractions could be predicted from dust and/or protein levels in the fractions, together with the allergen content and moisture levels in the bulk material. The models showed that for both HSP and STI, their levels in any particular fractions were influenced by both the dust level in the fraction and the amount of extractable allergen in the bulk material. The regressions equations are shown below, the  $p$  values of all regressions coefficients were less than 0.05.

$$\text{Log (HSP}_{\text{fraction}}) = 0.89[\text{log (Dust}_{\text{fraction}})] + 1.19[\text{log (HSP}_{\text{bulk}})] - 2.208[\text{Log (Moisture}_{\text{bulk}})] - 0.65, R_{\text{multiple}} = 0.96, \quad (1)$$

$$\text{Log (STI}_{\text{fraction}}) = 0.48[\text{log (Dust}_{\text{fraction}})] + 1.09[\text{log (STI}_{\text{bulk}})] - 1.73, R_{\text{multiple}} = 0.86, \quad (2)$$

$$\text{Log (soluble protein in a fraction)} = 0.68[\text{log (Dust}_{\text{fraction}})] - 0.22, R_{\text{multiple}} = 0.84, \quad (3)$$

- (1) The model did not include the levels of soluble protein in fractions;
- (2) The model did not include either moisture or levels of soluble protein in fractions;
- (3) The model did not include levels of moisture or levels of soluble protein in bulks.

We also calculated the dustiness tests as percentage of aerolysed total protein and the two allergens from the dustiness testing, i.e., expressed as percentages of the amount of extractable protein or specific allergen from the individual bulks. This use of percentage aerolysed data should eliminate the influence of the widely different protein and allergens concentrations in the bulks on the relative amounts of aerolysed material found in the different fractional sizes. Although the sample size is small, there is no evidence from this analysis that the two pelletized husk samples, as received, led to lower aerolysation in comparison with the soya samples in a chipped form. Sample EN1 gave significantly (Analysis of Variance  $p < 0.05$ ) higher aerolysed percentages of both allergens STI and HSP in all three sized fractions compared with the other soya samples. This finding was not reflected in the aerolysed soluble protein results where samples EN2 (a pelletised husk product) and EN3 (a chipped soya product) tended to be higher ( $p < 0.05$ ) than the other bulk products in the three fractions.

Coefficients of variation for each variable from the triplicate runs, particularly for the allergens, are substantially more than 10% that is the upper limit for acceptance for gravimetric results in standard rotating drum dustiness testing (see Table 2). Whether the wider variation within triplicates, especially for allergen measurements, reflects pre-analytical variable extraction efficiencies or other issues is currently unclear but can be substantially more than the within-batch analytical coefficients of variation for the measurements soluble protein and allergens of around 10–14%.

#### 4. Discussion

The intrinsic dustiness of a bulk product reflects its tendency to produce airborne dust during energetic handling. However, potential and different health risks may depend on the nature of the constituents of the dust and the distribution of particle size that defines how far along the respiratory tract the particles will penetrate and deposit.

The rotating drum dustiness test is one of two different procedures defined with European Standard EN 15051 [21] for testing under standard conditions the dustiness of bulk materials in health-related, particle size fractions. Such laboratory-based methods are not necessarily complete

predictors of likely exposure in real life, and it has been reported that results from the two methods in EN 15051 do not invariably show good agreement [29,30]. However, the use of such tests and the outcomes as shown in Table 2 may help in identifying those bulks of likely greater health risk when comparing relatively similar materials and handled by similar work-practices.

Therefore we decided to undertake drum dustiness testing of seven recent bulk soya imported and unloaded at docks in the UK and Ireland. However, we also decided to extend the standard methodology to include the levels of soluble protein and two major inhalation soya allergens in the inhalable, thoracic, and respirable health fractions [2,24–27]. The levels of readily extractable soluble protein and allergens in the bulk samples were also measured. We believe that this is the first time that dustiness testing has been extended to include major allergens besides gravimetric measurements as outcome measures. Methodological problems had precluded our initial aim of including endotoxin measurements in the aerolysed fractions from dustiness testing.

With the caveat concerning how completely the standardized tests reflect likely exposure in real life, the results in Table 2 may well reflect relative potential exposure and possible risk to health. There were wide differences between the dustiness of the seven soy bulks. Based on the classification given in ISO 15051 [21] they ranged from “very low” to “high” dustiness. Two bulks (EN1 and EN3) showed high levels of dustiness in the thoracic and respirable fractions with 230–300-fold higher gravimetric dust levels than in the lowest bulk. The levels of the two major allergens in the health fractions across the tested bulks did not correlate with the gravimetric dustiness levels. As might be expected, this is largely due to the different levels of readily extractable allergens in the various soya bulks. So while bulk EN3 shows the highest levels of gravimetric dust by a considerable margin in the respirable fraction, reflecting small particles that can penetrate deep into the lungs, the respirable fraction of EN1 shows 4-fold higher levels of STI and HSP and SC2 that appears a low “dusty material” from gravimetric results shows over 5-fold levels of HSP in comparison with EN3.

Two products within our study (EN2 and SC2) were pelletized husks but showed a varying degree of friability, presumably from compression within the container ships’ holds during transportation. Those handling UK bulk soya importations tend not to distinguish between husk or bean products, and there is a wider general view that “pelletisation” is necessarily associated with low dust exposure. From this data, husk products pelletized before transportation were not lower either in absolute or percentage levels of aerolysed protein or allergen in comparison with chipped bean products at the time of unloading or presumably further down the usage chain. However, these data confirm that soya husk contains, in comparison with bean product, significantly more on a per gram basis of the low molecular weight allergen that has been associated with asthma epidemics and strengthens reports that have noted high levels of endotoxin in soya husk [18].

Currently, we have no real health-based data in terms of interpreting the implications of these differences between soya bulks, and we are only measuring two, albeit major, allergens out of all the proven respiratory allergens in soya, as well as dust levels. Levels of even inert dust, e.g., dusts of little/no toxicity or not recognised as causing specific health effects such as sensitisation, may cause differential respiratory symptoms of irritancy or discomfort depending on the relative distribution of particle sizes and whether the exposed already suffers a pre-existing respiratory problem. In subjects already sensitized to major allergens, such as HSP and STI, the production of high levels of particles containing such allergens may lead to variable and different responses depending on the particle size and site of deposition. It is also worthwhile noting that some people could be priorly sensitised to STI via dietary products containing soya. For example, in the already sensitized individual, larger allergen-containing particles with sizes greater than the defined thoracic fraction may lead to symptoms restricted to eyes and nose, while high levels of allergen particles of a respirable size may be associated with presentation of a wider range of more severe symptoms within the lungs. Larger quantities of smaller, respirably sized particles containing allergen are also likely to be more buoyant and disperse through the wider environment. This may be relevant in terms of those reported asthma epidemics in harbour cities handling soya imports.

Although we have not undertaken a study of possible health effects related to these soya unloadings, the three samples (EN1, EN2, and EN3) unloaded at a single UK port had been accompanied by anecdotal complaints from dock-side workers about extreme levels of dustiness and respiratory symptoms in some of a firm's workers employed near the docks during the unloading of the initial bulk (EN1). Indeed, both EN1 and EN3 tested as some of the dustiest bulks and both EN1 and EN2 with the highest level of aerolyzed allergens. Bulks EN1, EN2, and EN3 were unloaded during an atmospheric monitoring exercise that showed significant levels of both allergens related to occupational activities and also at environmental sites outside the dock yard perimeter [19]. Bulk EN1 showed in the laboratory dustiness test a particularly significant propensity to aerolyse readily extractable allergens, which was not reflected in aerolysation of total protein.

Within a UK context, at least two pieces of regulatory guidance directly identify bulk soya: the risk of respiratory sensitisation and the need to control exposure to as low as reasonably practicable [31,32]. Perhaps the results in this paper indicate the need for more knowledge about, and characterisation of, bulk bio-hazardous dusts to inform control strategies that will limit air pollution and ensure the protection of health of both workers and the nearby general population. This concern is supported by the finding that the accepted practice of pelletisation of a powdered product at source is not necessarily always associated with no or low exposure to dust and allergens.

Levels of extractable endotoxin [33–35], which has been implicated with a range of health effects from inhalation of biological aerosols, were highly variable across the seven soya bulks, with high values associated with husks. The levels of endotoxin found in our bulk husks, and their higher levels relative to chipped beans are in agreement with Harris-Roberts [18], although the endotoxin levels in one of our chipped bean bulks was considerably higher than the range for soya bean endotoxin reported by Harris-Roberts. Our and Harris-Robert's findings concerning endotoxin levels in husks may possibly indicate a potential additional respiratory risk from this type of product, and the need to characterize the size of endotoxin associated particles in soya products in terms of health risk.

The use of drum dustiness testing has largely been to predict likely worker exposure, although they have been considered for incorporation in risk models for assessing inhalation exposure to nanoparticles [36]. While Brouwer found that dustiness testing was a major determinant of exposure in comparing dustiness and small-scale workplace simulations [22]. Heitbrink suggested that such laboratory tests need to be field validated, indicating there may be significant variability between laboratory tests and workplace exposures [37]. Therefore, we are cautious about over-interpreting the results from this study. However, we are not interested in predicting worker or environment exposure but more in gaining some indication of the propensity of different bulks to generate dust and allergen aerosols. That said, a paper is in preparation that will describe the air monitoring data from the second unloading (SC1 and SC2) and a comparison between the relevant dustiness tests described in this paper and the two unloadings with available air measurements.

## 5. Conclusions

Rotating drum testing of a limited number of soy imports has shown their widely different intrinsic dustiness in terms of important, health-based particle sizes, although in practice all these bulks would have been offloaded in the same manner. Moreover, the distribution of two major inhalation allergens in the fractions from the drum testing did not necessarily show similar distributions to those for gravimetric dust, such that a simple potential health risk model for lung irritancy or allergic responses is not obvious. Pelletised soya husk cannot be assumed to be a low dustiness material. These data reinforce the importance of actively controlling atmospheric pollution from the handling of all soya bulk products to as low as reasonably practicable in order to protect dock workers and nearby communities.

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**Author Contributions:** Howard J. Mason conceived the study with the help of Gareth Evans and Andrew Thorpe regarding the application of dustiness testing. Paul Roberts undertook the dustiness testing. Howard J. Mason performed the STI and protein measurements. Susana Gómez-Ollés and Marie-Jesus Cruz supplied the HSP analyses. Howard J. Mason initially drafted the paper. All authors contributed to the drafting and editing of the paper.

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