Graphical Abstract

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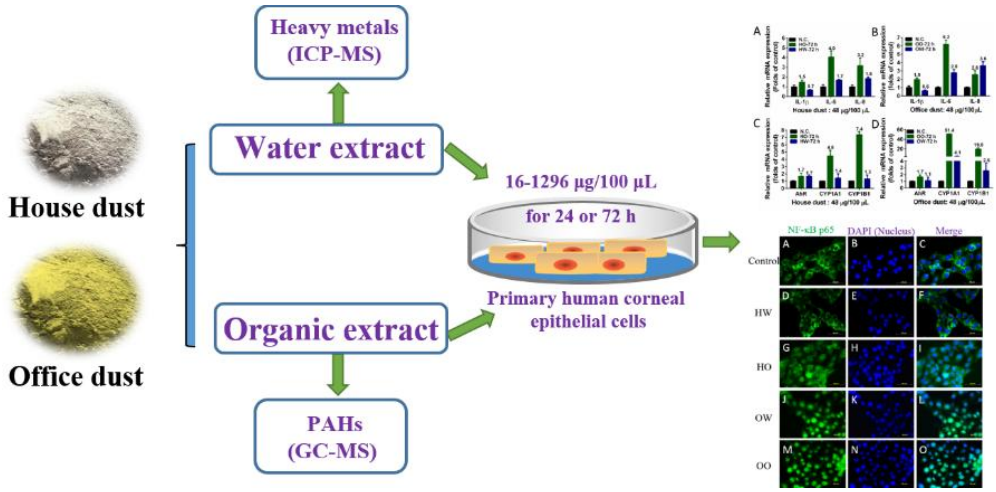

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Molecular mechanisms of dust-induced toxicity in primary human corneal epithelial cells: water and organic extract of office and house dust

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Keywords

Indoor dust; primary human corneal epithelial cells; oxidative stress; pro-inflammation; NF-κB activation

1. Introduction

The indoor environment is important for human health as people spend over 90% of their daily time indoors (Seaton et al., 1999). A large number of contaminants adsorb to particulate matter suspended in indoor air, which settles out as indoor dust (Maertens et al., 2004). Indoor dust is an important route of human exposure to contaminants (Wang et al., 2015). Numerous epidemiological data have associated the indoor dust exposures to human health issues, including cardiovascular, respiratory, and eye diseases (Gereda et al., 2000; Hansel et al., 2008). Evidence suggested that dust-induced adverse health effects may depend on contaminants in indoor dust. For instance, Kurt-Karakus (2012) showed the correlation between the carcinogenic risk for humans and Cr in indoor dust in Istanbul. Recently, concentration-dependent relationships between organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), in indoor dust and human health issues have also been established (Meeker et al., 2013; Wei et al., 2015).

Previous studies have addressed the effects of indoor dust on human respiratory system, immune system, and cardiovascular (Maertens et al., 2004; Riechelmann et al., 2007). However, few investigations focus on dust-induced eye damage. Human ocular surface epithelium (e.g., cornea and conjunctiva), covered by a tear film, acts as a protective barrier from external agents (e.g., dust, and diesel exhaust particles) (Tau et al., 2013). Ocular epithelial cells are continually exposed to environmental toxins, which may lead to adverse effect on eyes. For example, Japanese surveys showed that exposure to volcanic ash resulted in ocular symptoms including redness, discharge, foreign body sensation, and itching (Kimura et al., 2005).

Eye diseases induced by indoor air pollution (such as house and office) is also of concern, warranting more investigation on the impact of dust on corneal epithelial surface (West et al., 2013; Wolkoff et al., 2003). Mølhave et al (2012) found that exposure to office dust, even at concentrations of normal indoor environments resulted in inflammatory cell decrease in tear fluids, corneal epithelium defects, and break-up time decrease. Also, changes of foam formation in the eyes and tear film stability were also affected by dust exposures (Franck and Skov 1989; Pan et al., 2000). Our previous data showed that house dust suspension elicited oxidative stress and inflammation in human corneal epithelial (HCE) cells

(Xiang et al., 2016). However, the mechanisms of dust-induced adverse effects on human cornea are still not fully understood.

To further investigate the mechanism of dust-induced toxicity, organic extracts of dust has been often exposed to human cells and cell lines (Fang et al., 2015; Suzuki et al., 2013). Kang et al. (2010) found that exposure to organic dust extract significantly decreased the viability of human hepatocellular liver carcinoma cell line (HepG2) and human skin keratinocyte cell line (KERTr). More interestingly, a negative correlation was established between total PAH concentration and LC_{50} on HepG2 and KERTr cell lines. Besides, the aromatic hydrocarbon receptor (AhR) and its downstream genes (*CYP1A1* and *CYP1B1*), which can be metabolically activated by PAHs, were also induced by organic dust extract (Mahadevan et al., 2005). The activation of the AhR signal pathway is an important toxic mode of action for organic extracts of dust. On the other hand, heavy metals in water soluble fractions of dust also contributed to the toxic effects (Mohmand et al., 2015). Recently, Huang et al. (2015) compared the effects on HepG2, KERTr, and lung epithelial carcinoma (A549) cells after exposing to water extract of dust (250 to 2000 μg/100 μL). They found a progressive decrease of cell viability with elevating concentrations of water extracts of dust, and the inhibition effect on KERTr cell appeared earlier than HepG2 and A549 cells. Additionally, oxidative stress and pro-inflammatory responses are commonly observed after exposure to water and organic extracts (Ekstrand-Hammarstrom et al., 2013; Ghio et al., 2014). It has been suggested that the pro-inflammatory responses are, at least in part, driven by oxidative stress and redox-sensitive transcription factors (e.g., NF-κB), which initiates transcription of inflammatory mediators (*IL-1β*, *IL-6*, and *IL-8*) following exposure to particulate matter ($PM_{2.5}$) or dust extracts (Wei et al., 2011). Therefore, it is important to conduct toxicity test using both organic and water extracts of dust, and to compare their adverse effects on human corneas.

Various human cell line models, mainly cancer cells and immortalized cell lines, have been employed to elucidate the underlying molecular mechanism of dust-induced tissue specific toxicity (Kang et al., 2010; Riechelmann et al., 2007). However, published data showed that gene expression profiles between normal and neoplastic cells are strikingly diverse (Zhang et al., 1997). Proteomic study further revealed that, when compared to

primary cells, immortalized cell lines are characterized with deficient mitochondria, re-arranged metabolic pathways, suppressed metabolizing enzymes, and drastically upregulated cell cycle-associated functions (Pan et al., 2009). Furthermore, after exposure to $PM_{2.5}$, primary human cells showed higher sensitivity than cell lines (De Saint Jean et al., 2004; Ekstrand-Hammarstrom et al., 2013). In this context, it is preferable to employ primary human cells to investigate dust-induced toxicity.

In this study, the contaminants in indoor dust from residential houses and commercial offices in Nanjing, China were separated into two fractions, water and organic soluble. Presumably, organic contaminants are more soluble in organic extract while heavy metals are in water soluble fractions. Our objectives were: (1) to determine the concentrations of heavy metals in water extract and prolife of PAHs in organic extract, and (2) to compare the toxicity and cellular responses of primary human corneal epithelial cells after exposure to organic or water extracts of dust.

2. Materials and methods

2.1 Chemicals and Reagents

Cell culture plates and dishes were obtained from Corning Inc. (NY, USA); Cell culture medium were purchased from Life Technologies Inc. (CA, USA). Malondialdehyde (MDA) assay kit, and CCK-8 cell viability assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Human 8-Hydroxydeoxyguanosine (8-OHdG) ELISA kit was purchased from Yi Fei Xue Biotech. Co. (Nanjing, China). Reactive oxygen species (ROS) assay kit (DCFH-DA), superoxide dismutase (SOD) and catalase (CAT) assay kits, enhanced BCA protein assay kit, and cell lysis buffer were from Beyotime Institute of Biotechnology (Haimen, China). RQ1 RNase-free DNase was from Promega, USA. Chemical standards were from Aladdin Industrial Corporation (Shanghai, China) and J&K Scientific (Shanghai, China) with purity> 98%.

2.2 Indoor Dust sampling, Extraction and Characterization

Dust samples were collected from air conditioner filter of both residential house (n=16) and commercial offices (n=15) in Nanjing, China using a vacuum cleaner with a paper bag (Philips Fc8222, China). All dust samples were freeze-dried, and sieved through nylon

sieve (<100 μm) to remove fibrous fragments and large particles. All dust samples were then mixed into one composite sample as house and office dust. The particle size and total organic carbon (TOC) contents in dust were characterized (supporting information, SI).

Water and organic extracts were prepared from both dust samples (Fig. S1). For water extracts, 0.64 g of dust was dissolved into 40 mL Hank's balanced salt solution containing 3% antibiotic-antimycotic solution, sonicated at 4°C for 15 min for 4 times, and filtered through a 0.22 μm disposable sterile filter (Millipore, USA). Heavy metals in the dust and water extracts were determined by inductively coupled plasma mass spectrometry (ICP-MS) (NexION300X, PerkinElmer) after digestion using USEPA Method 3050B. For organic extracts, 1.6 g of dust was extracted with 160 mL n-hexane using sonication for 30 min for 2 times, and then concentrated by a rotatory evaporator (IKA®RV10, Germany) (He et al., 2016), reconstituted in 2 mL n-hexane, and filtered by 0.22 μm Nylon filter (ANPEL, China). An aliquot of 1 mL extract was used for chemical analysis, and the detailed process for PAHs analysis can be found in SI. An aliquot of 1 mL n-hexane extract was solvent-exchanged to 0.5 mL dimethyl sulfoxide. The toxicity equivalent concentrations of individual PAHs were calculated based on the toxic equivalency factor (TEF) with respect to benzo(a)pyrene values (Gao et al., 2015). The water and organic extracts, house dust (WH, OH) and office dust (WO, OO) were store at –20°C before cell assay.

Dust was acid digested using USEPA Method 3050B and analyzed for heavy metal by ICP-MS with duplicate analysis and check values (recovery of 91.7±2.3%). To minimize cross contamination, only glassware was used for sample extraction, storage, and analysis of organic contaminants. Prior to use, the glassware was scrupulously washed and heated at 450°C for 4 h. Method blanks, procedural blanks, and solvent blanks were included for quality control, and solvent blanks were analyzed every 8 samples on GC-MS. There was no PAHs being detected in blanks. The instrument limits of detection (LOD) were calculated as three times background noise level after running 7 solvent blanks. The LODs were 0.5±2.1 μg/kg for PAHs. Standard reference materials for dust (SRM2858) was also measured for method recovery, which were 87±5.1%–101±8.7% for PAHs.

2.3 Cell Culture and Cell Viability Assay

Primary human corneal epithelial (PHCE) cells derived from corneoscleral rims of a

healthy donor cornea were obtained from the Eye Hospital of Wenzhou Medical University. Cells were grown in Dulbecco's modified eagle medium supplemented with fetal bovine serum (FBS) (10%), epidermal growth factor (10 ng/mL) and 1% antibiotic-antimycotic solution at 37°C in a humidified incubator with 5% CO_2. Before exposure to dust extracts, PHCE cells were trypsinized and seeded into 6-well, 24-well, or 96-well plates overnight to allow attachment.

To determine the effects of different dust extract on cell viability, PHCE cells were replanted into a 96-well plate at density of 3×10^4 cells/100 μL/well. After overnight culture, the medium was replaced by 100 μL fresh medium containing 5 different concentrations of extract composites from dust (16, 48, 144, 432, or 1296 μg/100 μL). After 24 and 72 h incubation, cell viability was evaluated using CCK-8 cell viability assay kit. Changes in cell morphology was recorded by an inverted microscopy (TS-100, Nikon, Japan). LC_{50} was calculated by fitting cell viability data to a nonlinear regression curve [log(agonist) vs response] using Graphpad Prism Version 6 (Graphpad Software, USA).

2.4 Determination of Oxidative Stress Biomarkers: ROS, MDA, and 8-OHdG

The ROS was measured using an oxidation-sensitive fluorescent probe 2',7'-dichlorofluorescin diacetate (DCFH-DA) (Wang and Joseph 1999). Briefly, PHCE cells were seeded into 96-well black plates at a density of 3×10^4 cells/100 μL/well. After 24 h culture, the medium was replaced by 100 μL fresh culture medium containing different dust extracts and incubated for 72 h. Subsequently, cell culture medium was replenished by 10 μM DCFH-DA dissolved in FBS free medium and incubated for 30 min at 37 °C. The fluorescence value was recorded by fluorescence microplate reader (TECAN, USA) at 488 nm for excitation and 525 nm for emission wavelength. The ROS generation was expressed as the percentage of control group.

The MDA generation, a product of lipid peroxidation, were measured using a commercial kit to evaluate dust induced lipid peroxidation. In brief, 3×10^4 PHEC cells were seeded into 60 mm dishes overnight. The culture medium was replaced by fresh medium containing dust extracts at 48 μg/100 μL and incubated for additional 72 h. The levels of MDA was determined after reaction with thiobarbituric acid at a wavelength of 532 nm. The

MDA concentration was expressed as micromoles per milligram protein.

Another biomarker 8-OHdG was determined to evaluate oxidative DNA damage by an enzyme linked immunosorbent assay using an ELISA kit (CUSABIO, China). In brief, PHCE cells were seeded into 6-well plate at 5×10^5 cells/well overnight, and exposed to dust water/organic extracts (48 μg/100 μL) for 72 h. Determination of 8-OHdG was conducted according to the manufacturer's guideline.

2.5 Antioxidant Enzyme Activity Assays

Oxidative stress usually suppresses activity of antioxidant enzymes. To compare the effects of water and organic extracts of dust on antioxidant enzyme activity, PHCE cells were re-plated into a 6-well plate at 5×10^5 cells/well, and then treated with 48 μg/100 μL dust extracts for 72 h. After incubation, cells were sonicated at 4°C for 5 min and centrifuged at 10,000 g for 10 min at 4 °C. Supernatant was used to examine the activity of superoxide dismutase (SOD) and catalase (CAT) by commercial kits. Total protein contents were quantified using an enhanced BCA protein assay kit. Relative enzyme activity was represented as the fold of control group.

2.6 Xenobiotic-Metabolizing Enzymes and Pro-Inflammatory mediators Gene Expressions

AhR and its downstream genes (*CYP1A1* and *CYP1B1*), involved in xenobiotic metabolism, and pro-inflammatory mediator gene (e.g., *IL-1β, IL-6, and IL-8*) are always regulated by oxidative stress to elicit cytotoxicity (Ramadass et al., 2003; Reuter et al., 2010b).

To test the effects of dust extracts on xenobiotic-metabolizing enzymes and pro-inflammatory mediator gene expressions, the extracts were added to culture medium (containing 2% FBS) at 48 μg/100 μL and incubated with PHCE cells for 72 h. Cells treated with culture medium (containing 2% FBS and 0.1% DMSO) were served as control group. Subsequently, total RNA of PHCE cells was isolated using RNAiso Reagent (TaKaRa Biotech. Co., Japan). An aliquot of 1 μg total RNA was reverse transcribed to cDNA via a PrimeScript RT reagent kit (TaKaRa Biotech. Co., Japan). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using SYBR® Premix Ex Taq Ⅱ (Tli RNaseH Plus) (TaKaRa Biotech. Co., Japan) to analyze the expression levels of xenobiotic-metabolizing

enzyme and pro-inflammatory mediator genes. The detailed cycle description for RT-qPCR can be found in SI, and the specific primers are obtained from Harvard PrimerBank and listed in Table S1.

2.7 Immunostaining of NF-κB p65

NF-κB signal pathway is a classic and predominant molecular mechanism to regulate stimulus-induced stress. We hypothesized that dust extracts induced oxidative stress may be regulated by NF-κB signal pathway. To compare NF-κB signal pathway activation induced by dust extracts, PHCE cells were seeded on the coverslips for the immunofluorescent analysis of the subcellular localization of NF-κB p65. Briefly, PHCE cells cultured on coverslips were exposed to 48 μg/100 μL dust extracts for 72 h. After exposure, cells were washed twice with PBS, fixed in freshly prepared 4% paraformaldehyde for 15 min, and permeabilized with 0.1% Triton X-100 in PBS for 10 min. Subsequently, 5% normal goat serum diluted by PBS with 0.1% Tween-20 (PBST) was used to block non-specific sites for 1 h, followed by incubation with a rabbit monoclonal antibody against human NF-κB p65 (1:500; Abcam) overnight at 4°C. Cells was washed three times with PBST and incubated with a secondary FITC-conjugated goat anti-rabbit IgG (1:200; Jackson ImmunoResearch Laboratories, Inc.) for 1 h at 37 °C. Cell nuclei were counterstained by DAPI, which were visualized using a fluorescence microscope (Carl Zeiss Meditec, Inc., Germany).

2.8 Statistical Analysis

All experiments were carried out in triplicate. Statistical analyses were conducted using one-way ANOVA by PASW Statistics Version 18 (SPSS Inc., USA) and Graphpad Prism Version 6 (Graphpad Software, USA). Significant differences were set at $\alpha = 0.05$.

3. Results

3.1 Concentrations of Contaminants in Dust Extracts

The concentrations of heavy metals (i.e., Pb, Cu, Cd, Zn, Cr, Sb, Ni, Co, As, and Mn) in the dust and water extracts are shown in Table S2. The elemental compositions in house and office dust were similar, with both containing high levels of Zn, Cu, Pb, and Mn. However, Cr concentration (290 mg/ kg) in the office dust was ~5 times greater than that in the house dust (63.2 mg/ kg), while the office dust also had slightly higher levels of Pb, Cu,

Cd, Ni, Co, As, and Mn than that of the house dust. For water-soluble metals, levels of Zn, Cu, Ni, and Mn were much higher than those of other metal contents in the water extracts.

In addition to heavy metals, concentrations of 16 PAHs in organic extracts were quantified by GC-MS (Table S3). The total concentration of 16 PAHs in office dust was 25.3 mg/kg, which was >3 folds of that in house dust (7.94 mg/ kg). The concentration of high molecular weight (HMW, 4-to 6-rings) PAHs in office dust at 23.8 mg/ kg was greater than that in house dust at 6.36 mg/ kg, but concentration of low molecular weight (LMW, 2-to 3-rings) PAHs from both dust was similar at 1.48-1.58 mg/ kg. The concentrations of HMW PAHs were remarkably higher than those of LMW PAHs in dust samples (p<0.01), accounting for 80.1% and 99.8% of total PAHs in house dust and office dust. Carcinogenic PAHs in organic extracts was ~5 times (16.1 vs. 3.35 mg/g) higher in office than that in house dust.

Toxic equivalent quantity calculated for office and house dust based on their toxicity equivalent factor of the PAHs are shown in Table S3. The TEQs was 0.65 mg/ kg for house dust, while 3.53 mg/ kg for office dust. Benzo(a)pyrene was the highest carcinogenic contributor in office (34.6%) and house (43.8%) dust, followed by dibenzo(a,h)anthracene (23.4% for house dust and 32.6% for office dust. More interestingly, TEQ of all carcinogenic PAHs in office dust was 5.4 times higher than that in house dust (p<0.05) excluding naphthalene. In short, compared to house dust, office dust contained greater concentrations of heavy metals and PAHs, especially carcinogenic HMW PAHs.

3.2 Dust Extracts Decreased Cell Viability and Altered Cell Morphology

The cytotoxicity of PHCE cells after exposing to dust extracts was evaluated using CCK-8 assay. PHCE cells treated with house dust showed no significant cytotoxic effects at concentration ≤144 μg/100 μL after 24 h exposure (Fig. 1A). With prolonging exposure time to house dust (72 h), water extract showed greater toxicity at concentration ≤ 48 μg/100 μL than organic extract (Fig. 1B). More interestingly, cell viability treated with organic extract were dramatically decreased at ≥432 μg/100 μL (Fig. 1AB). For office dust, water extract at ≤ 48 μg/100 μL (Fig.1 CD) induced higher toxicity than organic extract, but at 432 μg/100 μL, organic extract showed much higher cytotoxic effects on PHCE cells than water extract (Fig.1 CD).

Based on the fitted curve (Fig. S2 AB), the LC_{50} of water and organic extracts of dust on PHCE cells was calculated (Table S4). Organic extract of office dust showed the lowest LC_{50} value (185 μg/100 μL after 24 h and 171 μg/100 μL after 72 h) among all extracts, which was significantly lower than HepG2 cells (230 μg/100 μL after 24 h) (Kang et al., 2010). The LC_{50} of water extracts after 72 h exposure was increased for house dust, indicating PHCE cells may initiate an adaptive cytoprotective response during the exposure.

In short, at ≤ 48 μg/100 μL, water extracts of dust exhibited higher toxicity, but at ≥432 μg/100 μL, organic extracts of dust did, especially for 72 h exposure. As such, a low (48 μg/100 μL) and a high (432 μg/100 μL) concentrations of dust were exposed to PHCE cells for 72 h for further assessment. It was found that organic dust extract caused majority of visible vacuolization and distorted membrane structure at 432 μg/100 μL (Fig. S3 EJ) implicating cell death (Gupta et al., 2013). To better understand different cellular responses to dust extracts at the same concentration, a lower concentration at 48 μg/100 μL was used in the subsequent study.

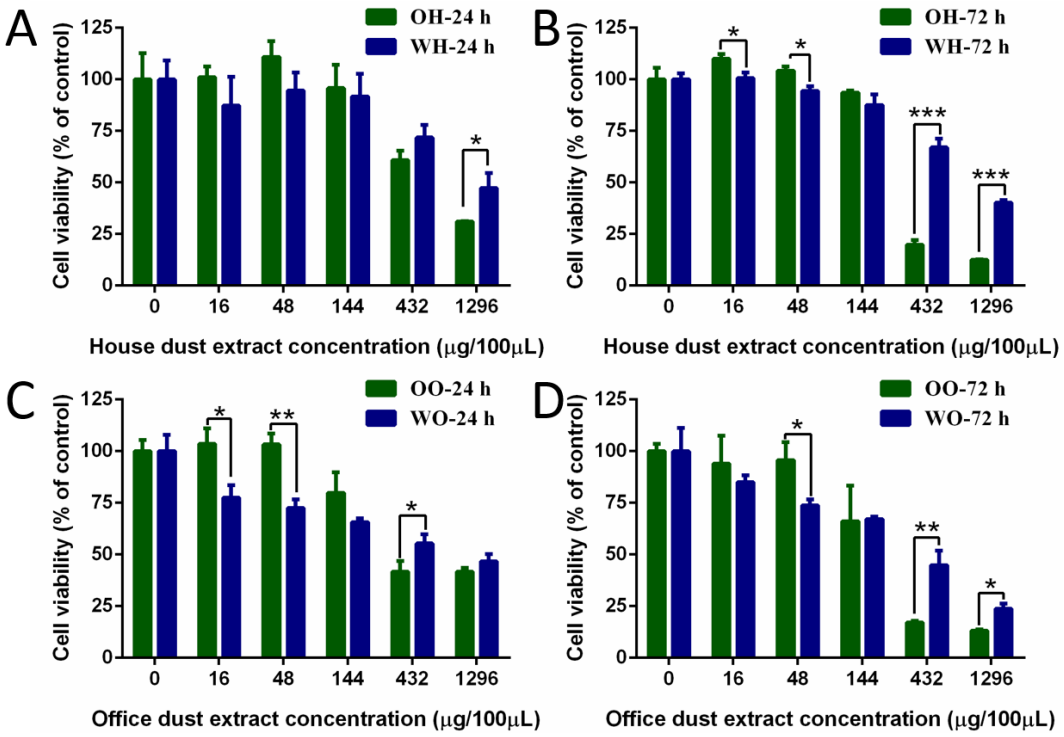

Figure 1 Characteristic cytotoxicity for PHCE cells exposed to dust extracts. Cell viability was determined using a CCK-8 assay after exposure to dust extracts for 24 h (AC) and 72 h (BD). Five concentrations at 16, 48, 144, 432, and 1296 μg/100 μL were tested. OH and OO stand for organic extracts of house and office dust and WH and WO stand for water extracts

of house and office dust. Error bar means standard deviation of three replicates.*p<0.05, **p<0.01, ***p<0.001.

3.3 Dust Extracts induced Oxidative Stress by Overproducing ROS, MDA and 8-OHdG

To compare the effects of dust on the oxidative stress, we determined the intracellular ROS production in PHCE cells after 72 h exposure to dust extract. The ROS levels in PHCE cells were significantly elevated after exposure. Organic extracts caused more ROS generation than water extracts, with office dust showing greater effects than house dust (Fig. 2A; p<0.05).

Excessive production of ROS usually results in oxidative damage to lipids and DNA. We measured the MDA contents, an important indicator of ROS-induced lipid peroxidation, in PHCE cells after 72 h exposure. We found that MDA production was increased from 1.86 to 2.56-4.34 μmol/mg protein after exposing to dust extract (Fig. 3B). The production of 8-OHdG as a measure of oxidative-stress induced DNA damage was also analyzed. The 8-OHdG level in PHCE cells exposed to water extract of house dust was the lowest at 260 pg/mL, while organic extract of office dust being highest at 398 pg/mL, both of which were significantly higher than the control cells (at 176 pg/mL). Collectively, organic extracts induced higher oxidative damage to PHCE cells than that of water extracts, with office dust inducing more oxidative toxicity than house dust.

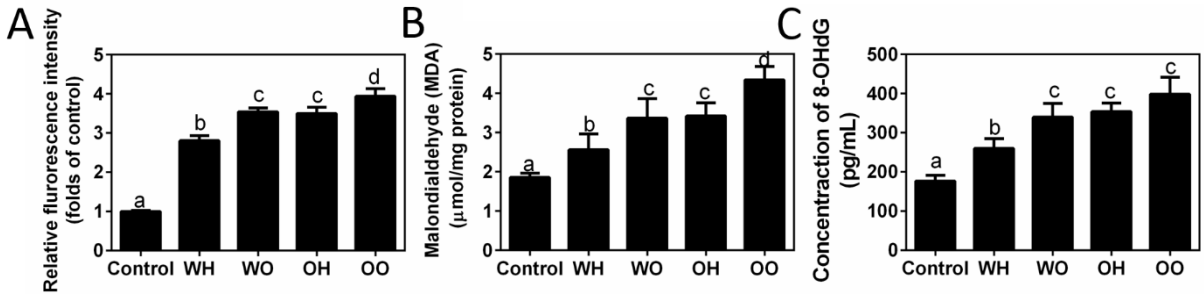

Figure 2 Comparison of dust induced oxidative stress in PHCE cells. Cultured PHCE cells were treated with 48 μg/100 μL of dust extracts for 72 h, with biomarkers of oxidative stress of ROS (A), MDA (B), and 8-OHdG (C) production being determined. OH and OO stand for organic extracts of house and office dust, and WH and WO stand for water extracts of house and office dust. Results are shown as mean ± SD of three replicates. Different letters indicate statistic difference at p<0.05.

3.4 Dust Extracts Suppressed Antioxidant Enzymes Activity

Anti-oxidative enzymes are important antioxidants to minimize oxidative stress induced toxic effects. To test the responses of antioxidant enzymatic defenses of PHCE cells to dust extracts, we evaluated the activity of SOD and CAT in PHCE cells, which were remarkably decreased after exposing to dust extracts (Fig. 3AB). Both SOD and CAT activity in PHCE cells was suppressed more by organic extract than water extract and by office dust than house dust.

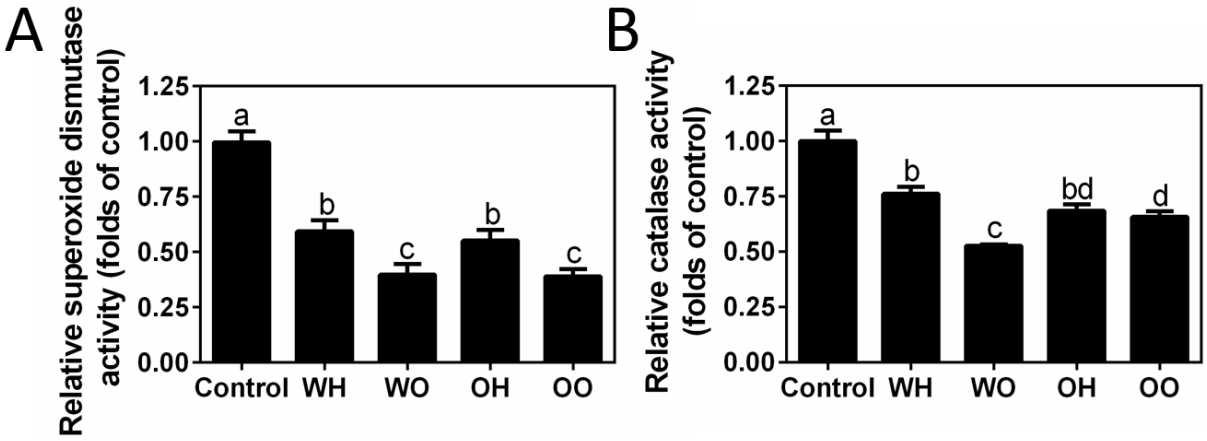

Figure 3 Effects of dust extracts on activity of antioxidant enzymes of superoxide dismutase (SOD; A) and Catalase (CAT; B) in PHCE cells following 72 h exposure at 48 μg/100 μL dust extract. OH and OO stand for organic extracts of house and office dust, and WH and WO stand for water extracts of house and office dust. Results are shown as mean ± SD of three replicates. Different letters indicate statistic difference at $p<0.05$.

3.5 Dust Extracts Altered Gene Expressions of Pro-Inflammatory Mediators and Xenobiotic-Metabolizing Enzymes

A significant induction of pro-inflammatory mediator gene expressions were observed in PHCE cells after 72 h of exposure to dust extracts (Fig. 4AB). The levels of *IL-1β, IL-6*, and *IL-8* in PHCE cells exposed to organic extracts were higher than those in water extracts with some exception. However, *IL-1β* was decreased to 0.6–0.7 fold of control cells after exposing to water extract of dust.

The expression of xenobiotic-metabolizing enzymes genes was also noted (Fig. 4CD).

After exposing to organic extract of house dust, the expression of *CYP1A1* and *CYP1B1* were elevated by 4.5 and 7.4 folds compared to control (Fig. 4C) and the increase was 51 and 20 folds after exposing to office dust (Fig. 4D). Interestingly, an upregulation of *CYP1A1* and *CYP1B1* gene expressions were also detected after exposing to water extracts of dust, with water extract of office dust showing greater gene expression than house dust. As an important upstream regulatory gene of *CYP1A1* and *CYP1B1*, the *AhR* mRNA levels were also slightly increased.

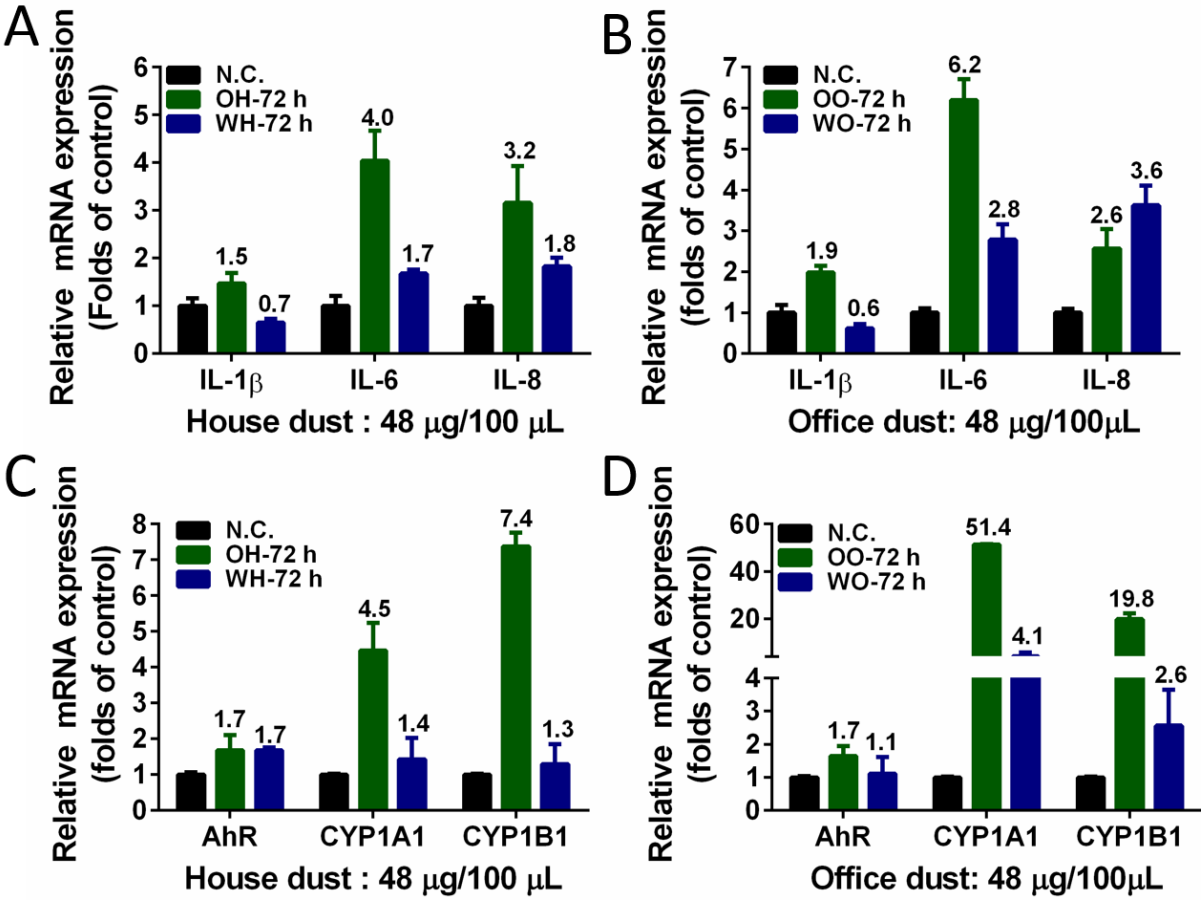

Figure 4 Dust-induced expression of pro-inflammatory mediators and xenobiotic-metabolizing enzymes gene in PHCE cells after 72 h exposure to dust exposure at 48 μg/100 μL. Results are shown as mean ± SD of three replicates. OH and OO stand for organic extracts of house and office dust, and WH and WO stand for water extracts of house and office dust. The numbers on each column represent mean of relative fold changes normalized to untreated group.

3.6 Dust Extracts induced NF-κB activation

NF-κB is a crucial upstream regulator of various genes involved in cellular responses

to diverse stimuli such as oxidative stress, cytokines, and free radicals. As the most commonly investigated subunit of NF-κB, p65 usually binds with NF-κB inhibitor protein IκB in the cytoplasm. Once activated by stimulation, IκB is phosphorylated and then degraded. Subsequently, the p65 subunit translocates to the cell nucleus to modulate transcription of NF-κB target genes. Both water (Fig. 5 JKL) and organic (Fig. 5 MNO) extracts of office dust elicited marked nuclear translocation of p65 in PHCE cells, whereas exposure to water extracts of house dust (Fig. 5 DEF) did not. However, green fluorescence was visualized in both cellular cytoplasm and nucleus in presence of organic extracts of house dust (Fig. 5 GHI), suggesting it also activated NF-κB p65 nuclear translocation. Nevertheless, it was weaker than that of office dust extracts.

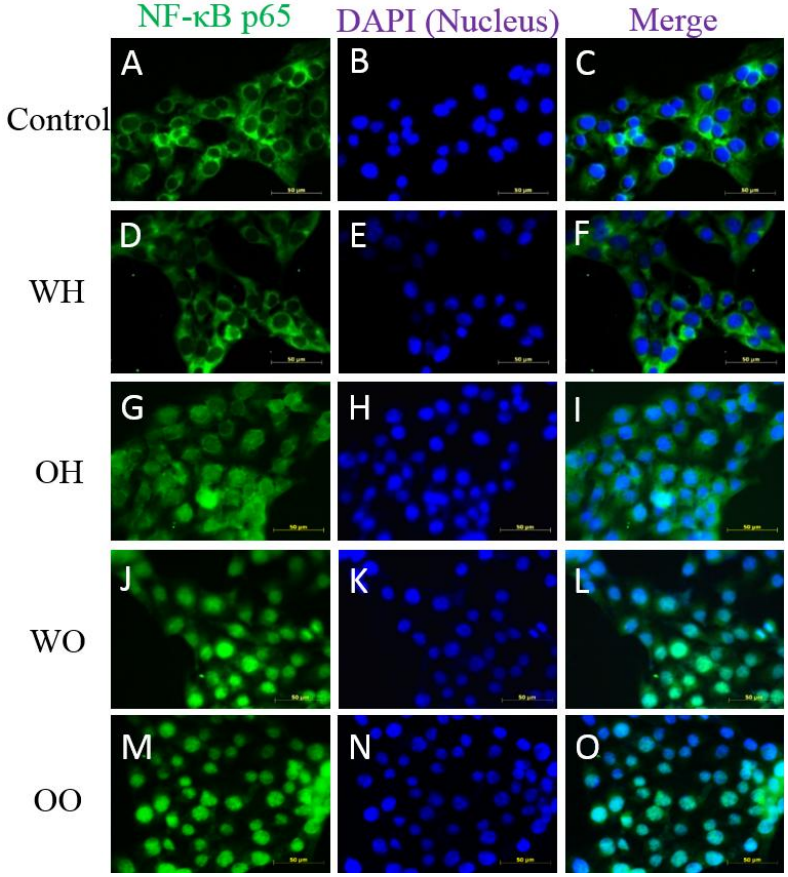

Figure 5 Effects of dust extracts on nuclear translocation of NF-κB p65 protein in PHCE cells. Cultured PHCE cells were treated with 48 μg/100 μL dust extracts. After 72 h exposure, the PHCE cells were incubated with p65 primary antibody and FITC-conjugated secondary antibody (A, D, G, J, M), and nuclei were counterstained with DAPI (B, E, H, K, N). The images were recorded (C, F, I, L, O) by a fluorescent microscopy and merged using

AxioVision Software. (Scale bar=50 μm). OH and OO stand for organic extracts of house and office dust, and WH and WO stand for water extracts of house and office dust.

4. Discussion

In this study, we compared the responses of PHCE cells after exposing to water and organic extracts of office and house dust. The parameters included contaminant concentrations (Tables S2, and S3), cell viability (Fig. 1), LC_{50} (Table S4; Fig. S2), cell morphology (Fig. S3), biomarkers of oxidative damage (Fig. 2), antioxidant enzymes activity (Fig. 3), mRNA expression of pro-inflammatory mediators and xenobiotic-metabolizing enzymes (Fig. 4), and NF-κB pathway activation (Fig. 5).

The mean concentrations of indoor dust ranged from below 10 to as high as 3200 $\mu g/m^3$ (Molhave et al., 2000). In many studies focusing on dust-induced cytotoxicity, dust ranging from 0.5–10000 μg/100 μL was used (Kang et al., 2010; Saraf et al., 1999). However, to our knowledge, there are no accurate data on HCE exposure to indoor dust. To evaluate ocular toxicity of dust, a NASA study exposed HCE tissue with authentic lunar dust at 10,000 μg/100 μL (Meyers et al., 2012). Recently, after exposing to house dust at 500–3000 μg/100 μL for 24 h, elevated levels of pro-inflammatory mediators IL-1β and IL-8, decreased HCE viability and reduced HCE thickness were observed in reconstituted HCE tissue (Cao et al., 2015). However, few studies focused on the effects of lower dust concentration on PHCE cells and the underlying mechanisms. Here, we used dust concentrations of 16 to 1296 μg/100 μL. Dust size is another critical factor for risk assessment of human exposure. Several studies suggested that dust particles <100 μm were retained most efficiently by skin and important for human exposure risk assessment (Cao et al., 2012). For study on the effects of dust on human eyes, existing studies also confirmed that dust <100 μm is more relevant to ocular exposures (Mølhave et al., 2002; Meyers et al., 2012). Therefore, dust with particle size <100 μm was selected in present study.

Dust-induced cytotoxicity usually depends on the concentrations and types of contaminants in the dust. In this study, the total concentrations of metals in office dust were significant higher than those in house dust ($p < 0.05$; Table S2). The frequent use of computers and printers in office may have contributed accumulation of heavy metals in office dust (Kurt-Karakus 2012). In addition, the concentrations of soluble metals in office dust

were higher than those in house dust, suggesting office dust may pose more health risks, since water-soluble metals are bioavailable and easily induce oxidative damage to membrane lipids, DNA, and proteins, which may eventually result in cell death (Huang et al., 2015).

Besides heavy metals, PAHs have received increased attention in recent decades for indoor environment pollution because some PAHs are carcinogenic (Armstrong et al., 2004). The mean concentrations of total PAHs in office dust (25.1 mg/ kg, Table S3) was higher than that reported in Hong Kong (6.18 mg/ kg) and Pearl River Delta (7.30 mg/ kg) (Kang et al., 2011). However, the PAH concentrations in house dust in present study were lower at 7.94 mg/ kg (Table S3), which was comparable to that in dust sample from 24 houses in Durham, USA (1.25–15.2 mg/kg) (Chuang et al., 1995), but higher than that from Kuwait (< 2.92 mg/kg) (Gevao et al., 2007). It was reported that the PAH concentration in dust increased with decreasing particle size, as small particles have a larger surface to volume ratio (Dong and Lee 2009). In the present study, PAHs contents in office sample were higher than those of house dust, which may be due to the fact that mean diameter for office sample was 20.9 μm, which was significantly smaller than that of house dust (58.5 μm). However, total organic carbon contents of house dust (15.6%) was similar to that of office dust (16.1%) (data not shown).

HMW-PAHs were predominant in dust samples, accounting for 94% and 80% of total PAHs in office and house dust. This result may be explained that carcinogenic 4-to 6-ring PAHs were primarily detected in organic extract of office dust, while 2-to 3-ring PAHs primarily exist in organic extract of house dust (Gao et al., 2015). The use of TEQs based on TEFs for PAHs was effective to evaluate the carcinogenic potency of PAH mixtures (Wang et al., 2013). In our study, benzo(a)pyrene was the highest carcinogenic contributor in office (35%) and house (44%) dust, while dibenzo(a,h)anthracene contributed 23% and 33% for office and house dust. The TEQs of the 8 carcinogenic PAHs for office dust was ~5.4 times higher than that of house dust, indicating its higher carcinogenic risk. Taken together, office dust was more contaminated with PAHs and showed high carcinogenic risk compared to house dust, which may result from tobacco smoking and lower clean frequency in offices than houses (Destaillats et al., 2008; Kang et al., 2010; Ren et al., 2006). Additionally, according to our records during sampling period, there were more computers, copy machines,

and leather products in offices than houses, which may also be a contributor of PAHs. Moreover, use of electromagnetic oven in house also decreases PAHs emission from combustion.

Previous studies showed that dust induced cytotoxicity and inflammation as well as cell growth inhibition in many immortalized cell lines and cancer cells (Kang et al., 2010; Riechelmann et al., 2007). However, hundreds of genes were expressed differently in normal and cancer cells (Zhang et al., 1997). Furthermore, cell lines are with deficient mitochondria, re-arranged metabolic pathways, drastically upregulated cell cycle-associated functions and largely suppressed metabolizing enzymes activity compared to primary cells (Pan et al., 2009). Most importantly, cell lines showed low sensibility to stimuli compared to primary cells (De Saint Jean et al., 2004). In present study, human primary corneal epithelial cells (PHCE) were employed to minimize the artifact since they more closely simulate in vivo condition. Dust extract decreased cell viability in a concentration-dependent manner. More interestingly, water extracts of dust induced higher cytotoxicity at $\leq$ 48 μg/100 μL. At 432 μg/100 μL, organic extracts showed much higher toxicity, particularly 72 h exposure (Fig. 1). This indicated that PHCE cells were more sensitive to water soluble metals at low concentrations, but at high concentrations, the toxic contaminants in organic extracts caused more cytotoxicity.

Yang et al. (2011) determined different responses of human lung epithelial cells to organic and water extract of PM, and they found organic extract induced greater ROS production. Consistent with their results, our data also showed the elevations of ROS, MDA, and 8-OHdG in PHCE cells after exposure to dust extract (Fig. 2), suggesting that dust extracts elicited oxidative stress in PHCE cells, probably attributing to PAHs and water soluble metals. Moreover, organic extracts induced greater production of these biomarkers (Fig. 2), indicating higher damage potential to cells including lipids and DNA. The antioxidant enzymes are always ready to detoxify the reactive intermediates or repair the resulting damage in biological system. For example, SOD catalyzes the dismutation of $O^{\cdot}_2$ to H_2O_2, and CAT protects cells by removal of H_2O_2. In our study, the activities of SOD and CAT were significantly suppressed by organic extracts of dust, followed by water extracts of dust (Fig. 3).

Oxidative stress often elicits activation of transcription factors including NF-κB and AP-1, leading to the transcription of pro-inflammatory mediators (e.g., *IL-1β, IL-6,* and *IL-8*) (Reuter et al., 2010a). Several corneal inflammatory diseases such as corneal inflammation, bullous keratopathy, and dry eye disease are also associated with oxidative stress (Shoham et al., 2008). The mRNA expressions of *IL-1β, IL-6,* and *IL-8* were up-regulated in the present study, indicating dust extracts elicited inflammatory responses. IL-1β acts as a key mediator in the pathogenesis of eye diseases, which could significantly promote the release of IL-6 and IL-8 in HCE cell lines (Cavet et al., 2011). IL-6 is involved in acute systemic inflammations and correlate with the severity of injury in corneal epithelium (Sotozono et al., 1997). IL-8, as a chemotactic signal attracting monocytes into the injury and infection site, participates corneal leukocytic infiltration and induces corneal neovascularization (Strieter et al., 1992). Moreover, the significant difference was observed between the two extracts for *IL-1β, IL-6*, and *IL-8* mRNA expression ($p<0.05$) for both office and house dust (Fig. 4 AB), suggesting that organic and water extracts resulted in different cellular responses, with organic extracts inducing greater inflammatory responses.

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in the regulation of biological responses to planar aromatic hydrocarbons such as PAHs, polyphenolics, and indoles (Stejskalova et al., 2011). This receptor regulates xenobiotic-metabolizing enzymes (CYP1A1, and CYP1B1) to subsequently generate active intermediates to elicit adverse health effects (Wincent et al., 2015). Additionally, AhR and CYP related genes have also been involved in tumor-promotion and enhanced oxidative stress (Mahadevan et al., 2005). Previous studies pointed out that AhR and its inducible genes (*CYP1A1*, and *CYP1B1*) can be activated by organic extract of dust, which may also participate in cell apoptosis and cytokine release (Andrysík et al., 2011). In present study, substantial increase in *CYP1A1* and *CYP1B1* expression was noted in PHCE cells treated with organic extract of dust (Fig. 4 CD), possibly attributing to the presence of PAHs (Misaki et al., 2008). Few investigations looked at the impact of water extract of dust on gene expression of *AhR*. Interestingly, a significant elevation of *CYP1A1* and *CYP1B1* expression in PHCE cells was also observed after exposed to water extract of office dust, but no changes for house dust, indicating water soluble office dust was sufficient to activate those genes.

Obviously, organic extracts showed higher potential to activate AhR pathway than water extracts, especially for office dust.

NF-κB is a redox-sensitive transcription factor. Activation of NF-κB subsequently initiates transcription of inflammatory mediators, leading to HCE cell apoptosis, necrosis, and disruption of barrier function (Cho et al., 1999; Kimura et al., 2008). It was found that soluble components of PM including metals and organic compounds are capable of generating ROS and activating NF-κB species (Jimenez et al., 2000). However, not all PHCE cells were observed with NF-κB p65 nuclear translocation after exposure to dust extracts (Fig. 5D). PHCE cells treated with organic extract of office dust showed stronger NF-κB p65 nuclear translocation than that of house dust (Fig. 5DM), probably due to the higher PAHs concentrations in office dust (Tian, 2009). For water extracts, office dust also induced visible NF-κB p65 nuclear translocation (Fig. 5J), which was consistent with literature that water-soluble PM activates NF-κB to cause inflammation in human cell lines (McNeilly et al., 2005; Wei et al., 2011). However, no evident nuclear translocation was observed in water extract of house dust (Fig. 5D), which may be due to the low concentration of soluble compounds (Pujalté et al., 2011). In short, dust extracts excluding water extract of house dust induced oxidative stress and inflammation via activation of NF-κB signal pathway.

5. Environmental Implication

In this study, we determined the concentrations of heavy metals in water extracts and 16 PAHs in organic extracts of house and office dust. Besides, we determined the molecular responses of PHCE cells after exposing to water and organic extracts of dust. Our data showed that office dust had higher concentrations of heavy metals and PAHs than that house dust. Both dust caused cytotoxicity, oxidative damage, inflammatory response, and activation of AhR inducible genes, with office dust being more potent. Excluding water extract of house dust, all dust extracts resulted in NF-κB activation. Consistent with their higher concentrations of heavy metals and/or PAHs, organic extracts had higher potential than water extracts, and office dust had greater potential than house dust to induce adverse effects in PHCE cells. Our data clearly demonstrated the importance to reduce the contaminant levels including heavy metals and PAHs in the dust to reduce their adverse impacts on human eyes.

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