DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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Centers for Disease Control
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National Institute for Occupational
Safety and Health (NIOSH)
1095 Willowdale Road
Morgantown, WV 26505-2888

March 12, 2008
HETA 2001-0445
Interim Letter IX

Mike Winkler, First Vice-President
Administrative and Residual Employees Union Local 4200
705 North Mountain Road, Suite A211
Newington, Connecticut 06111-1411

Dear Mr. Winkler:

Included with this letter is a hard copy of a poster to be presented by NIOSH personnel at the Society of Toxicology meeting to be held in Seattle, Washington on March 16-20, 2008. This presentation will report inflammatory effects in mice of floor dusts collected from the 2002 survey at the 25 Sigourney Street Building and associations of levels of endotoxin and glucan in dust with the inflammatory markers. The animal study is still ongoing, and the presentation is based on preliminary results.

The findings included in the presentation are as follows:

1. The exposure to the building floor dust produced dose-dependent inflammatory responses in mouse lung.
2. Although the levels of endotoxin and glucan in floor dust do not appear to be associated well with the markers of lung inflammation (since the significant association of the 2002 analytical results of endotoxin in dust with the inflammatory markers was driven by a single outlier), the polymyxin B experiment (polymyxin B: antibiotics which deactivates endotoxin activity) suggests that endotoxin may be an important inflammatory substance in dust in this animal model.

If you have any questions regarding the information provided in this interim letter, please do not hesitate to contact us at 1-800-232-2114.

Sincerely,

Park, Juhyeong

Ju-Hyeong Park, ScD, MPH, CIH
Environmental Health Scientist
Respiratory Disease Hazard Evaluation
and Technical Assistance Program
Field Studies Branch
Division of Respiratory Disease Studies

Use of a Mouse Model to Evaluate Pulmonary Inflammation Caused by Floor Dust from a Water-Damaged Building

S-H. Young, J. M. Cox-Ganser, M. Wolfarth, J.M. Antonini, V. Castranova, J-H. Park

National Institute for Occupational Safety and Health, Morgantown, WV

Introduction

Although the causes of building-related respiratory illness are still unclear, epidemiological research has indicated that fungi and endotoxin in floor dust are associated with such health risks. In the present study, we used a mouse model to evaluate pulmonary inflammation caused by floor dusts collected from the workstations of employees in a water-damaged office building. The dusts were tested in an endotoxin-sensitive strain of mouse - C3HeB/FeJ, and pulmonary inflammation was determined. We examined the correlation among markers of inflammation and levels of endotoxin and (1→3)-β-D-glucan, a major cell wall component of fungi.

Aims

1) To examine pulmonary inflammatory responses to increasing doses of floor dust from a water-damaged building.
2) To examine the correlations among markers of inflammation and levels of endotoxin and (1→3)-β-D-glucan.

Methods and Materials

Dust sample: Dust samples were collected from carpeted floors of workstations using backpack vacuum samplers with crevice tools. For each sampling location, a 2 m^2 floor area was vacuumed for 5 minutes. Each dust sample was analyzed for endotoxin and (1→3)-β-D-glucan.

Animals: Pathogen-free male endotoxin-sensitive strain of mouse - C3HeB/FeJ (number of mice=4 to 6 per dust sample) was used.

Exposure: Each mouse was treated with dust sample (1, 1.5, 2.5 or 3.5 mg/kg of body weight suspended in 40 µL of saline) by pharyngeal aspiration. Control mice received an equivalent volume of saline by aspiration.

Polymyxin B treatment: Dust was suspended in polymyxin B (PMB) solution (1mg/ml) and incubated at 37 °C for 30 min. PMB binds to and deactivates endotoxin.

Pulmonary Parameters:

At 18 hrs post aspiration, bronchoalveolar lavage (BAL) was done postmortem on lungs and the following inflammatory and lung injury markers were measured:

1. Lung inflammation
   Polymorphonuclear leukocytes (PMN) infiltration: Differential cells counts from total BAL cells.
2. Lung damage
   Lactate dehydrogenase (LDH) activity and albumin concentration from acellular BAL fluid.
3. Cytokine production
   Cytokines production : IL-12p70, TNF-α, IFN-γ, MCP-1, IL-10, and IL-6 in first acellular fraction of BAL fluid.

Disclaimer
The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Results

Dust-induced Pulmonary Inflammation- Dose-Response

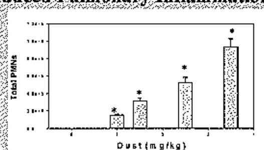

Figure 1, Total number of PMN after exposure to dust. One day post dust exposure, PMN was harvested from BAL fluid. There is a dose-dependent increase of PMN counts. Values are means ± S.E. * Significantly different than the mean value of saline control mice (p<0.05).

Lung Cell Injury

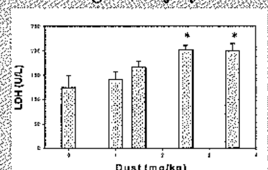

Figure 2, Lactate dehydrogenase (LDH) level in acellular BAL fluid after exposure to different doses of dust. There was a dose-dependent increase of LDH. Values are means ± S.E. * Significantly different than the mean value of saline control mice (p<0.05).

Cytokine Responses

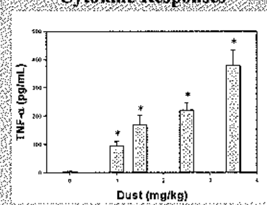

Figure 3, Pro-inflammatory cytokine TNF-α in the acellular BAL fluid of mice that were exposed to different doses of dust. Values are means ± SE. * Significantly different than the mean value of saline control mice (p<0.05).

Table 1, Pearson product moment correlations between analytes in dust and markers of inflammation. The cell contents are : r = Correlation Coefficient, p = P Value, and n = Number of Samples. Significant difference was set at p < 0.05 and was colored in yellow. <0.05 denotes that p-value is between 0.001 and 0.05.

		Alb	BAL cells	PMNs	TNF-α	IFN-γ	MCP-1	IL-6	Glucan	EU2007	EU2002
LDH	r	0.627	0.477	0.538	0.384	0.223	0.42	0.45	-0.0878	0.3494	0.0967
	p	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	0.336	0.097	0.23
	n	160	156	165	156	160	160	160	122	122	156
Alb	r		0.508	0.497	0.4	0.25	0.688	0.549	-0.144	-0.00200	0.0473
	p		<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	0.114	0.982	0.559
	n		155	156	158	156	168	168	122	122	156
BAL cells	r			0.947	0.573	0.126	0.48	0.542	-0.0807	0.0510	0.162
	p			<0.001	<0.001	0.119	<0.001	<0.001	0.377	0.57	<0.05
	n			155	155	155	155	155	122	122	155
PMNs	r				0.539	0.182	0.537	0.642	-0.138	0.3505	0.207
	p				<0.001	<0.05	<0.001	<0.001	0.129	0.930	<0.05
	n				155	155	155	155	122	122	155
TNF-α	r					0.202	0.619	0.757	-0.256	-0.0799	0.0369
	p					<0.05	<0.001	<0.001	<0.05	0.382	0.649
	n					158	156	156	122	122	156
IFN-γ	r						0.304	0.427	0.0149	0.174	0.197
	p						<0.001	<0.001	0.074	0.0553	<0.05
	n						160	160	122	122	156
MCP-1	r							0.780	-0.139	-0.0579	0.219
	p							<0.001	0.129	0.526	<0.05
	n							160	122	122	156
IL-6	r								-0.198	-0.00620	0.109
	p								<0.05	0.947	<0.05
	n								122	122	160
Glucan	r									0.450	0.092
	p									<0.001	0.314
	n									122	122
EU-2007	r										0.111
	p										0.222
	n										122

Table 2, Descriptive statistic for endotoxin measured in 2007 (EU-2007), endotoxin measured in 2002 (EU-2002), and (1→3)-β-D-glucan.

Column	Size	Mean	Std. Error	Range	Max	Min
EU-2007 (EU/mg)	25	38.260	5.944	267.790	272.000	4.210
EU-2002 (EU/mg)	86	16.807	3.000	119.969	120.000	0.0310
Glucan (ng/mg)	25	51.196	3.053	118.940	123.700	4.760

Polymyxin B Binds to Endotoxin and Decreases PMNs

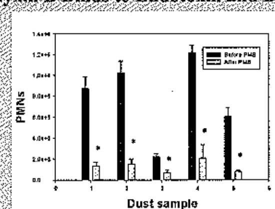

Figure 4, Total number of PMNs in BAL fluid of mice after exposure to 2.5 mg/kg bwt dust w/without polymyxin B (PMB) treatment. The PMB-treated dust was suspended in PMB solution and incubated at 37 °C for 30 min. Values are means ± S.E.
* Significantly different than the mean value of without PMB treatment mice (p<0.05).

Decreased TNF-α After Polymyxin B Treatment

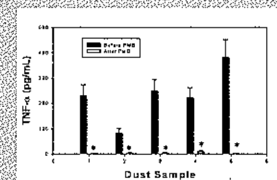

Figure 5, TNF-α production in BAL fluid of mice after exposure to 2.5 mg/kg bwt dust w/without polymyxin B (PMB) treatment. The PMB treated-dust was suspended in PMB solution and incubated at 37 °C for 30 min. Values are means ± S.E. * Significantly different than the mean value of without PMB treatment mice (p<0.05).

Correlation Between EU2007 and EU2002

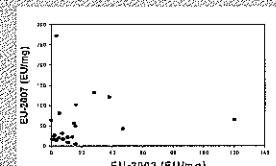

Figure 6, Scatter plot of EU2007 vs EU2002. These results were obtained by using the same LAL method but from 2 different labs. If we remove the two outliers from the graph, then EU2007 was significantly correlated with EU2002.

Summary and Conclusions

- Endotoxin in the floor dust was associated with markers of inflammation in these experiments. Endotoxin 2002 was significantly correlated with PMN, total BAL cells, IFN-γ, MCP-1, and IL-6. However, even at a very low endotoxin level inflammation still occurred. This suggested that another component in the dust also contributed to the overall inflammatory potency.
- The animal experiment was done in 2007-8. The collected dust samples were stored for 6 years in -80°C. EU-2002 data was 6 years old which may not reflect the actual endotoxin levels at the time of experiment. The correlations of EU-2002 with the markers were driven by an outlier. EU-2007 is a current endotoxin measurement, but was not correlated with markers of inflammation.
- Polymyxin B experiments indicated that endotoxin was a contributing factor in pulmonary inflammation in mice exposed to dust from a water-damaged building.
- β-Glucan measurement was not correlated with markers of inflammation. Possible reasons may include: 1. β-glucan is a weaker inflammatory agent than endotoxin, therefore some endotoxin contamination can mask the effect of β-glucan ; 2. The current available β-glucan measurement method is for detecting water soluble β-glucan, which was much less inflammatory than insoluble β-glucan.

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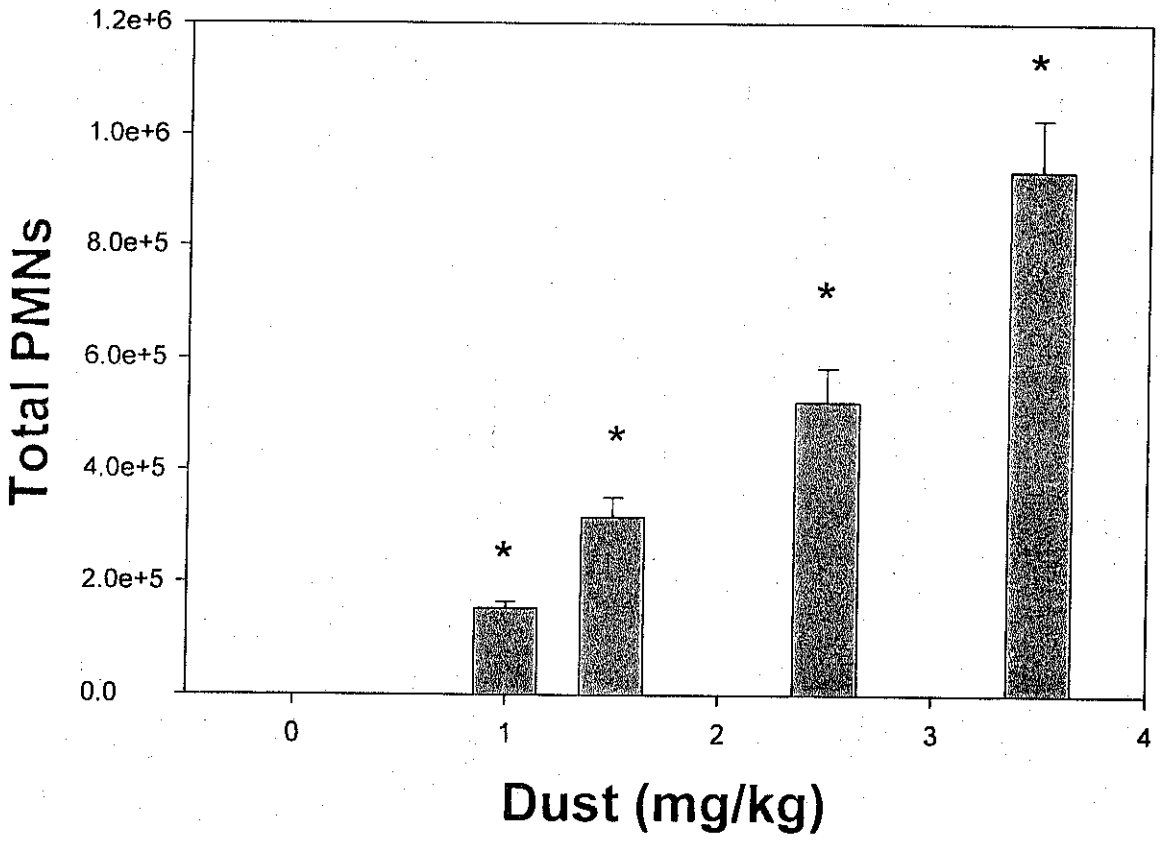

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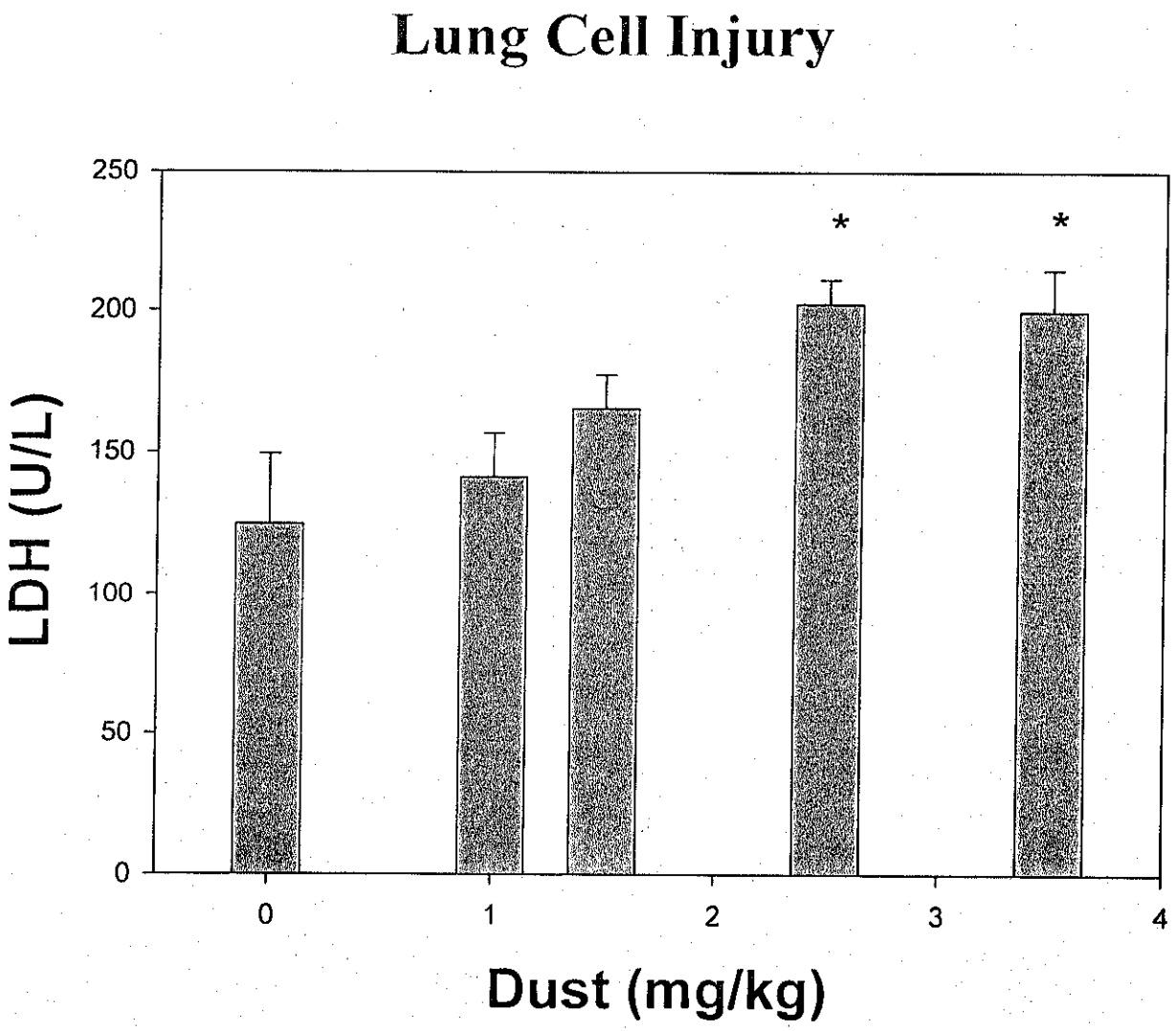

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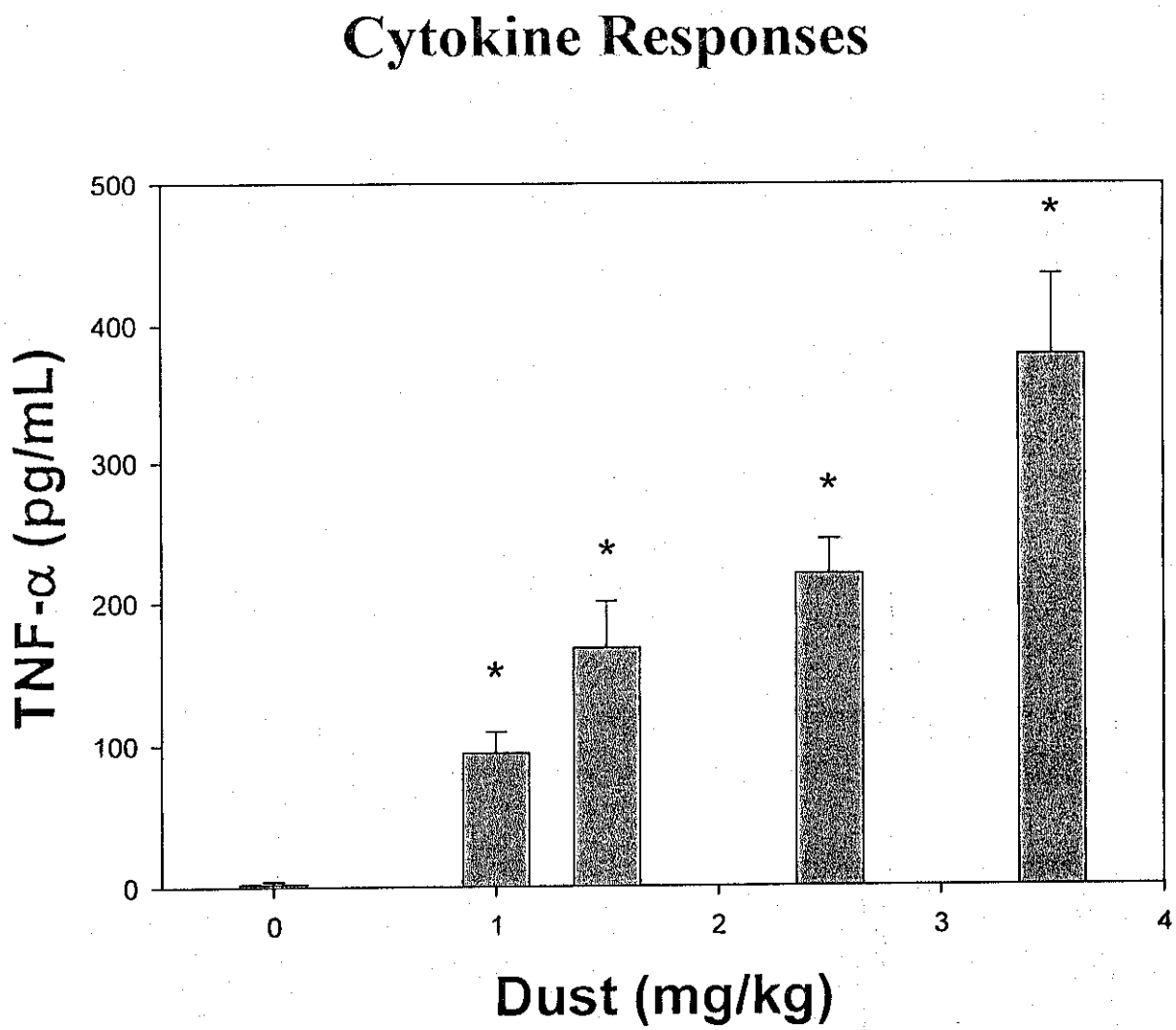

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	n	156	155	155	156	156	156	156	122	122	156
Ab	r		0.396	0.487	0.4	0.25	0.688	0.549	-0.144	-0.00209	0.0473
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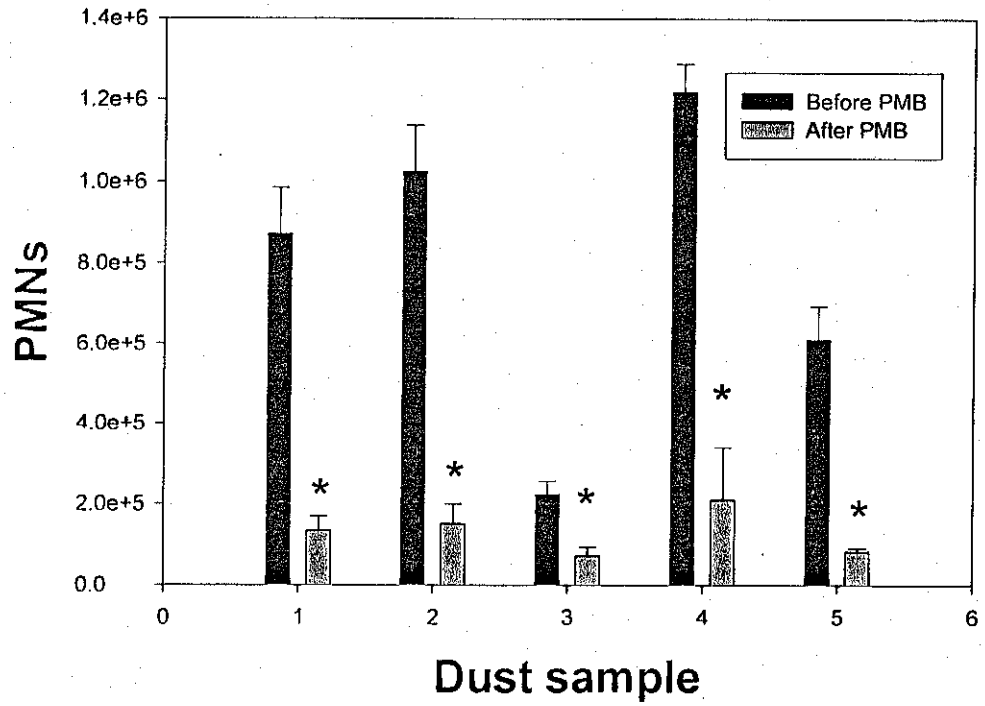

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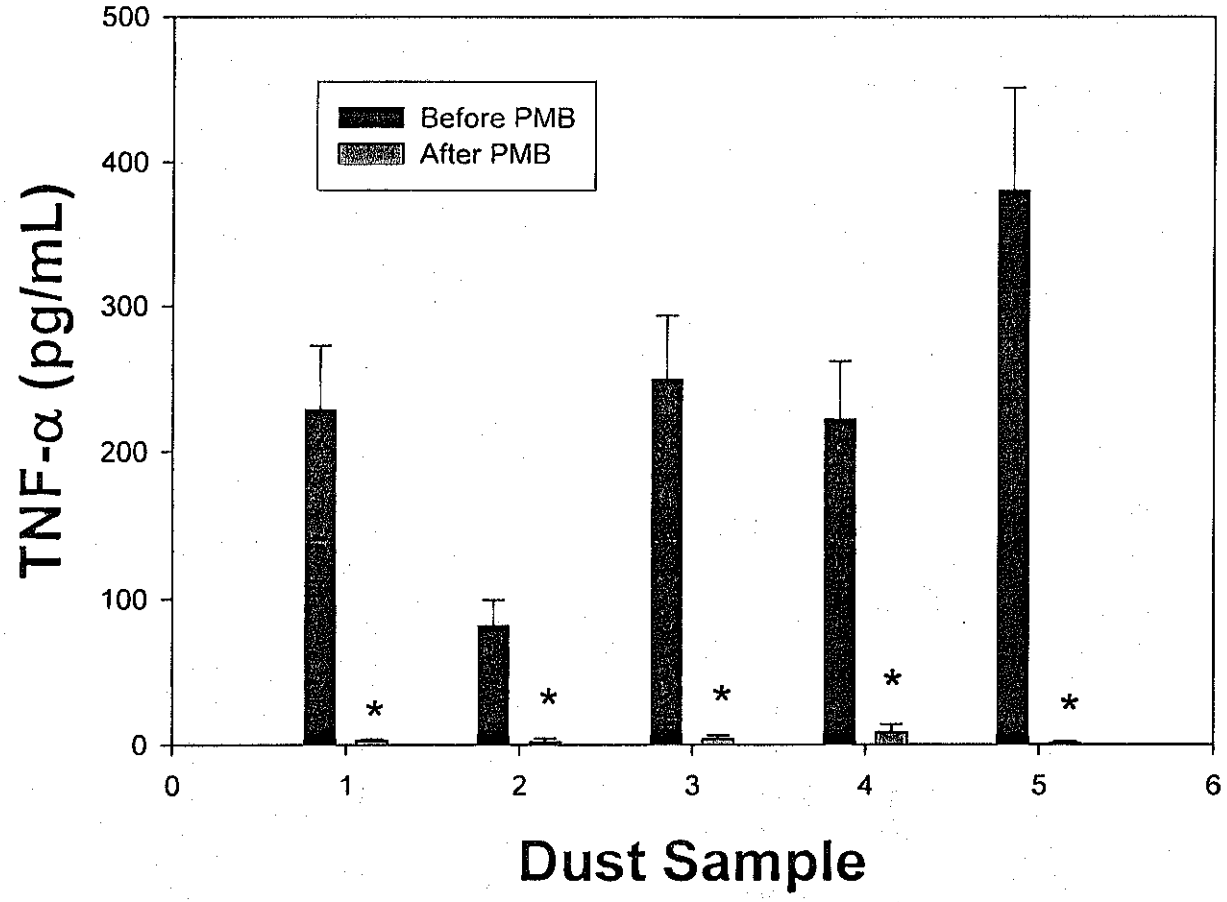

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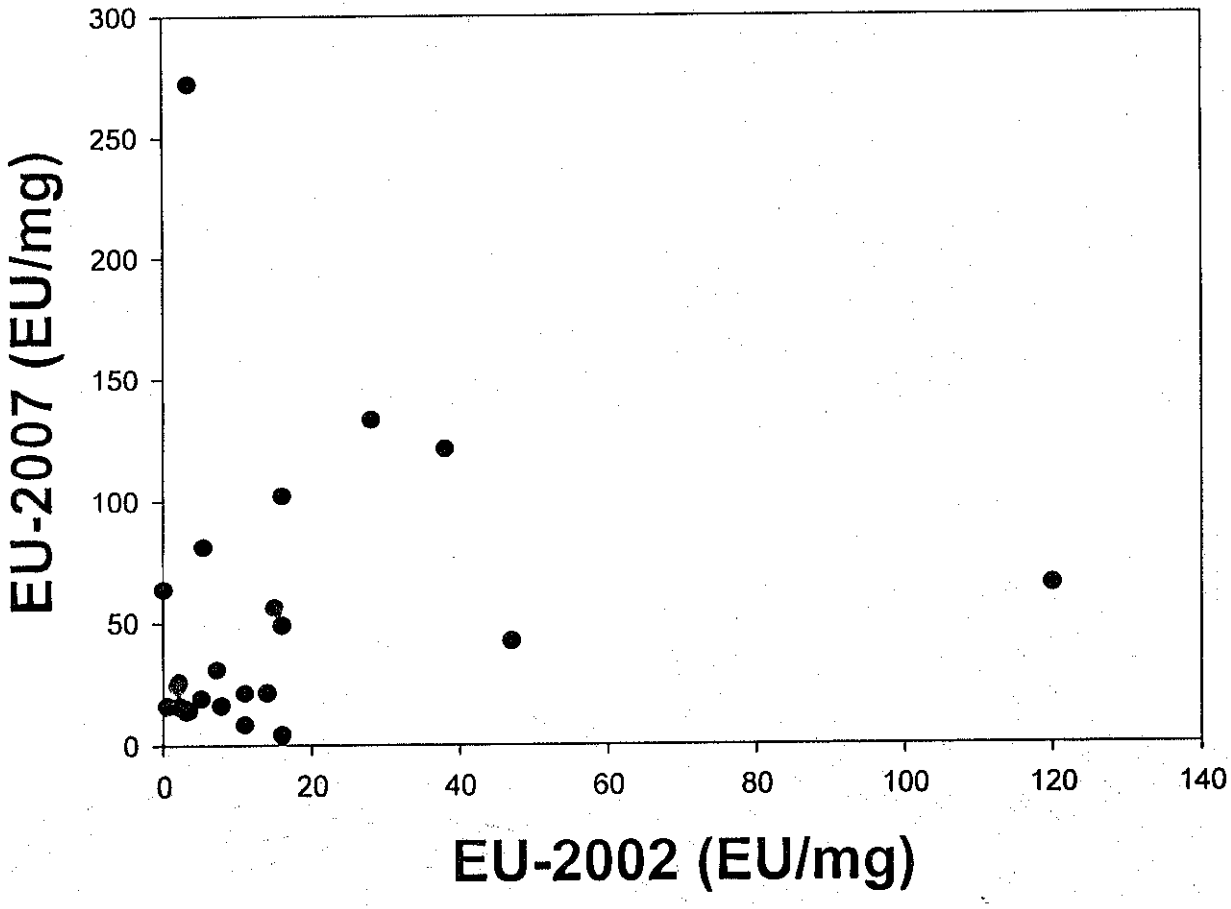

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