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CYTOTOXIC EFFECT OF ORGANIC DUST EXTRACTS FROM DIFFERENT WORKING ENVIRONMENTS: AN *IN VITRO* ASSAY

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Abstract: The evidence supporting organic dust as a cause of occupationally-related lung disease has substantially increased in the post two decades. The aim of this project was to test an assay which could be used to detect cytotoxic potential of organic dusts found in working environments. A cytotoxic assay with two cell lines was used: VERO and human A 549 carcinoma cells. Cells were exposed to extracts of dust samples collected from five different occupational environments with known adverse respiratory effects, using two different extraction protocols. Compared to water, alkaline soluble extract exerted the greater cytotoxic effect on the cells. With this assay it was possible to distinguish differences in the cytotoxic potential of the different types of organic dusts. The most aggressive dusts proved to be those with a high microbial content, such as compost and grain dust, which exerted an effect already after 2 hours of incubation at low concentrations.

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Key words: organic dust, cytotoxic effect, *in vitro*, A 549, VERO, cell lines.

INTRODUCTION

During the last two decades the evidence supporting organic dusts as a cause of occupationally-related lung diseases has greatly increased [9, 21, 27, 32, 40]. The cotton industry has been extensively investigated, and an association has been shown between an increased prevalence of byssinosis among the workers and the concentration of organic dust and endotoxin in the working environment [2, 12, 19, 30, 34].

Farmers are exposed to many different kinds of organic dusts when they harvest, work with grain, feed animals, etc. [26]. Cross-sectional studies have shown that farmers have an increased prevalence of lung diseases. Handling grain raises the risk of inflammatory airway disease which is usually a non-allergic reaction to organic components of grain dust and less often an allergic IgE- or IgG-mediated reaction [7, 39]. However, allergy to storage mites has been shown among farmers working with stored grain [16]. The risk of contracting lung diseases increases as farms become more industrialized, e.g. in swine confinement buildings [6, 15, 28]. It has not been possible with immunological studies [14, 18] to show an association between the symptoms and IgE response to farming related antigens. IgE antibodies to swine proteins are rarely demonstrated.

Recent investigations have shown an increased prevalence of respiratory complaints among recycling plant workers [3, 20, 33]. A Danish investigation among workers in a garbage-handling plant showed that as much as 8 out of 15 employers developed asthmatic symptoms during a period of 8 months [31].

To study the pathogenesis of organic dust related respiratory diseases, some *in vitro* investigations have

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been conducted. Johnson *et al.* [17] used a cytotoxic assay based on the release of ^{51}Cr from two types of cultured pulmonary endothelial cells and demonstrated that pure cotton bract tannin produced a dose-dependent lethal injury to both cell types. By comparing the cytotoxicity response curves of aqueous extracts of bract with those from pure cotton bract tannin they demonstrated that tannin was the major cytotoxin present in bract. Using light microscopy, they also found visible morphological changes at sublethal doses. Hoult and Tuxford [13] found that toxin produced by *Bacillus pumilus*, isolated from the air of 18 Lancashire cotton spinning mills, showed a cytopathic effect in VERO cells.

We have presented previously a new assay for quantitating the cytotoxic effect of *Bacillus* species on VERO cells [37]. The aim of this project was to test this assay as a method to detect the cytotoxic potential of organic dust found in working environments. The cytotoxic assay utilized two different cell lines against extracts of organic dust from different working environments. Only aqueous solubilization of organic dust without organic solvents as alcohols or DMSO was used in order to reproduce the respiratory environment as closely as possible. Many studies have used pyrogen-free water extracts of organic dusts [8, 11]. However, in a study of the components in the macromolecular organic dust collected from "sick-buildings", NH_4HCO_3 (pH 8.3) was used as the dilution medium [10]. In the present study both aqueous and alcaline extraction protocols were used since organic dust is a complex mixture. We wanted to see if the two extraction protocols could supplement each other.

MATERIALS AND METHODS

Dust samples. Dust samples were collected from five different working environments described in Table 1.

Extractions protocols. *Aqueous extraction*: 1:10 w/v crude dust suspended in pyrogen-free water was mixed vigorously with glass beads for 1 minute on a vortexmixer, or homogenized (waste pellets and compost) before freezing at -80ºC. It was thawed out and again mixed vigorously for 1 minute on a vortexmixer before freezing again, and this was repeated three times. After filtering through a fiberglass filter (Gelman Sciences type A/E Glass) the extract was freeze-dried before being dissolved in pyrogen-free water in the concentration of 1.25 g dust/ml. This final solution was filtered through a 0.2 μm filter (Schleicher & Schuell FP 030/3) and stored at -80ºC until use.

Alkaline extraction: With the alkaline extraction the dust was treated more gently but for a longer period. 1:10 w/v crude dust suspended in 0.125 M ammonium hydrogen carbonate (pH 8.3) was gently rotated (20 rounds/minute) for 18 hours at 4ºC. After filtering through a fiberglass filter (Gelman Sciences type A/E Glass) the extract was freeze-dried before being dissolved in pyrogen-free water in the concentration of 1.25 g dust/ml. This final solution was filtered through a 0.2 μm filter (Schleicher & Schuell FP 030/3) and stored at -80ºC until use.

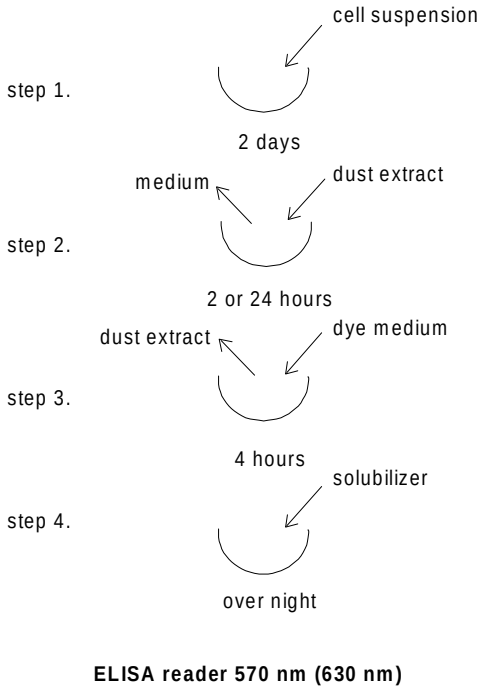

Figure 1. Working plan of cytotoxic assay. *Step 1:* Cells were seeded, 4 or 5×10^3 cells/well. *Step 2:* The medium was exchanged with medium including dust extract. *Step 3:* The medium was exchanged with medium including dye (15%). *Step 4:* Solubilizer was added to the dye-medium. Further information in the text.

Cell cultures. *Cultures of monkey kidney cells* (VERO from American Type Culture Collection; ATCC no: CCL 81 pass: 125-130) were grown in Earle's medium 199 containing 5% of fetal bovine serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml), at 37ºC, 5% CO_2. The content of lipopolysaccharide (LPS) in the serum was below 0.02 ng/ml as estimated with *Limulus* test by Gibco-BRL. For the assay 5×10^3 cells/well were seeded.

Table 1. Kinds of dust samples used in this study.

Dust from	Collected from
Crude cotton	Local cotton mill [34].
Grain	Open end of a grain auger-conveyor used for transportation of barley from a silo.
Swine confinement building	Swine confinement building with pellet-feeding.
Waste pellets	Garbage-handling plant [31].
Compost	Pile of organic household garbage, 5 weeks after drum [32].

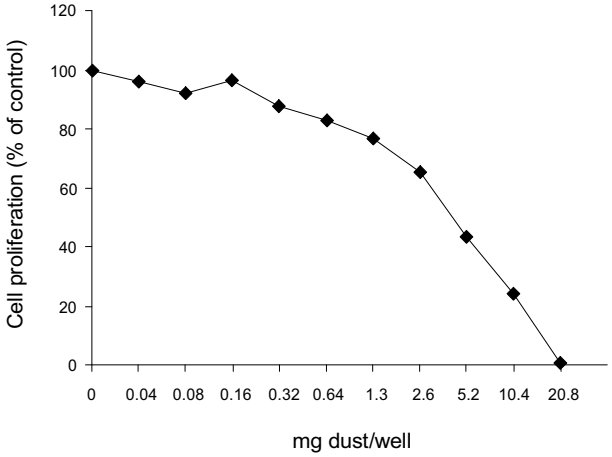

Figure 2. A dose-response plot of VERO cells exposed to the alkaline soluble extract (ASE) of crude cotton dust for 24 hours.

Cultures of human lung carcinoma cells (A 549, ATCC no: CCL 185 pass: 98-103) were grown in HAM's F 12K medium containing 10% of fetal bovine serum, penicillin (100 IU/ml) and streptomycin (100 μg/ml), at 37ºC, 5% CO_2. The content of LPS in the serum was below 0.02 ng/ml (estimated with *Limulus* test by Gibco-BRL). For the assay 4×10^3 cells/well were seeded.

Cytotoxic assay. The scheme of the assay is presented in Figure 1. CellTiter 96^{TM} Non-Radioactive Cell Proliferation/Cytotoxicity assay (Promega) was used. Cells were grown in microtiter plates (Greiner) for cell cultures for two days. On day 3, when the cells were in the log-phase, the medium was exchanged with extracts of dust in the concentrations of 0.04–20.8 mg/well (0.2–104 mg/ml) suspended in medium, and incubated 2 or 24 hours. At this time the cells were nearly confluent and the medium was exchanged once again with medium containing dye (15% tetrazolium-salt). Healthy cells digest and metabolize this salt to formazan blue which can be measured spectrophotometrically. After another 4 hours of incubation solubilizer was added to the dye-medium and the suspension was left overnight in darkness at room temperature and nearly 100% humidity. The results were read with a 570 nm messenger and 630 nm reference filter in an ELISA microplate reader (BIO-RAD 450), and a dose-response curve has been plotted as percent of nonexposed cells, which had been handled in the same way as the exposed cells (Fig. 2). In order to simplify the presentation and to stabilize the readings against random fluctuations, only two concentrations will be quoted for the rest of this paper. These are 0.48 mg dust/well and 15.6 mg dust/well. The measurement of 0.48 mg dust/well was the mean of 16 wells (8 readings at the concentration of 0.32 mg dust/well and 8 readings at the concentration of 0.64 mg dust/well). The measurement of 15.6 mg dust/well was the mean of 8 readings at the concentration of 10.4 mg dust/well and 8 readings at the concentration of 20.8 mg dust/well (see Figure 2).

Statistics. For significance testing, T-test and Mann-Whitney U - Wilcoxon Rank Sum W test (SPSS V 2.0) were used.

RESULTS

The monkey kidney cells (VERO) seemed to be more sensitive to the dust extracts than the human lung carcinoma cells (A 549). The alkaline soluble extract (ASE) showed a significantly greater effect than the water soluble extract (WSE) did for almost all kinds of dust.

Crude cotton dust. None of the extracts exerted a measurable cytotoxic effect in the human cell line, A 549. In VERO cells, after 2 hours of incubation with the high concentration of WSE a small increase in the number of cells was found compared to exposure with ASE which exerted a slight cytotoxic effect at the same concentration. After 24 hours of incubation there was a significant ($p < 0.001$) cytotoxic effect of both extracts in the VERO cells (Fig. 3).

Grain dust. The A 549 cells showed a weak cytotoxic reaction against WSE after 2 hours of incubation and a significantly greater reaction after 24 hours of incubation. The VERO cells showed significant cytotoxic reactions against both extracts after 2 hours of incubation ($p< 0.001$). This effect was seen over the whole range of dust concentrations (Fig. 4).

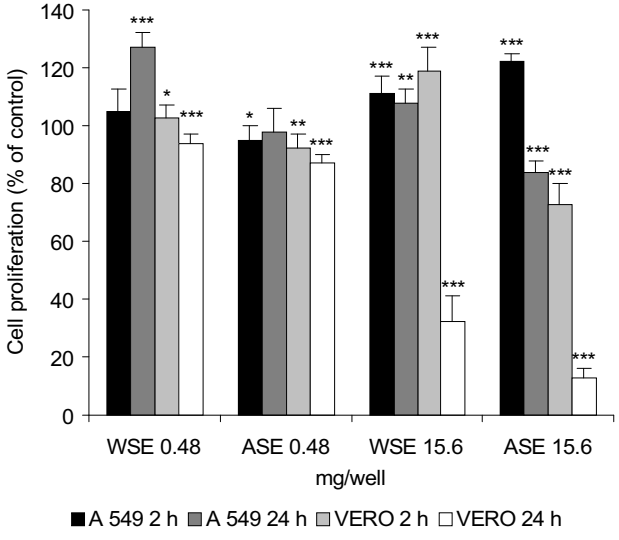

Figure 3. The cytotoxic effect of crude cotton dust. Viability expressed as % of unexposed cells ($\bar{x} \pm$ S.D.). * $p < 0.05$ group vs control, ** $p < 0.01$ group vs control.*** $p < 0.001$ group vs control.

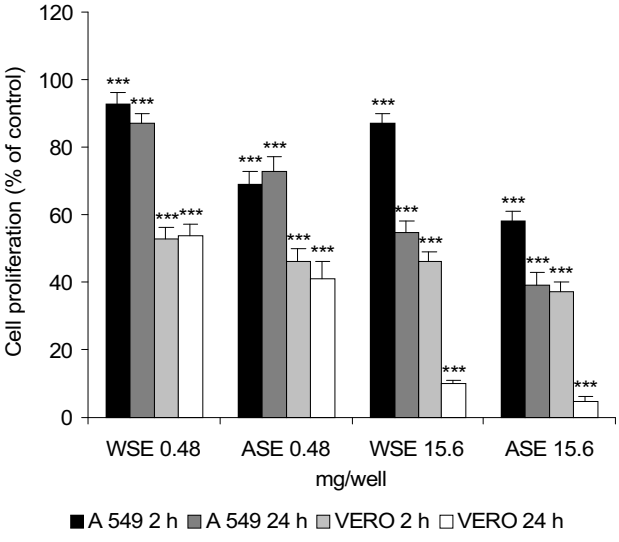

Figure 4. The cytotoxic effect of grain dust. Viability expressed as % of unexposed cells ($\bar{x} \pm$ S.D.). *** $p < 0.001$ group vs control.

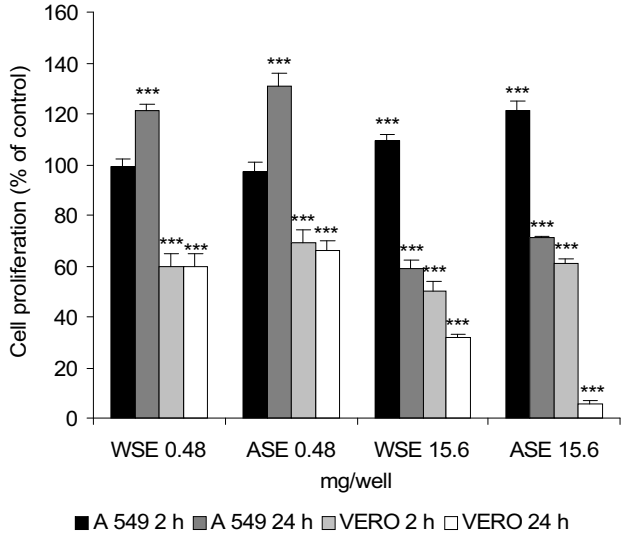

Figure 5. The cytotoxic effect of dust from a swine confinement building. Viability expressed as % of unexposed cells ($\bar{x} \pm$ S.D.). *** $p < 0.001$ group vs control.

Dust from swine confinement building. The water soluble and alkaline soluble extracts induced the same cytotoxic pattern in the cells. In A 549 cells there was a weak, although significant, proliferating reaction after 2 hours of incubation at the high concentrations (Fig. 5). After 24 hours of incubation there was also a weak, although significant, proliferating effect at the low concentrations of dust in contrast to a significant cytotoxic effect at the high concentrations (Fig. 5). The VERO cells showed a significant cytotoxic reaction ($p < 0.001$) after only 2 hours of incubation even at low dust concentrations.

Waste pellets. After 24 hours of incubation with extracts of waste pellets, A 549 cells showed no cytotoxic reaction against WSE. However, at that time a significant cytotoxic reaction against the strong concentration of ASE was seen (Fig. 6). The VERO cells were more sensitive and showed a weak reaction after 2 hours and significantly greater reactions after 24 hours of incubation against both extracts ($p < 0.001$). After incubation with the high concentration of waste pellet extract we found a stronger cytotoxic effect of ASE compared to WSE.

Compost. Both cell lines showed great cytotoxic reaction already at the low concentrations after 2 hours of incubation with both extracts. After 24 hours the activity of the cells had almost disappeared in the wells with the high concentrations of extracts. Activity was reduced by more than 50% at low concentrations (Fig. 7).

Comparing the different types of dust extracts showed that the most cytotoxic dust was compost and thereafter grain dust and dust from swine confinement building. Some of the organic dust extracts showed a growth promoting effect at low concentrations for 24 hours, i.e. swine confinement dust and crude cotton dust. These dusts also showed promotion of cell growth at high exposure concentrations for 2 hours.

DISCUSSION

The two cell lines were adherent epithelia cells which grew to confluence during the exposure and assay. These two cell lines were chosen because Hoult and Tuxford [13] have shown in them a cytopathic effect against toxin from *Bacillus pumilus*. A good and easy screening-test for the working environment was needed and therefore a preference was given to use available cell lines instead of primary cultures of human lung cells.

Cells were tested with extracts of five different types of dust and different patterns of the cytotoxic effect were found. These patterns could be caused by the various modes of operation of the heterogeneous types of dust. However, an earlier work demonstrated a cytotoxic potential of household waste during the composting process [24], which agreed with our knowledge of the composting rate and illnesses among garbage workers. We found a cytotoxic potential during the time of exposure, some of the dust extracts exerted a cytotoxic effect as soon as after 2 hours of incubation at the lower concentrations, while some of the other extracts only exerted an effect at higher concentrations after 24 hours of incubation.

The most aggressive type of dust extract seems to be compost. The cellular activity after 24 hours of incubation ranged from 84% to 1% in A 549 cells, which makes it possible to detect differences among the various dust types. These results suggest that this assay is a useful instrument for detecting the cytotoxic potential of organic

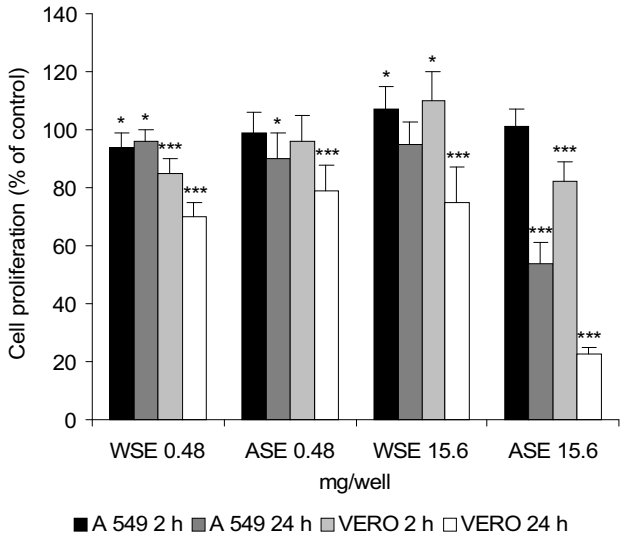

Figure 6. The cytotoxic effect of dust from waste pellets. Viability expressed as % of unexposed cells ($\bar{x} \pm$ S.D.). * $p < 0.05$ group vs control, *** $p < 0.001$ group vs control.

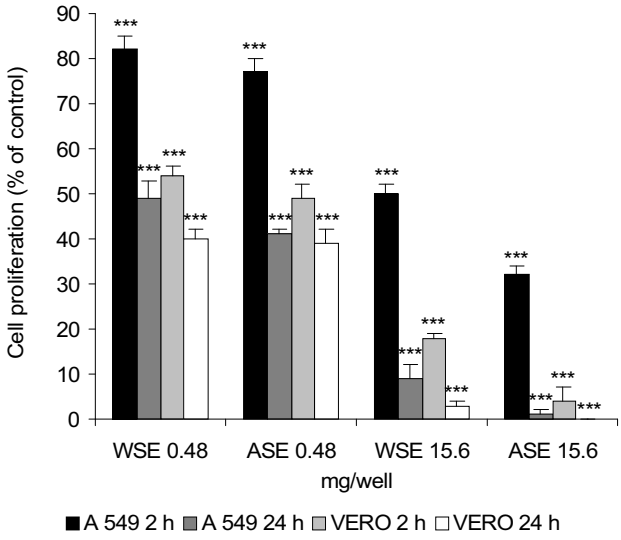

Figure 7. The cytotoxic effect of compost dust. Viability expressed as % of unexposed cells ($\bar{x} \pm$ S.D.). *** $p < 0.001$ group vs control.

dust found in working environments. VERO cells seems more sensitive towards the extracts than A 549 cells, and in this cell line incubation with 15.6 mg dust/well may be too strong to study organic dusts, because there was a strong effect after 24 hours of incubation with all extracts at this concentration. However, different cytotoxic potential was still found with various dust types. There is no obvious explanation why the kidney cells should be more sensitive to the dust than the lung cells, as found in this study. It might be an effect of the different content of serum in the growth mediums, to which toxic agents can bind. However, as long as the same ranking of the reactions towards the different dust types is found, both cell lines may be used to assay the cytotoxic potential.

Compost has a very high potential for microbial growth, especially during the middle part of the composting time due to the putrefactive process [5]. It was a limitation of this study that the concentration of microorganisms and other components in the dusts studied were not examined. However, the composition of microflora in compost from another batch was examined and it was found that 1/3 of the cells were fungi and that the number of cocci was larger than that of rod-shaped bacteria. Heat-treated cotton dust was also examined and a large number of different fungi comprising different species of *Aspergillus, Fusarium* and *Rhizopus* were found.

Organic dust is a complex mixture consisting of both Gram-positive and Gram-negative bacteria and their products (e.g. protein toxins and endotoxin), fungi and some chemical compounds. Organic dusts from different work places differ due to the different environmental conditions. However, if the same dust components cause adverse effects, the cytotoxic effect may be similar. Endotoxin from Gram-negative bacteria (LPS) is one of the components which has received the greatest attention among scientists working on organic dust and lung diseases [2, 19, 38]. In earlier studies [36] we tested our assay with pure LPS as well as LPS together with crude cotton dust extract. However, no effect of LPS in our assay was found, neither alone nor as an adjuvant. Also tested were other single components from organic dust [35]. However, the only toxins we found exerting an effect in our assay were toxins from *Bacillus* spp. and patulin, a mycotoxin [25]. Cotton dust mainly consists of bacteria and LPS, but fungi are also present [29].

When we examined the cytotoxicity of the extracts of the different types of dust, the greatest effect was seen in compost extracts. Compost contains many bacteria, actinomycetes and fungi in different concentrations and compositions, according to the composition of the raw compost and the stage of the composting process [1, 22, 33]. *Aspergillus fumigatus* is often present in large numbers [3].

Grain dust and dust from swine confinement buildings also exerted a significant cytotoxic effect on the cells. Grain dust, especially old and matured, as used in this assay, undoubtedly contained a large number of fungi, but the species composition was not determined. An investigation of airborne microbial contents in two types of swine confinement buildings in Quebec [4] showed a great content of fungi: *Scopulariopsis, Aspergillus, Penicillium* and *Candida* in the dust. We found no correlation between the total content of protein in the dust extracts and the cytotoxic effect (results not shown). The growth promoting effect of cotton and swine confinement dust observed at low concentrations, or at short exposing duration, might be explained as an effect of a stimulating

factor in the extracts that is obscured by the cytotoxic effect at higher doses or longer duration of exposures. This assay only examined whether the organic dust exerted a suppression of the proliferation of the cells. However, the cytokine release concurrently with the cytotoxic assay is presently being studied to see if there is any correlation between these two response parameters.

We found it necessary to supplement the WSE protocol with ASE extraction protocol, because the extracts with ammonium hydrogen carbonate (pH 8.3) as extraction medium showed a greater cytotoxic effect in both cell lines with nearly, but not all different types of organic dust. In earlier studies we have tested pure ammonium hydrogen carbonate alone in different concentrations and found no cytotoxic effect [23]. The greater effect of ASE extraction may be a result of the different treatment of the dust in the two extraction protocols. Another possibility might be that the organic dust contains at least one alkaline soluble cytotoxic component. Whether this component stems from fungi or elsewhere requires further investigation.

CONCLUSION

The presented assay was found to be a useful instrument for detecting the cytotoxic potential of organic dust found in different working environments. The assay makes it possible to show variation against both time of exposure and concentration of dust.

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REFERENCES

1. Breum NO, Nielsen EM, Nielsen BH: Bioaerosol exposure in collecting garden waste, recyclable materials and waste for incineration. *Ann Agric Environ Med* 1996, **3**, 27-32.

2. Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL: Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. *N Engl J Med* 1987, **317**, 605-610.

3. Clark CS, Rylander R, Larson L: Levels of Gram-negative bacteria, *Aspergillus fumigatus*, dust and endotoxin at compost plants. *Appl Environ Microbiol* 1983, **45**, 1501-1505.

4. Cormier Y, Tremblay G, Meriaux A, Brochu G, Lavoie J: Airborne microbial contents in two types of swine confinement buildings in Quebec. *Am Ind Hyg Assoc J* 1990, **6**, 304-309.

5. Crook B, Bardos RP, Lacey J: Domestic waste composting plants as sources of airborne micro-organisms. *UK Department of Environment, grant no. 1/MS/126/209/85.* 1988.

6. Donham KJ, Zavala DC, Merchant JA: Respiratory symptoms and lung function among workers in swine confinement buildings: A cross-sectional epidemiological study. *Arch Environ Health* 1984, **39**, 96-101.

7. DoPico GA, Reddan W, Flaherty D, Reed C, Tsiatis A: Health effects of occupational grain dust exposure. *Am J Ind Med* 1986, **10**, 298-299.

8. Fedan JS, Cahill JF, Franzak MS, Tucker JH, Weber KC, Morey P: Effects of a water extract of cotton bract (CBE) in isolated guinea-pig trachealis smooth muscle (GPT). **In:** Jacobs RR, Wakelyn PJ (Eds): Cotton Dust. *Proceedings of the Tenth Cotton Dust Research Conference, Beltwide Cotton Research Conference, Las Vegas, January 8-9, 1986*, 137-139. National Cotton Council, Memphis, TN 1986.

9. Fonn S, Groenveld HT, DeBeer M, Becklake MR: An environmental and respiratory health survey of workers in a grain mill in the Johannesburg area, South Africa. *Am J Ind Med* 1993, **24**, 387-400.

10. Gravesen S, Ipsen H, Skov P: Partial characterization of the components in the macromolecular organic dust (MOD) fraction and their possible role in the sick-building-syndrome (SBS). *Proceedings of International Conference on Indoor Air Quality and Climate*. Helsinki 1993.

11. Greenblatt GA, Ziprin RL: The effects of dust and bract extracts on alveolar macrophage prostaglandin production and cockroach hindgut muscle contraction. **In:** Jacobs RR, Wakelyn PJ (Eds): Cotton Dust. *Proceedings of the Tenth Cotton Dust Research Conference, Beltwide Cotton Research Conference, Las Vegas, January 8-9, 1986*, 86-89. National Cotton Council, Memphis, TN 1986.

12. Haglind P, Lundholm M, Rylander R: Prevalence of byssinosis in Swedish cotton mills. *Br J Ind Med* 1981, **38**, 138-143.

13. Hoult B, Tuxford AF: Toxin production by *Bacillus pumilus*. *J Clin Pathol* 1991, **44**, 455-458.

14. Iversen M, Dahl R: Specific antigens in dust from swine confinement buildings. *Am J Ind Med* 1994, **25**, 49-51.

15. Iversen M, Dahl R, Korsgaard J, Hallas T, Jensen EJ: Respiratory symptoms in Danish farmers: An epidemiological study of risk factors. *Thorax* 1988, **43**, 872-877.

16. Iversen M, Korsgaard J, Hallas T, Dahl R: Mite allergy and exposure to storage mites and house dust mites in farmers. *Clin Exp Allergy* 1990, **20**, 211-219.

17. Johnson CM, Hanson MN, Rohrbach MS: Endothelial cell cytotoxicity of cotton bracts tannin and aqueous cotton bracts extract: Tannin is the predominant cytotoxin present in aqueous cotton bracts extract. *Environmental Health Perspectives* 1986, **66**, 97-104.

18. Katila M, Mäntyjärvi RA, Ojanen T: Sensitization against environmental antigens and respiratory symptoms in swine workers. *Br J Ind Med* 1981, **38**, 334-338.

19. Kennedy SM, Christiani DC, Eisen EA, Wegman DH, Greaves IA, Olenchock SA, Ye T-T, Lu P-L: Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am Rev Respir Dis* 1987, **135**, 194-200.

20. Lundholm M, Rylander R: Occupational symptoms among compost workers. *J Occup Med* 1980, **22**, 256-257.

21. Malo J-L, Cartier A, Lárcheveque J, Trudeau C, Courteau J-P, Bherer L: Prevalence of occupational asthma among workers exposed to eastern white cedar. *Am J Respir Crit Care Med* 1994, **150**, 1697-1701.

22. Nakasaki K, Kato J, Akiyama T, Kubota H: Transformations of water soluble organic materials during thermophilic composting of sewage sludge. *J Ferment Technol* 1987, **65**, 675-681.

23. Roepstorff V, Sigsgaard T: Comparison of the effect of two different extraction protocols on the cytotoxicity of cotton dust extracts on cell cultures. **In:** Jacobs RR, Wakelyn PJ, Domelsmith LN (Eds): Cotton Dust. *Proceedings of the Eighteenth Cotton Dust Research Conference, Beltwide Cotton Research Conference, San Diego, January 7, 1994*, 355-357. National Cotton Council, Memphis, TN 1994.

24. Roepstorff V, Sigsgaard T: The cytotoxic potential of household waste during composting. *Waste Management & Research* 1996, in press.

25. Roepstorff V, Sigsgaard T: Cytotoxic effect of patulin on human pneumocytes, *in vitro*. **In:** Wakelyn PJ, Jacobs RR, Rylander R (Eds): Cotton and Other Organic Dusts. *Proceedings of the Twentieth Cotton and Other Organic Dusts Research Conference, Beltwide Cotton Conferences, Nashville, TN, January 11-12, 1996*, 323-327. National Cotton Council, Memphis, TN 1996.

26. Rylander R: Lung diseases caused by organic dusts in the farm environment. *Am J Ind Med* 1986, **10**, 221-227.

27. Rylander R, Donham KJ, Hjort C, Brouwer R, Heederik D: Effects of exposure to dust in swine confinement buildings - a working group report. *Scand J Work Environ Health* 1989, **15**, 309-312.

28. Rylander R, Essle N, Donham KJ: Bronchial hyperreactivity among pig and dairy farmers. *Am J Ind Med* 1990, **17**, 66-69.

29. Rylander R, Haglind P, Lundholm M: Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. *Am Rev Respir Dis* 1985, **131**, 209-213.

30. Rylander R, Lundholm M: Bacterial contamination of cotton and cotton dust and effects on the lung. *Br J Ind Med* 1978, **35**, 204-207.

31. Sigsgaard T, Bach B, Taudorf E, Malmros P, Gravesen S: Accumulation of respiratory disease among employees in a recently established plant for sorting refuse. *Ugeskr Læg* 1990, **152/35**, 2485-2488.

32. Sigsgaard T, Hansen I, Sherson D: Asthma, hyperreactivity, and ODTS among recycling workers. *Eur Respir J* 1992, **Suppl. 5**, 407s-408s.

33. Sigsgaard T, Malmros P, Nersting L, Petersen C: Respiratory disorders and atopy in Danish refuse workers. *Am J Respir Crit Care Med* 1994, **149**, 1407-1412.

34. Sigsgaard T, Pedersen OF, Juul S, Gravesen S: Respiratory disorders and atopy in cotton, wool, and other textile mill workers in Denmark. *Am J Ind Med* 1992, **22**, 163-184.

35. Sigsgaard T, Roepstorff V: Cytotoxic reaction of human pneumocytes and monkey kidney cells after exposure to organic dust and dust components. **In:** Wakelyn PJ, Jacobs RR, Rylander R (Eds): Cotton and Other Organic Dusts. *Proceedings of the Twentieth Cotton and Other Organic Dusts Research Conference, Beltwide Cotton Conferences, Nashville, TN, January 11-12, 1996*, 323-327. National Cotton Council, Memphis, TN 1996.

36. Sigsgaard T, Roepstorff V, Tuxford AF: Cytotoxicity of cotton dust and dust components to A549 and VERO cells. **In:** Jacobs RR, Wakelyn PJ, Domelsmith LN (Eds): Cotton Dust. *Proceedings of the Eighteenth Cotton Dust Research Conference, Beltwide Cotton Research Conference, San Diego, January 7, 1994*, 322-325. National Cotton Council, Memphis, TN 1994.

37. Sigsgaard T, Roepstorff V, Tuxford AF, Hoult B: Cytotoxicity of *Bacillus* species to VERO cells. **In:** Domelsmith LN, Jacobs RR, Wakelyn PJ (Eds): Cotton Dust. *Proceedings of the Seventeenth Cotton Dust Research Conference, Beltwide Cotton Research Conference, New Orleans, January 13-14, 1993*, 258-260. National Cotton Council, Memphis, TN 1993.

38. Smid T, Heederik D, Houba R, Quanjer PH: Dust- and endotoxin-related respiratory effects in the animal feed industry. *Am Rev Respir Dis* 1992, **146**, 1474-1479.

39. Von Essen SG, Thompson AB, Robbins RA, Jones KK, Dobry CA, Rennard SI: Lower respiratory tract inflammation in grain farmers. *Am J Ind Med* 1990, **17**, 75-76.

40. Zuskin E, Ivankovic D, Schachter EN, Witek TJ: A ten-year follow-up study of cotton textile workers. *Am Rev Respir Dis* 1991, **143**, 301-305.