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Atmospheric endocrine disruptors: A systematic review on oestrogenic and androgenic activity of particulate matter

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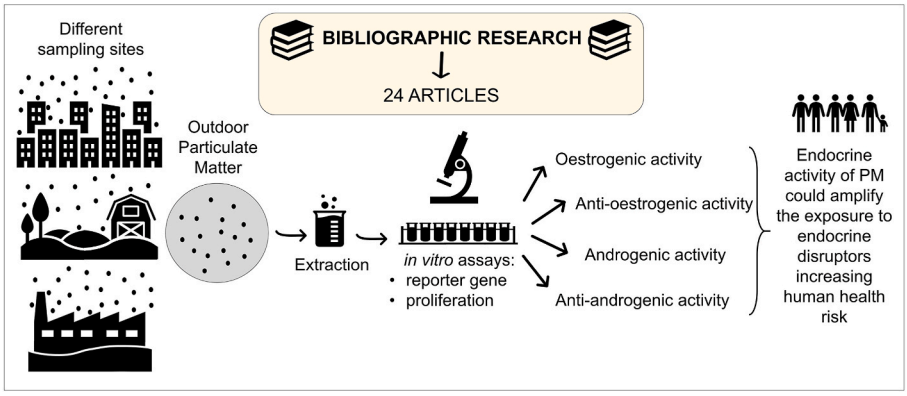
HIGHLIGHTS

- Endocrine activity of outdoor particulate matter assessed with *in vitro* assays.
- PM can induce oestrogenic, anti-oestrogenic, androgenic and anti-androgenic effects.
- Inhalation of PM could increase exposure to endocrine disruptors.
- Endocrine activity is difficult to predict using only pollutant concentrations.
- Bioassays useful to quantify health risk posed by endocrine disruptors on PM.

GRAPHICAL ABSTRACT



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ABSTRACT

The alarming human health effects induced by endocrine disruptors (ED) have raised the attention of public opinion and policy makers leading worldwide to regulations that are continuously improved to reduce exposure to them. However, decreasing the exposure levels is challenging because EDs are ubiquitous and exposure occurs through multiple routes. The main exposure route is considered ingestion, but, recently, the inhalation has been hypothesized as an important additional route. To explore this scenario, some authors applied bioassays to assess the endocrine activity of air. This review summarizes for the first time the applied methods and the obtained evidences about the *in vitro* endocrine activity of airborne particulate matter (PM) collected outdoor. Among the bioassay endpoints, (anti)oestrogenic and (anti)androgenic activities were selected because are the most studied endocrine activities. A total of 24 articles were ultimately included in this review. Despite evidences are still scarce, the results showed that PM can induce oestrogenic, antioestrogenic, androgenic and antiandrogenic effects, suggesting that PM has an endocrine disrupting potential that should be considered because it could represent a further source of exposure to EDs. Although it is difficult to estimate how much inhalation can contribute to the total burden of EDs, endocrine activity of PM may increase the human health risk. Finally, the results pointed out that the overall endocrine activity is difficult to predict from the concentrations of individual

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pollutants, so the assessment using bioassays could be a valuable additional tool to quantify the health risk posed by EDs in air.

1. Introduction

Endocrine disruptors (EDs) are defined as chemicals that interfere with the endocrine system affecting the production, release, transport, metabolism, binding, action or elimination of endogenous hormones, thus causing adverse health effects in an organism or in its progeny (Vieira et al., 2021). Many different compounds, both of natural or synthetic origin, are considered EDs by the scientific community based on their endocrine disrupting effects. EDs of natural origin include phytoestrogens (e.g. genistein and daidzein), mycotoxins (e.g. zearalenone), natural hormones themselves (e.g. oestradiol, testosterone) and their metabolites (e.g. estrone, estriol) (Pamplona-Silva et al., 2018; Vieira et al., 2021; Chen et al., 2022). EDs of synthetic origin include a vast multitude of chemicals with different characteristics and uses (Kabir et al., 2015; Metcalfe et al., 2022) such as

- polychlorinated biphenyls (PCBs): substances that were once widely used as coolants and lubricants in transformers, capacitors, and other electrical equipment, the use of which has been restricted in many countries but are still found in the environment as persistent compounds;
- pesticides (e.g. DDT, atrazine, chlorpyrifos): these are used in agriculture but also in the domestic environment. Although some have been banned or their use restricted in some countries, their presence in the environment is still high;
- polyfluoroalkyl substances (PFAS): used as surface coatings, they can be found in textiles but also in cosmetics;
- phthalates (e.g. diethylphthalate, dibutylphthalate, 2-ethylhexylphthalate, butylbenzylphthalate) used as plasticisers to give plastics flexibility, they are found in many plastic products but also in personal care products;
- bisphenols (e.g. bisphenol A and its substitutes): chemicals widely used in manufacturing polycarbonate plastics and epoxy resins used in many industries;
- parabens (e.g. methylparaben, butylparaben, benzylparaben): used as antimicrobial agents for food preservation, pharmaceuticals, personal care products;
- alkyl phenols (e.g. nonylphenol): due to their surface-active and antioxidant properties, they are present in pesticides, industrial oils, detergents;
- polycyclic aromatic hydrocarbons (PAHs): molecules produced by incomplete combustion processes of organic matter that can occur due to both natural occurrences and anthropogenic activities (e.g. fires, vehicle emissions, combustion in industrial plants, waste incineration);
- brominated flame retardants (e.g. polybrominated diphenyl ethers - PBDEs): these are included in electrical and electronic products, furniture and building materials;
- pharmaceuticals (e.g. ethinyl oestradiol, diethylstilbestrol - DES);
- polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF): pollutants released by combustion processes of natural or anthropogenic origin;
- metals (e.g. lead, mercury, arsenic, cadmium): found in cigarettes, in the environment as environmental contaminants and used in industrial processes (Kabir et al., 2015; Kiyama and Wada-Kiyama, 2015; Darbre, 2018; Yilmaz et al., 2020; Chen et al., 2022; Bertram et al., 2022; Metcalfe et al., 2022).

Numerous effects on human health have been associated with exposure to EDs. EDs appear to have the potential to interfere with the reproductive system by altering the structure and/or function of the female and male reproductive organs. Specifically, exposure to EDs has been associated with effects such as irregularities in the menstrual cycle, reduced fertility, polycystic ovary syndrome, endometriosis, early or late puberty, breast cancer, alterations in spermatozoa (decreased number, reduced mobility and abnormal morphology), cryptorchidism, hypospadias, prostate cancer and testicular cancer (Kabir et al., 2015; Yilmaz et al., 2020; Marlatt et al., 2022). Furthermore, numerous studies have highlighted the ability of some EDs to interfere with thyroid function potentially causing thyroid cancer and cognitive or behavioural deficits, while others have suggested that EDs may promote the onset of obesity and type II diabetes (Kabir et al., 2015; Yilmaz et al., 2020).

The alarming effects induced by EDs have led worldwide to development and adoption of numerous regulations that are continuously being improved in order to reduce human exposure to EDs. However, decreasing exposure levels is challenging because these pollutants are spread throughout the environment and humans can be exposed to EDs through multiple exposure routes, such as ingestion, inhalation, dermal contact and transfer through the placenta or breast milk. Among these, the main route of exposure to these substances is considered the ingestion of contaminated water and food. However, recently the inhalation has been recognized as an important additional exposure route, especially for volatile and semivolatile EDs (Annamalai and Namasivayam, 2015; Darbre, 2018). Indeed, EDs might be released in the air from everyday household products, from the materials of which furniture and buildings are made, agricultural pesticide aerosol treatments, industrial activities, waste incineration, vehicular traffic, and biomass combustion (Annamalai and Namasivayam, 2015; Kabir et al., 2015; Darbre, 2018; Yilmaz et al., 2020; Bertram et al., 2022).

Using chemical analytical methods individual EDs and subgroups of EDs with similar chemical structures have been found and quantified in the atmosphere (Annamalai and Namasivayam, 2015). However, using the analytical data, it might not be simple to estimate which hormones the complex mixture is able to interfere with (e.g. oestrogens, androgens, thyroidal hormones). In addition, knowing the concentrations of all the individual pollutants in the air, could not be enough to predict the cumulative effect of the complex ED mixture because the activity of all the individual compounds is not always known and unpredictable synergic or antagonistic effects could occur among the compounds. To overcome these limitations, novel approaches have been developed for the ED detection and for quantification of the overall endocrine activity of environmental matrices. These approaches are based on an effect directed analysis (i.e. can measure the integrated effect of all chemicals with the same mode of action) and include *in vitro* bioassays (Varticovski et al., 2022).

While these approaches have been extensively applied to study waters and were recently reviewed (Robitaille et al., 2022), the application of bioassays to assess the endocrine activity of air samples has been already performed by some authors but has not been reviewed yet.

This review summarizes the evidences about the *in vitro* endocrine activity of airborne particulate matter (PM) collected outdoor. The PM was selected as it is one of the most important air pollutants and it is the carrier of many pollutants that can be adsorbed on it, while, among the different *in vitro* effect-directed analyses on EDs, (anti)oestrogenic and (anti)androgenic activities were selected because are the most studied ones. In particular, the aims of the present review are: i) to describe methods applied to study the (anti)oestrogenic and (anti)androgenic activity of PM (extraction methods and assay types); ii) to show data about the (anti)oestrogenic activity of PM; iii) to show data about the (anti)androgenic activity of PM. The endocrine activity of PM has been discussed in comparison with the endocrine activity of gas phase and chemicals considered responsible for this activity have been listed as

reported in the selected articles.

2. Materials and methods

2.1. Search strategy and selection process

The review has been designed starting from January 2023 and following *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA, 2020; Page et al., 2021).

A literature search was performed in three databases: Pubmed, Embase and CINAHL on March 29, 2023. These databases were selected because they represent the three main sources of biomedical literature on the review topic. Detailed search strings are reported in the Supplementary materials and yielded a total of 3884 results in Pubmed, 1882 results in Embase and 7 results in Cinhal (total articles after de-duplication = 5480). These results were independently screened by two authors of the present review to select the articles that analysed airborne PM samples collected outdoor and assessed (anti)oestrogenic or (anti) androgenic activity using *in vitro* assays. The articles were included when they were written in English and met the following criteria: (i) the analysed samples were airborne PM collected in outdoor environments (e.g. articles analysing indoor PM were excluded), (ii) the PM samples were analysed using *in vitro* assays on cells or on yeasts (e.g. epidemiological studies and *in vivo* studies were excluded), (iii) the PM samples were analysed in order to assess at least one of these biological effects: oestrogenic activity, antioestrogenic activity, androgenic activity, antiandrogenic activity (e.g. articles about thyroid activity were excluded), (iv) the articles were full research articles (e.g. conference abstracts were excluded).

2.2. Data collection and synthesis

The following data were extracted for each article included in the analysis: reference, country in which PM samples were collected, sampling period, type of PM samples (aerodynamic diameter of PM and sampling site type), extraction methodologies of samples (extraction type and extraction solvents), number of PM samples, type of bioassay (cell or yeast model used), tested concentrations, endocrine activity results (agonistic and antagonistic effects of both oestrogenic or androgenic activity).

The collected data were presented in three different tables. In the first table, the methods used to analyse samples were synthetized reporting the extraction methods and the type of bioassay. In this table, all the articles included in the review were reported. These articles were later divided according to the endocrine effect analysed. In the second table, data collected on articles in which oestrogenic/antioestrogenic activity of PM was assessed were summarized, while in the third table data of articles focused on the androgenic/antiandrogenic activity of PM were reported. The articles that assessed both oestrogenic/antioestrogenic and androgenic/antiandrogenic activity were included in both tables. In the second and third table, the characteristics of PM samples (PM type, sampling site, sampling period, number of samples) and the endocrine activity induced by PM samples were summarized. The endocrine activity was reported in terms of agonistic and antagonistic effects and, when available, the results were reported also in terms of equivalent concentration of the reference hormone. In particular, for the estrogenic agonistic activity data were presented as estradiol equivalent quantity (EEQ), for the androgenic agonistic activity as dihydrotestosterone equivalent quantity (DHT-EQ), for the androgenic antagonistic activity as flutamide equivalent quantity (Flu-EQ). These values represent the biological effect induced by the total concentration of all the hormone-active compounds within the sample; this biological effect is equal to the effect induced by the corresponding concentration of the reference hormone. Where equivalent concentrations have been cited as numerical values in the articles, they were reported in the tables of this review; while where they have been shown only in graphic format within figures, they were extrapolated from the figures and reported together with an approximation symbol (≈). If available, the data on PM_{10} were preferentially reported as numerical values in the tables, while data on other size subfraction were only commented on. The results of the androgenic antagonistic activity were also presented as index of antiandrogenicity, which is the reciprocal value of the 25% or 50% inhibition concentration (IC25 or IC50, respectively).

Finally, the contribution of quantified chemicals was reported and discussed by comparing the biological effect, which was evaluated with effects-based tools (i.e. bioanalytical equivalents based on bioassay results - BEQbio), with the biological effect evaluated considering the concentrations of some pollutants that have been quantified in PM (i.e. bioanalytical equivalents based on the results of chemical analysis - BEQchem). The biological effects estimated considering chemical concentrations were quantified (through an additive approach) summing the biological effects induced by the concentrations of all chemicals found in the PM. The biological effect of each single chemical was assessed multiplying the concentration of the chemical by the relative potency factor of this chemical with respect to the reference compound (Novák et al., 2020; Nováková et al., 2020).

3. Results and discussion

3.1. Results of the selection process

The results of the search strategy and the selection process are reported in Fig. 1. A total of 24 articles were ultimately included in this review.

The articles of Kwon et al. (2012) and Mori et al. (2007) initially included were finally excluded for the following reasons. In the article of Kwon et al. (2012) the PM samples were analysed using an *in vitro* assay which measures the combined effects of oestrogenic and dioxin-like compounds so it was not possible to distinguish the oestrogenic activity from the dioxin-like activity. In the article of Mori et al. (2007) the PM was collected in subway stations that can not be considered proper outdoor environments.

Four articles not found through the bibliographic research with strings were retrieved and included in the present review (Lammel et al., 2011; Gea et al. 2023a, 2023b; Wooten et al., 2015). The articles of Lammel et al. (2011) and Wooten et al. (2015), were included because they were cited by two articles already included in the present review (Croes et al., 2016b; Smith et al., 2019, respectively), while the other two articles were retrieved while writing the review (they were published by the authors of the present review shortly after the bibliographic research was performed).

3.2. Applied methods for the assessment of endocrine activity of PM

3.2.1. PM extraction

The methods used to analyse the endocrine activity of PM samples are reported in Table 1. As can be seen, extraction was performed with Soxhlet, automatic extractors but also sonication and shaking. The most used solvents for the extraction of chemicals adsorbed on PM were dichloromethane and (cyclo)hexane:acetone. However, other solvents were used to perform extraction such as methanol but also simulated lung fluids and ultrapure water. In five articles, PM samples were extracted with different extraction solvents prior to the biological analysis, showing that the type of extraction solvents and/or fractionation could change the assay results.

In particular, the study of Clemons et al. (1998) assessed the oestrogenic activity of the crude extract of PM (extracted in dichloromethane) and of four different fractions of the crude extract (fraction A1 = non polar aliphatic fraction extracted with hexane; fraction A0 and A23-LH20 = non polar aromatic fractions extracted with dichloromethane and subjected to chromatography; fraction A45 = polar aromatic fraction extracted with methanol and methanol-water). The

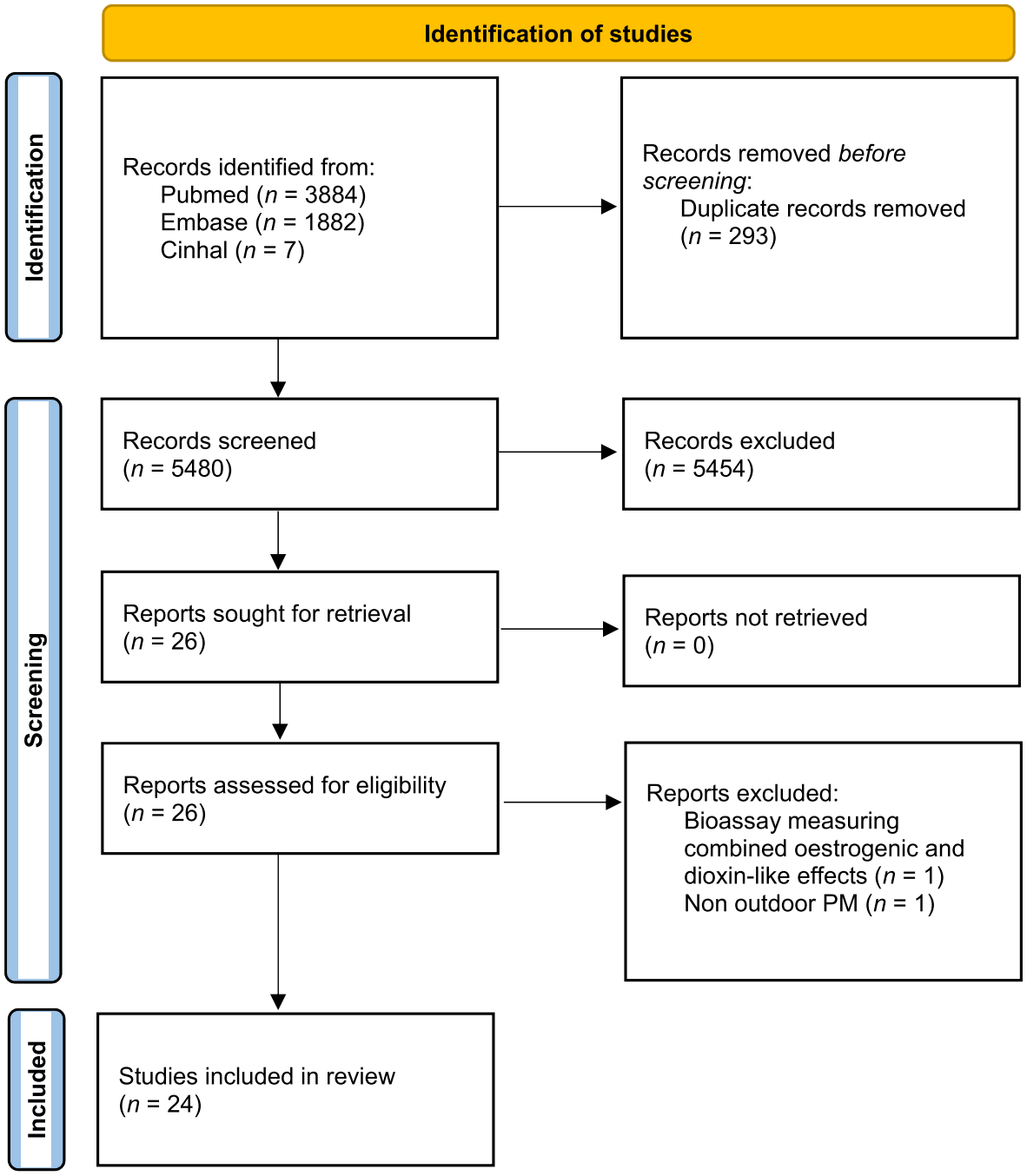

Fig. 1. Flowchart of the literature search and selection process (taken and modified from Page et al., 2021).

results showed that the crude extract and its non polar fractions (A1, A0, A23-LH20) induced a significant oestrogenic effect, while the polar fraction was not oestrogenic, suggesting that the oestrogenic activity of PM is mainly induced by non polar compounds. Interestingly, the crude extract activity was similar to the sum of the activity induced by the extract fractions. Also the study of Wang et al. (2004) evaluated the oestrogenic activity of the crude extract (extracted with dichloromethane-acetone) and of four of its different fractions (fraction X1 = aliphatic fraction extracted with silica column eluted with hexane, fraction X2 = aromatic fraction, silica column eluted with hexane-dichloromethane, X3 = moderately polar fraction, silica column eluted with dichloromethane, X4 highly polar fraction, silica column eluted with methanol). Contrarily to the previous article, in this second study, the highest oestrogenic activity was induced by the polar fraction and the activity of the crude extract was lower than the activity of the single fractions. In the third study (Matsumoto et al., 2005), the crude extract of PM (extracted with benzene:methanol 3:1) was further fractionated in three fractions: acidic, neutral and basic fractions. The results showed that the crude extract, the neutral fraction and the basic fraction did not induce a significant oestrogenic activity, while the acidic fraction induced a significant agonistic activity. In the fourth study (Croes et al., 2016a), PM samples were extracted with three different solvents [hexane: acetone (1:1), ethanol, acetonitrile]. The results demonstrated that samples extracted with hexane:acetone had a lower oestrogenic activity and a higher cytotoxicity, while samples extracted with acetonitrile had the lowest cytotoxicity but also a low oestrogenic activity. In conclusion, ethanol was identified as the best extraction solvent because samples extracted with it induced a high

Table 1
Methods applied to analyse the endocrine activity of PM samples: extraction methods and bioassays. AR = androgen receptor, ER = oestrogen receptor, N.A. = not available, N.E. = not evaluated, PM = particulate matter.

Reference	Extraction solvent	Extraction type	Cell/yeast model for estrogenic and antiestrogenic activity	Cell/yeast model for androgenic and antiandrogenic activity
Balaguer et al. (1996)	PM tested without extraction	N.A.	HeLa stably transfected with human ER and a luciferase reporter gene	N.E.
Clemons et al. (1998)	• Dichloromethane (crude extract) Fractionation: • Hexane (non-polar aliphatic fraction) (A1) • Dichloromethane (non-polar aromatic fraction) + chromatography: material eluted prior to naphthalene (A0) and after naphthalene (A23-LH20A23) • Methanol and methanol-water (polar aromatic fraction) (A45)	Soxhlet	MCF-7 transiently transfected with human ER and a luciferase reporter gene	N.E.
Wang et al. (2004)	• Dichloromethane - acetone (1:1) (crude extract) Fractionation (silica column): • Hexane (aliphatic fraction - X1) • Hexane:dichloromethane (aromatic fraction - X2) • Dichloromethane (moderately polar fraction - X3) • Methanol (highly polar fraction - X4)	Soxhlet	*Saccharomyces cerevisiae* stably transfected with human ERα and a β-galactosidase reporter gene	N.E.
Matsumoto et al. (2005)	• Benzene-methanol (3:1) (crude extract) Fractionation: • Acidic fraction • Neutral fraction • Basic fraction	Sonication	MCF-7 (proliferation assay)	N.E.
Klein et al. (2006)	N.A.[a]	N.A.[a]	BG1Luc4E2 (cells stably transfected with a luciferase reporter gene)	N.E.
Wenger et al. (2009)	Dichloromethane	Soxhlet	T47D.Luc (cells stably transfected with a luciferase reporter gene)	N.E.
Novák et al. (2009)	Dichloromethane	Buchi System B-811 automatic extractor	MVLN (cells stably transfected with a luciferase reporter gene)	*Saccharomyces cerevisiae* stably transfected with human AR and luciferase reporter gene
Kennedy et al. (2009)	Pre-extraction with acetone and hexane, extraction with hexane	N.A.	MCF7-BOS (proliferation assay)	N.E.
Lammel et al. (2011)	Dichloromethane	Buchi System B-811 automatic extractor	MVLN (cells stably transfected with a luciferase reporter gene)	*Saccharomyces cerevisiae* stably transfected with human AR and luciferase reporter gene
Novák et al. (2013)	Dichloromethane	Buchi System B-811 automatic extractor	MVLN (cells stably transfected with a luciferase reporter gene)	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Chen et al. (2013)	Hexane:acetone (1:1)	Sonication	T47D-Kbluc (proliferation assay) and T47D-Kbluc (cells stably transfected with a luciferase reporter gene)	N.E.
Novák et al. (2014)	Dichloromethane	Buchi System B-811 automatic extractor	MVLN (cells stably transfected with a luciferase reporter gene)	*Saccharomyces cerevisiae* stably transfected with human AR and luciferase reporter gene
Érseková et al. (2014)	Dichloromethane	Buchi System B-811 automatic extractor	MVLN (cells stably transfected with a luciferase reporter gene)	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Wooten et al. (2015)	Methanol	Extraction by shaking	MVLN (cells stably transfected with a luciferase reporter gene)	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Croes et al. (2016a)	• Hexane:acetone (1:1) • Ethanol • Acetonitrile	Accelerated Solvent Extractor	BG1Luc4E2 (stably transfected with a luciferase reporter gene)	N.E.
Croes et al. (2016b)	Hexane:acetone (1:1)	Accelerated Solvent Extractor	BG1Luc4E2 (cells stably transfected with a luciferase reporter gene)	T47D (cells stably transfected with a luciferase reporter gene)
Oziol et al. (2017)	Hexane:acetone (1:1)	Sonication	MELN (cells stably transfected with a luciferase reporter gene)	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Smith et al. (2019)	Methanol	Extraction by shaking	MVLN (cells stably transfected with a luciferase reporter gene)	N.E.
Novák et al. (2020)	Dichloromethane	Buchi System B-811 automatic extractor	HeLa9903 transfected with a luciferase reporter gene and human ERα expression construct	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Nováková et al. (2020)	• Dichloromethane (crude extract Fractionation: • Hexane-dichloromethane (non-polar fraction), • Dichloromethane (semi-polar fraction), • Dichloromethane-methanol (polar fraction)	Buchi System B-811 automatic extractor	HeLa9903 transfected with a luciferase reporter gene and human ERα expression construct	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Nováková et al. (2020)	Simulated lung fluids: • Artificial lysosomal fluid (ALF) • Simulated epithelial lung fluid (SELF)	24 h leaching through shaking (37 °C)	HeLa9903 transfected with a luciferase reporter gene and human ERα expression construct	MDA-kb2 (cells stably transfected with a luciferase reporter gene)
Gea et al. (2021)	Acetone:hexane (1:1)	Soxhlet	MELN (cells stably transfected with a luciferase reporter gene)	N.E.
Zhou et al. (2022)	Ultrapure water	Sonication	MCF-7 cells (proliferation assay)	N.E.
Gea et al. (2023a)	Acetone:cyclohexane (1:1)	Sonication	MELN (cells stably transfected with a luciferase reporter gene)	N.E.
Gea et al. (2023b)	Acetone:cyclohexane (1:1)	Sonication	MELN (cells stably transfected with a luciferase reporter gene)	N.E.

[a] Dann (1998). Ambient air measurements of polycyclic aromatic hydrocarbon (PAH), polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans in Canada (1987–1997). AAQD 97–92. Ottawa, Ontario, Canada: Analysis and Air Quality Division, Environment Canada.

oestrogenic activity, with a low cytotoxic effect. Finally, the study of Nováková et al. (2020), assessed the oestrogenic but also the androgenic activity of crude PM extracts (dichloromethane) and of three other fractions (non polar fraction extracted with hexane-dichloromethane, semi-polar fraction extracted with dichloromethane and a polar fraction extracted with dichloromethane-methanol). Similarly to Wang et al. (2004) the results showed that the crude extracts and the most polar fraction induced the highest oestrogenic and androgenic effect. In the study of Nováková et al. (2020) the PM extraction was performed also using two simulated lung fluids (artificial lysosomal fluid - ALF and simulated epithelial lung fluid - SELF). These extracts induced only a significant oestrogenic activity while no androgenic effect was detected. In addition, these extracts induced a lower oestrogenic activity with respect to organic extracts.

Taking together the results of these studies suggested that the extraction methods can greatly affect bioassay results; however, from the collected (scarce) evidences it seems difficult to define which is the best extraction solvent because the results are conflicting.

3.2.2. Endocrine activity evaluation: oestrogenic and androgenic activity assays

In order to assess the endocrine activity of PM, in the selected articles two types of assays were used: proliferation assays (oestrogenic activity) and reporter gene assays (oestrogenic and androgenic activity). The first assays are based on mammalian cell models that are able to proliferate after exposure with oestrogenic compounds, while the second ones are based on cells or yeasts transfected with reporter genes that are regulated by oestrogen or androgen receptors (ER or AR). As a consequence, the endocrine activity is detected as proliferation (proliferation assays) or as induction of the reporter genes, that encode for reporter proteins (luciferase or β-galactosidase) whose activity can be easily quantified with a luminescence or an absorbance measurement (reporter gene assays). Due to their characteristics, the proliferation assays are generally used only to assess the agonistic endocrine activity of samples while the reporter gene assays can evaluate both agonistic or antagonistic endocrine activity. Indeed, when the sample is tested together with the reference hormone (i.e. estradiol or dihydrotestosterone/testosterone), the agonist activity of the hormone can be inhibited by the antagonist activity of the sample (lower reporter gene induction with respect to the induction measured testing only the reference compound). The characteristics of these assays are schematically presented in Fig. 2, while the advantages/disadvantages are reported in details in previous articles (Mueller, 2004; Soto et al., 2006; Kiyama and Wada-Kiyama, 2015; Wagner et al., 2017; Wangmo et al., 2018; Gea et al., 2020). The concentrations of samples assessed using the assays are reported in Table S1 (Supplementary Materials).

3.3. Oestrogenic activity of PM

All the 24 articles included in the present review assessed the agonistic/antagonistic oestrogenic activity of PM; their results are reported in Table 2.

As regards the agonistic activity, a weak or a significant effect for at least one sample was found in 17 out of 24 articles with an EEQ ranging from 0.02 to 23 pg/m^3. The agonistic activity was found in samples collected in urban areas but also in industrial and even in rural areas showing that pollutants with an oestrogenic effect are widespread. Moreover, this activity was found not only in airborne PM but also in the gas phase of the atmosphere. From the comparison between PM and gas phase, Klein et al. (2006) and Oziol et al. (2017) reported that gas phase extracts frequently induced a higher effect with respect to PM extracts, while on the contrary Nováková et al. (2020) found a significant oestrogenic activity only in the PM phase and not in the gas one. Although some articles did not find a seasonal trend of the oestrogenic effect (Klein et al., 2006; Croes et al., 2016a; Zhou et al., 2022), Wang et al. (2004) reported a higher activity in foggy days and many authors showed a higher activity in colder periods (Croes et al., 2016b; Oziol et al., 2017; Novák et al., 2020; Nováková et al., 2020; Gea et al., 2023a; Gea et al., 2023b), suggesting that weather and climate conditions could influence the concentrations of oestrogenic pollutants in PM.

The agonistic activity was mainly attributed to the following pollutants: PAHs, bisphenol A, PCBs, organochlorine pesticides, phthalates,

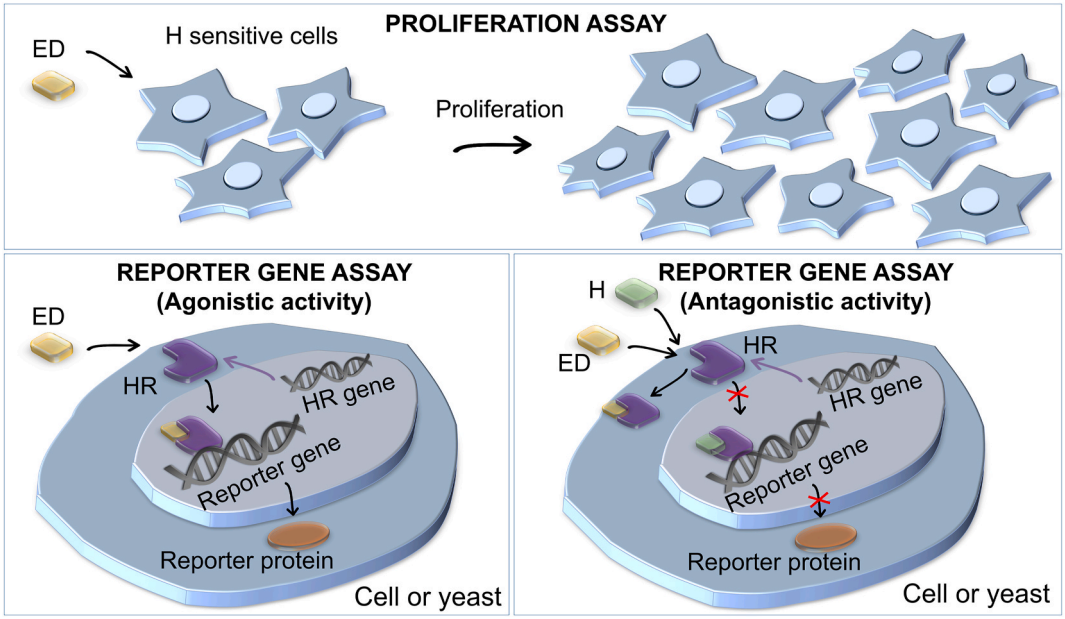

Fig. 2. Schematic representation of endocrine activity assays: proliferation assays and reporter gene assays (agonistic/antagonistic activity). H = hormone, ED = endocrine disruptor, HR = hormone receptor.

Table 2
Summary of the 24 articles in which agonistic/antagonistic oestrogenic activity of PM was assessed. ALF = artificial lysosomal fluid, E2 = oestradiol, EEQ = oestradiol equivalent quantity, IC = inhibition concentration, N.A. = not available, PCB = polychlorinated biphenyl, PM = particulate matter, SELF = simulated epithelial lung fluid, TSP = total suspended particles.

Reference	Country and period	PM type	Samples (*n*)	Agonistic activity	Antagonistic activity (co-treatment with E2)
Balaguer et al. (1996)	Canada (Ottawa), N.A.	TSP, 1 urban site	1	No activity	N.A.
Clemons et al. (1998)	Canada (Toronto), September 1990–January 1991	PM_{10}, 1 urban site	6 (crude extract + 4 chemical fractions)	Crude extract and non polar fractions: significant activity (EEQ = 0.2–1.0 mg/g or 5–23 pg/m^3); polar fraction: no activity	No activity
Wang et al. (2004)	China (Wuhan), March 2000	PM_{100}, 1 urban traffic site	11 (3 crude extracts: sunny day, two foggy days; fractionation of foggy extracts: 4 chemical fractions, 2 days)	Crude extract = significant activity, higher activity in foggy days (EEQ = 0.75–0.79 μg/g) than in sunny day (EEQ = 0.19 μg/g). Polar fraction: higher activity	No activity
Matsumoto et al. (2005)	Japan (Osaka), October 2000–March 2001	PM, 1 urban site	4 (crude extract + 3 chemical fractions)	Crude extract and acidic fraction = significant activity, neutral fraction and basic fraction = no activity	N.A.
Klein et al. (2006)	Canada (Toronto and Egbert), March 2000–August 2001	PM, 1 urban site and 1 rural site	6 (4 urban, 2 rural)	Significant activity. No difference between rural and urban sites. No difference between seasons	N.A.
Wenger et al. (2009)	Switzerland (Bern and Payerne), January–February 2006	PM_1, 1 urban site, 1 rural site	24 (12 from each site)	Significant activity. Urban EEQ = 4.5–23 ng/g; 0.08–1.25 pg/m^3; rural EEQ = 2.4–22 ng/g; 0.07–0.77 pg/m^3	N.A.
Novák et al. (2009)	Czech Republic (3 sites), July–August 2005	PM (ø<50 μm), site A: industries and pesticide contamination; site B: traffic, domestic heating, PCB contamination; site C: rural background	15 (6 site A, 8 site B, 1 site C)	No activity	Sites A and B: significant activity (higher activity in region B), site C: no activity. Index of antiestrogenicity (1/IC25)≈ 0.1–10 mL/m^3
Kennedy et al. (2009)	Australia (Queensland)	Passive sampling, 1 suburban site	1	No activity	N.A.
Lammel et al. (2011)	Czech Republic (Brno area), August 2007–February 2008	PM_{10} and size subfractions, 3 urban sites and 1 rural site	24 (4 sites, 6 size subfractions)	No activity	Significant activity. No differences for particle size and sites. Index of antiestogenicity (1/IC25) ≈ 10–<100 mL/m^3
Novák et al. (2013)	Bosnia and Herzegovina (Banja Luka area), July 2008	PM_{10}, 1 urban site, 1 industrial site and 1 rural site	6 (day sample and night sample for each site)	No activity	Significant activity (all samples). No differences between day and night samples. Higher activity in the industrial site. Index of antiestrogenicity (1/IC50)≈ <1–2.5 mL/m^3
Chen et al. (2013)	Taiwan, June 2005–January 2006	TSP, 1 urban site, 1 suburban site, 1 rural site	24 (8 samples for each site)	Significant activity (proliferation assay)	Significant activity (some samples partially blocked the E2 proliferation)
Chen et al. (2013)	Taiwan, June 2005–January 2006	TSP, 1 urban site, 1 suburban site, 1 rural site	24 (8 samples for each site)	Significant activity (reporter gene assay)	Significant activity (some samples)
Novák et al. (2014)	Czech Republic (6 localities), July 2007–February 2008	PM_{10} and 6 size subfractions, 6 sites (cement mill, stone quarry, small airport, traffic junction, village, town)	36 (6 sites, 6 size subfractions)	Weak activity in three size subfractions from cement mill.	Significant activity (21/36 samples). Index of antiestogenicity (1/IC25) ≈ <1000–<100′000 L/m^3
Érseková et al. (2014)	Lithuania, Slovakia, Romania, Serbia, March–August 2006	Passive sampling, 4 background sites, 4 urban/industrial sites	8 (one for each site)	No activity	Antagonistic activity: induced by 5 samples (2/5 from background sites). Index of antiestrogenicity (1/IC50) = 0.91–6.80 mL/m^3
Wooten et al. (2015)	United States (Southern High Plains near feedyards), N.A.	TSP, 5 feedyards	40 (4 downwind samples and 4 upwind samples for each feedyard)	Significant activity (all samples). No difference among the feedyards	N.A.
Croes et al. (2016a)	Belgium (Borgerhout), April 2013–January 2014	PM_{10}, 1 urban traffic site	36	Significant activity (some samples). No seasonal trend. Mean EEQ: 0.0507 pg/m^3; max EEQ = 0.195 pg/m^3	N.A.
Croes et al. (2016b)	Belgium (Borgerhout, Zelzate, Houtem), April 2013–January 2014	PM_{10}, 1 urban traffic site, 1 industrial site, 1 rural site	116 (37 urban traffic, 41 industrial, 38 rural)	Significant activity (some samples). Seasonal trend: in the industrial site higher activity in winter. No difference among sites. Median EEQ: industrial site = 0.0321 pg/m^3; urban site	N.A.

(*continued on next page*)

Table 2 (*continued*)

Reference	Country and period	PM type	Samples (*n*)	Agonistic activity	Antagonistic activity (co-treatment with E2)
				= 0.0359 pg/m^3; rural site = 0.0311 pg/m^3	
Oziol et al. (2017)	France (Paris), autumn 2011– winter 2012	TSP, 1 urban site	2 (1 site, 2 periods: autumn and winter)	Significant activity. Higher activity in winter than in autumn. EEQ = 0.02–0.18 pg/m^3	No activity
Smith et al. (2019)	United States (Southern High Plains near diaries), April 2013	TSP, 4 diaries	16 (2 upwind samples and 2 downwind samples for each diary)	No activity	N.A.
Novák et al. (2020)	Czech Republic (Brno), October 2009–October 2010	PM_{10} and size subfractions, 1 rural site and 1 urban traffic site	48 (2 sites, 4 seasonal samples, 6 size subfractions)	Significant activity. Higher activity in the finest subfractions. Higher activity in colder season. EEQ PM_{10} urban: spring = 0.15 pg/m^3; summer = <0.102 pg/m^3; autumn = 0.48 pg/m^3; winter = 4.91 pg/m^3; EEQ PM_{10} rural: spring = 0.1 pg/m^3; summer = 0.09 pg/m^3; autumn = 0.09 pg/m3; winter = 3.25 pg/m^3	No activity
Nováková et al. (2020)	Czech Republic (Ostrava, Kosetice), February 2016–February 2017	PM_{10} and size subfractions, 1 rural background site, 1 urban site	30 (urban winter, urban summer, rural winter; 10 sample types = crude extract + 3 chemical fractions, 6 size subfractions)	Significant activity. PM_{10} and the most polar fraction induced the highest effect. Higher activity testing the smaller size subfractions. PM_{10} EEQ: urban winter = 9.05 pg/m^3; urban summer = n.d. pg/m^3; rural winter = 0.828 pg/m^3	No activity
Nováková et al. (2020)	Czech Republic (Ostrava, Kosetice), February 2016–February 2017	PM_{10} and size subfractions, 1 rural background site and 1 urban site	23 (urban winter, urban summer, rural winter; ALF: PM_{10} and 5 size subfractions; SELF: 2 size subfractions; PM_{10} urban summer not evaluated)	Significant activity (both ALF and SELF samples). ALF PM_{10} EEQ: urban winter = 1.40 pg/m^3; rural winter = n.d.	N.A.
Gea et al. (2021)	Italy (Piedmont region), October 2017 (forest fire episode)	PM_{10} (1 urban site, 2 rural sites), $PM_{2.5}$ (1 rural site, 1 urban site)	5	Weak activity	Slight antagonistic activity (tested without co-exposure with E2).
Zhou et al. (2022)	China (Hangzhou), September 2016–February 2017	$PM_{2.5}$ (1 traffic site, 1 industrial site)	4 (2 sites, 2 seasons)	Significant activity (all samples). Higher activity induced by the traffic site, no seasonal differences	N.A.
Gea et al. (2023a)	Italy (Piedmont region), January 2019–December 2020	$PM_{2.5}$ (1 urban site, 1 traffic site, 1 rural site) PM_{10} (1 incinerator site)	24 (4 sites, 6 monthly pools for 2 years)	Significant activity (22/24 samples). Rural samples induced a lower activity. Higher activity in cold months. EEQ = 0.02–2.41 pg/m^3	N.A.
Gea et al. (2023b)	Italy (Piedmont region), January–December 2018	$PM_{2.5}$ (1 urban site, 1 traffic site, 2 rural sites); PM_{10} (1 incinerator site)	20 (5 sites, 4 monthly pools)	Significant activity (all samples). Higher activity in winter and autumn than in spring and summer. EEQ: 0.08–1.21 pg/m^3	N.A.

alkylphenols, PCDD/PCDF (Klein et al., 2006; Wenger et al., 2009; Croes et al., 2016b; Oziol et al., 2017; Novák et al., 2020; Gea et al., 2023b). The oestrogenic activity was found to be correlated with SO_2 (a marker for combustion processes), PAHs, PCDD/PCDF (Wenger et al., 2009; Croes et al., 2016b; Novák et al., 2020; Gea et al., 2023b). However, the contribution of chemicals quantified using chemical analysis to the total oestrogenic activity assessed using effect-based tools was low. Indeed, Wenger et al. (2009) reported that PAHs contributed only to 0.01–0.2% of the activity and Novák et al. (2020) and Nováková et al. (2020) reported that detected chemicals accounted on average to the 1.6% and to the 3.9% of the activity, respectively. This finding could be due to lack of data about the oestrogenic activity of airborne pollutants (i.e. lack of relative potency factor) but also to the lack of chemical quantification of the main endocrine disrupting pollutants in the samples. In addition, this could be explained considering that the cumulative effect of chemicals contained in the mixture can be due to synergic of antagonistic interactions, while the effect estimated considering the concentrations of some pollutants is quantified assuming that the biological effects of chemicals are additive. Anyway, this result underlined that the application of the chemical analysis without the assessment through effect-based tools could potentially underestimate the endocrine activity of PM.

The oestrogenic antagonistic activity of PM co-exposed with E2 was assessed in 11 articles, 6 of them reported that some samples induced significant anti-oestrogenic effects. Similarly to agonistic activity, the antagonistic activity was induced by samples collected in different areas (i.e. urban, industrial and rural sites) and was detected also in the gas phase (Novák et al., 2009; Lammel et al., 2011, Novák et al., 2013; Érseková et al., 2014; Novák et al., 2014). This activity was attributed to emissions from motorcycle, diesel engine, agriculture and to cross talk between aryl hydrocarbon receptor (AhR) and ER signalling pathways (Novák et al., 2009; Lammel et al., 2011, Novák et al., 2013, Novák et al., 2014). Moreover, the antagonistic activity was found to be correlated with concentration of PAHs (Novák et al., 2014).

3.4. Androgenic activity of PM

Table 3 shows the results reported in articles in which agonistic or antagonistic androgenic activity of PM was assessed. As can be seen, the interaction between chemicals adsorbed on PM and AR was less studied than the interaction with ER; indeed, only 10 articles take into consideration this aspect. Therefore, this endocrine disrupting effect should be further studied in order to collect stronger evidence.

According to the reported results, PM mainly induced an antagonistic activity on AR (showed in 7 articles), although two articles demonstrated that it can also cause an androgenic agonistic activity (Wooten et al., 2015, Nováková et al., 2020). The antagonistic activity ranged

Table 3
Summary of the 10 articles in which agonistic/antagonistic androgenic activity of PM was assessed. ALF = artificial lysosomal fluid, DHT = dihydrotestosterone, Flu-EQ = flutamide equivalent quantity, IC = inhibition concentration, N.A. = not available, PCB = polychlorinated biphenyl, PM = particulate matter, SELF = simulated epithelial lung fluid, TSP = total suspended particles.

Reference	Country and period	PM type	Samples (*n*)	Agonistic activity	Antagonistic activity (co-treatment with DHT or testosterone)
Novák et al. (2009)	Czech Republic (3 sites), July–August 2005	PM (≤50 μm), site A: industries and pesticide contamination, site B: traffic, domestic heating, PCB contamination, site C: rural background	15 (6 site A, 8 site B, 1 site C)	No activity	Site A significant activity, sites B and C no activity. Index of antiandrogenicity (1/IC25)≈ 1–100 mL/m^3
Lammel et al. (2011)	Czech Republic (Brno area), August 2007–February 2008	PM_{10} and size subfractions, 3 urban sites, 1 rural site	24 (4 sites, 6 size subfractions)	No activity	Activity mostly associated with gaseous phase (and not to particulate phase)
Novák et al. (2013)	Bosnia and Herzegovina (Banja Luka area), July 2008	PM_{10}, 1 urban site, 1 industrial site, 1 rural site	6 (day sample and night sample for each site)	No activity	Significant activity (all samples). No differences among sites. Significant difference between day and night only in the industrial site. Index of antiandrogenicity (1/IC50) ≈ 0.5–3 mL/m^3
Novák et al. (2014)	Czech Republic (6 localities), July 2007–February 2008	PM_{10} and 6 size subfractions, 6 sites (cement mill, stone quarry, small airport, traffic junction, village, town)	36 (6 sites, 6 size subfractions)	No activity	Significant activity (the largest size subfraction and the finest size subfraction from cement mill). Index of antiandrogenicity (1/IC25) = 0.14 and 0.12 mL/m^3
Érseková et al. (2014)	Lithuania, Slovakia, Romania, Serbia, March–August 2006	Passive sampling, 4 background sites, 4 urban/ industrial sites	8 (one for each site)	No activity	Significant activity (both background and urban sites). Highest activity induced by a background site. Index of antiandrogenicity (1/IC50) = 0.33–2.55 mL/m^3
Wooten et al. (2015)	United States (Southern High Plains near feedyards), N.A.	TSP, 5 feedyards	40 (4 downwind samples and 4 upwind samples for each feedyard)	No activity for upwind samples, significant activity for all the downwind samples. No difference among the 5 feedyards.	N.A.
Croes et al. (2016b)	Belgium (Borgerhout, Zelzate, Houtem), April 2013–January 2014	PM_{10}, 1 urban traffic site, 1 industrial site, 1 rural background site	30 (10 for each site)	No activity	N.A.
Oziol et al. (2017)	France (Paris), autumn 2011—winter 2012	TSP, 1 urban site	2 (1 site, 2 periods: autumn and winter)	No activity	Significant activity in the winter extract. Flu-EQ = 0.00739 μg/m^3
Novák et al. (2020)	Czech Republic (Brno), October 2009–October 2010	PM_{10} and size subfractions, 1 rural site, 1 urban traffic site	48 (2 sites, 4 seasonal samples, 6 size subfractions)	No activity	Significant activity (mainly in the finest size subfractions). Higher effect during autumn and winter. Flu-EQ PM_{10} urban: spring = 0.01 μg/m^3; summer = 0.0001 μg/m^3; autumn = 0.04 μg/m^3; winter = 0.02 μg/m^3; Flu-EQ PM_{10} rural: spring = 0.01 μg/m^3; summer = 0.003 μg/m^3; autumn = 0.02 μg/m^3; winter = 0.04 μg/m^3
Nováková et al. (2020)	Czech Republic (Ostrava, Kosetice), February 2016–February 2017	PM_{10} and size subfractions, 1 rural background site, 1 urban site	30 (urban winter, urban summer, rural winter; 10 sample types = crude extract + 3 chemical fractions, 6 size subfractions)	Significant activity. Crude extract and the most polar fraction induced the highest effect. Higher activity testing the smaller PM fractions. Higher activity in urban winter samples. PM_{10} DHT-EQ: urban winter = 25.6 pg/m^3; urban summer = 3.89 pg/m^3; rural winter = 1.60 pg/m^3	No activity
Nováková et al. (2020)	Czech Republic (Ostrava, Kosetice), February 2016–February 2017	PM_{10} and size subfractions, 1 rural background site, 1 urban site	23 (urban winter, urban summer, rural winter; ALF: PM_{10} and 5 size subfractions; SELF: 2 size subfractions; PM_{10} urban summer not evaluated)	No activity	N.A.

between 0.0001 and 0.04 μg/m^3 Flu-EQ, while the agonistic activity ranged between 1.60 and 25.6 pg/m^3 DHT-EQ. The antagonistic activity was also found in the gas phase of air. In fact, many studies highlighted a higher antiandrogenic activity in the gas phase with respect to the PM activity (Novák et al., 2009; Lammel et al., 2011, Novák et al., 2014; Oziol et al., 2017, Nováková et al., 2020). The antagonistic activity was mainly attributed to organochlorine pesticides and burning processes (e.g. diesel and wood combustion), but also to alkylphenols, bisphenol A, PAHs, phthalates, PCBs, parabens, brominated diphenyl ethers (Novák et al., 2009; Lammel et al., 2011, Novák et al., 2013, Novák et al., 2014; Érseková et al., 2014; Oziol et al., 2017, Novák et al., 2020). As shown for the estrogenic activity, the contribution of chemicals quantified through chemical analysis to the total antiandrogenic activity assessed using effect-based tools was low; the detected chemicals accounted on average to the 11% and to less than 2% of the activity (Novák et al., 2020; Nováková et al., 2020).

As regards the agonistic activity, a significant androgenic activity was induced by PM collected downwind of feedyards, this activity was attributed to synthetic and endogenous androgens (Wooten et al., 2015). Moreover, also PM collected in urban and background sites induced a significant androgenic activity that could be due to some PAHs; this activity is probably caused also by other (not quantified) pollutants, indeed, the quantified PAHs explained only the 0.85% of the detected effect (Nováková et al., 2020).

3.5. Study limitations

This review summarizes and discusses the evidences about the endocrine activity of PM currently reported in the scientific literature; although this is an emerging topic, the main limitation of this study is due to the still limited number of available articles that have considered this topic. 24 articles were finally included in this review, so the reported results should be considered with caution and further studies are needed to strengthen the evidence obtained so far. Moreover, the articles published to date have mainly focused on the oestrogenic and androgenic activity of PM, therefore, this review is focused on these two endocrine disrupting activities. However, the PM could potentially cause other endocrine disrupting activities, e.g. by interfering with thyroid hormones.

The main strength of this review is in relation to the contribution that airborne endocrine disruptors may have on the overall body burden of oestrogenic and androgenic activity and it is probable that many articles will consider the topic in the coming years.

4. Conclusion

Although evidences about this topic are still scarce, the results of the present review showed that PM can induce oestrogenic, antioestrogenic, androgenic and antiandrogenic effects, suggesting that PM has an endocrine disrupting potential. This potential should be taken into consideration because it could represent a further source of exposure to EDs. Indeed, according to these evidences, the inhalation may increase the exposure level to EDs, which is generally believed to be due to ingestion and dermal contact. Although it is difficult to estimate how much inhalation can contribute to the total burden of EDs, endocrine activity of PM may increase the human health risk (Gea et al., 2023b).

In addition, this review underlined that the endocrine activity of PM could be due to many groups of air pollutants; although some of them are already monitored using traditional analytical methods, the results of the considered articles pointed out that the overall endocrine activity is difficult to predict from the concentrations of individual pollutants. Therefore, the assessment of the overall endocrine disrupting potential using effect based tools seems to be a valuable additional tool to quantify as best as possible the health risk posed by the presence of EDs in air.

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CRediT authorship contribution statement

Marta Gea: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft. **Elisabetta Fea:** Resources, Supervision. **Letizia Racca:** Investigation, Visualization, Writing – review & editing. **Giorgio Gilli:** Supervision. **Paolo Gardois:** Investigation, Supervision, Writing – review & editing. **Tiziana Schilirò:** Conceptualization, Resources, Supervision, Writing – review & editing.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.140887.

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