Journal of Toxicology and Environmental Health, Part A

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: http://www.tandfonline.com/loi/uteh20

Comparative in Vitro Toxicity of Grape- and Citrus-Farm Dusts

Val Vallyathan , Donna Pack , Steve Leonard , Robert Lawson , Marc Schenker & Vince Castranova

To cite this article: Val Vallyathan , Donna Pack , Steve Leonard , Robert Lawson , Marc Schenker & Vince Castranova (2007) Comparative in Vitro Toxicity of Grape- and Citrus-Farm Dusts , Journal of Toxicology and Environmental Health, Part A, 70:2, 95-106, DOI: 10.1080/15287390600747825

To link to this article: https://doi.org/10.1080/15287390600747825

Published online: 24 Feb 2007.

Journal of Toxicology and Environmental Health, Part A, 70: 95–106, 2007

ISSN: 1528-7394 print / 1087-2620 online
DOI: 10.1080/15287390600747825

Taylor & Francis

Comparative in Vitro Toxicity of Grape- and Citrus-Farm Dusts

Val Vallyathan, Donna Pack, and Steve Leonard
Pathology and Physiology Branch, National Institute for Occupational Safety and Health, Health Effects Laboratory Division, Morgantown, West Virginia, USA

Robert Lawson and Marc Schenker
Department of Public Health Sciences, University of California, Davis, California, USA

Vince Castranova
Pathology and Physiology Branch, National Institute for Occupational Safety and Health, Health Effects Laboratory Division, Morgantown, West Virginia, USA

Agricultural workers are exposed to a variety of airborne dusts, including crystalline silica and other inorganic minerals. This study was designed to characterize the organic and inorganic components of agricultural dusts in California grape- and citrus-farm fields and to compare their cytotoxicity using in vitro toxicity bioassays as predictors of pathogenicity. Aerosolized dusts collected from farm fields were characterized by scanning-electron-microscopic energy-dispersive x-ray analysis, x-ray diffraction, trace metal analysis by plasma emission spectroscopy, and surface area measurements. As indicators of cytotoxicity, cell viability, release of alveolar enzymes activities (lactate dehydrogenase, N-acetyl glucosaminidase), production of reactive oxygen species (ROS), such as H2O2 and hydroxyl radical (OH), and lipid peroxidation were monitored after exposure of cells to grape- and citrus-farm dusts or inorganic components of these dusts. In addition, activation of nuclear factor κ B and activator protein-1 were evaluated at the peak time for response of 36 h postexposure. All toxicity studies were done in comparison with crystalline silica of similar particle size and diameter using the same mass concentrations as farm dusts. The results showed that inorganic minerals in the aerosolized farm dust fractions were mostly composed of aluminum silicates, crystalline silica, and free iron. Crystalline silica used in these studies was more cytotoxic than grape- and citrus-farm dusts. However, in general, citrus farm dust exhibited the greatest ability to generate ROS and induce lipid peroxidation. These results support human epidemiologic studies, reporting an increased incidence of pulmonary fibrosis in farm workers, by documenting the potential of farm dusts to induce oxidative stress and initiate disease development.

Accepted 16 January 2006.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Address correspondence to Val Vallyathan, PhD, 1095 Willowdale Road, NIOSH, Morgantown, WV 26505, USA. E-mail: vav1@cdc.gov

It is estimated that more than 5 million people work in the U.S. agricultural industry, involving a wide range of activities in diverse geographic and climatic conditions (ATS, 1998). Farmers and other agricultural workers in arid conditions have the potential for inhalation exposure to several toxic hazards, including crystalline silica, silicates, and pesticide residues and their fillers from soil, as well as organic dust, microorganisms, mycotoxins, and allergens from agricultural products (ATS, 1998; Schenker, 2000; Kirkhorn & Garry, 2000). As a result of these exposures, several types of acute and chronic respiratory diseases are well recognized as occupational hazards for U.S. farmers (Brackbill et al., 1994; ATS, 1998; Omland, 2002; LeVan et al., 2005). Among the various types of health hazards, occupational lung disease produced by the inhalation of inorganic mineral dusts in agricultural workers is one of increasing concern (Schenker, 2000). Several studies demonstrated a significantly increased risk for respiratory morbidity and mortality in agricultural workers (ATS, 1997; Monso et al., 2003). Exposure to inorganic minerals encountered at the workplace may produce alveolitis, diffuse interstitial fibrosis, focal macules, nodular fibrotic lesions, and small airway disease in workers (Schenker, 2000). This health hazard is being recognized with increasing frequency among agricultural workers in arid conditions.

Epidemiologic studies of farm workers documented the increased incidence and prevalence of respiratory functional impairments with restrictive disease symptoms in California grape and citrus workers (Gamsky et al., 1992a, 1992b). In a study of California rice farm workers, small irregular opacities with scores greater than 1/0 consistent with dust or fiber exposure were reported in 10% of workers (McCurdy et al., 1996). Farming in dry conditions is a dusty occupation, and dust levels often exceed the threshold limit value of 3 mg/m^3 (Atiemo et al., 1980; Nieuwenhuijsen et al., 1996, 1998, 1999; Lee et al., 2004). The respirable size fraction of dust generated

was reported to have a mass median diameter of <5 μm. While soil composition is predomininantly determined by geographic factors, it is also influenced by the use of agricultural chemicals with fillers and carriers for pesticides, such as talc, zeolite, kaolin, attapulgite, vermiculite, and other clay minerals.

High concentrations of crystalline silica, a potent fibrogenic dust, were found in farm dusts. In a study of 12 Alberta farms, silica content was shown to range from 0.8 to 17%, with other minerals, such as feldspar, talc, illite, kaolin, and calcite, being found at varying concentrations (Green et al., 1990). Therefore, in most agricultural work conditions, exposure to mixtures of minerals with varying concentrations of silica may be likely. Silicosis, silicate pneumoconiosis, and mixed dust pneumoconiosis in farm workers were reported in several case reports (Dynnik et al., 1981; Fennerty et al., 1993; Sherwin et al., 1979; Gylseth et al., 1984). In an autopsy study of California Central Valley Hispanic males, a high prevalence of simple pneumoconiosis with accumulation of mineral and carbonaceous dust was reported (Pinkerton et al., 2000). Granulomatous interstitial pneumonitis associated with the presence of birefringent polarizing material in pulmonary tissue of animals from California farms provides further evidence of the potential for inhalation of farm dust to induce pneumoconiosis (Brambilla et al., 1979).

Lung injury and fibrogenic response to dust generated during citrus and grape harvesting found that the grape-farm dust was more fibrogenic than the citrus-farm dust (Rajini et al., 1995). However, this study failed to provide any additional information on the components of dusts that elicited the fibrogenic response. Since the dust samples instilled intratracheally to the animals were identical in mass concentrations but contained different concentrations of silica and other minerals, the etiologic factors involved in the development of fibrogenic response to these dusts are not known. Furthermore, it is likely that the toxicity produce by silica will vary markedly from region to region due to the presence, concentration, and composition of other minerals and weathering effects on silica. Therefore, the purpose of the present study was to (1) investigate the cellular toxicity of grape- and citrus-farm dusts, (2) measure the inorganic minerals, organic dust fraction, endotoxin, and other potential etiologic agents, and (3) determine whether a relationship exists between the mineral content and other contributing factor(s) in the dusts with in vitro toxicity and reported human health risk.

MATERIALS AND METHODS

Using a high volume air sampler, aerosolized dust samples (250 L sample volume) were collected from table grape and orange harvesting work sites covered by a plastic canopy in the Central Valley of California. The pump was operated at 30 L/min and was fitted with a 20 × 25-cm polycarbonate filter with 0.4 μm pore size. The foliage was agitated several times during the 90 min per sample collection periods, and 2 samples were collected daily during the peak harvesting season for this study. Dust samples from the polycarbonate filters were sieved using a 10-μm-mesh filter to remove foliage. Stock crystalline silica (Min-U-Sil <5 μm, U.S. Silica, Berkeley Springs, WV) of 99.8% purity and diameter of less than 5 μm was used in all the studies as a positive control.

X-Ray Diffraction

Samples of citrus and grape harvest dusts were analyzed by x-ray diffraction for quartz, cristobalite, and tridymite according to NIOSH method 7500 (NIOSH, 1994) with some modifications. A 2-mg bulk sample was weighed onto FWS-B filters and was dissolved in tetrahydrofuran instead of ashing in a furnace. Standards and samples were run concurrently, and an external calibration curve was prepared from the integrated intensity, rather than using the suggested normalization procedure.

Trace Element Analysis by Plasma Emission Spectrometry

Samples were analyzed for 17 trace elements and metals after microwave digestion in 10 ml concentrated nitric acid. Digested samples were transferred to 50-ml volumetric flasks by rinsing with ASTM Type II water and brought up to 50 ml volume. Aliquots were then analyzed using a Thermo-Jarrel Ash ICAP-61 inductively coupled plasma emission spectrometer controlled by Thermospec software, according to NIOSH method 7300 (NIOSH, 1994).

Scanning Electron Microscopy and Concentration-Response Energy-Dispersive X-Ray Analysis

A third set of dust samples was analyzed by scanning electron microscopy (SEM) in combination with back-scattered imaging (BSI) and energy-dispersive x-ray analysis (EDXA), according to the methods described by Stettler et al. (1991). For these analyses a small amount of the sample (4–6 mg) was suspended in 100 ml of ultrapure (Type I) water with a drop of Aerosol OT surfactant and sonicated for 10 min. An aliquot of 5 ml was then filtered through a 25-mm-diameter polycarbonate filter with a pore size of 0.1 μm. The filter was then mounted on to carbon planchet. Using the SEM in the BSI mode, inorganic particles were identified and analyzed by EDXA. From each farm dust sample, 1000 or more particles from randomly selected fields of view were analyzed for elemental composition and mass median area diameter (MMAD).

Surface Area Measurements

Surface area measurements were made on 0.5-g samples by the nitrogen adsorption technique, using a surface analyzer

(model: Gemini 2360, Micrometric, Narcoss, GA), according to the technique of Brunauer et al. (1938). These measurements were done in a total number of 12 samples each for silica, citrus, and grape farm dusts.

Total Inorganic and Organic Contents of Farm Dusts

Total inorganic and organic contents in the citrus and grape dusts were estimated by muffle furnace ashing of dust samples at 400°C for 4 h. A sample of 500 mg was weighed, ashed, and the differences in weight of inorganic and organic materials were calculated.

Endotoxin Measurements

Grape- and citrus dust samples were analyzed for gram-negative bacterial endotoxin content, using the *Limulus* amebocyte assay with minor modifications (Kinetic-QCL, Bio Wittaker, Walkersville, MD). Dust samples were extracted in nonpyrogenic water for 60 min, centrifuged for 10 min at 1000 × g, and analyzed in duplicate for endotoxin. Results were expressed as endotoxin units (EU) per 100 mg dust.

Determination of Ferrous Iron as a Marker for Potential Oxidant Generation

Ferrous iron was determined by exposing 2-mg samples of farm dusts to 0.3 *M* sodium citrate, 0.3 *M* sodium bicarbonate, and 100 mg sodium dithionate according to the method of Roth et al. (1969). The samples were mixed, heated at 70°C for 30 min, centrifuged, treated with 1,10-phennthroline, and read in a spectrophotometer at 508 nm. Concentrations of ferrous iron in samples were calculated from a standard curve.

Isolation of Alveolar Macrophages

Alveolar macrophages (AMs) were collected by lavage from male specific-pathogen-free Sprague-Dawley rats weighing 250–275 g [Hla: (SD) CVF] (Hilltop, Scottdale, PA). The animals were housed at the National Institute for Occupational Safety and Health animal facility, which is approved by AAALAC. Rats were housed under temperature- and humidity-controlled conditions under a 12-h light/dark cycle and were allowed 1 wk of acclimatization before use in any experiment. They received food and water ad libitum.

All experiments were conducted under protocols approved by the NIOSH ACUC. Rats were anesthetized with an overdose of sodium pentobarbital (100 mg/kg body weight) and exsanguinated. The trachea was cannulated, and the lungs were lavaged repetitively 8 times with 10 ml cold Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution (HBSS) without phenol red (Life Technologies, Inc., Grand Island, NY). A total of 80 ml of lavage fluid was obtained from each animal, and lavages were centrifuged to sediment the cells. Sedimented cells were treated with hypotonic saline to lyse erythrocytes and then brought to isotonicity with hypertonic saline. Cells were then centrifuged and resuspended in HBSS containing 5.5 m*M* D-glucose. An aliquot of 100 μl cell suspension was then used to determine total and differential cell counts, using an electronic cell counter equipped with a cell sizer (Coulter Electronic, Multisizer II, Hialeah, FL) as described previously (Vallyathan et al., 1995). AMs represented 94–96% of the lavaged cells.

Cell Viability

AM viability was measured by the trypan blue dye exclusion method before and after exposure of the cells to different concentrations of farm dusts or crystalline silica. Viable cells were expressed as a percent of total cell counts.

In all the preliminary biologic studies, 3 dust samples each collected from grape- and citrus-working fields on different days showed a linear, concentration-dependent biologic response over mass concentrations of 100–1000 μg. The elemental composition and crystalline silica concentrations of these three dust samples were also comparable. Therefore, the three dust samples from orange harvesting fields were combined and mixed as one, as were the three dust samples from grape harvesting fields. These two combined mixed samples were used as representative samples from grape- and citrus farms for all the in vitro studies.

Lactate Dehydrogenase (LDH) Activity

To allow comparison with a potent fibrogenic dust and identify the probable culpable mineral in the farm dusts, all the biologic evaluations were made with both farm dusts and a well-characterized crystalline silica sample of the same respirable size range. Initially all the farm dust samples obtained on different collection days were evaluated at concentration levels ranging from 100 to 1000 μg. From these studies, optimal dust concentrations required to elicit various biological indices were determined. A concentration of 500 μg/ml of farm dust was then used in all the studies reported here. Using a cell count of 1×10^6 AMs, this concentration of agricultural dusts was found to be adequate to induce detectable biologic responses.

Lactate dehydrogenase (LDH) activity release from AMs exposed to grape- and citrus-farm dusts was measured as an index of cell membrane integrity. In these studies, 1×10^6 AMs in 1 ml HBSS were incubated with 500 μg dust for 1 h at 37°C with gentle mixing. At the termination of incubation, cell suspensions were centrifuged and supernatants were collected for LDH activity measurements. LDH activity was measured using a Cobas Fara II autoanalyzer and Roche LDH kits (Roche Diagnostic Systems, Inc., Montclair, NJ). Enzyme activity was monitored spectrophotometrically by monitoring the formation

of NADH by the enzyme-catalyzed reaction of pyruvate and NAD. LDH activity was expressed as units per liter of cell supernatant.

N-Acetyl-*N*-D-glucosaminidase (NAG)Activity

NAG activity was measured to monitor lysosomal enzyme release due to interaction of dust particles with AMs. In these studies, 1×10^6 AMs in 1 ml HBSS were incubated with 500 μg of dusts for 1 h at 37°C with gentle mixing. At the termination of incubation, cell suspensions were centrifuged and super-natants were collected for NAG activity measurements. NAG activity was assessed using a Boehringer Mannheim NAG kit (Boehringer Mannheim Corp., Indianapolis, IN) adapted to a Cobas Fara II autoanalyzer. An increase in absorbance due to the formation of 3-cresol purple from the substrate catalyzed by the enzyme reaction was monitored spectrophotometrically, and the results were expressed as units of NAG activity per liter of cell supernatant.

Hydrogen Peroxide (H_2O_2) Assay

H_2O_2, a reactive oxygen species intermediate, is released from AMs as a result of phagocytosis of dusts and the resulting stimulation of the cells (Shi et al., 1998). It is considered as a primary line of defense against microbial infection, but in particulate inhalation persistent H_2O_2 release from AMs is associated with potential toxicity (Shi et al., 1998). H_2O_2 was assayed with minor modifications using scopoletin (a fluorescent substrate for peroxidase), where H_2O_2 oxidizes scopoletin resulting in a loss of fluorescence as described earlier (Van Scott et al., 1984; Corbett, 1989). In a reaction mixture containing scopoletin and HEPES buffer, AMs were exposed to dusts for 20 min at 37°C, and after centrifugation 200 μl of each sample was read at 365 nm excitation and 450 nm emission wavelengths. Using a standard curve generated from known concentrations of H_2O_2, the decrease in fluorescence units was converted to pmoles of H_2O_2 released.

Electron Spin Resonance (ESR) Measurements

Electron spin resonance (ESR) measurements were made to examine the potential of farm dusts to generate short-lived hydroxyl radical (•OH). The spin trap 5,5-dimethyl-1-pyrroline oxide (DMPO), a paramagnetic compound, reacts with the short-lived •OH, producing a relatively long-lived spin adduct, which is monitored by ESR. The intensity of the signal represents the concentration of the radicals trapped, and the hyperfine splitting of the spin adduct is characteristic of the trapped radicals. In a test tube containing 1–20 mg farm dust and 100 μl of 1 *M* DMPO, the reaction was initiated by the addition of 100 μl of 0.1 *M* H_2O_2, mixed, centrifuged, and read in a Bruker EMX ESR spectrophotometer (Bruker Instruments, Inc., Billerica, MA), using a flat cell assembly. Hyperfine splittings were measured (0.1 G) directly from the magnetic field separation, using potassium tetraperoxo chromate and 1,1-diphenyl-2-picrylhydrazil as reference standards. An EPR DAP2 program was used for data acquisition and analysis. All the measurement parameters were as described earlier (Vallyathan et al., 1995).

Hydroxyl Radical (•OH) Generation From Macrophages

ESR spin trapping techniques was used to detect short-lived •OH radical from AMs exposed to agricultural dusts. For these studies, AMs (1×10^6) were mixed in a total volume of 0.5 ml PBS, pH 7.4, containing 200 m*M* DMPO and citrus or grape farm dust at concentrations of 1–10 mg/ml. Samples were mixed well, incubated for 2 min, filtered through a 0.45-μm filter, and transferred to a flat cell. ESR measurements were made within 5 min.

Measurements of Lipid Peroxidation in Macrophages

The cell membrane phospholipids are very vulnerable to oxidative damage by ROS, which results in increased permeability and cell death (Shi et al., 1998). Isoprostanes are produced by the nonenzymatic random oxidation of tissue phospholipids, and 8-isoprostane was shown to be a valuable specific marker of oxidative injury. A competitive enzyme-linked assay was used for determining 8-isoprostane in supernatant samples after exposing AMs to different concentrations of farm dusts or crystalline silica. An enzyme-linked immunosorbent assay (ELISA) method (Cayman Chemical, Ann Arbor, MI) was used, according to the manufacturer's protocol, to determine the concentration-dependent lipid peroxidation induced by farm dusts and crystalline silica. Five million cells were exposed to dusts for 1 h at 37°C, and the supernatant was separated and analyzed for 8-isoprostane in duplicate aliquots of 10-μl samples. The concentration of 8-isoprostane produced by the peroxidation of phospholipids in AMs was calculated from a standard curve and expressed as picograms per milliliter.

Activator Protein 1 (AP-1) Activity Assay

JB6 P+ mouse epidermal cells, stably transfected with the AP-1-luciferase reporter plasmid (JB6/AP/kB), were cultured in Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum (FBS), 2 m*M* L-glutamine, and 50 μg/ml gentamicin. The cells were grown to confluence, trypsinized, and suspended in Eagle's MEM containing 5% fetal bovine Solution (FBS). Viable cells, 5×10^4, were then transferred to 24-well plates and incubated at 37°C in a humidified cell culture incubator in a 5% atmosphere of CO_2. After

12 h, cells were cultured in Eagle's MEM containing 0.5% FBS for 12–24 h to minimize basal AP-1 activity and then were exposed to silica or farm dusts in the same medium to monitor AP-1 induction. After incubation for 12, 24, or 36 h, the cells were extracted with 200 μl of 1 × lysis buffer supplied in the luciferase assay kit by the manufacturer. AP-1 activity was measured using a Monolight luminometer, model 3010 (Analytical Luminescence Laboratory, Sparks, MD). AP-1 activity was expressed in relative units compared to controls.

Nuclear Factor κB (NF-κB) Assay

JB6 C141 NF-κB reporter cells were grown to confluence, trypsinized, suspended in 100 μl of 5% FBS MEM in a 96-well plate at a concentration of 8×10^3 viable cells, and incubated in a humidified incubator at 37°C in an atmosphere of 5% CO_2. Twelve to 24 h later, cells were cultured in 0.1% FBS MEM for 24 h to minimize basal activity and exposed to silica or farm dusts in the same medium. After incubation for 12, 24, or 36 h, luciferase activity was measured using a Monolight luminometer, model 3010 (Analytical Luminescence Laboratory, Sparks, MD). The results were expressed as relative NF-κB activity compared to controls.

Statistical Analysis

The data presented are the means ± SE of six or more separate experiments each performed in duplicate using same dust samples and AMs obtained from groups of rats lavaged and pooled. The differences between the controls and groups exposed to different dusts were determined using Student's *t*-test. A p value $<.05$ was considered statistically significant. In some cases statistical significance was determined by one-way analysis of variance (ANOVA) comparisons between groups.

RESULTS

Physical and Chemical Characteristics of the Dusts

Grape- and citrus-farm dusts, classified using an aerodynamic particle sizer, had a mean diameter of 2.22 μm for grape- and 2.72 μm for citrus-farm dusts, respectively (Table 1). Similar particle size distributions of grape- and citrus-farm dusts were observed (Figure 1). In all the aerosolized dust samples from both grape and citrus work sites, 95% of particles were less than 5 μm in diameter (Figure 1, a and b). Aerosolized bulk samples during harvesting from grape and citrus fields were ashed to determine the total inorganic and organic dust contents. The citrus farm samples had greater inorganic dust levels compared to grape farm dust (Table 2). SEM combined with EDXA showed that the inorganic makeup of the mineral inclusions in the farm dusts was mostly alu-minum silicates and crystalline silica. Analysis of bulk samples by x-ray diffraction showed that 12–14% of citrus inorganic dust was composed of crystalline silica (Table 2). This was further confirmed by particle number percent analysis using SEM in combination with BSI and EDXA, showing that the citrus-farm dust contained quantitative more crystalline silica (10.1 %) than the grape-farm dust (7.9 %) (Table 3). The mass median aerodynamic diameters of most of the mineral inclusions in the grape farm dust were comparable to those of citrus farm dust (Table 3). However, surface-area measurements by nitrogen adsorption showed a distinct difference between the grape- and citrus-farm dust samples. Grape farm dust samples had a greater surface area compared to citrus farm dust (Table 1). This was not reflected in the aerodynamic particle classification by the particle sizer.

Results obtained for analysis of grape- and citrus-farm dusts by plasma emission spectrometry for 17 trace elements and toxic metals (Al, As, Ba, Be, Cd, Ca, Cr, Co, Fe, Pb, Mg, Mn, Ni, Si, Na, Ti and V) revealed that the metals suspected to exert toxic health effects—Mn, Fe, Cu, and Zn—were the only ones found in moderately high concentrations (Table 4). There were no significant quantitative differences between grape- and citrus-farm dusts in any of the trace elements measured. All the trace metals suspected to possess biological toxicity, including total iron, were similar in both farm dusts.

Respirable farm dust samples analyzed for endotoxin showed the citrus farm dust contained significantly greater concentration of endotoxin than grape farm dust. Mean endotoxin concentrations in the citrus- and grape-farm dust were 182 EU/100 mg and 37 EU/100 mg, respectively (Table 2).

Cell viability studies, using mass dust concentrations of 50, 100, 250, 500, and 1000 μg/ml and 1×10^6 AMs during 1 h of exposure at 37°C, showed that crystalline silica was more cytotoxic to AMs than grape- and citrus-farm dusts (see

TABLE 1
Grape- and Citrus-Farm Dusts Particle Size Classification Using an Aerodynamic Particle Sizer and Surface Area Measurements Using Nitrogen Absorption

	Mean diameter (μm)	Geometric mean ± SD (μm)	Surface particle size ($\mu m^2/cm^2$)	Mass particle (mg/m^2)	Surface area (m^2/mg)
Grape-farm dust	2.22	1.87 ± 1.74	6.54	0.12	1.60 ± 0.29
Citrus farm dust	2.72	2.21 ± 1.84	7.62	0.09	1.13 ± 0.23

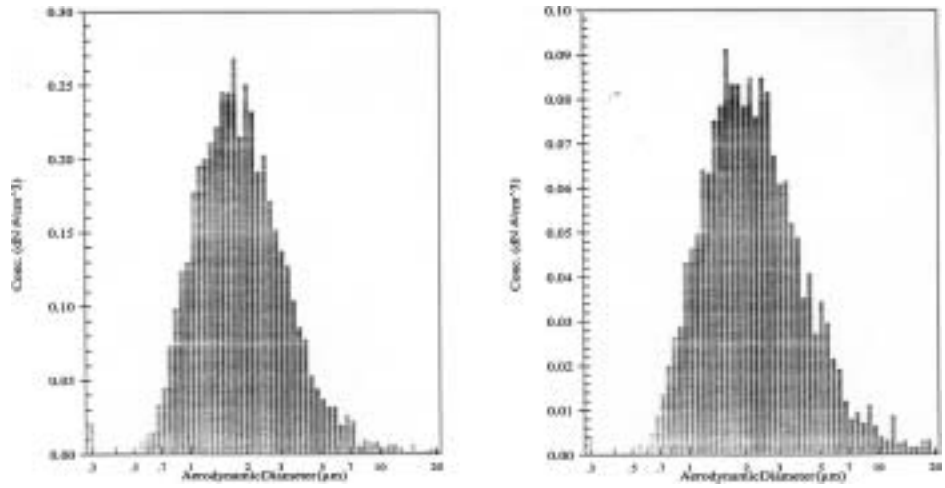

FIG. 1. Particle classification histograms by aerodynamic particle sizer showing the distribution patterns of (a) grape and (b) citrus farm dusts. Note that in both farm dusts, 95% of particles were less than 5 μm in diameter.

TABLE 2
Total Inorganic Minerals, Organic Materials, Crystalline Silica, Endotoxin, and Iron Levels Present in Citrus- and Grape-Farm Dusts as a percentage of Inorganic Dust

	Organic mg/100 mg (mean ± SD)	Inorganic mg/100 mg (mean ± SD)	Crystalline silica mg/100 mg (mean ± SD)	Endotoxin EU/100 mg (mean ± SD)	Iron μg/100 mg (mean ± SD)
Grape-farm	84.8 ± 1.59	15.2 ± 1.59	12.67 ± 0.58	37 ± 41	31.7 ± 1.75
dust Range	(83.0–86.1)	(13.9–17.0)	(12.0–13.0)	(12.0–84.0)	(29.0–33.0)
Citrus-farm	78.3 ± 1.05	21.8 ± 1.07	12.67 ± 1.15	182 ± 51	51.5 ± 9.61
dust Range	(77.3–79.4)	(20–22.7)	(12.0–14.0)	(144.0–241.0)	(38.0–63.0)

Figure 3). Leakage of the cytosolic enzyme (LDH) from AMs exposed to crystalline silica or to grape- or citrus-farm dusts showed a concentration-dependent response when 1×10^6 cells were exposed for 1 h (Figure 2a). Leakage of the lysosomal enzyme *N*-acetyl glucosaminidase (NAG), on the other hand, increased after silica exposures but not in response to farm dusts (Figure 2b).

Exposure of AMs to 500 μg/ml of grape or citrus farm dusts resulted in decreased cell viability to 67% and 63%, respectively, in comparison to 49% following exposure to crystalline silica (Figure 3). Responses to silica, grape- or citrus-farm dusts, or inorganic dusts from grape- or citrus-farm dusts were compared based on equal mass concentrations of of 500 μg/ml with 1×10^6 AMs (Figure 4). Crystalline silica, a highly fibrogenic dust, showed marked toxicity followed by grape- and citrus-farm dusts. Of interest, the inorganic mineral components without endotoxin obtained from grape- and citrus-farm dusts exhibited comparable toxicities to the total dust samples. Grape- and citrus-farm dusts and the inorganic components of these dusts exerted similar low levels of release of lysosomal enzyme NAG activity (Figure 5). Crystalline silica, on the other hand, exhibited a significantly greater potential to induce lysosomal enzyme release compared to grape- and citrus-farm dusts or the inorganic component of these dusts (Figure 5).

TABLE 3
X-Ray Analysis Classification, Mass Median Aerodynamic Diameter (MMAED), and Concentration by Number percent of Minerals Present in Grape- and Citrus-Farm Dusts

	Grape		Citrus	
Mineral	No. %	MMAED	No. %	MMAED
Silica	7.90	0.56	10.10	0.49
Al-silicates	79.7	0.65	76.50	0.60
Talc-like	0.70	2.32	0.90	1.79
Si-rich/Fe	3.40	0.65	3.90	0.70
Iron-rich silicates	2.90	0.35	3.40	0.32
Ti/Si/Fe	1.00	0.37	0.93	0.32
Al-rich silicates	0	0	0.10	0.34
Miscellaneous	0.85	0.60	0.90	1.25

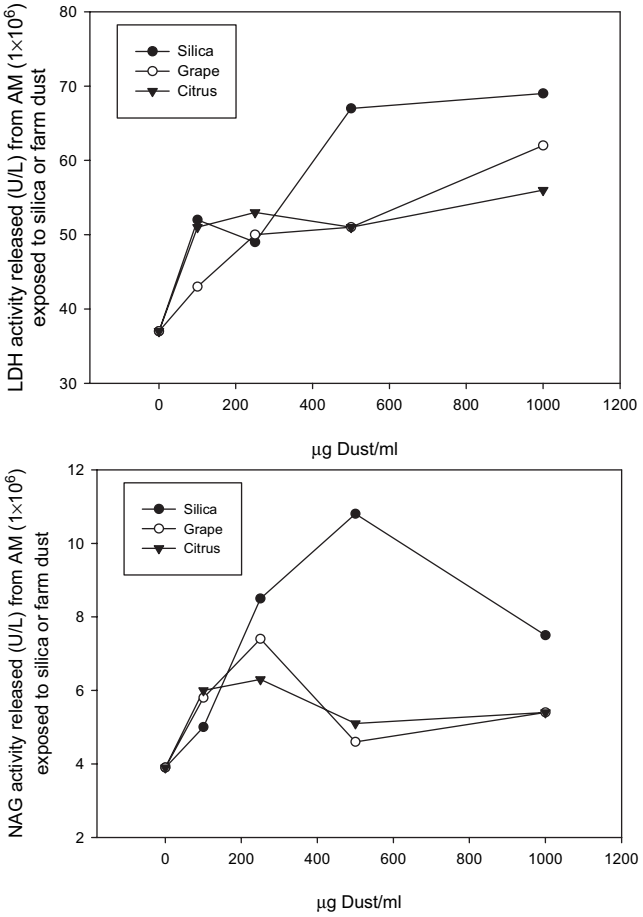

FIG. 2. (a) Concentration-response curves for LDH activity release from AMs resulting from exposure to different concentrations of crystalline silica or citrus-or grape-farm dusts. Values represent means ± SE (n=6). Significant difference from control ($p < .05$). (b) Concentration-response curves for NAG activity release from AMs resulting from exposure to different concentrations of crystalline silica or citrus- or grape-farm dusts. Values represent means ± SE (n=6). Significant difference from control ($p < .05$).

TABLE 4
Concentrations of Major Elements Present in Citrus- and Grape-Farm Dusts, Expressed as µg/g Dust

	Grape-farm dust	Citrus-farm dust
Sodium	2267	1600
Magnesium	13,667	11,667
Aluminum	36,000	29,667
Silicon	4100	4267
Calcium	25,667	18,667
Titanium	1900	1933
Vanadium	79	74
Chromium	66	57
Manganese	483	840
Iron	31,333	31,333
Cobalt	17	17
Nickel	30	70
Copper	1300	1400
Zinc	1300	1400
Arsenic	ND	100
Cadmium	3	20
Lead	55	46

Note. Farm dust samples were microwave digested in 10 ml concentrated nitric acid, transferred to volumetric flasks, diluted to 50 ml volume flask with ASTM Type II water, and analyzed using a Perkin-Elmer Optima 300 DV inductively coupled plasma spectrometer according to NIOSH Method 7300 (NIOSH, 1994).

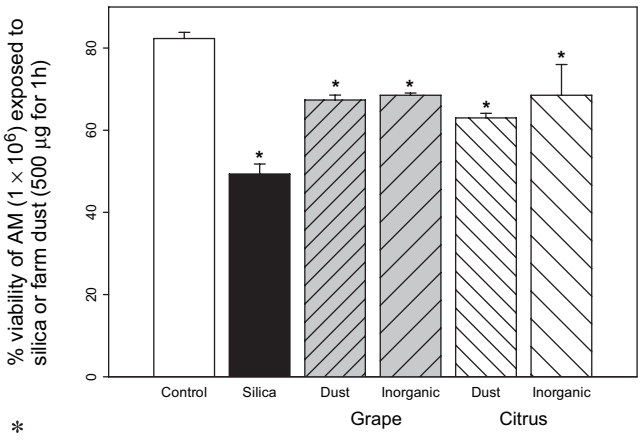

FIG. 3. Percent viability of AMs exposed to 500 µg crystalline silica, grape- or citrus-farm dusts, and their inorganic components. Viability was determined by trypan blue exclusion. Values represent means ± SE ($n = 6$). Significant difference from control ($p < .05$).

AMs exposed to grape- and citrus-farm dusts showed an enhanced ability to release H_2O_2, which was significantly greater than for crystalline silica (Figure 6). Compared to crystalline silica, grape-farm dust generated 40% more H_2O_2, while citrus-farm dust generated 19% more H_2O_2 than silica. In comparison with total farm dust samples, the inorganic component of dusts from grape- and citrus-farm dusts induced a 31% and 43% increase in the formation of H_2O_2 from AMs, respectively.

In parallel with these data, ESR studies provide correlative evidence for the potential of these farm dusts to generate ROS. Citrus farm dust generated a 2.4-fold stronger signal intensity compared to grape farm dust, (Figure 7).

Compared to crystalline silica and grape-farm dust, the potential of citrus-farm dust to generate •OH was 2.3-fold greater at a mass dust concentration of 10 mg/ml. The reaction mixture in the absence of farm dusts did not produce any detectable signals, whereas addition of farm dusts or silica generated a 1:2:2:1 quartet spin adduct signal (data not shown). The characteristic hyperfine splittings indicating a DMPO/OH adduct are considered evidence for •OH generation. Catalase,

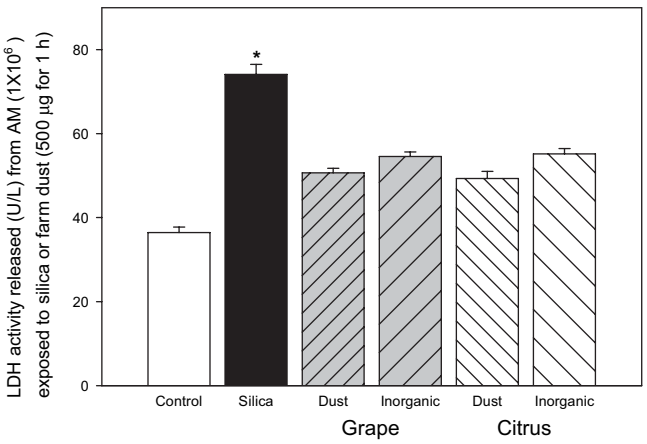

FIG. 4. LDH activity release from AMs exposed to crystalline silica, grape- or citrus-farm dusts, and their inorganic components. Values represent means ± SE (n=6). Significant difference from control ($p < .05$).

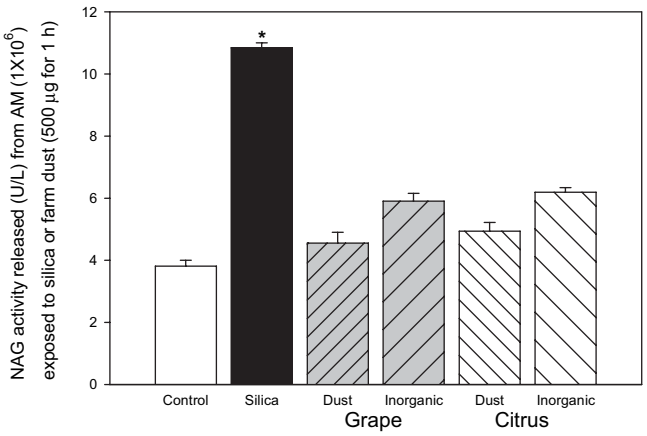

FIG. 5. NAG activity release from AMs exposed to crystalline silica, grape- or citrus-farm dusts, and their inorganic components. Values represent means ± SE (n = 6). Significant difference from control ($p < .05$).

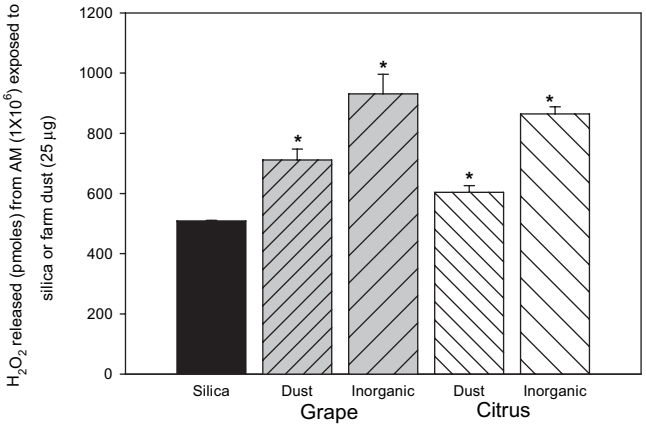

FIG. 6. H_2O_2 release from AMs exposed to 25 µg crystalline silica, grape- or citrus-farm dusts, or their inorganic components. Values represent means ± SE (n=6). Significant difference from control ($p < .05$).

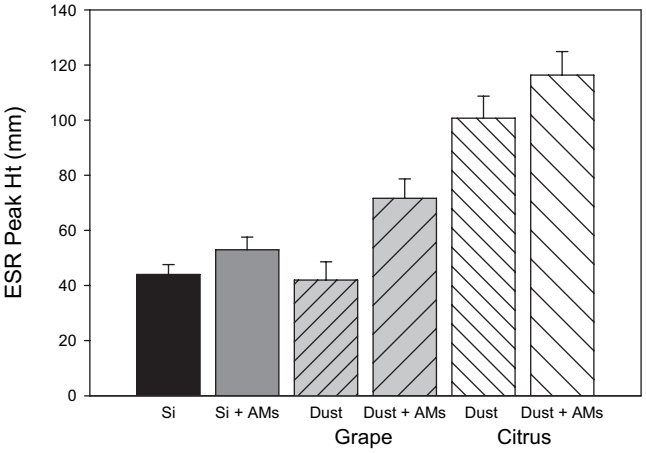

FIG. 7. Relative ESR intensity of •OH radical generation from crystalline silica, citrus- and grape-farm dusts plus H2O2, and from AMs exposed to silica or to citrus or grape dust. The concentrations of dusts in cell-free systems and with AMs were 10 mg/ml. Values represent means ± SE (n=3).

a specific H_2O_2-decomposing enzyme, was able to abolish all of the •OH generation from these dusts, and SOD was able to abolish more than 80% of •OH generation. Deferoxamine, a metal chelator, was able to inhibit almost 70% of •OH generation by the farm dusts (data not shown).

Generation of •OH From AMs Stimulated With Agricultural Dusts

The data show that AMs alone without farm dust did not generate any detectable spectrum, whereas AMs exposed to grape or citrus dusts generated a strong ESR signal in a concentration-dependent manner (data not shown). Furthermore, the citrus dust generated a stronger ESR spectrum than grape dust at all the concentrations tested (Figure 7 and Figure 8, d and g). This was consistent with the data without AMs using dusts plus H_2O_2 (Figure 7). The ESR signal generated was typical and consistent with the line shape of a DMPO–OH adduct, providing evidence of •OH generation. This was also verified by studies using the inhibition potential of SOD (data not shown), catalase (Figure 8, e and h), and deferoxamine (Figure 8, f and i). SOD inhibited the signal intensity produced by both grape and citrus dusts by 68%, catalase inhibited the signal intensity significantly by 83%, and deferoxamine inhibited it by 72%. These results suggest that •OH generation potential of these farm dusts in pulmonary cells might play a key role in cellular toxicity.

In both noncellular and cellular systems the potential of citrus-farm dust to generate •OH was significantly greater than that of grape-farm dust. This correlates with the greater level of available free iron in citrus-farm dust (51.5 ± 9.6 µg/100 mg) compared to (31.7 ± 1.7 µg/100 mg) grape-farm dust (Table 2).

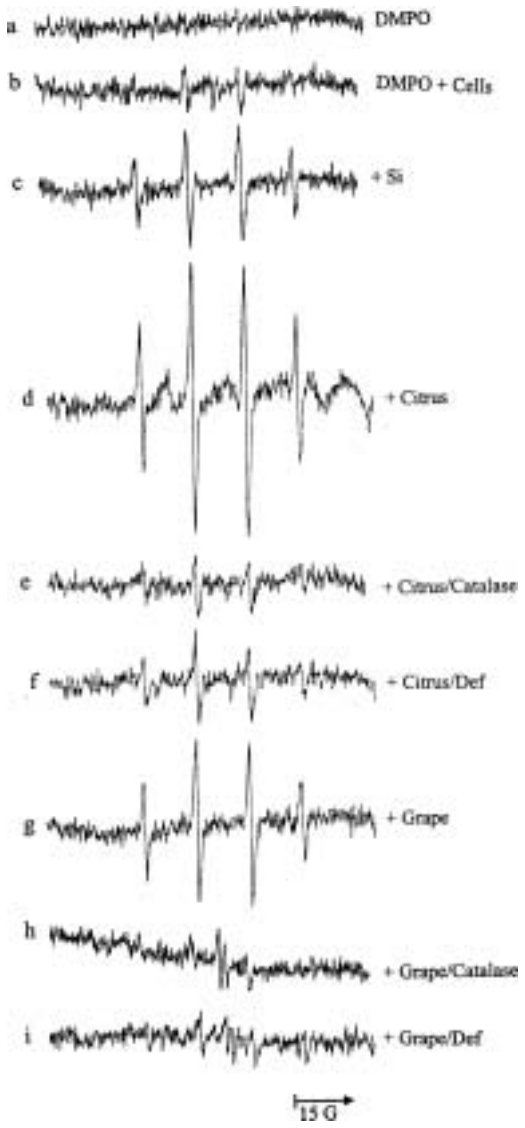

FIG. 8. Crystalline silica or grape- or citrus-farm dust-induced free radical generation from AMs and the effects of catalase and deferoxamine on OH radical generation. Samples were incubated for 5 min in a 37°C water bath before being measurements. ESR spectra were recorded 5 min after incubation at 37°C following the addition of dusts to cell suspension in a phosphate-buffered solution (pH 7.4) containing 200 m*M* DMPO, 1×10^6 rat AMs, and 1 mg/ml silica or grape- or citrus-farm dust. Deferoxamine (2 m*M*) was used to chelate metals. Catalase (2000 U/ml) was used to scavenge H2O2. The ESR spectrometer settings were: receiver gain, 5.0×10^4; time constant, 0.04 s; modulation amplitude, 1.0 G; scan time, 41 s; magnetic field, 3487 ± 100 G.

To examine whether the increased generation of ROS resulted in the oxidation of cellular lipids, the formation of isoprostanes produced by the non-enzymatic ROS-induced peroxidation was measured. Isoprostanes were shown to be the most sensitive, stable, and specific biomarkers of lipid peroxidation (Roberts & Morrow, 2000). Grape- and citrus-farm dusts produced a concentration-dependent increase in the lipid peroxidation by-product isoprostane from AMs exposed to

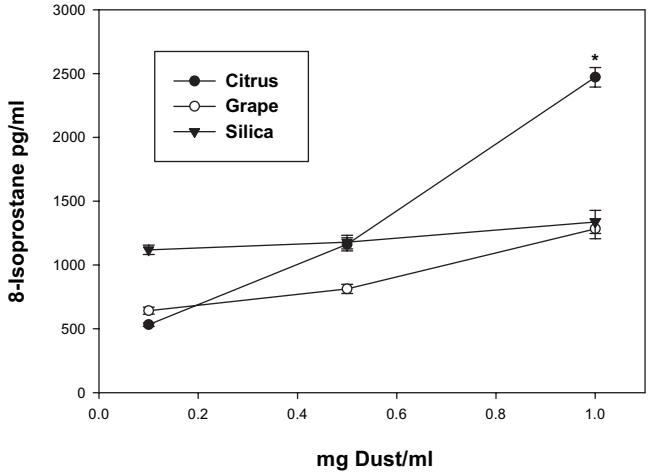

FIG. 9. Crystalline silica or grape- or citrus-farm dust-induced lipid peroxidation of AMs and formation isoprostanes. Data are mean ± SE (n=6). Significant from control ($p < .05$).

dusts for 1 h. However, the potential of citrus-farm dust to stimulate lipid peroxidation in AM was twofold greater than for grape-farm dust and crystalline silica (Figure 9). In comparison to crystalline silica, citrus-farm dust induced significantly greater lipid peroxidation in AMs in a concentration-dependent manner. The ability of citrus farm dust to oxidize lipids is almost twofold greater than crystalline silica, and this potential correlates with the ability to generate •OH radicals by citrus-farm dust compared to that of silica. This increased potential to induce lipid peroxidation by citrus-farm dust compared to grape-farm dust also correlates with the greater concentrations of free iron, which is conceivably the contributing factor in the increased generation of •OH and potential for lipid peroxidation.

AP-1 Activation

To explore the effects of crystalline silica and farm dusts on AP-1 activation, 5×10^4 JB6/AP/kB cells were exposed to 200 μg/ml farm dusts or silica for 12–36 h. A concentration-response study indicated that farm dusts or silica induced a concentration-dependent induction of AP-1, which peaked at 36 h. Grape-farm dust produced significant activation of AP-1, which was 5-fold above the basal level and 2.25-fold above the crystalline silica-induced activation of AP-1 (Figure 10). Citrus-farm dust induced a 3-fold increased activation above the basal level and 1.3-fold above the silica (Figure 10).

NF-κB Activation

To investigate the effects of crystalline silica and farm dusts on NF-κB induction, 8×10^3 JB6 C141 NF-κB-luciferase reporter cells were exposed to grape- and citrus-farm dusts or crystalline silica for varying lengths of time. A peak response was noted at 36 h at a maximal concentration of 200 μg/ml.

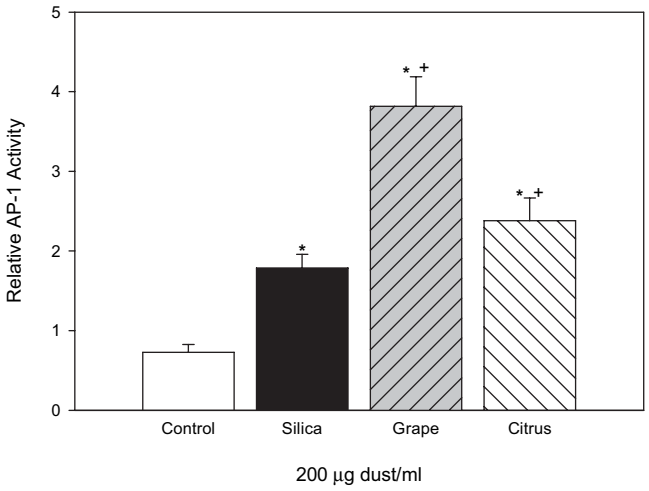

FIG. 10. Relative AP-1 induction and activation by crystalline silica or grape- or citrus-farm dusts in JB6 P+ cells. The cells were treated with 200 μg/ml of crystalline silica or grape- or citrus-farm dusts in 0.5% FBS for 36 h at 37°C, and relative AP-1 activity measured by luciferase assay. Asterisk indicates significant difference from control ($p < .05$). +, Significant difference from silica ($p < .05$).

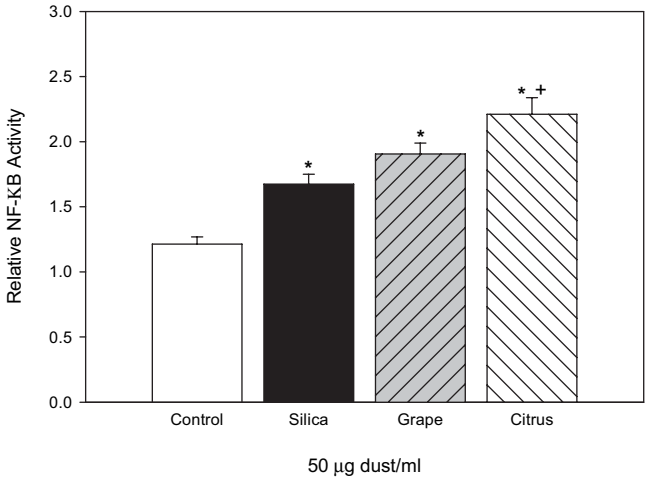

FIG. 11. Relative NF-κB induction and activation by crystalline silica or grape- or citrus-farm dusts in JB6 P+ cells. The cells were treated with 50 μg/ml of crystalline silica or grape- or citrus-farm dusts in 0.5% FBS for 36 h at 37°C, and relative NF-κB activity was measured by luciferase assay. Differences between control and silica or farm dusts were significant ($p < .05$). The difference between silica and grape dust was not significant, and the difference between silica and citrus farm dust was highly significant ($p < .05$). Values represent means ± SE (n=6).

Citrus-farm dust produced an induction of NF-kB of 1.9-fold above the basal level and grape farm dust (Figure 11). Crystalline silica was comparatively a weak activator for NF-κB and induced a 1.4-fold increase.

DISCUSSION

This study provides a systematic comparison of grape- and citrus-farm dusts in relation to cytotoxicity and potential to generate •OH, induce lipid peroxidation, and activate transcription factors AP-1 and NF-κB in their native respirable airborne form. In addition, studies were also carried out without the organic matter or endotoxin to compare the relative toxicity to the highly cytotoxic and fibrogenic mineral, crystalline silica. Furthermore, this study reports the differential toxicity of inorganic and organic components of farm dusts to evaluate their independent role in the pathogenesis of pulmonary disease. To further explore whether the surface area or total dust mass is more important in toxicity evaluations, data were analyzed based on surface area measurements.

Surface reactivity, chemical properties, and particle size and structure are well known to influence biological toxicity, inflammatory potential, and pathogenicity. Among these particle characteristics, surface area seems to be critical in toxicity of particles. Therefore, toxicity measurements were compared based on 1 mg mass with 1 mm^2 surface area measurements. On an equivalent surface area basis, citrus-farm dust showed greater toxicity compared to grape-farm dust. However, crystalline silica showed a greater toxicity based on surface area than either farm dust.

In agricultural workplaces, respiratory diseases, such as hypersensitivity pneumonitis, asthma, bronchitis, and organic dust syndrome, produced by organic dust exposure are well recognized and were reported in several studies (Schenker, 1998; Omland, 2002; Le Van et al., 2005). Although concern was raised regarding the respiratory hazards of inorganic dusts in agriculture (Schenker, 2000) and occasional case reports of mixed dust pneumoconiosis resulting from exposure to the inorganic components of agricultural dusts were reported in the literature, their potential toxicity and the correlation to specific etiologic factors remain unknown. In the California farming industry, it was estimated that up to 1.5 million people were employed in 1996 (Nieuwenhuijsen et al., 1996). In arid geographic conditions, such as in the Central Valley of California, farming is associated with exposure to numerous inorganic silicates in combination with high concentrations of silica. The magnitude of inorganic dust exposure reported in ambient air is high, but very little is known concerning the inorganic makeup of these particles and the biologic responses they elicit in cells.

Significant inorganic dust exposure in agricultural workers exhibiting mixed dust pneumoconiosis was reported in several case reports and in a recent autopsy study of Hispanic males in the farming county of California (Sherwin et al., 1979; Dynnik et al., 1981; Fennerty et al., 1993; Gylseth et al., 1984; Pinkerton et al., 2000). Sherwin et al. (1979), in a systematic study of farm workers lung tissues from the Central Valley of California, showed characteristic mixed dust-induced pulmonary lesions called silicate pneumoconiosis without silicosis. In this study involving five grape

workers, one farmer, and one rural resident, large numbers of birefringent silicate and silicon dioxide particles were identified in association with interstitial inflammation and fibrosis. Particles identified in lung tissues and particles in the soil were shown to have similar elemental composition. In another case report, several inorganic birefringent mineral particles, such as mica, talc, and silica, were identified by EDXA. The association of inorganic particles with fibrotic lesions implies that farm dusts may be the etiologic agents in pulmonary fibrosis (Gyleseth et al., 1984). Other case reports on silicosis in tractor drivers working on sandy soils (Dynnik et al., 1981) and silicosis in a Pakistani farm worker (Fennerty et al., 1993) provide supporting evidence implicating silica as a major potential hazard rather than silicate minerals.

Crystalline silica, a potent positive cytotoxic dust, was investigated in the present toxicity study in parallel with farm dusts. On an equivalent surface area basis it was highly cytotoxic in comparison with grape- and citrus-farm dusts. Note that the crystalline silica mass concentrations in farm dusts frequently range from 10 to 20%. Endotoxin levels apparently did not influence the cytotoxicity of farm dusts, since trypan blue exclusion, LDH activity, and NAG activity release in response to total dusts or inorganic fractions of farm dusts were comparable. Our studies indicate that on an equivalent mass basis, citrus-farm dust generated higher levels of H_2O_2 and $^{\bullet}OH$ radicals and induced more lipid peroxidation than crystalline silica. This enhanced potential to generate reactive species and induce lipid peroxidation may produce oxidative stress leading to the initiation of disease process in farm workers exposed to this dust. Furthermore, increased lipid peroxidation induced inactivation of proteins and activation of phopholipases leading to catalytic activity (Fruebis et al., 1992; McLean et al., 1993). In addition, lipid peroxides were found to be involved in modulating several cellular events, including inflammation, while isoprostanes, which in particular are known to be potential smooth muscle constrictors and mitogens, exert important biological actions (Rahman, 2004). The increased lipid peroxidation in response to exposure of AMs to farm dusts indicates oxidant stress is likely an important mechanism initiating the disease process in farmers exposed to these inorganic dusts. Enhanced generation of ROS was shown to induce activation of AP-1 and NF-κB transcription factors, which may play a critical role in disease development (Galter et al., 1994; Hayashi et al., 1993). AP-1 was more responsive to grape-farm dust, while NF-κB was activated more by citrus-farm dust. Differential regulation of AP-1 and NF-κB has been well documented in oxidative stress (Shrivastava & Agarwal, 1999). Further studies are required to elucidate signal transduction pathways involved in the activation of these transcription factors.

In addition to the increased levels of iron, other low-molecular-weight transition metals such as copper, zinc, manganese, and chromium are potential mediators of radical generation that are present in greater concentrations in grape and citrus farm dusts. Augmentation of pulmonary reactions resulting from inhalation of these trace metals and particles containing these metals are well documented.

In conclusion, in vitro studies are of significant value in predicting cytotoxicity and for elucidating mechanistic events involved in the pathogenesis of several pulmonary diseases induced by inorganic minerals (Brain, 1980; Vallyathan et al., 1988; Castranova & Vallyathan, 2000). Furthermore, the role of oxidants in the promotion of inflammation and fibrogenesis through signaling and activation of mediators, cytokines, and transcription factors is well documented in diseases produced by inorganic minerals (Vallyathan et al., 1992; Ding et al., 2002; Manning et al., 2002; Schins et al., 2002; Castranova, 2004). In this respect, crystalline silica, used in these studies as a positive fibrogenic dust, was shown to activate AP-1 and NF-κB by increasing the generation of ROS (Ding et al., 2001, 2002; Chen et al., 1998; Kang et al., 2000). Both these transcription factors were activated to a greater level by grape- and citrus-farm dusts compared to crystalline silica on equivalent mass basis. This study showed that the potential of farm dusts to release H_2O_2, generate $^{\bullet}OH$ radicals, induce lipid peroxidation, and activate transcription factors was significantly greater than that produced by crystalline silica on a mass basis. Therefore, data suggest that oxidative injury induced by grape- and citrus-farm dusts may play a central role in disease development in grape- and citrus-farm workers even when crystalline silica concentrations are not significantly high. However, the disease outcome to pulmonary fibrosis may be greatly influenced by higher concentrations of crystalline silica and other silicate minerals. Taken together, these results support human studies indicating that workers involved in grape and citrus farms in the arid working conditions of California have a greater propensity to develop fibrotic lung disease.

REFERENCES

American Thoracic Society (ATS) Committee of the Scientific Assembly on Environmental and Occupational Health. 1997. Adverse effects of crystalline silica exposure. *Am. J. Crit. Care Med*. 155:761–768.

American Thoracic Society (ATS) 1998. Respiratory health hazards in agriculture. *Am. J. Crit. Care Med*. 158:S1–S76.

Atiemo, M. A., Yoshida, K., and Zoerb, G. C. 1992. Dust measurements in tractor and combine cabs. *Trans. ASAE* 571–576.

Brackbill, R. M., Cameron, L. L., and Behrens, V. 1994. Prevalence of chronic diseases and impairments among US farmers. 1986–1990. *Am. J. Epidemiol*. 139:1055–1065.

Brain, J. D. 1992. Short-term in vitro and in vivo bioassays: their role in estimating the toxic potential of inhaled complex mixtures for humans. *Cell. Biol. Toxicol*. 8:123–132.

Brambilla, C., Abraham, J., Brambilla, E., Benirschke, K., and Bloor, C. 1979. Comparative pathology of silicate pneumoconiosis. *Am. J. Pathol*. 96:149–170.

Brunauer, S. P., Emmett, P. H., and Teller, E. T. 1938. Adsorption of gases in multi-molecular layers. *J. Am. Chem. Soc*. 60:309.

Castranova, V. 2004. Signaling pathways controlling the production of inflammatory mediators in response to crystalline silica exposure: role of reactive oxygen/nitrogen species. *Free Radical Biol. Med*. 37: 916–925.

Castranova, V., and Vallyathan, V. 2000. Silicosis and coal workers' pneumoconiosis. *Environ. Health Perspect*. 108:675–684.

Chen, F., Lu, Y., Demers, L. M., Rojanasakul, Y., Shi, X., Vallyathan, V., and Castranova, V. 1998. Role of hydroxyl radical in silica-induced Nf-kB activation in macrophages. *Ann. Clin. Lab. Sci*. 28:1–13.

Corbett, J. T. 1989. The scopoletin assay for hydrogen peroxide. A review and a better method. *J. Biochem. Biophys. Methods* 4:297–307.

Ding, M., Shi, X., Lu, Y., Huang, C., Leonard, S., Roberts, J., Antonini, J., Castranova, V., and Vallyathan, V. 2001. Induction of activator protein-1 through reactive oxygen species by crystalline silica in JB6 cells. *J. Biol. Chem*. 276:9108–9114.

Ding, M., Chen, F., Shi, X., Yucesoy, B., Mossman, B., and Valllyathan, V. 2002. Diseases caused by silica: Mechanisms of injury and disease development. *Int. Immunopharmacol*. 2:173–182.

Dynnik, V. I., Khzhniakova, L. N., Baranenko, A. A., Makotchenko, V. M., and Okseniuk, I. M. 1981. Silicosis in tractor drivers working on sandy soils on tree farms. *Gig. Tr. Prof. Zabol*. 12:26–28.

Fennerty, A. A., Hunter, A. M., Smith, A. P., and Pooley, F. D. 1993. Silicosis in a Pakistani farmer. *Br. Med. J*. 287:648–649.

Fruebis, J., Parthasarathy, S., and Steinberg, D. 1992. Evidence for a concerted reaction between lipid hydroperoxides and polypeptides. *Proc. Nat. Acad. Sci*. 89:10588–10592.

Galter, D., Sabine, M. I. H. M., and Droge, W. 1994. Distinct effects of glutathione disulphide on the nuclear transcription factor kB and the activator protein-1. *Eur. J. Biochem*. 221:639–648.

Gamsky, T. E., McCurdy, S. A., Samuels, S. J., and Schenker, M. B. 1992a. Reduced FVC among California grape workers. *Am. Rev. Respir. Dis*. 146:257–262.

Gamsky, T. E., Schenker, M. B., McCurdy, S. A., and Samuels, S. J. 1992b. Smoking, respiratory symptoms, and pulmonary function among a population of Hispanic farm workers. *Chest* 101:1361–1368.

Gylseth, B., Stettler, L., Mowe, G., Skaug, V., and Lexow, P. 1984. A striking deposition of mineral particles in the lungs of a farmer: a case report. *Am. J. Ind. Med*. 6:231–240.

Green, F. H. Y., Yoshida, K., Fick, G., Paul, J., High, A., and Green, W. F. 1990. Characterization of airborne mineral dust associated with farming activities in rural Alberta. *Can. Int. Occup. Environ. Health* 62:423–430.

Hayashi, T., Ueno, Y., and Okamoto. 1993. Oxidoreductive regulation of nuclear factor kB. Involvement of a cellular reducing catalyst thioredoxin. *J. Biol. Chem*. 268:11380–11388.

Kang, J. L., Go, H. Y., Hur, C. K., and Castranova, V. 2000. Silica-induced nuclear factor-kB activation involvement of reactive oxygen species and protein tyrosine kinase activation. *J. Toxicol. Environ. Health A* 6:27–46.

Kirkhorn, S. R., and Garry, V. F. 2000. Agricultural lung diseases. *Environ. Health Perspect*. 108(Suppl. 4): 705–712.

Lee, K., Lawson, R. J., Olenchock, S. A., Vallyathan, V., Southard, R. J., Thorne, P. S., Saiki, C., and Schenker, M. B. 2004. Personal exposures to inorganic and organic dust in manual harvest of California citrus and table grapes. *J. Occup. Environ. Hyg*. 8:505–514.

LeVan, T. D., Von Essen, S., Romberger, D. J., Lambert, P., Martinez, F. D., Vasquez, M. M., and Merchant, J. A. 2005. Polymorphisms in the CD14 gene associated with pulmonary function in farmers. *Am. J. Respir. Crit. Care Med*. 171:773–779.

Manning, C. B., Vallyathan, V., and Mossman, B. T. 2002. Diseases caused by asbestos: Mechanisms of injury and disease development. *Int. Immunopharmacol*. 2:191–200.

McLean, L. R., Hagaman, K. A., and Davidson, W. S. 1993. Role of lipid structure in the activation of phospholipase A2 by peroxidized phospholipids. *Lipids* 28:505–509.

McCurdy, S. A., Ferguson, T. J., Goldsmith, D. F., Parker, J. E., and Schenker, M. B. 1996. Respiratory health of California rice farmers. *Am. J. Respir. Crit. Care Med*. 153:1553–1559.

Monso, E., Schenker, M., Radon, K., Riu, E., Magarolas, R., McCurdy, S., Danuser, B., Iversen, M., Saiki, C., and Nowak, D. 2003. Region-related risk factors for respiratory symptoms in European and Californian farmers. *Eur. Respir. J*. 21:323–331.

Nieuwenhuijsen, M. J., Schenker, M. B., Samuels, S. J., Farrr, J. A., and Green, R. S. 1996. Exposure to dust, noise and pesticides, their determinants and the use of protective equipment among California farm operators. *Appl. Occup. Environ. Hyg*. 11:1217–1225.

Nieuwenhuijsen, M. J., Kruize, H., and Schenker, M. B. 1998. Exposure to dust and its particle size distribution in California agriculture. *Am. Ind. Hyg. Assoc. J*. 59:34–38.

Nieuwenhuijsen, M. J., Noderer, N. S., Schenker, M. B., Vallyathan, V., and Olenchock, S. 1999. Personal exposure to dust, endotoxin and crystalline silica in California agriculture. *Ann. Occup. Hyg*. 43:35–42.

NIOSH. 1994. *NIOSH manual of analytical methods*, 4th ed., Department of Human Health Services, Center for Disease Control and Prevention, National Institute for Occupational Safety and Health.

Omland, O. 2002. Exposure and respiratory health in farming in temperate zones—A review of the literature. *Ann. Agric. Environ. Med*. 9:119–136.

Pinkerton, K. E., Green, F. H. Y., Saiki, C., Vallyathan, V., Plopper, C. G., Gopal, V., Hung, D., Bahne, E. B., Lin, S. S., Menache, M. G., and Schenker, M. B. 2000. Distribution of particulate matter and tissue remodeling in the human lung. *Environ. Health Perspect*. 108: 1063–1069.

Rajini, P., Last, J. A., McCurdy, S. A., Lawson, R., Southard, R. J., Allamneni, K. P., and Witschi, H. 1995. Lung injury and fibrogenic response to dusts from citrus and grape harvests. *Inhal. Toxicol*. 7:363–376.

Rahman, I. 2004. Smoking-induced inflammation, injury, and disease: Molecular mechanisms. In *Oxygen/ nitrogen radicals, lung injury and disease*, eds. V. Vallyathan, V. Castranova, and X. Shi, pp. 211–255. New York: Marcel Dekker.

Roberts, L. J., and Morrow, J. D. 2000. Measurement of F2-isoprostanes as an index of oxidative stress in vivo. *Free Radical Biol. Med*. 28:505–513.

Roth, C. B., Jackson, M. L., and Syers, J. K. 1969. Deferration effect on structural ferrous–ferric iron ration and CES of vermiculites and soils. In *Clays and clays minerals, vol*. 17, pp. 253–264. Pergamon Press.

Schenker, M. 1998. Respiratory health hazards in agriculture. *Am. J. Respir. Crit. Care Med*. 158:S1–S76.

Schenker, M. 2000. Exposure and health effects from inorganic agricultural dusts. *Environ. Health Perspect*. 108(suppl. 4): 661–664.

Schins, R. P., Knaapen, A. M., Cakmak, G. D., Shi, T., Weishaupt, C., and Borm, P. J. 2002. Oxidant-induced DNA damage by quartz in alveiolar epithelial cells. *Mutat. Res*. 27:77–86.

Sherwin, R. P., Barman, M. L., and Abraham, J. L. 1979. Silicate pneumoconiosis of farm workers. *Lab. Invest*. 40:576–582.

Shi, X., Castranova, V., Halliwell, B., and Vallyathan, V. 1998. Reactive oxygen species and silica-induced carcinogenesis. *J. Toxicol. Environ. Health B* 1:181–197.

Shrivastava, A., and Agarwal, B. B. 1999. Antioxidants differentially regulate activation of nuclear factor-kappa B, activator protein-1, c-jun amino-terminal kinases, and apoptosis induced by tumor necrosis factor: Evidence that JNK and NF-kappa B activation are not linked to apoptosis. *Antioxidant Redox Signal*. 1:181–191.

Stettler, L. E., Platek, S. F., Riley, R. D., Mastin, J. P., and Simon, S. D. 1991. Lung particulate burdens of subjects from Cincinnati, Ohio urban area. *Scan. Microsc*. 5:85–92.

Vallyathan, V., Schwegler, D., Reasor, M., Stettler, L., Clere, J., and Green, F. H. Y. 1988. Comparative in vitro cytotoxicity and relative pathogenicity of mineral dusts. *Ann. Occup. Hyg*. 32:279–289.

Vallyathan, V., Mega, J. F., Shi, X., and Dalal, N. S. 1992. Enhanced generation of free radicals from phagocytes induced by mineral dusts. *Am. J. Respir. Cell Mol. Biol*. 6:404–413.

Vallyathan, V., Castranova, V., Pack, D., Leonard, S., Shumaker, J., Hubbs, A. F., Shoemaker, D. A., Ramsey, D. M., Pretty, J. R., and McLaurin, J. L. 1995. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation. Potential role of free radicals. *Am. J. Respir. Crit. Care Med*. 152:1003–1009.

Van Scott, M. R., Miles, P. R., and Castranova, V. 1984. Direct measurement of hydrogen peroxide release from rat alveolar macrophages: Artifactual effect of horseradish peroxidase. *Exp. Lung Res*. 6:103–114.