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Are the new atmospheric health risk substances EPFRs oxidatively toxic? : Results from a large urban road dust study in Northwest China

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Abstract:

Atmospheric particulate matter (PM) is an important factor of premature human mortality due to PM inhalation. However, the toxic and hazardous components contained within the atmospheric PM are still not fully identified. Environmental persistent free radicals (EPFRs), a novel environmental health risk substance in the atmosphere, have been hypothesized to be important contributors to human respiratory health risks due to their potential to enhance the oxidation potential of PM, but this hypothesis has not been compelling confirmed in the actual atmospheric environment. Therefore, the fugitive characteristics of EPFRs in dust and their potential oxidative toxicity were investigated by using road dust from a mention city in northwest China. The research results showed that the road dust in Xi'an is rich in C-centered EPFRs $((6.6 \pm 5.0) \times 10^{17}$ spins/g), and its half-life can reach 4.5 years. Water-insoluble dust was the main contributor (71%) to the oxidative toxicity of road dust, showing a rapid toxicity-producing process, while the rate of oxidative toxicity production was more constant for water-soluble dust. The contribution of the EPFRs dominant factor to the total dust oxidative toxicity was estimated to be 17% based on the positive matrix factorization (PMF) model, and up to 33% for water-insoluble dust oxidative toxicity. Metals and organic carbon were the main contributing components to the oxidative toxicity of the WS fraction. Summarily, this study demonstrated that the EPFRs are an important contributor to the oxidative toxicity of in actual atmospheric PM, and their oxidative toxicity is dependent on the type of free radicals, providing important insights into what other potentially toxic substances contribute to the oxidative toxicity of atmospheric PM.

Key Words:

Road dust; EPFRs; Oxidation potential; Potential health risks.

1. Introduction

Epidemiological and toxicological studies have shown that exposure to atmospheric particulate matter (PM) significantly contributes to cardiovascular disease, frequent respiratory disease^{1,2}, and the induction or exacerbation of diabetes mellitus³. Previously published studies have been typically focused on the surface area of PM and the organic components, transition metals, and microorganisms adsorbed on the PM surface^{4,5,6}. However, the subsequent studies have shown that known toxicants do not fully explain the toxicity produced by atmospheric PM. Thus, the unidentified and undiscovered toxicants or mechanisms of toxicity production have become the focus of research. In recent years, EPFRs have drawn great attention as a novel type of environmental risk substances and have been successively discovered in soil, water, and atmospheric environmental media^{7,8,9,10,11}. EPFRs in the environment are mainly divided into C-centered and O-centered radicals according to the size of the g-factor¹². EPFRs are a kind of radicals with lifetimes ranging from minutes to years and can be existed steadily in the environment¹³, which is proposed in comparison with short-lived radicals such as hydroxyl radicals ($\bullet\text{OH}$).

The primary origins of EPFRs in the atmospheric environment are vehicle exhaust emissions, industrial emissions, and emissions from combustion sources such as coal combustion. However, in recent years, several studies have been discovered that EPFRs can also be originated from road dust, and EPFRs produced from road dust contributes up to 27% in urban areas of northwest China¹⁴. Meanwhile, some research indicated that dust particles can carry EPFRs for long-range transport during dust storms¹⁵. The metallic minerals in dust particles provide the prerequisite for the formation and existence of EPFRs, such as Fe^{2+} , Cu^{2+} , Mn^{2+} , and Zn^{2+} . These metallic minerals possess paramagnetic characteristics and induce the probability to attach with the particles in the EPFRs generation process^{16,17,18}. Currently, the EPFRs generation mechanism with the involvement of transition metals described specifically as follows: aromatics adsorbed onto the surface of transition metal oxides, and then the electrons

of aromatics are transferred to transition metal ions, resulting the metal ions forming stable single-electron structures while being reduced^{19,20}. Meanwhile, some research also suggest that EPFRs can still be formed without the involvement of transition metals. For example, the coking process of organics to form graphite-like material could produce EPFRs, and this process is considered to be a main mechanism for the production of EPFRs in atmospheric PM²¹. The health effects of EPFRs, which are generated in large quantities through different mechanisms and attached to atmospheric particulate matter, have been hotly debated.

To date, the health risk and toxic mechanism of EPFRs in atmospheric PM is unclear. The paradoxical view is that EPFRs are long-lived free radicals but they are reactive. It is supposed that EPFRs play the role of catalysts to generate ROS. More research is being conducted to support the health risks of EPFRs. Meanwhile, in a laboratory simulation of EPFRs generation experiments, it was found that a single EPFR can catalyze the generation of 10 reactive oxygen species (ROS)^{22,23,24}; in studies of municipal solid waste incinerator fly ash, there was a significant correlation between EPFRs concentrations and hydroxyl and superoxide radical concentrations²⁵; EPFRs adsorbed on PM_{2.5} induced hydroxyl radical production in aqueous and hydrogen peroxide solutions, and the concentration of hydroxyl radical increased consistently with the time of induction²⁶. The major toxicity inducer EPFRs were found to produce ROS in human cells when the potential health effects of EPFRs in coal soot were investigated using the healthy human bronchial epithelial cell line 16HBE as a model²⁷. The respiratory exposure of humans to EPFRs present in the actual atmosphere is usually expressed in terms of the equivalent number of inhaled cigarettes, and studies have shown that the amount of EPFRs inhaled into the body per person in Xi'an is equivalent to smoking five cigarettes per day, and can be as high as about 23 cigarettes during winter haze²⁸. At present, the studies described above show that toxicity risk exists of EPFRs generated by laboratory simulations, but they have mostly focused on simulated EPFRs with a few ROS, and studies on the association between complex EPFRs and overall oxidative toxicity in actual atmospheric PM are lacking. The EPFRs

of actual atmospheric PM may differ from or be more complex than those generated by laboratory simulations, additionally, the oxidative toxicity of atmospheric PM cannot be represented a few ROS used in previous studies the overall. Therefore, in this study, in order to clarify the scientific questions of whether and how much oxidative toxicity of EPFRs in atmospheric PM, we investigated the oxidative toxicity characteristics of road dust and the fugacity characteristics of EPFRs in Xi'an, a large dusty city in northwest China, and explored the correlation analysis of ROS with EPFRs, as well as metal elements and carbonaceous fractions. The most important finding of this study is that EPFRs have important oxidative toxicity in road dust and the toxicity of EPFRs are determined by the type of free radicals.

2. Results and discussion

2.1 New environmental risk substances EPFRs in road dust

2.1.1 Concentrations and types of EPFRs in road dust

According to the electron paramagnetic resonance (EPR) analysis of the collected samples, it was determined that road dust contained EPFRs with a mass concentration range of $(1.4\sim40) \times 10^{17}$ spins/g, and an average mass concentration of $(6.6\pm5.0) \times 10^{17}$ spins/g, the specific concentration of samples shown in Table S1. As shown in Fig. 1 the level of concentration is comparable to the results that have been reported. However, compared with the concentration of EPFRs in PCP-contaminated soil (2.02×10^{18} spins/g), uncontaminated soil (7×10^{16} spins/g), asphaltene (6.7×10^{18} spins/g), and Xi'an atmospheric fine PM (2.65×10^{18} spins/g)^{28,29,30}, the EPFRs mass concentrations of samples are much lower. Compared with the mass concentrations of EPFRs in uncontaminated soil, the EPFRs mass concentrations of samples are much higher. Based on this result, converted to equivalent cigarette inhalation of EPFRs, the number of cigarettes inhaled by Xi'an citizens was 0.6 cigarettes per person per day and 219 cigarettes per year. Road dust can be raised repeatedly by external forces such as vehicle movements, increasing the risk of exposure to EPFRs from outdoor activities of urban

residents, and thus the health risks caused by road dust must be considered need to be taken into account especially in dusty cities in northwest China. As shown in Fig. 1, the city of Xi'an was divided into four regions based on the trajectories of metro lines 1 and 2, and found that the mass concentrations of EPFRs in road dust were significantly higher in region II, the eastern part of Xi'an, than in the other three regions. This is due to the concentration of coal thermal power plants, dense transportation hubs, and construction sites in region II. Among these, the transport and combustion emissions of coal used in thermal power plants are probably the most important source of EPFRs, along with an increase of mobile source emissions due to the large traffic flow and construction dust from building construction, both of which are non-negligible sources of EPFRs in road dust. There are various particle sizes of PM in road dust, and the mass concentrations of EPFRs in PM of different particle sizes are very different. In this research, the mass concentrations of EPFRs in road dust ($PM < 75 \mu m$) were found to be two orders of magnitude lower than those in $PM < 1 \mu m$, $PM 1.0-2.5 \mu m$, and $PM 2.5-10 \mu m$ ³¹. Road dust particles have a larger particle size, a smaller surface area, and a less porous surface than fine particles, resulting in fewer free radical formation sites, and a decrease in the concentration of EPFRs³².

Fig.1: Concentration of EPFRs in road dust samples in Xi'an and comparison with the concentration of EPFRs in different samples. (a) Spatial distribution map of EPFRs concentrations in road dust

139 samples in Xi'an. The spatial territory was divided into four regions according to geographical
140 locations (I. Northwestern region, II. Northeast region, III. Southwest region, IV. Southeast region).
141 The more red color in the figure represents the higher concentration of EPFRs in the region, and the
142 more blue color represents the lower concentration of EPFRs in the region. **(b)** Comparison of
143 EPFRs concentrations in different samples and road dust samples, where the red font represents road
144 dust samples. **(c)** Comparison of EPFRs concentrations in different particle sizes of atmospheric
145 particulate matter, where the red font represents road dust samples ($PM_{<75\ \mu m}$).

146 Based on the signal characteristics of EPFRs, it was found that the EPFRs of road
147 dust is mainly C-centered radical types. As we all know, the g factor is one of the most
148 important parameters for distinguishing types of EPFRs, and as shown in Fig. 2, the g
149 factors of road dust EPFRs range from 2.0024 to 2.0036 (mean: 2.0030 ± 0.0002), and
150 according to the g-factor classification principle, the unpaired electrons are closer to the
151 oxygen atoms, the g-factor is larger, and it can be determined that the types of EPFRs
152 in road dust in Xi'an are mainly C-centered radicals. Meanwhile, some research has
153 shown that C-centered radicals are relatively more active than O-centered radicals,
154 additionally, C-centered radicals are easily oxidized by reactive oxygen to be degraded
155 and to form semi-quinone and quinone oxygen radicals³¹. Another characteristic
156 information of EPFRs is the linewidth ΔH_{p-p} , which represents the relaxation time of
157 electrons returning from higher to lower energy levels after receiving energy transitions
158 and can reflect the structure of precursor molecules, however, the wider linewidth is
159 associated with organism-metal interactions¹³. The ΔH_{p-p} of road dust EPFRs was
160 mainly concentrated between 5.5 and 7.0 Gs (mean ΔH_{p-p} : 6.1 ± 0.6 Gs), and the
161 EPFRs spectral lines showed both symmetric and asymmetric types (Fig. S5),
162 indicating that many different types of radical species coexisted in the samples³². As
163 shown in Fig. 2, the EPFRs characteristic information g-factors and ΔH_{p-p} reported in
164 other literature were compared and analyzed with the results of this study, and it was
165 found that the information of multiple types and multiple sources of EPFRs engraved
166 in the spectrum, such as soil, e-cigarette, and asphalt were included in the range of road
167 dust characteristic information, indicating that the types and sources of EPFRs in road

dust are more abundant and complex. Such as, urban roads are mostly constructed based on asphalt, and the EPFRs contained in asphalt present a single, symmetric signal with a characteristic information g-factor of 2.0035 and ΔH_{p-p} of 5.1 G³⁰, and long-term wear and tear between cars and the ground cause asphalt to detach from the roadbed and turn into tiny particles that become ground dust, carrying asphalt EPFRs into the atmosphere.

Fig.2: The distribution of EPFRs concentration in road dust with g-factor and line width in Xi'an city. (a) The numbers in the grid represent the number of samples in the range of g-factor and ΔH_{p-p} . The darker blue color represents more samples. The blue boxes represent the range of g-factors and ΔH_{p-p} of EPFRs in soils and sediments. The green box represents the range of g-factors and ΔH_{p-p} of EPFRs in medical waste incinerates. The yellow box represents the range of g-factors and ΔH_{p-p} of EPFRs in soils contaminated with pentachlorophenol. The orange box represents the range of g-factors and ΔH_{p-p} of EPFRs in e-cigarettes. The red boxes represent the range sizes of g-factors and ΔH_{p-p} for EPFRs in asphaltene. (b) The number in the grid multiplied by 10^{17} represents the average mass concentration of EPFRs in all road dust samples in this grid, where the darker orange color represents the higher mass concentration of EPFRs.

2.1.2 Lifetime of EPFRs in road dust

To investigate the lifetime of EPFRs in road dust, EPR analysis was performed again on samples placed at room temperature and protected from light for 450 days, and it was found that EPFRs are decayable in road dust, and information on the

concentrations and types of EPFRs in samples after decay can be found in Tab.S2. Fig.3 shows the changes of different types of EPFRs before and after decay, and we found that more than 90% of the samples have different degrees of attenuation. The average half-life $t_{1/e}$ is 1650 days, and samples with >50% decay account for 52% of the total number of samples. The g-factor of the samples increased from 2.0030 to 2.0034 during the decay process, indicating that the type of EPFRs changed to a mixture of C-centered and O-centered radicals after the decay. On the contrary, the g-factor of EPFRs in the non-decaying type samples is decreasing, from 2.0028 to 2.0022, and most of these radicals are present inside the particles or confined in the solid matrix, making these radicals that remain inside unable to be oxidized in air and therefore more persistent existence⁷. The samples with high concentrations of EPFRs, indicated by the orange arrows in Fig. 3a, occurred at sampling sites in region II of the distribution, and the mass concentrations of EPFRs decreased rapidly after decay, indicating that the majority of the EPFRs before decay belonged to the type of rapid decay. The mass concentration of EPFRs in the samples indicated by the blue arrows increased rather than decreased. It was found that the sampling points of these samples were located near gas stations, where the traffic flow converges, and not only a large amount of primary EPFRs can be emitted, but also precursors such as PAHs, which can be oxidized by air to form O-centered secondary EPFRs³³, which can be confirmed by the change of g-factor before and after decay. The g-factor of road dust samples after decay is 0.0004 higher than the original samples, indicating that the oxidation of substances in road dust samples causes the type of EPFRs to change from C-centered radicals to O-centered radicals, resulting in the decomposition of C-centered radicals and thus increasing the proportion of O-centered radicals. Also comparing the changes of ΔH_{p-p} before and after the decay, it was found that the linewidth ΔH_{p-p} of the decayed sample was (6.0 ± 1.0) Gs, which was basically the same as the linewidth ΔH_{p-p} of the original sample EPFRs, which was consistent with the results reported by Gehling and Dellinger⁷.

The obtained EPR spectra were segmented by using the least squares method to fit

the spectra of different types of EPFRs, and the signal characteristics (g-factor and ΔH_{p-p}) and intensity of C-centered EPFRs (C-EPFRs) and O-centered EPFRs (O-EPFRs) were obtained, respectively³⁴. The results are shown in Fig. 3a and b. The types of EPFRs in road dust in Xi'an were mainly C-centered radical types (>80%) both before and after decay. After decay, C-centered EPFRs quantity decreased in 66% of the samples, while O-centered EPFRs quantity decreased in 34% of the samples, indicating that C-EPFRs have more reactivity than O-EPFRs. For example, the half-life $t_{1/e}$ of C-centered phenoxy radicals was 0.25-21 days, while the half-life $t_{1/e}$ of O-centered semiquinone radicals was high at 21-417 days⁷.

Fig.3: Mass concentration changes of EPFRs in road dust samples before and after decay and EPR spectra. (a) The orange arrows in the figure represent the samples with great mass concentration of EPFRs in dust. (b) The blue arrows in the figure represent the samples with increased mass concentration of EPFRs in dust after decay. (c) Signal plots of EPFRs before and after decay of sample #11 after correction. (d) Signal plots of different types of EPFRs before and after decay of sample #11 after correction. (e) Percentage plot of samples with different half-lives, where others represent samples with increased mass concentration of EPFRs after 450 d.

2.2 Oxidative toxicity of road dust

The study used the consumption rate of the reducing substance dithiothreitol (DTT) in simulated lung fluid contains road dust samples to represent its oxidative toxicity

level, and the results showed that the collected road dust was oxidatively toxic. As shown in Fig. 4, the total oxidative toxicity of road dust (Total-DTT) was (0.34 ± 0.08) $\mu\text{mol}/\text{min}/\text{g}$, which is smaller than both the oxidative toxicity of atmospheric $\text{PM}_{2.5}$ (0.151 ± 0.02 $\text{nmol}/\text{min}/\mu\text{g}$) and organic aerosols (0.036 ± 0.022 $\text{nmol}/\text{min}/\mu\text{g}$) in the southeastern United States³⁵ and also smaller than the oxidative toxicity of local atmospheric $\text{PM}_{2.5}$ in Xi'an (0.12 $\text{nmol}/\text{min}/\mu\text{g}$)³⁶. Such results are primarily related to the particle size distribution and chemical composition of the PM, with road dust containing more coarse particles of relatively low toxicity. In the study of the oxidative toxicity of atmospheric PM in Italy, it was found that the local coarse particles increased significantly after the impact of Saharan dust storms, while the rate of DTT depletion by PM_{10} was (3.1 ± 1.7) $\mu\text{mol}/\text{min}/\text{g}$, which was significantly lower than the rate of DTT depletion in $\text{PM}_{2.5}$ (9.2 ± 5.2) $\mu\text{mol}/\text{min}/\text{g}$ ³⁷. In this study, the oxidative toxicity of road dust was divided into water-soluble toxicity (WS-DTT) and water-insoluble toxicity (WIS-DTT), and the results as Fig. 4b showed that the consumption rates of WS-DTT and WIS-DTT were (0.1 ± 0.03) $\mu\text{mol}/\text{min}/\text{g}$ and (0.24 ± 0.08) $\mu\text{mol}/\text{min}/\text{g}$, respectively, with WIS- DTT accounted for 71% of the Total-DTT consumption rate, indicating that the water-insoluble fraction was the main contributor to the oxidative toxicity of road dust. Verma et al. similarly found that water-insoluble fractions deplete DTT at a faster rate than water-soluble fractions when studying the oxidative toxicity level in different fractions of atmospheric PM, and emphasized that the oxidative toxicity of PM is related to the organic carbon (OC) content³⁸.

Fig.4: (a) Plot of DTT consumption rate variation in road dust samples in Xi'an. (b) The boxes in the graph represent the 25th, 50% and 75% percentiles, and the numbers represent the mean values.

The water-soluble and water-insoluble fractions of road dust not only differ in the proportion of oxidative toxicity but also in the kinetic characteristics of the released toxicity. In this study, road dust samples were tested for a longer period of time than in previous studies using DTT for oxidative toxicity, up to 180 min (previous studies: 40 min). The results of the study are shown in Fig. 5. We divide the whole process into three parts according to the characteristics of the consumption rate of Total-DDT with time. Part I is the period of rapid DTT consumption, part II is the period of slow DTT consumption, and part III is the period of smooth DTT consumption. The water-insoluble fraction in road dust has been greater than the consumption of DTT by the water-soluble fraction during the whole DTT consumption period, but the consumption ratio of DTT by the water-insoluble fraction decreases with the increase of time, which indicates that the oxidative toxic substances of the water-insoluble fraction in road dust have the characteristics of rapid release of toxicity and irreversible characteristics. In contrast, the oxidative toxicity of the water-soluble fraction has maintained a stable release characteristic, indicating that the toxic-producing substances present in the water-soluble fraction from a cyclic process of oxidative toxicity production, and it is

known that transition metals and quinones can continuously produce ROS through the redox cyclic process²⁴. The majority of EPFRs in the atmospheric PM is present in the water-insoluble fraction³⁶, and it is speculated that the rapid rate of DTT consumption in the water-insoluble fraction of road dust may be attributed to the C-centered EPFRs attached to the particle surface and actively catalyze reacting to produce ROS from oxygen molecules⁷, showing a rapid kinetic process of ROS generation. However, as the external EPFRs are consumed, the internal EPFRs are difficult to change in a short period of time due to the adsorption location and their own structure, which leads to the slow DTT consumption rate of water-insoluble fraction in the later stage.

Fig.5: Plots of DTT consumption in road dust samples with time. Where k_{Total} , k_{WS} , and k_{WIS} (pmol/min) represent the consumption rates of Total-DDT, WS-DDT, and WIS-DDT, respectively. The pie charts represent the ratio of DTT consumption to Total-DDT consumption in road dust without fractions.

2.3 Contribution of novel environmental risk substances EPFRs to ROS

In this study, it was found that EPFRs contribute to the oxidative toxicity of road dust and that the toxicity of EPFRs is related to their type. The oxidative toxicity of

road dust is the result of a combination of toxicity factors, which definitely include the contribution of carbon fractions and metals. To explain the oxidative toxicity of road dust, we analyzed the content of OC, EC, and 53 metals in the samples (Fig. S6 and S7), and classified the main 18 metal elements in metals according to their magnetic properties: anti-magnetic metals (Cu, Zn, Cd, Pb), anti-ferromagnetic metals (Ti, Cr, V, Mn), paramagnetic metals (Al, Ca, K, Mg, Na, Cs, Ba), and ferromagnetic metals (Fe, Co, Ni). We assumed that EPFRs, OC, EC, and metals all have positive contributions to the oxidative toxicity of road dust particles, and in this study, the contributions of different components to the oxidative toxicity of road dust were quantitatively evaluated based on the positive matrix factorization (PMF) model (Fig. S8, 9, 10), and three main factors were identified: metal element-dominated factors, EPFRs-dominated factors, and composite factors.

As shown in Fig. 6, it was found that Total-DTT and WIS-DTT showed a weak correlation with the concentrations of EPFRs after decay, but not with any other components, indicating that EPFRs may contribute to the oxidative toxicity of road dust. To concretely investigate the contribution of EPFRs to oxidative toxicity in road dust, a correlation analysis was performed between different types of EPFRs and DTT consumption rates in road dust before and after decay (Tab. S3), and there was no correlation between the original EPFRs, whether C-centered EPFRs or O-centered EPFRs, and Total-DTT and WIS-DTT. However, the decayed C-centered EPFRs showed a weak correlation with Total-DTT and WIS-DTT ($r_1=0.25$, $r_2=0.23$, $p<0.05$), this result indicates that different types of EPFRs contribute differently to the oxidative toxicity of road dust, where C-centered EPFRs are more reactive and easily react with O_2 to form ROS, while converting themselves to more stably existing EPFRs⁷. In order to investigate the relationship between oxidative toxicity of road dust and metal elements, the study did a correlation analysis between the DTT consumption rate of road dust samples and metal element concentrations (Tab.S5) and found that the Total-DTT, WIS-DTT, and anti-magnetic metal concentrations showed weak correlations, specifically with metals Zn and Pb. Meanwhile, the PMF model results showed that the

contribution of EPFRs-dominated factors to the Total-DTT and WIS-DTT was 17.3% and 33.3%, respectively, but the contribution of Metals-dominated factors was 40.6% and 47.5%, respectively. It indicates that metals is the main contributor to the Total-DTT and WIS-DTT, whereas EPFRs is one of the important contributing components to the oxidative toxicity of road dust. Studies generally suggest that EPFRs can catalyze O_2 to $\bullet O_2^-$ through electron transfer and disproportionation reactions with hydrogen ions to produce hydrogen peroxide, whereas $\bullet OH$ are generated through Fenton reactions involving iron ions³⁹.

Fig. 6: Schematic representation of the contribution of different components to oxidative toxicity

and the amount of contribution in road dust in Xi'an city. **(a)** Blue represents no correlation; orange represents correlation and **. At the 0.01 level (two-tailed), the correlation is significant; *. At the 0.05 level (two-tailed), the correlation is significant. **(b)** Contribution of different fractions of road dust samples to Total-DTT consumption rate as a percentage. **(c)** Contribution of different fractions to the consumption rate of WS-DTT in road dust samples. **(d)** Contribution of different fractions to the consumption rate of WIS-DTT in road dust samples.

The oxidative toxicity of the water-soluble road dust was mainly contributed by carbonaceous component and metals. As shown in Fig. 6, the WS-DTT was positively correlated with carbonaceous components (TC and OC: $r_1 = 0.253$, $r_2 = 0.263$, $p < 0.05$), indicating that OC contributed to the oxidative toxicity of the water-soluble fraction of road dust, which is consistent with previous findings that water-soluble organics include quinones and HULIS in the fraction are primarily responsible for the WS-DTT^{35,40,41}. There was also a weak correlation with the metal elements Al, K, and Mg, however no correlation between the WS-DTT and EPFRs, either with the total mass concentration of EPFRs before and after decay or with the concentration of different types of EPFRs before and after decay. Previous correlation studies have shown that EPFRs are mainly present in the WIS fraction, and therefore EPFRs are not expected to be the main contributor to the WS-DTT. By PMF model, the study determined that EPFRs were a factor in the oxidative toxicity of water-soluble road dust with a percentage of 17.9%. It's worth noting that EPFRs signals have been detected in HULIS fractions and that EPFRs are more unstable and active²¹. Highway PM_{2.5} are found to generate mainly •OH in the aqueous phase. The •OH show a good correlation with EPFRs, implying the role of EPFRs in aqueous •OH generation⁴². The isolated Metals-dominated factor contributed 20% of the WS-DTT, but the metals actually contributed much more than that (Fig. S9), as more metal elements were present in the complex-type factor (62% of the WS-DTT contribution). The complex-type factor is a composite contribution of OC and metals, and probably due to their interaction in producing oxidative toxicity. The contribution of metals to WS-DTT in this study was expected to be in the range of 20%-

80%, and assuming a 50/50 contribution of metals and OC in the composite factor, the final contribution of meats to WS-DTT was 51%, which is similar to the results of the contribution of transition metals (Cu and Mn) to DTT activity ($55\pm 13\%$) found in a previous study by Lyu et al⁴³.

3. Materials and methods

3.1 Road dust sample collection

The roads in Xi'an, China, were divided and sampling points were set up, with a total of 134 sampling points, and the specific division method and sampling point locations are shown in Fig. S1. Sampling was performed at the set sampling points using a vacuum cleaner (V10, Mi, China)^{44,45}, and the collected samples were placed on tin foil in sealed bags for storage, transported back to the laboratory, equilibrated in a desiccator for 24 h, sieved through 200 mesh, and stored in a refrigerator at -20°C until analysis, as detailed in Text S1.

3.2 Sample pretreatment and EPFRs analysis

In this study, the road dust samples were pretreated by the dispersion method in order to facilitate accurate placement of the PM on quartz filter membranes (see Text 1 for specific information). A 20 mg road dust sample was weighed into a glass vial, 0.5 ml of methanol (chromatographic pure, Fisher, USA) was added for shaking and dispersion, and 100 μl of the mixture was pipetted in a uniform drop onto a pre-cut quartz filter membrane (28 $\times$ 5 mm) immediately after shaking well. The quartz filter membrane containing road dust was then rotary evaporated for 30 min using a rotary evaporator (RE100-Pro, Beijing Dalong Xingchuang Experimental Instrument Co., Ltd.), in order to completely evaporate the methanol from the mixture while also allowing the road dust to be uniformly distributed on the quartz filter membrane.

The EPR was set as follows: magnetic field intensity 330-345 mT; detection time 60 s; modulation amplitude 0.20000 mT; the number of detections 1; microwave intensity 8.0 mW. The obtained EPR spectra were analyzed using the method shown in

Text S2. The EPR spectra were resolved using the method shown in Text S2. EPR was performed on standards (inner diameter: 3.0 mm, outer diameter: 4.0 mm; Freiberg Instruments Inc., Germany) containing Mg^{2+} and Cr^{3+} before and after the determination of each batch of samples simultaneously and was used to calibrate the absolute spin amount and g-factor of the samples. Signal processing of EPFR uses the software developed by our research group (Fig.S3). The mass concentration of EPFRs in road dust (spins/g) was calculated as follows²⁸:

$$S_{EPFRs} = S_{Cr^{3+}} \cdot \frac{A_{EPFRs}}{A_{Cr^{3+}}} \cdot m_{dust}^{-1} \quad (1)$$

Where, $S_{Cr^{3+}}$ represents the total spin amount of chromium standard, A_{EPFRs} and $A_{Cr^{3+}}$ represent the area of the integral curve in the EPR absorption curves of the sample and chromium standard, respectively, and m_{dust} represents the road dust mass (g). In this study, the EPR absorption curve area was expressed as the peak-to-peak distance multiplied by the line width squared.

To determine the decay behavior of EPFRs in road dust, the road dust samples after EPR detection were placed at room temperature (temperature $25^{\circ}C \pm 5^{\circ}C$ and relative humidity of $60 \pm 5\%$) and analyzed for EPR again after an interval of 450 days. A first-order decay model was used to fit the decay data and calculate the 1/e lifetime of the radical ($t_{1/e}$)³¹. The linear equation of the model was:

$$\ln(c/c_0) = -kt \quad (2)$$

$$t_{1/e} = 1/k \quad (3)$$

To evaluate the magnitude of potential health risks to Xi'an residents from EPFRs in atmospheric PM, we used the equivalent number of cigarettes to represent the daily exposure to EPFRs per person. The method for converting the daily concentration of EPFRs in $PM_{2.5}$ inhaled by adults to the number of cigarettes is shown in Equation 4:

$$N_{cig} = \frac{(C_{EPFRs} \cdot V \cdot C_{PM} \cdot \eta)}{(RC_{cig} \cdot C_{tar})} \quad (4)$$

Where, N_{cig} denotes the number of cigarettes; C_{EPFRs} (spins/g) denotes the mass concentration of EPFRs in road dust; V denotes the amount of air inhaled by adults per

day ($20 \text{ m}^3/\text{day}$)⁴⁶; C_{PM} denotes the mean of atmospheric PM mass concentration in Xi'an during the sampling period ($104 \text{ }\mu\text{g}/\text{m}^3$); η denotes indicates the percentage of EPFRs in PM from road dust (27%)¹⁴; RC_{cig} indicates the concentration of free radicals in cigarette tar ($4.75 \times 10^{16} \text{ spins/g}$)⁴⁷; C_{tar} indicates the amount of tar per cigarette (0.013 g/cig)²⁴.

3.3 Oxidation toxicity analysis of road dust

The rate of DTT consumption in the simulated lung fluid was used to express the ability of road dust to produce oxidative toxicity. The basic principle is that when DTT is used to measure the oxidative toxicity of atmospheric PM, the ROS produced by the PM oxidizes DTT to DTT-disulfide compounds⁴⁸. The reaction of particle-generated ROS continues, causing DTT to be consumed continuously, which is terminated when DTNB is added at regular intervals. The remaining DTT concentration is obtained by measuring the absorbance of the solution after the reaction, calculating^{49,50} the rate of DTT consumption by particles is, and the rate of DTT consumption within 40 min satisfies zero-level kinetic relationship.

The road dust samples ($1 \text{ mg}/\text{mL}$) were vortexed for 5 min using a vortex mixer (MX-S, SCILOGEX, America). 1 mL of the mixture and 0.5 mL of DTT (0.5 mM, 98%, Sigma-Aldrich, prepared with 0.1 M phosphate buffer pH=7.4) were taken from each of the six centrifuge tubes, and the tubes with the mixture and DTT were placed in a water bath at 37°C in a water bath nitrogen blower (YY-DCY-12S, Yunyan Instrument, China) and 1 mL of TCA (2%, w/v) was added at 0, 5, 10, 15, 25, and 40 min, and filtered with a $0.45 \text{ }\mu\text{m}$ filter tip (Jinteng Instrument, China). After filtration, 0.5 mL of filtrate plus 0.5 mL of DTNB (0.5 mM, 98%, Sigma-Aldrich, prepared with 0.1 M phosphate buffer pH=7.4) and 2 mL of Tris-HCl buffer (0.08 M Tris-HCl containing 4 mM EDTA, pH adjusted to 8.9 by adding HCl dropwise) were removed from each centrifuge tube. The sample mixture was filtered immediately after preparation to obtain water-soluble samples for DTT consumption rate experiments, which distinguishes the WS-DTT experimental protocol from Total-DTT. Finally, a UV-vis spectrophotometer was used to measure the absorbance of the samples at 412 nm

(Abs₄₁₂) (AquaLog, HORIBA, Japan). The concentration of DTT in the sample solution was quantified by Abs₄₁₂, and the DTT consumption rate was calculated based on the remaining DTT concentration in the samples at different reaction times. To minimize potential interference during the experiment, the entire procedure was carried out under as light-proof conditions as possible, while ensuring that all samples were assayed within 2 hours.

3.4 Analysis of other components

3.4.1 Metal element analysis

The study was conducted to detect the concentrations of Total-Metal and WS-Metals in road dust using an inductively coupled plasma-mass spectrometry (ICP-MS) instrument (iCAP-RQ, Thermo Fisher, USA). The detection of total metals in road dust samples was handled by first weighing 100 mg of road dust samples and adding a certain amount of HNO₃, HCl, HF, and H₂O₂ to the digestion, and after the digestion was completed, all acid solutions were sublimated until almost dry. After the digestion tube was cooled to room temperature, deionized water was added to dissolve the solution, and the solution was transferred to a volumetric flask and the supernatant was measured by ICP-MS.

The detection of metal elements in the water-soluble road dust fraction was performed by weighing 40 mg of road dust sample, adding 8 ml of ultrapure water and sonicating for 1 h (temperature not exceeding 30°C during ultrasound), filtering with a 0.45 µm Teflon filter tip, and finally obtaining the supernatant to determine the content of water-soluble metal elements by ICP-MS.

The main operating parameters of ICP-MS were: RF power: 1550 W, atomization gas flow rate: 1.05 L/min, cooling gas flow rate: 14.0 L/min, auxiliary gas flow rate: 0.75 L/min, sampling cone aperture: 1.0 mm, interception cone aperture: 0.7 mm, scanning mode: peak hopping, integration time: 0.5 s, injection time: 30 s.

3.4.2 OC/EC analysis

The samples were pretreated (Text S3) and then analyzed by OC/EC analyzer

(DRI-2001A, Sunset, America) using the IMPROVE-A protocol to quantify the carbonaceous fraction using thermoluminescence. The OC components volatilize and decompose, some directly into CO₂, others into small OC molecules, and all organic molecules are converted into CO₂ when they enter the MnO₂ catalytic furnace. The generated CO₂ is measured by a non-dispersive infrared detector, and the signal is quantified based on the signal of standard methane gas. The signal size of the sample is quantified by the signal of the standard methane gas. After the OC stage, the elemental carbon (EC) stage is entered, where a helium-oxygen mixture (2% O₂) is introduced and heating is continued to increase the temperature, producing different ECs in different stages. It is worth noting that a part of the OC is converted to EC in the OC phase due to the coking of OC, also known as the carbon coking (OPC). The part of EC should also be counted in the OC. To calculate the amount of OPC generated, the intensity of transmitted light during the warming process of the sample is measured by using a laser at a wavelength of 635 nm, and due to the generation of OPC, the intensity of transmitted light will first decrease and then increase, and when its value agrees with the initial signal value, this time point is the splitting point between OC and EC. Therefore, the total organic carbon TOC is defined as TOC = OC + OPC; the total elemental carbon TEC is defined as TEC = EC - OPC; TC = OC + EC.

3.5 Statistical analysis

To investigate the relationship between road dust oxidative toxicity and EPFRs in terms of statistical presence, Pearson correlation analysis (strong: $r > 0.7$, moderate: $0.5 < r < 0.7$, weak: $r < 0.5$) and analysis of variance (ANOVA) tests were performed in the corresponding statistical analyses. All statistical data were processed using the data processing software statistical program (SPSS, version 25.0, IBM, USA). The EPA PMF5.0 model was also used to obtain the percentage contribution of the different components of road dust to DTT consumption^{51,52,53,54}. In this study, the concentrations of EPFRs, OC, EC, Na, Mg, Al, Ca, K, Fe, Pb, Zn, Mn, Ba, Cu, Si, As, Ni, Ti, V, Cr, and Cd from 134 road dust samples in Xi'an were input into the EPA PMF 5.0 model. In the model, the signal-to-noise ratio (S/N) ranges from 1 - 10 for "Strong", from 0.5

- 1 for "Weak", and from 0 - 0.5 for "Bad". Jaeckels et al⁵⁴ describe in detail how to use PMF 5.0 and the specific steps and requirements for data entry. When the concentration of a component is below the detection limit of the method, the concentration of the component is replaced by half of the MDL, and the uncertainty is calculated using the formula: $Unc. = K \times Conc$, where Unc. is the uncertainty, Conc is the concentration of the component, and K is the analytical uncertainty; the missing value is replaced by the median of the measured value of the component, and the uncertainty is 4 times the median of the component to reduce the influence on the model results. The rotation of the model results is controlled by the FPEAK and FKEY values, and the detailed basic operation and analysis of the results are described in the model specification and other literature^{55,56}.

Author contributions.

Hao Li and Qingcai Chen designed the experiments, data analysis and prepared the paper with the contributions from all co-authors; Chao Wang and Ruihe Wang designed the experiments and provided financial support; Xiqi Yang participated in the sample collection; Dyussenova Ainur participated in writing the paper; Yuqin Wang is responsible for PMF model analysis.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version.

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