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Can oestrogenic activity in air contribute to the overall body burden of endocrine disruptors?

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ABSTRACT

Endocrine disruptors (EDCs) are emerging contaminants that are harmful to health. Human exposure occurs mainly through ingestion or dermal contact, but inhalation could be an additional exposure route; therefore, this study was conducted to evaluate the oestrogenic activity of airborne particulate matter (PM). Outdoor PM was collected for a year in five Italian sites and extracted with organic solvents (four seasonal extracts/site). The oestrogenic activity was assessed using a gene reporter assay (MELN), and the risk to human health through inhalation was quantified using the results. Moreover, extracts were analysed to assess cytotoxicity (WST-1 and LDH assays) on human bronchial cells (BEAS-2B). The extracts induced a significant cytotoxicity and oestrogenic activity. Oestrogenic activity showed a seasonal trend and was correlated with concentrations of benzo(a)pyrene and toxic equivalency factor. Although a low inhalation cancer risk was found, this study confirmed that oestrogenic activity in air could contribute to overall health risks due to EDC exposure.

1. Introduction

Outdoor air pollution is a great environmental health problem. Numerous epidemiological, clinical and toxicological studies have demonstrated that air pollution causes adverse effects in humans, such as respiratory symptoms, cardiovascular effects and lung cancer (Rojas-Rueda et al., 2021). Moreover, exposure to air pollution was also associated with other adverse effects, particularly effects on fertility, pregnancy, newborns and children (Nyadanu et al., 2022; Wang et al., 2021).

Particulate matter (PM) is among the most important proxy indicators of outdoor air pollution. It can be defined as a mixture of fine solid or liquid droplets suspended in the air with a heterogeneous composition, including elemental and organic carbon, biological components (e.g., allergens and microbial compounds), inorganic components (e.g., trace metals, nitrates, sulfates, ammonium), and organic compounds (e.g., polycyclic aromatic hydrocarbons) (Kim et al., 2015; Peixoto et al., 2017). This mixture could cause different biological responses, so evaluating PM using effect-based tools, such as in vitro assays, is important for assessing the cumulative risk of PM exposure on human health. Numerous in vitro assays have been applied to test the cytotoxicity, mutagenicity, genotoxicity and inflammatory response induced by PM (Bonetta et al., 2019; Gea et al., 2021; Marangon et al., 2021). However, few studies have been performed to test the oestrogenic activity of this environmental matrix by performing different in vitro assays and obtaining different results according to sampling site and sampling period (Chen et al., 2013; Croes et al., 2016a, 2016b; Gea et al., 2023; Matsumoto et al., 2005; Novák et al., 2020; Nováková et al., 2020; Oziol et al., 2017; Wang et al., 2004; Wooten et al., 2015; Zhou et al., 2022).

Oestrogenic compounds are ubiquitous in the environment and can induce oestrogenic effects, even at very low concentrations, causing reproductive and developmental disorders in humans and animals (Pamplona-Silva et al., 2018). In humans, exposure to oestrogenic compounds causes numerous effects, including cancer of hormone-sensitive organs (e.g., breast, prostate, testis), early puberty and reduced fertility (Kabir et al., 2015; Pamplona-Silva et al., 2018; Yilmaz et al., 2020). These compounds can interfere with the function of

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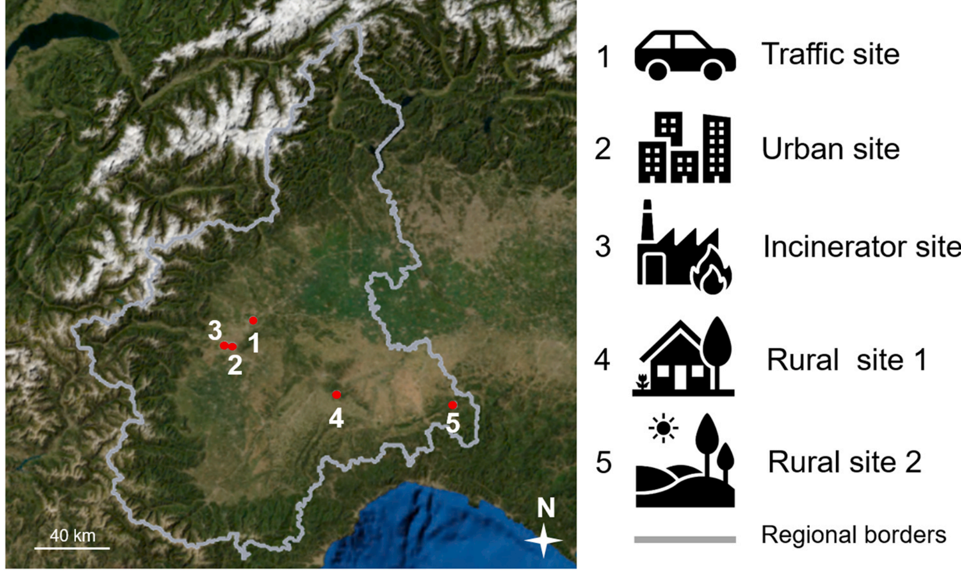


Fig. 1. Sites at which particulate matter (PM) was sampled. The grey line identifies the regional borders of the Piedmont region, northwest Italy.

natural oestrogens, i.e., estrone, oestradiol and estriol (Kiyama and Wada-Kiyama, 2015), so they are classified as endocrine disrupting chemicals (EDCs), which are "exogenous substances or mixtures that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub) populations" (Zoeller et al., 2012). EDCs are ubiquitous in the environment, and they have been found in water, soil, food and air. Their presence has been extensively monitored to assess oral and dermal exposure, but their presence in air has been less studied. In recent years, a growing concern has been the potential adverse effects caused by inhalation of these compounds (Darbre, 2018).

The aim of this study was to assess the oestrogenic activity of PM organic extracts. PM_{10} (ø <10 μm) and $PM_{2.5}$ (ø <2.5 μm) were collected over a year at five different sites with different pollution sources (an urban area with high traffic incidence, an urban area with low traffic incidence, an urban area near an incinerator and two rural areas). Filters were pooled and extracted to obtain four seasonal extracts for each site. Oestrogenic activity was assessed using a gene reporter assay on MELN cells, and the results were used to quantify the risk for human health associated with exposure to oestrogenic compounds adsorbed on PM. Moreover, the extracts were analysed to assess the cytotoxicity (WST-1 and LDH assays) on human bronchial epithelial cells (BEAS-2B). Finally, monthly concentrations of PM_{10}, $PM_{2.5}$, and four polycyclic aromatic hydrocarbons (PAHs) at the five sites were collected from the Regional Agency for Environmental Protection of Piedmont (ARPA) website and compared with the oestrogenic activity and cytotoxicity results.

2. Materials and methods

2.1. PM sampling and extraction

Sampling was performed at the following monitoring stations in northwestern Italy (Fig. 1):

- traffic site: urban site with high traffic level (city of Settimo T.se, altitude = 201 m);
- urban site: urban site with moderate traffic level (city of Torino, altitude = 243 m);
- incinerator site: urban site near an incinerator (city of Torino, altitude = 262 m);
- rural site 1: rural site (rural village of Vinchio (AT), altitude = 250 m);
- rural site 2: rural site (rural village of Dernice (AL), altitude = 580 m).

PM_{10} (incinerator site) and $PM_{2.5}$ (traffic, urban and rural sites) were daily sampled on quartz-fibre filters (Ø = 47 mm) with a low volume sampler (flow 2.3 m^3/h) over a year. Filters were pooled to obtain one sample for each season, and each pool was extracted through ultrasounds using acetone/cyclohexane (1:1) (Macrì et al., 2023). A rotary evaporator was used to evaporate the solvent, and the organic-extractable compounds were resuspended in dimethyl sulfoxide (DMSO) and stored at − 20 °C.

The concentrations of PM_{10}, $PM_{2.5}$, benzo(a)pyrene (BaP), benzo(a) anthracene (BA), benzo(b+j + k)fluoranthene (BF), and indeno (1,2,3 – cd)pyrene (IP) at the five sites were collected on the ARPA website (Regional Agency for Environmental Protection of Piedmont, 2022).

PAH concentrations were used to calculate the toxic equivalency factor (TEF), which expresses the toxicity of PAH mixtures as BaP equivalents (Gea et al., 2023; Nisbet and LaGoy, 1992; Samburova et al., 2017).

2.2. Cytotoxicity evaluation

The cytotoxic effect of PM organic extracts on the human bronchial epithelial cell line BEAS-2B was assessed using a WST-1 assay (which measures the mitochondrial activity of cells) and a LDH assay (which measures the integrity of the plasma membrane). The assays were performed as previously described by Gea et al. (2021, 2023) using Cell Proliferation Reagent WST-1 (Roche) and the Cytotoxicity Detection Kit PLUS (Roche). BEAS-2B cells were seeded in 96-well plates (5×10^3 cells/well) and cultured overnight. Then, the cells were exposed to organic extracts (5, 10, 25, 50 m^3/mL) for 24 h, 48 h or 72 h.

For the WST-1 assay, after exposure, the content of each well was replaced with a solution of WST-1 dye (dilution 1:10, 100 μL/well), and the cells were incubated for 2 h (37 °C, 5% CO_2). Finally, the absorbance of each well was measured at 440 nm (Infinite Reader M200 Pro, Tecan). Cells exposed to DMSO were used as a negative control.

For the LDH assay, after exposure, 100 μL/well of the Reaction Mixture was added, and the 96-well plate was incubated for 15 min (room temperature). Then, Stop Solution (50 μL/well) was added, and the absorbance was measured at 492 nm (Infinite Reader M200 Pro, Tecan). Cells exposed to DMSO were used as a negative control, while cells exposed to DMSO and lysed with lysis solution were used as a

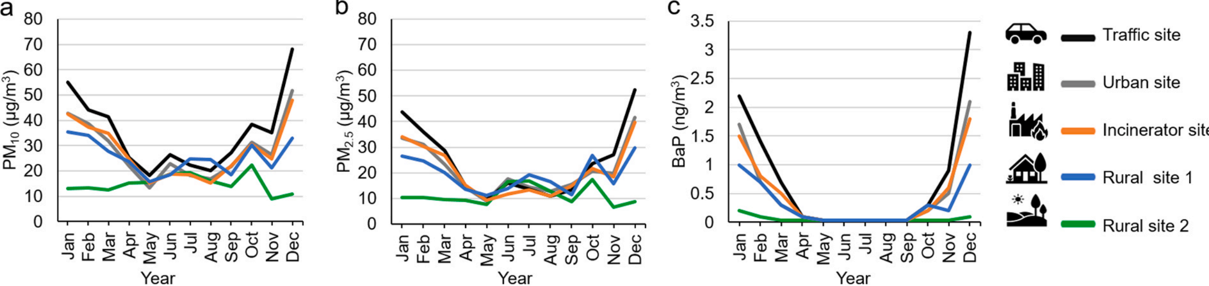


Fig. 2. Seasonal trend in air pollutant concentrations at the five sites: a) PM_{10}, b) $PM_{2.5}$, and c) BaP. BaP = benzo(a)pyrene, PM = particulate matter.

positive control.

All experiments were performed in quadruplicate (four wells for each experimental condition), and data were expressed as a percentage of viability with respect to the negative control (WST-1 assay; % cell viability of negative control = 100%) or as a percentage of LDH release with respect to the negative control (LDH assay; % LDH release of negative control = 0%; % LDH release of positive control = 100%).

2.3. Oestrogenic activity evaluation

Oestrogenic activity was assessed with the luciferase gene reporter assay using the One-Glo Luciferase Assay System (Promega) and MELN cell line that was kindly provided by Dr. P. Balaguer (INSERM, Montpellier, France) (Balaguer et al., 1999; Gea et al., 2021). For three days, cells were cultured in test medium (supplemented with dextran-coated charcoal-treated foetal bovine serum), seeded in 96-well plates (4×10^4 cells/well) and cultured overnight. The next day, the cells were exposed to PM organic extracts (1–50 m^3/mL) and incubated for 20 h. At the end of the incubation, One-Glo Reagent (100 µL/well) was added, and the luminescence was measured by a luminometer (Infinite Reader M200 Pro, Tecan). Cells exposed to DMSO were used as a negative control, and eight concentrations of 17β-oestradiol (E2) (10^{-12} - 10^{-8} M) were tested as a standard positive curve. The extracts (dose of 10 or 25 m^3/mL) were also tested in combination with tamoxifen (an oestrogen receptor antagonist, 10^{-6} M) and in combination with E2 (10^{-10} M) to confirm whether the effect was due to oestrogen receptor activation and to study the interaction of extracts with E2. All experiments were performed in quadruplicate (four wells for each experimental condition). The oestrogenic activity was expressed as relative luciferase activity and was calculated as the percentage of activity induced by the treatment with respect to the activity induced by the positive control, E2 10^{-8} M (relative luciferase activity of E2 10^{-8} M = 100%). The oestrogenic activity of the extracts was also evaluated by determining the E2 equivalent concentration (EEQ). The EEQ was calculated using the concentrations of E2 and extracts at which 50% of the biological effect was achieved (EC50) through the formula:

$$EEQ \quad \left(\frac{pg}{m^3}\right) = \frac{\left[E2EC50\left(\frac{pg}{mL}\right)\right]}{\left[extract \quad EC50\left(\frac{m^3}{mL}\right)\right]}$$

In the experimental conditions, the detection limit was equal to 0.006 pg/m^3.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 28.0. The normality of data was assessed using the Shapiro—Wilk test. The Kruskal—Wallis test and post hoc Tukey's test were applied to compare the pollutant levels among the different sites. The Kruskal—Wallis test and post hoc Dunnett's test were used to assess a significant decrease in % cell viability after PM exposure with respect to the negative control in the WST-1 assay. Moreover, the same statistical analysis was applied to assess a significant increase in % LDH release after PM exposure with respect to the negative control in the LDH assay. For WST-1 and LDH assays, the biological effect was considered significant when, at the same dose, a statistically significant difference with respect to the negative control was detected for all exposure times (24, 48, 72 h). Regarding the MELN gene reporter assay, the EC50 of E2 and PM extracts was calculated by dose–response curves, which were estimated through a probit regression between the relative luciferase activity and log transformed concentrations of E2 or PM extracts. The seasonal trend of EEQ was assessed by comparing the EEQ of winter-autumn extracts vs. the EEQ of spring-summer extracts using the Mann—Whitney assay. Finally, Spearman's rho test was applied to assess a potential correlation between the oestrogenic activity induced by the seasonal extracts of the five sites (expressed as EEQ) and the seasonal mean concentration of BaP or the seasonal mean TEF value in the same sites. Data were considered significant with a p value < 0.05.

2.5. Risk assessment of cancer through inhalation

To quantify the risk associated with exposure to oestrogenic compounds adsorbed on PM, an inhalation cancer risk assessment was performed using the EEQ measured in the present study using the approach reported by ISPRA (2016). The assessment was performed assuming that the mean annual EEQ measured at each site was equal to the mean air concentration of E2 at each site. To quantify the exposure, for each site, the mean annual EEQ was used as the estimate of the E2 air concentration and was multiplied by the daily amount of inhaled air per body mass unit (IE).

$$E = EEQ \times IE$$

E = exposure to PM oestrogenic compounds (mg/kg/day).

EEQ = E2 equivalent concentrations found in the present study (mg/m^3).

IE = daily amount of inhaled air per body mass unit (m^3/kg/day).

To quantify the IE, the adult and child exposures were added using the following formula.

$$IE = \frac{Ia \times Ed \times Ey \times Ea}{BWa \times MEy \times 365\frac{days}{year}} + \frac{Ic \times Ed \times Ey \times Ec}{BWc \times MEy \times 365\frac{days}{year}}$$

Ia/Ic = adult/child inhalation rate (residential exposure 0.9/0.7 m^3/h).

Ed = daily exposure (24 h/day).

Ey = year exposure (350 days/year).

Ea/Ec = adult/child exposure (adult = 24 years; child = 6 years).

BWa/BWc = adult/child body weight (adult = 70 kg; child = 15 kg).

MEy = years of exposure (70 years).

Then, the incremental probability of a person developing cancer over a lifetime (R) was estimated by multiplying the results of exposure to PM oestrogenic compounds (E) by the inhalation cancer slope factor (SF) of E2 (E2 SF = 39 kg day/mg) (OEHHA, 1992).

$$R = E \times SF$$

E = exposure to PM oestrogenic compounds (mg/kg/day).

Table 1
Annual mean concentrations of air pollutants in the five investigated sites in comparison with the Italian or WHO annual values (Italian Legislative Decree 155/2010; WHO, 2021). BA = benzo(a)anthracene, BaP = benzo(a)pyrene, BF = benzo(b+j+k)fluoranthene, IP = indeno(1,2,3 - cd) pyrene, N.A. = guideline value not available, PM = particulate matter, TEF = toxic equivalency factor, WHO = World Health Organization.

	Traffic site	Urban site	Incinerator site	Rural site 1	Rural site 2	Italian limit	WHO guideline value
PM_{10} (μg/m^3)	36	28	28	26	15	40	15
$PM_{2.5}$ (μg/m^3)	24	21	20	19	11	25	5
BaP (ng/m^3)	0.7	0.5	0.5	0.3	0.1	1	N.A.
BA (ng/m^3)	0.6	0.4	0.4	0.2	0.1	N.A.	N.A.
BF (ng/m^3)	1.7	1.2	1.2	0.8	0.2	N.A.	N.A.
IP (ng/m^3)	0.8	0.6	0.4	0.4	0.1	N.A.	N.A.
TEF (ng/m^3)	1.0	0.7	0.7	0.4	0.1	N.A.	N.A.

Table 2
Results obtained by the WST-1 assay and LDH assay, which were performed to test the effects of seasonal PM organic extracts collected at the five sites on BEAS-2B cells (tested doses = 5, 10, 25, 50 m^3/mL). The results are expressed as the lowest doses of PM extracts (m^3/mL) that induced a significant* viability decrease (WST-1 assay) or a significant* % of LDH release (LDH assay) with respect to control cells for all exposure times (24 h, 48 h, 72 h). - = no tested dose induced a significant effect with respect to control cells, * = $p < 0.05$, Kruskal—Wallis followed by Dunnett's post hoc test vs. control cells.

Lowest doses of PM extacts which induced a significant* effect on BEAS-2B (m^3/mL)										
	Traffic		Urban		Incinerator		Rural 1		Rural 2	
	WST-1	LDH	WST-1	LDH	WST-1	LDH	WST-1	LDH	WST-1	LDH
Winter	10	25	25	25	25	25	25	25	50	-
Spring	50	50	50	50	50	-	25	25	-	-
Summer	50	-	50	-	50	-	50	-	-	-
Autumn	10	25	10	25	10	25	10	25	50	-

SF = cancer slope factor (kg day/mg).

3. Results and discussion

3.1. Air pollution data

The levels of PM_{10}, $PM_{2.5}$ and benzo(a)pyrene at the five sites during the year are reported in Fig. 2. Overall, the pollutant levels were characterized by a seasonal trend, as the concentrations were higher in cold months (autumn-winter) than in warm months (spring-summer). This seasonal trend is peculiar to the investigated area and is due to climatic and geographic conditions (Robotto et al., 2022; Gea et al., 2021).

The statistical analysis, performed to compare the pollutant levels among the different sites, suggested that in autumn-winter, the sites were characterized by different PM_{10} and $PM_{2.5}$ levels (Kruskal—Wallis test $p < 0.05$). In particular, rural site 2 showed the lowest pollution levels (post hoc Tukey $p < 0.05$ rural site 2 vs. all the other sites), while the other four sites (traffic site, urban site, incinerator site, rural site 1) were characterized by similar pollution levels. A statistically significant difference was found only when the PM_{10} levels measured at the traffic site were compared with the PM_{10} levels measured at rural site 1 in autumn-winter, suggesting a slight difference between these two sites (post hoc Tukey $p < 0.05$ traffic site vs. rural site 1).

The PM_{10} and $PM_{2.5}$ concentrations measured in spring-summer were not significantly different among the five sites (Kruskal—Wallis test $p > 0.05$), suggesting that the level of pollution at the five sites is comparable during warm months.

Table 1 shows the annual averages of the concentrations of PM_{10}, $PM_{2.5}$ and PAHs at the five sites. At all five sites, the concentrations of the three legislated pollutants did not exceed the annual limits set by the Italian legislation (Legislative Decree 155/2010, European Commission Directive 2004/107/EC; European Commission Directive, 2008/50/EC). However, the $PM_{2.5}$ levels were above the WHO guideline value at all sites, and four out of five sites also exceeded the PM_{10} WHO guideline value (WHO, 2021). The high pollution levels are not surprising; the provinces of the five sites are located in the Padana Plain, an area surrounded by mountains where the geographical and climatic conditions reduce pollutant dispersion and promote severe air pollution episodes (Bonetta et al., 2019; Robotto et al., 2022).

3.2. Cytotoxicity results

The results obtained by the WST-1 and LDH assays are reported in Table 2 and in Figs. S1–S8 in the Supplementary Materials.

The results obtained by the summer and spring samples tested with the WST-1 assay showed that all extracts, except for rural extract 2, induced a significant decrease in cell viability only at the highest dose (50 m^3/mL) (Fig. S1 = WST-1 results obtained for the summer extracts; Fig. S2 = WST-1 results obtained for the spring extracts); moreover, the spring sample collected in rural site 1 also induced a significant effect at 25 m^3/mL. Therefore, in spring and summer, rural site 2 showed the least cytotoxic effect (no significant effect), while a similar effect was detected at the other sites except for rural site 1, which was the most cytotoxic in these seasons. The extract during the winter season with the greatest cytotoxic effect was the traffic extract, which induced a significant decrease in cell viability starting from 10 m^3/mL (Fig. S3 = WST-1 results of winter extracts). Among the other samples, urban extract, incinerator extract and rural extract 1 showed a decrease in cell viability starting at 25 m^3/mL, while rural extract 2 induced a significant decrease in cell viability only at the highest dose (50 m^3/mL). Finally, among the autumn extracts, traffic extract, urban extract, incinerator extract and rural site 1 showed a significant effect starting from 10 m^3/mL, while for the other seasons, rural extract 2 was the least cytotoxic sample (significant effect only at 50 m^3/mL) (Fig. S4 = WST-1 results of autumn extracts).

No significant LDH release was detected for all the summer samples, while a significant effect on the plasma membrane was induced by the spring samples from the traffic site, urban site and rural site 1 (Fig. S5 = LDH results of summer extracts, Fig. S6 = LDH results of spring extracts). The effect was detected starting at 50 m^3/mL for traffic and urban sites,

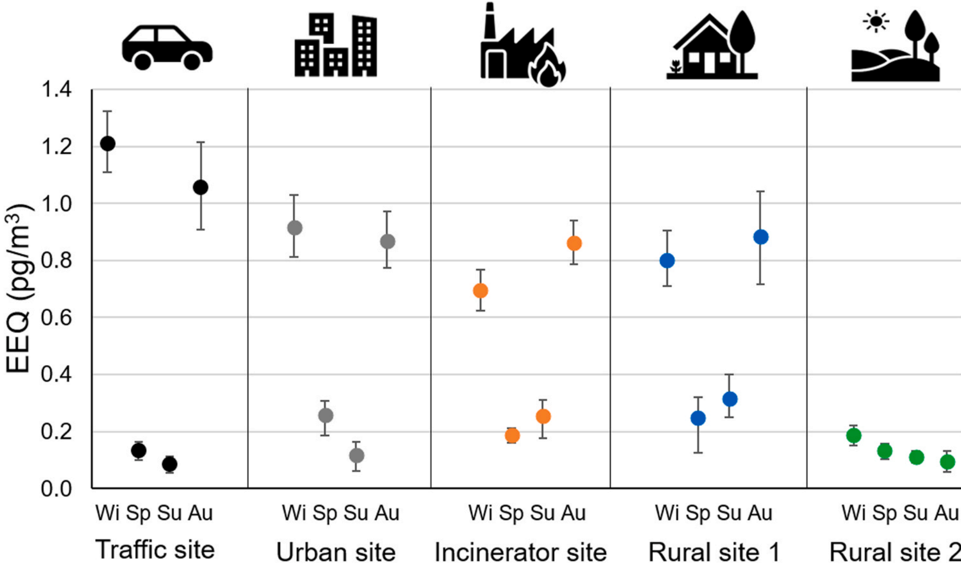


Fig. 3. Oestrogenic activity induced by seasonal PM organic extracts collected at the five sites. Data are reported as the means ± 95% confidence intervals. Au = autumn extract, EEQ = 17β-oestradiol equivalent concentrations, PM = particulate matter, Sp = spring extract, Su = summer extract, Wi = winter extract.

whereas rural extract 1 induced a significant effect starting at 25 m^3/mL, confirming the results obtained using the WST-1 assay for spring samples. Similar to the WST-1 results, the LDH results showed that winter-autumn samples induced a higher effect than that of spring-summer extracts; for both seasons, traffic extract, urban extract, incinerator extract and rural extract 1 induced a significant LDH release starting from 25 m^3/mL (Fig. S7 = LDH results of winter extracts, Fig. S8 = LDH results of autumn extracts). As also demonstrated using the WST-1 assay, rural extract 2 was the least cytotoxic sample; all the seasonal extracts of this site did not induce any significant effect on plasma membrane integrity.

Overall, the WST-1 and LDH results showed a strong seasonal trend for all sites, that could be explained considering that the release of air pollutants is higher (mainly for domestic heating) and the dispersion of pollutants is lower during autumn and winter seasons (climatic situation of the Padana Plain). Moreover, interestingly, despite the different pollution sources (traffic, urban sources, and waste incineration), traffic extracts, urban extracts and incinerator extracts generally showed similar effects for all seasons. Since these three sites are located near each other, this result suggests that cytotoxic pollutants spread in this area, which leads to a similar biological effect. The different effects induced by the two rural sites are probably due to the geographical location; rural site 2 is located at a higher altitude and on the edge of the Padana Plain, while rural site 1 is located at a lower altitude and in a hilly area within the Padana Plain. In addition, the differences between the two rural sites could result from different pollution sources. At rural site 1, many home heating systems are based on biomass combustion, which could represent an important source of air pollutants (Regional Agency for Environmental Protection of Piedmont, 2018).

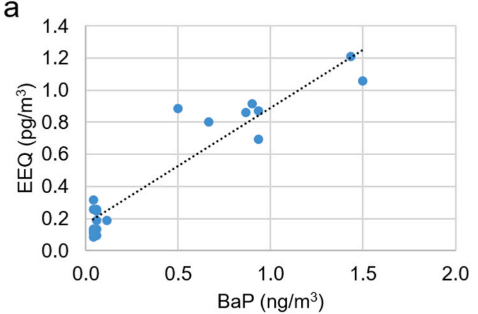
3.3. Results for oestrogenic activity and risk assessments

The oestrogenic activity of PM extracts was detected using the luciferase gene reporter assay. The results, expressed as EEQ, are reported in Fig. 3. The highest EEQ was measured for the traffic site in winter (mean EEQ = 1.21 pg/m^3), and in accordance with the cytotoxicity results, all the seasonal samples collected at rural site 2 showed a low EEQ, confirming the low pollution levels at this site.

Overall, the oestrogenic activity measured in our study (range of mean EEQ: 0.08–1.21 pg/m^3) corresponds with other studies in which oestrogenic activity was measured in PM organic extracts collected outdoors (Croes et al., 2016a: EEQ of PM_{10} extracts = no effect – 0.2 pg/m^3; Croes et al., 2016b: EEQ of PM_{10} extracts = 0.03–0.04 pg/m^3; Gea et al., 2023: EEQ of PM_{10} and $PM_{2.5}$ extracts: 0.02–2.41 pg/m^3; Novák et al., 2020: EEQ of PM_{10} extracts = 0.09–4.91 pg/m^3; Nováková et al., 2020: EEQ of PM_{10} extracts = $<$0.0047–1.40 pg/m^3; Oziol et al., 2017: EEQ of total suspended particle extracts = 0.02 – ≈0.2 pg/m^3; Wenger et al., 2009: EEQ of PM_1 extracts = 0.07–1.25 pg/m^3). In contrast, our results are different from the studies by Érseková et al. (2014) and Novák et al. (2009); Novák et al. (2013); Novák et al. (2014), in which no oestrogenic activity was detected in PM extracts. In these studies, some PM samples induced significant antiestrogenic activity when tested with E2. This result is partially concordant with the present study, in which a dose of each PM extract (10 m^3/mL or 25 m^3/mL) was tested in combination with E2 (10^{-10} M) to study the interaction between extracts and E2. The results showed that the oestrogenic activity of E2 was significantly increased by 11 extracts, remained unchanged by the addition of 7 extracts (winter traffic, winter incinerator, winter rural 1, spring urban, summer incinerator, autumn rural 1, and autumn rural 2 extracts) and was decreased by two extracts (winter rural 2 and summer rural 1 extracts), suggesting that the interaction between E2 and PM extracts on oestrogen receptors is complex and can result in unpredictable results (Fig. S9 in Supplementary Materials).

In the present study, a dose of each PM extract (10 m^3/mL or 25 m^3/mL) was also tested in combination with tamoxifen (an oestrogen receptor antagonist, 10^{-6} M) to confirm whether the observed effect induced by the extracts was due to oestrogen receptor activation. The results showed that tamoxifen decreased the oestrogenic activity induced by all the extracts; however, for many extracts, significant oestrogenic activity with respect to tamoxifen alone was detectable, suggesting that extract components and tamoxifen compete for oestrogen receptors (Fig. S9 in Supplementary Materials).

After the results were analysed together, significantly higher oestrogenic activity was detected in winter and autumn than in spring and summer ($p < 0.05$ Mann—Whitney test winter-autumn EEQ vs. spring-summer EEQ); therefore, an overall seasonal trend was found for this biological effect. Previous studies did not observe this trend (Croes et al., 2016a, 2016b; Klein et al., 2006; Zhou et al., 2022). However, similar to the present study, a seasonal trend of the oestrogenic activity of outdoor PM was found by Oziol et al. (2017), in which a significantly lower oestrogenic activity was found in autumn (high external mean



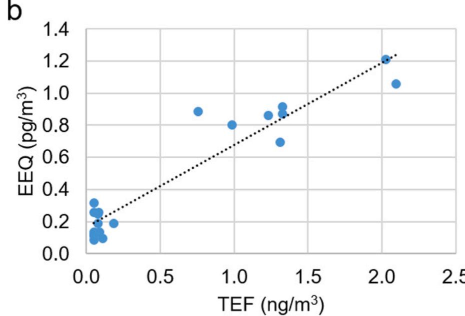


Fig. 4. Comparison between the oestrogenic activity induced by the seasonal extracts of the five sites (expressed as EEQ) and the seasonal mean concentrations of BaP and TEF in the five sites. a) BaP, Spearman's Rho coefficient = 0.798; $p < 0.001$, b) TEF, Spearman's Rho coefficient = 0.751, $p < 0.001$.

temperature) than in winter (low external mean temperature). The seasonal trend could be due to a higher emission during cold seasons of oestrogenic compounds, such as PAHs. Use of domestic heating and traffic emissions are higher in the cold period than in the summer vacation period. In addition, during winter, the weather conditions favour the presence of semivolatile organic compounds in PM and not in the gaseous phase.

A significant correlation was found between the oestrogenic activity induced by the seasonal extracts of the five sites (expressed as EEQ) and the seasonal mean concentration of BaP and TEF in the five sites (Fig. 4; Spearman's Rho coefficient: Bap = 0.798; $p < 0.001$; TEF = 0.751, $p < 0.001$), suggesting that the oestrogenic activity of PM samples might result from the PAHs adsorbed on PM. However, as reported by Darbre (2018), the endocrine-disrupting activity of outdoor air can result from other chemicals, such as pesticides, bisphenol A, alkylphenols, polybrominated diphenyl ethers, polychlorinated biphenyls and polychlorinated dibenzodioxins/dibenzofurans, which are not routinely monitored in PM.

The overall results showed that PM can be a source of exposure to EDCs with an oestrogenic effect; however, it is difficult to estimate how important the contribution of air exposure to the total burden due to EDCs is and whether the EDCs of air can threaten human health. To quantify the risk associated with exposure to oestrogenic compounds adsorbed on PM, an inhalation cancer risk assessment was performed using the EEQ measured in the present study using an approach reported by ISPRA (2016). The assessment was performed assuming that the mean annual EEQ measured at each site was equal to the mean air concentration of E2 at each site. The incremental probabilities of a person developing cancer over a lifetime (R) due to exposure to the mean annual EEQ found in the present study were below 1 case in 100,000,000 people for all five sites (Table S1 in Supplementary Materials). This result suggests that the oestrogenic activity of PM extracts should not be considered a threat to human health. However, it is important to highlight that the total exposure to oestrogenic compounds is the sum of exposures to compounds by all exposure routes. Therefore, since we are mainly exposed to oestrogenic compounds by ingestion and dermal contact (Darbre, 2018), the oestrogenic compounds in air is a further source of exposure, which may increase the total exposure level, contributing to an increase in risk.

4. Conclusion

Research on the risk due to EDCs has focused on exposure through ingestion and dermal contact routes; however, EDCs were also found in indoor and outdoor air. Considering that we constantly breathe air and that indoor air quality is influenced by outdoor air quality, assessing the potential of outdoor air to induce endocrine-disrupting effects is important. In the present study, a significant oestrogenic effect was induced by the organic extracts of outdoor PM sampled at different sites, demonstrating that outdoor air can exert endocrine-disrupting activity. Although the risk assessment analysis showed that the cancer risk associated with inhalation of PM oestrogenic compounds was low, this study confirmed that inhalation exposure is a potential exposure route to EDCs that can contribute to the overall health risk due to EDC exposure.

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CRediT authorship contribution statement

Marta Gea: Investigation, Data curation, Formal analysis, Writing – original draft, Visualization, Validation. **Manuela Macrì**: Investigation, Data curation, Writing – review & editing. **Daniele Marangon**: Investigation. **Francesco Antonio Pitasi**: Investigation. **Marco Fontana**: Supervision. **Sara Bonetta**: Writing – review & editing, Validation. **Tiziana Schilirò**: Conceptualization, Supervision, Resources, Writing – review & editing, Project administration. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2023.104232.

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