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# Differing estrogen activities in the organic phase of air particulate matter collected during sunny and foggy weather in a Chinese city detected by a recombinant yeast bioassay

Jingxian Wang[a,c,*], Ping Xie[a,1], Ying Xu[a], Antonius Kettrup[b], Karl-Werner Schramm[b]

[a] *Donghu Experimental Station of Lake Ecosystems, The State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, P.R. China*

[b] *GSF-National Research Centre of Environment and Health, Institute of Ecological Chemistry, Ingolstädter Landstr.1, D-85764 Neuherberg, Germany*

[c] *The Graduate School of the Chinese Academy of Sciences, P.R. China*

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**Abstract**

Total air suspended particles ($PM_{100}$) collected from an urban location near a traffic line in Wuhan, China, were examined for estrogen using a recombinant yeast bioassay. Wuhan, located at the central part of China, is the fourth biggest city in China with 7 million populations. Today, Wuhan has developed into the biggest city and the largest traveling center of central China, becoming one of the important bases of industry, education and research. Wuhan is right at the confluent point of Yangzi River, the third longest river in the world, and its largest distributary Hanjiang, with mountains and more than 100 lakes in downtown area. Therefore, by its unique landscape, Wuhan has formed clear four seasons with relatively long winter and summer and short spring and autumn. Foggy weather usually happen in early spring. The yeast line used in this assay stably expresses human estrogen receptor-alpha. Weak but clear estrogenic activities were detected in the organic phase of crude extracts of air particle materials (APM) in both sunny and foggy weather by 0.19–0.79 μg E2/g $PM_{100}$ which were statistically significantly elevated relative to the blank control responding from 20% to 50% of the maximum E2 response, and the estrogenic activity was much higher in foggy weather than in sunny weather. The estrogenic activities in the sub-fractions from chromatographic separation of APM sampled in foggy days were also determined. The results indicated that the responses of the fractions were obviously higher than the crude extracts. Since there is no other large pollution source nearby, the estrogenic material was most likely from vehicle emissions, house heating sources and oil fumes of house cooking. The GC/MS analysis of the $PM_{100}$ collected under foggy weather showed that there were many phenol derivatives, oxy-PAHs and resin acids which have been reported as environmental estrogens. These results of the analysis of estrogenic potency in sunny and foggy weather in a subtropical city of China indicate that further studies are required to investigate the actual risks for the associated health and atmospheric system.



*Keywords:* Air pollution; China; Cooking; Estrogenic receptor-alpha; Meteorology; Particulate matter; Smoke; Vehicle emissions

*Corresponding author. The State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, P.R. China. Tel./fax: +86-27-8764-7622.

*E-mail addresses:* wangjx@ihb.ac.cn (J. Wang), xieping@ihb.ac.cn (P. Xie).

[1] Also correspondence to.

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## 1. Introduction

Evidence has been accumulating indicating that humans, and domestic and wildlife species have suffered adverse health consequences from exposure to environmental chemicals that interact with the endocrine system (Adams, 1995; Golden et al., 1998). These chemicals have been described as "exogenous estrogens" that cause adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function (Weybridge UK, 1996). Epidemiological studies have indicated an association between atmospheric particulate pollution and adverse health effects (Dockery et al., 1993; Pope et al., 1995; Lippmann and Ito, 1995). Reproductive studies conducted on both male and female populations indicated the prevalence of low birth weight and premature births and decreases in semen quality when exposed to high levels of air pollution (Radim et al., 1996). Clouds and fog play an important role as processors of atmospheric aerosols and soluble gases. Concerns are increasing about adverse effects of fog on respiratory function and airway response in asthmatics (Honma et al., 2000; Fontana et al., 2002). Urban air particulate material (APM) is a complex mixture of chemicals released from vehicular and industrial sources, together with oil fumes from house cooking and re-entrained particles from roads. A study has discovered estrogenic activities present in air particulate material using in vitro gene expression assays (Clemons et al., 1998). However, little information is available on the changes of estrogenic activities in APM under different weather conditions. The main purpose of this study was to examine estrogenic activities in the crude organic extract and chemical fractions derived from urban APM associated with sunny and foggy weather.

The estrogen bioassay employed in this study involves the use of a genetically modified yeast strain to determine the ability of a test agent to trans-activate the estrogen receptor. This yeast strain contains a human estrogen receptor gene (ER alpha) and a reporter gene coding for the β-galactosidase. Test agents with estrogenic activity activate the receptor gene in yeast strain and subsequently result in the production β-galactosidase. The estrogenic activity of chemicals is quantified by determining the activity of the β-galactosidase (Routledge and Sumpter, 1996). It is a sensitive, rapid and inexpensive assay for estrogen-modulating activity (Gaido et al., 1997; Graumann et al., 1999). These characteristics make it an ideal candidate for primary screening purposes. In this study, we improved the testing procedure in a single 96-well plate to examine the estrogenic activity associated with the crude organic extract and chemical fractions derived from urban APM ($PM_{100}$) and determined the compositions of chemicals found within these extracts.

## 2. Materials and methods

### 2.1. Materials

17β-estrodiol (E2, 99%) and ethynylestradiol (EE2, 98%) were obtained from Sigma-Aldrich, Steinheim, Germany. Bisphenol-A (BPA, 97%) was purchased from Acros Organics, NJ, USA. And *p*-nonyphenol (NP, 98.4%) was from Sigma-Aldrich Laborchemikalien GMBH, Seelze. All solvents were of HPLC grade from LGC Promochem, Germany and were used without further purification. Other chemicals of high purity available were purchased from Merck, Darmstadt, Germany and Fluka, Neu-Ulm, Germany. Yeast Nitrogen Base and Bacto agar were obtained from Difco, Augsburg, Germany. Oxalyticase was kindly donated by Dr. Li You, Chemical Industry Institute of Toxicology, NC, USA.

### 2.2. Air particulate collection and fractionation

Air particulate materials were collected with an Anderson $PM_{100}$ hi-vol air sampler (Beijing Analytic Instrumental Work). The sampling was carried out on 16–18 March 2000 near the Donghu Lake sampling site on Bayi Road in downtown Wuchang, Wuhan, China. The meteorologic measurements for the sampling days are listed in Table 1. The area mainly consists of inhabitant districts, schools and institutes. There was no industrial pollution source nearby apart from a traffic road crossing with a flow of 1000 vehicles $h^{-1}$ of the vehicles recorded running on the road $1\,h^{-1}$ during the sampling days, 15% of which were buses and trucks, 60% were small cars and 25% were motor bicycles and tricycles. No heavy traffic happened on both sunny and foggy days. Two sampling sites were set at distances of 20 and 50 m from the road, respectively. On the other side of the sampling sites was a five-story office building at distances of 50 and 80 m from the two sampling sites. Apart from these, the surroundings were of mainly trees and bungalows. Further distances were mostly less than five-story residential buildings, school buildings and office buildings, including small-scale heating boilers. At each sampling site, particulate materials were collected for 20 h at a rate of $1.13\,m^3$/min on a glass fiber filter ($250 \times 200$ mm). The glass fiber filters were heated in 250 °C oven for 4 h before sampling, to remove the organics on the filters. A total of six filters were obtained and two filters were taken simultaneously each day. Two filters of the same date collected from the two sampling sites were combined into a composite sample. The first two sampling days were foggy whereas the subsequent one sampling day was sunny. Thus we got one sample to represent APM on a sunny day and two samples to represent APM at foggy days. The composite samples were extracted with

Table 1
Meteorologic data of sampling days

| Sampling dates in 2000 | 16, March | 17, March | 18, March |
|---|---|---|---|
| Range of temperature (°C) | 6.0–17.0 | 7.8–17.9 | 12.2–20.8 |
| Average temperature (°C) | 10.8 | 13.1 | 15.0 |
| Average air pressure (kPa) | 101.42 | 101.12 | 100.96 |
| Average relative humidity (%) | 89 | 70 | 75 |
| Average total cloud cover | 5.0 | 10.0 | 6.7 |
| Average wind velocity (m/s) | 0.3 | 0.8 | 0.8 |
| Weather phenomenon | Foggy | Foggy | Sunny |

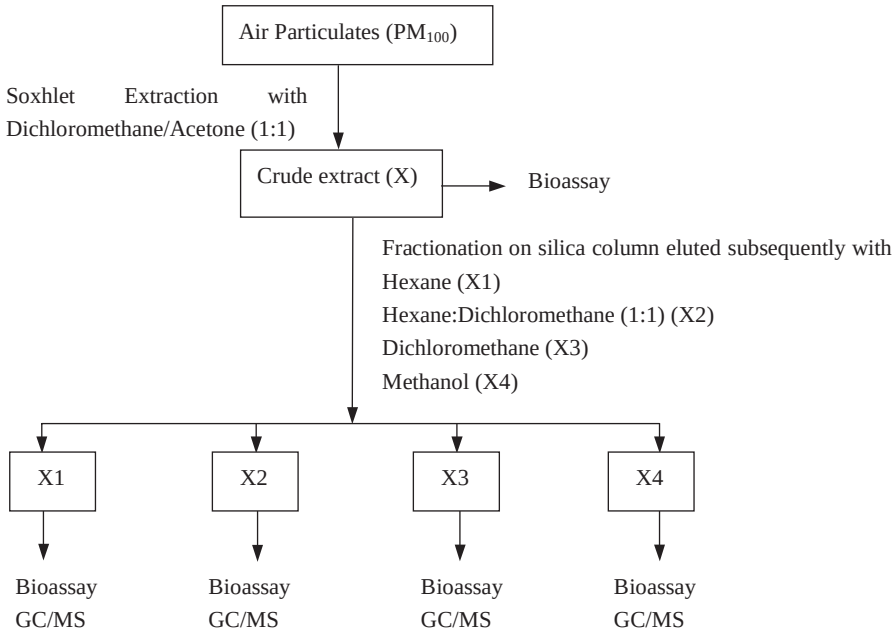


Fig. 1. Scheme of the sample fractionation procedure for air particulate samples

dichloromethane and acetone (1:1) for 24 h using a Soxhlet apparatus to afford crude extracts that were fractionated according to procedure which was a slightly modified version of that described by Clemons et al. (1998).

The fractionation process is summarized in Fig. 1. The crude extract of the combined filters was evaporated to 2 mL carefully onto a column containing 20 g silica. The column was eluted with hexane (120 mL) to yield an aliphatic fraction (X1), followed by the mixture of hexane and dichloromethane (1:1, 120 mL) to afford an aromatic fraction (X2), next by dichloromethane (120 mL) to provide moderately polar compounds (X3) and finally by methanol (120 mL) to produce highly polar compounds (X4).

### 2.3. Chemical analysis

Chemical analysis of sub-fraction samples was performed by gas chromatography – mass spectrometry. The instruments used were a Hewlett-Packard 5890 series II gas chromatograph coupled with Finnigan SSQ 7000 Mass Selective Detector. Helium was used as the carrier gas with head pressure 25−psi. The conducting parameters of mass spectrometry analysis were: ionization mode, EI; ionization energy, 70ev; resolution, 1000; ion source temperature, 150 °C. A 60 m DB-5ms column was used with the following temperature program: initial temperature, 60 °C; program rate, 5 °C $min^{-1}$; finial temperature, 300 °C; hold at 300 °C for 10 min. The transfer line temperature was 280 °C. Data were

acquired in full scan mode. Following the subtraction of background spectra, the mass spectrum of each chromatographic peak was obtained by library search. Peaks were tentatively identified using the NIST library with a fit $\geqslant 60\%$. Peaks with fits $<60\%$ were suppressed from the list.

### 2.4. Transformed oestrogen receptor gene yeast system

The strain used in this assay constitutively expresses the gene for the human estrogen receptor. On a separate expression plasmid, the estrogen-responsive sequences control the expression of the *Lac-Z* reporter gene. Estrogenic agents interacting with the receptor leads to the binding of the receptor with the promoter of the reporter resulting in the production of β-galactosidase. The activity of β-galactosidase results in a color reaction, which was measured by assaying the absorbency at 420 nm with a microplate reader. Absorbency at 600 nm was used to measure cell density and vitality.

### 2.5. Recombinant yeast bioassay procedure

Yeast (*Saccharomyces cerevisiae*) stably transfected with the human estrogen receptor-alpha (hER) gene and expression plasmids carrying an ERE, and the reporter gene *LacZ* encoding the enzyme β-galactosidase were used and selective medium was prepared following the prescription of Routledge and Sumpter (1996). Recombinant estrogen sensitive yeast cells were stored in 15% DMSO at $-80\,^{\circ}C$ and grown as required at $30\,^{\circ}C$ in selective media. All chemicals have a purity of $>97\%$ and none was re-purified prior to use. After the organic phase of $PM_{100}$ samples was extracted, the following experiment was carried out at least two times and each time all samples were evaluated in triplicate. Each test sample was serially diluted and added in DMSO for a total of 5–7 concentrations such that the concentration of DMSO did not exceed 1% in the recombinant yeast medium whose cell density of the culture at 600 nm wave length $OD_{600}$ was 0.50 when the medium was determined (Wang and Xu, 2005). Serial dilutions of test samples were combined with 100 μL of medium, containing yeast, per well in 96-well optically flat-bottom microtiter plates and were incubated at $30\,^{\circ}C$ with vigorous orbital shaking (130 rpm) on a titer plate shaker for 2 h, and then the cell density of the culture was measured at 600 nm wave length. Each plate contained a negative control (DMSO) as well as a standard curve for 17β-estradiol (positive control).

After measuring the $OD_{600}$ of the culture, 100 μL of β-galactosidase composite solution was added in the culture. The solution was made by 2 mg/mL *o*-nitrophenol-β-D-galactopyranoside (*o*NPG) (22 mL), 0.1% SDS (110 μL), 50 mM β-mercaptoethanol (29.7 μL), 200 U/mL oxalyticase (Corvallis) (11 μL) and Z-buffer (10.9 mL). Then the assays were incubated at $30\,^{\circ}C$ on a titer plate shaker for another 1 h. The reactions were terminated by the addition of 100 μL $Na_2CO_3$ (1 M) and the $OD_{420\,nm}$ of the assay solutions was determined (Wang and Xu, 2005).

The calculation of the β-galactosidase activity and EC50 was based on a previously published method (Wang et al., 2003).

## 3. Results and discussion

### 3.1. Dose–response relationship of four chemicals in a single-plate yeast bioassay

The estrogenic activities of 17β-estradiol (E2), ethynylestradiol (EE2), bisphenol-A (BPA) and *p*-nonyphenol (NP) were determined as the representatives of natural and synthetic steroids using the single-plate yeast fast screening bioassay and the results are shown in Fig. 2. The EC50 of the compounds and their comparison with references are listed in Table 2. Because of the use of different laboratories, there are some differences in screening procedures of the yeast bioassay. But in most cases, at least more than 1 day is needed for detection (Routledge and Sumpter, 1996; Arnold et al., 1996; Coldham et al., 1997; Miller et al., 2001; Boever et al., 2001). As an improvement of the screening procedure, we were able to accomplish the detection of a batch of samples within 4–6 h. Results in Table 2 show that the EC50-based ratios of the estrogenic activities of the compounds in the study were comparable to values found with another yeast construct (Gaido et al., 1997; Rehmann et al., 1999; Murk et al., 2002).

### 3.2. Estrogenic activities in crude and fractionated extracts of air particulate material $PM_{100}$

APM from a downtown sampling site (Bayi Road, Wuhan, China) was extracted and assessed using recombinant gene yeast to investigate their estrogenic activities (Fig. 3). The crude fraction responded from 20% to 50% of the maximum E2 response suggesting the presence of partial ER agonists. Two blank glass fiber filters were combined into one composite as a blank reference and were treated following the same procedure of the environmental samples. No estrogenic activity was found in blank glass fiber filters response to yeast culture (data not shown). On the basis of the β-galactosidase activity observed with the three APM crude extracts, the inductive efficiencies of samples Aa and Ab collected in foggy days were three times higher than sample A collected on the sunny day. The E2 EC50 values are reported in grams per liter to facilitate direct comparison to the APM EC50 values that are expressed in grams of $PM_{100}$. The estrogenic activities expressed by

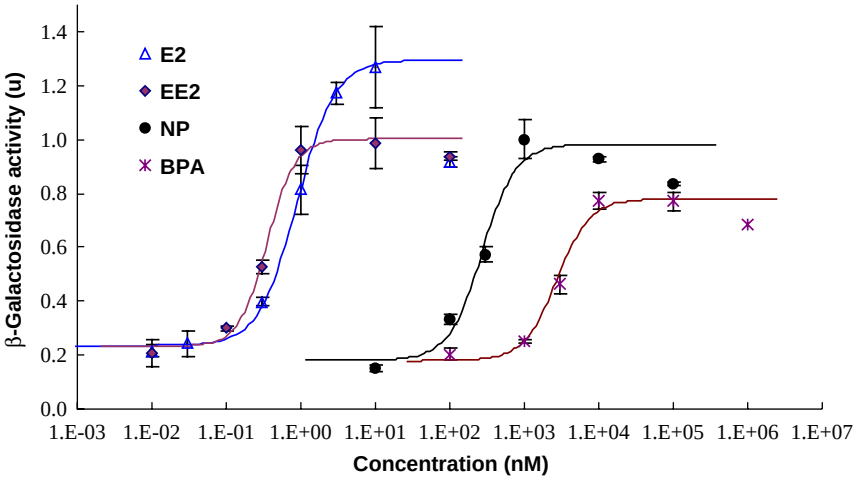


Fig. 2. Dose-response curves of four estrogenic chemicals in response to recombinant yeast bioassay

Table 2
EC50 values of estrogenic chemicals and their comparison to reports from literature by recombinant yeast bioassay

| Compounds | EC50 (nM) | EC50 of reference (nM) |
|---|---|---|
| E2 | 0.889 | 0.22[a], 1.5[b] |
| EE2 | 0.356 | 0.08[c] |
| NP | 272.4 | 1100[a], 175.4[c] |
| BPA | 3168 | 3400[a], 104000[b], 10000[c] |

*Note*:
[a]Gaido et al. (1997);
[b]Rehmann et al. (1999);
[c]Murk et al. (2002).

EC50 in Table 3 showed that Aa and Ab possessed more potent E2 equivalence of 0.79 and 0.75 of μg E2/g $PM_{100}$ than A of 0.19 μg of E2/g $PM_{100}$. Therefore, both of the EC50 values and β-galactosidase activities of APM on foggy days were higher than those the sunny day.

The APM sampled on foggy days were chemically separated yielding four fractions: Ab1, nonpolar aliphatics, Ab2, aromatic fraction, Ab3, polar compounds and Ab4, highly polar compounds (Fig. 1), and were assessed similar to the crude extracts by yeast bioassay. The results of sample Ab are shown in Fig. 4. The result of sample Aa (not presented here) was similar to that of Ab. There was only a small difference among the four fractions, and the polar fraction tended to have higher response activity (Fig. 4). It should be noted, however, that the response of the crude extract was obviously lower than the fractions. No significant synergistic or antagonistic interactions were observed following co-treatment with E2 by 0.30 ng/mL and either the crude extracts or their fractions (data not shown). The results indicate that the chemicals in the APM were not acting synergistically or antagonistically with E2. Certainly, we still could not simply make a conclusion about significant synergistic or antagonistic interactions existing in air particulate matter by the above-mentioned phenomenon; the reason seems to be complicated and further studies are needed.

Although in vitro assays are simple, cost-effective and consequently capable of screening large numbers of samples, they cannot replace in vivo models. The pharmacokinetic and pharmacodynamic interactions that can occur in vivo are not taken into account; therefore, in vitro tests should be used to complement in vivo testing. In addition, in vitro assays may not take into consideration other mechanisms of action that may lead to adverse effects. Moreover, important factors such as critical lifecycle or sensitive developmental windows and the effects of bioconcentration and bioaccumulation cannot be accurately modeled using in vitro assay systems. Accordingly, a mere yeast-based bioassay cannot find the causes of estrogenic activities in APM. However, results from in vitro assays indicate potential mechanisms of action that could be used to refine in vivo studies. Therefore, additional in vitro and in vivo studies are warranted to further address the complexity of mechanisms of estrogen generation in APM and the possible health effects under different weather conditions.

### 3.3. *Organic constituent of $PM_{100}$ air particulate material in foggy weather*

Understanding the effects of fogs on atmospheric chemistry and ecological health requires detailed information on their chemical composition. Although inorganic compounds have been studied extensively in

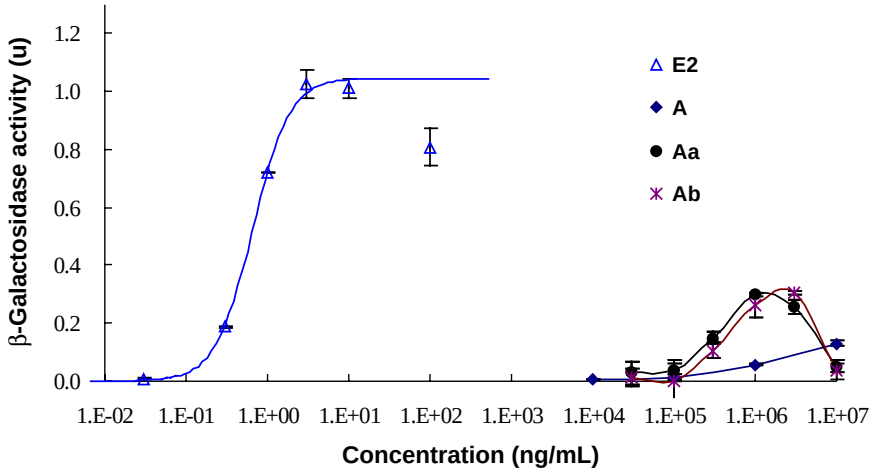


Fig. 3. The estrogenic activities of air-suspended particles $PM_{100}$ in response to recombinant yeast bioassay. A standard E2 curve was produced by analysis of 0.01–100 ng/mL E2 in selective media for estrogenic activity, against which the extracts were compared. A represents the APM collected on the sunny day. Aa and Ab represent the APM collected during foggy weather.

Table 3
EC50 values and E2 equivalences of crude extracts of air particulate material ($PM_{100}$)

| Samples | Sampling dates in 2000 | Weather phenomenon | Weight of particles (g) | EC50 (mg/mL) | E2 equivalence (μg E2/g $PM_{100}$) |
|---|---|---|---|---|---|
| A | 18 March | Sunny | 1.0202 | 1.2348 | 0.19 |
| Aa | 16 March | Foggy | 1.1294 | 0.3054 | 0.79 |
| Ab | 17 March | Foggy | 0.7102 | 0.3191 | 0.75 |

*Note*: E2 equivalence is computed by comparison to E2.

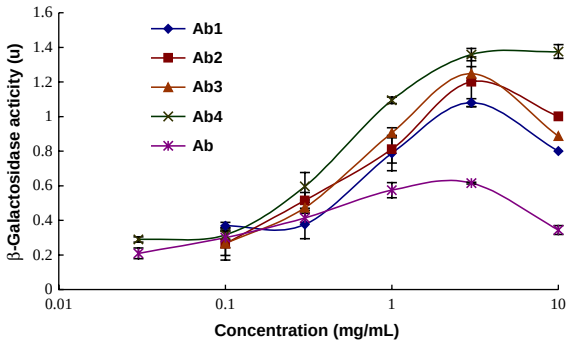


Fig. 4. The estrogenic activities of different fractions of the crude extract of sample Ab collected during foggy weather in response to recombinant yeast bioassay. Ab is the crude extract, Ab1 is nonpolar aliphatic fraction, Ab2 is aromatic fraction, Ab3 is polar fraction, Ab4 is highly polar fraction.

fog waters (Herckes et al., 2002b; Minami and Ishizaka, 1996), relatively little is known about the organic constituents (Kiss et al., 1999; Herckes et al., 2002a). The organic compounds identified in the extract of the APM that were obtained in foggy weather are listed in Table 4. It should be noted that the list is not exhaustive but just an indicator of the large number of compounds present. Any chromatographic compounds found in common between the procedural blank and sample reports were not included since these compounds are derived in part from the solvents and glass fiber filters used in the extraction procedures. For the identified chemicals, position isomers were treated as though they were the same chemicals because mass spectra for position isomers were virtually indistinguishable with the instrumentation used in this study.

The main compounds present in the APM in foggy weather were dominant by long-chain *n*-alkanes, PAHs and oxy-PAHs and resin acids. Studies have demonstrated that most chemicals with estrogenic activities contain phenolic groups (Routledge et al., 1998; Schultz et al., 2000; Tong et al., 1998), and PAH and derivatives also have been considered as possible endocrine disrupters (Anstead and Kym, 1995; Chang and Liao, 1987; Hwang et al., 1992). Consequently, the oxy–PAHs, phenol derivatives and the large amount of resin acids most likely contributed to the estrogenic activities

Table 4
Organic composition of air particulate material $PM_{100}$ under foggy weather

| Compounds | |
|---|---|
| ***n-Alkanes*** | **Resin acids** |
| Tetramethyl-Heptadecane | 2-Oxo-octadecanoic acid, methyl ester |
| Dimethyl-Tridecane | Methyl-pentadecanoic acid, methyl ester |
| Tetratetracontane | 9,12-Octadecadienoic acid, 2,3- dihydroxy -propyl ester |
| Heptadecane | 8-Octadecenoic acid, methyl ester |
| Heneicosane | 13- Octadecenoic acid, methyl ester |
| Pentatriacontane | Methyl-heptadecanoic acid, methyl ester |
| Tetratetracontane | 9-Octadecenoic acid, 2,3-dihydroxypropyl ester |
| Trimethyl-octahydro-Naphthalenone, | Propyl-heptadecanoic acid, methyl ester |
| Trifluoromethyl-Dioxolane | 3-Oxo-octadecanedioic acid, dimethyl ester |
| Methyl-Heneicosane | Hexadecanoic acid, 2-hydroxy-1- (hydroxy -methyl) ethyl ester |
| Pentadecylcyclohexane | Oleic acid, 3-hydroxypropyl ester |
| Tritetracontane | |
| Cyclotetracosane | |
| Dotriacontane | |
| Diheptyl-Pentadecane | |
| Tetracosane | ***N-compounds*** |
| Pentadecane | Acetamide, N-methyl-N-[4-[4-methoxyl-1-hexahydropyridyl1]-2-butynyl] |
| Hexadecane | Butyl-nitrobenzene |
| Eicosyl-Cyclohexane | Methyl-propanamide |
| Methyl-Eicosane | Hexadecanamide |
| Decyl-Tetracosane | Octadecanamide |
| | 9-Octadecenamide |
| | 4-Nitro-5-hydroxy-1,2-dimethylindole |
| ***PAHs and oxy-PAH*** | |
| 4H-1-Benzopyran-4-one, 2-(3,4-dihydroxyphenyl)-6,8-di-.beta.-D-gluopyra | |
| 6H,8H-Benzo[10,11]chryseno[1,12-cd]pyran-6,8-dione | |
| Dimethyl-cholestanone | ***Other compounds*** |
| Trihydroxy-9,10-anthracenedione | 1-Doctriacontanol |
| Fluoranthene | Dimethylethyl -ethyl-phenol |
| 6-Oxabicyclo[3.2.1]octan-7-one, 1,5-dimethyl-8-[2- [3-(1-methylethy)phen | 1-Heneicosyl formate |
| | 1-Docosanol, formate |
| Triphenylene | Dibromo-dodecane |
| Perylene | Fluoro-dodecane |
| Clonitazene | Tricosene |
| *N*-Methyl-*N*-(1-oxododecyl)-glycine | Tridecenal |
| Dimethylethyl -methyl-benzenethiol | Dodecalactone |
| Lanostanol | Tetramethylheptadecan-4-olide |
| Cholesterol | Butyl dodecanoate |
| Cedrol | |

in the APMs. A meat-cooking tracer — cholesterol that was identified indicated that one of the polluted source was cooking emissions. The occurrence of bromine in polluted air is commonly assumed to be governed by two sources, natural particulate Br and vehicle exhausts (Lammel, 1995). Therefore, the compound dibromodo-decane detected in the APM (Table 4) surely came from the traffic line nearby. Our recent work has discovered estrogenic activities in emissions from petroleum and diesel-fired vehicle and coal-fired stove (Wang et al., 2003). Accordingly, the presence of estrogenic activity in urban APM may come from vehicle exhausts, house-heating releases and oil fumes from house cooking.

Since there was no other large pollution source near the sampling sites, and the meteorologic measurements for the sampling days were similar (Table 1), the presence of higher estrogenic activities in APM on foggy days than on a sunny day seems, to be because of to the interactions or changes among particles affected by changes of natural environmental conditions,

especially the different weather phenomenon. Particulate air pollution comes from a mixture of particles that vary in size, composition, and origin. Fine particles with aerodynamic diameters that are equal to or less than 10 μm are the largest concern to human health because they can readily enter the lungs and become trapped by the alveoli. There is a strong concensus that a significant and consistent association exists between mortality and air particulates with mean aerodynamic diameters below 10 μm (Wordley et al., 1997; Hong et al., 2002). Humidity, dilution ratio and residence time were found to affect the particle size distribution. Under specific humidity, temperature and dilution conditions, a large number of fine particles or ultrafine particles were formed (Shi and Harrison, 1999). The organic fraction of urban airborne particulate material has been evaluated with regard to the mutagenic and genotoxic activity of the compound found in various extracts (Hannigan et al., 1998; Gundel et al., 1993). In both $PM_{2.5}$ and fog waters, the average concentrations of combined organic compounds were generally 4–5 times higher than those of free forms of inorganic compounds (Zhang and Anastasio, 2003). Furthermore, secondary organic aerosols form in the atmosphere following photooxidation of tropospheric species and formation of secondary organic compounds of low vapor pressure. Alternatively, the secondary organics can condense on existing inorganic aerosol, forming an outer organic film encompassing an inner aqueous or solid core (Murphy et al., 1998; Posfai et al., 1998). Thus, foggy weather may help to generate more fine particles and form more combined organic compounds, as well as secondary aerosols. On the other hand, some kinds of compounds are rapidly transformed in water drops and particles during exposure to ozone and sunlight (McGregor and Anastasio, 2001). All these reasons may partly explain why particles collected in sunny weather possessed lower estrogenic activity than collected on foggy days.

In summary, estrogenic activities are present in air particulate material on both sunny and foggy days measured by a recombinant yeast bioassay, and higher estrogenic activities were found in APM collected on foggy days than in sunny weather. Since a mere yeast-based bioassay cannot find the complex mechanism and the potential health consequence, further in vitro and in vivo bioassays are needed. As the pollutants in fog water and particle samples exert influences on various ecosystems, the chemistry of fog has been an important topic of investigation during the past few decades. Although the health impact of estrogenic activity being enhanced during foggy weather condition is unknown, the presence of these activities may contribute to and exacerbate adverse health effect evoked by APM. Further study on the detailed mechanisms and kinetics for the production of estrogens in foggy weather is warranted and the potential toxicological significance should be clarified.

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## References

Adams, N.R., 1995. Organizational and activational effects of phyto-estrogens on the reproductive tract of the ewe. Proceedings of the Society for Experimental Biology and Medicine 208, 87–91.

Anstead, G.M., Kym, P.R., 1995. Benz[a]anthracene diols: predicted carcionogenicity and structure-estrogen receptor binding affinity relationships. Steroids 60, 383–394.

Arnold, S.F., Robinson, M.K., Notides, A.C., Guillette Jr., L.J., McLachlan, J.A., 1996. A yeast estrogen screen for examining the relative exposure of cells to natural and xenoestrogens. Environmental Health Perspectives 104, 544–548.

Boever, P.D., Demare, W., Vanderperren, E., Cooreman, K., Bossier, P., Verstraete, W., 2001. Optimization of a yeast estrogen screen and its applicability to study the release of estrogenic isoflavones from a soygerm powder. Environmental Health Perspectives 109, 691–697.

Chang, C.S., Liao, S.S., 1987. Topographic recognition of cyclic hydrocarbons and related compounds by receptors for androgens, estrogens, and gluco corticoids. Journal of Steroid Biochemistry 27, 123–131.

Clemons, J.H., Allan, L.M., Marvin, C.H., Wu, Z., McLarry, B.E., Bryant, D.W., et al., 1998. Evidence of estrogen- and TCDD-like activities in crude and fractioned extracts of $PM_{10}$ air particulate material using in vitro gene expression assays. Environmental Science and Technology 32, 1853–1860.

Coldham, N.G., Dave, M., Sivapathasundaram, S., McDonnell, D.P., Connor, C., Sauer, M.J., 1997. Evaluation of a recombinant yeast cell estrogen screening assay. Environmental Health Perspectives 105, 734–742.

Dockery, D.W., Pope, C.A., Xu, X., Spengler, J.D., Ware, J.H., Fay, M.E., et al., 1993. An association between air pollution and mortality in six US cities. The New England Journal of Medicine 329, 1753–1759.

Fontana, G.A., Lavorini, F., Pistolesi, M., 2002. Review "Water aerosols and cough". Pulmonary Pharmacology & Therapeutics 15, 205–211.

Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J., et al., 1997. Evaluation of chemicals with

endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicology and Applied Pharmacology 143, 205–212.

Golden, R.J., Noller, K.L., Titus-Ernstoff, L., Kaufman, R.H., Mittendorf, R., Stillman, R., Reese, E.A., 1998. Environmental endocrine modulators and human health: an assessment of the biological evidence. Critical Reviews of Toxicology 28, 109–227.

Graumann, K., Breithofer, A., Jungbauer, A., 1999. Monitoring of estrogen mimics by a recombinant yeast assay: synergy between natural and synthetic compounds? Science of the Total Environment 225, 69–79.

Gundel, L.A., Daisey, J.M., de Caravalho, L.R.F., Kado, N.Y., Schuetzle, D., 1993. Polar organic matter in airborne particles chemical charaterization and mutagenic activity. Environmental Science and Technology 27, 2112–2119.

Hannigan, M.P., Cass, G.R., Penman, B.W., Crespi, C.L., Lafleur, A.L., Busby, W.F., et al., 1998. Bioassay-directed chemical analysis of Los Angeles airborne particulate matter using a human cell mutagenic activity. Environmental Science and Technology 32, 3502–3514.

Herckes, P., Hannigan, M.P., Trenary, L., Lee, T., Collett, JL., 2002a. Organic compounds in radiation fogs in Davis (California). Atmospheric Research 64, 99–108.

Herckes, P., Wortham, H., Mirabel, P., Millet, M., 2002b. Evolution of the fogwater composition in Strasbourg (France) from 1990 to 1999. Atmospheric Research 64, 53–62.

Hong, Y.-C., Lee, J.-T., Kim, H., Ha, E.-H., Schwartz, J., Christiani, D.C., 2002. Effects of air pollutants on acute stroke mortality. Environmental Health Perspectives 110, 187–191.

Honma, S., Tanaka, H., Teramoto, S., Igarashi, T., Ase, S., 2000. Effects of naturally occurring acid fog on inflammatory mediators in airway and pulmonary functions in asthmatic patients. Respiratory Medicine 94, 935–942.

Hwang, K.J., Carlso, K.E., Anstead, G.M., Katzenellenbogen, J.A., 1992. Donor–acceptor tetrahydrochrysenes inherent by fluorescent, high affinity ligands for the estrogen receptor: binding and fluorometric assay of receptor. Biochemistry 31, 11536–11545.

Kiss, G., Varga, B., Galambos, I., Krivacsy, Z., Hansson, H.C., Alsberg, T., et al., 1999. Investigation on water soluble of organic compounds in fog droplets and interstitial aerosol particles. Journal of Aerosol Science 30 (Suppl 1), S267–S268.

Lammel, G., 1995. Particulate and fog- and cloud-water bromide in polluted air. Atmospheric Environment 29, 3257–3262.

Lippmann, M., Ito, K., 1995. Separating the effects of temperature and season on daily mortality from those of air pollution in London: 1965-1972. Inhalation Toxicology 7, 85–98.

McGregor, K.G., Anastasio, C., 2001. Chemistry of fog waters in California's central valley: 2. Photochemical transformations of amino acids and alkyl amines. Atmospheric Environment 35, 1091–1104.

Miller, D., Wheals, B.B., Beresford, N., Sumpter, J.P., 2001. Estrogenic activity of phenolic additives determined by an in Vitro yeast bioassay. Environmental Health Perspectives 109, 133–138.

Minami, Y., Ishizaka, Y., 1996. Evaluation of chemical composition in fog water near the summit of a high mountain in Japan. Atmospheric Environment 30, 3363–3376.

Murk, A.J., Legler, J., van Lipzig, M.M.H., Meerman, J.H.N., Belfroid, A.C., Spenkelink, A., et al., 2002. Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environmental Toxicology and Chemistry 21, 16–23.

Murphy, D.M., Thomson, D.S., Mahoney, M.J., 1998. In situ measurements of organics, meteoritic material, mercury, and other elements in aerosols at 5 to 19 kilo meters. Science 282, 1664–1669.

Pope C.A.III., Thun, M.J., Namboodiri, M.M., Dockery, D.W., Evans, J.S., Speizer, F.E., et al., 1995. Particulate air pollution as a predictor of mortality in a prospective study of US adults. American Journal of Respiratory and Critical Care Medicine 151, 669–674.

Posfai, M., Xu, A.F., Anderson, J.R., Buseck, P.R., 1998. Wet and dry sizes of atmosphere aerosol particles: an AFM-TEM study. Geophysical Research Letters 25, 1907–1910.

Radim, J.S., Ivan, B., Blanka, B., Jan, D., Donald, H., Frantisek, K., et al., 1996. Teplice program—the impact of air pollution on human health. Environmental Health Perspectives 104 (Suppl 4), 699–714.

Rehmann, K., Schramm, K.-W., Kettrup, A., 1999. Applicability of a yeast oestrogen screen for the detection of oestrogen-like activities in environmental samples. Chemosphere 38, 3303–3312.

Routledge, E.J., Praker, J., Ashby, J., Sumpter, J.P., 1998. Some alkylhydroxy benzoates preservatives (parabens) are estrogenic. Toxicology and Applied Pharmacology 153, 12–19.

Routledge, E.J., Sumpter, J.P., 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry 15, 241–248.

Schultz, T.W., Seward, J.R., Sinks, G.D., 2000. Estrogenicity of benzophenones evaluated with a recombinant yeast assay: comparison of experimental and rules-based predicted activity. Environmental Toxicology and Chemistry 19, 301–304.

Shi, J.P., Harrison, R.M., 1999. Investigation of ultrafine particle formation during diesel exhaust dilution. Environmental Science and Technology 33, 3730–3736.

Tong, W., Lowis, D.R., Perkins, R., Chen, Y., Welsh, W.J., Goddette, D.W., et al., 1998. Evaluation of quantitative-structure-activity relationship methods for large-scale prediction of chemical binding to estrogen receptor. Journal of Chemical Informatics and Computer Sciences 38, 669–677.

Wang, J., Wu, W., Henkelmann, B., You, L., Kettrup, A., Schramm, K.-W., 2003. Presence of estrogenic activity from emission of fossil fuel combustion as detected by a recombinant yeast bioassay. Atmospheric Environment 37, 3225–3235.

Wang, J., Xu, Y., 2005. Comparison of three recombinant gene yeast bioassay for screening xenoestrogenic compounds. Acta Hydrobiologica Sinica 29 (1), in press (in Chinese).

Weybridge, UK, MRC Institute for Environment and Health, 1996. European Workshop on the Impact of Endocrine Disrupters on Human Health and Wildlife, 2–4 December, p.125.

Wordley, J., Walters, S., Ayres, J.G., 1997. Short term variations in hospital admissions and mortality and particulate air pollution. Occupational and Environmental Medicine 54, 108–116.

Zhang, Q., Anastasio, C., 2003. Free and combined amino compounds in atmospheric fine particles ($PM_{25}$) and fog waters from Northern California. Atmospheric Environment 37, 2247–2258.