A novel approach for a toxicity prediction model of environmental pollutants

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Contents

Abstract

There are myriad environmental pollutants on the earth, and a large amount of new environmental pollutants may be produced in future. The identification of newly emerging pollutants predicted from limited information is important in human health risk management. From the viewpoints of cost and ethics, development of two effective approaches, instead of the conventional animal experiment, is expected. One is toxicogenomics, representing the DNA microarray analysis; and the other is *in silico* approaches based on the quantitative structure-activity relationship (QSAR). Toxicogenomics has been widely used for sensitively and quickly elucidating the molecular and cellular actions of chemicals and other environmental stressors resulting in biological damage. QSAR is a potential tool for predicting the activity and properties of chemicals, including their physicochemical attributes, health effects, ecotoxicity, and biological activity. In this study, I attempted to develop new and efficient toxicity prediction models for the myriad environmental pollutants including those in automobile emissions.

Toward this goal, I tried to combine toxicogenomics with QSAR. 64 chemicals/particulates detected in automobile emissions were selected; and the DNA microarray method was used to examine their effect on gene expression in human lung cells. The results showed that the expression of various genes was altered in cells exposed to PAHs, nitroarenes or quinones. Furthermore, these 64 chemicals/particulates were divided into some groups reflecting the physicochemical characteristics of these compounds by using hierarchical clustering analysis of the gene expression data. Then, IL-8, as a well-known proinflammatory cytokine involved in allergic inflammation induced by automobile emissions, was selected to develop an in *silico* prediction model by utilizing

the QSAR for IL-8 gene expression. Furthermore, I validated the prediction model according to known data from previous reports. As a result, this prediction model showed high accuracy in predicting up-regulation of the IL-8 gene. These results suggest that the prediction model using QSAR based on the gene expression from toxicogenomics may have great potential in predictive toxicology of environmental pollutants.

1.Introduction

Concern about the toxicity of chemicals released into the environment has been increasing recently. Many chemicals are suspected to have hazardous effects, but evaluation of their toxicity is still difficult and challenging. One of these difficulties is that certain chemicals are reported to have an adverse effect on organisms despite giving negative results in conventional toxicity tests. Thus, a new technique is desirable in order to evaluate the effects of chemicals on human health.

Recently, the impact of ambient fine particulate matter (PM2.5, particulate matter with an aerodynamic diameter < or = 2.5 μm) on health and the environment has become a big issue. It has been reported that an increase in PM2.5 is associated with the mortality and morbidity from respiratory and cardiovascular diseases [1,2]. In Japan, an environmental quality standards for PM2.5 was established in 2009, and so many people are now interested in the effects of PM2.5 on health. Diesel exhaust particles (DEP) are well known as one of the most important components of ambient PM2.5. The development of emission-reduction technologies in recent years has produced considerable reduction in the particle concentration in diesel emissions; however, there is a possibility that unexpected toxic substances are now being produced in diesel emissions owing to new technologies and types of fuel [3]. Therefore, it is necessary to understand the toxicity of automobile emission consequential to these new technologies and fuels.

Animal exposure studies can play an important role in evaluating the toxicity of environmental pollutants including those in automobile emissions. However, because these pollutants are of great variety, it is impossible to understand in a cyclopedic manner their toxicity by such studies only. Furthermore, an animal exposure study is a fairly long-term process and involves huge cost. In addition, the use of animal studies should be reduced from the view point of animal welfare. In the industrial world, one aim in the toxicological

evaluation of chemicals is a reduction in, refining, and replacing animal testing, especially in the context of the new EU chemical policy REACH (Registration, Evaluation, Authorisation and Restriction of CHemicals) [4]. This new legal framework also supports the development of alternative methods to animal experimentation, encouraging the improvement and/or design of new methodological strategies for the toxicological evaluation of chemical compounds. In light of this background, there is a real need for new approaches for rational estimation of the toxicity of new environmental pollutants without the use of experimental animals.

Recent advances in molecular biology have provided a technique for a better understanding of the responses of organisms to chemicals; and this emerging field is known as toxicogenomics. Toxicogenomics is defined as an integration of genomics (transcriptomics, proteomics, metabolomics) and toxicology. For example, the DNA microarray can be used to explore the gene expression profiles of organisms in response to certain chemicals. The DNA microarray method is a powerful tool to determine the comprehensive changes in gene expression induced by various chemicals. By this technique, many researchers can detect the toxic reaction to chemical compounds as changes in gene expression. It is said that a change in gene expression is "an early warning marker" of toxicity, because gene expression data provide useful information to predict the toxicity of chemicals before the phenotype is manifested [4-6].

On the other hand, as a screening method to replace animal tests, an *in silico* toxicity prediction based on the quantitative structure-activity relationship (QSAR) is meaningful. This QSAR approach, which elucidates the relationship between the chemical structure and biological activity of a compound of interest, has been in use over a long period of time. Several *in silico* toxicity prediction systems with QSAR have been developed. For the prediction of the toxicity including mutagenicity of candidate drugs for development,

QSAR is utilized widely in the pharmaceutical industry [7-11]. Therefore, the fusion between toxicogenomics and QSAR may provide a high-accuracy toxicity prediction model for various chemical compounds.

In this study, gene expression in a human lung epithelial cell line treated with 64 chemicals/particles related to diesel emissions was examined by use of the DNA microarray method. In addition, oxidative activity and *in vitro* cytotoxicity were measured to supplement the gene expression data. Based on the data obtained, an *in silico* gene expression prediction model was constructed to predict the toxicity of unknown chemicals.

2. Materials and methods

2.1. Treatment with DEP and chemicals

It was unrealistic to analyze the gene expression profiles elicited by all chemicals included in the diesel emissions in this study, because there are a great many chemicals in these emissions. Therefore, priority was given to just 64 chemicals/particulates (Table 1; refer to the MSAT program of the U.S. EPA [5,6] and the ACES program of the HEI [3]. The DEPs, SRM 2975 (Industrial Forklift), were purchased from the National Institute of Standards and Technology (Gaithersburg, MD, USA). Other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). DEP and the various chemicals were dissolved and sonicated in DMSO.

2.2. DTT assay

Oxidative activity was determined in triplicate by conducting the dithiothreitol (DTT) assay, which is used for the quantitative measurement of reactive oxygen species (ROS) formation *in vitro* [7]. All samples were prepared at arbitrary concentrations in 250 mM Tris-HCl buffer (pH 8.9). Briefly, 10 μl of 20 mM DTT solution and 1 ml of a test sample including a blank (250 mM Tris-HCl buffer only), both containing DMSO (final concentration, 0.1%), were mixed in tubes and incubated for 30 min at 37 °C in a water bath. Then 16.6 μl of 20 mM DTNB was added to this mixture to develop the yellow color. After color development, samples (50 μl) were placed in microtiter wells, and the absorbance was measured at 405 nm with a microplate reader (Microplate reader Model 680, Bio-Rad, USA).

2.3. Cell culture

Cell lines A549 (human lung) and HL-60 (human leukemia) were purchased from the American Type Culture Collection (CCL 185 line; Rockville, MD, USA). These cells were kept at 37°C in a humidified incubator under 5% CO_2 in air and grown in DMEM culture medium containing 10 μg/ml gentamicin supplemented with 10% FBS until they had reached 80–90% confluence.

2.4. Cytotoxicity assays

After treatment with chemicals, cytotoxicity was assessed as cell viability by using 4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt (WST-8), a novel tetrazolium salt, provided in a commercially available kit. HL-60 cells were plated in 96-well microtiter plates at a density of 5×10^3 cells per well, and each plate was incubated for 48 hours at 37 °C in 5% CO_2. Two days after the seeding, the cells were exposed to DEP or various chemicals for 48 hours. The live cell count was assayed by using a Cell Counting Kit-8 (Dojin, Kumamoto, Japan) according to the instructions provided by the manufacturer, and the absorbance of each well was measured at 450 nm with a microtiter plate reader. Cell viability was calculated as the ratio of viable treated cells to viable untreated cells.

2.5. DNA microarray analysis

A549 cells (1×10^6) were seeded into each of several dishes. Two days after the seeding, the cells were exposed to DEP or various chemicals for 4 hours. Final concentrations used were 1 μM and 10 μM for chemicals and 30 μg/ml and 100 μg/ml for DEP. Control cells were treated with the same concentration of DMSO. After the exposure, total RNA was extracted from the cells by using an RNeasy mini kit (QIAGEN, Hikden, Germany) according to the manufacturer's protocol, eluted with RNase-free water, and stored at -

80°C prior to use. RNA concentrations were determined with a spectrophotometer (GeneQuant, Amersham Biosciences, Piscataway, NJ, USA), and analyzed for quantity and quality by using a bio-analyzer (Agilent Technologies, USA). Total RNA was used for the synthesis of fluorescent cRNA with an Agilent Quick Amp Labeling Kit (Agilent Technologies, USA), and the Cy3-labeled cRNA was combined with and hybridized to Agilent 4×44K Human Oligo Microarrays (Agilent Technologies, USA) according to the manufacturer's protocol. After hybridization, the slides were washed and scanned with an Agilent microarray scanner. The scanned images for each slide were analyzed by using Feature Extraction software version 9.5.3.1 (Agilent Technologies, USA). The obtained data were then analyzed by using GeneSpring GX 10.0 software (Agilent Technologies, USA). The data were normalized by the per-chip normalization method, and filtering of the data was performed by using flags (present, absent, and marginal).

2.6. Construction of the *in silico* prediction model

Of the 64 chemicals/particles examined, the 54 chemicals were classified into 2 groups based on the gene expression of IL-8. One was the up-regulation class; and the other, the down-regulation class. Successively, 372 physico-chemical descriptors of the chemicals were calculated by the use of ADMEWORKS (Fujitsu, JAPAN). Then, some of these descriptors related to IL-8 expression were chosen, and a prediction model was constructed by using the ADMEWORKS.

3. Results

3.1. Oxidative activity of the selected 64 chemicals/particles related to diesel emissions

The oxidative activity was evaluated by conducting the DTT assay. As a result, quinones and metals showed oxidative activity (Fig. 1 and Table 2). Especially, anthraquinone, copper (I), copper (II), and nickel (II) showed a high DTT consumption rate (Fig. 1 and Table 2). On the one hand, PAHs, nitroarenes, phthalates, nitrophenols, and particles (except for DEP) had little or no oxidative activity.

3.2. Cytotoxicity of the 64 chemicals related to diesel emissions

The cytotoxicity was evaluated in terms of the half maximal (50%) inhibitory concentration (IC_{50}). The IC_{50} values of the chemicals are shown in Table 3. The viability of cells exposed to 11 chemicals (9-methyl anthracene, 1-nitropyrene, 3-nitro benzanthrone, 1,2-naphthoquinone, 9,10-phenanthraquinone, p-benzoquinone, bis[2-ethylhexyl] phthalate, copper(I) chloride, copper(II) chloride, nickel(II) chloride, zinc(II) chloride) was less than 50%. For other chemicals, no cytotoxicity was seen at the concentration of 10 μM or 100 μM.

3.3. Analysis of gene ontology features in DNA microarray data

The DNA microarray analysis was performed by using total RNA from A549 cells treated or not singly with 64 chemicals/particles related to diesel emissions. The results are summarized in Tables 4-8. At first, chemical-elicited changes in global gene expression changes were evaluated. Fig. 3 shows an individual gene expression of each material in the form of a line graph. If the influence of the material was associated with a change in gene expression, the gene expression should be changed to a greater extent by PAHs, nitroarenes, quinones, and phthalates than by other groups.

Next, 8 gene classifications related to the health effects of diesel exhaust or DEP were chosen, and analyzed based on the Gene Ontology (GO) Consortium (http://www.geneontology.org/). These classifications of GO used for analysis are shown in Table 4. The results of the evaluation of standard deviations (SDs) are given in Table 5.

There was a tendency for high SD values in all classification categories and in all of the groups of chemicals/particles tested. Among the PAHs, many of them showed high values in the "Catalytic activity," "Oxygen and reactive oxygen species metabolism," and "Immune response" categories. Many nitroarenes showed high values in the classification "Response to DNA-damaging stimulus;" and many phthalates, high values in the "Humoral immune response" and "Antioxidant activity" classifications. As to quinones, many of them showed high values in all classification groups except the "Humoral immune response." Many nitrophenols showed high values in the "Humoral immune response" category. For metals, many showed high values in all classification groups except "Response to DNA-damaging stimulus." In the particles group except for DEP, many showed high values in the "Cell death" category. In the case of DEPs high values were found for the "Oxygen and reactive oxygen species metabolism" and "Immune response" categories. Among the chemicals/particles tested, 7-methyl benzo[a]pyrene, 1,2-naphthoquinone, 9,10-phenanthraquinone, 4-nitrophenol, and F-DEP30 showed high values in more than half of the classification groups.

Next, the number of genes whose expression was =2-fold increased as compared with their expression levels in the vehicle group was assessed (Table 6). Overall, there was a tendency for a high number of genes increased in expression in any classification category and by any of the chemicals/particles tested. Of the groups, PAHs and nitroarenes showed high numbers in the "Oxygen and reactive oxygen species metabolism" and "Response to DNA- damaging stimulus" categories. Quinones showed high numbers in all classification

categories; and phthalates, nitrophenols, metals, and particles, in the features classified as "Oxygen and reactive oxygen species metabolism," "Humoral immune response," and "Antioxidant activity." In each material, 7-methyl benzo[a]pyrene, 1,2-naphthoquinone, 9,10-phenanthraquinone, 3-methyl-4-nitrophenol, gold colloid (5 nm), and F-DEP100 showed high values in many classification categories.

Next, the number of genes whose expression was =0.5-fold decreased was assessed (Table 7). Overall, there was a tendency for high numbers of genes decreased in expression in either classification category and in all test groups except for particles. PAHs showed high numbers in the "Oxygen and reactive oxygen species metabolism," "Humoral immune response," and "Antioxidant activity" categories; and nitroarenes, in the "Oxygen and reactive oxygen species metabolism" and "Antioxidant activity" ones. Quinones showed high numbers in the classification categories of "Catalytic activity" and "Inflammatory response;" whereas phthalates showed high ones in the classification of "Oxygen and reactive oxygen species metabolism." Nitrophenols showed high numbers in the classification of "Oxygen and reactive oxygen species metabolism," "Inflammatory response," "Immune response," and "Cell death" designations. Metals showed high numbers only in the "Inflammatory response" category. As to specific chemicals, benzo[k]fluoranthene, fluorene, 7-methyl benzo[a]pyrene, 4-nitrophenol, and 3-methyl-4-nitrophenol showed high numbers in many classification categories.

3.4. Analysis of arbitrarily selected gene expression

Attention was next paid to certain genes whose expression was affected by diesel exhaust or DEP. Changes in their expression are shown in Table 8.

Cytochrome P450s (CYPs) are highly inducible by PAHs, and some metabolites of CYP enzymes are known to be related to carcinogenesis. Thus, it is necessary to pay attention to these genes as possible oncogenes.

Oxidative stress is assumed to be one of the factors contributing to the adverse effects of DEP. In previous studies, organic extracts of DEP were shown to provoke oxidative stress on alveolar macrophages, alveolar type II cells [8,9], and endothelial cells [10]. Reactive oxygen species (ROS) such as peroxide, which induces oxidative stress, are known to induce the oxidation of various molecules comprising the living body, such as proteins, lipids, and DNA. The threat of ROS damage is countered by coordinated cellular responses that modulate the expression of sets of gene products, one of which is heme oxygenase-1 (HO-1), which, along with other inducible enzymes, constitutes a cellular adaptive resistance pathway for defense against various oxidants. An organic extract of DEP was shown to induce HO-1 gene expression in alveolar macrophages [9] and macrophage cell lines [11,12] as an early response to oxidative stress. Thus, HO-1 was chosen as an oxidative stress marker.

Many studies have suggested that DEP induces the production of inflammatory markers in human lung epithelial cells [13,14] and that exposure to diesel emissions augments endotoxin-induced pulmonary inflammation [15] and allergic airway inflammation in a mouse asthma model [16,17]. Inflammatory cytokines and chemokines play important roles in these inflammatory responses [18]. Thus, certain inflammation-related genes (IL-1ß, IL-6, IL-8, and GM-CSF) were also selected for study.

Table 8 indicates that CYP1A1 and CYP1B1 were up-regulated by most PAHs and particles. CYP3A4 was up-regulated by nitrophenols, most metals, and particles. HO-1 was down-regulated by almost all materials. IL-1ß was up-regulated by some quinones. IL-6 was down-regulated by most PAHs. IL-8 was down-regulated by most PAHs and

nitroarenes. On the other hand, it was up-regulated by quinones, phthalates, nitrophenols, metals, and particles. GM-CSF was up-regulated by some nitroarenes, quinones, phthalates, nitrophenols, metals, and particles. The changes in IL-8 gene expression are also depicted in graphic form (Fig 3).

3.5. Cluster analysis of DNA microarray data

A critical question in toxicogenomics is whether gene expression information may be used to reveal chemical-specific signature patterns. Therefore, several computational analyses were used to determine whether treatment with different chemicals results in distinguishable gene expression patterns. Application of hierarchical cluster analysis [19] confirmed that individual chemicals could be distinguished by the gene expression (Fig. 4).

As a result, the 64 chemicals/particles were classified into 4 groups (Groups Ⅰ ~Ⅳ) by using cluster analysis. Furthermore, Groups Ⅱ and Ⅳ could each be divided into 2 subgroups (Group Ⅱ-a, -b and GroupⅣ-a, -b, respectively). These groups mainly included the following chemicals/particles: Group Ⅰ, PAHs; Group Ⅱ-a, nitroarenes; Group Ⅱ-b, quinones and phthalates; GroupⅢ, metals and nitrophenols; GroupⅣ-a, particles except for DEP; and GroupⅣ-b, DEP.

3.6. Construction of the *in silico* prediction model for IL-8

IL-8 is a well-known inflammatory cytokine involved in allergic inflammation [20], and its expression is up-regulated by exposure of animals to diesel emissions or to treatment with DEP *in vitro* [26-30]. Although many reports suggest that diesel emission affects allergic responses, it is not clear what components of DEP are responsible for it. Therefore, as a final part of this study the relationship between IL-8 gene expression and DEP was

examined by developing, with the use of toxicogenomics and QSAR, a prediction model for IL-8 gene expression elicited by various chemicals found in diesel exhaust.

Materials with a chemical structural formula are suitable for QSAR analysis, and so the 54 chemicals were assigned to 2 classes, *i.e.*, up-regulation class and down-regulation class, by using ADMEWORKS, which is a chemical compound toxicity prediction system, and the IL-8 gene expression data obtained from the DNA microarray (Table 8 and Fig. 3). As a result, the following model was built:

$$y = -0.57\,[\mathrm{WTPT3}] + 0.44\,[\mathrm{MOLC4}] + 0.31\,[\mathrm{V5CH}] + 0.30\,[\mathrm{SYMM2}] + 0.19\,[\mathrm{S3C}] - 0.15\,[\mathrm{CRB\_LEADL}] - 0.02\,[\mathrm{OPERA\_RULEI}]$$

$y>0$; down-regulation, $y<0$; up-regulation

Table 9 shows the 7 descriptors used in this prediction model and their degree of contribution to the IL-8 gene expression; and Table 10, the values of these descriptors of all 54 chemicals. If the absolute value of the contribution degree is large, the chemical is closely linked to variability of IL-8 gene expression in A549 cells. Furthermore, a positive value for the contribution degree is related to down-regulation of the cytokine; and a negative one, to up-regulation of it. The IL-8 gene expression in A549 cells treated with any chemicals was considered to be predictable by this model by knowing the chemical structures. The rate of classification of the 54 chemicals except for DEP by this model was 92%.

3.7. Validation of the *in silico* prediction model

The prediction model of IL-8 gene expression was validated by reference to previous reports indicating that some chemicals changed the IL-8 gene expression level in A549 cells in the same manner as found in this present study. It is generally thought that IL-8 is related to inflammation [21] or oxidative stress [22]. Therefore, it is thought that this cytokine may be up-regulated by pro-inflammatory compounds and oxidants. Therefore,

chlorobenzene [23], sodium sulfite [24], and sphingosine-1-phosphate [25] as pro-inflammatory compounds, and paraquat [26] as an oxidant, were chosen for study (Table 11).

On the other hand, it is generally thought that IL-8 expression may be down-regulated by anti-inflammatory compounds and antioxidants. Therefore, dexamethasone [27] as an anti-inflammatory compound and ß-carotene [28] and theaflavin [29] as antioxidants were also examined (Table 12). In addition, it is well known that NF-κB, a transcription factor, plays an important role in inflammation [30]. Since it is reported that isohelenin, an NF-κB inhibitor [31,32] down-regulates IL-8 at the mRNA level in A549 cells [33], it was chosen as an NF-κB inhibitor for validation of the prediction model of IL-8 (Table 12). The results of these evaluations are shown in Tables 11 and 12.

The prediction model using IL-8 and all compounds chosen based on previous reports showed 75% accuracy. The prediction of up-regulation by this model was 100% accurate; and that of down-regulation, 50% by it. There was thus no discrepancy between previous data and the prediction of up-regulation of IL-8. However, although it was previously reported that IL-8 gene is down-regulated by dexamethasone and theaflavin, this model predicted up-regulation by these compounds.

4. Discussion

4.1. Cytotoxicity and oxidative activity

Of the 64 chemicals/particles assessed for cytotoxicity, cell viability was less than 50% in the case of 11 of them. Among the test groups, quinones had strong cytotoxicity. The cell injury mechanism of quinones is classified roughly into 2 types. One is Arylation/Alkylation by covalent bonding with intracellular sulfhydryl groups. The other is oxidative stress caused by superoxide derived from a redox cycle. Indeed, the oxidative activity of quinones assessed by the DTT assay was high (Table 2). This result does not contradict the above mentioned cytotoxicity assay results. However, quinones did not up-regulate HO-1 gene expression as assessed by the DNA microarray method, indicating that intracellular oxidative stress did not induce expression of this gene at the mRNA level. To account for this lack of induction, it will be necessary to examine basic characteristics of HL-60 cells and A549 cells, including their antioxidative ability.

Naphthalene did not induce cytotoxicity, whereas 1,2-naphthoquinone did do so. This finding means that structural characteristics of quinones with oxo groups play an important role in the cytotoxicity. Pyrene did not induce cytotoxicity, but 1-nitropyrene was cytotoxic. Similarly, this difference shows the importance of the nitro group in the cytotoxicity. Therefore, unstable chemicals with some functional groups may be more toxic than stable chemicals having no functional groups. This toxicity may be caused by the fact that the structurally unstable chemicals tend to react with important biomolecules.

As for metals, copper, nickel, and zinc induced cytotoxicity. Copper and nickel showed high oxidative activity in the DTT assay (Table 2); however, zinc did not show it. Other metals (*e.g.*, iron) without cytotoxicity showed higher oxidative activity than zinc. Therefore, cytotoxicity cannot be explained simply only by the oxidative activity of a

chemical. Thus complicated mechanisms may be involved in cytotoxicity, including intracellular metabolic activation of chemicals.

It is necessary to pay attention to some materials for which IC_{50} values could not be obtained. HL-60 cells, which are said to be susceptible to various chemicals, were used for the cytotoxicity assays. However, the possibility exists that the reactivity to specific materials differs according to the cell line used. On the other hand, particles did not induce cytotoxicity. These materials actually increased the apparent cell viability at maximum concentrations. In this assays, viable cells are finally determined by absorbance. Therefore, coalescence of particles may increase absorbance, making accurate measurement of absorbance difficult. For this reason, it cannot be asserted that particles did not induce cytotoxicity. Evaluation of materials was made to the extent that they could be dissolved in medium (0.1% DMSO) in this assays. If their concentrations could be raised with a different solvent, the possibility of showing the cytotoxicity of these insoluble materials may be possible.

4.2. Efficient exploitation of gene expression analysis for toxicity evaluation

The analysis based on the GO Consortium revealed that the effect of quinones on gene expression was very big. A variety of quinones have been identified as DEP components [34,35]. Quinones themselves have toxicological properties, allowing them to serve as alkylating agents and to interact with, for example, flavoproteins to generate reactive oxygen species (ROS), which can induce biological injury [46-48]. A recent study showed that PQ recruits inflammatory cells, such as eosinophils and neutrophils, into the lungs *in vivo* along with the lung expression of pro-inflammatory molecules such as IL-5 and eotaxin [36]. More recently, it was also demonstrated that PQ aggravates antigen-related airway inflammation in mice and that PQ also has an adjuvant activity for the production

of antigen-specific immunoglobulins [37]. These studies suggest that quinones may be key compounds involved in the enhancing effects of DEP on allergic airway diseases.

Many compounds up-regulated or down-regulated CYP gene expression. It is known that CYP is induced by PAHs. The present results show that CYP1A1 and CYP1B1 were up-regulated by many PAHs. Thus, it may be thought that these gene expression changes reflect the characteristics of the materials tested. However, many PAHs showed a tendency to down-regulate the CYP1A2 gene expression. It is known that CYP1A2 is specifically expressed in the liver, so it may be necessary to pay attention to the influence of PAHs on the liver. Nitroarenes showed a tendency to down-regulate the expression of CYP1A1, CYP1B1, and CYP1A2 genes. If a nitro group is attached to a PAH, its metabolic pathway may be altered. Although metals showed a tendency to down-regulate the CYP1A1, CYP1B1, and CYP1A2 genes, there was a tendency for them to up-regulate the CYP3A4 gene expression. It is known that CYP3A4 is induced by dexamethasone, which is an anti-inflammatory compound in mammals. Thus, it may be thought that these metals are related to the inflammatory response. Previous studies reported that diesel exhaust or DEP induce oxidative stress [13-15]. However, almost all compounds showed a tendency to down-regulate the HO-1 gene expression, which is a marker of oxidative stress. It may be that these materials did not induce oxidative stress or that their concentration diesel exhaust or DEP is higher than that used for testing these materials. On the other hand, there is an opinion that A549 cells show strong resistance to oxidative stress. Thus, it may be that the oxidative stress of these materials cannot be detected precisely with these cells. Indeed, the oxidative activity of quinones, metals, and DEP assessed by the DTT assay was high (Table 2). Therefore, when oxidative stress induced by diesel exhaust or DEP is evaluated, it may be necessary to examine it by using other cell lines. IL-1ß, an inflammatory cytokine, was up-regulated by 1,2-naphthoquinone, 9,10-phenanthraquinone, and 0.020-

nm latex particles. In addition, phthalates and some DEP showed a tendency to up-regulate its gene expression. There were no remarkable changes when PAHs, nitroarenes, and metals were tested. However, oxidative stress is important to the inflammatory response by IL-1ß [38]. As mentioned above, A549 cell resistance to oxidative stress may have concealed the change in IL-1ß gene expression. IL-8 is also an important inflammatory cytokine, and DEP up-regulated IL-8 gene expression, a result supported by previous reports [39,40]. In our study, IL-8 was down-regulated by most PAHs and nitroarenes. On the other hand, it was up-regulated by quinones, phthalates, nitrophenols, and metals. However, it was earlier reported that IL-8 is up-regulated by PAHs in human lung epithelial cells [41,42]. This discrepancy may have been caused by the difference in the experimental conditions such as treatment time between those reports and this study. Because I confirmed that IL-8 gene expression was most strongly up-regulated by DEP treatment for 4 hours in our experimental environment in preliminary experiments (data not shown), I fixed the treatment time at 4 hours.

The 64 chemicals/particles were classified into 6 groups (Groups Ⅰ ~Ⅳ, with 2 of them each divided into 2 subgroups) by using cluster analysis. Group Ⅰ mainly included PAHs, so it was thought that the gene expression changes were strongly related to the chemical structure of compounds. Group Ⅱ-a included nitroarenes and 4 kinds of PAH. Two compounds among these PAHs possess a methyl group, and so it was thought that PAHs possessing a nitro groups or methyl group should be classified in this group. Group Ⅱ-b mainly included quinones and phthalates, and Group Ⅲ mainly included metals, nitrophenols, some quinones, and phthalates. Thus, these classifications depended on the gene expression that reflected the chemical structure of the materials. Group Ⅳ-a mainly included particles except for DEP; and Group Ⅳ-b, mainly DEP. So these classifications

depended on the biological properties of compounds, not their chemical structure. By this cluster analysis, the classification of the 64 chemicals into appropriate groups reflected the chemical structure of these compounds. If more information about the physicochemical properties of compounds can be accumulated, valuable information may become available to predict the influence of unknown materials in the future.

Much interesting information from the DNA microarray data was obtained by comparing the effects of the test materials on individual genes; however, the entirety of their effects on human health cannot be determined in this way. In the future, it will be necessary to obtain protein expression data as much as possible in addition to the DNA microarray data.

4.3. The potential of the *in silico* prediction model

In this prediction model, WTPT3, CRB_LEADL, and OPERA_RULEI were related to up-regulation of IL-8 gene expression. Since the contribution degree of WTPT3 was the highest, we considered WTPT3 to be the most important descriptor related to up-regulation of IL-8 gene expression. WTPT3 refers to the sum of atom indexes for all heteroatoms. The atom index means the number of the bond order between arbitrary atom pairs; in other words, it indicates the structural environment around the heteroatoms. In our analysis, the IL-8 gene expression in the A549 cells was down-regulated by PAHs and up-regulated by quinones, phthalates, and metals. Reflecting this, the WTPT3 values of the quinones, phthalates, and metals were larger than those of the PAHs. As PAHs are chemical compounds that consist of fused aromatic rings and do not contain heteroatoms, these results may be considered reasonable. CRB_LEADL means the count of rotatable bonds. CRB_LEADL values for the phthalates were high. Numerous rotatable bonds indicate that such a molecule can assume the shape of various stereoisomers. In fact, the phthalates are

known to form several stereoisomers. Since the IL-8 gene expression was strongly up-regulated by phthalates in our analysis, the type of stereoisomer may be important for up-regulation of the IL-8 gene expression. OPERA_RULEI is a value that reflects the "rule of five" of Lipinski, which is related to oral bioavailability [43]. The significance of it in this model, based on the data from the *in vitro* assay, is unknown. Since there was no great distinction among chemicals in terms of their OPERA_RULEI value, the contribution degree of this descriptor might be low. The role of this descriptor in the up-regulation of IL-8 may be considered to be complementary.

MOLC4, V5CH, SYMM2, and S3C were related to down-regulation of IL-8 gene expression. Among these descriptors, MOLC4 showed the highest contribution degree; therefore, it could be the most important descriptor related to the down-regulation of IL-8 gene expression. MOLC4 refers to the total of the pass weight about atom pairs that are 2 bonds in distance from one another. The term "pass" means the shortest distance between 2 arbitrary atoms, and the pass weight means the weighted value of the pass. The MOLC4 values of PAHs and nitroarenes, which compounds down-regulated IL-8 gene expression, tended to be high. In particular, the MOLC4 values of the chemicals that had more than 5 benzene rings, such as benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene, indeno[123-cd]pyrene, and 7-methyl benzo[a]pyrene, were high; and the average of their MOLC4 values was 5.436. V5CH means the total of the pass weight about atom pairs that are 5 bonds away from each other; and S3C, the total of the pass weight about a 3rd order cluster. SYMM2 refers to the geometrical symmetry of the pass. A low value for SYMM2 means that the molecular symmetric property is large. For some chemicals that down-regulated the gene expression, their V5CH values were equal to or less than 0.1. Furthermore, there was no remarkable difference among the chemicals regarding SYMM2

and S3C, either. As the contribution degree of these descriptors was low, the contribution of these descriptors to down-regulation of IL-8 may also be considered complementary.

In terms of IL-8 gene expression, WTPT3 and MOLC4 are the most important descriptors, showing the topological information about the chemicals. High values of WTPT3 for 2 of the compounds (dexamethasone and theaflavin) may have confused the prediction of IL-8 gene expression, and it may be that WTPT3 might have been overestimated in our model. On the other hand, it is reasonable that the values of MOLC4, which is thought to contribute to down-regulation, were high for dexamethasone, theaflavin, ß-carotene, and isohelenin. These results suggest that there is still room for improvement of the model formula to be able to reflect down-regulation of IL-8 even when WTPT3 is high. In the future, it will be necessary to accumulate data by analyzing many compounds with diverse structures, and to continuously rebuild a prediction model to obtain higher accuracy. In brief, the property of unchangeability of the molecule may be important for affecting IL-8 gene expression.

Until now, there have not been many studies evaluating the toxicity of chemicals by means of *in silico* and *in vitro* assays. A U.S. Environmental Protection Agency (EPA) report notes the need to leverage *in vitro* assays using human cell lines and computational toxicology in their "Strategic Plan for Evaluating the Toxicity of Chemicals" [44]. Although our toxicity prediction model, which fuses toxicogenomics and QSAR, is still in the trial phase, it may be a step in the right direction for future assessment of the toxicology of environmental pollutants.

5. Conclusion

The analysis of gene expression with a systems biology approach will provide a more comprehensive insight into the biological effects of unknown materials or complex mixtures and will improve risk assessment of the same. Furthermore, this present study showed that the construction of a new toxicity prediction model for environmental pollutants based on QSAR and gene expression data might be useful to understand the various biological reactions such as not only mutagenicity, as in traditional toxicology, but also inflammation and other toxicological responses.

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Abbreviations

PM2.5: particulate matter with an aerodynamic diameter < or = 2.5 μm

DEP: diesel exhaust particles

REACH: Registration, Evaluation, Authorisation and Restriction of CHemicals

QSAR: quantitative structure-activity relationship

U.S. EPA: United States Environmental Protection Agency

MSAT: mobile source sir toxics

HEI: the health effects institute

ACES: the advanced collaborative emissions study

DMSO: dimethyl sulfoxide

DTT: dithiothreitol

ROS: reactive oxygen species

DTNB: 2-nitrobenzoic acid

WST-8: 4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt

CYP: cytochrome P450

PAH: polyaromatic hydrocarbon

HO-1: heme oxygenase-1

IL-1ß: Interleukin-1ß

IL-6: interleukin-6

IL-8: interleukin-8

GM-CSF: granulocyte macrophage colony-stimulating factor

Table 1. List of the 64 chemicals/particles related to diesel emissions.

No.	Material	Abbreviation
Polyaromatic hydrocarbons		(PAH)
1	Naphthalene	Nap
2	Fluoranthene	Flu
3	Benzo[k]fluoranthene	BkF
4	Acenaphthylene	Acl
5	Pyrene	Pyr
6	Benzo[a]pyrene	BaP
7	Acenaphthene	Ace
8	Benz[a]anthracene	BaA
9	Dibenzo[a,h]anthracene	DBahA
10	Fluorene	Fle
11	Phenanthrene	Phe
12	Chrysene	Chr
13	Benzo[ghi]perylene	BghiP
14	Anthracene	Ant
15	Benzo[b]fluoranthene	BbF
16	Indeno[123-cd]pyrene	IDP
17	Perylene	Per
18	Benzo[c]phenanthrene	BcPhe
19	9-methyl anthracene	9-MAnt
20	1-methyl fluorene	1-MFle
21	7-methyl benzo[a]pyrene	7-MBaP
22	3,6-dimethyl phenanthrene	3,6-DMPhe
Nitroarenes		
23	1-nitropyrene	1-NP
24	3-nitrobiphenyl	3-NBP
25	2-nitrobiphenyl	2-NBP
26	2-nitrofluorene	2-NFle
27	3-nitrobenzanthrone	3-NBA
28	2-nitronaphthalene	2-NNap
29	1-nitronaphthalene	1-NNap
30	9-nitrophenanthrene	9-NPhe
31	3-nitrophenanthrene	3-NPhe
32	9-nitroanthracene	9-NAnt
33	6-nitrochrysene	6-NChr
Quinones		
34	1,2-naphthoquinone	1,2-NQ
35	9,10-phenanthraquinone	9,10-PQ
36	Antraquinone	AQ
37	p-benzoquinone	1,4-BQ
Phthalates		
38	Butylbenzyl phthalate	BBPt
39	Diethyl phthalate	DEPt
40	Dibutyl phthalate	DBPt
41	Bis[2-ethylhexyl] phthalate	DEHPt
Nitrophenols		
42	4-nitrophenol	4-NPh
43	3-methyl-4-nitrophenol	3-M-4-NPh
Metals		
44	Copper(I)	CuCl
45	Copper(II)	$CuCl_2$
46	Iron(II)	$FeCl_2$
47	Iron(III)	$FeCl_3$
48	Aluminum(III)	$AlCl_3$
49	Nickel(II)	$NiCl_2$
50	Zinc(II)	$ZnCl_2$
51	Chromium(II)	$CrCl_2$
52	Chromium(III)	$CrCl_3$
53	Platinum(II)	$PtCl_2$
54	Platinum(IV)	$PtCl_4$
Particles		
55	Latex particle (0.020 mm)	LP0.02
56	Latex particle (0.115 mm)	LP0.115
57	Latex particle (1.01 mm)	LP1.01
58	Gold Colloid (5 nm)	Au5
59	Gold Colloid (10 nm)	Au10
60	Gold Colloid (20 nm)	Au20
61	DEP (100 μg/ml)	DEP100
62	DEP (30 μg/ml)	DEP30
63	Forklift-DEP (100 μg/ml)	F-DEP100
64	Forklift-DEP (30 μg/ml)	F-DEP30

Table 2. Concentrations at 50% consumption of DTT.

Material	Concentrations
Quinone	unit : μM
1,2-NQ	6.76
9,10-PQ	2.79
AQ	0.28
1,4-BQ	22.65
Metal	unit : μM
CuCl	0.034
$CuCl_2$	0.021
$FeCl_2$	6.07
$FeCl_3$	1.44
$NiCl_2$	0.095
Particle	unit : μg/ml
DEP100	3.43
F-DEP100	2.47

Table 3. IC_{50} values of the 64 chemicals/particles.

	Material	IC_{50}	Concentrations for the DNA microarray analysis
	PAHs		
1	Nap	>100	10 μM
2	Flu	>100	10 μM
3	BkF	>10	10 μM
4	Acl	>100	10 μM
5	Pyr	>100	10 μM
6	BaP	>100	10 μM
7	Ace	>100	10 μM
8	BaA	>100	10 μM
9	DBahA	>10	10 μM
10	Fle	>100	10 μM
11	Phe	>100	10 μM
12	Chr	>10	10 μM
13	BghiP	>10	10 μM
14	Ant	>10	10 μM
15	BbF	>100	10 μM
16	IDP	>10	10 μM
17	Per	>10	10 μM
18	BcPhe	>100	10 μM
19	9-MAnt	98	10 μM
20	1-MFle	>100	10 μM
21	7-MBaP	>10	10 μM
22	3,6-DMPhe	>100	10 μM
	Nitroarenes		
23	1-NP	7.18	1 μM
24	3-NBP	>100	10 μM
25	2-NBP	>100	10 μM
26	2-NFle	>100	10 μM
27	3-NBA	5.86	1 μM
28	2-NNap	>100	10 μM
29	1-NNap	>100	10 μM
30	9-NPhe	>100	10 μM
31	3-NPhe	>10	10 μM
32	9-NAnt	>100	10 μM
33	6-NChr	>10	10 μM

	Material	IC_{50}	Concentrations for the DNA microarray analysis
	Quinones		
34	1,2-NQ	6.91	10 μM
35	9,10-PQ	0.27	1 μM
36	AQ	>10	10 μM
37	1,4-BQ	49.37	10 μM
	Phthalates		
38	BBPt	>10	10 μM
39	DEPt	>10	10 μM
40	DBPt	>10	10 μM
41	DEHPt	6.76	10 μM
	Nitrophenols		
42	4-NPh	>10	10 μM
43	3-M-4-NPh	>100	10 μM
	Metals		
44	CuCl	19.88	10 μM
45	$CuCl_2$	37.56	10 μM
46	$FeCl_2$	>100	10 μM
47	$FeCl_3$	>100	10 μM
48	$AlCl_3$	>100	10 μM
49	$NiCl_2$	60.53	10 μM
50	$ZnCl_2$	88.63	10 μM
51	$CrCl_2$	>100	10 μM
52	$CrCl_3$	>100	10 μM
53	$PtCl_2$	>100	10 μM
54	$PtCl_4$	>100	10 μM
	Particles		
55	LP0.02	>10	100 μg/ml
56	LP0.115	>10	100 μg/ml
57	LP1.01	>10	100 μg/ml
58	Au5	>10	1 μg/ml
59	Au10	>10	1 μg/ml
60	Au20	>10	1 μg/ml
61	DEP100	>1	100 μg/ml
62	DEP30	-	30 μg/ml
63	F-DEP100	>1	100 μg/ml
64	F-DEP30	-	30 μg/ml

Table 4. List of the Gene Ontology features.

Gene Ontology	Accession	Number of genes	Definition
Catalytic activity	GO:0003824	3776	Catalysis of a biochemical reaction at physiological temperatures. In biologically catalyzed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic.
Oxygen and reactive oxygen species metabolism	GO:0006800	62	The chemical reactions and pathways involving dioxygen (O_2), or any of the reactive oxygen species, e.g. superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (-OH).
Inflammatory response	GO:0006954	261	The immediate defensive reaction (by vertebrate tissue) to infection or injury caused by chemical or physical agents. The process is characterized by local vasodilation, extravasation of plasma into intercellular spaces, and accumulation of white blood cells and macrophages.
Immune response	GO:0006955	572	immune system process that functions in the calibrated response of an organism to a potential internal or invasive threat.
Humoral immune response	GO:0006959	52	An immune response mediated through a body fluid.
Response to DNA-damaging stimulus	GO:0006974	317	Any response that results in a change in the state or activity of a cell (in terms of movement, secretion, enzyme production, gene expression, etc.) due to damage to its DNA caused by a stimulus such as an environmental insult or errors during metabolism.
Cell death	GO:0008219	890	A biological process that results in permanent cessation of all vital functions of a cell.
Antioxidant activity	GO:0016209	33	Inhibition of the reactions brought about by dioxygen (O_2) or peroxides. Usually the antioxidant is effective because it can itself be more easily oxidized than the substance protected. The term is often applied to components that can trap free radicals, thereby breaking the chain reaction that normally leads to extensive biological damage.

Table 5. Evaluation of standard deviations.

No.	Material	Catalytic activity	Oxygen and reactive oxygen species metabolism	Inflammatory response	Immune response	Humoral immune response	Response to DNA-damaging stimulus	Cell death	Antioxidant activity
	PAH								
1	Nap	0.467	0.498	0.912	0.679	0.977	0.395	0.480	0.414
2	Flu	0.511	0.527	0.866	0.725	0.703	0.474	0.505	0.466
3	BkF	0.562	0.715	0.783	0.793	1.160	0.385	0.462	0.427
4	Acl	0.458	0.285	0.741	0.614	0.983	0.314	0.488	0.248
5	Pyr	0.522	1.016	0.772	0.686	0.537	0.387	0.505	0.336
6	Bap	0.514	0.506	0.514	0.744	0.599	0.364	0.436	0.366
7	Ace	0.576	0.704	0.661	0.684	0.608	0.381	0.514	0.379
8	BaA	0.475	0.630	0.829	0.814	1.136	0.365	0.488	0.391
9	DBahA	0.508	0.959	1.041	0.820	0.555	0.339	0.436	0.372
10	Fle	0.482	1.001	1.105	0.816	0.723	0.354	0.453	0.341
11	Phe	0.413	0.586	0.649	0.676	0.503	0.353	0.407	0.326
12	Chr	0.507	0.580	0.712	0.700	0.591	0.347	0.461	0.427
13	BghiP	0.464	1.042	0.724	0.810	0.610	0.339	0.480	0.337
14	Ant	0.485	0.451	0.477	0.702	0.888	0.392	0.377	0.304
15	BbF	0.425	0.771	0.614	0.604	0.559	0.313	0.421	0.446
16	IDP	0.463	0.415	0.757	0.629	0.492	0.293	0.446	0.472
17	Per	0.393	0.290	0.537	0.593	0.422	0.288	0.383	0.362
18	BcPhe	0.453	0.917	0.655	0.605	0.674	0.346	0.394	0.405
19	9-MAnt	0.400	0.468	0.648	0.613	0.957	0.315	0.382	0.422
20	1-MFle	0.394	0.415	0.463	0.584	0.528	0.314	0.389	0.363
21	7-MBaP	0.593	0.867	0.733	0.816	0.731	0.557	0.587	0.659
22	3,6-DMPhe	0.492	0.581	0.605	0.667	0.528	0.542	0.438	0.646
	Nitroarene								
23	1-NP	0.450	0.865	0.611	0.593	0.535	0.438	0.458	0.902
24	3-NBP	0.446	0.715	0.785	0.659	0.412	0.398	0.453	0.387
25	2-NBP	0.522	0.409	0.512	0.699	0.737	0.448	0.521	0.462
26	2-NFle	0.486	0.390	0.528	0.642	0.620	0.401	0.429	0.461
27	3-NBA	0.430	0.834	0.680	0.559	0.622	0.407	0.471	0.508
28	2-NNap	0.396	0.520	0.535	0.769	0.763	0.306	0.384	0.356
29	1-NNap	0.467	0.476	0.681	0.748	0.616	0.369	0.437	0.394
30	9-NPhe	0.419	0.327	0.654	0.632	0.554	0.330	0.401	0.397
31	3-NPhe	0.429	0.269	0.671	0.586	0.557	0.313	0.405	0.322
32	9-NAnt	0.403	0.784	0.721	0.682	0.535	0.305	0.440	0.361
33	6-NChr	0.495	0.275	0.583	0.699	0.645	0.369	0.396	0.366
	Quinone								
34	1,2-NQ	0.569	0.782	0.891	0.801	0.987	0.415	0.576	1.138
35	9,10-PQ	0.651	0.754	1.311	0.872	1.029	0.677	0.696	0.958
36	AQ	0.493	0.880	0.913	0.740	0.909	0.343	0.517	0.807
37	1,4-BQ	0.517	0.565	0.771	0.600	0.951	0.378	0.524	0.752
	Phthalate								
38	BBPt	0.488	0.859	0.715	1.023	2.668	0.360	0.475	0.980
39	DEPt	0.450	0.662	0.660	0.771	1.001	0.340	0.516	1.139
40	DBPt	0.483	0.478	0.861	0.670	1.015	0.324	0.467	0.704
41	DEHPt	0.486	0.474	0.757	0.747	1.234	0.475	0.429	0.756
	Nitrophenol								
42	4-NPh	0.530	0.748	0.955	0.667	1.137	0.361	0.548	0.738
43	3-M-4-NPh	0.438	0.723	0.621	0.662	1.203	0.331	0.460	0.793
	Metal								
44	CuCl	0.440	0.535	0.657	0.653	1.114	0.317	0.485	0.799
45	$CuCl_2$	0.416	0.472	0.755	0.661	1.102	0.279	0.508	0.937
46	$FeCl_2$	0.409	0.427	0.622	0.594	0.920	0.341	0.443	0.734
47	$FeCl_3$	0.400	0.434	0.602	0.606	0.916	0.313	0.466	0.758
48	$AlCl_3$	0.400	0.437	0.700	0.611	0.786	0.311	0.439	0.759
49	$NiCl_2$	0.383	0.386	0.564	0.949	0.777	0.267	0.408	0.739
50	$ZnCl_2$	0.456	0.502	0.881	0.794	1.353	0.307	0.491	0.712
51	$CrCl_2$	0.509	0.665	0.824	0.647	1.086	0.360	0.439	0.731
52	$CrCl_3$	0.441	0.838	0.735	0.752	1.142	0.313	0.512	0.729
53	$PtCl_2$	0.564	0.396	0.784	0.721	0.871	0.403	0.586	0.762
54	$PtCl_4$	0.450	0.917	0.690	0.620	0.837	0.285	0.477	0.814
	Particle								
55	LP0.02	0.496	0.540	0.847	0.674	0.871	0.339	0.635	0.826
56	LP0.115	0.472	0.544	0.787	0.788	1.124	0.407	0.608	0.760
57	LP1.01	0.490	0.546	0.927	0.743	0.906	0.342	0.590	0.789
58	Au5	0.498	0.585	0.817	0.685	0.889	0.344	0.536	0.829
59	Au10	0.489	0.586	0.879	0.592	0.867	0.338	0.498	0.792
60	Au20	0.505	0.851	0.850	0.680	0.885	0.507	0.546	0.776
61	DEP30	0.469	0.765	0.805	0.735	0.837	0.333	0.520	0.800
62	DEP100	0.493	0.579	0.806	0.813	0.846	0.387	0.476	0.769
63	F-DEP30	0.564	0.932	1.013	0.803	1.219	0.415	0.564	0.865
64	F-DEP100	0.466	0.918	0.766	0.605	0.856	0.309	0.419	0.740

:Top 10 chemicals of the each Gene Ontology feature in terms of SD value.

Table 6. Number of genes with =2-fold increase in expression.

No.	Material	Catalytic activity	Oxygen and reactive oxygen species metabolism	Inflammatory response	Immune response	Humoral immune response	Response to DNA-damaging stimulus	Cell death	Antioxidant activity
	PAH								
1	Nap	50	0	6	7	2	4	14	0
2	Flu	54	0	10	9	1	4	9	1
3	BkF	71	1	9	16	2	2	8	0
4	Acl	36	0	4	9	1	2	8	0
5	Pyr	68	1	5	15	1	4	17	0
6	Bap	63	0	5	10	0	3	7	0
7	Ace	64	1	7	8	0	3	11	0
8	BaA	64	1	8	14	3	1	12	0
9	DBahA	60	1	9	12	0	1	10	0
10	Fle	53	2	16	16	1	1	13	0
11	Phe	37	1	3	6	0	4	8	0
12	Chr	59	3	10	16	0	2	13	1
13	BghiP	38	1	6	8	1	1	10	0
14	Ant	51	0	6	10	1	3	9	0
15	BbF	50	2	7	5	0	1	8	1
16	IDP	48	0	7	8	0	0	6	1
17	Per	31	0	3	8	0	4	6	0
18	BcPhe	27	1	3	8	1	0	2	0
19	9-MAnt	21	0	6	11	2	0	2	0
20	1-MFle	27	0	3	11	1	0	5	0
21	7-MBaP	98	3	10	23	2	4	19	3
22	3,6-DMPhe	48	0	7	15	1	2	5	1
	Nitroarene								
23	1-NP	33	1	6	15	2	4	8	1
24	3-NBP	32	1	11	18	0	3	9	0
25	2-NBP	76	0	5	24	3	5	17	0
26	2-NFle	72	1	5	15	3	4	13	1
27	3-NBA	50	1	7	15	2	6	18	1
28	2-NNap	42	1	7	20	2	3	13	0
29	1-NNap	72	1	10	31	3	2	16	0
30	9-NPhe	45	0	8	16	1	2	11	0
31	3-NPhe	51	0	7	18	1	2	10	0
32	9-NAnt	40	3	11	18	0	2	15	1
33	6-NChr	71	1	10	32	4	3	14	1
	Quinone								
34	1,2-NQ	132	6	27	46	8	4	33	2
35	9,10-PQ	152	6	39	60	8	11	62	5
36	AQ	80	4	25	27	5	2	17	1
37	1,4-BQ	70	2	16	17	5	3	20	1
	Phthalate								
38	BBPt	72	2	12	22	4	2	14	2
39	DEPt	60	2	10	27	6	2	14	3
40	DBPt	71	1	18	24	6	3	15	1
41	DEHPt	50	1	12	16	5	3	11	1
	Nitrophenol								
42	4-NPh	82	3	14	16	7	1	15	1
43	3-M-4-NPh	58	3	10	15	7	1	9	2
	Matal								
44	CuCl	48	2	10	15	6	0	12	1
45	$CuCl_2$	46	1	12	13	6	0	14	2
46	$FeCl_2$	54	2	13	16	8	4	14	1
47	$FeCl_3$	46	2	12	17	6	2	9	1
48	$AlCl_3$	40	2	14	14	5	0	8	1
49	$NiCl_2$	32	1	10	14	6	0	9	1
50	$ZnCl_2$	54	2	15	19	6	0	14	1
51	$CrCl_2$	59	2	13	12	6	2	9	1
52	$CrCl_3$	48	3	10	19	6	1	13	1
53	$PtCl_2$	70	1	11	20	5	3	22	1
54	$PtCl_4$	57	3	12	13	5	0	12	2
	Particle								
55	LP0.02	83	1	19	19	5	1	31	2
56	LP0.115	65	1	16	22	7	2	21	1
57	LP1.01	60	1	12	18	3	2	18	1
58	Au5	81	4	24	26	6	2	21	2
59	Au10	72	2	17	17	5	0	16	1
60	Au20	77	2	18	25	6	2	18	1
61	DEP30	73	2	14	24	4	2	14	1
62	DEP100	69	1	12	24	5	3	17	1
63	F-DEP30	90	4	20	21	6	4	19	2
64	F-DEP100	64	2	13	17	5	2	10	1

:Top 3 chemicals of the each Gene Ontology category.
:Top 4 and 5 chemicals of each Gene Ontology category.

Table 7. Number of genes whose expression was =0.5-fold decreased.

No.	Material	Catalytic activity	Oxygen and reactive oxygen species metabolism	Inflammatory response	Immune response	Humoral immune response	Response to DNA-damaging stimulus	Cell death	Antioxidant activity
	PAHs								
1	Nap	33	2	6	15	2	2	12	0
2	Flu	55	3	4	14	2	1	22	1
3	BkF	42	1	8	17	3	4	18	1
4	Acl	29	1	3	4	1	1	4	0
5	Pyr	41	2	8	14	3	4	12	0
6	Bap	29	2	6	12	3	1	11	0
7	Ace	26	1	7	10	3	2	12	0
8	BaA	40	2	7	14	3	3	10	0
9	DBahA	28	2	3	6	1	0	9	0
10	Fle	38	2	6	15	5	2	10	1
11	Phe	21	1	2	7	2	1	6	0
12	Chr	30	1	6	11	4	1	11	0
13	BghiP	26	2	6	11	3	0	11	0
14	Ant	15	1	1	9	2	1	4	0
15	BbF	15	1	2	6	2	1	5	0
16	IDP	18	1	3	5	2	0	6	0
17	Per	23	1	3	8	1	0	8	1
18	BcPhe	38	2	6	8	2	2	10	1
19	9-MAnt	33	2	4	10	2	1	8	1
20	1-MFle	30	2	3	7	1	1	9	1
21	7-MBaP	93	3	4	12	1	13	19	0
22	3,6-DMPhe	60	2	4	8	0	10	17	1
	Nitroareness								
23	1-NP	50	2	3	6	0	6	11	1
24	3-NBP	37	2	3	6	0	2	10	1
25	2-NBP	61	1	4	5	1	7	17	0
26	2-NFle	52	1	5	5	1	2	11	0
27	3-NBA	31	1	1	3	1	1	11	0
28	2-NNap	20	1	3	4	0	0	5	1
29	1-NNap	36	0	1	4	0	5	8	0
30	9-NPhe	40	1	4	7	1	0	12	1
31	3-NPhe	25	1	4	3	0	0	5	1
32	9-NAnt	23	0	5	3	1	0	5	1
33	6-NChr	41	0	3	6	1	4	7	0
	Quinones								
34	1,2-NQ	65	0	7	13	2	5	14	0
35	9,10-PQ	74	0	2	7	0	9	12	0
36	AQ	40	0	7	4	0	0	14	0
37	1,4-BQ	63	1	9	11	2	1	15	0
	Phthalates								
38	BBPt	30	2	4	7	0	0	9	0
39	DEPt	26	2	4	5	0	0	6	0
40	DBPt	30	0	5	8	1	0	10	0
41	DEHPt	36	1	4	4	0	0	8	0
	Nitrophenols								
42	4-NPh	68	1	9	15	2	6	24	0
43	3-M-4-NPh	59	2	9	19	3	2	19	0
	Metals								
44	CuCl	53	1	8	15	2	2	18	0
45	$CuCl_2$	33	1	6	8	1	1	7	0
46	$FeCl_2$	32	0	6	10	0	0	9	0
47	$FeCl_3$	45	0	7	11	0	0	16	0
48	$AlCl_3$	38	0	7	9	0	1	8	0
49	$NiCl_2$	25	0	4	7	0	0	6	0
50	$ZnCl_2$	30	0	6	7	0	1	10	0
51	$CrCl_2$	25	0	5	9	0	2	8	0
52	$CrCl_3$	29	0	5	10	0	0	8	0
53	$PtCl_2$	59	0	5	8	1	6	19	0
54	$PtCl_4$	29	0	7	6	0	0	7	0
	Particles								
55	LP0.02	45	0	6	8	1	2	13	0
56	LP0.115	41	0	8	7	0	0	11	0
57	LP1.01	36	0	6	6	1	0	12	0
58	Au5	30	0	4	3	0	0	8	0
59	Au10	35	0	5	4	0	1	9	0
60	Au20	25	0	5	5	0	0	7	0
61	DEP30	31	0	4	4	0	2	8	0
62	DEP100	25	0	3	3	0	0	4	0
63	F-DEP30	43	1	4	5	0	2	17	1
64	F-DEP100	32	0	3	4	0	2	7	0

Top 3 chemicals ( ) and Top 4 and 5 chemicals ( ) affecting each Gene Ontology category.

Table 8. Arbitrarily selected genes whose expression was affected by the 64 chemicals/particles.

No.	Material	CYP 1A1	1A2	1B1	3A4	HO-1	IL-1ß	IL-6	IL-8	GM-CSF
	PAHs									
1	Nap		-0.51	-0.11		-0.31	-0.14		-0.32	
2	Flu		-0.85	-1.46		-0.36	0.13		-0.72	
3	BkF	4.27	-0.94	2.47		-0.17	0.28	-1.22	-0.86	
4	Acl		-0.54	0.21		-0.20			-0.56	
5	Pyr		-0.69	-1.66		-0.31	0.30	-1.06	-0.13	
6	Bap	3.87	-0.85	2.14		-0.32	0.18	-1.04	-0.97	
7	Ace	-0.45	-0.89	-0.25		-0.32	-0.14	-0.63	-1.10	
8	BaA	3.88	-0.79	2.10		-0.19	0.19		-0.68	
9	DBahA	4.04	-0.72	2.24		0.06	0.13		-0.58	1.65
10	Fle	-0.39	-0.51	-1.17		-0.17	0.03	-1.06	-0.44	
11	Phe	-0.33	-0.51	-1.20		-0.26	0.10	-0.6	-0.32	
12	Chr	3.24	-0.14	1.83		-0.05	0.19	-1.09	-0.37	
13	BghiP	-0.20	-0.56	-0.18		-0.18	0.04	-1.16	-0.48	
14	Ant		-0.31	-1.16		-0.22	0.22		-0.46	
15	BbF	4.15	-0.58	2.31		-0.07	0.07		-0.64	
16	IDP	4.04	-0.35	2.21		0.24	-0.06	-1.13	-0.12	
17	Per		-0.64	0.10		-0.51	-0.42		-1.26	
18	BcPhe	0.07	-0.60	-0.09		-0.52	0.07		-1.22	
19	9-MAnt	-0.42	-0.50	-1.73		-0.57	-0.07	-0.88	-0.47	
20	1-MFle	-0.22	-0.59	-1.71		-0.47	0.18		-1.05	0.84
21	7-MBaP	4.13	-1.30	2.20		-0.23	0.42	0.04		
22	3,6-DMPhe	0	-1.28	-1.15		-0.47	0.40	0	-1.25	1.19
	Nitroarenes									
23	1-NP	-0.05	-1.47	-1.25		-0.69	0.33	-0.45	-0.98	1.10
24	3-NBP	-0.20	-1.02	-1.98		-0.90	0.24	-0.56	-1.01	
25	2-NBP	0.04	-1.02	-0.01		-0.70	0.14	0.19	-1.06	
26	2-NFle	0.41	-0.76	0.30		-0.74	0.02	-0.09	-1.42	0.86
27	3-NBA	-0.61	-1.09	-0.96		-0.83	0.33	-0.06	-0.97	
28	2-NNap	0.31	-0.98	-0.17		-0.80	0.34		-1.09	
29	1-NNap	0.03	-1.01	-0.01		-0.89	0	0.04	-0.88	
30	9-NPhe	-0.61	-1.12	-0.82		-1.07	0.52	-0.04	-0.14	1.47
31	3-NPhe	0.06	-1.23	-0.28		-1.11	0.07		-0.23	
32	9-NAnt	-0.35	-1.30	-1.76		-0.53	-0.16		0.35	1.86
33	6-NChr	2.38	-1.32	1.77		-0.62	0.24	0.60	-1.21	1.26
	Quinones									
34	1,2-NQ	2.79	-0.61	1.86		-1.77	1.04	-0.36	0.98	2.16
35	9,10-PQ	-0.27	-0.41	-0.17		-1.36	1.55	0.22	3.08	3.24
36	AQ		-0.53	-1.36		-2.30	0.60		1.98	2.60
37	1,4-BQ	1.25	-0.62	1.65		-1.54	0.55	-0.66	1.49	2.02
	Phthalates									
38	BBPt	0.56	0.14	0.04		-1.60	0.68		1.03	1.66
39	DEPt	0.38	-0.12	-0.10		-1.67	0.65	-0.67	1.20	1.50
40	DBPt	0.01	0.11	0.10		-1.86	0.56	-0.86	1.15	1.21
41	DEHPt		2.13	0.06		-1.28	0.32		1.85	2.18
	Nitrophenols									
42	4-NPh	-0.31	-0.32	0.13	1.14	-1.17	-0.33		0.86	0.90
43	3-M-4-NPh	4.42	-0.26	2.64	1.19	-1.23	0.25	-1.32	0.25	0.84
	Metals									
44	CuCl	-0.33	-0.66	0.16	0.82	-1.42	0.05		0.92	1.11
45	$CuCl_2$	-0.17	-0.12	0.13	1.04	-1.36	0.16		1.22	1.52
46	$FeCl_2$	-0.14	-0.40	0.13	1.34	-1.21	0.14	-0.36	0.95	1.22
47	$FeCl_3$	-0.18	-0.18	0.16	1.34	-1.38	0.05	-0.69	1.09	0.95
48	$AlCl_3$	-0.20	0.54	0.11	1.34	-1.31	0.01	-0.72	1.16	1.06
49	$NiCl_2$	-0.28	-0.39	0.08	1.49	-1.41	-0.21	-0.48	0.90	
50	$ZnCl_2$	-0.17	0.08	0.09	1.47	-1.32	0.25		1.07	
51	$CrCl_2$		-0.03	-0.08	1.33	-1.30	0.25		0.95	
52	$CrCl_3$	0.02	-0.07	-0.05	1.50	-1.34	-0.04		0.94	
53	$PtCl_2$	0	-0.36	-0.53	1.66	-1.33	-0.17		1.09	
54	$PtCl_4$	-0.55	-0.31	-0.17	1.03	-1.35	0.07		0.95	1.35
	Particles									
55	LP0.02	2.09	0.01	0.94	1.03	-1.01	1.82		2.01	4.04
56	LP0.115	-0.80	-0.12	-0.30	1.33	-1.71	0.68		2.09	2.26
57	LP1.01	-0.07	2.15	0.10	1.97	-1.71	0.23		1.72	2.30
58	Au5	2.47	0.38	1.97	2.39	-1.67	0.13		1.45	2.33
59	Au10	1.32	0.36	1.32	1.39	-1.61	0.19	-0.81	1.36	2.15
60	Au20	0.93	-0.26	1.01	1.66	-1.72	0.63	-0.64	1.62	2.53
61	DEP30	3.13	-0.32	2.39	1.40	-1.34	0.79		1.48	2.69
62	DEP100	3.50	-0.51	2.61	2.00	-1.45	0.70	-0.72	1.79	1.83
63	F-DEP30	2.78	-0.21	2.06	1.54	-1.63	0.39	-0.53	1.63	2.08
64	F-DEP100	2.42	-0.59	2.42	1.45	-1.34	0.49	-0.74	1.76	1.44

Table 9. List of the descriptors related to IL-8 gene expression.

Descriptor	Abbreviation	Contribution degree	Relationship with IL-8 gene expression
Sum of atom indexes for all heteroatoms	WTPT3	-0.57	up-regulation
Path-2 molecular connectivity	MOLC4	0.44	down-regulation
5th order chain MC valence	V5CH	0.31	down-regulation
Geometrical symmetry	SYMM2	0.30	down-regulation
3rd order cluster MC Simple	S3C	0.19	down-regulation
Count of rotatable bonds	CRB_LEADL	-0.15	up-regulation
The rule based on Lipinski's rule	OPERA_RULEI	-0.02	up-regulation

Table 10. Descriptor values of 54 chemcals.

No.	Material	WTPT3	MOLC4	V5CH	SYMM2	S3C	CRB_LEADL	OPREA_RULEI
	PAH							
1	Nap	0	2.23	0	0.30	0.33	0	1
2	Flu	0	4.13	0.03	0.38	0.82	0	1
3	BkF	0	5.21	0.03	0.30	1.16	0	0
4	Acl	0	3	0.04	0.50	0.61	0	1
5	Pyr	0	3.93	0	0.25	0.89	0	1
6	Bap	0	4.97	0	0.25	1.16	0	0
7	Ace	0	3.30	0.06	0.50	0.61	0	1
8	BaA	0	4.35	0	0.28	0.94	0	1
9	DBahA	0	5.38	0	0.18	1.21	0	0
10	Fle	0	3.49	0.04	0.54	0.61	0	1
11	Phe	0	3.26	0	0.36	0.61	0	1
12	Chr	0	4.35	0	0.28	0.94	0	1
13	BghiP	0	5.59	0	0.18	1.38	0	0
14	Ant	0	3.31	0	0.29	0.67	0	1
15	BbF	0	5.17	0.03	0.40	1.09	0	0
16	IDP	0	5.83	0.03	0.45	1.38	0	0
17	Per	0	4.93	0	0.20	1.10	0	0
18	BcPhe	0	4.31	0	0.33	0.88	0	1
19	9-MAnt	0	3.69	0	0.27	0.80	0	1
20	1-MFle	0	3.93	0.04	0.57	0.81	0	1
21	7-MBaP	0	5.41	0	0.24	1.37	0	0
22	3,6-DMPhe	0	4.27	0	0.38	1.18	0	1
	Nitroarene							
23	1-NP	7.53	4.26	0	0.42	1.33	1	1
24	3-NBP	7.53	3.09	0	0.67	0.83	2	1
25	2-NBP	7.55	3.06	0	0.73	0.77	2	1
26	2-NFle	7.53	3.85	0.04	0.63	1.11	1	1
27	3-NBA	10.04	4.79	0	0.38	1.45	1	1
28	2-NNap	7.52	2.59	0	0.62	0.83	1	1
29	1-NNap	7.54	2.56	0	0.85	0.77	1	1
30	9-NPhe	7.52	3.60	0	0.41	1.04	1	1
31	3-NPhe	7.52	3.63	0	0.41	1.11	1	1
32	9-NAnt	7.54	3.61	0	0.41	1.05	1	1
33	6-NChr	7.52	4.63	0	0.43	1.32	1	1
	Quinone							
34	1,2-NQ	4.97	2.61	0	0.58	0.73	0	1
35	9,10-PQ	4.98	3.75	0	0.31	0.93	0	1
36	AQ	5.03	3.75	0	0.31	0.93	0	1
37	1,4-BQ	4.93	1.47	0	0.38	0.58	0	1
	Phthalate							
38	BBPt	10.56	5.10	0	0.61	0.95	9	1
39	DEPt	10.46	2.99	0	0.69	0.74	6	1
40	DBPt	10.63	4.52	0	0.50	0.74	10	1
41	DEHPt	10.63	7.58	0	0.43	1.15	16	0
	Nitrophenol							
42	4-NPh	9.78	1.69	0	0.60	0.79	1	1
43	3-M-4-NPh	9.83	2.15	0	0.73	0.99	1	1
	Metal							
44	CuCl	4	0	0	0.50	0	0	1
45	$CuCl_2$	6.83	0.52	0	0.67	0	0	1
46	$FeCl_2$	6.83	0.52	0	0.67	0	0	1
47	$FeCl_3$	9.46	3.96	0	0.50	0.58	0	1
48	$AlCl_3$	9.46	3.96	0	0.50	0.58	0	1
49	$NiCl_2$	6.83	0.52	0	0.67	0	0	1
50	$ZnCl_2$	6.83	0.52	0	0.67	0	0	1
51	$CrCl_2$	6.83	0.52	0	0.67	0	0	1
52	$CrCl_3$	9.46	3.96	0	0.50	0.58	0	1
53	$PtCl_2$	6.83	0.52	0	0.67	0	0	1
54	$PtCl_4$	12	4.07	0	0.40	2	0	1

Table 11. List of chemicals up-regulating IL-8 gene expression and prediction results.

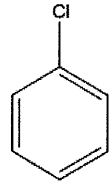
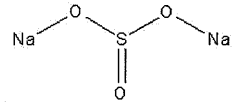
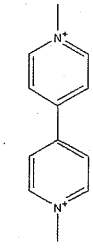
Chemical	Chlorobenzene	Sodium sulfite	Sphingosine-1-phosphate	Paraquat
Molecular Formula	C_6H_5Cl	Na_2SO_3	$C_{18}H_{38}NO_5P$	$C_{12}H_{14}Cl_2N_2$
Function	pro-inflammatory	pro-inflammatory	pro-inflammatory	oxidant
Structural Formula				
WTPT3	2.257772	15.092514	17.507229	6.229206
MOLC4	1.731071	0.457245	6.805808	3.336656
V5CH	0	0	0	0
SYMM2	0.571429	0.666667	0.4	0.357143
S3C	0.288675	0.288675	2.032065	0.910684
CRB_LEADL	0	2	17	1
OPREA_RULEI	1	1	1	1
Calculation Result	-0.31898185	-8.46669683	-9.04847266	-1.97234592
Prediction	up-regulation	up-regulation	up-regulation	up-regulation
Previous Report (Reference)	up-regulation (Lehmann et al.,2008)	up-regulation (Yang et al.,2009)	up-regulation (Milara et al.,2009)	up-regulation (Bianchi et al.,1993)

Table 12. List of chemicals down-regulating IL-8 gene expression and prediction results.

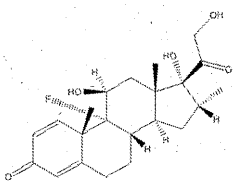
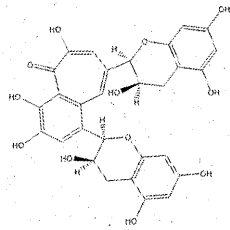
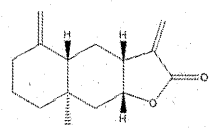
Chemical	Dexamethasone	β -carotene	Theaflavin	Isohelenin
Molecular Formula	$C_{22}H_{29}FO_5$	$C_{40}H_{56}$	$C_{29}H_{24}O_{12}$	$C_{15}H_{20}O_2$
Function	anti-inflammatory	anti-oxidant	anti-oxidant	NF-kB inhibitor
Structural Formula				
WTPT3	14.579576	0	28.937641	5.316734
MOLC4	9.749151	13.275419	10.128259	6.233736
V5CH	0.058926	0	0	0.034021
SYMM2	0.464286	0.35	0.243902	0.705882
S3C	3.613039	3.962761	3.804071	1.887041
CRB_LEADL	2	10	2	0
OPREA_RULEI	1	0	0	1
Calculation Result	-3.49670161	5.19910895	-11.54207732	0.27315436
Prediction	up-regulation	down-regulation	up-regulation	down-regulation
Previous Report (Reference)	down-regulation (Stoeck et al.,2000)	down-regulation (Yeh et al.,2009)	down-regulation (Aneja et al.,2004)	down-regulation (Mazor et al.,2000)

Fig. 1. Evaluation of the oxidative activity by use of the DTT assay. Oxidative activity was measured in triplicate by use of the DTT assay described under "Materials and methods." The DTT consumption of chemicals was determined after a 10-min incubation, with the DTT consumption of the blank having been subtracted. Values are shown as the mean ± SEM (n = 3). Anthraquinone, copper(I), copper(II), and nickel(II) showed a high DTT consumption rate.

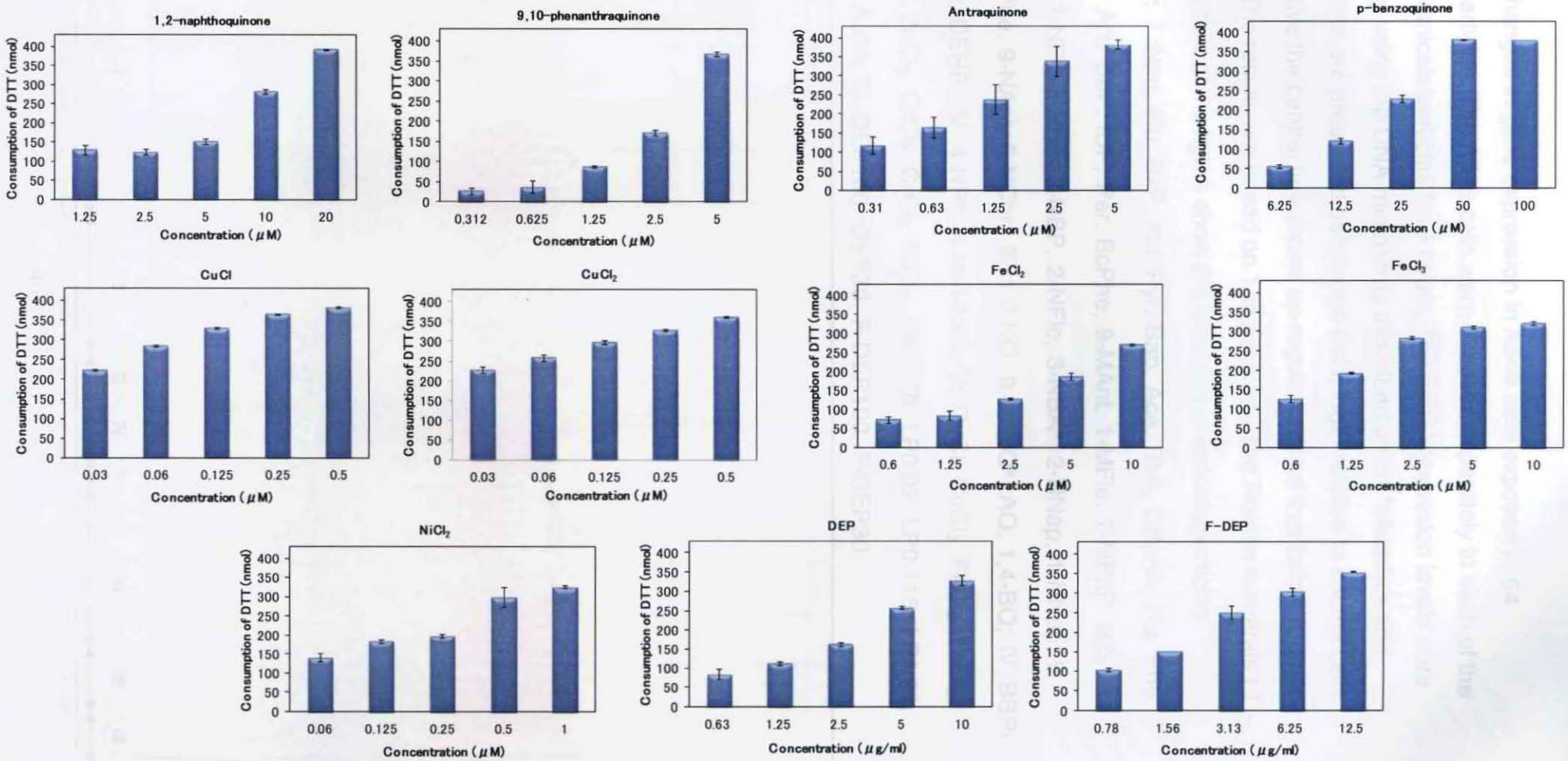


Fig. 2. Global changes in gene expression in A549 cells exposed to 64 chemicals/particles. The A549 cells were exposed separately to each of the indicated chemicals/particles for 4 hours. The gene expression levels were measured by using the DNA microarray described under "Materials and methods." Data are presented as change (fold, log2) relative to control cells. The part above the central line shows up-regulation; and that below it, down-regulation. The results are based on 1 experiment. The Roman numerals (Ⅰ ~ Ⅷ) at the bottom of the figure show the following chemicals/particles sequentially: Ⅰ:Nap, Flu, BkF, Acl, Pyr, Bap, Ace, BaA, DBahA, Fle, Phe, Chr, BghiP, Ant, BbF, IDP, Per, BcPhe, 9-MAnt, 1-MFle, 7-MBaP, 3,6-DMPhe; Ⅱ:1-NP*, 3-NBP, 2-NBP, 2-NFle, 3-NBA*, 2-NNap, 1-NNap, 9-NPhe, 3-NPhe, 9-NAnt, 6-NChr; Ⅲ:1,2-NQ, 9,10-PQ*, AQ, 1,4-BQ; Ⅳ:BBPt, DEPt, DBPt, DEHPt; Ⅴ:4-NPh, 3-M-4-NPh; Ⅵ:CuCl, $CuCl_2$, $FeCl_2$, $FeCl_3$, $AlCl_3$, $NiCl_2$, $ZnCl_2$, $CrCl_2$, $CrCl_3$, $PtCl_2$, $PtCl_4$; Ⅶ:LP0.02, LP0.115, LP1.01, Au5, Au10, Au20; Ⅷ:DEP100, DEP30, F-DEP100, F-DEP30.

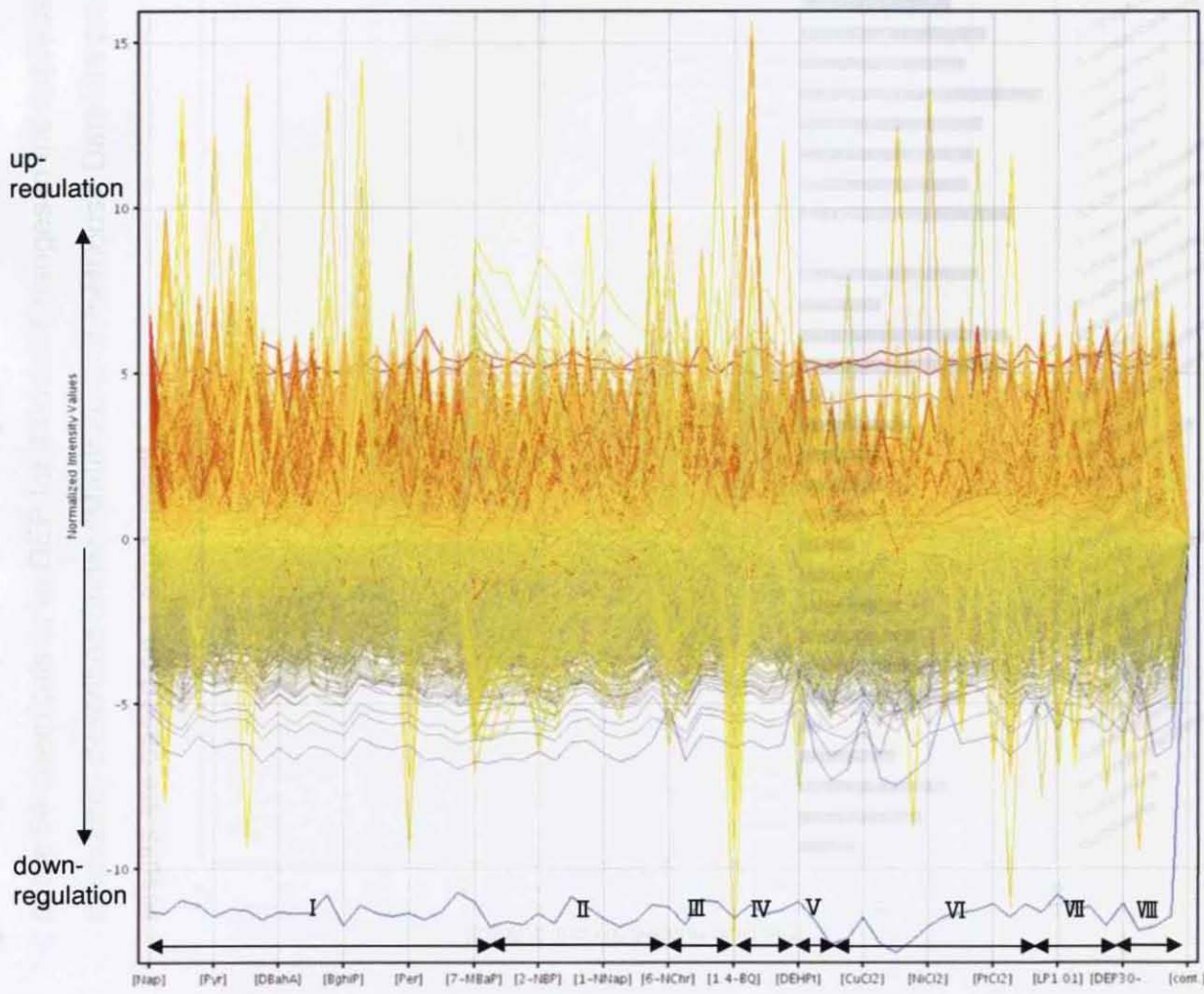


Fig. 3. IL-8 gene expression induced by the 54 chemicals and DEP in A549 cells. The A549 cells were exposed separately to each of the 54 chemicals or to DEP for 4 hours. Changes in the expression level of the IL-8 gene were measured by using the DNA microarray described under "Materials and methods." Data are presented as change (fold, log2) relative to control cells. The results are based on 1 experiment.

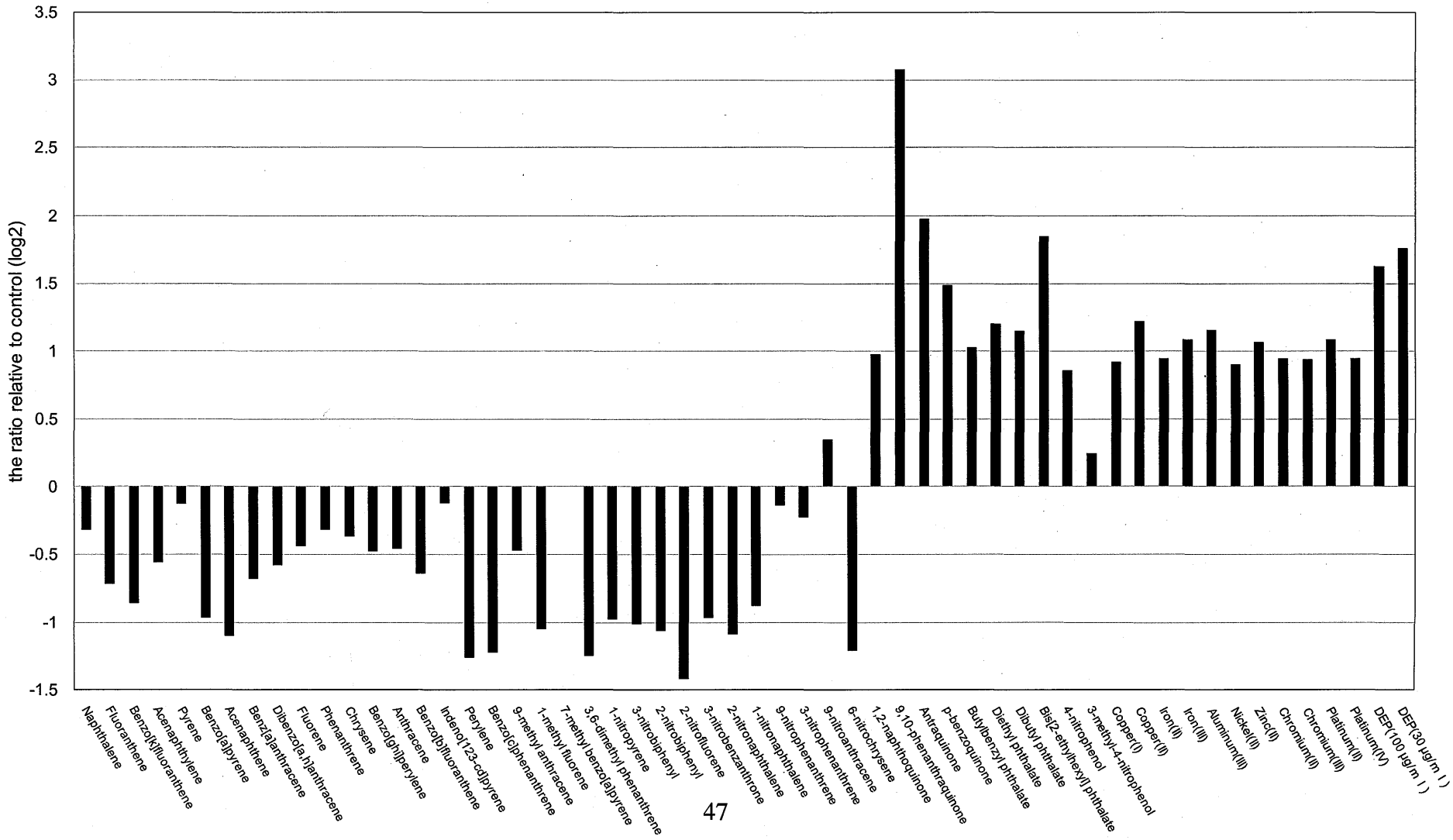


Fig. 4. Hierarchical clustering analysis of 64 chemicals/particles based on the gene expression profiles. These chemicals/particles were classified into 4 groups (Groups Ⅰ ~Ⅳ). Furthermore, Groups Ⅱ and Ⅳ were classified into 2 subgroups (Group Ⅱ -a, -b and Group Ⅳ-a, -b, respectively).