National Institute of
Environmental Health Sciences

PROTOCOL FOR SYSTEMATIC REVIEW OF INFLAMMATION-BASED ATHEROSCLEROSIS ASSOCIATED WITH EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

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ACRONYMS

Agency for Toxic Substances and Disease Registry	ATSDR
Analysis of variance	ANOVA
Benchmark dose	BMD
C-reactive protein	CRP
Cardiovascular disease	CVD
Carotid intima media thickness	CIMT
Conflict of interest	COI
Division of Translational Toxicology	DTT
Environmental Protection Agency	EPA
Good laboratory practice	GLP
Grades of Recommendation, Assessment, Development, and Evaluation	GRADE
Hazard identification	Hazard ID
Health Assessment Workspace Collaborative	HAWC
High-performance liquid chromatography with tandem mass spectroscopy	HPLC-MS/MS
Hydroxypyrene	OHP
Integrative Health Assessments Branch	IHAB
Interleukin-6	IL-6
Lowest-observed-effect level	LOEL
National Health and Nutrition Examination Survey	NHANES
National Heart, Lung, and Blood Institute	NHLBI
National Institute for Occupational Safety and Health	NIOSH
National Institute of Environmental Health Sciences	NIEHS
National Institutes of Health	NIH
National Toxicology Program	NTP
No-observed-effect level	NOEL
Not applicable	NA
Not reported	NR
Office of Health Assessment and Translation	OHAT
Organization for Economic Co-operation and Development	OECD
Polychlorinated biphenyl	PCB
Polycyclic aromatic hydrocarbon	PAH
Population, Exposure, Comparators, and Outcomes	PECO

PROTOCOL TO EVALUATE THE EVIDENCE OF INFLAMMATION-BASED ATHEROSCLEROSIS ASSOCIATED WITH POLYCYCLIC AROMATIC HYDROCARBON EXPOSURE

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Summary: The National Institute of Environmental Health Sciences (NIEHS) is conducting a systematic review to evaluate the evidence for inflammation-based atherosclerosis associated with exposure to polycyclic aromatic hydrocarbons.

BACKGROUND AND SIGNIFICANCE

Background

Inflammation has long been understood as a beneficial immune response responsible for restoring tissue architecture and function after infection or tissue injury. However, biomedical research conducted in the past two decades has identified persistent inflammation as a key factor in the development of a myriad of chronic diseases, including cardiovascular disease, cancer, renal disease, autoimmune disease (such as type 2 diabetes), and diseases of aging such as Alzheimer's disease (reviewed in Manabe (2011)). The failure to resolve inflammation, the progression from acute to chronic inflammation, and the co-existence of acute and chronic inflammatory responses may all contribute to persistent inflammation linked to disease (Nathan and Ding 2010).

Cardiovascular disease encompasses many diseases of the heart or blood vessels, and an important underlying condition of cardiovascular disease is atherosclerosis (Goff *et al.* 2014). Atherosclerosis, characterized by the buildup of plaques (deposits of fat and other bloodborne substances) in the arterial walls, causes a narrowing of the arteries and a subsequent restriction of blood flow. Atherosclerosis is a significant public health concern and is one of the dominant conditions underlying cardiovascular disease, including heart attack and stroke. A recent American Heart Association report found strong associations between the preclinical measures of atherosclerosis (e.g., carotid intima-media thickness) and mortality from cardiovascular disease (Benjamin *et al.* 2018). From an economic perspective, the cost of atherosclerosis-related diseases is significant. Cardiovascular disease in the United States alone was estimated to have cost $555 billion in 2016, and this number is expected to double by 2035 (American Heart Association 2017). Although the exact cause of atherosclerosis is unclear, there is a well-established role of chronic inflammation in the disease process (Ross 1999, Pearson *et al.* 2003, Rosenfeld and Campbell 2011, Soehnlein and Libby 2021).

There is also a growing body of evidence suggesting a role of environmental exposures in a wide range of diseases that involve inflammation. Persistent inflammation from prolonged exposure to stimuli, such as certain environmental exposures, can lead to chronic inflammation, long-term elevation of inflammatory mediators, and tissue damage (Gorman *et al.* 2004, Zakynthinos and Pappa 2009, Donath and Shoelson 2011, Hanahan and Weinberg 2011, Wyss-Coray and Rogers 2012, Schwarze *et al.* 2013). Herein, we set out to evaluate the evidence for an association between inflammation-based atherosclerosis and polycyclic aromatic hydrocarbons (PAHs), a relevant representative environmental exposure.

PAHs are a class of chemicals that are released during the incomplete burning of substances such as coal, oil, tobacco, wood, and charbroiled or smoked foods. The primary pathways of exposure to PAHs for most of the U.S. population are inhalation of tobacco smoke, wood smoke, and ambient air, and consumption of certain foods (ATSDR 1995). In addition to these well-established sources of exposures, there is growing concern that climate change is increasing the frequency and severity of extreme weather events, and climate change -related increases in wildfires are impacting the nature and frequency of human exposure to PAHs (Messier *et al.* 2019). In the United States, PAH metabolites were found in the urine of most participants in studies documented in the Fourth National Report on Human Exposure to Environmental Chemicals, suggesting widespread exposure to PAHs (Centers for Disease Control and Prevention (CDC) 2015). PAH exposure has been linked to cancer and other adverse health

effects of the respiratory and cardiovascular systems (Kim *et al*. 2013, Alshaarawy *et al*. 2016), and a recent study of U.S. adults found an association between levels of PAH biomarkers and cardiovascular disease (Alshaarawy *et al*. 2016).

Evidence from human observational studies suggests an association between PAH exposure and inflammation (Clark *et al.* 2012, Alshaarawy *et al.* 2013). This association is supported by preclinical studies that suggest PAHs may promote inflammation by increasing proinflammatory cells in atherosclerotic plaques (Curfs *et al.* 2004, Curfs *et al.* 2005). A growing body of evidence suggests that activation of the inflammatory pathway is mediated by three key biomarkers: fibrinogen, C-reactive protein (CRP), and interleukin-6 (IL-6) (Libby and King 2015, Ridker 2016); however, the extent to which PAHs affect this pathway that ultimately leads to atherosclerosis and cardiovascular disease remains unclear.

Significance

A key premise of this systematic review is that common biological pathways or shared mechanisms, such as inflammation, initiate the development or drive the progression of multiple diseases. Understanding environmental triggers of common disease mechanisms is essential to developing and improving public health strategies to combat the myriad of chronic and costly diseases. In this regard, characterizing key biomarkers of diseases triggered by environmental exposures that drive inflammation could profoundly impact early detection of disease and potentially provide an impetus for improved disease treatments. This theoretical framework encompasses the goals of this systematic review and stems from discussions with the National Institute of Environmental Health Sciences (NIEHS) Strategic Plan Cross-Divisional Implementation Planning Committee on Inflammation. The committee is keenly interested in work that contributes to the identification of markers of environmentally-induced inflammation and has identified the need for a systematic review of evidence to identify environmental triggers of inflammation that lead to atherosclerosis.

The Division of Translational Toxicology (DTT) at NIEHS will conduct a systematic review of the evidence for an association between inflammation-based atherosclerosis and PAH exposures based on the problem formulation and scoping efforts identifying PAHs as relevant environmental exposures for a potential association. If the literature base is sufficient, this review will reach level-of-evidence conclusions for hazard identification. If the evaluation is too limited to support hazard conclusions, the evaluation will include a synthesis of the evidence that exposure to PAHs may be associated with atherosclerosis and atherosclerotic-related cardiovascular effects and will identify areas of consistency and uncertainty in the evidence.

Thus, the value and significance of this proposed systematic review are threefold: First, the systematic review will evaluate the evidence for inflammation-based atherosclerosis associated with exposures to PAHs. Second, it will test the hypothesis that PAHs are linked to atherosclerosis through an inflammatory pathway. Third, it will identify biomarkers that may be of interest in the design of high or medium throughput screening assays for chemicals that might cause cardiotoxicity through an inflammation pathway. Building capabilities to evaluate cardiovascular toxicity is an area of interest within the DTT, and this systematic review would inform future efforts.

OVERALL OBJECTIVE AND SPECIFIC AIMS

Objective

The objective of this review is to evaluate the evidence for an association between inflammation-based atherosclerosis and environmental exposure to PAHs and will include hazard identification and level-of-concern statements if the data are sufficient to support such conclusions. In addition, a high-level overview of the nature and extent of the literature informing evolving sources of PAH exposures associated with atherosclerosis and cardiovascular disease—specifically exposure sources related to climate change, such as biomass burning and wildfires, will be included. The objective will be addressed by completing the specific aims for the proposed evaluation as outlined below. The systematic review will be based on guidance outlined in the Office of Health Assessment and Translation (OHAT) *Handbook for Conducting a Literature-based Health Assessment* (NTP 2019).

Specific Aims

- Identify literature that assessed inflammation and atherosclerosis following PAH exposures in human, animal, and in vitro/mechanistic studies.
- Identify literature that assessed cardiovascular effects following sources of PAH exposures due to climate change-related wildfires and biomass burning.
- Extract data on potential long-term health effects from relevant studies.
- Assess the internal validity (risk of bias) of individual studies using pre-defined criteria.
- Summarize the extent and types of health effects evidence available.
- Describe limitations of the systematic review and of the evidence base and identify areas of uncertainty, data gaps, and research needs on long-term health effects of environmental exposures.

Dependent on the extent and nature of the available evidence:

- Synthesize the evidence using a narrative approach or meta-analysis (if appropriate) considering limitations on data integrating such as study design heterogeneity.
- Rate confidence in the body of evidence for human and animal studies separately according to one of four statements: high, moderate, low, or very low/no evidence available.
- Translate confidence ratings into level of evidence of health effects for human and animal studies separately according to one of four statements: high, moderate, low, or inadequate.
- Combine the level-of-evidence ratings for human and animal data to reach one of five possible hazard identification conclusions: known, presumed, suspected, not classifiable, or not identified to be a hazard to humans.

The evaluation will integrate evidence of inflammation-based atherosclerosis effects associated with acute or chronic PAH exposures from human studies across a broad range of study design types along with controlled exposure animal studies and mechanistic/in vitro studies based on the problem formulation efforts that identified PAHs as a relevant environmental exposure for inflammation-based atherosclerosis. This assessment will not evaluate the broader polycyclic aromatic compounds (PACs), which would include heterocyclic compounds containing other atoms besides carbon and hydrogen (e.g., nitrogen, oxygen, or sulfur) within the ring structure. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes in vitro and in vivo laboratory studies directed at cellular, biochemical, and molecular mechanisms that explain how a chemical produces specific adverse health effects. As a result of the problem formulation/scoping efforts as well as consultation with subject matter experts, the in vitro mechanistic markers will focus on: (1) fibrinogen, (2) C-reactive protein (CRP), and (3) Interleukin-6 (IL-6) (Libby and King 2015, Ridker 2016). These markers are thought to play important roles in the inflammatory response and/or the prediction of cardiovascular disease. IL-6 is an inflammatory cytokine that induces the production of acute phase reactants in the liver, such as fibrinogen and CRP, which increase in response to cytokine activation. Elevated fibrinogen or CRP levels in plasma or serum are both associated with increased cardiovascular risk (with CRP being the best-established marker for cardiovascular disease). Depending on the extent of these studies, technical advisors and subject matter experts will be consulted to consider quality and relevance to the **P**opulation, **E**xposure, **C**omparators, and **O**utcomes (PECO) statement.

PECO Statement

PECO statements were developed as an aid to identify search terms and inclusion/exclusion criteria as appropriate for addressing the overall research question (inflammatory atherosclerosis from acute/chronic PAH exposure) for the systematic review (Higgins and Green 2011).The PECO statements are listed below for human (Table 1), animal (Table 2), and in vitro/mechanistic (Table 3) studies.

Table 1. Human PECO (Population, Exposure, Comparator, and Outcome) Statement	
PECO Element	**Evidence**
Population	Humans without restriction as to age or sex, geographic location, or life stage at exposure or outcome assessment
Exposure	Acute or chronic PAH exposure based on: • Known dose or concentration in an experimental protocol • Diagnostic biomonitoring data (e.g., markers in plasma or urine) • Environmental detection (e.g., air, soil) No restriction on whether exposure is accidental or intentional
Comparators	For controlled and uncontrolled studies, comparable populations not exposed to the environmental exposure; and case series-reports, no comparable populations
Outcomes	Atherosclerosis: The following outcome measures were considered for atherosclerosis and related diseases (e.g., coronary heart disease, carotid artery disease, peripheral artery disease, and chronic kidney disease): • Fatal events (e.g., all-cause mortality, cardiovascular disease deaths, and other vascular deaths) • Non-fatal events (e.g., acute coronary syndromes such as myocardial infarction, unstable angina pectoris, and cerebrovascular events such as stroke and aneurysm) • Direct measurments of disease severity (e.g., digital subtraction angiograms for peripheral arterial disease; carotid intima-media thickness) • Indirect mesasures of disease severity (e.g., ankle-brachial pressure index, limb weakness, walking distance for claudicants) • Corroboration by assessment of direct (in hospital, in clinic) or indirect observation of symptoms of atherosclerosis Inflammation: The following outcome measures were considered for inflammation: • Biomarkers of inflammation (e.g., cytokines, chemokines, eiconsoids, and prostanoids) • Inflammatory gene expression of fibrinogen, CRP, and IL-6 • Immune response from clotting factors (e.g., platelets), leukocyte infiltrate, differential white blood cells, and immunophenotyping (e.g., T cells, myeloid)

Table 2. Animal PECO (Population, Exposure, Comparator, and Outcome) Statement	
PECO Element	**Evidence**
Population	Without restriction as to species, age, sex, or life stage at exposure or outcome assessment
Exposure	Acute or chronic exposure to PAHs based on administered dose or concentration or biomonitoring data (e.g., urine, blood, or other specimens)
Comparators	Comparable untreated animal subjects or animals exposed to vehicle-only treatment
Outcomes	Atherosclerosis: The following outcome measures were considered for atherosclerosis and related diseases (e.g., coronary artery disease, carotid artery disease, peripheral artery disease, and chronic kidney disease): • Mortality • Non-fatal events including acute coronary syndromes such as myocardial infarction, unstable angina pectoris, and cerebrovascular events such as stroke • Effects on direct measurement of disease severity (e.g., artery/aorta lesion area/volume; aortic calcification; carotid intima-media thickness, coronary artery calcium; score, brachial artery index) • Indirect tests of disease severity Inflammation: The following outcome measures were considered for inflammation: • Biomarkers of inflammation (e.g., cytokines, chemokines, eicosanoids, and prostanoids) • Inflammatory gene expression of fibrinogen, CRP, and IL-6 • Immune response from clotting factors (e.g., platelets), leukocyte infiltrate, differential white blood cells, and immunophenotyping (e.g., T cells, myeloid)

Table 3. In Vitro/Mechanistic PECO (Population, Exposure, Comparator, and Outcome) Statement	
PECO Element	**Evidence**
Population	Human or animal cells, tissues, or model systems with in vitro exposure regimens
Exposure	PAHs based on administered dose or concentration
Comparators	Comparable cells or tissues exposed to vehicle-only treatment or untreated controls
Outcomes	Inflammation: The following outcome measures were considered for inflammation: • Biomarkers of inflammation (e.g., cytokines, chemokines, eicosanoids, and prostanoids) • Inflammatory gene expression of fibrinogen, CRP, and IL-6 • Immune response from clotting factors (e.g., platelets), leukocyte infiltrate, differential white blood cells, and immunophenotyping (e.g., T cells, myeloid)

The overall objective, PECO statements, and strategy to synthesize study results were based on a series of problem formulation steps beginning with detailed input from scientific and clinical experts with backgrounds in toxicology, atherosclerosis, and systematic review.

An additional PECO statement (**Table 4**) was developed as an aid to identify search terms and inclusion/exclusion criteria that are appropriate to survey the literature that informs climate-change associated exposure to PAHs that could contribute to inflammation-based atherosclerosis and other cardiovascular diseases.

Table 4. Population, Exposure, Comparator, and Outcome (PECO) Statement for Climate associated CVD

PECO Element	Evidence
Population	Humans; no restrictions on age, sex, geographic location, or life stage at exposure or outcome assessment
Exposure	Wildfires, PAH exposures due to wildfires
Comparators	Comparable populations not exposed to wildfires, comparable populations exposed to a lower level of wildfires, populations before and after wildfires
Outcomes	Cardiovascular diseases and risk factors (no restrictions)

METHODS

Step 1. Problem Formulation

Nomination History

IHAB proposes to examine the evidence that exposure to PAHs contributes to inflammation that ultimately leads to atherosclerosis and to identify markers of the inflammation involved. Part of the problem formulation efforts included refinement of the research question to focus on a relevant environmental exposure. The process of identifying potential exposures for this evaluation included the development of a systematic evidence map to identify environmental exposures that could move forward for the systematic review. The rationale and process for developing the systematic evidence map is outlined in the accompanying systematic review protocol entitled, "Evidence Map of Inflammation-Based Atherosclerosis Associated with Environmental Exposures." Briefly, review of the inflammation-based atherosclerosis literature identified several relevant exposures including smoking, air pollution, metals, PAHs, and polychlorinated biphenyls (PCBs). Associations between some of the exposures identified in the systematic evidence map protocol, such as air pollution and smoking, have already been identified as risk factors for various cardiovascular diseases, including atherosclerosis (Adar *et al.* 2013, McEvoy *et al.* 2015, Shah *et al.* 2015, Hackshaw *et al.* 2018) and were not considered for this systematic review. Also, the cardiovascular effects of PCBs are the subject of other agency systematic reviews (U.S. EPA 2019). Therefore, after consultation with subject matter experts, PAHs were selected as an example exposure that would add value to the existing literature that characterizes relevant environmental exposures that may be associated with the development or exacerbation of atherosclerosis through an inflammation pathway.

This systematic review will focus on PAH compounds with unsubstituted aromatic rings (e.g., benzo[a]pyrene, chrysene, naphthalene), which are generally formed by the combustion of organic materials (Wickliffe *et al.* 2014), as well as mixtures with unsubstituted PAHs and metabolites of PAHs. Several PAHs align with the Environmental Protection Agency (EPA) and Agency for Toxic Substances and Disease Registry (ATSDR) lists of priority PAHs and are: (1) prevalent at National Priorities List hazardous waste sites, (2) primary contributors to human exposure, (3) suspected to be harmful, and (4) likely representative of other PAHs (Mumtaz *et al.* 1996). In addition, given the priority of these PAHs at EPA and ATSDR, they and similar PAHs are likely to have more research information available for evidence synthesis.

The proposed focus for this evaluation is on a single inflammation-based health effect—atherosclerosis, or the buildup of plaques on artery walls leading to restricted blood flow—among a range of health effects potentially

associated with inflammation resulting from exposure to environmental substances. The focus on a single health effect is proposed for several reasons: (1) to facilitate direct comparison of evidence supporting or opposing the role of environmental substances in promoting inflammation that leads to the health effect (in this case, atherosclerosis), (2) to identify and evaluate the evidence for specific markers of inflammation linked to the health effect, and (3) to select a health effect with a manageable database of relevant studies.

In addition to discussion with NIEHS/DTT scientists with expertise on inflammation and atherosclerosis, the Integrative Health Assessments Branch (IHAB) has solicited input from scientists at other federal agencies working on inflammation as well as atherosclerosis health effects, including scientists at EPA, the National Heart, Lung, and Blood Institute (NHLBI), and the National Institute for Occupational Safety and Health (NIOSH).

Step 2. Search of Databases and Select Studies for Inclusion

Literature Search Strategy for PAH Exposure and Inflammation-based Atherosclerosis

Search terms were developed to identify all relevant published evidence that addresses the research question on inflammation-based atherosclerosis potentially associated with environmental exposures by (1) using the search term "atherosclerosis" and related synonyms + the search terms for "inflammation" and related synonyms + general environmental exposure search terms (see **Appendix 1** for search strategy by database). A test set of relevant studies was used to ensure that the search terms retrieved 100% of the test set. The following six electronic databases were searched using a search strategy tailored for each database by an informationist on the evaluation team (details presented in **Appendix 1**). No language restrictions or publication year limits were imposed. The literature search will be updated for a final time approximately 90–120 days prior to peer review.

Databases Searched

- Cochrane Library
- EMBASE
- PubMed
- Scopus
- Toxline
- Web of Science

Literature Search Strategy for Climate Change-associated PAH Exposures and Inflammation-based Atherosclerosis

A second literature search was conducted to survey the available evidence that human exposures to PAHs may be evolving due to increased biomass burning and wildfires due to climate change that could contribute to an increased incidence of inflammation-based atherosclerosis. Three electronic databases (listed below) along with gray literature sources were searched for relevant studies using a search strategy tailored for each database by an informationist on the evaluation team. No language restrictions or publication year limits were imposed. Search details, search terms by database, and search results are provided in Appendix 2.

Databases Searched

- PubMed
- Scopus
- Web of Science

Searching Other Resources

The following methods will be used to find studies that would not be identified through the electronic database searches. Studies will be evaluated against the same inclusion and exclusion criteria as used for screening records retrieved from the electronic search. Relevant studies identified through these steps will be marked as "provided from other sources" in a study selection flow diagram. Manual searching will be conducted by:

- Reviewing the reference lists of relevant reviews or reports
- Reviewing commentaries or letters on specific studies to consider whether they contain content that meets inclusion criteria
- Searching the reference lists of all included studies after the full-text review

Per the expanded hierarchy of evidence for human studies, original papers may include non-peer-reviewed studies, for example, reports from U.S. military observational studies, as well as uncontrolled studies, case series, case reports, or social media. In all instances, the paper or social media source must document PAH exposures that relate to (1) inflammation, (2), atherosclerosis, or (3) both. *In addition, for the secondary literature search, sources must also document PAH exposures related to climate change-associated wildfires or biomass burning.*

Unpublished Data

This evaluation will only include publications that have been publicly disclosed and are available to the public so that they can be transparently reviewed and evaluated.

Screening Process

References retrieved from the literature search will be screened for relevance and eligibility using DistillerSR®, a web-based systematic review software program with structured forms and procedures to ensure standardization of the process.[1] Search results will first be consolidated in EndNote reference management software and duplicate articles will be removed prior to uploading the references into DistillerSR®.

Evidence Selection Criteria

To be eligible for inclusion, studies must comply with the type of evidence specified by the PECO statements (Table 1, Table 2, and Table 3). Inclusion and exclusion criteria based on the PECO statements are detailed in Table 4. These criteria will be used to screen articles for relevance and eligibility at both the title-and-abstract and full-text-screening stages. In addition to criteria defining the relevant PECO, Table 4 defines criteria for relevant publication types (e.g., the report must contain original data). Studies that do not meet these criteria will be excluded. Some articles may be categorized as possible supportive material if they appear inappropriate for inclusion but appear to contain relevant background information.

Table 4. Inclusion and Exclusion Criteria to Determine Study Eligibility

	Inclusion Criteria	Exclusion Criteria (or Blank if None)
Population (Human Studies or Experimental Model Systems)		
Human	No restrictions on sex, age, or life stage at exposure or outcome assessment	
Animal	No restrictions on sex, age, species, or life stage at exposure or outcome assessment	Animal observational/wildlife studies
In vitro/ mechanistic	Mechanistic studies will be restricted to human or animal cells, tissues or model systems with in vitro exposure regimens that examine the markers fibrinogen, CRP, or IL-6	Studies in non-animal organisms

[1]DistillerSR® (https://www.evidencepartners.com/products/distillersr-systematic-review-software/) is a proprietary project management tool for tracking studies through the screening process and storing data extracted from these studies using user-customized forms.

Table 4. Inclusion and Exclusion Criteria to Determine Study Eligibility		
	Inclusion Criteria	**Exclusion Criteria (or Blank if None)**
Exposure		
Human	Any PAH exposure based on: • Known dose or concentration in an experimental protocol • Diagnostic biomonitoring data (e.g., PAHs, or biomarkers in plasma or urine) • Environmental detection (e.g., air, soil) • Second-hand tobacco smoke (i.e., environmental tobacco exposure)	• Non-PAH exposures (e.g., drugs, diet, stress) • Multiple exposures that include both PAH and non-PAH exposures in which the PAH components were not measured and analyzed separately (e.g., PM2.5 where the PAH component was not measured • Smoking (primary exposure)
Animal	Any PAH exposure based on: • Known dose or concentration in an experimental protocol	• Non-PAH exposures (e.g., drugs, diet, stress) • Multiple exposures that include both PAH and non-PAH exposures in which the PAH components were not measured and analyzed separately (e.g., PM2.5 where the PAH component was not measured
In vitro/ mechanistic	Any PAH exposure based on: • Known dose or concentration in an experimental protocol	• Non-PAH exposures (e.g., drugs, diet, stress) • Multiple exposures that include both PAH and non-PAH exposures in which the PAH components were not measured and analyzed separately (e.g., PM2.5 where the PAH component was not measured)
Comparators		
Human	Humans without a PAH exposure	
Animals	Comparable untreated animal subjects or animals exposed to vehicle-only treatment	
In vitro/ mechanistic	Study must include vehicle-only control group	
Outcomes		
Human	Atherosclerosis:	

Table 4. Inclusion and Exclusion Criteria to Determine Study Eligibility		
	Inclusion Criteria	**Exclusion Criteria (or Blank if None)**
	• Mortality • Non-fatal events including acute coronary syndromes such as myocardial infarction, unstable angina pectoris, and cerebrovascular events such as stroke • Effects on direct measurement of disease severity (e.g., digital subtraction angiograms for peripheral arterial disease) • Indirect tests of disease severity (e.g., ankle-brachial pressure index) Inflammation: • Biomarkers of inflammation (e.g., cytokines, chemokines, adhesion molecules, lipoproteins) • Histopathology (e.g., blood cells)	
Animal	Atherosclerosis: • Mortality • Non-fatal events including acute coronary syndromes such as myocardial infarction, unstable angina pectoris, and cerebrovascular events such as stroke • Effects on direct measurement of disease severity (e.g., artery/aorta lesion area/volume; aortic calcification; carotid intima-media thickness, coronary artery calcium; score, brachial artery index) • Indirect tests of disease severity Inflammation: • Biomarkers of inflammation (e.g., cytokines, chemokines, adhesion molecules, lipoproteins) • Histopathology (e.g., blood cells)	
In vitro/ mechanistic	Inflammation: • Biomarkers of inflammation (e.g., cytokines, chemokines, adhesion molecules, lipoproteins) • Histopathology (e.g., blood cells)	
Publication Type (e.g., specify any language restrictions, use of conference abstracts)		
Human, animal, or vitro mechanistic	• Report must contain original data in whole or in part relevant to the aims of this evaluation • Reference to the original report must be in the public domain, i.e., rule out classified documents • May be written, video, or social media report; for source data not written, there must be a contemporaneous written description of original data by an independent medical expert, which must also include a description of assessment methodology	• Articles with no original data (e.g., editorial or review[1]) • Studies published in abstract form only (e.g., grant awards, conference abstracts) • Retracted articles

[1]Relevant reviews are used as background and for reference scanning.

Outcome measures

Based on scoping activities that identified fibrinogen, CRP, and IL-6 as key markers of inflammation in atherosclerosis-related diseases, inflammation outcome measures for animal and in vitro studies will be limited to these three markers (Libby and King 2015, Ridker 2016). For human and animal studies, standard clinical measures of mortality, non-fatal events, and both direct and indirect measures of disease severity will be included. The predictive value of outcomes will be considered further in deciding whether to downgrade evidence for indirectness when rating the confidence in the body of evidence (Figure 1).

Multiple publications of same data

If we identify multiple publications with overlapping data for the same study (e.g., publications reporting subgroups, additional outcomes, exposures outside the scope of an evaluation, or longer follow-up) by examining author affiliations, study designs, cohort name, enrollment criteria, and enrollment dates, if necessary and if possible, study authors will be contacted to clarify any uncertainty about the independence of two or more articles. In the case of multiple publications, this review will include all publications on the study, but will select one study to use as the primary study. The other references will be considered as secondary publications, annotated clearly to show the relationship to the primary record during data extraction. In general, the primary study will be the publication with the longest follow-up, or for studies with equivalent follow-up periods, the most recent study or one with the largest number of cases. Relevant original data from all publications of the study will be included, although if the same outcome is reported in more than one report, duplicate data will be excluded.

Title/Abstract Review

There will be two independent screeners for all of the three streams of human, animal, and mechanistic evidence. The screeners will be trained using project-specific written instructions that reflect the criteria outlined in Table 4. The screening process will begin with an initial pilot phase to improve clarity of the inclusion and exclusion instructions and/or to improve accuracy and consistency among screeners. If changes to the inclusion criteria are made based on the pilot phase, they will be documented in a protocol amendment along with the date any modifications are made and the logic for the changes. The trained screeners will then conduct a title-and-abstract screen of the search results, including any results of manual searches, to determine whether a reference meets the inclusion or exclusion criteria.

Studies that are not excluded based on the title and abstract will be screened through a full-text review. In case of screening conflicts, screeners will independently review their screening results to confirm the inclusion/exclusion decision and, if needed, discuss discrepancies with the other screener. If a true disagreement exists between screeners, the study will pass to the full-text review.

The same approach was used for the title/abstract screening of studies identified in the search for literature informing climate change-associated PAH exposures and human cardiovascular health effects. Because this search was intended to survey available literature rather than generate an exhaustive database of literature, studies identified as relevant at the title/abstract level did not proceed to full-text review.

Full-Text Review

After completion of the title/abstract screen, full-text articles will be retrieved for those studies that either clearly meet the inclusion criteria or where eligibility to meet the inclusion criteria is unclear. Full-text review will be independently conducted by the two screeners who participated in the title/abstract screening. Any disagreements will be resolved by discussion through consultation with members of the Systematic Review Subcommittee team and technical advisors.

Tracking study eligibility and reporting the flow of information

The main reason for exclusion at the full-text-review stage will be annotated and reported in a study selection flow diagram in the final report (using reporting practices outlined in Moher *et al.* (2009)). The following reasons for exclusion will be documented: (1) does not evaluate a PAH exposure; (2) does not contain reliable data on

inflammation-based atherosclerosis; (3) lacks original data describing inflammation-based atherosclerosis, for example, a review; or (4) is a conference abstract or other brief report lacking detailed methods and results.

Release of the list of included studies

The list of included studies will be posted on the NTP website once screening has been completed and prior to completion of the draft systematic review.

Step 3. Data Extraction

Data Extraction Process and Data Warehousing

Data extraction will be managed using the Health Assessment Workspace Collaborative (HAWC), an open-source and freely available web-based interface application.[2] The data extraction results for included studies will be visualized and made publicly available in Excel format upon publication of the final systematic review on the HAWC Project website.

The extracted data will be used to help summarize study designs and findings, facilitate assessment of risk of bias, and/or conduct statistical analyses during evidence synthesis. The number of elements or collection of information on a specific element may be revised following the identification of important study details from individual studies included in the review (Appendix 3 and Appendix 4). Data extraction will be performed by one member of the evaluation team and checked by a second member for completeness and accuracy. Any discrepancies in data extraction will be resolved by discussion or consultation with a third member of the evaluation team. Information that is inferred, converted, or estimated during data extraction will be marked as annotated, for example, using brackets [n = 10]. A member of the review team or contractors will attempt to contact authors of included studies to obtain missing data considered important for evaluating key study findings (e.g., level of data required to conduct a meta-analysis). The evaluation report will note that an attempt to contact study authors was unsuccessful if study researchers do not respond to an email or phone request within one month of the attempt to contact.

Step 4. Quality Assessment of Individual Studies

Internal validity or risk of bias will be assessed for individual studies using a tool developed by IHAB that outlines a parallel approach to evaluating risk of bias from human, animal, and mechanistic studies to facilitate consideration of risk of bias across evidence streams with common terms and categories. The risk-of-bias tool comprises a common set of 11 questions that are answered based on the specific details of individual studies to develop risk-of-bias ratings (using the four options shown in Table 5 and the 11 questions listed in Table 6) for each study. Study design determines the subset of questions that should be used to assess risk of bias for an individual study. For example, risk-of-bias questions applicable to all experimental study designs include a question on randomization of exposure that would not be applicable to observational study designs. Therefore, a similar set of questions will be used across experimental study designs (experimental animal, human uncontrolled, and human controlled studies; there are no human prospective "trials," only observational studies).

Studies will be independently assessed by two assessors who answer all applicable risk-of-bias questions with one of the four options listed in Table 5 following pre-specified criteria detailed in Appendix 4. The criteria describe aspects of study design, conduct, and reporting required to reach risk-of-bias ratings for each question and specify factors that can distinguish among ratings (e.g., what separates "definitely low" from "probably low" risk of bias). The instructions and detailed criteria will be tailored to the specific evidence stream and type of human study designs. Risk of bias will be assessed at the outcome level because study design or method specifics may increase the risk of bias for some outcomes and not others within the same study.

[2] HAWC (Health Assessment Workspace Collaborative): A Modular Web-based Interface to Facilitate Development of Human Health Assessments of Chemicals (https://hawcproject.org/portal/).

Table 5. Answers to the Risk-of-Bias Questions Result in One of Four Risk-of-Bias Ratings	
++	***Definitely Low*** risk of bias: There is direct evidence of low risk-of-bias practices.
+	***Probably Low*** risk of bias: There is indirect evidence of low risk-of-bias practices or it is deemed that deviations from low risk-of-bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias.
 NR	***Probably High*** risk of bias: There is indirect evidence of high risk-of-bias practices (indicated with "-") or there is insufficient information provided about relevant risk-of-bias practices (indicated with "NR" for not reported). Both symbols indicate probably high risk-of-bias.
--	***Definitely High*** risk of bias: There is direct evidence of high risk-of-bias practices.

Risk-of-Bias Assessment Process

Two assessors will be trained using the criteria in Appendix 5 with an initial pilot phase undertaken to improve clarity of criteria that distinguish between adjacent ratings and to improve consistency among assessors. The two assessors may also be screeners, one analyst and one independent non-expert.

All assessors involved in the risk-of-bias assessment will be trained on the same set of studies and asked to identify potential ambiguities in the criteria used to assign ratings for each question. Any ambiguities and rating conflicts will be discussed relative to opportunities to refine the criteria to distinguish between adjacent ratings more clearly. If major changes to the risk-of-bias criteria are made based on the pilot phase (i.e., those that would likely result in revision of response), they will be documented in a protocol amendment along with the date modifications were made and the logic for the changes. It is also expected that information about confounding, exposure characterization, outcome assessment, and other important issues may be identified during or after data extraction, which can lead to further refinement of the risk-of-bias criteria (Sterne *et al.* 2014).

Table 6. Questions in Risk-of-Bias Assessment and Applicability by Study Design

	Experimental Animal[1]	Human Controlled Trials[2]	Cohort	Case-Control	Cross-Sectional[3]	Case Series
1. Was administered dose or exposure level adequately randomized?	X	X				
2. Was allocation to study groups adequately concealed?	X	X				
3. Did selection of study participants result in the appropriate comparison groups?			X	X	X	
4. Did study design or analysis account for important confounding and modifying variables?			X	X	X	X
5. Were experimental conditions identical across study groups?	X	X				
6. Were research personnel blinded to the study group during the study?	X	X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	X
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X
9. Can we be confident in the outcome assessment (including blinding of outcome assessors)?	X	X	X	X	X	X
10. Were all measured outcomes reported?	X	X	X	X	X	X
11. Were there no other potential threats to internal validity?	X	X	X	X	X	X

[1]Experimental animal studies are controlled exposure studies. Non-human animal observational studies can be evaluated using the design features of observational human studies such as cross-sectional study design.

[2]Human controlled trials are studies in humans with controlled exposure (e.g., randomized controlled trials, non-randomized experimental studies)

[3]Cross-sectional studies include population surveys with individual data (e.g., National Health and Nutrition Examination Survey, NHANES) and surveys with aggregate data (i.e., ecological studies).

After assessors have independently made risk-of-bias determinations for a study across all risk-of-bias questions, the two assessors will compare their results to identify discrepancies and attempt to resolve them. Any remaining discrepancies will be considered by the project lead and, if needed, other members of the Systematic Review Subcommittee and/or technical advisors. The final risk-of-bias rating for each question will be recorded along with a statement of the basis for that rating. The risk-of-bias assessment of included studies will be part of the study summaries released in materials for the systematic review that will be released as part of the final report.

Missing Information for Risk of Bias Assessment

Staff involved with this systematic review will attempt to contact authors of included studies by email to obtain missing information considered critical for evaluating risk of bias that cannot be inferred from the study. If additional information or data are received from study authors, risk-of-bias judgments will be modified to reflect the updated study information. If reviewers do not receive a response from the authors by one month of the contact attempt, a risk-of-bias response of "NR" for "not reported; probable high risk of bias" will be used, and a note made in the data extraction files that an attempt to contact the authors was unsuccessful.

Step 5. Organization and Confidence Rating in Bodies of Evidence

The Systematic Review Subcommittee, analysts, and advisors will consider the collection of studies on inflammation-based atherosclerosis health outcomes as bodies of evidence and will develop overall confidence ratings in these bodies of evidence using a modification of the GRADE framework (Grades of Recommendation, Assessment, Development, and Evaluation). Procedures for grouping inflammation-based atherosclerosis effects, considering quantitative or narrative synthesis, and developing confidence ratings for this evaluation are described below.

Health Outcome and Endpoint Grouping

The main category for inflammation-based atherosclerosis health outcomes includes all atherosclerosis effects. After the data are collected, physiological and behavioral data will be grouped for related endpoints. Technical advisors and subject matter experts will be consulted to determine (1) endpoints that can be grouped as similar or related endpoints and (2) whether downgrades are warranted based on the reliability or quality of specific endpoints or groups of endpoints for determining health effects.

Considerations for Pursuing a Narrative or Quantitative Evidence Synthesis

Preliminary findings of the human evidence suggest that within- and between-group heterogeneity is so high that only a narrative analysis—and not a quantitative analysis or meta-analysis—is appropriate for evidence integration. Summaries of main characteristics for each included study will be compiled and reviewed by two reviewers to determine comparability between studies and to identify data transformations necessary to ensure comparability. The main characteristics considered across all eligible studies will include the following:

Human Studies

- Study design (e.g., cross-sectional, cohort; age and gender in study group and comparators)
- Details on how participants were classified into exposure groups
- Details on source of exposure data (e.g., questionnaire, area monitoring, biomonitoring)
- Health outcome(s) reported, whether self-reported or evaluated using independent physiological, functional, or cognitive tests
- Subset of health outcomes reported using independent tests of post-exposure pathology, whether or not clinically observable
- Conditioning variables in the analysis (e.g., variables considered confounders)
- Type of data (e.g., continuous or dichotomous), statistics presented in paper, access to raw data
- Variation in degree of risk of bias at individual study level

Animal Studies

- Experimental design (e.g., acute, chronic)
- Animal model used (e.g., species, strain, sex, genetic background)
- Age of animals (e.g., at start of treatment, mating, and/or pregnancy status)
- Developmental stage of animals at treatment and outcome assessment
- Dose levels, frequency of treatment, timing, duration, and exposure route
- Health outcome(s) reported
- Type of data (e.g., continuous or dichotomous), statistics presented in paper, access to raw data
- Variation in degree of risk of bias at individual study level

More detailed guidance on evaluating heterogeneity, transforming or normalizing data to ensure comparability, and the process for determining whether a meta-analysis will be pursued is provided in the OHAT *Handbook for Conducting a Literature-based Health Assessment* (http://ntp.niehs.nih.gov/go/38673, see Step 5). In addition to screening and assessment by at least two independent personnel, we expect to solicit input from topic-specific experts to help assess whether studies are too heterogeneous for meta-analysis. Situations where including a study may not be appropriate are those in which (1) data on exposure or outcome are too different to be combined, (2) concerns about high risk of bias exist, or (3) other circumstances may indicate that averaging study results would not produce meaningful results.

Stratified Analyses, Meta-Regression, and Publication Bias

If there is significant study-level heterogeneity, we may conduct stratified analyses or multivariate meta-regression to assess how much heterogeneity can be explained by taking into account both within- and between-study variance (Vesterinen *et al.* 2014). Multivariate meta-regression approaches are especially useful for assessing the significance of associations in between-study design characteristics. These approaches are considered most suitable if there are at least 6 to 10 studies for a continuous variable and at least 4 studies for a categorical variable.

If there are sufficient studies to conduct a meta-analysis, we will assess potential publication bias by developing funnels and performing Egger regression on the estimates of effect size. In addition, if these methods suggest that publication bias is present, we will use trim-and-fill methods to predict the effect of the hypothetical "missing" studies (Vesterinen *et al.* 2014).

Confidence Rating: Assessment of Body of Evidence

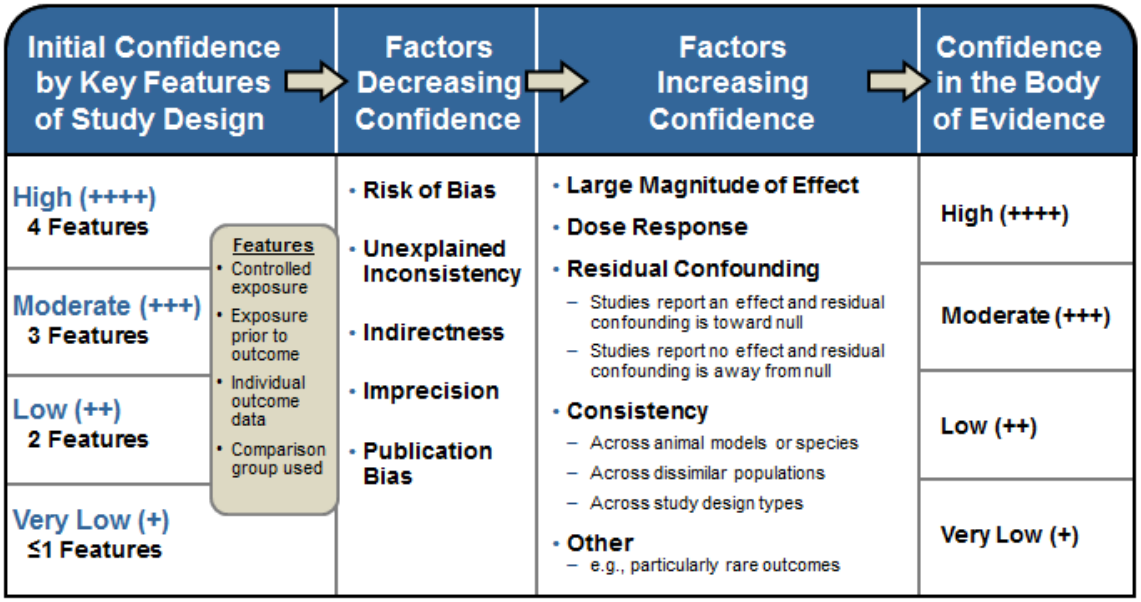
The certainty of evidence within groups of human studies will be graded using the GRADE system for rating the confidence in the body of evidence (Guyatt *et al.* 2011) as used by IHAB (Rooney *et al.* 2014). More detailed guidance on reaching confidence ratings in the body of evidence as "high," "moderate," "low," or "very low" is provided in the OHAT *Handbook for Conducting a Literature-based Health Assessment* (http://ntp.niehs.nih.gov/go/38673, see Step 5) (NTP 2019).

In brief, available studies on a particular outcome will be initially grouped by key study design features, and each grouping of studies will be given an initial confidence rating by those features. This initial rating (column 1 of Figure 1) will be downgraded for factors that decrease confidence in the results (column 2 of Figure 1 [risk-of-bias, unexplained inconsistency, indirectness or lack of applicability, imprecision, and publication bias]) and upgraded for factors that increase confidence in the results (column 3 of Figure 1 [large magnitude of effect, dose response, consistency across study designs/populations/animal models or species, consideration of residual confounding, and other factors that increase our confidence in the association or effect]).

The reasons for downgrading (or upgrading) confidence may not be due to a single domain of the body of evidence. If a decision to downgrade is borderline for two domains, the body of evidence will be downgraded once in a single domain to account for both partial concerns based on considering the key drivers of the strengths or weaknesses. Similarly, the body of evidence will not be downgraded twice for what is essentially the same

limitation (or upgraded twice for the same asset) that could be considered applicable to more than one domain of the body of evidence. Consideration of consistency across study designs, human populations, or animal species is not included in the GRADE guidance (Guyatt *et al.* 2011); however, it will be considered here in the modified version of GRADE used by IHAB (Rooney *et al.* 2014).

Figure 1. Assessing Confidence in the Body of Evidence



Confidence ratings will be independently assessed by the analyst-contractors, and the Systematic Review Subcommittee. Any discrepancies will be resolved by consensus and in consultation with technical advisors as needed. Confidence ratings will be summarized in evidence profile tables (see Table 7 for the general format).

Relevance of Animal Models to Inflammation-Based Atherosclerosis and Human Health

- *Rats, mice, and other mammalian model systems:* Noting differences in human and animal metrics to observe symptoms and test for effects, we will consider several alternatives to extract physiological data from animal studies so that, to the extent possible, animal data can be integrated with human data into a single evidence stream. Most animal models of atherosclerosis rely on diet or genetic modification and not just environmental exposures (Kapourchali *et al.* 2014). In addition, these animal models may use methods of exposure that, while experimentally pragmatic (e.g., intratracheal instillations), may be more limited to reflect physiologically relevant exposures (Araujo 2011). For these reasons, any studies meeting inclusion criteria conducted in non-human mammals will be downgraded one level for indirectness.
- *Birds, reptiles, amphibians, fish, and other non-mammalian vertebrate model systems:* Most cell types are relatively consistent across vertebrate systems. However, use of these model systems to address human health is not as well established as use of the mammalian model systems (WHO 2012). For this reason, any studies meeting inclusion criteria conducted in non-mammalian vertebrates will be downgraded one level for indirectness.

Inflammation-based Atherosclerosis

For the evaluation of inflammation-based atherosclerosis health effects, all outcomes and effects related to atherosclerosis at the individual and population level will be considered.

Environmental Exposure

- *Human studies:* All exposure levels and scenarios encountered in the human studies (e.g., general population, occupational settings) will be considered direct and not downgraded. As noted above, a key inclusion criterion is that exposure at any level must be documented.
- *Dose levels used in animal studies:* There will be no downgrade for dose level used in experimental animal studies. We recognize that the level of dose or exposure is an important factor when considering the relevance of animal findings to human health. In addition, in IHAB's process, the relevance of the dose or exposure level occurs after hazard identification as part of reaching a "level of concern" conclusion.
- *Route of administration in animal studies:* All of the most commonly used routes of administration including inhalation, oral and dermal routes will be considered direct for the purposes of establishing confidence ratings. Other routes (intraperitoneal, subcutaneous) will be considered but not prioritized. We recognize that some of these exposure routes may be relevant only for certain human subpopulations. However, for this review, this consideration occurs after identifying inflammation-based atherosclerosis as part of reaching a "level-of-concern" conclusion.

In Vitro/Mechanistic Studies

- As noted by IHAB (Rooney *et al.* 2014), the framework described above to develop confidence ratings applies only to human and animal studies. Although there is no analogous model to develop confidence ratings for other relevant data such as outcomes from in vitro, mechanistic, cellular, or genomic studies, we will group our findings as to "established" and "emerging." The proposed approach to consider other relevant data, including in vitro studies, is described separately in a later section of this document in Step 7 (see "Consideration of Mechanistic Data").

Table 7. Evidence Profile Table Format

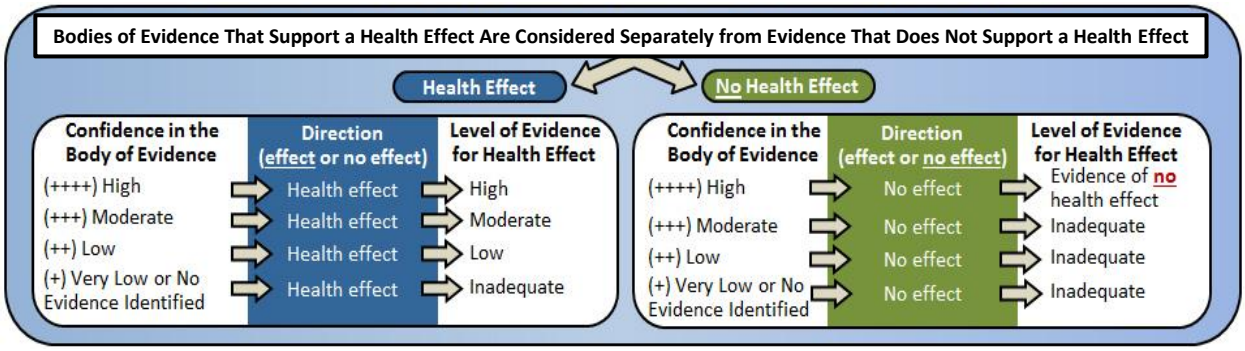
Example of the type of information that will be in an evidence profile for atherosclerosis health outcomes

Body of Evidence	Risk of Bias	Unexplained Inconsistency	Indirectness	Imprecision	Publication Bias	Magnitude	Dose Response	Residual Confounding	Consistency Across Species/ Model	FINAL RATING
Evidence stream (human or animal)	Serious or not serious	Serious or not serious	Serious or not serious	Serious or not serious	Detected or undetected	Large or not large	Yes or no	Yes or no	Yes or no	**Final rating**
(# studies) initial rating	Describe trend Describe key questions Describe issues	Describe results in terms of consistency Explain apparent inconsistency (if it can be explained)	Discuss use of upstream indicators or populations with less relevance	Discuss ability to distinguish treatment from control Describe confidence intervals	Discuss factors that might indicate publication bias (e.g., funding, lag)	Describe magnitude of response	Outline evidence for or against dose response	Address whether there is evidence that confounding would bias toward null	Describe cross-species, model, or population consistency	**High, Moderate, or Low**

Step 6. Preparation of Draft Level-of-Evidence Statement

The confidence ratings will be translated into draft level of evidence of health effects for each type of health outcome separately, according to one of four statements: (1) high; (2) moderate; (3) low; or (4) inadequate (Figure 2). The descriptor "evidence of no health effect" will be used to indicate confidence that the substance is not associated with a health effect. Because of the inherent difficulty in proving a negative, the conclusion "evidence of no health effect" will be reached only when there is high confidence in the body of evidence.

Figure 2. Translation of Confidence Ratings into Evidence of Health Effect Conclusions



Evidence Descriptors	Definition
High Level of Evidence	There is high confidence in the body of evidence for an association between environmental exposures and the health outcome(s)
Moderate Level of Evidence	There is moderate confidence in the body of evidence for an association between environmental exposures and the health outcome(s)
Low Level of Evidence	There is low confidence in the body of evidence for an association between exposure to environmental exposures and the health outcome(s), or no data are available
Inadequate Evidence	There is insufficient evidence available to assess if environmental exposures are associated with the health outcome(s)
Evidence of No Health Effect	There is high confidence in the body of evidence that environmental exposures are not associated with the health outcome(s)

Step 7. Integration of Evidence to Develop Conclusions of Inflammation-Based Atherosclerosis Health Effects

Finally, the level-of-evidence ratings for human and animal data will be integrated with consideration of in vitro/mechanistic data to reach one of five possible categories of evidence of inflammation-based atherosclerosis health effect: (1) known, (2) presumed, (3) suspected, (4) not classifiable, or (5) not identified to be an inflammation-based atherosclerosis effect in humans (Figure 3).

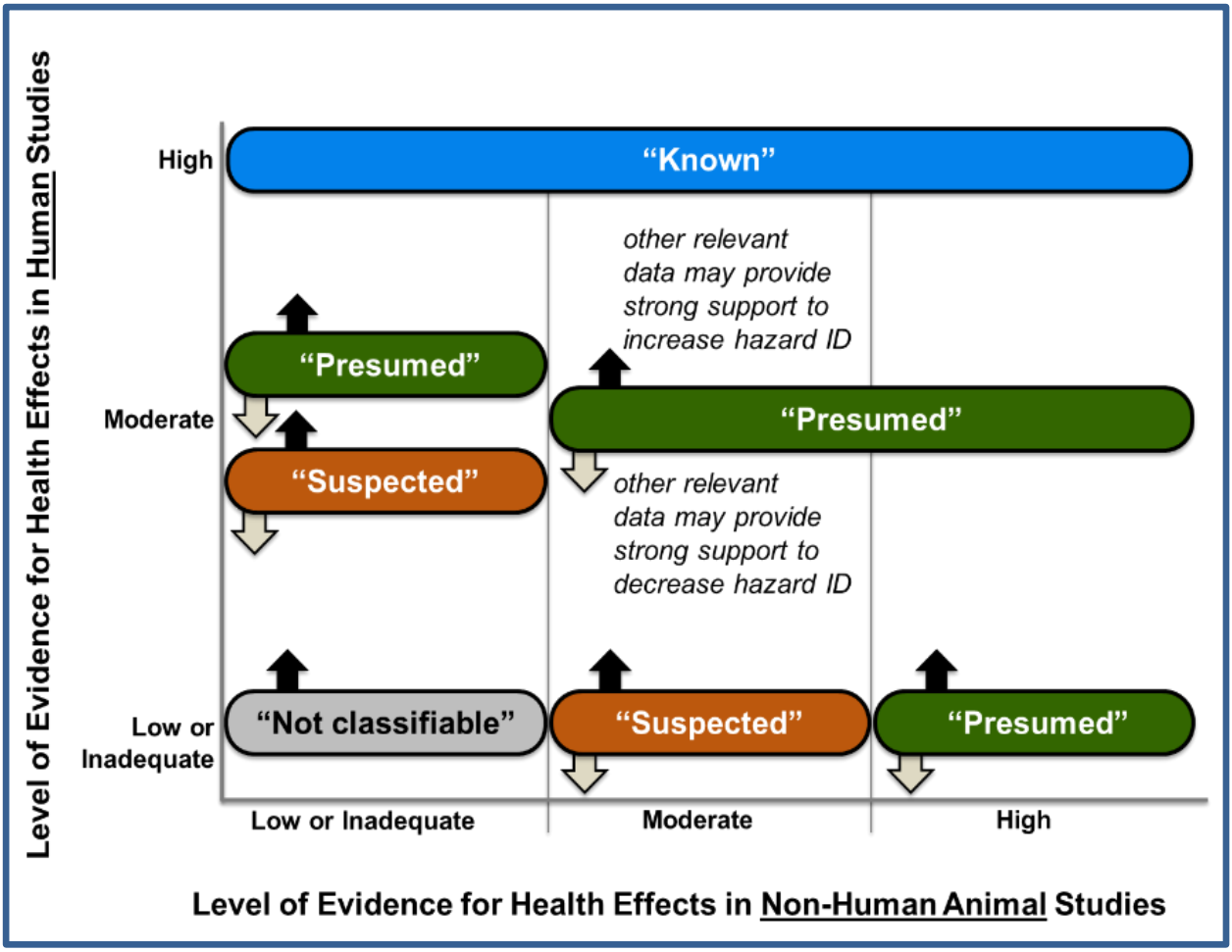
Consideration of Human and Animal Data

Initial hazard identification conclusions will be reached by integrating the highest level-of-evidence conclusion for inflammation-based atherosclerosis health effect(s) on an outcome basis for the human and animal evidence streams. Hazard identification conclusions may be reached on groups of biologically related outcomes or functionally related outcomes, as well as more specific endpoints if data are available to make more specific conclusions. If the data support an inflammation-based atherosclerosis effect, the level-of-evidence conclusion for human data from Step 6 for that health outcome will be considered together with the level of evidence for animal data to reach one of four initial hazard identification conclusions as to the evidence of inflammation-based

atherosclerosis effects in humans: known, presumed, suspected, or not classifiable. If either the human or animal evidence stream is characterized as "inadequate evidence," conclusions will be based on the remaining evidence stream alone (which is equivalent to treating the missing evidence stream as "low" in Figure 3).

If the human level-of-evidence rating of "evidence of no health effect" from Step 6 is supported by a similar level-of-evidence rating for animal evidence for no health effect, the hazard identification conclusion would be "not identified to be an inflammation-based atherosclerosis effect observed in humans."

Figure 3. Hazard Identification Scheme for Inflammation-based Atherosclerosis Effects



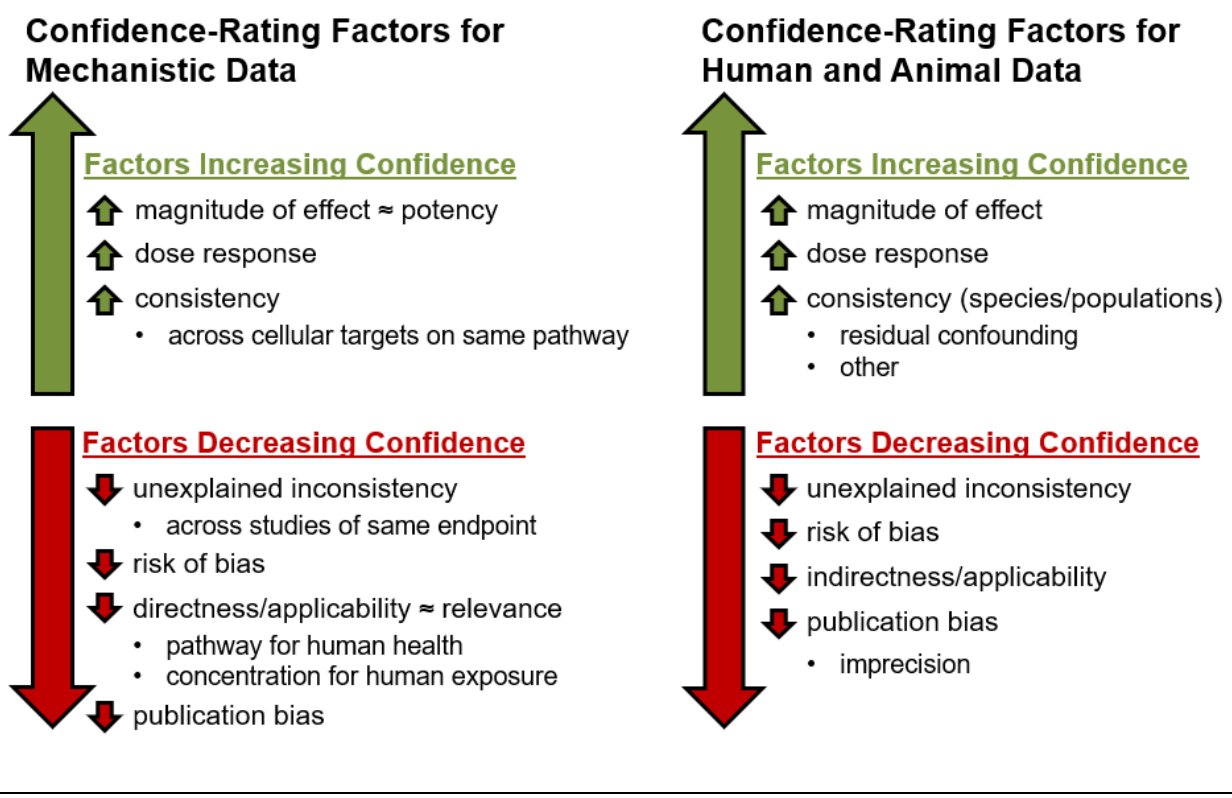
Consideration of Mechanistic Data

There is no requirement to consider mechanistic or mode-of-action data to reach a hazard identification conclusion regarding inflammation-based atherosclerosis health effects. However, when available, this and other relevant supporting types of evidence may be used to raise (or lower) the category of the hazard identification conclusion. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes in vitro and in vivo laboratory studies directed at cellular, biochemical, genetic, and molecular mechanisms that explain how a chemical produces particular adverse effects.

For the evaluation of inflammation-based atherosclerosis associated with PAHs, we are interested in mechanistic or in vitro measures that may support the biological plausibility of corresponding health outcomes reported from in vivo studies in animals or humans.

The strength of the support or opposition presented by the other relevant data is evaluated using the guidance presented in Figure 4. The factors outlined for increasing or decreasing confidence in that the mechanistic data support biological plausibility are conceptually similar to those used to rate confidence in bodies of evidence for human or animal in vivo studies. Evaluations of the strength of evidence provided by mechanistic data are made on an outcome-specific basis based on discussion by the evaluation team including the Systematic Review Subcommittee, and in consultation with technical advisors as needed.

Figure 4. Factors Considered in Evaluating the Support for Biological Plausibility



If mechanistic data provide strong support for biological plausibility of the relationship between exposure and the health effect, the hazard identification conclusion may be upgraded (indicated by black "up" arrows in Figure 3) from that initially derived by considering the human and non-human animal evidence together.

If mechanistic data provide strong opposition for biological plausibility of the relationship between exposure and the health effect, the hazard identification conclusion may be downgraded (indicated by gray "down" arrows in Figure 3) from that initially derived by considering the human and non-human animal evidence together.

SYSTEMATIC REVIEW: OUTLINE

This systematic review of the association between PAHs and inflammation-based atherosclerosis will include the following information:

Protocol for Systematic Review: Inflammation-based Atherosclerosis Associated with Polycyclic Aromatic Hydrocarbon Exposures

Introduction

This section will provide a brief background on the topic.

Methodology

This section will provide a brief overview of the methodologies used in the review process, including the:

- Research question
- Search strategy used to identify and retrieve studies
- Process for selecting studies
- Methods of data extraction
- Methods used to assess risk of bias of included studies
- Methods used to synthesize the data of included studies
- Methods used to evaluate confidence in the body of evidence
- Methods used to reach hazard identification conclusions for evidence of health effects

Results

This section will include the results from the systematic review of the evidence of inflammation-based atherosclerosis associated with environmental exposures. Results will be presented in tables or figures if possible. The results from the included studies will be discussed by outcome. This will include a description of:

- The number of studies identified that examined inflammation-based atherosclerosis health effects
- A summary of the results and risk-of-bias assessment for each included study (including files in downloadable format)
- Description of results and ratings for confidence in the bodies of evidence for inflammation-based atherosclerosis where there are data linked to environmental exposure using GRADE as performed by IHAB
- Evidence profiles for inflammation-based atherosclerosis where there are data linked to environmental exposures
- Presentation of level of evidence and draft hazard identification conclusions for inflammation-based atherosclerosis where there are data linked to environmental exposures

Discussion

The discussion will provide a summary of the review findings, including a discussion of any gaps identified in the evidence and any suggestions of areas for further research. Any important limitations of the review will be described and their impact on the available evidence will be discussed.

Conclusion

This will present the conclusion of the review.

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ABOUT THE PROTOCOL

Contributors

Systematic Review Subcommittee Team

The Systematic Review Subcommittee teams will be composed of federal staff and contractor staff. Federal staff members will do a self-evaluation for conflict of interest. Epidemiologists and toxicologists on this evaluation team should have at least three years of experience and/or training in reviewing studies, including summarizing studies and critical review (e.g., assessing study quality and interpreting findings). Experience in evaluating toxicological or clinical studies involving inflammation and/or atherosclerosis will be preferred. Team members should have at least a master's degree or equivalent in epidemiology, toxicology, translational development of novel compounds, or a related field.

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Contract Support

Contractors listed below are anticipated to provide support necessary to complete the literature searches, study selection, data extraction, and risk-of-bias assessment.

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Subject Matter Experts

Subject matter experts were consulted for the initial development of the concept and identification of key biomarkers as part of the problem formulation efforts. Subject matter experts were selected for their experience with inflammation-based atherosclerosis and/or PAHs and systematic review procedures. Service as a subject matter expert does not necessarily indicate that an advisor has read the entire protocol or endorses the final document.

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Sources of Support

[None]

Protocol History and Revisions

Date	Activity or Revision
August 23, 2019	Draft date
June 16, 2023	Internal review and revision (editorial)
July 7, 2023	Posting

APPENDICES

Appendix 1. Systematic Review Literature Search Strategy

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
Cochrane Library	(((artery OR renal heart OR angina OR arrhythmia OR arrhythmias) AND plaque) OR Arteriosclerosis OR ((arterial OR carotid OR coronary) AND plaque) OR Atherogenesis OR atheroma OR atheromas OR Atherosclerosis OR Atherosclerotic OR fatty-streak OR fatty-streaks OR fibroatheroma OR fibroatheromas OR foam-cell OR foam-cells OR Peripheral-Arterial-Disease OR Peripheral-Arterial-Diseases OR Peripheral-Artery-Disease OR Peripheral-Artery-Diseases OR proatherogen* OR pro-atherogen*)
Embase	(('arteries':ti,ab OR 'artery':ti,ab OR 'arterial':ti,ab OR 'carotid':ti,ab OR 'coronary':ti,ab OR 'heart':ti,ab OR 'peripheral':ti,ab OR 'renal':ti,ab OR 'stenosis':ti,ab) AND 'plaque*':ti,ab) OR 'Atherogenesis':ti,ab OR 'atheroma*':ti,ab OR 'Atherosclerosis':ti,ab OR 'Atherosclerotic-plaque*':ti,ab OR 'fatty-streak*':ti,ab OR 'fibroatheroma*':ti,ab OR 'foam-cell*':ti,ab OR 'Peripheral-Arterial-Disease*':ti,ab OR 'Peripheral-Artery-Disease*':ti,ab OR 'proatherogen*':ti,ab OR 'pro-atherogen*':ti,ab OR 'Atherogenesis'/exp OR 'atheroma'/exp OR 'atheromatosis'/exp OR 'Atherosclerosis'/exp OR 'Atherosclerotic-plaque'/exp OR 'fatty-streak'/exp OR 'fibroatheroma'/exp OR 'foam-cell'/exp OR 'peripheral-arterial-disease'/exp) AND ('aromatic-hydrocarbon'/exp OR 'polycyclic-aromatic-hydrocarbon'/exp OR 'polycyclic-aromatic-hydrocarbons'/exp OR 'polycyclic-aromatic-compound'/exp OR 'xylene'/exp OR 'Anthracene'/exp OR 'Azulene'/exp OR 'Fluorene'/exp OR 'Phenanthrene'/exp OR 'Pyrene'/exp OR 'acenaphthene'/exp OR 'acenaphthylene'/exp OR 'chrysene'/exp OR 'coronene'/exp OR 'fluoranthene'/exp OR 'perylene'/exp OR "3,4-benzopyrene":ti,ab OR "benzo(a)pyrene":ti,ab OR "benzo-a-pyrene":ti,ab OR 'aromatic-hydrocarbons':ti,ab OR 'PAHs':ti,ab OR 'polycyclic-aromatic-hydrocarbons':ti,ab OR 'xylene':ti,ab OR "Benz(a)Anthracene":ti,ab OR "Benz(a)Anthracenes":ti,ab OR 'Anthracene':ti,ab OR 'Anthracenes':ti,ab OR 'Azulene':ti,ab OR 'Azulenes':ti,ab OR 'Benzocycloheptene':ti,ab OR 'Benzocycloheptenes':ti,ab OR 'Fluorene':ti,ab OR 'Fluorenes':ti,ab OR 'Indene':ti,ab OR 'Indenes':ti,ab OR 'Naphthacene':ti,ab OR 'Naphthacenes':ti,ab OR 'Naphthalene':ti,ab OR 'Naphthalenes':ti,ab OR 'Phenalene':ti,ab OR 'Phenalenes':ti,ab OR 'Phenanthrene':ti,ab OR 'Phenanthrenes':ti,ab OR 'Pyrene':ti,ab OR 'Pyrenes':ti,ab OR "1,10-(o-Phenylene)pyrene":ti,ab OR "1,2:3,4-Dibenzopyrene":ti,ab OR "1,2:5,6-Dibenzanthracene":ti,ab OR "1,2:5,6-Dibenzoanthracene":ti,ab OR "1,2-Benz(a)anthracene":ti,ab OR "1,2-Benzanthracene":ti,ab OR "1,2-Benzoanthracene":ti,ab OR "1,2-Benzpyrene":ti,ab OR "3,4:9,10-Dibenzopyrene":ti,ab OR "3,4-Benz(a)pyrene":ti,ab OR "3,4-Benzo(a)pyrene":ti,ab OR "3,4-Benzofluoranthene":ti,ab OR "3,4-Benzpyrene":ti,ab OR "6,7-Benzopyrene":ti,ab OR "7H-Dibenzo(c,g)carbazole":ti,ab OR "B(a)P":ti,ab OR "Benz(a)pyrene":ti,ab OR "Benz(e)acephenanthrylene":ti,ab OR "Benz(j)fluoranthene":ti,ab OR "Benzo(a)anthracene":ti,ab OR "Benzo(b)fluoranthene":ti,ab OR "Benzo(d,e,f)chrysene":ti,ab OR "Benzo(j)fluoranthene":ti,ab OR "Benzo(k)fluoranthene":ti,ab OR "Benzo(rst)pentaphene":ti,ab OR "Dibenz(a,h)anthracene":ti,ab OR "Dibenz(a,h)antracene":ti,ab OR "Dibenz(a,i)pyrene":ti,ab OR "Dibenz(a,j)acridine":ti,ab OR "Dibenzo(a,e)pyrene":ti,ab OR "Dibenzo(a,h)anthracene":ti,ab OR

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
	"Dibenzo(a,h)pyrene":ti,ab OR "Dibenzo(a,i)pyrene":ti,ab OR "Dibenzo(a,l)pyrene":ti,ab OR "Dibenzo(b,def)chrysene":ti,ab OR "Dibenzo(d,e,f,p)chrysene":ti,ab OR "Dibenzo(def,p)chrysene":ti,ab OR "Indeno(1,2,3-cd)pyrene":ti,ab OR '5-Methylchrysene':ti,ab OR 'Benzanthracene':ti,ab OR 'Benzanthrene':ti,ab OR 'Benzoanthracene':ti,ab OR 'Indenopyrene':ti,ab OR 'Naphthanthracene':ti,ab OR 'Tetraphene':ti,ab OR '3-methylcholanthrene':ti,ab OR 'acenaphthene':ti,ab OR 'acenaphthylene':ti,ab OR 'albocarbon':ti,ab OR 'anthracin':ti,ab OR 'benzacenaphthene':ti,ab OR 'benzfluoranthene':ti,ab OR 'benzindene':ti,ab OR 'benzofluoranthene':ti,ab OR 'benzoperylene':ti,ab OR 'benzophenanthrene':ti,ab OR 'benzopyrene':ti,ab OR 'benzperylene':ti,ab OR 'benzphenanthrene':ti,ab OR 'benzpyrene':ti,ab OR 'beta-pyrene':ti,ab OR 'beta-pyrine':ti,ab OR 'bibenzene':ti,ab OR 'binaphthylene':ti,ab OR 'biphenyl':ti,ab OR 'biphenylenemethane':ti,ab OR 'biphenyleneoxide':ti,ab OR 'biphenylylene-sulfide':ti,ab OR 'butylanthracene':ti,ab OR 'butylchrysene':ti,ab OR 'butyldibenzothiophene':ti,ab OR 'butylnaphthalene':ti,ab OR 'butylphenanthrene':ti,ab OR 'chrysene':ti,ab OR 'coronene':ti,ab OR 'dezodorator':ti,ab OR 'dibenzanthracene':ti,ab OR 'dibenzoanthracene':ti,ab OR 'dibenzofuran':ti,ab OR 'dibenzonaphthalene':ti,ab OR 'dibenzopyrene':ti,ab OR 'dibenzothiophene':ti,ab OR 'dihydroacenaphthalene':ti,ab OR 'dimethylanthracene':ti,ab OR 'dimethylbenzaanthracene':ti,ab OR 'dimethylchrysene':ti,ab OR 'dimethyldibenzothiophene':ti,ab OR 'dimethylfluoranthene':ti,ab OR 'dimethylfluorene':ti,ab OR 'dimethylnaphthalene':ti,ab OR 'dimethylphenanthrene':ti,ab OR 'dimethylpyrene':ti,ab OR 'diphenyl':ti,ab OR 'diphenylenemethane':ti,ab OR 'diphenyleneoxide':ti,ab OR 'Diphenylene-sulfide':ti,ab OR 'ethylanthracene':ti,ab OR 'ethylchrysene':ti,ab OR 'ethyldibenzothiophene':ti,ab OR 'ethylenenaphthalene':ti,ab OR 'ethylfluoranthene':ti,ab OR 'ethylfluorene':ti,ab OR 'ethylnaphthalene':ti,ab OR 'ethylphenanthrene':ti,ab OR 'ethylpyrene':ti,ab OR 'fluoranthene':ti,ab OR 'idryl':ti,ab OR 'methylanthracene':ti,ab OR 'methylchrysene':ti,ab OR 'methyldibenzothiophene':ti,ab OR 'methylenebiphenyl':ti,ab OR 'methylethylnaphthalene':ti,ab OR 'methylfluoranthene':ti,ab OR 'methylfluorene':ti,ab OR 'methylnaphthalene':ti,ab OR 'methylphenanthrene':ti,ab OR 'methylpyrene':ti,ab OR 'monomethylanthracene':ti,ab OR 'monomethylchrysene':ti,ab OR 'monomethyldibenzothiophene':ti,ab OR 'monomethylfluoranthene':ti,ab OR 'monomethylfluorene':ti,ab OR 'monomethylnaphthalene':ti,ab OR 'monomethylphenanthrene':ti,ab OR 'monomethylpyrene':ti,ab OR 'naphtalinum':ti,ab OR 'naphthaline':ti,ab OR 'naphthalinum':ti,ab OR 'naphthyleneethylene':ti,ab OR 'paranaphthalene':ti,ab OR 'periethylenenaphthalene':ti,ab OR 'perylene':ti,ab OR 'phenantrin':ti,ab OR 'phenylbenzene':ti,ab OR 'phenylenepyrene':ti,ab OR 'polycyclic-aromatic-compound':ti,ab OR 'polycyclic-aromatic-hydrocarbon':ti,ab OR 'propylanthracene':ti,ab OR 'propylchrysene':ti,ab OR 'propylfluorene':ti,ab OR 'propylnaphthalene':ti,ab OR 'propylphenanthrene':ti,ab OR 'propylpyrene':ti,ab OR 'tetramethylanthracene':ti,ab OR 'tetramethyldibenzothiophene':ti,ab OR 'tetramethylnaphthalene':ti,ab OR 'tetramethylphenanthrene':ti,ab OR 'tetra-olive-N2G':ti,ab OR 'tetrosin-LY':ti,ab OR 'thiafluorene':ti,ab OR 'trimethylanthracene':ti,ab OR 'trimethylchrysene':ti,ab OR 'trimethyldibenzothiophene':ti,ab OR 'trimethylfluoranthene':ti,ab OR 'trimethylfluorene':ti,ab OR 'trimethylnaphthalene':ti,ab OR 'trimethylphenanthrene':ti,ab OR 'trimethylpyrene':ti,ab OR 'xenene':ti,ab OR "Methylbenz(a)anthracene":ti,ab OR "Naphtho(2,3)pyrene":ti,ab OR 'Acenaphthenone':ti,ab OR 'Acenaphthenequinone':ti,ab OR 'Methylanthraquinone':ti,ab OR "1,4-Anthraquinone":ti,ab OR "9,10-Anthraquinone":ti,ab OR "1,4-Benzoquinone":ti,ab OR "9-Fluorenone":ti,ab OR 'Naphthacenequinone':ti,ab OR "1,2-Naphthoquinone":ti,ab OR "1,4-

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
	Naphthoquinone":ti,ab OR 'Perinaphthenone':ti,ab OR "9,10-Phenanthrenequinone":ti,ab OR 'Xanthone':ti,ab OR 'Antracene':ti,ab OR 'Anthanthrene':ti,ab OR "Benzo[c]fluorene":ti,ab OR "Dibenzo[a,e]fluoranthene":ti,ab OR "Cyclopenta(cd)pyrene":ti,ab OR "Cyclopenta(D,E,F)chrysene":ti,ab OR "Naphtho[2,3-E]pyrene":ti,ab OR "Benz[j]aceanthrylene":ti,ab OR 'Dinitropyrene':ti,ab OR 'Nitrochrysene':ti,ab OR "Benzo(j,k)fluorene":ti,ab OR "1-Nitropyrene":ti,ab OR "Benzo(c)fluorene":ti,ab OR "Dibenzo(a,e)fluoranthene":ti,ab OR "Naphtho(2,3-E)pyrene":ti,ab OR "Benz(j)aceanthrylene":ti,ab OR “o-Phenylenepyrene”:ti,ab OR 189-55-9:rn OR 189-64-0:rn OR 191-30-0:rn OR 192-65-4:rn OR 193-39-5:rn OR 194-59-2:rn OR 205-82-3:rn OR 205-99-2:rn OR 207-08-9:rn OR 224-42-0:rn OR 226-36-8:rn OR 3697-24-3:rn OR 50-32-8:rn OR 53-70-3:rn OR 56-55-3:rn OR 120-12-7:rn OR 129-00-0:rn OR 132-64-9:rn OR 132-65-0:rn OR 191-24-2:rn OR 192-97-2:rn OR 206-44-0:rn OR 208-96-8:rn OR 218-01-9:rn OR 3-methylcholanthrene:rn OR 83-32-9:rn OR 85-01-8:rn OR 86-73-7:rn OR 91-20-3:rn OR 92-52-4:rn)
PubMed/MEDLINE	(((arteries[tiab] OR artery[tiab] OR arterial[tiab] OR carotid[tiab] OR coronary[tiab] OR heart[tiab] OR peripheral[tiab] OR renal[tiab] OR stenosis[tiab]) AND plaque*[tiab]) OR Arteriosclerosis[Mesh:NoExp] OR Atherogenesis[tiab] OR atheroma*[tiab] OR Atherosclerоses[tiab] OR Atherosclerosis[mh] OR Atherosclerosis[tiab] OR Atherosclerotic-plaque*[tiab] OR fatty-streak*[tiab] OR fibroatheroma*[tiab] OR foam-cell*[tiab] OR Peripheral-Arterial-Disease*[tiab] OR Peripheral-Artery-Disease*[tiab] OR plaque,-atherosclerotic[mh] OR proatherogen*[tiab] OR pro-atherogen*[tiab]) AND ("3,4-benzopyrene"[tiab] OR "benzo(a)pyrene"[mh] OR "benzo(a)pyrene"[tiab] OR "benzo-a-pyrene"[tiab] OR aromatic-hydrocarbons[tiab] OR hydrocarbons,-aromatic[mh:noexp] OR PAHs[tiab] OR polycyclic-aromatic-hydrocarbons[tiab] OR Polycyclic-hydrocarbons,-aromatic[mh:noexp] OR xylene[tiab] OR xylenes[mh] OR "Benz(a)Anthracene"[tiab] OR "Benz(a)Anthracenes"[tiab] OR Anthracene[tiab] OR Anthracenes[tiab] OR Azulene[tiab] OR Azulenes[tiab] OR Benzocycloheptene[tiab] OR Benzocycloheptenes[tiab] OR Fluorene[tiab] OR Fluorenes[tiab] OR Indene[tiab] OR Indenes[tiab] OR Naphthacene[tiab] OR Naphthacenes[tiab] OR Naphthalene[tiab] OR Naphthalenes[tiab] OR Phenalene[tiab] OR Phenalenes[tiab] OR Phenanthrene[tiab] OR Phenanthrenes[tiab] OR Pyrene[tiab] OR Pyrenes[tiab] OR "1,10-(o-Phenylene)pyrene"[tiab] OR "1,2:3,4-Dibenzopyrene"[tiab] OR "1,2:5,6-Dibenzanthracene"[tiab] OR "1,2:5,6-Dibenzoanthracene"[tiab] OR "1,2-Benz(a)anthracene"[tiab] OR "1,2-Benzanthracene"[tiab] OR "1,2-Benzoanthracene"[tiab] OR "1,2-Benzpyrene"[tiab] OR "3,4:9,10-Dibenzopyrene"[tiab] OR "3,4-Benz(a)pyrene"[tiab] OR "3,4-Benzo(a)pyrene"[tiab] OR "3,4-Benzofluoranthene"[tiab] OR "3,4-Benzpyrene"[tiab] OR "6,7-Benzopyrene"[tiab] OR "7H-Dibenzo(c,g)carbazole"[tiab] OR "B(a)P"[tiab] OR "Benz(a)pyrene"[tiab] OR "Benz(e)acephenanthrylene"[tiab] OR "Benz(j)fluoranthene"[tiab] OR "Benzo(a)anthracene"[tiab] OR "Benzo(b)fluoranthene"[tiab] OR "Benzo(d,e,f)chrysene"[tiab] OR "Benzo(j)fluoranthene"[tiab] OR "Benzo(k)fluoranthene"[tiab] OR "Benzo(rst)pentaphene"[tiab] OR "Dibenz(a,h)anthracene"[tiab] OR "Dibenz(a,h)antracene"[tiab] OR "Dibenz(a,i)pyrene"[tiab] OR "Dibenz(a,j)acridine"[tiab] OR "Dibenzo(a,e)pyrene"[tiab] OR "Dibenzo(a,h)anthracene"[tiab] OR "Dibenzo(a,h)pyrene"[tiab] OR "Dibenzo(a,i)pyrene"[tiab] OR "Dibenzo(a,l)pyrene"[tiab] OR "Dibenzo(b,def)chrysene"[tiab] OR "Dibenzo(d,e,f,p)chrysene"[tiab] OR

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
	"Dibenzo(def,p)chrysene"[tiab] OR "Indeno(1,2,3-cd)pyrene"[tiab] OR 189-55-9[rn] OR 189-64-0[rn] OR 191-30-0[rn] OR 192-65-4[rn] OR 193-39-5[rn] OR 194-59-2[rn] OR 205-82-3[rn] OR 205-99-2[rn] OR 207-08-9[rn] OR 224-42-0[rn] OR 226-36-8[rn] OR 3697-24-3[rn] OR 50-32-8[rn] OR 53-70-3[rn] OR 56-55-3[rn] OR 5-Methylchrysene[tiab] OR Benzanthracene[tiab] OR Benzanthrene[tiab] OR Benzoanthracene[tiab] OR Indenopyrene[tiab] OR Naphthanthracene[tiab] OR o-Phenylenepyrene[tiab] OR Tetraphene[tiab] OR 120-12-7[rn] OR 129-00-0[rn] OR 132-64-9[rn] OR 132-65-0[rn] OR 191-24-2[rn] OR 192-97-2[rn] OR 206-44-0[rn] OR 208-96-8[rn] OR 218-01-9[rn] OR 3-methylcholanthrene[tiab] OR 83-32-9[rn] OR 85-01-8[rn] OR 86-73-7[rn] OR 91-20-3[rn] OR 92-52-4[rn] OR acenaphthene[tiab] OR acenaphthylene[tiab] OR albocarbon[tiab] OR anthracin[tiab] OR benzacenaphthene[tiab] OR benzfluoranthene[tiab] OR benzindene[tiab] OR benzofluoranthene[tiab] OR benzoperylene[tiab] OR benzophenanthrene[tiab] OR benzopyrene[tiab] OR benzperylene[tiab] OR benzphenanthrene[tiab] OR benzpyrene[tiab] OR beta-pyrene[tiab] OR beta-pyrine[tiab] OR bibenzene[tiab] OR binaphthylene[tiab] OR biphenyl[tiab] OR biphenylenemethane[tiab] OR biphenyleneoxide[tiab] OR biphenylylene-sulfide[tiab] OR butylanthracene[tiab] OR butylchrysene[tiab] OR butyldibenzothiophene[tiab] OR butylnaphthalene[tiab] OR butylphenanthrene[tiab] OR chrysene[tiab] OR coronene[tiab] OR dezodorator[tiab] OR dibenzanthracene[tiab] OR dibenzoanthracene[tiab] OR dibenzofuran[tiab] OR dibenzonaphthalene[tiab] OR dibenzopyrene[tiab] OR dibenzothiophene[tiab] OR dihydroacenaphthalene[tiab] OR dimethylanthracene[tiab] OR dimethylbenzaanthracene[tiab] OR dimethylchrysene[tiab] OR dimethyldibenzothiophene[tiab] OR dimethylfluoranthene[tiab] OR dimethylfluorene[tiab] OR dimethylnaphthalene[tiab] OR dimethylphenanthrene[tiab] OR dimethylpyrene[tiab] OR diphenyl[tiab] OR diphenylenemethane[tiab] OR diphenyleneoxide[tiab] OR Diphenylene-sulfide[tiab] OR ethylanthracene[tiab] OR ethylchrysene[tiab] OR ethyldibenzothiophene[tiab] OR ethylenenaphthalene[tiab] OR ethylfluoranthene[tiab] OR ethylfluorene[tiab] OR ethylnaphthalene[tiab] OR ethylphenanthrene[tiab] OR ethylpyrene[tiab] OR fluoranthene[tiab] OR idryl[tiab] OR methylanthracene[tiab] OR methylchrysene[tiab] OR methyldibenzothiophene[tiab] OR methylenebiphenyl[tiab] OR methylethylnaphthalene[tiab] OR methylfluoranthene[tiab] OR methylfluorene[tiab] OR methylnaphthalene[tiab] OR methylphenanthrene[tiab] OR methylpyrene[tiab] OR monomethylanthracene[tiab] OR monomethylchrysene[tiab] OR monomethyldibenzothiophene[tiab] OR monomethylfluoranthene[tiab] OR monomethylfluorene[tiab] OR monomethylnaphthalene[tiab] OR monomethylphenanthrene[tiab] OR monomethylpyrene[tiab] OR naphtalinum[tiab] OR naphthaline[tiab] OR naphthalinum[tiab] OR naphthyleneethylene[tiab] OR paranaphthalene[tiab] OR periethylenenaphthalene[tiab] OR perylene[tiab] OR phenantrin[tiab] OR phenylbenzene[tiab] OR phenylenepyrene[tiab] OR polycyclic-aromatic-compound[tiab] OR polycyclic-aromatic-hydrocarbon[tiab] OR propylanthracene[tiab] OR propylchrysene[tiab] OR propylfluorene[tiab] OR propylnaphthalene[tiab] OR propylphenanthrene[tiab] OR propylpyrene[tiab] OR tetramethylanthracene[tiab] OR tetramethyldibenzothiophene[tiab] OR tetramethylnaphthalene[tiab] OR tetramethylphenanthrene[tiab] OR tetra-olive-N2G[tiab] OR tetrosin-LY[tiab] OR thiafluorene[tiab] OR trimethylanthracene[tiab] OR trimethylchrysene[tiab] OR trimethyldibenzothiophene[tiab] OR trimethylfluoranthene[tiab] OR trimethylfluorene[tiab] OR trimethylnaphthalene[tiab] OR trimethylphenanthrene[tiab] OR trimethylpyrene[tiab] OR xenene[tiab] OR "Methylbenz(a)anthracene"[tiab] OR "Naphtho(2,3)pyrene"[tiab] OR Acenaphthenone[tiab] OR Acenaphthenequinone[tiab] OR Methylanthraquinone[tiab] OR

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
	"1,4-Anthraquinone"[tiab] OR "9,10-Anthraquinone"[tiab] OR "1,4-Benzoquinone"[tiab] OR "9-Fluorenone"[tiab] OR Naphthacenequinone[tiab] OR "1,2-Naphthoquinone"[tiab] OR "1,4-Naphthoquinone"[tiab] OR Perinaphthenone[tiab] OR "9,10-Phenanthrenequinone"[tiab] OR Xanthone[tiab] OR Antracene[tiab] OR Anthanthrene[tiab] OR "Benzo[c]fluorene"[tiab] OR "Dibenzo[a,e]fluoranthene"[tiab] OR "Cyclopenta(cd)pyrene"[tiab] OR "Cyclopenta(D,E,F)chrysene"[tiab] OR "Naphtho[2,3-E]pyrene"[tiab] OR "Benz[j]aceanthrylene"[tiab] OR Dinitropyrene[tiab] OR Nitrochrysene[tiab] OR "Benzo(j,k)fluorene"[tiab] OR "6-Nitrochrysene"[tiab] OR "1-Nitropyrene"[tiab] OR "Benzo(c)fluorene"[tiab] OR "Dibenzo(a,e)fluoranthene"[tiab] OR "Naphtho(2,3-E)pyrene"[tiab] OR "Benz(j)aceanthrylene"[tiab])
Scopus	(((arteries OR artery OR arterial OR carotid OR coronary OR heart OR peripheral OR renal OR stenosis) AND plaque*) OR Atherogenesis OR atheroma* OR Atherosclerоses OR Atherosclerosis OR Atherosclerotic-plaque* OR fatty-streak* OR fibroatheroma* OR foam-cell* OR Peripheral-Arterial-Disease* OR Peripheral-Artery-Disease* OR proatherogen* OR pro-atherogen*) AND ("3,4-benzopyrene" OR "benzo(a)pyrene" OR "benzo-a-pyrene" OR aromatic-hydrocarbons OR PAHs OR polycyclic-aromatic-hydrocarbons OR xylene OR "Benz(a)Anthracene" OR "Benz(a)Anthracenes" OR Anthracene OR Anthracenes OR Azulene OR Azulenes OR Benzocycloheptene OR Benzocycloheptenes OR Fluorene OR Fluorenes OR Indene OR Indenes OR Naphthacene OR Naphthacenes OR Naphthalene OR Naphthalenes OR Phenalene OR Phenalenes OR Phenanthrene OR Phenanthrenes OR Pyrene OR Pyrenes OR "1,10-(o-Phenylene)pyrene" OR "1,2:3,4-Dibenzopyrene" OR "1,2:5,6-Dibenzanthracene" OR "1,2:5,6-Dibenzoanthracene" OR "1,2-Benz(a)anthracene" OR "1,2-Benzanthracene" OR "1,2-Benzoanthracene" OR "1,2-Benzpyrene" OR "3,4:9,10-Dibenzopyrene" OR "3,4-Benz(a)pyrene" OR "3,4-Benzo(a)pyrene" OR "3,4-Benzofluoranthene" OR "3,4-Benzpyrene" OR "6,7-Benzopyrene" OR "7H-Dibenzo(c,g)carbazole" OR "B(a)P" OR "Benz(a)pyrene" OR "Benz(e)acephenanthrylene" OR "Benz(j)fluoranthene" OR "Benzo(a)anthracene" OR "Benzo(b)fluoranthene" OR "Benzo(d,e,f)chrysene" OR "Benzo(j)fluoranthene" OR "Benzo(k)fluoranthene" OR "Benzo(rst)pentaphene" OR "Dibenz(a,h)anthracene" OR "Dibenz(a,h)antracene" OR "Dibenz(a,i)pyrene" OR "Dibenz(a,j)acridine" OR "Dibenzo(a,e)pyrene" OR "Dibenzo(a,h)anthracene" OR "Dibenzo(a,h)pyrene" OR "Dibenzo(a,i)pyrene" OR "Dibenzo(a,l)pyrene" OR "Dibenzo(b,def)chrysene" OR "Dibenzo(d,e,f,p)chrysene" OR "Dibenzo(def,p)chrysene" OR "Indeno(1,2,3-cd)pyrene" OR 189-55-9 OR 189-64-0 OR 191-30-0 OR 192-65-4 OR 193-39-5 OR 194-59-2 OR 205-82-3 OR 205-99-2 OR 207-08-9 OR 224-42-0 OR 226-36-8 OR 3697-24-3 OR 50-32-8 OR 53-70-3 OR 56-55-3 OR 5-Methylchrysene OR Benzanthracene OR Benzanthrene OR Benzoanthracene OR Indenopyrene OR Naphthanthracene OR o-Phenylenepyrene OR Tetraphene OR 120-12-7 OR 129-00-0 OR 132-64-9 OR 132-65-0 OR 191-24-2 OR 192-97-2 OR 206-44-0 OR 208-96-8 OR 218-01-9 OR 3-methylcholanthrene OR 83-32-9 OR 85-01-8 OR 86-73-7 OR 91-20-3 OR 92-52-4 OR acenaphthene OR acenaphthylene OR albocarbon OR anthracin OR benzacenaphthene OR benzfluoranthene OR benzindene OR benzofluoranthene OR benzoperylene OR benzophenanthrene OR benzopyrene OR benzperylene OR benzphenanthrene OR benzpyrene OR beta-pyrene OR beta-pyrine OR bibenzene OR binaphthylene OR biphenyl OR biphenylenemethane OR biphenyleneoxide OR biphenylylene-sulfide OR butylanthracene OR butylchrysene OR butyldibenzothiophene

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
	OR butylnaphthalene OR butylphenanthrene OR chrysene OR coronene OR dezodorator OR dibenzanthracene OR dibenzoanthracene OR dibenzofuran OR dibenzonaphthalene OR dibenzopyrene OR dibenzothiophene OR dihydroacenaphthalene OR dimethylanthracene OR dimethylbenzaanthracene OR dimethylchrysene OR dimethyldibenzothiophene OR dimethylfluoranthene OR dimethylfluorene OR dimethylnaphthalene OR dimethylphenanthrene OR dimethylpyrene OR diphenyl OR diphenylenemethane OR diphenyleneoxide OR Diphenylene-sulfide OR ethylanthracene OR ethylchrysene OR ethyldibenzothiophene OR ethylenenaphthalene OR ethylfluoranthene OR ethylfluorene OR ethylnaphthalene OR ethylphenanthrene OR ethylpyrene OR fluoranthene OR idryl OR methylanthracene OR methylchrysene OR methyldibenzothiophene OR methylenebiphenyl OR methylethylnaphthalene OR methylfluoranthene OR methylfluorene OR methylnaphthalene OR methylphenanthrene OR methylpyrene OR monomethylanthracene OR monomethylchrysene OR monomethyldibenzothiophene OR monomethylfluoranthene OR monomethylfluorene OR monomethylnaphthalene OR monomethylphenanthrene OR monomethylpyrene OR naphtalinum OR naphthaline OR naphthalinum OR naphthyleneethylene OR paranaphthalene OR periethylenenaphthalene OR perylene OR phenantrin OR phenylbenzene OR phenylenepyrene OR polycyclic-aromatic-compound OR polycyclic-aromatic-hydrocarbon OR propylanthracene OR propylchrysene OR propylfluorene OR propylnaphthalene OR propylphenanthrene OR propylpyrene OR tetramethylanthracene OR tetramethyldibenzothiophene OR tetramethylnaphthalene OR tetramethylphenanthrene OR tetra-olive-N2G OR tetrosin-LY OR thiafluorene OR trimethylanthracene OR trimethylchrysene OR trimethyldibenzothiophene OR trimethylfluoranthene OR trimethylfluorene OR trimethylnaphthalene OR trimethylphenanthrene OR trimethylpyrene OR xenene OR "Methylbenz(a)anthracene" OR "Naphtho(2,3)pyrene" OR Acenaphthenone OR Acenaphthenequinone OR Methylanthraquinone OR "1,4-Anthraquinone" OR "9,10-Anthraquinone" OR "1,4-Benzoquinone" OR "9-Fluorenone" OR Naphthacenequinone OR "1,2-Naphthoquinone" OR "1,4-Naphthoquinone" OR Perinaphthenone OR "9,10-Phenanthrenequinone" OR Xanthone OR Antracene OR Anthanthrene OR "Benzo[c]fluorene" OR "Dibenzo[a,e]fluoranthene" OR "Cyclopenta(cd)pyrene" OR "Cyclopenta(D,E,F)chrysene" OR "Naphtho[2,3-E]pyrene" OR "Benz[j]aceanthrylene" OR Dinitropyrene OR Nitrochrysene OR "Benzo(j,k)fluorene" OR "1-Nitropyrene" OR "Benzo(c)fluorene" OR "Dibenzo(a,e)fluoranthene" OR "Naphtho(2,3-E)pyrene" OR "Benz(j)aceanthrylene")
Toxline	(((artery OR renal heart OR angina OR arrhythmia OR arrhythmias) AND plaque) OR Arteriosclerosis OR ((arterial OR carotid OR coronary) AND plaque) OR Atherogenesis OR atheroma OR atheromas OR Atheroscleroses OR Atherosclerosis OR Atherosclerotic OR fatty-streak OR fatty-streaks OR fibroatheroma OR fibroatheromas OR foam-cell OR foam-cells OR Peripheral-Arterial-Disease OR Peripheral-Arterial-Diseases OR Peripheral-Artery-Disease OR Peripheral-Artery-Diseases OR proatherogen* OR pro-atherogen*) AND (189-55-9 OR 189-64-0 OR 191-30-0 OR 192-65-4 OR 193-39-5 OR 194-59-2 OR 205-82-3 OR 205-99-2 OR 207-08-9 OR 224-42-0 OR 226-36-8 OR 3697-24-3 OR 50-32-8 OR 53-70-3 OR 56-55-3 OR 120-12-7 OR 129-00-0 OR 132-64-9 OR 132-65-0 OR 191-24-2 OR 192-97-2 OR 206-44-0 OR 208-96-8 OR 218-01-9 OR 83-32-9 OR 85-01-8 OR 86-73-7 OR 91-20-3 OR 92-52-4)

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
Web of Science	(((arteries OR artery OR arterial OR carotid OR coronary OR heart OR peripheral OR renal OR stenosis) AND plaque*) OR Atherogenesis OR atheroma* OR Atheroscleroses OR Atherosclerosis OR Atherosclerotic-plaque* OR fatty-streak* OR fibroatheroma* OR foam-cell* OR Peripheral-Arterial-Disease* OR Peripheral-Artery-Disease* OR proatherogen* OR pro-atherogen*) AND ("3,4-benzopyrene" OR "benzo(a)pyrene" OR "benzo-a-pyrene" OR aromatic-hydrocarbons OR PAHs OR polycyclic-aromatic-hydrocarbons OR xylene OR "Benz(a)Anthracene" OR "Benz(a)Anthracenes" OR Anthracene OR Anthracenes OR Azulene OR Azulenes OR Benzocycloheptene OR Benzocycloheptenes OR Fluorene OR Fluorenes OR Indene OR Indenes OR Naphthacene OR Naphthacenes OR Naphthalene OR Naphthalenes OR Phenalene OR Phenalenes OR Phenanthrene OR Phenanthrenes OR Pyrene OR Pyrenes OR "1,10-(o-Phenylene)pyrene" OR "1,2:3,4-Dibenzopyrene" OR "1,2:5,6-Dibenzanthracene" OR "1,2:5,6-Dibenzoanthracene" OR "1,2-Benz(a)anthracene" OR "1,2-Benzanthracene" OR "1,2-Benzoanthracene" OR "1,2-Benzpyrene" OR "3,4:9,10-Dibenzopyrene" OR "3,4-Benz(a)pyrene" OR "3,4-Benzo(a)pyrene" OR "3,4-Benzofluoranthene" OR "3,4-Benzpyrene" OR "6,7-Benzopyrene" OR "7H-Dibenzo(c,g)carbazole" OR "B(a)P" OR "Benz(a)pyrene" OR "Benz(e)acephenanthrylene" OR "Benz(j)fluoranthene" OR "Benzo(a)anthracene" OR "Benzo(b)fluoranthene" OR "Benzo(d,e,f)chrysene" OR "Benzo(j)fluoranthene" OR "Benzo(k)fluoranthene" OR "Benzo(rst)pentaphene" OR "Dibenz(a,h)anthracene" OR "Dibenz(a,h)antracene" OR "Dibenz(a,i)pyrene" OR "Dibenz(a,j)acridine" OR "Dibenzo(a,e)pyrene" OR "Dibenzo(a,h)anthracene" OR "Dibenzo(a,h)pyrene" OR "Dibenzo(a,i)pyrene" OR "Dibenzo(a,l)pyrene" OR "Dibenzo(b,def)chrysene" OR "Dibenzo(d,e,f,p)chrysene" OR "Dibenzo(def,p)chrysene" OR "Indeno(1,2,3-cd)pyrene" OR 189-55-9 OR 189-64-0 OR 191-30-0 OR 192-65-4 OR 193-39-5 OR 194-59-2 OR 205-82-3 OR 205-99-2 OR 207-08-9 OR 224-42-0 OR 226-36-8 OR 3697-24-3 OR 50-32-8 OR 53-70-3 OR 56-55-3 OR 5-Methylchrysene OR Benzanthracene OR Benzanthrene OR Benzoanthracene OR Indenopyrene OR Naphthanthracene OR o-Phenylenepyrene OR Tetraphene OR 120-12-7 OR 129-00-0 OR 132-64-9 OR 132-65-0 OR 191-24-2 OR 192-97-2 OR 206-44-0 OR 208-96-8 OR 218-01-9 OR 3-methylcholanthrene OR 83-32-9 OR 85-01-8 OR 86-73-7 OR 91-20-3 OR 92-52-4 OR acenaphthene OR acenaphthylene OR albocarbon OR anthracin OR benzacenaphthene OR benzfluoranthene OR benzindene OR benzofluoranthene OR benzoperylene OR benzophenanthrene OR benzopyrene OR benzperylene OR benzphenanthrene OR benzpyrene OR beta-pyrene OR beta-pyrine OR bibenzene OR binaphthylene OR biphenyl OR biphenylenemethane OR biphenyleneoxide OR biphenylylene-sulfide OR butylanthracene OR butylchrysene OR butyldibenzothiophene OR butylnaphthalene OR butylphenanthrene OR chrysene OR coronene OR dezodorator OR dibenzanthracene OR dibenzoanthracene OR dibenzofuran OR dibenzonaphthalene OR dibenzopyrene OR dibenzothiophene OR dihydroacenaphthalene OR dimethylanthracene OR dimethylbenzaanthracene OR dimethylchrysene OR dimethyldibenzothiophene OR dimethylfluoranthene OR dimethylfluorene OR dimethylnaphthalene OR dimethylphenanthrene OR dimethylpyrene OR diphenyl OR diphenylenemethane OR diphenyleneoxide OR Diphenylene-sulfide OR ethylanthracene OR ethylchrysene OR ethyldibenzothiophene OR ethylenenaphthalene OR ethylfluoranthene OR ethylfluorene OR ethylnaphthalene OR ethylphenanthrene OR ethylpyrene OR fluoranthene OR idryl OR methylanthracene OR methylchrysene OR methyldibenzothiophene OR methylenebiphenyl OR methylethylnaphthalene OR

Systematic Review: Literature Search Strategy	
Database	**Search Terms**
	methylfluoranthene OR methylfluorene OR methylnaphthalene OR methylphenanthrene OR methylpyrene OR monomethylanthracene OR monomethylchrysene OR monomethyldibenzothiophene OR monomethylfluoranthene OR monomethylfluorene OR monomethylnaphthalene OR monomethylphenanthrene OR monomethylpyrene OR naphtalinum OR naphthaline OR naphthalinum OR naphthyleneethylene OR paranaphthalene OR periethylenenaphthalene OR perylene OR phenantrin OR phenylbenzene OR phenylenepyrene OR polycyclic-aromatic-compound OR polycyclic-aromatic-hydrocarbon OR propylanthracene OR propylchrysene OR propylfluorene OR propylnaphthalene OR propylphenanthrene OR propylpyrene OR tetramethylanthracene OR tetramethyldibenzothiophene OR trimethyldibenzothiophene OR trimethylfluoranthene OR trimethylfluorene OR trimethylnaphthalene OR trimethylphenanthrene OR trimethylpyrene OR xenene OR "Methylbenz(a)anthracene" OR "Naphtho(2,3)pyrene" OR Acenaphthenone OR Acenaphthenequinone OR Methylanthraquinone OR "1,4-Anthraquinone" OR "9,10-Anthraquinone" OR "1,4-Benzoquinone" OR "9-Fluorenone" OR Naphthacenequinone OR "1,2-Naphthoquinone" OR "1,4-Naphthoquinone" OR Perinaphthenone OR "9,10-Phenanthrenequinone" OR Xanthone OR Antracene OR Anthanthrene OR "Benzo[c]fluorene" OR "Dibenzo[a,e]fluoranthene" OR "Cyclopenta(cd)pyrene" OR "Cyclopenta(D,E,F)chrysene" OR "Naphtho[2,3-E]pyrene" OR "Benz[j]aceanthrylene" OR Dinitropyrene OR Nitrochrysene OR "Benzo(j,k)fluorene" OR "1-Nitropyrene" OR "Benzo(c)fluorene" OR "Dibenzo(a,e)fluoranthene" OR "Naphtho(2,3-E)pyrene" OR "Benz(j)aceanthrylene")tetramethylnaphthalene OR tetramethylphenanthrene OR tetra-olive-N2G OR tetrosin-LY OR thiafluorene OR trimethylanthracene OR trimethylchrysene OR

Appendix 2. Search Strategy for Climate Change-associated PAH Exposures and Cardiovascular Diseases

This search was intended to provide a survey of relevant literature and was not meant to be an exhaustive database search. To achieve this goal and maximize relevance, forest fire terms were searched in conjunction with atherosclerosis and cardiovascular disease (CVD) health effects terms. General terms "fire", "flame", "smoke", and "climate change" were excluded because they returned many potentially irrelevant results.

Search Summary

Source	Unique Results
PubMed	128
Web of Science	139
Scopus	155
Unique References after De-duplication	**219**

Search Strategies

Database: PubMed

Date of Search: 10/07/2021
Limits: None

Set	Search Strategy for PubMed	Results
#1 Health: Atherosclerosis	(((arteries[tiab] OR artery[tiab] OR arterial[tiab] OR carotid[tiab] OR coronary[tiab] OR heart[tiab] OR peripheral[tiab] OR renal[tiab] OR stenosis[tiab]) AND plaque*[tiab]) OR Arteriosclerosis[Mesh:NoExp] OR Atherogenesis[tiab] OR atheroma*[tiab] OR Atherosclerosis[tiab] OR Atherosclerosis[mh] OR Atherosclerosis[tiab] OR Atherosclerotic-plaque*[tiab] OR fatty-streak*[tiab] OR fibroatheroma*[tiab] OR foam-cell*[tiab] OR Peripheral-Arterial-Disease*[tiab] OR Peripheral-Artery-Disease*[tiab] OR plaque,-atherosclerotic[mh] OR proatherogen*[tiab] OR pro-atherogen*[tiab])	220,017
#2 Health: CVD - Cardiotoxicity	OR ("Angiomatosis"[tiab] OR "Angiomatosis"[mh] OR "Aortic Diseases"[mh] OR "Aortic valve calcification"[tiab] OR "aortic valve disease"[tiab] OR "aortic valve diseases"[tiab] OR "aortic valve injuries"[tiab] OR "Aortic Valve Injury"[tiab] OR "arrhythmia"[tiab] OR "arrhythmias"[tiab] OR "Arrhythmias, Cardiac"[mh] OR "Arrhythmogenic right ventricular dysplasia"[mh] OR "arterial thromboembolism"[tiab] OR "Atrial fibrillation"[tiab] OR "Atrial rupture"[tiab] OR "Atrial tachycardia"[tiab] OR "Atrial thrombosis"[tiab] OR "Bradyarrhythmia"[tiab] OR "Bradycardia"[mh] OR "bradycardia"[tiab] OR "Bradydysrhythmias"[tiab] OR "Capillary Leak Syndrome"[tiab] OR "Capillary Leak Syndrome"[mh] OR "Cardiac valve disease"[tiab] OR "Cardiomyopathies"[mh] OR "Cardiomyopathies"[tiab] OR "Cardiomyopathy"[tiab] OR "cardiomyopathy, dilated"[mh] OR "Cardiotoxicity"[tiab] OR "Cardiotoxicity"[mh] OR "cardiovascular disease"[tiab] OR "cardiovascular diseases"[tiab] OR "cardiovascular diseases"[mh] OR "coronary artery disease"[tiab] OR "death, sudden, cardiac"[mh] OR "Embolism and Thrombosis"[mh] OR "Embolism"[tiab] OR "Endocardial fibrosis"[tiab] OR "endomyocardial fibrosis"[tiab] OR "endomyocardial fibrosis"[mh] OR "Heart block"[mh] OR "Heart block"[tiab] OR "Heart Diseases"[tiab] OR "heart disease"[tiab] OR "Heart Diseases"[mh] OR "Heart Failure"[tiab] OR	3,069,098

Set	Search Strategy for PubMed	Results
	"Heart Failure"[mh] OR "Heart valve calcification"[tiab] OR "Heart Valve Diseases"[tiab] OR "Heart Valve Diseases"[mh] OR "Hypertension"[mh] OR "hypertension"[tiab] OR "Hypotension"[mh] OR "Hypotension"[tiab] OR "Ischaemia"[tiab] OR "Ischemia"[tiab] OR "Ischemia"[mh] OR "long qt syndrome"[tiab] OR "long qt syndrome"[mh] OR "Mitral valve calcification"[tiab] OR "Mitral valve disease"[tiab] OR "Mitral valve hypoplasia"[tiab] OR "Myocardial calcification"[tiab] OR "Myocardial fibrosis"[tiab] OR "Myocardial hypoxia"[tiab] OR "Myocardial Infarction"[tiab] OR "myocardial infarction"[mh] OR "Myocardial ischaemia"[tiab] OR "Myocardial Ischemia"[tiab] OR "Myocardial Ischemia"[mh] OR "Myocarditis"[mh] OR "Myocarditis"[tiab] OR "pericardial disease"[tiab] OR "Pericardial fibrosis"[tiab] OR "Pericarditis"[tiab] OR "Pericarditis"[mh] OR "Peripheral Vascular Diseases"[mh] OR "Pregnancy Complications, Cardiovascular"[mh] OR "Prehypertension"[tiab] OR "Prehypertension"[mh] OR "Pulmonary Heart Disease"[mh] OR "Pulmonary Veno Occlusive Disease"[tiab] OR "Pulmonary Veno-Occlusive Disease"[mh] OR "QT interval"[tiab] OR "QT prolongation"[tiab] OR "QT/QTc interval"[tiab] OR "QTc interval"[tiab] OR "right ventricular dysplasia"[tiab] OR "sudden cardiac death"[tiab] OR "Superior Vena Cava Syndrome"[tiab] OR "Superior Vena Cava Syndrome"[mh] OR "Systolic dysfunction"[tiab] OR "Tachyarrhythmia"[tiab] OR "Tachyarrhythmias"[tiab] OR "Tachydysrhythmias"[tiab] OR "Thrombosis"[tiab] OR "thrombosis"[mh] OR "ventricle dysfunction"[tiab] OR "ventricle failure"[tiab] OR "Ventricular arrhythmia"[tiab] OR "ventricular disfunction"[tiab] OR "ventricular dysfunction"[tiab] OR "ventricular dysfunction"[mh] OR "Ventricular dyssynchrony"[tiab] OR "ventricular enlargement"[tiab] OR "ventricular failure"[tiab] OR "ventricular hypertrophy"[tiab] OR "Ventricular hypoplasia"[tiab])	
#3 Forest Fire	AND ("Wildfires"[Mesh] OR “biomass burning”[tiab] OR “Bushfire*”[tiab] OR “Forest Fire*”[tiab] OR “Wild fire*”[tiab] OR “Wildfire*”[tiab] OR “Wildland fire*”[tiab])	6,048
Total	(#1 OR #2) AND #3	**128**

Database: Web of Science
Date of Search: 10/11/2021
All terms searched in Topic (i.e., title, abstract, or keywords)
Limits: Limited to SCI-Expanded and SSCI Indexes

Set	Search Strategy for Web of Science	Results
#1 Health: Atherosclerosis	Topic = (((arteries OR artery OR arterial OR carotid OR coronary OR heart OR peripheral OR renal OR stenosis) AND plaque*) OR Atherogenesis OR atheroma* OR Atherosclerosis OR Atherosclerotic-plaque* OR fatty-streak* OR fibroatheroma* OR foam-cell* OR Peripheral-Arterial-Disease* OR Peripheral-Artery-Disease* OR proatherogen* OR pro-atherogen*)	238,355
#2 Health: CVD - Cardiotoxicity	OR Topic = ("Angiomatosis" OR "Aortic valve calcification" OR "aortic valve disease" OR "aortic valve diseases" OR "aortic valve injuries" OR "Aortic Valve Injury" OR "arrhythmia" OR "arrhythmias" OR "arterial thromboembolism" OR "Atrial fibrillation" OR "Atrial rupture" OR "Atrial tachycardia" OR "Atrial thrombosis" OR "Bradyarrhythmia" OR "bradycardia" OR "Bradydysrhythmias" OR "Capillary Leak Syndrome" OR "Cardiac valve disease" OR "Cardiomyopathies" OR "Cardiomyopathy" OR "Cardiotoxicity" OR "cardiovascular disease" OR "cardiovascular diseases" OR "coronary artery disease" OR "Embolism" OR "Endocardial fibrosis" OR "endomyocardial fibrosis" OR "Heart block" OR "Heart Diseases" OR "heart disease" OR "Heart Failure" OR "Heart valve calcification" OR "Heart Valve Diseases" OR "hypertension" OR "Hypotension" OR "Ischaemia" OR "Ischemia" OR "long qt syndrome" OR "Mitral valve calcification" OR "Mitral valve disease" OR "Mitral valve hypoplasia" OR "Myocardial calcification" OR "Myocardial fibrosis" OR "Myocardial hypoxia" OR "Myocardial Infarction" OR "Myocardial ischaemia" OR "Myocardial Ischemia" OR "Myocarditis" OR "pericardial disease" OR "Pericardial fibrosis" OR "Pericarditis" OR "Prehypertension" OR "Pulmonary Veno Occlusive Disease" OR "QT interval" OR "QT prolongation" OR "QT/QTc interval" OR "QTc interval" OR "right ventricular dysplasia" OR "sudden cardiac death" OR "Superior Vena Cava Syndrome" OR "Systolic dysfunction" OR "Tachyarrhythmia" OR "Tachyarrhythmias" OR "Tachydysrhythmias" OR "Thrombosis" OR "ventricle dysfunction" OR "ventricle failure" OR "Ventricular arrhythmia" OR "ventricular disfunction" OR "ventricular dysfunction" OR "Ventricular dyssynchrony" OR "ventricular enlargement" OR "ventricular failure" OR "ventricular hypertrophy" OR "Ventricular hypoplasia")	1,933,250
#3 Forest Fire	AND TOPIC = (“biomass burning” OR “Bushfire*” OR “Forest Fire*” OR “Wild fire*” OR “Wildfire*” OR “Wildland fire*”)	31,974
Results	(#1 OR #2) AND #3	139

Database: Scopus
Date of Search: 10/07/2021
All terms searched in: Title/Abstract, select Index terms
Limits: None

Set	Search Strategy for Scopus	Results
#1 Health: Atherosclerosis	(TITLE-ABS(((arteries OR artery OR arterial OR carotid OR coronary OR heart OR peripheral OR renal OR stenosis) AND plaque*) OR Atherogenesis OR atheroma* OR Atherosclerosis OR Atherosclerotic-plaque* OR fatty-streak* OR fibroatheroma* OR foam-cell* OR Peripheral-Arterial-Disease* OR Peripheral-Artery-Disease* OR proatherogen* OR pro-atherogen*))	203,529
#2 Health: CVD - Cardiotoxicity	OR (TITLE-ABS (angiomatosis) OR INDEXTERMS (angiomatosis) OR INDEXTERMS ({Aortic Diseases}) OR TITLE-ABS ({Aortic valve calcification}) OR TITLE-ABS ({aortic valve disease}) OR TITLE-ABS ({aortic valve diseases}) OR TITLE-ABS ({aortic valve injuries}) OR TITLE-ABS ({Aortic Valve Injury}) OR TITLE-ABS (arrhythmia) OR TITLE-ABS (arrhythmias) OR INDEXTERMS ({Arrhythmias, Cardiac}) OR INDEXTERMS ({Arrhythmogenic right ventricular dysplasia}) OR TITLE-ABS ({arterial thromboembolism}) OR TITLE-ABS ({Atrial fibrillation}) OR TITLE-ABS ({Atrial rupture}) OR TITLE-ABS ({Atrial tachycardia}) OR TITLE-ABS ({Atrial thrombosis}) OR TITLE-ABS (bradyarrhythmia) OR INDEXTERMS (bradycardia) OR TITLE-ABS (bradycardia) OR TITLE-ABS (bradydysrhythmias) OR TITLE-ABS ({Capillary Leak Syndrome}) OR INDEXTERMS ({Capillary Leak Syndrome}) OR TITLE-ABS ({Cardiac valve disease}) OR INDEXTERMS (cardiomyopathies) OR TITLE-ABS (cardiomyopathies) OR TITLE-ABS (cardiomyopathy) OR INDEXTERMS ({cardiomyopathy, dilated}) OR TITLE-ABS (cardiotoxicity) OR INDEXTERMS (cardiotoxicity) OR TITLE-ABS ({cardiovascular disease}) OR TITLE-ABS ({cardiovascular diseases}) OR INDEXTERMS ({cardiovascular diseases}) OR TITLE-ABS ({coronary artery disease}) OR INDEXTERMS ({death, sudden, cardiac}) OR INDEXTERMS ({Embolism and Thrombosis}) OR TITLE-ABS (embolism) OR TITLE-ABS ({Endocardial fibrosis}) OR TITLE-ABS ({endomyocardial fibrosis}) OR INDEXTERMS ({endomyocardial fibrosis}) OR INDEXTERMS ({Heart block}) OR TITLE-ABS ({Heart block}) OR TITLE-ABS ({Heart Diseases}) OR TITLE-ABS ({heart disease}) OR INDEXTERMS ({Heart Diseases}) OR TITLE-ABS ({Heart Failure}) OR INDEXTERMS ({Heart Failure}) OR TITLE-ABS ({Heart valve calcification}) OR TITLE-ABS ({Heart Valve Diseases}) OR INDEXTERMS ({Heart Valve Diseases}) OR INDEXTERMS (hypertension) OR TITLE-ABS (hypertension) OR INDEXTERMS (hypotension) OR TITLE-ABS (hypotension) OR TITLE-ABS (ischaemia) OR TITLE-ABS (ischemia) OR INDEXTERMS (ischemia) OR TITLE-ABS ({long qt syndrome}) OR INDEXTERMS ({long qt syndrome}) OR TITLE-ABS ({Mitral valve calcification}) OR TITLE-ABS ({Mitral valve disease}) OR TITLE-ABS ({Mitral valve hypoplasia}) OR TITLE-ABS ({Myocardial calcification}) OR TITLE-ABS ({Myocardial fibrosis}) OR TITLE-ABS ({Myocardial hypoxia}) OR TITLE-ABS ({Myocardial Infarction}) OR INDEXTERMS ({myocardial	2,768,015

Set	Search Strategy for Scopus	Results
	infarction}) OR TITLE-ABS ({Myocardial ischaemia}) OR TITLE-ABS ({Myocardial Ischemia}) OR INDEXTERMS ({Myocardial Ischemia}) OR INDEXTERMS (myocarditis) OR TITLE-ABS (myocarditis) OR TITLE-ABS ({pericardial disease}) OR TITLE-ABS ({Pericardial fibrosis}) OR TITLE-ABS (pericarditis) OR INDEXTERMS (pericarditis) OR INDEXTERMS ({Peripheral Vascular Diseases}) OR INDEXTERMS ({Pregnancy Complications, Cardiovascular}) OR TITLE-ABS (prehypertension) OR INDEXTERMS (prehypertension) OR INDEXTERMS ({Pulmonary Heart Disease}) OR TITLE-ABS ({Pulmonary Veno Occlusive Disease}) OR INDEXTERMS ({Pulmonary Veno-Occlusive Disease}) OR TITLE-ABS ({QT interval}) OR TITLE-ABS ({QT prolongation}) OR TITLE-ABS ({QT/QTc interval}) OR TITLE-ABS ({QTc interval}) OR TITLE-ABS ({right ventricular dysplasia}) OR TITLE-ABS ({sudden cardiac death}) OR TITLE-ABS ({Superior Vena Cava Syndrome}) OR INDEXTERMS ({Superior Vena Cava Syndrome}) OR TITLE-ABS ({Systolic dysfunction}) OR TITLE-ABS (tachyarrhythmia) OR TITLE-ABS (tachyarrhythmias) OR TITLE-ABS (tachydysrhythmias) OR TITLE-ABS (thrombosis) OR INDEXTERMS (thrombosis) OR TITLE-ABS (ventricle AND dysfunction) OR TITLE-ABS ({ventricle failure}) OR TITLE-ABS ({Ventricular arrhythmia}) OR TITLE-ABS ({ventricular disfunction}) OR TITLE-ABS ({ventricular dysfunction}) OR INDEXTERMS ({ventricular dysfunction}) OR TITLE-ABS ({Ventricular dyssynchrony}) OR TITLE-ABS ({ventricular enlargement}) OR TITLE-ABS ({ventricular failure}) OR TITLE-ABS ({ventricular hypertrophy}) OR TITLE-ABS ({Ventricular hypoplasia}))	
#3 Forest Fire	AND TITLE-ABS(“biomass burning” OR “Bushfire*” OR “Forest Fire*” OR “Wild fire*” OR “Wildfire*” OR “Wildland fire*”)	39,574
Results	(#1 OR #2) AND #3	155

Appendix 3. Data Extraction Elements for Human Studies

HUMAN	
Funding	Funding source(s)
	Reporting of conflict of interest by authors (*reporting bias)
Subjects	Study population name/description
	Dates of study and sampling time frame
	Geography (country, region, state, etc.)
	Demographics (sex, race/ethnicity, age or life stage at exposure and at outcome assessment)
	Number of subjects (target, enrolled, n per group in analysis, and participation/follow-up rates) (*missing data bias)
	Inclusion/exclusion criteria/recruitment strategy (*selection bias)
	Description of reference group (*selection bias)
Methods	Study design (e.g., prospective or retrospective cohort, nested case-control study, cross-sectional, population-based case-control study, intervention, case report)
	Length of follow-up (*information bias)
	Health outcome category, e.g., cardiovascular
	Health outcome, e.g., blood pressure (*reporting bias)
	Diagnostic or methods used to measure health outcome (*information bias)
	Confounders or modifying factors and how considered in analysis (e.g., included in final model, considered for inclusion but determined not needed (*confounding bias)
	Substance name and Chemical Abstracts Service number
	Exposure assessment (e.g., blood, urine, hair, air, drinking water, job classification, residence, administered treatment in controlled study) (*information bias)
	Methodological details for exposure assessment (e.g., high-performance liquid chromatography with tandem mass spectrometric [HPLC-MS/MS] detection, limit of detection) (*information bias)
	Statistical methods (*information bias)
Results	Exposure levels (e.g., mean, median, measures of variance as presented in paper, such as standard deviation, standard error of the mean, 75th/90th/95th percentile, minimum/maximum); range of exposure levels, number of exposed cases
	Statistical findings (e.g., adjusted β, standardized mean difference, adjusted odds ratio, standardized mortality ratio, relative risk) or description of qualitative results. When possible, IHAB will convert measures of effect to a common metric with associated 95% confidence intervals. Most often, measures of effect for continuous data are expressed as mean difference, standardized mean difference, and percent control response. Categorical data are typically expressed as odds ratio, relative risk (also called risk ratio), or β values, depending on what metric is most commonly reported in the included studies and on IHAB's ability to obtain information for effect conversions from the study or through author query.

HUMAN	
	If not presented in the study, statistical power can be assessed during data extraction using an approach that can detect a 10% to 20% change from response by control or referent group for continuous data, or a relative risk or odds ratio of 1.5 to 2 for categorical data, using the prevalence of exposure or prevalence of outcome in the control or referent group to determine sample size. For categorical data where the sample sizes of exposed and control or referent groups differ, the sample size of the exposed group will be used to determine the relative power category. Recommended sample sizes to achieve 80% power for a given effect size, i.e., 10% or 20% change from control, will be compared to sample sizes used in the study to categorize statistical power as "appears to be adequately powered" (sample size for 80% power met), somewhat underpowered (sample size is 75% to <100% of number required for 80% power), "underpowered" (sample size is 50% to <75% of number required for 80% power), or "severely underpowered" (sample size is <50% of number required for 80% power).
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, non-monotonic)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, and statistical result conversions, etc.

Items marked with an asterisk (*) are examples of items that can be used to assess internal validity/risk of bias

Appendix 4. Data Extraction Elements for Animal Studies

ANIMAL	
Funding	Funding source(s)
	Reporting of COI by authors (*reporting bias)
Animal Model	Sex
	Species
	Strain
	Source of animals
	Age or life stage at start of dosing and at health outcome assessment
	Diet and husbandry information (e.g., diet name/source)
Treatment	Chemical name and Chemical Abstracts Service number
	Source of chemical
	Purity of chemical (*information bias)
	Dose levels or concentration (as presented and converted to mg/kg bw/d when possible)
	Other dose-related details, such as whether administered dose level was verified by measurement, information on internal dosimetry (*information bias)
	Vehicle used for exposed animals
	Route of administration (e.g., oral, inhalation, dermal, injection)
	Duration of dosing (e.g., minutes, hours)
Methods	Study design (e.g., single acute treatment)
	Guideline compliance (i.e., use of EPA, Organisation for Economic Co-operation and Development (OECD), NTP or another guideline for study design, conducted under good laboratory practice (GLP) guideline conditions, non-GLP but consistent with guideline study, non-guideline peer-reviewed publication)
	Number of animals per group (and dams per group in developmental studies) (*missing data bias)
	Randomization procedure, allocation concealment, blinding during outcome assessment (*selection bias)
	Method to control for litter effects in developmental studies (*information bias)
	Use of negative controls and whether controls were untreated, vehicle-treated, or both
	Report on data from positive controls—was expected response observed? (*information bias)
	Endpoint health category (e.g., reproductive)
	Endpoint (e.g., infertility)
	Diagnostic or method to measure endpoint (*information bias)
	Statistical methods (*information bias)
Results	Measures of effect at each dose or concentration level (e.g., mean, median, frequency, and measures of precision or variance) or description of qualitative results. When possible, IHAB will convert measures of effect to a common metric with associated 95% confidence intervals. Most often, measures of effect for continuous data will be expressed as mean difference, standardized mean difference, and percent control response. Categorical data will be expressed as relative risk (also called risk ratio).

ANIMAL	
	No Observed Effect Level (NOEL), Lowest Observed Effect Level (LOEL), benchmark dose (BMD) analysis, statistical significance of other dose levels, or other estimates of effect presented in paper. **Note:** The NOEL and LOEL are highly influenced by study design, do not give any quantitative information about the relationship between dose and response, and can be subject to author's interpretation (e.g., a statistically significant effect may not be considered biologically important). Also, a NOEL does not necessarily mean zero response. Ideally, the response rate at specific dose levels is used as the primary measure to characterize the response.
	If not presented in the study, statistical power can be assessed during data extraction using an approach that assesses the ability to detect a 10% to 20% change from control group's response for continuous data, or a relative risk or odds ratio of 1.5 to 2 for categorical data, using the outcome frequency in the control group to determine sample size. Recommended sample sizes to achieve 80% power for a given effect size, i.e., 10% or 20% change from control, will be compared to sample sizes used in the study to categorize statistical power as "appears to be adequately powered" (sample size for 80% power met), "somewhat underpowered" (sample size is 75% to <100% of number required for 80% power), "underpowered" (sample size is 50% to <75% of number required for 80% power), or "severely underpowered" (sample size is <50% of number required for 80% power).
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, non-monotonic)
	Data on internal concentration, toxicokinetics, or toxicodynamics (when reported)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, and statistical result conversions, etc.

Items marked with an asterisk (*) are examples of items that can be used to assess internal validity/risk of bias.

Appendix 5. Risk-of-Bias Criteria

The OHAT risk-of-bias tool (version date January 2015 and available at http://ntp.niehs.nih.gov/go/38673) reflects OHAT's current best practices and provides the detailed discussion and instructions for the risk-of-bias practices used in this evaluation. The OHAT tool uses a single set of questions (also called "elements" or "domains") to assess risk of bias across various study types to facilitate consideration of conceptually similar potential sources of bias across the human and animal evidence streams with a common terminology. Individual risk-of bias questions are designated as only applicable to certain study designs (e.g., cohort studies or experimental animal studies), and a subset of the questions to apply to each study design (Table 6).

The specific criteria used to assess risk of bias for this evaluation are outlined below for human/observational studies and for experimental animal studies.

Observational Studies (Human Studies)

Cohort Studies

1. Was administered dose or exposure level adequately randomized? [NA]

2. Was allocation to study groups adequately concealed? [NA]

3. Did selection of study participants result in the appropriate comparison groups?

Definitely Low Risk of Bias (++)
• Direct evidence that subjects (both exposed and non-exposed) were similar (e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age and health status), recruited within the same time frame, and had similar participation/response rates, • **Note:** A study will be considered low risk of bias if baseline characteristics of groups differed, but these differences were considered as potential confounding or stratification variables (see question #4)
Probably Low Risk of Bias (+)
• Indirect evidence that subjects (both exposed and non-exposed) were similar (e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age and health status), recruited within the same time frame, and had similar participation/response rates, • **OR** differences between groups would not appreciably bias results
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that subjects (both exposed and non-exposed) were not similar, recruited within very different time frames, or had very different participation/response rates, • **OR** there is insufficient information provided about the comparison group including a different rate of non-response without an explanation (record "NR" as basis for answer)
Definitely High Risk of Bias (--)
• Direct evidence that subjects (both exposed and non-exposed) were not similar, recruited within very different time frames, or had very different participation/response rates

4. Did study design or analysis account for important confounding and modifying variables?

Definitely Low Risk of Bias (++)
• Direct evidence that appropriate adjustments or explicit considerations were made for the variables listed below as potential confounders and/or effect measure modifiers in the final analyses through the use of statistical models to reduce research-specific bias including standardization, matching, adjustment in multivariate model, stratification, propensity scoring, or other methods that were appropriately justified. Acceptable consideration of appropriate adjustment factors includes cases when the factor is not included in the final adjustment model because the author conducted analyses that indicated it did not need to be included.

- **AND** there is direct evidence that primary covariates and confounders were assessed using valid and reliable measurements,
- **AND** there is direct evidence that other exposures anticipated to bias results were not present or were appropriately measured and adjusted for. In occupational studies or studies of contaminated sites, other chemical exposures known to be associated with those settings were appropriately considered.
- **Note**: The following variables should be considered as potential confounders and/or effect measure modifiers for the relationship between exposure and outcomes: age, gender, race/ethnicity, smoking (if not the main source of PAHs in the study), body mass index, alcohol consumption, and variables that represent socioeconomic status (e.g., educational level, household income) based on prior reports of associations with exposure levels and outcomes involving environmental exposures (Piarulli *et al.* 2005, Margeirsdottir *et al.* 2010, Alshaarawy *et al.* 2016).
- **Note**: The following variables should be considered as potential confounders and/or effect measure modifiers for the relationship between exposure and disease outcomes: diabetes, cholesterol, hypertension, lipid levels, and systolic blood pressure (Rodondi *et al.* 2010).
- **Note**: Exposure to other known or suspected PAH sources and/or non-PAH sources of inflammation or atherosclerosis should be considered as co-exposures (e.g., population living need coal power generation station).

Probably Low Risk of Bias (+)

- Indirect evidence that appropriate adjustments were made,
- **OR** it is deemed that not considering or only considering a partial list of covariates or confounders in the final analyses would not appreciably bias results,
- **AND** there is evidence (direct or indirect) that covariates and confounders considered were assessed using valid and reliable measurements,
- **OR** it is deemed that the measures used would not appreciably bias results (i.e., the authors justified the validity of the measures from previously published research),
- **AND** there is evidence (direct or indirect) that other co-exposures anticipated to bias results were not present or were appropriately adjusted for,
- **OR** it is deemed that co-exposures present would not appreciably bias results.
- **Note**: this includes insufficient information provided on co-exposures in general population studies.

Probably High Risk of Bias (-) or (NR)

- Indirect evidence that the distribution of important covariates and known confounders differed between the groups and was not appropriately adjusted for in the final analyses,
- **OR** there is insufficient information provided about the distribution of known confounders (record “NR” as basis for answer),
- **OR** there is indirect evidence that covariates and confounders considered were assessed using measurements of unknown validity,
- **OR** there is insufficient information provided about the measurement techniques used to assess covariates and confounders considered (record “NR” as basis for answer),
- **OR** there is indirect evidence that there was an unbalanced provision of additional co-exposures across study groups, which were not appropriately adjusted for,
- **OR** there is insufficient information provided about co-exposures in occupational studies or studies of contaminated sites where high exposures to other chemical exposures would have been reasonably anticipated (record “NR” as basis for answer).

Definitely High Risk of Bias (--)

- Direct evidence that the distribution of important covariates and known confounders differed between the groups, confounding was demonstrated, and was not appropriately adjusted for in the final analyses,
- **OR** there is direct evidence that covariates and confounders considered were assessed using non-valid measurements,

• **OR** there is direct evidence that there was an unbalanced provision of additional co-exposures across study groups, which were not appropriately adjusted for.

5. Were experimental conditions identical across study groups? [NA]

6. Were the research personnel blinded to the study group during the study? [NA]

7. Were outcome data complete without attrition or exclusion from analysis?

Definitely Low Risk of Bias (++)
• Direct evidence that loss of subjects (i.e., incomplete outcome data) was adequately addressed and reasons were documented when human subjects were removed from a study. • **Note**: Acceptable handling of subject attrition includes: very little missing outcome data; reasons for missing subjects unlikely to be related to outcome (for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across study groups, with similar reasons for missing data across groups, • **OR** missing data have been imputed using appropriate methods and characteristics of subjects lost to follow-up or with unavailable records are described in identical way and are not significantly different from those of the study participants.
Probably Low Risk of Bias (+)
• Indirect evidence that loss of subjects (i.e., incomplete outcome data) was adequately addressed and reasons were documented when human subjects were removed from a study, • **OR** it is deemed that the proportion lost to follow-up would not appreciably bias results. This would include reports of no statistical differences in characteristics of subjects lost to follow-up or with unavailable records from those of the study participants. Generally, the higher the ratio of participants with missing data to participants with events, the greater potential there is for bias. For studies with a long duration of follow-up, some withdrawals for such reasons are inevitable.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that loss of subjects (i.e., incomplete outcome data) was unacceptably large and not adequately addressed, • **OR** there is insufficient information provided about numbers of subjects lost to follow-up (record “NR” as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that loss of subjects (i.e., incomplete outcome data) was unacceptably large and not adequately addressed. • **Note**: Unacceptable handling of subject attrition includes: reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across study groups; or potentially inappropriate application of imputation.

8. Can we be confident in the exposure characterization?

Definitely Low Risk of Bias (++)
• Direct evidence that exposure was consistently assessed (i.e., under the same method and time frame) using well-established methods that directly measure exposure in blood, serum, or plasma, • **OR** exposure was assessed using less-established methods that directly measure exposure and are validated against well-established methods, • **AND** exposure was assessed in a relevant time-window for development of the outcome, • **AND** there is sufficient range or variation in exposure measurements across groups to potentially identify associations with health outcomes, • **AND** there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.

Probably Low Risk of Bias (+)
• Indirect evidence that the exposure was consistently assessed using well-established methods that directly measure exposure, • **OR** exposure was assessed using indirect measures (e.g., drinking water levels and residency, questionnaire or occupational exposure assessment by a certified industrial hygienist) that have been validated or empirically shown to be consistent with methods that directly measure exposure (i.e., inter-methods validation: one method vs. another), • **AND** exposure was assessed in a relevant time-window for development of the outcome. Current exposure measures will be considered relevant for any outcome, • **AND** there is sufficient range or variation in exposure measurements across groups to potentially identify associations with health outcomes (at a minimum from high exposure or ever exposed from low exposure or never exposed), • **AND** there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the exposure was assessed using poorly validated methods that directly measure exposure, • **OR** there is evidence that the exposure was assessed using indirect measures that have not been validated or empirically shown to be consistent with methods that directly measure exposure (e.g., questionnaire, job-exposure matrix or self-report without validation), • **OR** there is insufficient information provided about the exposure assessment, including validity and reliability, but no evidence for concern about the method used (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that the exposure was assessed using methods with poor validity, • **OR** evidence of exposure misclassification (e.g., differential recall of self-reported exposure).

9. Can we be confident in the outcome assessment?

Definitely Low Risk of Bias (++)
• Direct evidence that the outcome was assessed using well-established methods (e.g., gold standard at the time or best-available method), • **AND** subjects had been followed for the same length of time in all study groups, • **AND** there is direct evidence that the outcome assessors (including study subjects, if outcomes were self-reported) were adequately blinded to the study group or exposure level, and it is unlikely that they could have broken the blinding prior to reporting outcomes. • **NOTE** Well-established methods will depend on the outcome, but examples of such methods may include doctor diagnosis of atherosclerosis (e.g., carotid intima-media thickness, ankle-brachial pressure index, blood pressure/flow, blood tests for cholesterol, electrocardiograms, cardiac catheterization/angiograms, stress tests [exercise with measure of heart rate/blood pressure, etc.]) or doctor diagnosis obtained from medical records (NIH 2018).
Probably Low Risk of Bias (+)
• Indirect evidence that the outcome was assessed using acceptable methods (i.e., deemed valid and reliable but not the gold standard), • **AND** subjects had been followed for the same length of time in all study groups, • **OR** it is deemed that the outcome assessment methods used would not appreciably bias results, • **AND** there is indirect evidence that the outcome assessors (including study subjects, if outcomes were self-reported) were adequately blinded to the study group, and it is unlikely that they could have broken the blinding prior to reporting outcomes, • **OR** it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results, which is more likely to apply to objective outcome measures.

• **NOTE**: Acceptable, but not ideal assessment methods will depend on the outcome, but examples of such methods may include proxy reporting of outcomes such as and mining of data collected for other purposes. Proxy reporting (e.g., parental reporting of days sick or doctor diagnosis) of other types of disease, colds, etc. should be considered on a case-by-case basis with consideration of whether or not there is empirical evidence as to the reliability of proxy reporting for that outcome.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the outcome assessment method is an insensitive instrument (e.g., self-reported status of atherosclerosis), • **OR** the length of follow-up differed by study group, • **OR** there is indirect evidence that it was possible for outcome assessors (including study subjects if outcomes were self-reported) to infer the study group prior to reporting outcomes, • **OR** there is insufficient information provided about blinding of outcome assessors (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that the outcome assessment method is an insensitive instrument, • **OR** the length of follow-up differed by study group, • **OR** there is direct evidence for lack of adequate blinding of outcome assessors (including study subjects if outcomes were self-reported), including no blinding or incomplete blinding.

10. Were all measured outcomes reported?

Definitely Low Risk of Bias (++)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
Probably Low Risk of Bias (+)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** analyses that had not been planned in advance (i.e., retrospective unplanned subgroup analyses) are clearly indicated as such and deemed that unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g., appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** and there is indirect evidence that unplanned analyses were included that may appreciably bias results, • **OR** there is insufficient information provided about selective outcome reporting (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g., subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.

11. Were there no other potential threats to internal validity?

There are no environmental-exposure-specific additions to the risk-of-bias questions for this evaluation. This question will be used to examine individual studies for appropriate statistical methods (e.g., confirmation of

homogeneity of variance for analysis of variance (ANOVA) and other statistical tests that require normally distributed data). It will also be used for risk-of-bias considerations that do not fit under the other questions.

Cross-sectional and Case Series Studies

1. Was administered dose or exposure level adequately randomized? [NA]

2. Was allocation to study groups adequately concealed? [NA]

3. Did selection of study participants result in the appropriate comparison groups? [NA to Case series]

Definitely Low Risk of Bias (++)
• Direct evidence that subjects (both exposed and non-exposed) were similar, e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age and health status), recruited within the same time frame, and had similar participation/response rates. • **Note**: A study will be considered low risk of bias if baseline characteristics of groups differed, but these differences were considered as potential confounding or stratification variables (see question #4).
Probably Low Risk of Bias (+)
• Indirect evidence that subjects (both exposed and non-exposed) were similar, e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age and health status), recruited within the same time frame, and had similar participation/response rates, • **OR** differences between groups would not appreciably bias results.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that subjects (both exposed and non-exposed) were not similar, recruited within very different time frames, or had very different participation/response rates, • **OR** there is insufficient information provided about the comparison group including a different rate of non-response without an explanation (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that subjects (both exposed and non-exposed) were not similar, recruited within very different time frames, or had very different participation/response rates.

4. Did study design or analysis account for important confounding and modifying variables?

Definitely Low Risk of Bias (++)
• Direct evidence that appropriate adjustments or explicit considerations were made for the variables listed below as potential confounders and/or effect measure modifiers in the final analyses through the use of statistical models to reduce research-specific bias including standardization, matching, adjustment in multivariate model, stratification, propensity scoring, or other methods that were appropriately justified. Acceptable consideration of appropriate adjustment factors includes cases when the factor is not included in the final adjustment model because the author conducted analyses that indicated it did not need to be included, • **AND** there is direct evidence that primary covariates and confounders were assessed using valid and reliable measurements, • **AND** there is direct evidence that other exposures anticipated to bias results were not present or were appropriately measured and adjusted for. In occupational studies or studies of contaminated sites, other chemical exposures known to be associated with those settings were appropriately considered. • **Note**: The following variables should be considered as potential confounders and/or effect measure modifiers for the relationship between exposure and outcomes: age, gender, race/ethnicity, smoking (if not the main source of PAHs in the study), body mass index, alcohol consumption, and variables that represent socioeconomic status (e.g., educational level, household income) based on prior reports of associations with exposure levels and outcomes involving environmental exposures (Piarulli *et al.* 2005, Margeirsdottir *et al.* 2010, Alshaarawy *et al.* 2016).

- **Note**: The following variables should be considered as potential confounders and/or effect measure modifiers for the relationship between exposure and disease outcomes: diabetes, cholesterol, hypertension, lipid levels, and systolic blood pressure (Rodondi *et al.* 2010).
- **Note**: Exposure to other known or suspected PAH sources and/or non-PAH sources of inflammation or atherosclerosis should be considered as co-exposures (e.g., population living need coal power generation station).

Probably Low Risk of Bias (+)

- Indirect evidence that appropriate adjustments were made,
- **OR** it is deemed that not considering or only considering a partial list of covariates or confounders in the final analyses would not appreciably bias results,
- **AND** there is evidence (direct or indirect) that covariates and confounders considered were assessed using valid and reliable measurements,
- **OR** it is deemed that the measures used would not appreciably bias results (i.e., the authors justified the validity of the measures from previously published research),
- **AND** there is evidence (direct or indirect) that other co-exposures anticipated to bias results were not present or were appropriately adjusted for,
- **OR** it is deemed that co-exposures present would not appreciably bias results.
- **Note**: this includes insufficient information provided on co-exposures in general population studies.

Probably High Risk of Bias (-) or (NR)

- Indirect evidence that the distribution of important covariates and known confounders differed between the groups and was not appropriately adjusted for in the final analyses,
- **OR** there is insufficient information provided about the distribution of known confounders (record "NR" as basis for answer),
- **OR** there is indirect evidence that covariates and confounders considered were assessed using measurements of unknown validity,
- **OR** there is insufficient information provided about the measurement techniques used to assess covariates and confounders considered (record "NR" as basis for answer),
- **OR** there is indirect evidence that there was an unbalanced provision of additional co-exposures across study groups, which were not appropriately adjusted for,
- **OR** there is insufficient information provided about co-exposures in occupational studies or studies of contaminated sites where high exposures to other chemical exposures would have been reasonably anticipated (record "NR" as basis for answer).

Definitely High Risk of Bias (--)

- Direct evidence that the distribution of important covariates and known confounders differed between the groups, confounding was demonstrated, and was not appropriately adjusted for in the final analyses,
- **OR** there is direct evidence that covariates and confounders considered were assessed using non-valid measurements,
- **OR** there is direct evidence that there was an unbalanced provision of additional co-exposures across study groups, which were not appropriately adjusted for.

5. Were experimental conditions identical across study groups? [NA]

6. Were the research personnel blinded to the study group during the study? [NA]

7. Were outcome data complete without attrition or exclusion from analysis?

Definitely Low Risk of Bias (++)

- Direct evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.

Probably Low Risk of Bias (+)
• Indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that exclusion of subjects from analyses was not adequately addressed, • **OR** there is insufficient information provided about why subjects were removed from the study or excluded from analyses (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that exclusion of subjects from analyses was not adequately addressed. • **Note**: Unacceptable handling of subject exclusion from analyses includes: reason for exclusion likely to be related to true outcome, with either imbalance in numbers or reasons for exclusion across study groups.

8. Can we be confident in the exposure characterization?

Definitely Low Risk of Bias (++)
• Direct evidence that exposure was consistently assessed (i.e., under the same method and time frame) using well-established methods that directly measure exposure, or the exposure in blood, serum, or plasma (e.g., 1-OHP), • **OR** exposure was assessed using less-established methods that directly measure exposure and are validated against well-established methods, • **AND** exposure was assessed in a relevant time-window for development of the outcome. Current exposure measures will be considered relevant for any outcome, • **AND** there is sufficient range or variation in exposure measurements across groups to potentially identify associations with health outcomes, • **AND** there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.
Probably Low Risk of Bias (+)
• Indirect evidence that the exposure was consistently assessed using well-established methods that directly measure exposure), • **OR** exposure was assessed using indirect measures (e.g., drinking water levels and residency, questionnaire or occupational exposure assessment by a certified industrial hygienist) that have been validated or empirically shown to be consistent with methods that directly measure exposure (i.e., inter-methods validation: one method vs. another), • **AND** exposure was assessed in a relevant time-window for development of the outcome. Current exposure measures will be considered relevant for any outcome, • **AND** there is sufficient range or variation in exposure measurements across groups to potentially identify associations with health outcomes (at a minimum from high exposure or ever exposed from low exposure or never exposed), • **AND** there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the exposure was assessed using poorly validated methods that directly measure exposure, • **OR** there is direct evidence that the exposure was assessed using indirect measures that have not been validated or empirically shown to be consistent with methods that directly measure exposure (e.g., a job-exposure matrix or self-report without validation) (record "NR" as basis for answer), • **OR** there is insufficient information provided about the exposure assessment, including validity and reliability, but no evidence for concern about the method used (record "NR" as basis for answer).

Definitely High Risk of Bias (--)
• Direct evidence that the exposure was assessed using methods with poor validity, • **OR** evidence of exposure misclassification (e.g., differential recall of self-reported exposure).

9. Can we be confident in the outcome assessment?

Definitely Low Risk of Bias (++)
• Direct evidence that the outcome was assessed using well-established methods (the gold standard), • **AND** there is direct evidence that the outcome assessors (including study subjects, if outcomes were self-reported) were adequately blinded to the exposure level, and it is unlikely that they could have broken the blinding prior to reporting outcomes. • **NOTE** Well-established methods will depend on the outcome, but examples of such methods may include doctor diagnosis of atherosclerosis (e.g., carotid intima-media thickness, ankle-brachial index, blood pressure/flow, blood tests for cholesterol, electrocardiograms, cardiac catheterization/angiograms, stress tests [exercise with measure of heart rate/blood pressure, etc.]) or doctor diagnosis obtained from medical records (NIH 2018).
Probably Low Risk of Bias (+)
• Indirect evidence that the outcome was assessed using acceptable methods, • **OR** it is deemed that the outcome assessment methods used would not appreciably bias results, • **AND** there is indirect evidence that the outcome assessors were adequately blinded to the exposure level, and it is unlikely that they could have broken the blinding prior to reporting outcomes, • **OR** it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results (including that subjects self-reporting outcomes were likely not aware of reported links between the exposure and outcome lack of blinding is unlikely to bias a particular outcome).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the outcome assessment method is an insensitive instrument (e.g., self-reporting status of atherosclerosis), • **OR** there is indirect evidence that it was possible for outcome assessors to infer the exposure level prior to reporting outcomes (including that subjects self-reporting outcomes were likely aware of reported links between the exposure and outcome), • **OR** there is insufficient information provided about blinding of outcome assessors (record “NR” as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that the outcome assessment method is an insensitive instrument, • **OR** there is direct evidence that outcome assessors were aware of the exposure level prior to reporting outcomes (including that subjects self-reporting outcomes were aware of reported links between the exposure and outcome).

10. Were all measured outcomes reported?

Definitely Low Risk of Bias (++)
• Direct evidence that all of the study’s measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
Probably Low Risk of Bias (+)
• Indirect evidence that all of the study’s measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** analyses that had not been planned in advance (i.e., retrospective unplanned subgroup analyses) are clearly indicated as such and deemed that unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g., appropriate analyses of an unexpected effect). This would include

outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** and there is indirect evidence that unplanned analyses were included that may appreciably bias results, • **OR** there is insufficient information provided about selective outcome reporting (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g., subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.

11. Were there no other potential threats to internal validity?

There are no environmental-exposure-specific additions to the risk-of-bias questions for this evaluation. This question will be used to examine individual studies for appropriate statistical methods (e.g., confirmation of homogeneity of variance for ANOVA and other statistical tests that require normally distributed data). It will also be used for risk-of-bias considerations that do not fit under the other questions.

Case Control Studies

1. Was administered dose or exposure level adequately randomized? [NA]

2. Was allocation to study groups adequately concealed? [NA]

3. Did selection of study participants result in the appropriate comparison groups?

Definitely Low Risk of Bias (++)
• Direct evidence that cases and controls were similar (e.g., recruited from the same eligible population including being of similar age, gender, ethnicity, and eligibility criteria other than outcome of interest as appropriate), recruited within the same time frame, and controls are described as having no history of the outcome, • **Note**: A study will be considered low risk of bias if baseline characteristics of groups differed, but these differences were considered as potential confounding or stratification variables (see question #4).
Probably Low Risk of Bias (+)
• Indirect evidence that cases and controls were similar (e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age), recruited within the same time frame, and controls are described as having no history of the outcome, • **OR** it is deemed differences between cases and controls would not appreciably bias results.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that controls were drawn from a very dissimilar population than cases or recruited within very different time frames, • **OR** there is insufficient information provided about the appropriateness of controls including rate of response reported for cases only (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that controls were drawn from a very dissimilar population than cases or recruited within very different time frames.

4. Did study design or analysis account for important confounding and modifying variables?

Definitely Low Risk of Bias (++)
• Direct evidence that appropriate adjustments were made for the variables listed below as potential confounders and/or effect measure modifiers in the final analyses through the use of statistical models to reduce research-specific bias including standardization, matching of cases and controls, adjustment in multivariate model, stratification, propensity scoring, or other methods were appropriately justified, • **AND** there is direct evidence that primary covariates and confounders were assessed using valid and reliable measurements, • **AND** there is direct evidence that other exposures anticipated to bias results were not present or were appropriately measured and adjusted for.
Probably Low Risk of Bias (+)
• Indirect evidence that appropriate adjustments were made, • **OR** it is deemed that not considering or only considering a partial list of covariates or confounders in the final analyses would not appreciably bias results, • **AND** there is evidence (direct or indirect) that covariates and confounders considered were assessed using valid and reliable measurements, • **OR** it is deemed that the measures used would not appreciably bias results (i.e., the authors justified the validity of the measures from previously published research), • **AND** there is evidence (direct or indirect) that other co-exposures anticipated to bias results were not present or were appropriately adjusted for, • **OR** it is deemed that co-exposures present would not appreciably bias results. • **Note**: this includes insufficient information provided on co-exposures in general population studies. • **Note**: The following variables should be considered as potential confounders and/or effect measure modifiers for the relationship between exposure and outcomes: age, gender, race/ethnicity, smoking (if not the main source of PAHs in the study), body mass index, alcohol consumption, and variables that represent socioeconomic status (e.g., educational level, household income) based on prior reports of associations with exposure levels and outcomes involving environmental exposures (Piarulli *et al.* 2005, Margeirsdottir *et al.* 2010, Alshaarawy *et al.* 2016). • **Note**: The following variables should be considered as potential confounders and/or effect measure modifiers for the relationship between exposure and disease outcomes: diabetes, cholesterol, hypertension, lipid levels, and systolic blood pressure (Rodondi *et al.* 2010). • **Note**: Exposure to other known or suspected PAH sources and/or non-PAH sources of inflammation or atherosclerosis should be considered as co-exposures (e.g., population living need coal power generation station).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the distribution of important covariates and known confounders differed between cases and controls and was not investigated further, • **OR** there is insufficient information provided about the distribution of known confounders in cases and controls (record "NR" as basis for answer), • **OR** there is indirect evidence that covariates and confounders considered were assessed using measurements of unknown validity, • **OR** there is insufficient information provided about the measurement techniques used to assess covariates and confounders considered (record "NR" as basis for answer), • **OR** there is indirect evidence that there was an unbalanced provision of additional co-exposures across cases and controls, which were not appropriately adjusted for, • **OR** there is insufficient information provided about co-exposures in occupational studies or studies of contaminated sites where high exposures to other chemical exposures would have been reasonably anticipated (record "NR" as basis for answer).

Definitely High Risk of Bias (--)
• Direct evidence that the distribution of important covariates and known confounders differed between cases and controls, confounding was demonstrated, but was not appropriately adjusted for in the final analyses, • **OR** there is direct evidence that covariates and confounders considered were assessed using non-valid measurements, • **OR** there is direct evidence that there was an unbalanced provision of additional co-exposures across cases and controls, which were not appropriately adjusted for.

5. Were experimental conditions identical across study groups? [NA]

6. Were the research personnel blinded to the study group during the study? [NA]

7. Were outcome data complete without attrition or exclusion from analysis?

Definitely Low Risk of Bias (++)
• Direct evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
Probably Low Risk of Bias (+)
• Indirect evidence that exclusion of subjects from analyses was adequately addressed, and reasons were documented when subjects were removed from the study or excluded from analyses.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that exclusion of subjects from analyses was not adequately addressed, • **OR** there is insufficient information provided about why subjects were removed from the study or excluded from analyses (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that exclusion of subjects from analyses was not adequately addressed. • **Note**: Unacceptable handling of subject exclusion from analyses includes: reason for exclusion likely to be related to true outcome, with either imbalance in numbers or reasons for exclusion across study groups.

8. Can we be confident in the exposure characterization?

Definitely Low Risk of Bias (++)
• Direct evidence that exposure was consistently assessed (i.e., under the same method and time frame) using well-established methods that directly measure exposure, or the exposure in blood, serum, or plasma (e.g., 1-OHP), • **OR** exposure was assessed using less-established methods that directly measure exposure and are validated against well-established methods, • **AND** exposure was assessed in a relevant time-window for development of the outcome. Current exposure measures will be considered relevant for any outcome, • **AND** there is sufficient range or variation in exposure measurements across groups to potentially identify associations with health outcomes, • **AND** there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.
Probably Low Risk of Bias (+)
• Indirect evidence that the exposure was consistently assessed using well-established methods that directly measure exposure, • **OR** exposure was assessed using indirect measures (e.g., drinking water levels and residency, questionnaire or occupational exposure assessment by a certified industrial hygienist) that have been validated or empirically shown to be consistent with methods that directly measure exposure (i.e., inter-methods validation: one method vs. another),

- **AND** exposure was assessed in a relevant time-window for development of the outcome. Current exposure measures will be considered relevant for any outcome,
- **AND** there is sufficient range or variation in exposure measurements across groups to potentially identify associations with health outcomes (at a minimum from high exposure or ever exposed from low exposure or never exposed),
- **AND** there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.

Probably High Risk of Bias (-) or (NR)

- Indirect evidence that the exposure was assessed using poorly validated methods that directly measure exposure,
- **OR** there is direct evidence that the exposure was assessed using indirect measures that have not been validated or empirically shown to be consistent with methods that directly measure exposure (e.g., a job-exposure matrix or self-report without validation) (record "NR" as basis for answer),
- **OR** there is insufficient information provided about the exposure assessment, including validity and reliability, but no evidence for concern about the method used (record "NR" as basis for answer).

Definitely High Risk of Bias (--)

- Direct evidence that the exposure was assessed using methods with poor validity,
- **OR** evidence of exposure misclassification (e.g., differential recall of self-reported exposure).

9. Can we be confident in the outcome assessment?

Definitely Low Risk of Bias (++)

- Direct evidence that the outcome was assessed in cases (i.e., case definition) and controls using well-established methods (the gold standard),
- **AND** subjects had been followed for the same length of time in all study groups,
- **AND** there is direct evidence that the outcome assessors (including study subjects, if outcomes were self-reported) were adequately blinded to the exposure level when outcome was assessed in cases (i.e., case definition) and controls.
- **NOTE** Well-established methods will depend on the outcome, but examples of such methods may include doctor diagnosis of atherosclerosis (e.g., carotid intima-media thickness, ankle-brachial pressure index, blood pressure/flow, blood tests for cholesterol, electrocardiograms, cardiac catheterization/angiograms, stress tests [exercise with measure of heart rate/blood pressure, etc.]) or doctor diagnosis obtained from medical records (NIH 2018).

Probably Low Risk of Bias (+)

- Indirect evidence that the outcome was assessed in cases (i.e., case definition) and controls using acceptable methods),
- **AND** subjects had been followed for the same length of time in all study groups,
- **OR** it is deemed that the outcome assessment methods used would not appreciably bias results,
- **AND** there is indirect evidence that the outcome assessors were adequately blinded to the exposure level when reporting outcomes,
- **OR** it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results (including that subjects self-reporting outcomes were likely not aware of reported links between the exposure and outcome or lack of blinding is unlikely to bias a particular outcome).
- **NOTE** Acceptable, but not ideal assessment methods will depend on the outcome, but examples of such methods may include proxy reporting of outcomes and mining of data collected for other purposes. Proxy reporting disease should be considered on a case-by-case basis with consideration of whether or not there is empirical evidence as to the reliability of proxy reporting for that outcome.

Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the outcome was assessed in cases (i.e., case definition) using an insensitive instrument (e.g., self-reported status of atherosclerosis), • **OR** there is insufficient information provided about how cases were identified (record "NR" as basis for answer), • **OR** there is indirect evidence that it was possible for outcome assessors to infer the exposure level prior to reporting outcomes (including that subjects self-reporting outcomes were likely aware of reported links between the exposure and outcome), • **OR** there is insufficient information provided about blinding of outcome assessors (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that the outcome was assessed in cases (i.e., case definition) using an insensitive instrument, • **OR** there is direct evidence that outcome assessors were aware of the exposure level prior to reporting outcomes (including that subjects self-reporting outcomes were aware of reported links between the exposure and outcome).

10. Were all measured outcomes reported?

Definitely Low Risk of Bias (++)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
Probably Low Risk of Bias (+)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** analyses that had not been planned in advance (i.e., retrospective unplanned subgroup analyses) are clearly indicated as such and deemed that unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g., appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** and there is indirect evidence that unplanned analyses were included that may appreciably bias results, • **OR** there is insufficient information provided about selective outcome reporting (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g., subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.

11. Were there no other potential threats to internal validity?

There are no environmental-exposure-specific additions to the risk-of-bias questions for this evaluation. This question will be used to examine individual studies for appropriate statistical methods (e.g., confirmation of homogeneity of variance for ANOVA and other statistical tests that require normally distributed data). It will also be used for risk-of-bias considerations that do not fit under the other questions.

Experimental Animal Studies

1. Was administered dose or exposure level adequately randomized?

Definitely Low Risk of Bias (++)
• Direct evidence that animals were allocated to any study group including controls using a method with a random component, • **AND** there is direct evidence that the study used a concurrent control group as an indication that randomization covered all study groups. • **Note**: Acceptable methods of randomization include: referring to a random number table, using a computer random number generator, coin tossing, or shuffling cards (Higgins and Green 2011). • **Note**: Restricted randomization (e.g., blocked randomization) to ensure particular allocation ratios will be considered low bias. Similarly, stratified randomization approaches that attempt to minimize imbalance between groups on important prognostic factors (e.g., body weight) will be considered acceptable.
Probably Low Risk of Bias (+)
• Indirect evidence that animals were allocated to any study group including controls using a method with a random component (i.e., authors state random allocation, without description of method), • **AND** evidence that the study used a concurrent control group as an indication that randomization covered all study groups, • **OR** it is deemed that allocation without a clearly random component would not appreciably bias results.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that animals were allocated to study groups using a method with a non-random component, • **OR** indirect evidence that there was a lack of a concurrent control group, • **OR** there is insufficient information provided about how cells were allocated to study groups (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that animals were allocated to study groups using a non-random method including judgment of the investigator, the results of a laboratory test or a series of tests, • **OR** direct evidence that there was a lack of a concurrent control group.

2. Was allocation to study groups adequately concealed?

Definitely Low Risk of Bias (++)
• Direct evidence that at the time of assigning study groups the research personnel did not know what group animals were allocated to, and it is unlikely that they could have broken the blinding of allocation until after assignment was complete and irrevocable. • **Note**: Acceptable methods used to ensure allocation concealment include sequentially numbered treatment containers of identical appearance or equivalent methods.
Probably Low Risk of Bias (+)
• Indirect evidence that at the time of assigning study groups the research personnel did not know what group animals were allocated to and it is unlikely that they could have broken the blinding of allocation until after assignment was complete and irrevocable, • **OR** it is deemed that lack of adequate allocation concealment would not appreciably bias results.

robably High Risk of Bias (-) or (NR)
• Indirect evidence that at the time of assigning study groups it was possible for the research personnel to know what group animals were allocated to, or it is likely that they could have broken the blinding of allocation before assignment was complete and irrevocable, • **OR** there is insufficient information provided about allocation to study groups (record "NR" as basis for answer).

Probably High Risk of Bias (-) or (NR)
Definitely High Risk of Bias (--)
• Direct evidence that at the time of assigning study groups it was possible for the research personnel to know what group animals were allocated to, or it is likely that they could have broken the blinding of allocation before assignment was complete and irrevocable.

3. Did selection of study participants result in the appropriate comparison groups? [NA]

4. Did study design or analysis account for important confounding and modifying variables? [NA]

5. Were experimental conditions identical across study groups?

Definitely Low Risk of Bias (++)
• Direct evidence that same vehicle was used in control and experimental animals, • **AND** direct evidence that non-treatment-related experimental conditions were identical across study groups (i.e., the study report explicitly provides this level of detail).
Probably Low Risk of Bias (+)
• Indirect evidence that the same vehicle was used in control and experimental animals, • **OR** it is deemed that the vehicle used would not appreciably bias results, • **AND** identical non-treatment-related experimental conditions are assumed if authors did not report differences in housing or husbandry.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the vehicle differed between control and experimental animals, • **OR** authors did not report the vehicle used (record "NR" as basis for answer), • **OR** there is indirect evidence that non-treatment-related experimental conditions were not comparable between study groups.
Definitely High Risk of Bias (--)
• Direct evidence from the study report that control animals were untreated, or treated with a different vehicle than experimental animals, • **OR** there is direct evidence that non-treatment-related experimental conditions were not comparable between study groups.

6. Were the research personnel blinded to the study group during the study?

Definitely Low Risk of Bias (++)
• Direct evidence that the research personnel were adequately blinded to study group, and it is unlikely that they could have broken the blinding during the study. Methods used to ensure blinding include central allocation; sequentially numbered treatment containers of identical appearance; sequentially numbered animal cages; or equivalent methods.
Probably Low Risk of Bias (+)
• Indirect evidence that the research personnel were adequately blinded to study group, and it is unlikely that they could have broken the blinding during the study, • **OR** it is deemed that lack of adequate blinding during the study would not appreciably bias results. This would include cases where blinding was not possible, but research personnel took steps to minimize potential bias, such as restricting the knowledge of study group to veterinary or supervisory personnel monitoring for overt toxicity, or randomized husbandry or handling practices (e.g., placement in the animal room, necropsy order).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the research personnel were not adequately blinded to study group, • **OR** there is insufficient information provided about blinding to study group during the study (record "NR" as basis for answer).

Definitely High Risk of Bias (--)
• Direct evidence that the research personnel were not adequately blinded to study group.

7. Were outcome data complete without attrition or exclusion from analysis?

Definitely Low Risk of Bias (++)
• Direct evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study, • **OR** missing data have been imputed using appropriate methods (ensuring that characteristics of animals are not significantly different from animals retained in the analysis). • **Note**: Acceptable handling of attrition includes: very little missing outcome data; reasons for missing animals unlikely to be related to outcome (or for survival data, censoring unlikely to be introducing bias); missing outcome data balanced in numbers across study groups, with similar reasons for missing data across groups; missing outcomes is not enough to impact the effect estimate.
Probably Low Risk of Bias (+)
• Indirect evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study, • **OR** it is deemed that the proportion lost would not appreciably bias results. This would include reports of no statistical differences in characteristics of animals removed from the study from those remaining in the study.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that loss of animals was unacceptably large and not adequately addressed, • **OR** there is insufficient information provided about loss of animals (record "NR" as basis for answer).
Definitely High Risk of Bias (--)
• Direct evidence that loss of animals was unacceptably large and not adequately addressed. • **Note**: Unacceptable handling of attrition or exclusion includes: reason for loss is likely to be related to true outcome, with either imbalance in numbers or reasons for loss across study groups.

8. Can we be confident in the exposure characterization?

Definitely Low Risk of Bias (++)
• Direct evidence that the environmental exposure was independently characterized and purity confirmed generally as ≥98%, • **AND** that exposure was consistently administered (i.e., with the same method and time frame) across treatment groups, • **AND** for dietary or drinking water studies that information is provided on consumption or internal dose metrics to confirm expected exposure levels sufficiently to allow discrimination between exposure groups, • **AND** if internal dose metrics are available, there is evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished, • **AND** if internal dose metrics are available, the study used spiked samples to confirm assay performance.
Probably Low Risk of Bias (+)
• Indirect evidence that the environmental exposure was appropriately characterized and purity confirmed generally as ≥98% (i.e., the supplier of the chemical provides documentation of the purity of the chemical), • **OR** direct evidence that purity was independently confirmed as ≥95% and it is deemed that impurities of up to 5% would not appreciably bias results, • **AND** that exposure was consistently administered (i.e., with the same method and time frame) across treatment groups, • **AND** for dietary or drinking water studies no information is provided on consumption or internal dose metrics,

• **AND** if internal dose metrics are available, there is indirect evidence that most of the exposure data measurements are above the limit of quantitation for the assay such that different exposure groups can be distinguished.
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the exposure (including purity of the test substance and compliance with the treatment, if applicable) was assessed using poorly validated methods, • **OR** there is insufficient information provided about the validity of the exposure assessment method, but no evidence for concern (record "NR" as basis for answer), • **AND** if internal dose metrics are available, there is indirect evidence that most of the exposure data measurements are below the limit of quantitation for the assay such that different exposure groups cannot be distinguished.
Definitely High Risk of Bias (--)
• Direct evidence that the exposure (including purity of the test substance and compliance with the treatment, if applicable) was assessed using poorly validated methods.

9. Can we be confident in the outcome assessment?

Definitely Low Risk of Bias (++)
• Direct evidence that the outcome was assessed using well-established methods (e.g., gold standard), • **AND** assessed at the same length of time after initial exposure in all study groups, • **AND** there is direct evidence that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding prior to reporting outcomes.
Probably Low Risk of Bias (+)
• Indirect evidence that the outcome was assessed using acceptable methods (i.e., deemed valid and reliable but not the gold standard), • **AND** assessed at the same length of time after initial exposure in all study groups, • **OR** it is deemed that the outcome assessment methods used would not appreciably bias results, • **AND** there is indirect evidence that the outcome assessors were adequately blinded to the study group, and it is unlikely that they could have broken the blinding prior to reporting outcomes, • **OR** it is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results, which is more likely to apply to objective outcome measures. • **NOTE** For some outcomes, particularly histopathology assessment, outcome assessors are not blind to study group as they require comparison to the control to appropriately judge the outcome, but additional measures such as multiple levels of independent review by trained pathologists can minimize potential bias. • **NOTE** Acceptable assessment methods will depend on the outcome, but examples of such methods may include: objectively measured antibody or cytokine concentrations with diagnostic methods using commercial kits with some variation, but ability to discriminate between the high dose treatment and control group (or indirect evidence that the assay could have detected a difference based on responses to a positive control).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that the outcome assessment method is an insensitive instrument, • **OR** the length of time after initial exposure differed by study group, • **OR** there is indirect evidence that it was possible for outcome assessors to infer the study group prior to reporting outcomes without sufficient quality control measures, • **OR** there is insufficient information provided about blinding of outcome assessors (record "NR" as basis for answer).

Definitely High Risk of Bias (--)
• Direct evidence that the outcome assessment method is an insensitive instrument, • **OR** the length of time after initial exposure differed by study group, • **OR** there is direct evidence for lack of adequate blinding of outcome assessors, including no blinding or incomplete blinding without quality control measures.

10. Were all measured outcomes reported?

Definitely Low Risk of Bias (++)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported. This would include outcomes reported with sufficient detail to be included in meta-analysis or fully tabulated during data extraction and analyses had been planned in advance.
Probably Low Risk of Bias (+)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** analyses that had not been planned in advance (i.e., retrospective unplanned subgroup analyses) are clearly indicated as such and deemed that unplanned analyses were appropriate and selective reporting would not appreciably bias results (e.g., appropriate analyses of an unexpected effect). This would include outcomes reported with insufficient detail such as only reporting that results were statistically significant (or not).
Probably High Risk of Bias (-) or (NR)
• Indirect evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have been reported, • **OR** and there is indirect evidence that unplanned analyses were included that may appreciably bias results, • **OR** there is insufficient information provided about selective outcome reporting (record "NR" as answer basis).
Definitely High Risk of Bias (--)
• Direct evidence that all of the study's measured outcomes outlined in the protocol, methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. In addition to not reporting outcomes, this would include reporting outcomes based on composite score without individual outcome components or outcomes reported using measurements, analysis methods or subsets of the data (e.g., subscales) that were not pre-specified or reporting outcomes not pre-specified, or that unplanned analyses were included that would appreciably bias results.

11. Were there no other potential threats to internal validity?

Definitely Low Risk of Bias (++)
• There is direct evidence that other exposures anticipated to bias results were not present or were appropriately measured and adjusted for.
Probably Low Risk of Bias (+)
• There is evidence (direct or indirect) that other co-exposures anticipated to bias results were not present or were appropriately adjusted for.
Probably High Risk of Bias (-) or (NR)
• There is indirect evidence that there was an unbalanced provision of additional co-exposures across the primary study groups, which were not appropriately adjusted for.
Definitely High Risk of Bias (--)
• There is direct evidence that there was an unbalanced provision of additional co-exposures across the primary study groups, which were not appropriately adjusted for.

There are no other environmental-exposure-specific additions to the risk-of-bias questions for this evaluation. This question will be used to examine individual studies for appropriate statistical methods (e.g., confirmation of homogeneity of variance for ANOVA and other statistical tests that require normally distributed data). It will also be used for risk-of-bias considerations that do not fit under the other questions.