Impact of indoor air pollution on DNA damage and chromosome stability: A systematic review

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Abstract

Indoor air pollution is becoming a rising public health problem and is largely resulting from the burning of solid fuels and heating in households. Burning these fuels produces harmful compounds, such as particulate matter regarded as a major health risk, particularly affecting the onset and exacerbation of respiratory diseases. As exposure to polluted indoor air can cause DNA damage including DNA strand breaks as well as chromosomal damage, in this paper, we aim to provide an overview of the impact of indoor air pollution on DNA damage and genome stability by reviewing the scientific papers that have used the comet, micronucleus, and γ-H2AX assays. These methods are valuable tools in human biomonitoring and for studying the mechanisms of action of various pollutants, and are readily used for the assessment of primary DNA damage and genome instability induced by air pollutants by measuring different aspects of DNA and chromosomal damage. Based on our search, in selected studies (*in vitro*, animal models, and human biomonitoring), we found generally higher levels of DNA strand breaks and chromosomal damage due to indoor air pollutants compared to matched control or unexposed groups. In summary, our systematic review reveals the importance of the comet, micronucleus, and γ-H2AX assays as sensitive tools for the evaluation of DNA and genome damaging potential of different indoor air pollutants. Additionally, research in this particular direction is warranted since little is still known about the level of indoor air pollution in households or public buildings and its impact on genetic material. Future studies should focus on research investigating the possible impact of indoor air pollutants in complex mixtures on the genome and relate pollutants to possible health outcomes.

Keywords: Indoor air quality; Genome damage; Comet assay; Micronucleus assay; γ-H2AX assay; Health risk

1. Introduction

Indoor air pollution refers to the presence of harmful pollutants within enclosed spaces, such as homes, offices, schools, kindergartens, subway stations, or any other living environments, and as such poses a significant threat to human health given that a considerable amount of time is spent indoors (Bruce et al. 2012; Xu and Hao 2017; Manisalidis et al. 2020; Tran et al. 2020; Chen et al. 2020; Yang et al. 2023; US EPA 2024; Vilcins et al. 2024). These pollutants can originate from various sources, including tobacco smoke (e.g. passive smoking and third-hand smoking), household items such as cleaning products, building materials, curtains, carpets, electronic devices (e.g. computers), and inadequate ventilation, to name only a few. Common indoor pollutants include particulate matter (PM), particularly $PM_{2.5}$ (particles with a diameter less than or equal to 2.5 μm), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), flame retardants (FR), carbon monoxide (CO), ozone (O_3), formaldehyde, toxic metals (e.g., lead, cadmium, arsenic, nickel), pesticides, radon, asbestos, as well as biological contaminants such as dust mites, moulds, pollen, microorganisms, and viruses (Ren et al. 2006; Bastl et al. 2017; Rivas et al. 2019; Vardoulakis et al. 2020; Gopalakrishnan and Jeyanthi 2022; Jakovljević et al. 2022; NIH 2023; US EPA 2023). Besides, current research points to the new emerging pollutants being micro- and nano-plastics in the air we breathe (Bhatia et al. 2024; Kek et al. 2024).

Prolonged exposure to these pollutants can lead to non-communicable diseases including respiratory problems such as the development and exacerbation of asthma, allergies, stroke, ischaemic heart disease, chronic obstructive pulmonary disease (COPD), lung cancer, and other long-term health issues. Additionally, indoor air pollution has been linked to headaches, fatigue, and more severe conditions like cardiovascular disease and even respiratory infections (Brunekreef and Holgate 2002; Tran et al. 2020; Samet et al. 2022; Kumar et al. 2023). Indoor air pollution was responsible for an estimated 3.2 million deaths per year in 2020, including over 237.000 deaths of children under the age of five, while the combined effects of outdoor and indoor air pollution are associated with 6.7 million premature deaths annually (WHO 2023).

Vulnerable populations, such as children, adolescents, the elderly, and individuals with pre-existing health conditions, might be even more susceptible to these health impacts. Children are particularly vulnerable to the adverse effects of indoor air pollution due to their developing respiratory and immune systems. This risk is also given by children's anatomical features, their

longer life expectancy in which the risk is expressed, and the ratio of smaller body size/volume and inhaled air volume. Besides, children are particularly susceptible since their respiratory system and internal organs are in the developmental stage, thus potentially leading to a weaker immune system. They inhale a greater amount of polluted air due to their higher breathing rate, tendency to breathe through their mouths and their proximity to ground-level pollutants. In addition, children are more physically active and are susceptible to increased exposure due to their exploratory nature (hand-to-mouth behaviour). Exposure also differs during the prenatal period, when they are exposed to pollutants while in the mother's uterus. Thus, exposure to indoor pollutants can have various negative impacts on children, including respiratory and developmental issues, allergies, cognitive effects, and effects on the immune system (Moya et al. 2004; EEA 2023; Castel et al. 2023; Tran et al. 2023).

One should not forget that there is also an apparent link between outdoor and indoor air pollution that lies in the exchange of air between these two environments (Leung 2015) since indoor and outdoor air contains the same pollutants, but in different proportions and amounts (Rosário Filho et al. 2021). Outdoor pollutants, such as gases (O_3, nitrogen dioxide (NO_2), VOCs), PM, and pollen can enter indoor spaces through ventilation, open windows, and doors, making even more complex mixtures of air pollutants present from indoor activities, such as cooking, heating, and smoking if not properly ventilated. Moreover, pollutants from vehicle emissions, industrial activities, and natural sources can infiltrate indoor spaces, impacting the overall indoor air quality (Rosário Filho et al. 2021; Mohammadi and Calautit 2022). Therefore, understanding this link is crucial because addressing outdoor air quality issues can indirectly impact indoor air quality and vice versa. Thus, proper reduction of pollutant sources both indoors and outdoors contributes to creating healthier living environments.

In addition to the adverse health effects mentioned above, exposure to air pollution has been associated with genotoxic effects. Certain pollutants, such as PM and chemicals present in tobacco smoke or household products, may lead to a generation of DNA damage, including DNA single- (SSBs) and double-strand breaks (DSBs), DNA adducts as well as oxidative stress within the body. Subsequently, oxidative stress can cause additional damage to cellular components, such as proteins, lipids, and DNA (Pizzino et al. 2017; Juan et al. 2021; Aramouni et al. 2023). Persistent DNA damage can compromise genome stability, increasing the risk of mutations and chromosomal abnormalities and this instability may contribute to the development of various health conditions,

including cancer which is usually manifested with a delay of several years or even decades after the initial exposure, consequently causing a significant financial and social burden, especially in aging populations (Viegas et al. 2017). Besides, there is a growing body of evidence indicating that breathing polluted air impairs the immune system's ability to regulate inflammation, subsequently leading to adverse health outcomes (Fandiño-Del-Rio et al. 2021; Lim et al. 2022). Research in this field continues to explore the specific ways indoor air pollutants affect genome stability, emphasizing the importance of reducing exposure to maintain genome integrity and overall health.

Both the comet and micronucleus assays, as well as the γ-H2AX assay, are valuable tools in human biomonitoring (Sánchez-Flores et al. 2015; Sommer et al. 2020; Gajski et al. 2020, 2022, 2024; Azqueta et al. 2020; Milić et al. 2021; Nersesyan et al. 2022; Gerić et al. 2024; Ladeira et al. 2024) and are readily used for the assessment of DNA damage and genome instability related to exposure to air pollutants. These assays provide insights into the genotoxicity of pollutants by measuring different aspects of DNA and chromosomal damage (Fenech 2007; Rahmanian et al. 2021; Gajski and Gerić 2022; Collins et al. 2023; Gajski et al. 2024; Ladeira et al. 2024). The comet assay, also known as the single-cell gel electrophoresis assay, detects primary DNA damage, such as DNA SSBs and alkali-labile sites in individual cells of different types (Gerić et al. 2018; Gajski et al. 2019b, a; Azqueta et al. 2020; Møller et al. 2020; Collins et al. 2023). On the other hand, the micronucleus assay evaluates chromosomal damage by detecting the presence of micronuclei (MNi), nucleoplasmic bridges, and nuclear buds along with the cytotoxic and cytostatic effects, and is particularly useful for assessing chromosomal breakage, aneuploidy, and other chromosomal abnormalities (Fenech 2007; Kopjar et al. 2010; Gajski et al. 2018, 2024; Nersesyan et al. 2022). In addition, H2AX histone phosphorylation represents an early event in the cellular response against DNA DSBs and plays a central role in sensing and repairing DNA damage. Hence, the analysis of H2AX phosphorylated (γ-H2AX) histone is used as a biomarker of DNA DSBs and genomic instability (Gerić et al. 2014; Sánchez-Flores et al. 2015; Kopp et al. 2019). These assays are considered complementary, as they measure different genotoxic endpoints. Although *in vitro* genotoxicity tests cannot quantitatively predict hazards to humans and wildlife, they enable the estimation of the relative genotoxic potency and the health risk of the tested compounds as well as the assessment of the susceptibility of different cell models.

Since exposure to indoor air can cause DNA strand breaks and consequently induce chromosomal damage, both the comet and micronucleus assays as well as the γ-H2AX assay represent useful tools to quantify the extent of such damage by detecting abnormalities within the cell's genome. The application of these assays in studies on indoor air pollution could contribute to understanding the genotoxic effects of pollutants at a cellular level and, in turn, shed light on the potential onset of non-communicable diseases including cancer (Bonassi et al. 2007, 2021). Therefore, the present paper will give an overview of the impact of indoor air pollution on DNA damage and genome stability evaluated by the comet, micronucleus, and γ-H2AX assays both *in vitro* and *in vivo* as well as in human biomonitoring studies.

2. Search strategy

We conducted a literature search to identify relevant papers using scientific databases. The databases included were PubMed (www.pubmed.com) and Web of Science (www.webofscience.com) up to March 2024. The following search terms were used in our literature search: indoor air pollution and comet assay or micronucleus assay or γ-H2AX assay. From our search, we omitted non-English publications, papers older than the year 2000, review papers and meta-analyses, and papers done on plants and in occupational settings leaving the ones done *in vitro* on both animal and human cells, studies done on animals as well as humans in indoor settings.

Our search retrieved 102 articles in total from both databases. Following the screening of titles and abstracts, and after excluding duplicates, non-English communications, papers older than the year 2000, reviews and meta-analyses, and those that were regarded as out of scope we were left with 32 papers for inclusion in the present review for the impact of indoor air pollution on genome damage using comet, γ-H2AX and micronucleus assays. Other relevant related original and review papers were also checked in the reference lists of papers found in the search and those papers have also been included in the present review where appropriate. A detailed description of the selected studies using comet, γ-H2AX, and micronucleus assays can be found in Tables 1 and 2, respectively, and in the following chapters.

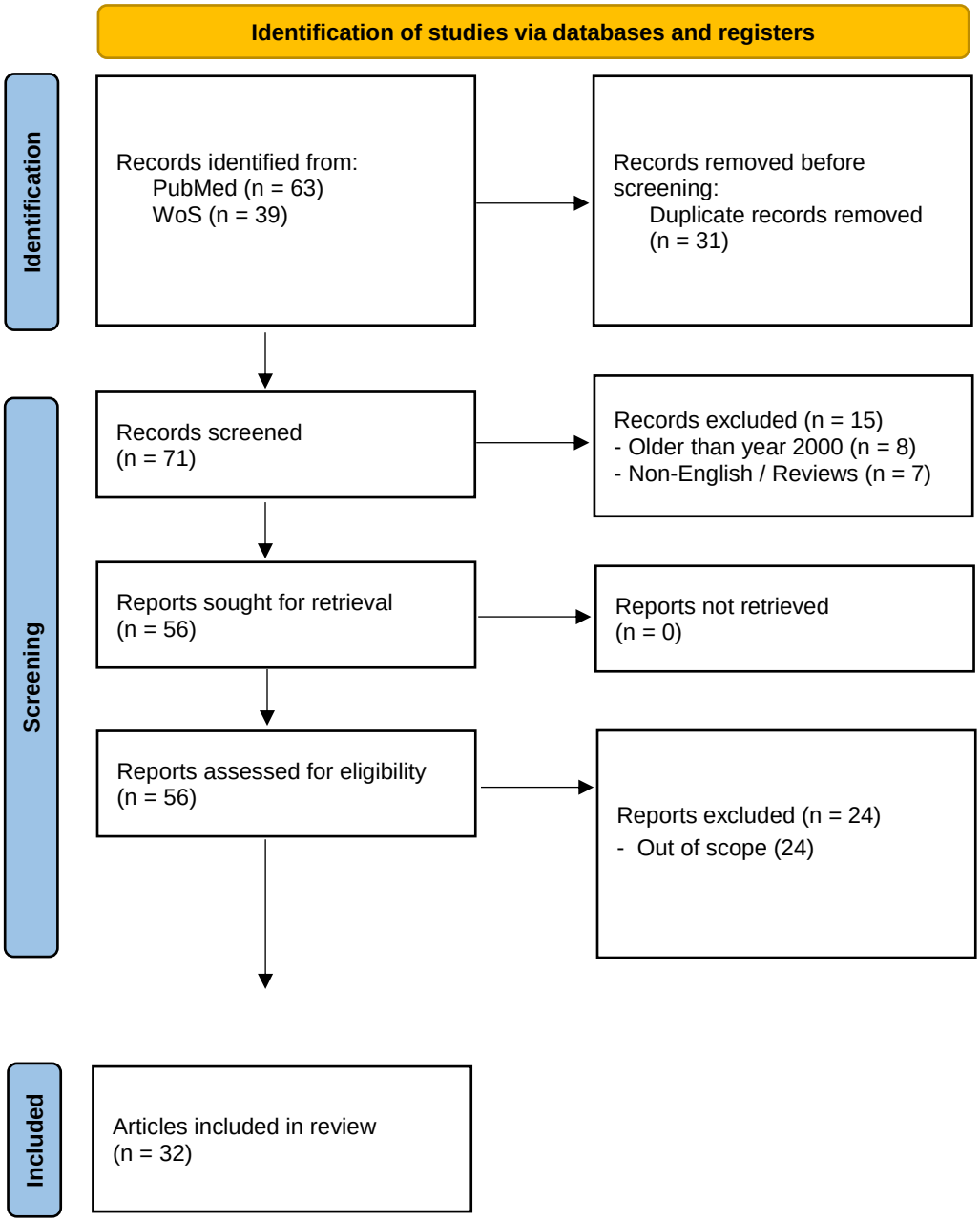

Fig. 1. PRISMA Flow diagram of systematic review for indoor air pollutants.

3. Effects of indoor air pollution *in vitro*

In total, 12 studies are dealing with *in vitro* exposure to indoor air pollutants using the comet, γ-H2AX, and micronucleus assays to determine their possible genotoxic potential. Plumejeaud et al. (2018) analysed potentially harmful elements in house dust for genotoxicity indicating that five gastric extracts induced dose-dependent genotoxicity in human adenocarcinoma gastric (AGS) cells. Third-hand smoke exposure caused replication stress and impaired transcription in human lung cells (hPFs and BEAS-2B) in addition to increased formation of MNi, a marker of genomic instability (Sarker et al. 2020). Besides, Salgaonkar et al. (2016) showed DNA damaging effect of both biomass and cigarette smoke extracts in peripheral blood mononuclear cells with the highest DNA damage for cigarette smoke followed by cow dung smoke and the lowest one for sawdust and wood smoke. This particular study also showed the ability of *N, N'*-diacetylchitobiose (*N*-acetylglucosamine dimer with anti-inflammatory, antimicrobial, anti-angiogenic, immune-stimulating, antioxidant, and DNA damage protecting ability) in alleviating the harmful effects of indoor air pollutants. Particles (of relevance for sub-Saharan Africa) derived from the combustion of less energy-dense fuels had a higher PAH content and were more cytotoxic in bronchial epithelial (BEAS-2B) cells whereas the least energy-dense and cheapest fuel also induced pro-inflammatory effects in THP-1 derived macrophages although they all induced concentration-dependent genotoxicity (McCarrick et al. 2024). Silica particles in the presence or absence of O_3 induced both DNA damage and increased MNi frequency in human lung carcinoma (A549) cells and normal human (Hs27) fibroblasts with more pronounced genotoxic effects in A549 cells (Colafarina et al. 2022). Furthermore, VOCs (toluene and benzene air mixtures), ultrafine particles (UFPs) from domestic wood stoves, and subway station particles (PM_{10}) can induce DNA damage (both SSBs and DSBs), and oxidative stress in A549 cells with subway particles being even more genotoxic compared to street particles (Karlsson et al. 2004; Pariselli et al. 2009; Marabini et al. 2017).

On the contrary, airborne VOCs emitted from pine wood and oriented strand boards (terpenes and aldehydes), as well as candlelight combustion particles (CP), failed to induce significant genotoxic effects (Gminski et al. 2010; Skovmand et al. 2017) although the latter did increase intracellular levels of reactive oxygen species (ROS) (Skovmand et al. 2017). Chlorophenols, chlorocatechols, and chloroguaiacols were shown to induce DNA base oxidation

in human lymphocytes (Michałowicz and Majsterek 2010) with the pyrimidine bases being more oxidized compared to purines. The obtained results also revealed that chlorinated catechols displayed a higher oxidative potential in comparison to chlorophenols and chloroguaiacols. Szeto et al. (2009) showed that DNA damage, including strand breaks, was induced in human lymphocytes after treatment with both aqueous and ethanolic extracts of incense smoke which is a common traditional and ceremonial practice in Southeast Asian countries. These data indicate the potential adverse health effects of such exposure in worshippers and particularly in temple workers who are exposed more often.

4. Effects of indoor air pollution on animal models

Our search retrieved two studies dealing with indoor air pollutant exposure in animals. Gustavino et al. (2014) investigated the genotoxic effects induced by exposure to radioactive radon (221–26,000 Bq/m^3) in wild cricket (*Dolichopoda geniculata* and *Dolichopoda laetitiae*) populations sampled from caves, as a natural indoor space, in central Italy where varying concentrations of radon were present. Cave crickets were also tested as possible bioindicators of the genotoxic potential of contaminated residential and confined environments. A statistically significant increase of DNA damage was found in all groups of individuals from each cave, for both haemocytes and brain cells. The obtained results indicate that cave crickets represent a reliable tool for the detection of genotoxic potential induced by radioactive contamination of confined environments and can be proposed as a possible bioindicator system for air pollution related to indoor exposure.

Insects are an interesting model in toxicology and could partially replace vertebrates in such studies, avoiding to some extent the ethical issues related to this type of research. Although the extrapolation of the data obtained in such models to higher animals could be difficult there are still many advantages that insects as a model can provide in this type of study such as inexpensive breeding that does not require much space or time, the possibility of large-scale experiments at a low-cost and minimization of inter-individual variability allowing for more reliable statistical analyses. As insects are the largest group of invertebrates, they can be widely used in toxicological and ecotoxicological research (Augustyniak et al. 2016; Gajski et al. 2019b; Macrì et al. 2023).

In another study by Skovmand et al. (2017), the authors aimed to compare the pulmonary effects of CP with two benchmark diesel exhaust particles (A-DEP and SRM2975). Intratracheal (*i.t.*) instillation of CP (5 mg/kg body weight) in C57BL/6n mice produced a significant influx of alveolar macrophages and polymorphonuclear leukocytes and increased concentrations of proteins and lactate dehydrogenase activity in the bronchoalveolar fluid. Lower levels of these markers of inflammation and cytotoxicity were observed after *i.t.* instillation of the same dose of A-DEP or SRM2975. The *i.t.* instillation of CP did not generate oxidative damage to DNA in lung tissue, measured as DNA strand breaks and human 8-oxoguanine glycosylase-sensitive sites by the comet assay, although the group treated with a higher dose (5 mg/kg) of CP displayed a slight increase in levels of DNA strand breaks compared to the control. The authors concluded that pulmonary exposure to particles from burning candles is associated with inflammation and cytotoxicity in the lungs.

Mice as well as other rodents have been widely used as animal models for the evaluation of the DNA damaging effects and genome stability of a variety of chemicals using both the comet and micronucleus assays (Hayashi 2016; Gajski et al. 2019a). There are several very specific guidelines for both *in vitro* and *in vivo* genotoxicity testing using those assays (Tice et al. 2000; Hartmann et al. 2003). Multiple organs of mice such as blood, liver, kidney, brain, lungs, and bone marrow have been used for genotoxicity testing of a large range of chemicals and several published OECD test guidelines (OECD 2016a, b) summarize the basics and limitations, the principle of the method, verification of the laboratory's proficiency, historical control data, and a detailed description of the methods.

5. Effects of indoor air pollution on humans

The search retrieved 19 studies that evaluated the potential impact of human exposure to indoor air pollutants. Exposure included PM, gases, biomass, and wood smoke as well as indoor radon exposure. The studies were performed largely in Europe (Denmark, Italy, Poland, Portugal, Romania, Slovenia, and Sweden) and India followed by studies in Brazil, Ethiopia, Malaysia, Mexico, and Russia. Since indoor UFPs consist of a combination of ambient particles that readily penetrate buildings and infiltrate indoor air, a Denmark (Copenhagen) study by Vinzents et al. (2005) found that the combined outdoor and indoor exposures to UFPs were identified as

independent and significant predictors of purine oxidation levels in DNA. However, these exposures did not serve as predictors for strand breaks.

Several studies were done on children exposed to indoor air pollutants. Zani et al. (2020) failed to find a significant correlation between DNA damage measured by the comet assay in salivary leukocytes in children living in Brescia (Italy) and measured air pollutants (PM_{10}, $PM_{2.5}$, NO_2, CO, sulphur dioxide (SO_2), benzene, and O_3) with the estimates for indoor exposure (stove, fireplace, parental smoking, etc.). On the contrary, in children from the same area, a statistically significant increase in the parameters of the buccal MN assay was noticed although there was no apparent association between MNi frequency and the indoor and outdoor exposure variables investigated via the questionnaire (Ceretti et al. 2014). A comparable result was found by Sopian et al. (2020) indicating significantly higher concentrations of indoor air pollutants (PM_1, $PM_{2.5}$, PM_{10}, NO_2, and SO_2) in schools in the vicinity of an industrial park compared to control schools. There was a significant association between PM_{10}, SO_2, NO_2, and MNi frequency in buccal cells. The authors concluded that proximity to industrial areas negatively impacts indoor air quality in schools thereby increasing the potential risk of genotoxicity and worsening respiratory health among children exposed to industrial air pollution. In addition, higher levels of indoor air pollutants ($PM_{2.5}$ and PAHs), urinary 1-OHP, DNA adducts, and cytogenetic damage in epithelial cells were observed in rural areas of Janów and Złoty Potok compared to the urban site of Dąbrowa Górnicza (Poland) (Błaszczyk et al. 2022).

Combustion of different biomass fuels produces various pollutants that may cause serious health effects in exposed populations. Pandey et al. (2005) found significantly higher levels of DNA damage in the lymphocytes of biomass fuel users compared to liquefied petroleum gas among rural Indian women. Similar effects were found in studies done by Mondal et al. (2011, 2010), Mukherjee et al. (2014, 2013), and Musthapa et al. (2004) with higher DNA damage (both SSBs and DSBs), chromosomal damage, and oxidative stress in either buccal cells or lymphocytes of Indian women cooking with biomass (wood, dung, crop residues). Besides, biomass users showed a higher percentage of cells expressing oxidative DNA damage marker 8-oxoguanine and lower percentages of BER proteins OGG1 and APE1. In the same population, ROS generation increased while the level of superoxide dismutase was significantly depleted. The concentrations of PM were higher in biomass-using households which positively correlated with ROS and

negatively with BER protein expressions while ROS generation was positively correlated with 8-oxoguanine and negatively with BER proteins (Mukherjee et al. 2014).

Mexican (San Luis Potosí) study showed that the intervention program offers an acceptable risk reduction to those families that use biomass for food cooking based on the significantly lower values of measured DNA damage and urinary 1-OHP levels before and after the intervention program that included removal of indoor soot adhered to roofs and internal walls, paving the dirt floors, and introduction of a new wood stove with a metal chimney that ejects smoke outdoors (Torres-Dosal et al. 2008). In two studies from Denmark, exposure to wood smoke did not affect markers of oxidative stress, DNA damage, cell adhesion, cytokines, or microvascular function in atopic subjects (Forchhammer et al. 2012) while even high inhalation exposure to wood smoke was associated with only limited systemic effects on markers of DNA damage, oxidative stress, inflammation, and monocyte activation in subjects living in a reconstructed Viking Age house, with indoor combustion of wood for heating and cooking (Jensen et al. 2014).

Radon as a naturally occurring, colourless, and odourless radioactive gas can be found in homes and other buildings as another important indoor air pollutant (Kreuzer and McLaughlin 2010; Gopalakrishnan and Jeyanthi 2022). Sinitsky and Druzhinin, (2014) found elevated frequencies of micronucleus assay parameters in the lymphocytes of children at a boarding school located in the Tashtagolsky district of the Kemerovo region (Russia) an area with high radon concentrations (626.0 Bq/m^3) compared to children living in the Zarubino village (Kemerovo region) with low radon levels (91.5 Bq/m^3). A similar scenario was identified by Bilban and Vaupotič (2001) in Slovenian schools with elevated indoor radon concentrations (up to 7000 Bq/m^3). There was an increase in cytogenetic damage (chromosomal aberrations and MNi) in children exposed to higher radiation compared to children from schools with indoor radon concentrations below 400 Bq/m^3. Additionally, Linhares et al. (2018) found increased levels of DNA damage and MNi in buccal epithelial cells of individuals chronically exposed to indoor radon in a volcanic area (Furnas volcano, Azores, Portugal) with a hydrothermal system (Ribeira Quente village) compared to those inhabiting a non-hydrothermal area (Ponta Delgada city). The observed association between chronic exposure to indoor radon and the occurrence of chromosome damage in human oral epithelial cells evidences the usefulness of biological surveillance to assess mutations involved in pre-carcinogenesis in hydrothermal areas, reinforcing the need for further studies with human populations living in these areas. Furthermore, in Lucrécia (Brazil) a city with

a high cancer rate, higher indoor radon, and gamma emitters (U, K, and Th) concentrations, as well as other potential pollutants, were measured compared to Natal, having a significant impact on the MNi frequency and other nuclear abnormalities in exfoliated buccal cells (Marcon et al. 2017). Additionally, a statistically significant increase was observed in terms of DNA damage and MNi frequency in women residing in radon priority area Băiţa-Ştei (Romania) exposed to different levels of indoor radon concentrations (Dicu et al. 2022). The authors concluded that an increased radiosensitivity of lymphocytes, as well as slower repair kinetics, may be associated with exposure to higher indoor radon concentrations.

6. Discussion, future directions, and conclusions

Air pollution is currently one of the major issues in environmental and public health and has been recognized by leading world authorities as a risk factor associated with adverse health outcomes (Boogaard et al. 2019; Lelieveld et al. 2020; EEA 2023; US EPA 2024). Both, outdoor and indoor air pollution are categorized by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1) (IARC 2015). The most common air pollutants in indoor air include PM of different sizes, O_3, NO_2, CO, and SO_2. Indoor spaces are common places of exposure to poor air quality and are difficult to monitor and regulate. Indoor air pollution can be linked to households; the release of gases or particles into the air is the primary cause of indoor air quality problems. Regarding indoor air, one major concern is biomass smoke since it contains many health-damaging chemicals, including PM of various sizes, CO, oxides of nitrogen, formaldehyde, acrolein, benzene, toluene, styrene, 1,3-butadiene, and PAHs (Rivas et al. 2019; Vardoulakis et al. 2020).

In this review paper, we visualized the complex landscape of genotoxicity data analysis from the comet, micronucleus, and γ-H2AX assays, utilizing an open-source R package UpSetR. Given that such datasets are inherently complex, often containing numerous variables and intricate relationships, traditional visualization methods may fall short in capturing the full scope of interactions present within these datasets. UpSet analysis comprehensively explores the intersections and relationships between multiple variables simultaneously. As shown in Figure 2, we identified intersections between DNA damage parameters and various agents such as PM, PAHs, radon, etc. The plot revealed the frequency of the two most common intersections, exposure

to radon and secondary DNA damage expressed as the frequency of MNi (N=3) and exposure to $PM_{2.5}$ and PM_{10} from biomass smoke and primary DNA damage expressed as tail intensity (TI), tail length (TL), and Olive tail moment (OTM) (N=3). By exclusively incorporating studies (N=28) reporting statistically significant results regarding DNA damage resulting from exposure to various indoor air pollutants, our analysis highlights the current state of research regarding indoor air pollution and potentially serves as a tool for pinpointing gaps in research or areas requiring further examination. Visualization of such relationships suggests avenues for integrating additional parameters of DNA damage with various stressors to enhance understanding in this field.

Based on the results of our search, we concluded that there is a smaller number of studies evaluating the effects of indoor air pollutants on DNA damage and genome stability compared to research conducted on outdoor air pollutants. Nevertheless, so far, data indicate that common indoor air pollutants such as PM, PAHs, VOCs, and radon, as well as biological contaminants, can cause adverse effects on DNA and chromosomal levels. Since all three methods included in the review, comet, micronucleus, and γ-H2X, can serve as predictive indicators of cancer (Paull et al. 2000; Martin and Bonner 2006; Bonassi et al. 2007, 2021; Valdiglesias et al. 2013), it can be concluded from the results of the evaluated studies that exposure to significant amounts of indoor air pollutants, especially over an extended period of time in everyday life, has the potential to influence the initiation and promotion of cancer. As both outdoor and indoor air pollution are intertwined, here we aim to propose certain measures to mitigate indoor air pollution. However, it should be kept in mind that due to the differences in physical and chemical properties of pollutants, the proposed measures may not be uniformly effective for different types of pollutants (e.g. for gases or PM), so a combined approach is needed. We believe that following such practices, one can significantly reduce indoor air pollution and create a healthier living environment.

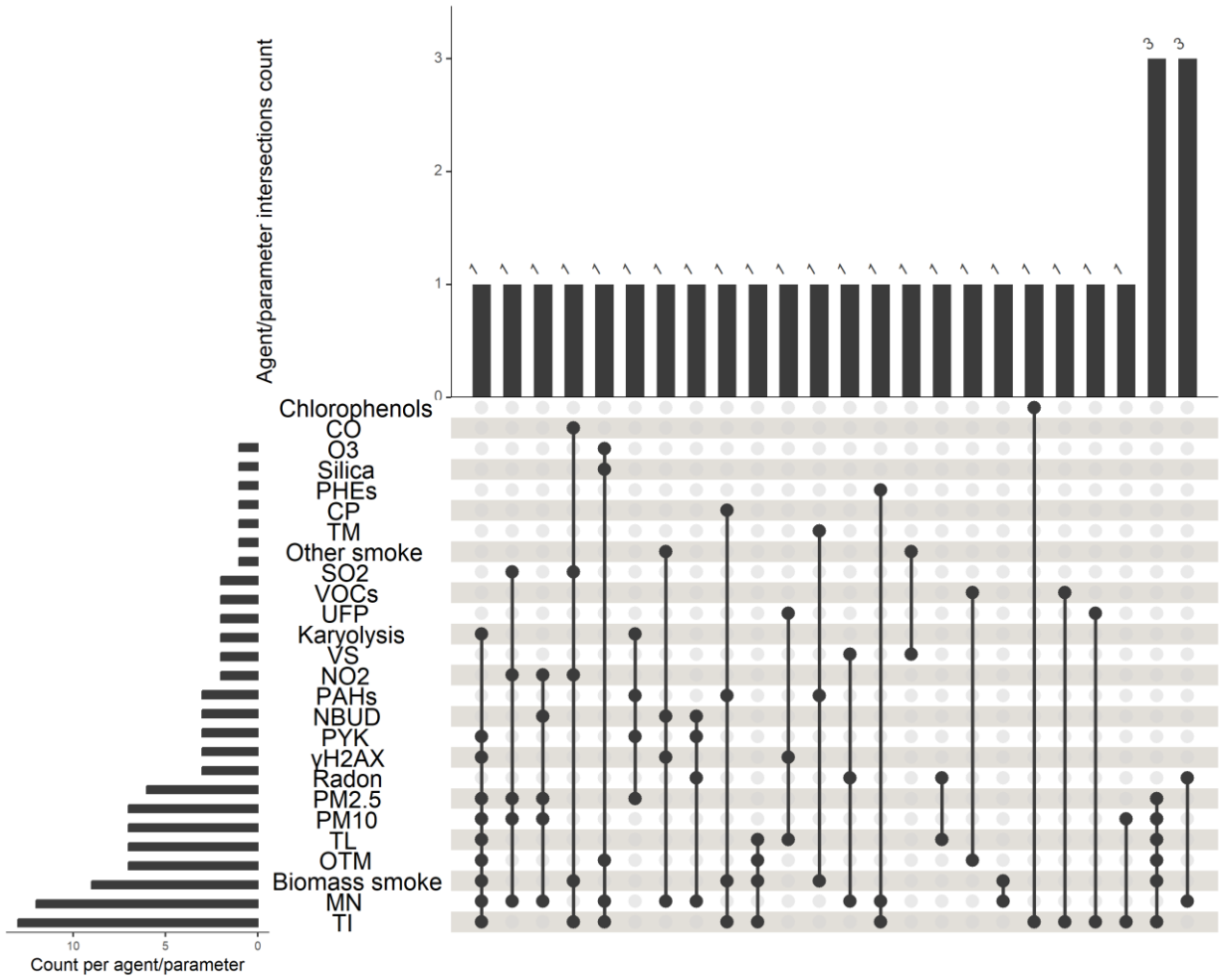

Fig. 2. Exploring complex interactions in genotoxicity data. The figure illustrates the application of UpSet plots in the analysis of genotoxicity interactions where each UpSet plot displays a matrix of intersecting sets, with the intersections represented by connected bars. The horizontal bars correspond to different agents or DNA damage parameters within the dataset, while the vertical bars represent the intersections between these sets. The length of each vertical bar indicates the size of the intersection, providing insights into the frequency and distribution of interactions throughout the studies. Only studies (N=28) that found statistically significant results regarding DNA damage resulting from exposure to a particular stressor were included in the analysis. Through the systematic arrangement of bars, it is possible to identify both common and rare interactions.

- Ventilation: Ensure proper ventilation by opening windows and using exhaust fans to allow fresh air circulation and remove pollutants. However, avoid such ventilation when warning of high outdoor pollution.
- Regular Maintenance of Heating, Ventilation, and Air Conditioning (HVAC) System: In residential homes or apartment units with such installations, regular maintenance and timely replacement of filters should be carried out by a designated qualified person.
- Reduction of Indoor Asbestos Exposure: To safely address asbestos found in older homes, particularly in insulation, paints, and floor tiles, professional remediation or removal by trained contractors is necessary when materials are damaged or renovation plans might disturb them.
- Lead Abatement: Lead-based paint is commonly found in homes built before the 1970s and its use in many countries remains legal. It may exist on various surfaces like window frames and walls, cautioning against its disturbance, and advising professional intervention for its safe removal.
- Reduce Smoking: Avoid smoking indoors (traditional combustible tobacco products as well as non-combustible alternatives), as tobacco smoke is a significant indoor air pollutant.
- Regular Cleaning: Keep indoor spaces and air ducts clean to reduce dust, mould, and other potential pollutants. Vacuum with a HEPA filter and clean surfaces regularly.
- Proper Venting of Appliances: Ensure that stoves, heaters, and other appliances are properly vented to the outdoors to prevent the build-up of indoor pollutants.
- Designing Interior Spaces: Keep in mind that the large number of curtains and carpets, as well as the number of connected electronic devices, can have an impact on pollution levels; avoid keeping electronic devices turned on if it is not necessary.
- Control Humidity: Maintain optimal indoor humidity levels (ideally between 30 – 50%) to prevent mould growth and reduce the presence of dust mites.
- Air Purifiers: Use air purifiers with HEPA filters to capture and remove airborne particles and pollutants.
- Limit VOCs: Choose low-VOC or VOC-free products such as paints, cleaning supplies, and furnishings to minimize their emissions.

- Avoid Synthetic Fragrances: Minimize the use of air fresheners, scented candles, and synthetic fragrances, which can contribute to indoor air pollution, especially the ones that can react with ozone to form particles and formaldehyde (for example, a pine or citrus scent).
- Avoid Appliances with Incomplete Combustion: Avoid open fires such as fireplaces, and solid fuel stoves.
- Monitor Radon Levels: Test for radon, a naturally occurring radioactive gas, and implement measures to reduce elevated levels if detected. In such a case, it is necessary to ventilate the interior regularly.
- Indoor Plants: Consider using indoor plants such as peace lily (*Spathiphyllum wallisii*), corn plant (*Dracaena fragrans*), fern arum (*Zamioculcas zamiifolia*), weeping fig (*Ficus benjamina*), or spider plant (*Chlorophytum comosum*), as they can help improve air quality by naturally filtering certain pollutants but avoid over-watering as this may promote growth of microorganisms which can affect individuals prone to allergies.

Since there is rather limited information on the possible DNA damaging effects as well as on the adverse effects on human health imposed by indoor air pollutants, research in this direction is warranted. Future studies should focus on research regarding the possible DNA damaging impact of indoor air pollutants both as a single compound and in complex mixtures, since toxicity data for a single compound may not be sufficient for the prediction of toxicity in a complex living environment. The presence of air pollutants in different amounts and with different modes of action suggests the need to study the connection between genotoxic components in the mixture and the resulting effects, considering the mode of action of each component by itself. Additionally, human biomonitoring research should focus on the adverse effects of indoor air pollutants in sufficiently sized cohorts with an emphasis on more vulnerable populations such as children, adolescents, the elderly, and individuals with pre-existing health conditions who may be more susceptible to these effects.

The European Union (EC 2021) has developed a Green Deal to reach zero pollution by 2050, and its current action plan targets to decrease limit values for outdoor air pollutants. Other actions will be oriented to the improvements in indoor design, use of safe materials, reduction of smoking, reliable heating and cooling systems, issue of policies to control indoor air, and campaigns to raise

awareness. In the UK (Duffield and Bunn 2023), there is also no legislation for indoor air quality, but outdoor air quality measures are set to indirectly reduce indoor air pollution. In 2019, the strategy for raising awareness and reducing indoor air pollution was issued, while in 2023, alignment with EU set limits was made. US EPA (2022) highlighted several strategic research areas and encouraged solutions-driven research for a better understanding of air pollution and climate change and their impacts on human health and ecosystems such as improvements of measurements and modelling techniques for characterization of air quality concentrations and exposure, human health impacts of air pollution, and climate change.

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Conflict of interest

There is nothing to disclose.

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Table 1. The use of the comet assay for the evaluation of DNA damage after exposure to indoor air pollutants.

Study	Type of study	Model used	Exposure	Agent/Stressor	Concentration range	Parameters tested (Response)	Main findings/comments
Karlsson et al. 2005 (Sweden)	*In vitro*	A549	Particles from a subway station and a nearby urban street	PM_{10}	Cells were exposed for 4 h to 5, 10, 20, or 40 μg/cm^2 (9-70 μg/mL) of particle suspension mixture	TI ↑ compared to control 8-oxoG ↑ subway particles compared to control	The ability to induce DNA damage was observed in both types of particles, in a concentration-dependent manner. Subway particles were found to be more potent at every concentration.
Pandey et al. 2005 (India)	Human biomonitoring	Isolated lymphocytes N = 70 biomass users (exposed) age: 38.94 ± 1.15 N = 74 LPG users (control) age: 36. 50 ± 1.15	Biomass smoke	Biomass smoke	–	TI ↑, OTM ↑, TL ↑ compared to control	All the measured parameters consistently revealed a significantly higher level of DNA damage in the BMF users group compared to the reference group. Exposure to BMF smoke induced more DNA strand breaks concerning age groups and the length of exposure.
Vinzents et al. 2005 (Denmark)	Human biomonitoring	Mononuclear cells isolated from venous blood N=15 (exposed and control), 10 males (25.3 ± 3.5 years) and 5 females (25.4 ± 1.5 years)	UFPs while cycling in traffic and spending time indoors	$PM_{2.5}$ and PM_{10}, CO, NO_X and NO_2	Cumulated UFP exposure outdoor bicycling bicycling: 3.01 (2.25 – 4.44) time outdoors 1.54 (0.68 – 3.28) time outdoors 10.5 (5.86 – 16.7) indoor bicycling time outdoors 1.42 (0.52 – 2.41) time outdoors 9.20 (6.15 – 13.1)	SB Ø, FPG ↑ compared to indoor bicycling	The combined outdoor and indoor exposures to UFPs were identified as independent and significant predictors of purine oxidation levels in DNA. However, these exposures did not serve as predictors for DNA strand breaks.
Torres-Dosal et al. 2008 (Mexico)	Human biomonitoring	PBL N = 20 Age: 5 – 35 (10 children aged 5 – 17 years, 10 adults aged 20 – 35 years)	Biomass smoke	CO and PAHs	COHb before: 4.9 ± 4.3% after: 1.0 ± 0.14 % 1-OHP before: 6.72 ± 3.58 μmol/mol Cr after: 4.80 ± 3.29 μmol/mol Cr	TM ↓ compared to exposure levels before the implementation of the risk reduction program	DNA damage in people exposed before the risk reduction program was higher than when the program was introduced. A significant positive correlation was obtained between urinary 1-OHP and DNA damage in blood cells before the intervention, while with the reduction of PAHs, such a trend was not reported.
Pariselli et al. 2009 (Italy)	*In vitro*	A549	VOCs	Toluene and Benzene	Toluene 0.25 ± 0.06 ppm Benzene 0.28 ± 0.03 ppm	TI ↑ for benzene, Ø for toluene, TI ↑ for the binary mixture compared to control	The genotoxic effects caused by toluene were seen after 3 h of exposure but diminished after 24 h. The effects of the binary mixture persisted for 24 hours.
Szeto et al. 2009 (Hong Kong)	*In vitro*	Cryopreserved isolated lymphocytes N = 4	Incense smoke	Incense smoke extracts	Incense smoke PBS or ethanol extracts: 10^{-2}, 10^{-3}, 10^{-4}, or 10^{-5} from initial extract	VS (0–4) ↑ for two samples (PBS and ethanol extracts) and ethanolic extract of the third sample out of six samples tested compared to control	DNA damage induction was dependent on the type of incense stick and the extract solvent.

Gminski et al. 2010 (Germany)	*In vitro*	A549	VOC mixtures emitted from pine wood and OSB and their main constituents	Terpenes: α-pinene, β-pinene, Δ^3-carene, camphene, limonene Aldehydes: Pentanal, Hexanal, 2-Heptenal, 2-Octenal	1. Pine wood panel emissions: Sum VOCs: 10.11 – 79.05 mg/m^3 2. OSB panel emissions: Sum VOCs: 7.25 – 81.8 mg/m^3 3. Selected terpenes and aldehydes: α-pinene: 1 – 1800 mg/m^3, Δ^3-carene: 5 – 600 mg/m^3, Hexanal: 2 – 2000 mg/m^3, 2-Heptenal: 10 – 250 mg/m^3, 2-Octenal: 10 – 250 mg/m^3 for 1 – 28 days	OTM Ø for pine wood panel and OSB VOC compared to control, OTM ↑ for 2-Heptenal and 2-Octenal, OTM Ø for the three VOCs compared to control	VOC mixtures emitted from pine wood and OSB did not result in adverse effects in A549. Selected single compounds at higher concentrations caused genotoxic effects but slight cytotoxic effects were also observed. Despite those results, indoor VOC emissions from these materials should not be considered a risk for humans.
Michałowicz and Majsterek 2010 (Poland)	*In vitro*	Isolated lymphocytes N = 3 age: 27 – 35	Chlorophenols and their derivatives	2,4,5-TCP, PCP, 4,6-DCG, TeCG, 4,5-DCC, and TeCC	0.2, 1 and 5 µg/ml	TI (EndoIII) ↑ and TI (FPG) ↑ compared to control	The oxidation of pyrimidine bases was more pronounced compared to purines.
Mondal et al. 2010 (India)	Human biomonitoring	AEC, BEC, Isolated lymphocytes N = 132 biomass users (exposed) age: 28 – 42 N = 85 LPG users (control) age: 27 – 42	Biomass smoke	PM_{10} and $PM_{2.5}$	Biomass: PM_{10}: 625 ± 150 µg/m3 $PM_{2.5}$: 312 ± 85 µg/m3 LPG: PM_{10}: 129 ± 42 µg/m3 $PM_{2.5}$: 77 ± 29 µg/m3	isolated lymphocytes: TI ↑, TL ↑, OTM ↑ compared to LPG users, AEC and BEC: MN ↑, 'broken egg' ↑, BN ↑, karyorrhexis ↑, karyolysis ↑, pyknosis ↑; isolated lymphocyte and AEC: γH2AX↑, Mre11↑, Ku70↑ compared to LPG users	In comparison to LPG users, individuals using all three types of biomass fuel exhibited significantly higher comet assay descriptors. Among the biomass users, dung cake users had the highest tail % DNA, which was significantly greater than that of wood users and crop residue users.
Mondal et al. 2011 (India)	Human biomonitoring	BEC N = 85 biomass users (exposed) age median: 35 (20 – 42) N = 76 LPG users (control) age median: 34 (21 – 41)	Biomass smoke	PM_{10} and $PM_{2.5}$	Biomass users PM_{10}: 351 ± 176 µg/m^3 $PM_{2.5}$: 198 ± 97 µg/m^3 LPG users PM_{10}: 119 ± 42 µg/m^3 $PM_{2.5}$: 66 ± 29 µg/m^3	TI ↑, TL ↑, OTM ↑ compared to LPG users NDF ↑ compared to LPG users	Dung users had significantly higher TI compared to wood and crop residue users. Users having separate kitchens had lower comet assay descriptors compared to users lacking spare kitchens. A positive association between PMs and DNA damage was observed.

Forchhammer et al. 2012 (Denmark)	Human biomonitoring	PBMC N = 20 age: 19 – 55	Wood smoke	$PM_{2.5}$, CO and 9 PAHs	$PM_{2.5}$: (clean air 14 ± 8, low exposure 220 ± 49, high exposure 354 ± 148 µg/m^3) CO: (clean air 0 ± 0, low exposure 9.85 ± 3.54, high exposure 16.05 ± 4.74 ppm) PAHs: (clean air 0.1 ± 0.2 – 0.7 ± 0.6, low exposure 23 ± 12 – 329 ± 249, high exposure 25 ± 8 – 342 ± 284 ng/m^3)	SB Ø, EndoIII Ø, FPG Ø	The findings suggest that a 3-hour exposure to wood smoke particles, even at concentrations considerably higher than ambient levels, is not linked to noticeable DNA damage.
Mukherjee et al. 2013 (India)	Human biomonitoring	Sputum cells N = 56 biomass users (exposed) age median: 34 (26 – 42) N = 49 LPG users (control) age median: 33 (25 – 41)	Biomass smoke	PM_{10} and $PM_{2.5}$	Cooking hours (biomass vs LPG) PM_{10}: 389.7 vs 104.5 µg/m^3 $PM_{2.5}$: 206.6 vs 61.0 µg/m^3 Noncooking hours (biomass vs LPG) PM_{10}: 116.7 vs 62.3 µg/m^3 $PM_{2.5}$: 62.5 vs 35.4 µg/m^3 urine *t,t*-MA ↑ in biomass users	TI ↑, TL ↑, OTM ↑ compared to control	In comparison to the control group, biomass users exhibited a fourfold increase in TI, 37% higher TL, and five times more TM. Multivariate logic regression showed an association between comet assay descriptors, PMs, and urinary *t,t*-MA.
Gustavino et al. 2014 (Italy)	*In vivo* (animal study)	Haemocytes and brain cells N=34 *(Dolichopoda* specimens sampled from 7 locations) N=4 (control)	Radiation	Radon	Minimal hourly concentration of 221±35 Bq/m^3 – 25997±520 Bq/m^3	TL ↑, TI Ø, TM Ø compared to control	The regression analysis of comet data revealed a notable dose-effect increase specifically for brain cells, with a significant association observed only in the context of radon exposure. TL exhibits a distinct rise with increasing doses, whereas TI and TM values do not display the same trend.
Jensen et al. 2014 (Denmark)	Human biomonitoring	PBMC N = 11 age: 23 ± 2	Wood smoke	$PM_{2.5}$, CO and NO_2	$PM_{2.5}$: 471 ± 60 µg/m^3 CO: 7.6 ± 0.7 ppm NO_2:140 – 154 µg/m^3	SB Ø, hOGG1 Ø, FPG Ø compared to levels before the exposure.	No difference was observed in the levels of DNA damage before and after residing in the house with open indoor fire in terms of SB, hOGG1-sensitive sites, and FPG-sensitive sites.
Mukherjee et al. 2014 (India)	Human biomonitoring	Sputum cells and AEC N= 80 biomass users (exposed) age median: 34 (22 – 42) N=70 LPG users (control) age median: 33 (23 – 40)	Biomass smoke	PM_{10} and $PM_{2.5}$	Biomass users PM_{10}: 454.3 ± 256.5µg/m^3 $PM_{2.5}$: 247.2 ± 99.6 µg/m^3 LPG users PM_{10}: 126.9 ± 32.1µg/m^3 $PM_{2.5}$: 59.2 ± 14.2 µg/m^3	TI ↑, TL ↑, OTM ↑ compared to LPG users 8-oxoG ↑, OGG1 ↓, APE1 ↓ compared to LPG users	An increase in oxidative DNA damage and reduction in BER protein expression. Multivariate logistic regression indicated an association between cooking with biomass and DNA damage.
Salgaonkar et al. 2016	*In vitro*	PBMC	BSE and CSE	CO, SO_2, NO and NO_2	BSE CO: 1.5 – 6798.0 ppm SO_2: 0.0 – 11.0 ppm	TI ↑ for CSE and BSE (cow dung), TI Ø for BSE (sawdust and wood)	The DNA damage was highest for cigarette smoke followed by cow dung smoke and was least for sawdust and wood smoke. Pre-treatment with N,N'-

(India)					NO: 0.0 – 16.5 ppm NO_2: 0.0 – 37.66 ppm CSE CO: 98.6 – 5865.0 ppm SO_2: 0.0 – 53.3 ppm NO: 2.6 – 128.6 ppm NO_2: 0.0 – 0.36 ppm for 1 h	compared to control	diacetylchitobiose showed protective properties to CSE and BSE exposure *in vitro*.
Marabini et al. 2017 (Italy)	*In vitro*	A549	Wood smoke	UFP extracts (carbon, water soluble ions, anhydrosugars, elements, PAHs)	UFP: 25 – 100 µg/mL concentrations for 24 h	TL ↑ compared to control γ-H2AX ↑ compared to control	All UFPs demonstrated the induction of DNA damage after a 24-hour treatment, as evidenced by the alkaline comet test. While no statistical difference was detected among the samples, all of them exhibited a significant increase in DSBs compared to the control.
Skovmand et al. 2017 (Denmark)	*In vitro* and *In vivo* (animal study)	A549 Lung tissue N = 45 (C57BL/6n mice)	Candlelight CP and two benchmark DEP(A-DEP and SRM2975)	PAHs	A549 CP, A-DEP and SRM2975: 0 – 100 µg/ml C57BL/6n mice CP group: low (LCP; 0.5 mg/kg) and high (HCP; 5 mg/kg) dose A-DEP and SRM2975 groups: dose of 5 mg/kg.	A549 alkaline DNA lesions/10^6 bp Ø C57BL/6n mice alkaline DNA lesions/10^6 bp Ø, hOGG1 DNA lesions/10^6 bp Ø compared to control	*In vitro*: exposure to up to 100 µg/ml of CP did not lead to a significant increase in DNA damage. *In vivo*: there was no significant difference in DNA damage between the groups, although the HCP group displayed a slight increase in levels of DNA strand breaks compared to the control.
Plumejeaud et al. 2018 (Portugal)	*In vitro*	AGS	Ingestion of PHEs in house dust	Al, Zn, Cu, Pb, Mn, Ba, Ni, Cr, Sn, V, As, Co, Sb, Mo, Ga, Cd	Gastric extracts: 0.067 – 0.53 g/L (2 h/ 24 h)	TI ↑ compared to control C–MNed ↑, C+MNed ↑, compared to control	Following a 2-hour exposure to gastric extracts, there was an observed dose-dependent increase in TI and MNed cells across all sites. Cu was likely the predominant PHE inducing primary DNA damage in this context.
Zani et al. 2020 (Italy)	Human biomonitoring	Human saliva leukocytes N = 152 age: 3 – 6	Airborne pollutants	PM_{10}, $PM_{2.5}$, NO_2, CO, SO_2, benzene and O_3	– (estimates for indoor exposures based on interviews with parents)	TI Ø, VS Ø compared to volunteers from different schools or residence	No significant correlation was identified between indoor air pollution (passive smoking or indoor fire) and DNA damage in the saliva leukocytes of children. Ambient O_3 levels 2 days before sampling were associated with an increase in DNA damage.
Colafarina et al. 2022 (Italy)	*In vitro*	Hs27 and A549	Fine silica particles in the presence or absence of O_3	silica, silica + O_3	silica (40 µg/h), silica + O_3 (120 ppb): 48 h, 72 h	A549 TI ↑, OTM ↑, compared to control Hs27 TI ↑, OTM ↑, for silica	There was significant cytotoxic and genotoxic damage at 48 and 72 h, in both A549 and Hs27, compared to the control. DNA strand breaks did not differ in Hs27 cells after 48 h of exposure to both

						TI ↑, for silica + O_3 at 72 h A549 and Hs27: MN ↑, compared to control	pollutants, therefore the synergistic or additive effects were difficult to assess.
Dicu et al. 2022 (Romania)	Human biomonitoring	PBMC N = 38 (exposed) age: 49.3 ± 9.0 N = 38 (control) age: 46.6 ± 10.5	Radiation	Radon	Annual effective dose exposed: 10.3 ± 13.1 mSv control: 4.1 ± 2.7 mSv	comet assay lesion score and tail factor ↑, challenge assay ↑, repair ↓ compared to control MN ↑, serum 8-OHdG Ø, compared to control	Higher DNA damage and lower DNA repair capacity were found in the exposed group.
McCarrick et al. 2024 (Ethiopia/ Sweden)	*In vitro*	BEAS-2B and THP-1	CP derived from biomass fuels (eucalyptus charcoal, eucalyptus wood, and cow dung) and DEP (*NIST 2975*) as reference particles	PAHs 21 parent PAHs 14 alkyl-PAHs 20 nitro-PAHs 10 oxy-PAHs 6 DBT-PAHs	BEAS-2B 5 – 25 µg/mL (dung & wood) 5 – 100 µg/mL (charcoal, DEP) for 4 h and 24 h. THP-1 24 h at concentrations from 10 – 100 µg/mL	BEAS-2B TI ↑ (24 h exposure) wood and dung ≥ 10 µg/mL charcoal and DEP ≥ 25 µg/mL compared to control THP-1 TI ↑ (24 h exposure) wood and charcoal at 100 µg/mL DEP ≥ 10 µg/mL	The highest mass fraction of total particulate PAHs was observed in particles originating from wood (3219 ng/mg), with dung (618 ng/mg), charcoal (135 ng/mg), and DEP (118 ng/mg) following in descending order. All CPs induced concentration-dependent genotoxicity in BEAS-2B irrespective of PAH content. In THP-1 cells, a similar trend was observed for wood and DEP only.

Abbreviations: ↑, significant increase; ↓, significant decrease; Ø, no effect; ≥, greater than or equal to; 1-OHP, 1-hydroxypyrene; 2,4,5-TCP, 2,4,5-trichlorophenol; 4,5-DCC, 4,5-dichlorocatechol; 4,6-DCG, 4,6-dichloroguaiacol; 8-oxoG, 8-oxoguanine, A549, human basal alveolar epithelial cell adenocarcinoma; AEC, airway epithelial cells; AGS, adherent human adenocarcinoma gastric stomach cells; APE1, alkyl-PAHs, alkylated polycyclic aromatic hydrocarbons, apurinic/apyrimidinic site endonuclease 1; BEAS-2B, immortalized human bronchial epithelial cells; BEC, buccal epithelial cells; BER, base excision repair; BMF, biomass fuel; BN, binucleated; BSE, biomass smoke extract; CO, cabron monoxide; COHb, carboxyhemoglobin; CP, combustion particles; CSE, cigarette smoke extract; DBT-PAHs, dibenzothiophenes particle bound polycyclic aromatic hydrocarbons; DEP, diesel exhaust particles; DMEM, Dulbecco's Modified Eagle Medium; DNA, deoxyribonucleic acid; DS, damage score; DSB, double-stranded break; EndoIII, endonuclease III; FPG, formamidopyrimidine DNA glycosylase; HCP, high combustion particles; hOGG1, human 8-oxoguanine DNA N-glycosylase 1; Hs27, human skin fibroblasts; Ku70, endogenous nuclear protein (69.9 kDa); LCP, low combustion particles; LPG, liquid petroleum gas; MN, micronucleus; MNed, micronucleated; Mre11, meiotic recombination 11; NDF, nuclear diffusion factor; nitro-PAHs, nitrated polycyclic aromatic hydrocarbons; NO_2, nitrogen dioxide; NO_X, nitrogen oxides; O_3, ozone; OGG1, 8-oxoguanine DNA N-glycosylase 1; OSB, oriented strand board; OTM, Olive tail moment, oxy-PAHs, oxygenated polycyclic aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PCP, pentachlorophenol; PHE, potentially harmful elements; PM, particulate matter; PM_{10}, particulate matter with an aerodynamic diameter less than or equal to 10 micrometers; $PM_{2.5}$, particulate matter with an aerodynamic diameter less than or equal to 2.5 micrometers; ppb, parts per billion; ppm, parts per million; SB, strand breaks; SD, standard deviation; SO_2, sulfur dioxide; SRM, standard reference material; TeCC, tetrachlorocatechol; TeCG, tetrachloroguaiacol; THP-1, human peripheral blood monocyte derived macrophages, TI, tail intensity; TL, tail length; TM, tail moment; t,t,-MA, trans,trans-muconic acid; UFP, ultrafine particles; VOC, volatile organic compound; VS, visual scoring; γH2AX, phosphorylated histone H2AX

Table 2. The use of the micronucleus assay for the evaluation of genome damage after exposure to indoor air pollutants.

Study	Type of study	Model used	Exposure	Agent/Stressor	Concentration range (air pollutants)	Parameters tested (response)	Main findings/comments
Bilban and Vaupotič 2001 (Slovenia)	Human biomonitoring	PBL N = 85 (exposed), age: 8 – 12, N = 20 (control), age: 8 – 12	Indoor radiation	Radon	Effective dose from radon exposure exposed: 7 – 11 mSv/year control: 0.7 mSv/year	MNi ↑, total aberrations ↑, chromatid breaks ↑, chromosomal breaks ↑, acentric fragments Ø, dicentrics Ø compared to the control group	More cytogenetic damage was found compared to the control group. There was a significant correlation between age and cytogenetic damage, in the exposed group. Binuclear cells with 2-4 MNi per cell were found in the exposed group
Musthapa et al. 2004 (India)	Human biomonitoring	PBL N = 121 biomass and kerosene users, N = 58 LPG users (control),	Biomass smoke	Mixture of pollutants	-	MNed cells and cells with CA: cow dung ↑, cow dung/wood ↑, wood ↑, kerosene Ø compared to LPG users	Increased cytogenetic damage was found among biofuel users compared to control. Cow dung users exhibited the highest frequencies of CA and MN. The length of exposure to biomass smoke was associated with the cytogenetic results.
Mondal et al. 2010 (India)	Human biomonitoring	AEC, BEC, isolated lymphocytes N = 132 biomass users, age: 28 – 42, N = 85 LPG users (controls), age: 27 – 42	Biomass smoke	PM_{10} and $PM_{2.5}$	Biomass: PM_{10}: 625 ± 150 μg/m^3 $PM_{2.5}$: 312 ± 85 μg/m^3 LPG: PM_{10}: 129 ± 42 μg/m^3 $PM_{2.5}$: 77 ± 29 μg/m^3	AEC and BEC: MN ↑, 'broken egg' ↑, BN ↑, karyorrhexis ↑, karyolysis ↑, pyknosis ↑; isolated lymphocytes: TI ↑, TL ↑, OTM ↑ compared to LPG users, isolated lymphocyte and AEC: γH2AX↑, Mre11↑, Ku70↑ compared to LPG users	The highest MN frequencies were found in women using cow dung for cooking fuel. MN descriptors were associated with the kitchen position and years of cooking with biomass fuel. The exposed population showed aberrant expression of DNA repair proteins, generation of ROS, and depletion of antioxidant defence.
Ceretti et al. 2014 (Italy)	Human biomonitoring	BEC N = 181, age: 4.35 ± 0.9	Urban air pollution	CO, NO_2, SO_2, benzene, O_3, PM_{10} and $PM_{2.5}$	1-week average range (mg/m^3).: PM_{10}: 46.8 – 112.3 $PM_{2.5}$: 32.8 – 93.1 NO_2: 55.6 – 87.0 CO, SO_2, benzene, O_3 levels were bellow permissible limits *pollutants on the sampling day, 2-week, and 3-week averages were also given	MN ↑, NBUD ↑, BNed ↑, basal cells ↑, condensed chromatin cells Ø, karyorrhectic cells Ø, pyknotic cells Ø, karyolytic cells Ø, cells without nucleus Ø for an increase of PM_{10}, $PM_{2.5}$, and NO_2	Linear regression models showed a modest increase in measured DNA damage related to PM_{10}, $PM_{2.5}$, and NO_2, whereas no clear pattern was observed related to a particular biomarker or exposure window. The MN frequency was higher than that observed in pooled analysis (N = 321) of healthy children ("without important exposure")
Sinitsky and Druzhinin 2014 (Russia)	Human biomonitoring	Blood cells N = 60 (exposed), age: 8 –17, N = 60 (control), age: 8 – 18	Indoor radiation	Radon	Activity of radon (mean) exposed: 626 Bq/m^3 control: 91.5 Bq/m^3	MNi ↑, MNed ↑ NPBed Ø, NBUDed Ø, PI ↑ compared to the control group	A significantly higher number of MNi and PI were found in the radon-exposed group compared with the control group.

Marcon et al. 2017 (Brazil)	Human biomonitoring	BEC N = 35 (exposed), age: 18 – 45, N = 35 (control), age: 18 – 45	Indoor radon and gamma emitters, contaminated tap water	Indoor radon, uranium (U), potassium (K) and thorium (Th) + Toxic metals, radiation, and cyanobacteria in tap water	Estimated annual effective dose from all radioactive contaminants: 6.3 mSv/year (CI95% 4.3 – 8.2) (exposed) 1.3 mSv/year (CI95% 1.0 – 1.6) (control)	MN↑, NBUD↑, BN↑, pyknosis ↑	Middle to high radiation exposure levels along with water contamination might be associated with high genotoxicity biomarkers and higher cancer rates in the exposed city.
Linhares et al. 2018 (Portugal)	Human biomonitoring	BEC N = 33 (exposed), age: 42.2 ± 2.5, N = 49 (control), age: 35.5 ± 1.4	Indoor radon in a hydrothermal area	Radon	Activity of radon (mean) exposed: 115 Bq/m^3 control: 47 Bq/m^3	MNed ↑, karyorrhexis Ø, karyolitic Ø, pyknosis Ø, compared to the control group	Poisson regression, with adjustment for age, gender, smoking status, and alcohol consumption showed that a higher frequency of MNed was associated with exposure to indoor radon. RAPD profiles using one primer showed no exclusive bands to one of the groups.
Plumejeaud et al. 2018 (Portugal)	*In vitro*	AGS	Ingestion of PHEs in house dust	Al, Zn, Cu, Pb, Mn, Ba, Ni, Cr, Sn, V, As, Co, Sb, Mo, Ga, Cd	Gastric extracts: 0.067 – 0.53 g/l (2 h/ 24 h)	C–MNed ↑, C+MNed ↑, compared to control TI↑, compared to control	After exposure to bioaccessible fractions of potentially harmful elements an increase in TI and MNed across all sites was observed. Pb was the predominant PHE inducing chromosome-damaging effect.
Sarker et al. 2020 (US)	*In vitro*	BEAS-2B, hPFs, hTERT-HCA2	Cigarette smoke	Thirdhand smoke	THS: nicotine 950 ng/ml, NNK 0.56 ng/ml, and nicotelline 1.52 ng/ml dilutions: 1.25 %, 2.5 %, 5 %, 10 %, 20 % (12 h/ 24 h/ 48 h)	MNed/NBUDed ↑ (in BEAS-2B cells with 5% THS), compared to a mock exposed control RPA ↑, ATR ↑, ATM ↑, CHK1 ↑, and BRCA1 ↑, NER ↓, γH2AX ↑, 53BP1 ↑, in BEAS-2B cells compared to a mock exposed control	Exposure to THS *in vitro* activates key DNA damage response proteins, induces DNA double-strand breaks, inhibits RNA synthesis, and blocks NER.
Sopian et al. 2020 (Malaysia)	Human biomonitoring	BEC N = 101, (exposed), age: 10 – 11 N = 75 (control), age: 10 – 11	Air pollution in proximity to industrial zone (classrooms and homes)	PM_1, $PM_{2.5}$, PM_{10}, NO_2, SO_2	Exposed schools/homes ($\mu g/m^3$): PM_1: 46.90 ± 15.64 $PM_{2.5}$: 48.82 ± 15.89 PM_{10}: 69.73 ± 19.72/ 108.39 ± 94.11 NO_2: 31.91 ± 44.27/ 20.78 ± 37.81 SO_2: 197.59 ± 98.93/ 141.96 ± 59.85 Control schools/homes ($\mu g/m^3$): PM_1: 28.50 ± 4.47 $PM_{2.5}$: 29.75 ± 5.36 PM_{10}: 36.00 ± 6.44/ 46.15 ± 41.29 NO_2: < 0.01/ < 0.01 SO_2: 72.01 ± 49.98/ 6.89 ± 30.04	MN ↑, compared to the control group	Significantly higher concentrations of indoor air pollutants were found in schools in the vicinity of an industrial park compared to control schools. Regression analysis shows that NO_2 was the most significant air pollutant predictor to induce MN formation.

Błaszczyk et al. 2022 (Poland)	Human biomonitoring	BEC N = 93 age: 6.3 ± 0.9	Industrial air pollution and biomass smoke (kindergartens)	$PM_{2.5}$, Σ15 PAHs, BaP	Industrial city $PM_{2.5}$: 28.2 ± 3.1 (18.5 – 42.4 μg/m^3) Σ15 PAHs: 38.8 (14.6 – 72.9 ng/m^3) BaP: 3.7 ± 0.8 (1.2 – 7.5 ng/m^3) Rural sites $PM_{2.5}$: 31.9 ± 3.3 and 34.4 ± 88.4 (16.4 – 82.2 μg/m^3) Σ15 PAHs: 45.3 ± 9.4 and 58.3 ± 11.8 (25.0 –120.3 ng/m^3) BaP: 5.7 ± 1.6 and 6.4 ± 1.8 (2.2 – 15.9 ng/m^3)	MN Ø, BUDs Ø between the sites, BN ↑ in industrial city, pyknotic cells ↑ compared to one rural site and ↓ to the other rural site, karyolitic ↑ compared to one rural site and ↓ to the other rural site mutagenicity of organic PM extract Ø, urinary mutagenicity Ø between the sites, BPDE-1-DNA adducts ↓ in industrial city	No differences between urinary 1-OHP between the sites. Multiple linear regression analysis indicated that DNA adducts were related to the residency site studied and the coal used for heating and cooking.
Colafarina et al. 2022 (Italy)	*In vitro*	A549 and Hs27	Fine silica particles in the presence or absence of O_3	silica, silica + O_3	silica (40 μg/h), silica + O_3 (120 ppb): 48 h, 72 h	A549 and Hs27: MN ↑, compared to control A549 TI ↑, OTM ↑, compared to control Hs27: TI ↑, OTM ↑, for silica TI ↑, for silica + O_3 at 72 h	There was significant cytotoxic and genotoxic damage at 48 and 72 h, in both A549 and Hs27, compared to the control. Strand breaks did not differ in Hs27 cells after 48 h of exposure to both pollutants, therefore the synergistic or additive effects were difficult to assess.
Dicu et al. 2022 (Romania)	Human biomonitoring	PBMC N = 38 (exposed) age: 49.3 ± 9.0 N = 38 (control) age: 46.6 ± 10.5	Radiation	Radon	Annual effective dose exposed: 10.3 ± 13.1 mSv control: 4.1 ± 2.7 mSv	MN ↑, compared to control comet assay lesion score and tail factor ↑, challenge assay ↑, repair ↓, serum 8-OHdG Ø, compared to control	Higher DNA damage and lower DNA repair capacity were found in the exposed group.

Abbreviations: ↑, statistically significant increase, ↓, statistically significant decrease; Ø, no effect; <, less than; γH2AX, phosphorylated histone H2AX; A549, human basal alveolar epithelial cell adenocarcinoma; AEC, airway epithelial cells; AGS, adherent human adenocarcinoma gastric stomach cells; BEAS-2B, transformed non-tumorigenic human lung epithelial cells; BEC, buccal epithelial cells; BN, binucleated cells; C–MNed, chromosome breakages and micronucleated cells; C+MNed, chromosome losses and micronucleated cells; CA, chromosomal aberrations; Hs27, human normal fibroblasts; hTERT-HCA2, hTERT-immortalized skin fibroblast cells; Ku70,endogenous nuclear protein (70kDa), LPG, liquefied petroleum gas; MN, total number of micronuclei; MNed, number of cells with micronuclei; Mre11, meiotic recombination; NBUD, total number of nuclear buds; NBUDed, number of cells with nuclear buds; NER – nucleotide excision repair; NNK, Nicotine-derived nitrosamine ketone (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NPB, total number of nuclear bridges; NPBed, number of cells with nuclear bridges; OTM, Olive tail moment; PBL, peripheral blood lymphocytes; PHEs, potentially harmful elements; PI, proliferation index; PM_1, particulate matter with an aerodynamic diameter ≤ 1 μm; $PM_{2.5}$, particulate matter with an aerodynamic diameter ≤ 2.5 μm; PM_{10}, particulate matter with an aerodynamic diameter ≤ 10 μm; THS, thirdhand cigarette smoke; TI, tail intensity; TL, tail length; TM, tail moment;