NTP Evaluation Concept: Inflammation-based Atherosclerosis Associated with Environmental Exposures

Project Leader

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Background and Rationale

Inflammation is a cellular response to chemical damage, physical damage, or infection that can be part of a healthy restorative process or contribute to further tissue injury and disease when the response becomes chronic. The extent to which environmental exposures ultimately lead to adverse health effects through an inflammatory pathway remains unclear despite growing evidence for a role of the environment in a wide range of diseases that involve inflammation.

Acute inflammation is initiated by cells at the site of injury, mainly resident macrophages and dendritic cells that release inflammatory mediators such as cytokines and acute phase proteins responsible for vasodilation, increased permeability of blood vessels, and migration of leukocytes. The immune cells at the site of tissue damage operate in a complex network with the epithelium to produce and release a range of inflammatory signaling molecules including cytokines, chemokines, leukotrienes, and adhesion molecules (Ross 1999, Germolec *et al.* 2010). Depending on the extent of the injury or infection, the reaction orchestrated in this acute inflammatory response may be sufficient to remove the damaged cells or infectious agent and resolve the inflammation.

In contrast, persistent inflammation from prolonged exposure to stimuli can lead to chronic inflammation, long-term elevation of inflammatory mediators, and tissue damage. Chronic inflammation is reported to contribute to numerous health effects including multiple cardiovascular and pulmonary conditions, cancer, arthritis, autoimmune diseases, diabetes, and neurological disorders such as Alzheimer's disease (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).

The Office of Health Assessment and Translation (OHAT) proposes to examine the evidence that environmental substances contribute to inflammation that ultimately leads to health effects and to identify biomarkers of the inflammation involved. This evaluation concept stems from discussions with the NIEHS Strategic Plan Cross-Divisional Implementation Planning Committee on Inflammation. The committee is strongly interested in work that would contribute to the identification of biomarkers of environmentally-induced inflammation and identified the need for an evaluation of the available evidence that environmental triggers of inflammation lead to health effects.

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 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 biomarkers of inflammation linked to the health effect, and 3) to select a health effect with a manageable database of relevant studies.

Atherosclerosis

Atherosclerosis was selected as the endpoint because of the significant public health impact of the disease and the role of atherosclerosis as one of the dominant conditions underlying cardiovascular

disease and stroke. There is a well-established role for inflammation in the disease process leading to atherosclerosis (Ross 1999, Pearson *et al.* 2003, Rosenfeld and Campbell 2011). Environmental xenobiotics with reported associations to atherosclerosis include bisphenol A (BPA), diesel exhaust particles, particulate matter, persistent organic pollutants (POPs), phthalates, metals, and second hand tobacco smoke (Brook and Rajagopalan 2010, Kallio *et al.* 2010, Lind and Lind 2011, Lind *et al.* 2012, Schwarze *et al.* 2013). For many of the xenobiotics there is evidence for an association with both inflammation and atherosclerosis.

In addition to xenobiotics, there are also a number of infectious agents that reportedly contribute to atherosclerosis including cytomegalovirus (CMV), *Chlamydia pneumoniae, Porphyromonas gingivalis, Helicobacter pylori*, hepatitis C virus (HCV), human immunodeficiency virus (HIV), and influenza A virus (Rosenfeld and Campbell 2011, Tufano *et al.* 2012). While not the main focus of the evaluation, data linking infectious agents to atherosclerosis through an inflammatory mechanism will be used to support potential inflammatory biomarkers and pathways linking exposure to inflammation and ultimately to atherosclerosis (i.e., data on potential inflammatory biomarkers can be compared between xenobiotics and infectious agents to evaluate support for the association with atherosclerosis).

Key Issues

There are two major challenges for this evaluation: (1) addressing the extent to which conclusions can be integrated across the variety of environmental exposures with data on inflammation that may contribute to atherosclerosis, and (2) selecting inflammatory biomarkers associated with atherosclerosis (i.e., biomarkers with some evidence of a link to atherosclerosis will be included in the evaluation and biomarkers without that link will be excluded). The identification of inflammatory biomarkers associated with health effects is complicated by the need to distinguish chronic inflammation from acute inflammation, which is part of a healthy response to resolve tissue damage and initiate healing.

Specific Aims

To the extent possible, if the data support the analyses, the overall objective is to develop hazard identification conclusions as well as level-of-concern[1] conclusions that environmental substances contribute to inflammation that leads to atherosclerosis. This objective will be addressed by answering the key questions (KQ) for the proposed evaluation as outlined in Table 1. An additional objective is to evaluate the evidence for specific biomarkers of environmentally-induced inflammation linked to atherosclerosis (see proposed approach below for planned analyses to address this second objective).

Table 1: Key Questions (KQ)	
KQ1	What is our confidence in the body of evidence from human studies for the association between exposure to environmental substances and inflammation that may contribute to atherosclerosis?
KQ2	What is our confidence in the body of evidence from animal studies for the association between exposure to environmental substances and inflammation that may contribute to atherosclerosis?
KQ3	How does the evidence from other relevant studies (e.g., mechanistic or *in vitro* studies) support or refute the biological plausibility of the association between exposure to environmental substances and inflammation that may contribute to atherosclerosis?

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[1] OHAT is the process of updating the guidance on how hazard identification conclusions will be used to reach level-of-concern conclusions. Updated draft guidance for reaching level-of-concern conclusions is anticipated for release in 2015.
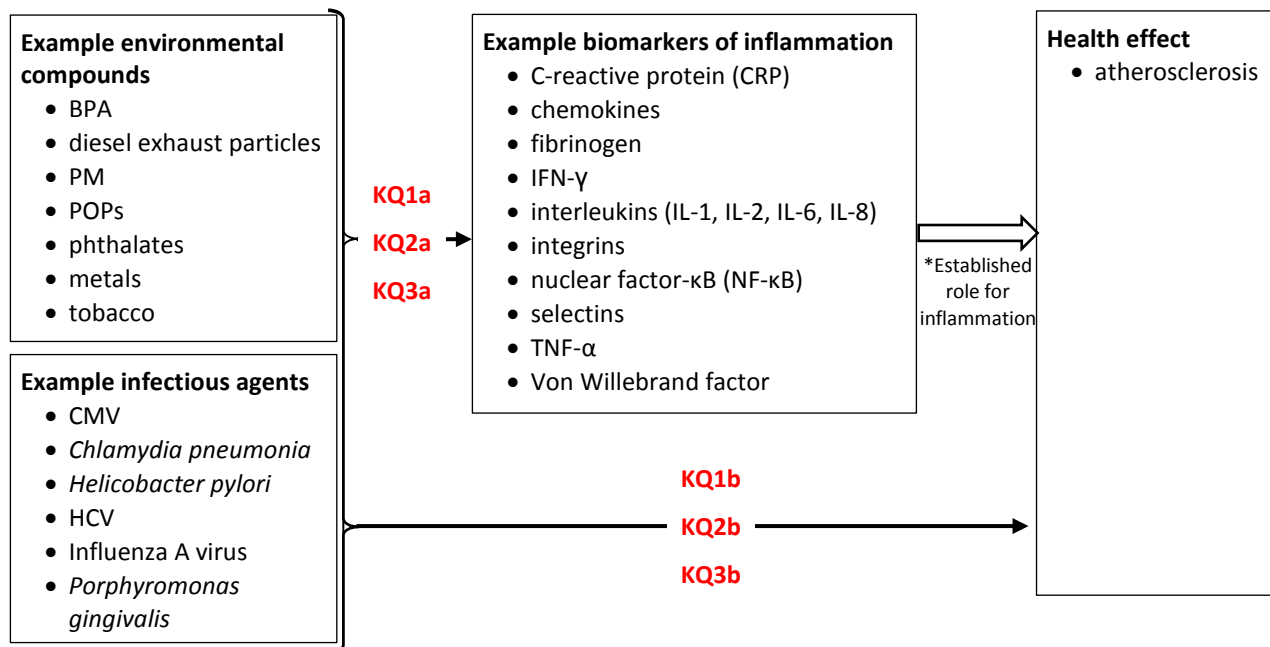
Step 1

The evaluation will proceed stepwise, by first assessing the aggregate data for all exposures and developing confidence conclusions on the body of evidence that environmentally-induced inflammation is associated with the development or exacerbation of atherosclerosis. Ratings will indicate confidence that the study findings accurately reflect the true association between exposure and effect.

Step 2

Confidence conclusions would also be developed for individual substances with a sufficient literature base. These substances would then move forward for the development of exposure-specific hazard identification conclusions ("known", "presumed", "suspected", or "not classifiable") by integrating evidence from human, animal, and other relevant studies (e.g., mechanistic or *in vitro* studies) for the association between environmentally-induced inflammation and atherosclerosis. To the extent warranted for a particular substance, the evaluation would also reach a level-of-concern opinion on whether the substance may be of concern for inducing inflammation that contributes to atherosclerosis given what is known about current human exposure levels.

Figure 1 illustrates how the key questions address the two steps linking exposure and the health effect through inflammation. For each evidence stream (human, animal, other relevant data) the evaluation will examine the evidence that environmental exposure (a) contributes to inflammation and (b) contributes to atherosclerosis. The degree to which the data address the necessary temporal association such that inflammation precedes the development of atherosclerosis will also be considered. For example, some support that environmentally-induced inflammation contributes to atherosclerosis would be provided by an exposure that is linked to both inflammation and to atherosclerosis. Additional data supporting the temporal sequence (i.e., exposure→inflammation→atherosclerosis) would increase the confidence in relationship that environmentally-induced inflammation is associated with the development or exacerbation of atherosclerosis.

Figure 1: Analytical framework

Example environmental compounds
- BPA
- diesel exhaust particles
- PM
- POPs
- phthalates
- metals
- tobacco

KQ1a
KQ2a
KQ3a

Example biomarkers of inflammation
- C-reactive protein (CRP)
- chemokines
- fibrinogen
- IFN-γ
- interleukins (IL-1, IL-2, IL-6, IL-8)
- integrins
- nuclear factor-κB (NF-κB)
- selectins
- TNF-α
- Von Willebrand factor

*Established role for inflammation

Health effect
- atherosclerosis

Example infectious agents
- CMV
- *Chlamydia pneumonia*
- *Helicobacter pylori*
- HCV
- Influenza A virus
- *Porphyromonas gingivalis*

KQ1b
KQ2b
KQ3b

*Note: Atherosclerosis was selected as the health effect because there is a well-established role for inflammation in the disease process leading to atherosclerosis. The evaluation will not review the evidence that inflammation contributes to atherosclerosis.

Proposed Approach

OHAT will continue to work with the cross-divisional committee on inflammation as we further refine the focus and objectives for this project. In addition to discussion with NIEHS/NTP scientists with expertise on inflammation and associated health effects, OHAT has solicited input from scientists at other federal agencies working on inflammation as well as cardiovascular health effects including scientists at the Environmental Protection Agency (EPA), National Heart, Lung, and Blood Institute (NHLBI), and the National Institute for Occupational Safety and Health (NIOSH).

The proposed evaluation would include two complementary approaches: 1) a systematic review to evaluate the evidence that environmental substances contribute to inflammation that results in atherosclerosis and 2) development of an adverse outcome pathway (AOP) for environmental influences on inflammation-based atherosclerosis to aid in the identification of inflammatory biomarkers, uncertainties, and data gaps.

OHAT Approach

The evidence that environmental substances contribute to inflammation that results in atherosclerosis will be evaluated using the OHAT approach to systematic review and evidence integration (http://ntp.niehs.nih.gov/go/38673). After additional steps to refine the project and develop a protocol, the protocol will be posted and other key milestones in the evaluation will be announced on the NTP listserv (e.g., posting list of included studies). Data management will be conducted in a manner that permits public sharing of the exploratory literature search results as well as data extracted from studies in a database format when the monograph is finalized following peer-review. Sharing of data in this format should facilitate future updates to this evaluation conducted by NTP or other organizations.

Adverse outcome pathway

An AOP is a conceptual framework that represents and displays the knowledgebase for a toxicological response concerning the linkage between a molecular initiating event (e.g., direct molecular interaction between a toxicant and a specific biomolecule) and an adverse outcome at the organism level or population level (Ankley *et al.* 2010, OECD 2012)(see supplemental material for additional background on AOPs).

Development of an AOP for environmental influences on inflammation-based atherosclerosis is proposed as part of this evaluation to provide another tool to organize and integrate all levels of toxicity data in addition to a literature-based systematic review. The AOP is anticipated to be useful in identifying data gaps and uncertainties in the pathway linking environmental exposures to inflammation and then to atherosclerosis. This will include a clear presentation of the experimental support and the strength of the evidence for each of the events in the AOP and the associated linkages as well as a narrative discussion of the confidence in the AOP.

The AOP will be used to address the second objective of the evaluation, and to evaluate the evidence for specific biomarkers (either individually or as a collection of cellular or organ-level responses) of environmentally-induced inflammation linked to atherosclerosis. The confidence conclusions on the AOP and the strength of evidence conclusions on the events within the AOP will be used to inform the evaluation of specific biomarkers.

The development of the AOP will be conducted in an effort to take advantage of the observations on the effects of a wide range of compounds on biological pathways from the Tox21 program. It is anticipated that this approach may be particularly useful in identifying potential molecular initiating events and cellular responses that may be biomarkers of inflammation that may be part of the Tox21 dataset. Of particular interest will be data from the Diversity Plus Panel of BioMap Systems from BioSeek, which includes multiple high throughput assay systems relevant for inflammation and inflammatory diseases.

The use of an AOP to help organize and explore the high throughput data for inflammatory markers may also provide novel suggestions of chemicals with the potential for inflammatory activity based on the high throughput data.

Significance

The proposed evaluation will reach confidence conclusions on the body of evidence that environmentally-induced inflammation is associated with the development or exacerbation of atherosclerosis. With a sufficient literature base for any particular substance, the evaluation will reach exposure-specific hazard identification conclusions for the association between environmentally-induced inflammation and atherosclerosis. To the extent data warrant for a particular substance, the evaluation would also reach a level-of-concern opinion on whether the substance may be of concern for inducing inflammation that contributes to atherosclerosis given what is known about current human exposure levels.

The confidence conclusions on specific biomarkers or potential groups of biomarkers of environmentally-induced inflammation associated with atherosclerosis should facilitate the selection and use of biomarkers in future studies of environmentally-induced inflammation. The development of an AOP for environmental influences on inflammation-based atherosclerosis is also expected to inform the identification of biomarkers of inflammation and to identify data gaps and contribute to research recommendations. The conclusion on atherosclerosis also have the potential to lay the groundwork as an approach for evaluating the contribution of environmentally-induced inflammation to a wider range of health effects, potentially starting with cardiovascular health effects in general.

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SUPPLEMENTAL MATERIAL

Additional background on adverse outcome pathways

An adverse outcome pathway (AOP) is a conceptual framework that represents and displays the knowledgebase for a toxicological response concerning the linkage between a molecular initiating event (e.g., direct molecular interaction between a toxicant and a specific biomolecule) and an adverse outcome at the organism-level or population level (Figure 1) (Ankley *et al.* 2010, OECD 2012). Every AOP begins with the molecular initiating events in which a substance interacts with a biological target (Figure S1, anchor 1). The initiating events lead to a sequential series of events that span different levels of biological organization (cellular, organ) that lead to an adverse outcome (Figure S1, anchor 2).
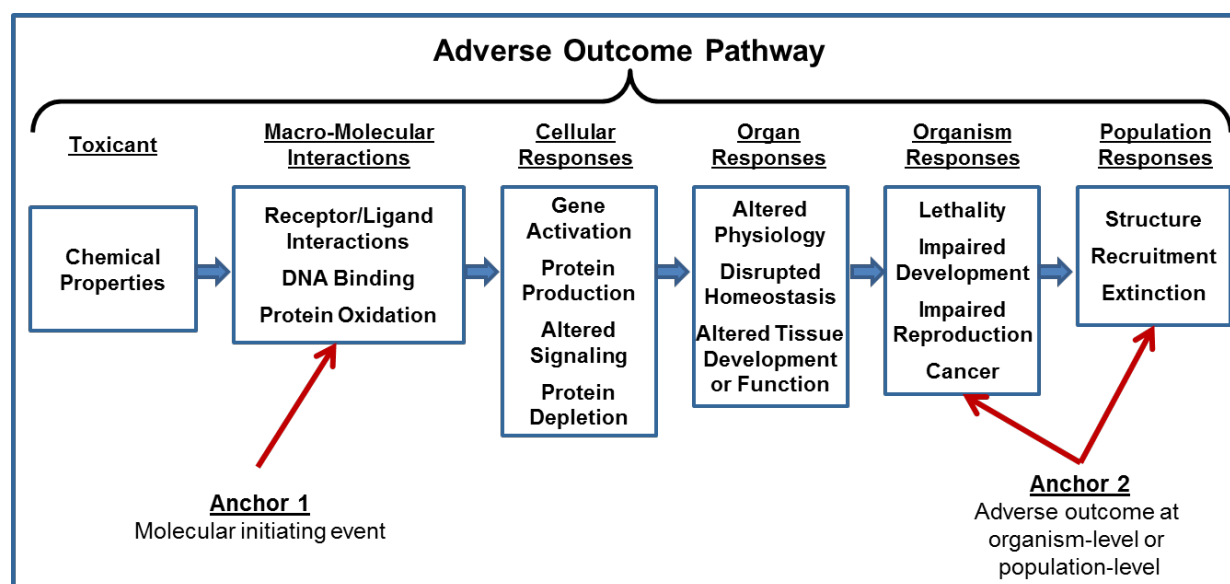

Figure S1: An Adverse Outcome Pathway (AOP) is a conceptual framework that displays the knowledgebase for a toxicological response and shows the linkage between a molecular initiating event in which a substance interacts with a biological target (anchor 1) and an adverse outcome at the organism or population level (anchor 2). The framework illustrates the sequential series of events leading to the outcome that span different levels of biological organization (i.e., cellular and organ) and identifies the strength of the evidence supporting each relationship (e.g., "plausible linkage with limited data") (Ankley *et al.* 2010, OECD 2012).

An AOP can be used to display a relatively complete knowledgebase or to identify data gaps and hypothetical linkages. The intermediate events connecting the initiating event to the outcome can comprise different levels of complexity that may include multiple organ interactions depending on the extent of the biological information available and the endpoint modeled (OECD 2012). Relationships between sequential levels of biological organization may be causal, mechanistic or inferential and are identified as such (e.g., "plausible linkage with limited data" and "established mechanistic linkage with quantitative data" (Ankley *et al.* 2010)).

A critical aspect of recent guidance on development of an AOP (OECD 2012) is the assessment and review of the AOP to gauge the reliability and robustness of the model. This can be accomplished at multiple steps during development of the AOP and relies on a clear presentation of the experimental support and the strength of the evidence for each of the events in the AOP and the associated linkages.