Environmental Pollution 264 (2020) 114790

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Exposure to wood smoke particles leads to inflammation, disrupted proliferation and damage to cellular structures in a human first trimester trophoblast cell line☆

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ARTICLE INFO

Article history:
Received 4 March 2020
Received in revised form
8 May 2020
Accepted 9 May 2020
Available online 11 May 2020

Keywords:
Wood burning
PAHs
Placenta
Trophoblast
Cytotoxicity
Mitochondria

ABSTRACT

The ongoing transition to renewable fuel sources has led to increased use of wood and other biomass fuels. The physiochemical characteristics of biomass combustion derived aerosols depends on appliances, fuel and operation procedures, and particles generated during incomplete combustion are linked to toxicity. Frequent indoor wood burning is related to severe health problems such as negative effects on airways and inflammation, as well as chronic hypoxia and pathological changes in placentas, adverse pregnancy outcome, preterm delivery and increased risk of preeclampsia. The presence of combustion-derived black carbon particles at both the maternal and fetal side of placentas suggests that particles can reach the fetus. Air pollution particles have also been shown to inhibit trophoblast migration and invasion, which are vital functions for the development of the placenta during the first trimester. In this study we exposed a placental first trimester trophoblast cell line to wood smoke particles emitted under Nominal Burn rate (NB) or High Burn rate (HB). The particles were visible inside exposed cells and localized to the mitochondria, causing ultrastructural changes in mitochondria and endoplasmic reticulum. Exposed cells showed decreased secretion of the pregnancy marker human chorionic gonadotropin, increased secretion of IL-6, disrupted membrane integrity, disrupted proliferation and contained specific polycyclic aromatic hydrocarbons (PAHs) from the particles. Taken together, these results suggest that wood smoke particles can enter trophoblasts and have detrimental effects early in pregnancy by disrupting critical trophoblast functions needed for normal placenta development and function. This could contribute to the underlying mechanisms leading to pregnancy complications such as miscarriage, premature birth, preeclampsia and/or fetal growth restriction. This study support the general recommendation that more efficient combustion technologies and burning practices should be adopted to reduce some of the toxicity generated during wood burning.

Abbreviations: DGI, Dekati gravimetric impactor; ER, endoplasmatic reticulum; EPA, environmental protection agency; FBS, fetal bovine serum; GC-MS, gas chromatography-mass spectrometry; HB, high burn rate; HIC, high income countries; hCG, human chorionic gonadotropin; ICP-MS, inductively coupled plasma-mass spectrometry; IL-6, interleukin-6; LIC, low income countries; MeOH, methanol; NB, nominal burn rate; PM, particulate matter; $PM_{2.5}$, PM of aerodynamic diameter <2.5 μm; PBS, phosphate-buffered saline; PAHs, polycyclic aromatic hydrocarbons; PTFE, polytetrafluoroethylene; TEM, transmission electron microscopy.

☆ This paper has been recommended for acceptance by Wen Chen.

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https://doi.org/10.1016/j.envpol.2020.114790

1. Introduction

Countries such as Sweden, with ample wood supplies, burn more biomass fuel per capita than most other countries in the world (Aguilar, 2014). Over the past few decades, the transition towards more renewable energy sources have led to a steady increase in the use of wood and other biomass fuels all over Europe (Wagner et al., 2010; Hoogeveen et al., 2013). During the same period, the knowledge, awareness and concern about the health issues related to traffic-induced air pollution have increased dramatically around the world. This has resulted in different interventions to lower traffic emissions, such as vehicle emission controls and low emission zones (Burns et al., 2019). Emissions from large-scale combustion for heat- and power production have been subject to some regulation, but wood smoke from households is rarely regulated (Burns et al., 2019). It is recognized that wood burning in the form of stoves, fireplaces, wildfires and agricultural fires emits significant quantities of harmful pollutants, and the evidence is clear that exposure to wood smoke causes inflammation in the respiratory system, and increases the incidence of asthma and allergic diseases (Allen et al., 2008; Orozco-Levi et al., 2006; Rokoff et al., 2017). However, there is a lack of knowledge regarding non-respiratory outcomes. In the light of recent intense wildfires burning in e.g. the US and Australia, that are linked to climate change (Flannigan, 2000) and that generates massive smoke plumes that can travel around the globe, there is an urgent need for research into both short- and long-term health impacts from wood smoke particle exposure.

Frequent indoor wood burning is related to severe health problems, and 4.3 million people die from indoor air pollution each year according to WHO (G.B.D. Risk Factor Collaborators, 2018). The lack of chimneys in houses in many Low Income Countries (LIC) causes high levels of air pollution, and the indoor levels can be more than 100-fold higher compared to those present outdoors in a typical residential area in a High Income Country (HIC). Combustion processes and type of wood also differ between LIC and HIC. Most of the knowledge on health effects from lower doses in HIC settings comes from exposure chamber studies and intervention studies. Chamber studies on healthy human subjects exposed to wood smoke suggest negative effects on the airways (Stockfelt et al., 2012), and on markers of inflammation, blood homeostasis, and lipid peroxidation (Barregard et al., 2006) already at relatively low exposure levels. Surprisingly, few population-based epidemiological studies examining the health impact of wood smoke in ambient air have been conducted. However, intervention studies conducted in Montana, USA, where old furnaces were replaced with newer heating technology, showed improved health measured as less respiratory symptoms (Noonan, 2012). We have previously shown that acute exposure to wood smoke particles generated during incomplete combustion causes reduced levels of immune cells in human lungs, indicating that the particles are cytotoxic (Muala et al., 2015). Moreover, these particles have a higher relative (per mass) toxicity than those generated during more efficient combustion (KocbachBolling et al., 2009; Tapanainen et al., 2012), further highlighting the importance of the combustion process when it comes to health risks. Metals and polycyclic aromatic hydrocarbons (PAHs) that are present both inside and outside of soot particles have been strongly linked to toxicity (Costa and Dreher, 1997; Bostrom et al., 2002) and the toxicity of PAHs in cell assays is enhanced when they are adsorbed onto a soot particle surface, compared to PAHs individually applied in suspension (Dilger et al., 2016). Burning of biomass fuels for cooking and heating has been shown to be a major global source of PAHs and its emission contributes to high levels of indoor exposure for women and children in LIC (Kim, 2011). In addition, particles generated from a Norwegian wood stove was shown to induce higher levels of DNA damage than seen for particles originating from traffic (Danielsen, 2009). Overall, it is clear that emissions from biomass combustion have an adverse effect on public health, but more knowledge is needed to understand and assess these effects (Sigsgaard et al., 2015).

Traffic-derived particles have been shown in numerous studies to have adverse effects on e.g. respiratory health, cardiovascular disease and autoimmune diseases (Zhao et al., 2019; Loxham, 2019; Zhang et al., 2019), but also on pregnancy outcome (Xue, 2019). We and others have demonstrated an association between combustion-related air pollution and the risk of preeclampsia during pregnancy (Pedersen et al., 2017; Malmqvist, 2013). Studies have found a link between ambient air pollution and effects in placentas on the molecular level, with changes to DNA methylation, mitochondrial DNA content and methylation, microRNA expression and nitrative/oxidative stress (Luyten et al., 2018; Saenen et al., 2019). Molecular consequences for the developing fetus has also been observed, seen as elevated levels of liver enzymes (Pejhan et al., 2019), lipids (Heydari et al., 2020) and cortisol (Khamirchi et al., 2020) in the cord blood of newborn babies. Neonate mice exposed *in utero* to traffic-derived particles show reduced metabolic activity, dysregulated gene expression and DNA hypomethylation in cardiomyocytes (Goodson, 2019), resulting in alterations of the developing heart that could predispose to heart disease. When it comes to indoor wood and stove emissions it has been shown that it is linked to chronic hypoxia and pathological changes in placentas (Dutta et al., 2018). But it is also associated with a risk of adverse pregnancy outcome (Amegah, 2014), preterm delivery (Wylie et al., 2014), increased perinatal mortality (Weber et al., 2020) and a 2-fold higher risk of preeclampsia (Agrawal and Yamamoto, 2015). Furthermore, *in utero* exposure to PAHs results in reduced fetal and child growth (Tang et al., 2006). The placenta is a natural barrier between the mother and fetus, and is exposed to environmental stressors throughout pregnancy including air pollution that enters the mother's lungs and circulation. Recently a study demonstrated the presence of combustion-derived black carbon particles at both the maternal and fetal side of placentas, suggesting that particles can be transported from the mother through the placental tissue to reach the fetus (Bove et al., 2019). In addition, transplacental transport of benzo(α)pyrene, a common PAH in wood burning emissions, has been demonstrated to occur from the maternal side to the fetal side in the human placenta perfusion model (Mathiesen, 2009). The developing fetus is highly susceptible to exposure-related alterations that can affect both metabolism and development, and in the end also the pregnancy outcome. Furthermore, adverse pregnancy outcomes such as preeclampsia and intrauterine growth restriction are associated with incomplete placentation, which may be due to reduced invasion ability of the extravillous trophoblasts in early pregnancy. Studies have shown that exposure to particulate matter of size <2.5 μm in aerodynamic diameter ($PM_{2.5}$), or PAHs and metals carried by $PM_{2.5}$, can inhibit trophoblast migration and invasion, as well as causing cell cycle arrest (Li and Loch-Caruso, 2007; Liu et al., 2016; Qin et al., 2017).

There is currently a lack of data regarding the effect of well characterized wood smoke particles on placental cells, and to address this we exposed placental first trimester trophoblast cells to collected wood smoke particles emitted under two different burning conditions in a Nordic wood stove. We examined cellular responses and particle cytotoxicity. The experiments were conducted within a larger project that also assessed impact from traffic related particles (Familari et al., 2019; Nääv, 2020), thus providing comparable cell responses.

2. Material and method

2.1. Combustion setup and particle collection

The fuels used in this study were wood logs from four different species; silver birch (*Betula pendula*), quaking aspen (*Populus tremula*), Norway spruce (*Picea abies*), and Scots pine (*Pinus sylvestris*). These four species can be regarded as representative of the fire wood used as biomass fuel in the Nordic countries. Small-scale wood combustion in residential settings in Sweden accounts for ~10 TWh annually, corresponding to ~ two million tonnes of dry wood (fire wood) (Swedish Energy Agency, "Energy in Sweden 2019", official statistics). The combustion procedure used has previously been described in Nyström et al. (2017). The combustion setup consisted of a typical natural draft 9 kW Nordic wood stove installed in a laboratory setup to ensure consistent and controlled combustion and sampling conditions. A flue gas fan was used to maintain a constant low pressure of 10 Pa, to simulate a chimney draft found in real-life residential applications. The wood stove was, as previously stated, operated in two different combustion modes: Nominal Burn rate (NB) and High Burn rate (HB). To achieve nominal burn rate, the operation instructions from the manufacturer were followed, stating that each batch should not exceed 2.5 kg of wood, divided on three wood logs. The wood logs were ignited by placing them on a glowing bed of embers from at least one previous batch, to avoid cold start conditions, and then opening a grate in the bottom to enable air to pass through the glowing embers and around the wood logs. After 3–4 min the wood logs were fully ignited and the grate was closed. The sampling from the flue gas channel started when the logs were added onto the glowing embers and stopped when the last flame had extinguished. The same procedure was carried out for the HB with the difference that five smaller wood logs were used with a combined weight of 3.5 kg, thus slightly overloading the stove. The variations in burn rate between NB and HB were illustrated by differences in both average excess O_2 in emissions (12% for NB and 7% for HB) and shorter episodes of air-starved conditions (O_2 <2%) in the HB case (Nyström et al., 2017). During sampling of aerosol in the flue gas channel, the particles were diluted with air through a porous tube dilutor with the dilution ratio of 1:70, and further collected onto polytetrafluoroethylene (PTFE) filters using a five stage Dekati Gravimetric Impactor (DGI). Particles with an aerodynamic diameter of <1 μm were selected by pooling stages 1–3 from the DGI. Based on the assumption that regardless of wood type, a wood stove operated by the same person would most of the time perform with the same burn rate, particles collected from all four wood types were pooled for each combustion mode (NB and HB). After pooling, the collected particles were extracted from the PTFE filters using a pure methanol (MeOH) extraction protocol as follows. The filters were cut into 2 cm-squares and put into 120 ml MeOH in a 250 ml Erlenmeyer flask. The flask was sonicated under foil cover for 60 min in an ultrasonic bath at a temperature below 30 °C. The MeOH extract was transferred to a clean flask, and another 120 ml MeOH was added to the cut up filters. The sonication procedure was repeated and the new extract was added to the previous. The whole procedure was then repeated for a third time. The resulting extract was divided into aliquots in PCR-clean 1.5 ml Eppendorf tubes. The MeOH was then evaporated in a vacuum centrifuge evaporator (SpeedVac HT-4X, GeneVac Technologies). Each Eppendorf tube was numbered and weighed both before being filled with MeOH extract and after evaporation. The numbered and weighed Eppendorf tubes, containing powdered dry material, were carefully sealed and stored at room temperature until use. With regards to contaminating microbial products in the collected particles, we have unpublished data supporting that wood smoke particles of <1 μm emitted under high effect fires in a Nordic wood stove using non-mouldy wood contains no microbial products such as endotoxins or ergosterol. Moreover, due to the hot smoke, the use of pure methanol, sterile plastic and glass ware, and working in a LAF-bench during the process of preparing the particles, we consider the resulting particles sterile and suitable for cell culture work.

2.2. Metal and PAHs analysis of NB and HB wood smoke particles

Dried NB and HB wood smoke particles were analyzed for their PAHs and metal content. The particles were analyzed by gas chromatography-mass spectrometry (GC-MS) for the presence of PAHs (Strandberg et al., 2018) and by inductively coupled plasma-mass spectrometry (ICP-MS) for the metal composition. Metals analyzed were: aluminium (Al), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), thallium (Tl), vanadium (V) and zinc (Zn). For the metal analysis, ~10 mg particles were digested in 1 ml concentrated nitric acid at 70 °C for 16 h. After dilution with Milli-Q water, the metal concentrations were determined by ICP-MS (iCAP Q, Thermo Scientific, Bremen, GmbH) in collision cell mode with kinetic energy discrimination and using helium as collision gas. The detection limits (calculated as three times the standard deviation of the blank) for each metal were: 0.05 ng for Mn, Ni, As, Cd, Tl, Pb; 0.06 ng for Ba; 0.07 ng for Cr, Cu; 0.08 ng for V; 0.09 ng for Co; 0.15 ng for Zn; 0.70 ng for Fe and 2.7 ng for Al.

2.3. Dispersion of NB and HB particles

After collection, the wood smoke particles were stored as powdered material in Eppendorf tubes and required sonication for resuspension prior to exposure experiments. This is standard procedure in air pollution research when using cell cultures (LasagniVitar et al., 2018). For this, 1.0 mg of dried wood smoke particles (NB or HB) were dissolved in 1 ml of cell medium (see below). The particles were subjected to indirect ultra-sonication at 4 °C and at 120 W for 15 min using an Ultrasonic Cleaner water bath (Mettler Electronics), followed by direct sonication at room temperature at 50 W, 0.05 cycle, 20% amplitude for 60 s using an UP50H Ultrasonic Processor (Hielscher Ultrasound Technology). The particle immersions were divided into aliquots and used directly at desired concentrations, or stored at 4 °C until use. Stored aliquots were subjected to an additional direct sonication step followed by vortex before every exposure experiment. Cell medium without particles underwent the same procedure and was used in the same volumes to expose control cells.

2.4. Cell culture

We used the commercially available human first trimester trophoblast cell line HTR-8 (HTR-8/SVneo (ATCC® CRL-3271™), lot number 64275781) to do exposure experiments with wood smoke particles (NB and HB). The HTR-8 cell line was derived by transfecting cells from chorionic villi explants from placentas of 6–12 weeks of gestation and exhibit a variety of markers characteristic of extravillous invasive trophoblast cells (www.lgcstandards-atcc.org). HyClone RPMI-1640 medium (Fisher Scientific) was used for culturing, supplemented with 5% fetal bovine serum (FBS) (Life Technologies), 100 μg/ml streptomycin and 100 U/ml penicillin (Fisher Scientific). Incubation was performed at 37 °C in a humidified 5% CO_2 incubator. Cells were seeded at 3×10^5 cells per well in 6-well plates or at 1×10^4 cells per well in 96-well plates, if not otherwise stated. Cells were incubated over night after being seeded, and thereafter subjected to particle exposure at 50, 500, 1000, 5000 and 10000 ng/ml for 48 h. The range of doses used in

this study was based on calculations and estimations from two previous studies (Familari et al., 2019; Nääv, 2020), where the highest dose of 10000 ng/ml is suggested to correspond to a level of PM2.5 air pollution of 50 μg/m (Hoogeveen et al., 2013). As a reference, WHO has a target of 25 $\mu g/m^3$ for short term exposure (24 h). Cell culture supernatants were harvested at 48 h and analyzed for protein secretion. Extended cultures for 7 days were used for cell number counts.

2.5. Cell culture supernatant analysis

The cell culture supernatants were analyzed for cellular secretion of human chorionic gonadotropin (hCG), progesterone and interleukin-6 (IL-6) after 48 h exposure. The hCG and progesterone analyses were performed at the Clinical Biochemistry Laboratory at Lund University Hospital, Sweden. For the human IL-6 analysis, we used a human IL-6-specific ELISA (Invitrogen) according to manufacturer's protocol, and all samples were run as duplicates.

2.6. Transmission electron microscopy analysis of cellular particle uptake

HTR-8 cells plated in 6-well plates were exposed to 500 ng/ml of NB or HB particles for 48 h. The cells were washed with phosphate-buffered saline (PBS), trypsinated (0.25% trypsin) and pelleted by centrifugation. The cells were fixed for 2 h at room temperature in fixative (1.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M Sörensen buffer pH 7.2), washed and then stored overnight at 4 °C in Sörensen buffer. The fixed samples were prepared for ultrathin sectioning and subjected to transmission electron microscopy (TEM) analysis as reviewed in (Carlemalm, 1990). Microscopy was performed on duplicate sections from cells harvested from 1 well per particle type and time point.

2.7. Cytotoxicity

To evaluate the cell cytotoxicity of the wood smoke particles, we used the CellTox Green® cytotoxicity assay (Promega) according to manufacturer's protocol to measure changes in membrane integrity. Shortly, the assay uses a cyanine dye that is excluded from viable cells but binds to the DNA of compromised nonviable cells, and the fluorescent signal that is produced is proportional to cytotoxicity. Cells plated in 96-well plates were exposed to NB or HB particles for 48 h. The CellTox green dye included in the assay was diluted 1/500 in complete cell medium and applied to cells. After 15 min of incubation at room temperature, fluorescence was measured at 485 nm/535 nm using a VICTOR (Hoogeveen et al., 2013) 1420 Multilabel Counter (PerkinElmer) plate reader.

2.8. PAHs analysis of exposed cells

After exposure to 10 000 ng/ml of NB or HB particles in 6-well plates for 48 h, cells were harvested and the cell pellet lysed with 500 μl of cell lysis buffer (Qiagen), followed by sonicated for 30 min. To each sample, 500 μl of water (MilliQ) and 40 ng of internal standards (1 ng/μl) containing the 16 U.S. Environmental Protection Agency (EPA) priority PAHs (Dr Ehrenstorfer, Augsburg, Germany) were added. Samples were extracted twice with 2 ml of dichloromethane, the extractions pooled and evaporated to near dryness. To each of the samples, 40 ng of recovery standard and 100 μl hexane was added, and samples were transferred to HPLC glass vials (Agilent Technologies). Analysis was performed on a GC-MS 7890 (Agilent Technologies) as described earlier (Strandberg et al., 2018) for triplicates of each group.

2.9. Statistical analysis

Statistical analyses were performed using Origin 8 software (Microcal Northampton, USA). Differences between groups were evaluated using one-way ANOVA followed by post hoc Tukey test, and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Characterization of wood burning particles

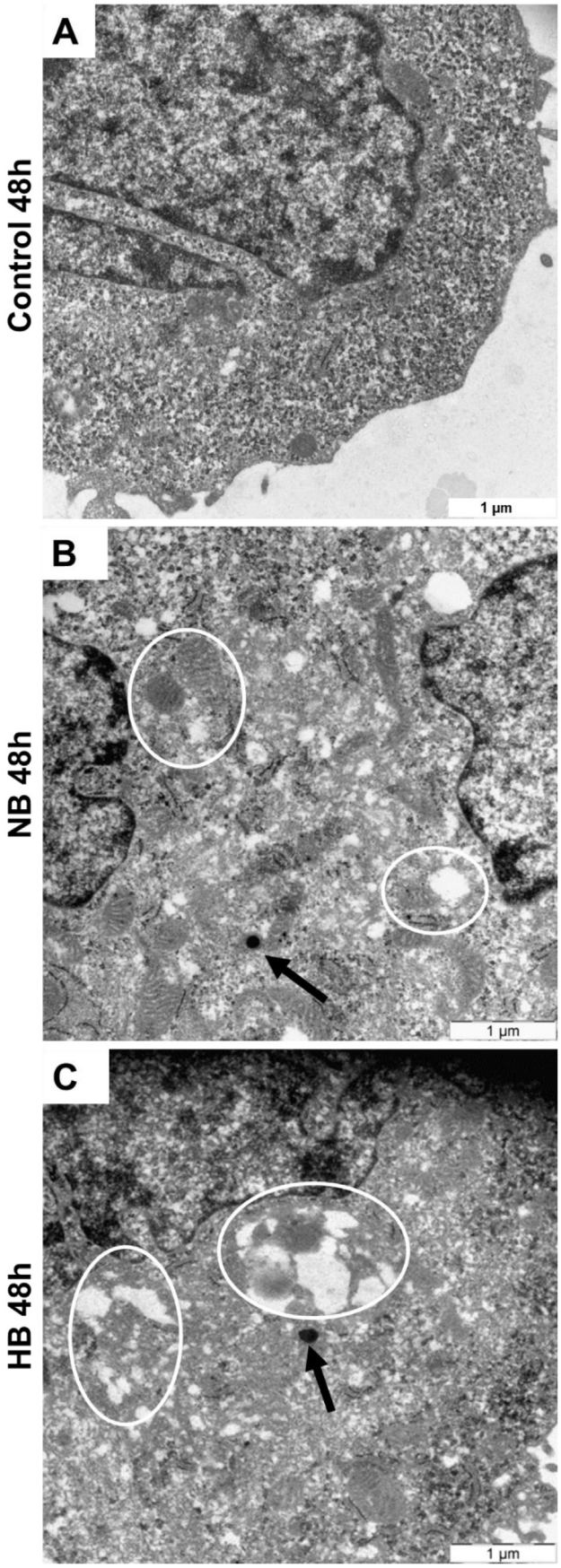
The pooled NB particle samples consisted of material from combustion of the different wood fuels distributed as: 33% from birch tests, 17% from aspen tests, 30% from spruce tests, and 20% from pine tests. The particles from the pooled HB samples had a somewhat different composition, distributed as: 7% from birch tests, 27% from aspen tests, 16% from spruce tests, and 50% from pine tests. Both NB and HB wood smoke particles were analyzed for the presence of 32 different PAHs, which can be viewed in Table S1. The HB particles contained 3.5 times more total amount of PAHs per mg (2792 ng/mg) compared to what was found in the NB particles (764 ng/mg). The distribution of low molecular weight (LMW, 2–4 aromatic rings) and high molecular weight (HMW, 5–6 aromatic rings) PAHs differed between the NB and HB particles, where the NB particles contained more LMW PAHs (73% LMW vs 27% HMW) and the HB particles more HMW PAHs (44% LMW vs 56% HMW). For example, the content of benzo(a)pyrene, a HMW PAH and a well-known group 1 carcinogen (WHO, 2006), was 7 times higher in the HB particles than in the NB particles. The two types of wood smoke particles were also analyzed for their metal composition, looking at 14 different metals. We found that they contained similar levels of total amounts of metals (1080 and 1215 ng/mg particles, respectively). Some metals were more abundant in NB particles (cadmium and chromium) while others were more abundant in HB (aluminium, barium, copper, iron and manganese), with zinc being the most prevalent metal in both (Table S2).

3.2. Wood smoke particles localizes to mitochondria

The cell line HTR-8, with a phenotype that is characteristic for extravillous invasive trophoblast cells, was used for *in vitro* exposure experiments. Cellular uptake of wood smoke particles after exposure was evaluated by TEM. After 48 h of exposure to 500 ng/ml of particles, both NB and HB particles were visible inside cells and both were localized to the mitochondria (Fig. 1). Unexposed control cells showed mitochondria and endoplasmic reticulum (ER) with normal morphology (Fig. 1A). Cells exposed to NB or HB particles displayed ultrastructural changes to both the mitochondria and the ER, with vacuolization, swelling of membranes and disruption of cristae (Fig. 1B and C).

3.3. Secretion of pregnancy-related hormones and pro-inflammatory IL-6

To investigate any effects of wood smoke particle exposure on markers related to placenta biology, HTR-8 cells were exposed to NB or HB particles for 48 h at concentrations ranging from 50 to 10000 ng/ml. Markers secreted by trophoblast cells that were examined were hCG (pregnancy marker), IL-6 (pro-inflammatory) and progesterone (pregnancy hormone). The HTR-8 cells showed a significant 27% drop in secreted hCG compared to controls already at ≥50 ng/ml ($p < 0.0001$) NB- or HB-exposure (Fig. 2A and B), as well as a significant 2-fold increase in IL-6 production at ≥500 ng/ml ($p < 0.0001$) (Fig. 2C and D). In addition, there was no significant changes in the progesterone production after exposure to NB



particles compared to controls (Fig. 2E), while there was a significant increase ($p < 0.05$) for the HB particles at higher concentrations, 5000 ng/ml (Fig. 2F).

3.4. Membrane integrity is affected by exposure to wood smoke particles

To evaluate any effects of wood smoke particle exposure on cell membrane integrity in exposed cells, we used the CellTox™ Green Cytotoxicity assay. After 48 h of exposure to NB particles, the cells showed significant cytotoxicity at ≥50 ng/ml ($p < 0.001$) compared to controls (Fig. 3A). Exposure to HB particles for 48 h resulted in a small increase in cytotoxicity at lower particle doses, although not significant compared to the control cells (Fig. 3B). However, when leaving cultures for a longer period of time, 7 days, there was a significant 40% drop in cell numbers in the HB cultures already at ≥50 ng/ml particles ($p < 0.01$) (Fig. 3D). For the NB cultures there was a 27% drop in cell numbers at 50 ng/ml ($p < 0.001$), which increased to 40% drop ($p < 0.0001$) at 500 ng/ml and 5000 ng/ml (Fig. 3C).

3.5. PAHs detected in exposed cells

HTR-8 cells seeded in 6-well plates were exposed to 10 000 ng/ml of NB or HB particles for 48 h, and thereafter the harvested cell lysates were analyzed for the presence of PAHs (Table 1). The level of PAHs found in cell lysate ranged from 0.4 ng (benzo(g,h,i)perylene) to 22.9 ng (phenanthrene) for NB exposed cells, and from 1 ng (benzo(g,h,i)perylene) to 20.6 ng (phenanthrene) for NB exposed cells. For 3 PAHs there were significantly higher levels ($p < 0.0001$) in cells exposed to NB or HB particles compared to untreated cells; phenanthrene, fluoranthene and pyrene. All these three are LMW PAHs and detected at higher concentrations compared to the HMW PAHs (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene). There was a significantly higher total amount of PAHs in cells exposed to NB ($p < 0.01$) or HB ($p < 0.05$) compared to untreated cells (Table 1).

4. Discussion

Our results demonstrate that particles derived from wood burning can enter the cell and localize to the mitochondria in trophoblast cells with a phenotype of extravillous invasive trophoblasts. This is a cell type that is critical to the early stages of placenta development. A functional placenta is vital for normal fetal development and for a normal progression of the pregnancy. During placentation, extravillous trophoblasts penetrate and invade the uterine wall where they transform the spiral arteries to establish the utero-placental blood circulation, to assure the nutrient and gas supply to the developing fetus (Velicky, 2016). Severe pregnancy complications, such as preeclampsia and fetal growth restriction are associated with abnormal function of the extravillous invasive trophoblasts, resulting in shallow invasion and decreased blood flow to the placenta. A recent study investigating if ambient carbon particles could be transported to the fetus during pregnancy, showed that the particles were present in the placenta

Fig. 1. Cellular uptake of wood smoke particles and localization to mitochondria. TEM analysis of cells exposed to a single dose of 500 ng/ml of NB or HB particles for 48 h. (**A**) Unexposed cells had mitochondria with normal morphology. Cells exposed to NB particles (**B**) or HB particles (**C**) had particles localized to the mitochondria (black arrow), which demonstrated vacuolization, swelling and cristae disruption (white oval). Scale bar = 1 µm. TEM – transmission electron microscopy; NB – nominal burn; HB – high burn.

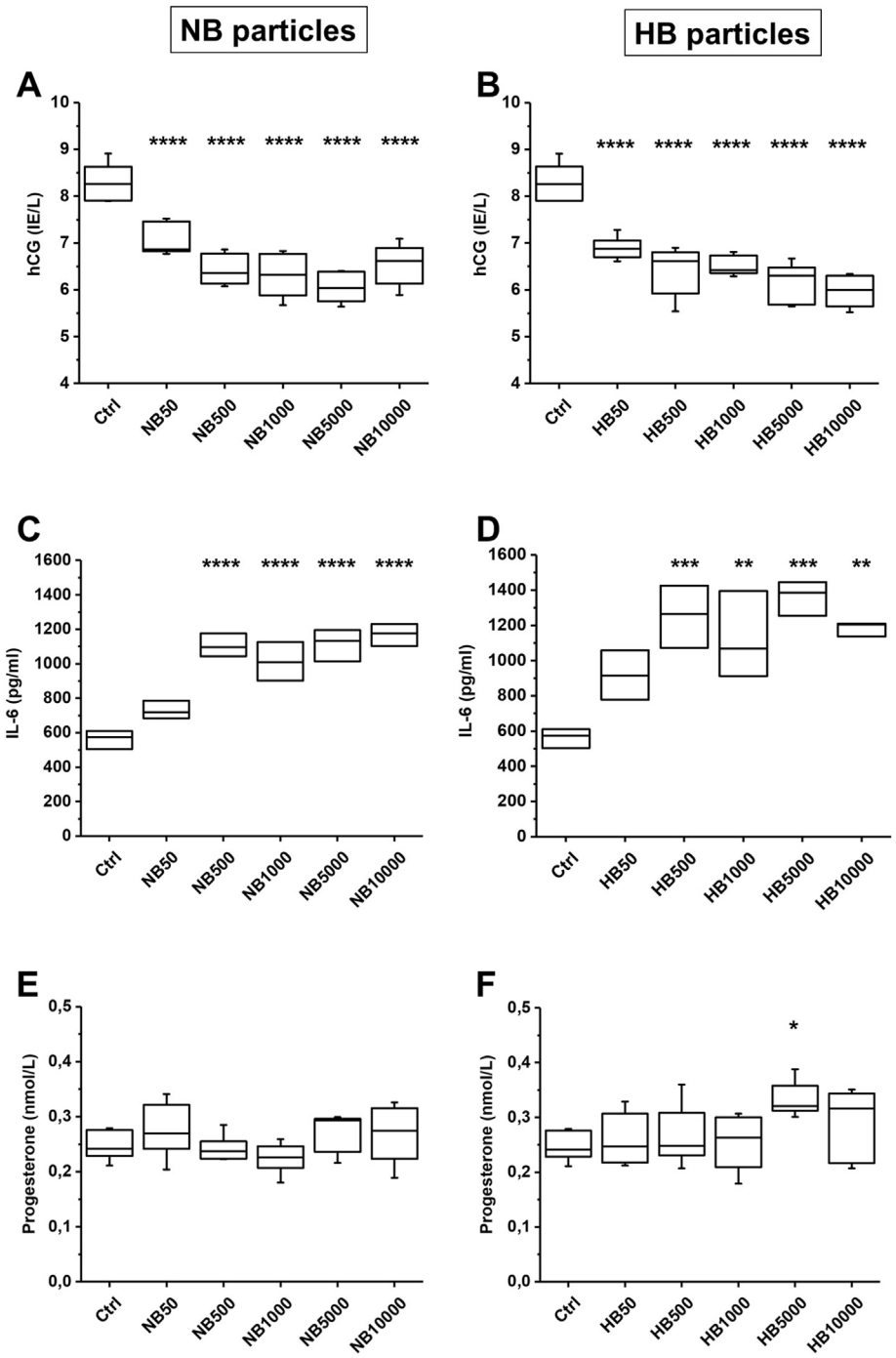


Fig. 2. Exposure to wood smoke particle induce changes in hCG and IL-6 secretion. HTR-8 cells were exposed for 48 h to 50–10000 ng/ml of NB or HB particles. (**A-B**) Levels of hCG in supernatant after 48 h of exposure. The y-axis scale starts at 4 IE/L. Data is presented as box plots with median values and 25th and 75th percentile, and with whiskers indicating min-max (n = 6). (**C-D**) Levels of IL-6 in supernatant after 48 h of exposure. Data is presented as box plots with median values and min-max (n = 3). (**E-F**) Levels of progesterone in supernatant after 48 h of exposure. Data is presented as box plots with median values and 25th and 75th percentile, and with whiskers indicating min-max, n = 6. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. NB – nominal burn; HB – high burn; hCG – human chorionic gonadotropin.

at week 12 of gestation and were accumulating on the fetal side of the placenta (Bove et al., 2019). This means particles can reach the placenta already during the early stages of pregnancy, potentially exposing the fetus to toxic compounds during a period when the fetus is highly vulnerable. Particles could also penetrate the extravillous trophoblasts at time of placentation, and potentially have detrimental effects causing oxidative stress, mitochondrial damage and energy storage depletion, as have been demonstrated for lung fibroblasts (Bove et al., 2018). This would have consequences for trophoblast cell function and a negative effect on migration and invasion during the placentation, as have been demonstrated *in vitro* (Li and Loch-Caruso, 2007; Liu et al., 2016; Qin et al., 2017). Furthermore, exposure to air pollution during this critical period results in placental global DNA hypomethylation (Janssen et al., 2013), and exposure during the early stages of pregnancy could also have detrimental effects on health later in life

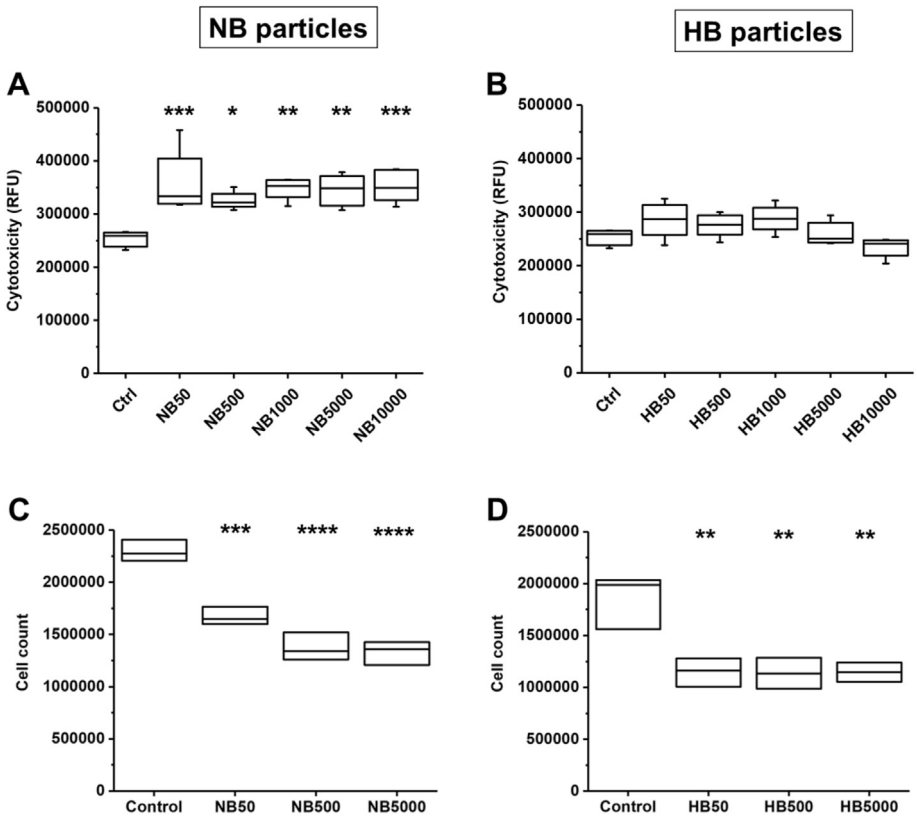


Fig. 3. Exposure to wood smoke particles causes changes in membrane integrity and reduction in cell numbers. Cytotoxicity after 48 h of NB exposure at 50–10000 ng/ml (**A**) or 48 h of HB exposure at 50–10000 ng/ml (**B**). Data is presented as box plots with median values and 25th and 75th percentile, and with whiskers indicating min-max, n = 5. Cell counts from 7-days cultures for cells exposed to NB at 50–5000 ng/ml (**C**) or HB at 50–5000 ng/ml (**D**). Data is presented as box plots with median values and min-max (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. NB – nominal burn; HB – high burn.

Table 1
Detection of PAHs in cells after 48 h exposure to 10 000 ng/ml NB or HB particles.

PAHs	Control (ng)	NB (ng)	HB (ng)
phenanthrene	2.0 ± 1.2	22.9 ± 1.4****	20.6 ± 1.5****
anthracene	1.2 ± 0.7	1.2 ± 0.5	1.0 ± 0.4
fluoranthene	2.7 ± 0.6	12.3 ± 0.5****	11.6 ± 1.6****
pyrene	2.8 ± 0.8	13.9 ± 0.4****	11.6 ± 1.4****
benzo(b)fluoranthene	4.2 ± 1.1	3.2 ± 1.3	1.5 ± 1.3
benzo(k)fluoranthene	2.6 ± 1.1	3.4 ± 3.2	4.7 ± 6.5
benzo(a)pyrene	2.6 ± 1.1	1.1 ± 1.0	1.0 ± 1.1
dibenzo(a,h)anthracene	1.9 ± 1.1	0.9 ± 0.5	1.8 ± 2.4
benzo(g,h,i)perylene	2.7 ± 0.9	0.4 ± 0.3	1.0 ± 0.8
Total PAHs	**22.9 ± 8.4**	**59.3 ± 8.5****	**54.8 ± 12.1***

Data is presented as mean ± SD and n = 3. *p < 0.05, **p < 0.01, ****p < 0.0001. PAHs – polycyclic aromatic hydrocarbons; NB – nominal burn; HB – high burn.

for the child, on e.g. growth and mental health (Pagliaccio et al., 2020; Rundle et al., 2019). In pregnant rabbits exposed to aerosolized diesel exhaust, diesel nanoparticles were found in the maternal circulation, in trophoblast cells as well as in fetal blood, demonstrating transplacental transfer of particles (Valentino et al., 2016). This resulted in reduced placental function and growth retardation in the pups. But the exposure also resulted in metabolic modifications in the second generation after exposure, where grand-dam exposure resulted in decreased cholesterol and increased triglyceride levels in plasma, demonstrating negative health effects through generations.

We showed that wood smoke particles localizes to the mitochondria and cause structural damage. We also detected several PAHs originating from the particles inside exposed cells. The exposure to PM, from both combustion modes, caused a down-regulation of the pregnancy marker hCG and an increase in the pro-inflammatory cytokine IL-6. The levels of hCG in early pregnancy is important for the maintenance of the corpus luteum in the ovaries, which allows for secretion of progesterone during the first trimester. Also, hCG has been suggested to contribute to the local maternal immunotolerance and to facilitate trophoblast invasion (Kayisli, 2003). Increased levels of pro-inflammatory cytokines in the fetoplacental compartment has been associated with adverse pregnancy outcomes in humans (Vrachnis et al., 2010), and improper activation is linked to a disruption of trophoblast function needed for a normal pregnancy (Challis et al., 2009). Interleukin-6 is used as a marker of inflammation during pregnancy and maternal immune activation results in increased IL-6 production by trophoblast cells in the placenta (Hsiao and Patterson, 2011). Elevated IL-6 during pregnancy affects normal fetal brain development and results in behavioral abnormalities in the offspring (Wu, 2017). Interleukin-6 has also been shown to increase in the circulation due to acute air pollution exposure (van Eeden et al., 2001). The pregnancy hormone progesterone was not affected by NB exposure, and only to a low extent by HB exposure. However, it is not until week 12 that the trophoblasts become the major producer of progesterone (Schneider, 1993), up until then progesterone is produced by the corpus luteum. The HTR-8 cells represents a first trimester extravillous trophoblast and may therefore not yet produce progesterone at any significant levels, which may explain the low levels we detected that were close to the detection limit of the assay. Exposure to wood smoke particles also disrupted membrane

integrity and reduced the number of cells in the cultures, indicating disrupted proliferation. Overall, the wood smoke particles appear to have an effect on the HTR-8 cells that are similar to what we have observed previously for traffic-related $PM_{2.5}$, however, they seem to be already at low doses more cytotoxic and to induce a greater reduction in proliferation (Familari et al., 2019; Nääv, 2020). When comparing the wood smoke particles with the traffic-derived $PM_{2.5}$ from Malmö, Sweden (Familari et al., 2019; Nääv, 2020), NB and HB particles contained ~25- and 100-times more PAHs, respectively, while the Malmö particles contained ~1.5-times more metals.

The knowledge is still limited regarding short- and long-term effects from wood smoke exposure on human health (Sigsgaard et al., 2015; Black, 2017). However, studies on health effects associated with smoke exposure during the 2008 wildfires in northern California, USA, has shown an association between the level of exposure and respiratory illnesses (Reid et al., 2016). A study conducted on rhesus macaque monkeys that were exposed to wood particles during infancy from the very same wildfires, has shown that the monkeys develop immune dysregulation and have 20% smaller lungs compared to controls born one year later (Black et al., 2017b). The differences started to show when the monkeys came into adolescence and continues as they mature, demonstrating long-term effects from early life exposure. Recent years intense wildfires around the world generate massive smoke plumes that travels around the globe, exposing millions of people to wood particles from these fires, and climate change is expected to increase the frequency and intensity of wildfires in the future (Flannigan, 2000). These fires also offer an opportunity to study the effect on human health, both short- and long-term and during pregnancy.

We have previously shown that the total amount of PM emission during HB in general is more than double compared to NB, although with rather similar composition (Nyström et al., 2017). However, there were some differences in particle properties seen at the time between the two modes, such as an increased fraction of soot and PAHs in the HB particles. In the present study, the analysis showed that the total PAHs content was 3.5-times higher in the HB particles compared to the NB, and with some of the individual PAHs showing up to 8 times higher levels in the HB particles. The total amount of metal was similar, but 5 different metals were more abundant in the HB particles and two in NB. The HB particles seemed to elicit a stronger pro-inflammatory IL-6 response than NB, but NB particles had a stronger cytotoxic effect on the cells. However, the cells exposed to HB particles demonstrated a more dramatic fall in proliferation already at the lowest level of exposure, indicating a stronger cytotoxic effect. It has been concluded that less efficient biomass combustion results in a higher relative (per mass) toxicity of the particles than for those generated during more efficient combustion (KocbachBolling et al., 2009; Tapanainen et al., 2012). Moreover, acute exposure to wood smoke particles from incomplete combustion has been shown to have an immediate impact on the cardiovascular system by increasing central arterial stiffness and heart rate (Unosson et al., 2013). The PAHs detected in the exposed cells showed higher concentrations of LMW PAHs than of HMW PAHs. It is possible that HMW PAHs were not dissolved in the medium to the same extent as the LMW PAHs, and therefore not as accessible to the cells. Or the HMW PAHs were broken down to other metabolites. However, the level of PAHs found in the cells were in general rather low, so the conclusion at this point is only that PAHs from the particles are present inside the cells after exposure.

Our study has some limitations that warrants a comment. Firstly, we show that particles derived from wood burning can enter the cell and localize to the mitochondria in trophoblast cells, causing structural damage. But we acknowledge that our study lacks experimental evidence to demonstrate mitochondrial functional damage caused by the exposure. Secondly, the actual exposure level for the placenta is unknown, making it difficult to determine physiological exposure doses. The doses used in our study were based on previous calculations (Familari et al., 2019), estimating that pregnant women are exposed to 50–500 ng of traffic-derived PM2.5 per day (Malmö, Sweden). Since exposure to wood smoke particles often are intermittent but at higher doses, both in Sweden and definitely in LIC (indoor level ~900 $\mu g/m^3$ (WHO, 2014)), we decided to expand the dose range upwards to 10000 ng/ml. Also, 10000 ng/ml for the PAHs analysis of exposed cells (Table 1) was used to secure that the levels inside the cells would be above the detection limit of the assay. We also acknowledge that the levels of hCG, IL-6, cytotoxicity and cell counts were all affected already at the lowest dose of 50 ng/ml, suggesting that we could have expanded the dose range downwards including 0.5 and 5 ng/ml.

Taken together, these results suggest that wood smoke particles can enter trophoblasts and have detrimental effects early in pregnancy by disrupting critical trophoblast functions important for normal placenta development and function. This could contribute to the underlying mechanisms that can lead to pregnancy complications such as miscarriage, premature birth, preeclampsia and/or fetal growth restriction. This study therefore support the general recommendation that more efficient combustion technologies and burning practices should be adopted to reduce some of the toxicity generated during wood burning.

Funding sources

This work was supported by the Research Council FORTE, Sweden [grant no. 2016-00572] and the Swedish Research Council, Sweden [grant no. 621-2012-3802]. The funding source(s) had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Data availability statement

Materials, data and associated protocols are available upon request.

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.

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Lena Erlandsson: Conceptualization, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Robert Lindgren:** Investigation, Writing - original draft, Writing - review & editing. **Åsa Nääv:** Investigation, Writing - review & editing. **Annette M. Krais:** Resources, Investigation, Writing - review & editing. **Bo Strandberg:** Resources, Investigation, Writing - review & editing. **Thomas Lundh:** Resources, Investigation, Writing - review & editing. **Christoffer Boman:** Resources, Writing - original draft, Writing - review & editing. **Christina Isaxon:** Conceptualization, Funding acquisition, Resources, Investigation, Writing - original draft, Writing - review & editing. **Stefan R. Hansson:** Conceptualization, Funding acquisition, Resources, Writing - original draft, Writing - review & editing. **Ebba Malmqvist:** Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing.

Acknowledgement

The authors would like to acknowledge Eva Hansson for valuable technical assistance during the analysis. Lund University Bio-Imaging Center (LBIC), Lund University is acknowledged for providing experimental resources regarding TEM.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114790.

References

Agrawal, S., Yamamoto, S., 2015. Effect of indoor air pollution from biomass and solid fuel combustion on symptoms of preeclampsia/eclampsia in Indian women. Indoor Air 25, 341–352. https://doi.org/10.1111/ina.12144.
Aguilar, F.X., 2014. Wood Energy in Developed Economies: Resource Management, Economics and Policy. Taylor and Francis.
Allen, R.W., et al., 2008. Changes in lung function and airway inflammation among asthmatic children residing in a woodsmoke-impacted urban area. Inhal. Toxicol. 20, 423–433. https://doi.org/10.1080/08958370801903826.
Amegah, A.K., et al., 2014. Household air pollution from solid fuel use and risk of adverse pregnancy outcomes: a systematic review and meta-analysis of the empirical evidence. PloS One 9, e113920. https://doi.org/10.1371/journal.pone.0113920.
Barregard, L., et al., 2006. Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal. Toxicol. 18, 845–853. https://doi.org/10.1080/08958370600685798.
Black, C., et al., 2017. Wildfire smoke exposure and human health: significant gaps in research for a growing public health issue. Environ. Toxicol. Pharmacol. 55, 186–195. https://doi.org/10.1016/j.etap.2017.08.022.
Black, C., et al., 2017. Early life wildfire smoke exposure is associated with immune dysregulation and lung function decrements in adolescence. Am. J. Respir. Cell Mol. Biol. 56, 657–666. https://doi.org/10.1165/rcmb.2016-0380OC.
Bostrom, C.E., et al., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect. 110 (Suppl. 3), 451–488. https://doi.org/10.1289/ehp.110-1241197.
Bove, H., et al., 2018. Combustion-derived particles inhibit in vitro human lung fibroblast-mediated matrix remodeling. J. Nanobiotechnol. 16, 82. https://doi.org/10.1186/s12951-018-0410-x.
Bove, H., et al., 2019. Ambient black carbon particles reach the fetal side of human placenta. Nat. Commun. 10, 3866. https://doi.org/10.1038/s41467-019-11654-3.
Burns, J., et al., 2019. Interventions to reduce ambient particulate matter air pollution and their effect on health. Cochrane Database Syst. Rev. 5, CD010919. https://doi.org/10.1002/14651858.CD010919.pub2.
Carlemalm, E., 1990. Lowicryl resins in microbiology. J. Struct. Biol. 104, 189–191.
Challis, J.R., et al., 2009. Inflammation and pregnancy. Reprod. Sci. 16, 206–215. https://doi.org/10.1177/1933719108329095.
Costa, D.L., Dreher, K.L., 1997. Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. Environ. Health Perspect. 105 (Suppl. 5), 1053–1060. https://doi.org/10.1289/ehp.97105s51053.
Danielsen, P.H., et al., 2009. Oxidative damage to DNA and repair induced by Norwegian wood smoke particles in human A549 and THP-1 cell lines. Mutat. Res. 674, 116–122. https://doi.org/10.1016/j.mrgentox.2008.10.014.
Dilger, M., et al., 2016. Toxicity of wood smoke particles in human A549 lung epithelial cells: the role of PAHs, soot and zinc. Arch. Toxicol. 90, 3029–3044. https://doi.org/10.1007/s00204-016-1659-1.
Dutta, A., et al., 2018. Household air pollution and chronic hypoxia in the placenta of pregnant Nigerian women: a randomized controlled ethanol Cookstove intervention. Sci. Total Environ. 619–620, 212–220. https://doi.org/10.1016/j.scitotenv.2017.11.091.
Familari, M., et al., 2019. Exposure of trophoblast cells to fine particulate matter air pollution leads to growth inhibition, inflammation and ER stress. PloS One 14, e0218799. https://doi.org/10.1371/journal.pone.0218799.
Flannigan, M.D., et al., 2000. Climate change and forest fires. Sci. Total Environ. 262, 221–229. https://doi.org/10.1016/s0048-9697(00)00524-6.
G.B.D. Risk Factor Collaborators, 2018. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1923–1994. https://doi.org/10.1016/S0140-6736(18)32225-6.
Goodson, J.M., et al., 2019. In utero exposure to diesel exhaust is associated with alterations in neonatal cardiomyocyte transcription, DNA methylation and metabolic perturbation. Part. Fibre Toxicol. 16, 17. https://doi.org/10.1186/s12989-019-0301-9.
Heydari, H., et al., 2020. Maternal exposure to ambient air pollution during pregnancy and lipid profile in umbilical cord blood samples; a cross-sectional study. Environ. Pollut. 261, 114195. https://doi.org/10.1016/j.envpol.2020.114195.
Hoogeveen, Y., Asquith, M., Jarosinska, D., Henrich, T., 2013. Natural Resources and Human Well-Being in a Green Economy.
Hsiao, E.Y., Patterson, P.H., 2011. Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. Brain Behav. Immun. 25, 604–615. https://doi.org/10.1016/j.bbi.2010.12.017.
Janssen, B.G., et al., 2013. Placental DNA hypomethylation in association with particulate air pollution in early life. Part. Fibre Toxicol. 10, 22. https://doi.org/10.1186/1743-8977-10-22.
Kayisli, U.A., et al., 2003. Human chorionic gonadotropin contributes to maternal immunotolerance and endometrial apoptosis by regulating Fas-Fas ligand system. J. Immunol. 171, 2305–2313. https://doi.org/10.4049/jimmunol.171.5.2305.
Khamirchi, R., et al., 2020. Maternal exposure to air pollution during pregnancy and cortisol level in cord blood. Sci. Total Environ. 713, 136622. https://doi.org/10.1016/j.scitotenv.2020.136622.
Kim, K.H., et al., 2011. A review of diseases associated with household air pollution due to the use of biomass fuels. J. Hazard Mater. 192, 425–431. https://doi.org/10.1016/j.jhazmat.2011.05.087.
Kocbach Bolling, A., et al., 2009. Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. Part. Fibre Toxicol. 6, 29. https://doi.org/10.1186/1743-8977-6-29.
Lasagni Vitar, R.M., et al., 2018. Diesel exhaust particles (DEP) induce an early redox imbalance followed by an IL-6 mediated inflammatory response on human conjunctival epithelial cells. Exp. Eye Res. 171, 37–47. https://doi.org/10.1016/j.exer.2018.03.005.
Li, C.S., Loch-Caruso, R., 2007. Sodium arsenite inhibits migration of extravillous trophoblast cells in vitro. Reprod. Toxicol. 24, 296–302. https://doi.org/10.1016/j.reprotox.2007.06.002.
Liu, L., et al., 2016. Benzo(a)pyrene inhibits migration and invasion of extravillous trophoblast HTR-8/SVneo cells via activation of the ERK and JNK pathway. J. Appl. Toxicol. 36, 946–955. https://doi.org/10.1002/jat.3227.
Loxham, M., et al., 2019. The health effects of fine particulate air pollution. BMJ 367, l6609. https://doi.org/10.1136/bmj.l6609.
Luyten, L.J., et al., 2018. Air pollution and the fetal origin of disease: a systematic review of the molecular signatures of air pollution exposure in human placenta. Environ. Res. 166, 310–323. https://doi.org/10.1016/j.envres.2018.03.025.
Malmqvist, E., et al., 2013. Gestational diabetes and preeclampsia in association with air pollution at levels below current air quality guidelines. Environ. Health Perspect. 121, 488–493. https://doi.org/10.1289/ehp.1205736.
Mathiesen, L., et al., 2009. Transport of benzo[alpha]pyrene in the dually perfused human placenta perfusion model: effect of albumin in the perfusion medium. Basic Clin. Pharmacol. Toxicol. 105, 181–187. https://doi.org/10.1111/j.1742-7843.2009.00431.x.
Muala, A., et al., 2015. Acute exposure to wood smoke from incomplete combustion–indications of cytotoxicity. Part. Fibre Toxicol. 12, 33. https://doi.org/10.1186/s12989-015-0111-7.
Nääv, Å., et al., 2020. Urban PM2.5 induces cellular toxicity, hormone dysregulation, oxidative damage, inflammation and mitochondrial interference in the HRT8 trophoblast cell line. Front. Endocrinol. https://doi.org/10.3389/fendo.2020.00075.
Noonan, C.W., et al., 2012. A rural community intervention targeting biomass combustion sources: effects on air quality and reporting of children's respiratory outcomes. Occup. Environ. Med. 69, 354–360. https://doi.org/10.1136/oemed-2011-100394.
Nyström, R., et al., 2017. Influence of wood species and burning conditions on particle emission characteristics in a residential wood stove. Energy Fuels 31. https://doi.org/10.1021/acs.energyfuels.6b02751, 5514-4424.
Orozco-Levi, M., et al., 2006. Wood smoke exposure and risk of chronic obstructive pulmonary disease. Eur. Respir. J. 27, 542–546. https://doi.org/10.1183/09031936.06.00052705.
Pagliaccio, D., et al., 2020. Prenatal exposure to polycyclic aromatic hydrocarbons modifies the effects of early life stress on attention and Thought Problems in late childhood. J. Child Psychol. Psychiatry Allied Discip. https://doi.org/10.1111/jcpp.13189.
Pedersen, M., et al., 2017. Impact of road traffic pollution on pre-eclampsia and pregnancy-induced hypertensive disorders. Epidemiology 28, 99–106. https://doi.org/10.1097/EDE.0000000000000555.
Pejhan, A., et al., 2019. Exposure to air pollution during pregnancy and newborn liver function. Chemosphere 226, 447–453. https://doi.org/10.1016/j.chemosphere.2019.03.185.
Qin, Z., et al., 2017. Fine particulate matter exposure induces cell cycle arrest and inhibits migration and invasion of human extravillous trophoblast, as determined by an iTRAQ-based quantitative proteomics strategy. Reprod. Toxicol. 74, 10–22. https://doi.org/10.1016/j.reprotox.2017.08.014.
Reid, C.E., et al., 2016. Differential respiratory health effects from the 2008 northern California wildfires: a spatiotemporal approach. Environ. Res. 150, 227–235. https://doi.org/10.1016/j.envres.2016.06.012.
Rokoff, L.B., et al., 2017. Wood stove pollution in the developed world: a case to raise awareness among pediatricians. Curr. Probl. Pediatr. Adolesc. Health Care 47, 123–141. https://doi.org/10.1016/j.cppeds.2017.04.001.
Rundle, A.G., et al., 2019. Prenatal exposure to airborne polycyclic aromatic hydrocarbons and childhood growth trajectories from age 5-14years. Environ. Res. 177, 108595. https://doi.org/10.1016/j.envres.2019.108595.
Saenen, N.D., et al., 2019. Air pollution-induced placental alterations: an interplay of oxidative stress, epigenetics, and the aging phenotype? Clin. Epigenet. 11, 124.

https://doi.org/10.1186/s13148-019-0688-z.
Schneider, M.A., et al., 1993. The timing of placental competence in pregnancy after oocyte donation. Fertil. Steril. 59, 1059–1064. https://doi.org/10.1016/s0015-0282(16)55928-7.
Sigsgaard, T., et al., 2015. Health impacts of anthropogenic biomass burning in the developed world. Eur. Respir. J. 46, 1577–1588. https://doi.org/10.1183/13993003.01865-2014.
Stockfelt, L., et al., 2012. Effects on airways of short-term exposure to two kinds of wood smoke in a chamber study of healthy humans. Inhal. Toxicol. 24, 47–59. https://doi.org/10.3109/08958378.2011.633281.
Strandberg, B., et al., 2018. Evaluation of polyurethane foam passive air sampler (PUF) as a tool for occupational PAH measurements. Chemosphere 190, 35–42. https://doi.org/10.1016/j.chemosphere.2017.09.106.
Tang, D., et al., 2006. PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort. Environ. Health Perspect. 114, 1297–1300. https://doi.org/10.1289/ehp.8939.
Tapanainen, M., et al., 2012. Efficiency of log wood combustion affects the toxicological and chemical properties of emission particles. Inhal. Toxicol. 24, 343–355. https://doi.org/10.3109/08958378.2012.671858.
Unosson, J., et al., 2013. Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans. Part. Fibre Toxicol. 10, 20. https://doi.org/10.1186/1743-8977-10-20.
Valentino, S.A., et al., 2016. Maternal exposure to diluted diesel engine exhaust alters placental function and induces intergenerational effects in rabbits. Part. Fibre Toxicol. 13, 39. https://doi.org/10.1186/s12989-016-0151-7.
van Eeden, S.F., et al., 2001. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am. J. Respir. Crit. Care Med. 164, 826–830. https://doi.org/10.1164/ajrccm.164.5.2010160.
Velicky, P., et al., 2016. Function and control of human invasive trophoblast subtypes: intrinsic vs. maternal control. Cell Adhes. Migrat. 10, 154–162. https://doi.org/10.1080/19336918.2015.1089376.
Vrachnis, N., et al., 2010. Intrauterine inflammation and preterm delivery. Ann. N. Y. Acad. Sci. 1205, 118–122. https://doi.org/10.1111/j.1749-6632.2010.05684.x.
Wagner, F., et al., 2010. Baseline Emission Projections and Further Cost-Effective Reductions of Air Pollution Impacts in Europe - A 2010 Perspective.
Weber, E., et al., 2020. Household fuel use and adverse pregnancy outcomes in a Ghanaian cohort study. Reprod. Health 17, 29. https://doi.org/10.1186/s12978-020-0878-3.
WHO, 2006. International Agency for Research on Cancer [Accessed on Oct 10, 2006];List of All Agents Evaluated to Date.
WHO, 2014. WHO Guidelines for Indoor Air Quality: Household Fuel Combustion. World Health Organization.
Wu, W.L., et al., 2017. The placental interleukin-6 signaling controls fetal brain development and behavior. Brain Behav. Immun. 62, 11–23. https://doi.org/10.1016/j.bbi.2016.11.007.
Wylie, B.J., et al., 2014. Impact of biomass fuels on pregnancy outcomes in central East India. Environ. Health 13, 1. https://doi.org/10.1186/1476-069X-13-1.
Xue, T., et al., 2019. Association between pregnancy loss and ambient PM2.5 using survey data in Africa: a longitudinal case-control study, 1998-2016. Lancet Planet Health 3, e219–ee225. https://doi.org/10.1016/S2542-5196(19)30047-6.
Zhang, J.J., et al., 2019. Health effects of air pollution: what we need to know and to do in the next decade. J. Thorac. Dis. 11, 1727–1730. https://doi.org/10.21037/jtd.2019.03.65.
Zhao, C.N., et al., 2019. Emerging role of air pollution in autoimmune diseases. Autoimmun. Rev. 18, 607–614. https://doi.org/10.1016/j.autrev.2018.12.010.