Space health effects informed through application of the Adverse Outcome Pathway framework

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Abstract

The scientific evidence required to make policy decisions that protect human health can be challenging to organize. Primary research is often silo-ed between different agency repositories, the pace of publication is unflagging and wide-spread interdisciplinary collaboration can be logistically difficult. Since 2012, the Organisation for Economic Co-operation and Development (OECD) adverse outcome pathway (AOP) framework has provided solutions for some of the challenges of supplying relevant and accessible scientific data for evidence-based decision-making. Development of AOPs is guided by a crowd-sourced approach in which progressions of adverse outcomes (AO) are distilled into pathways containing only the essential key events (KEs) and the causal key event relationships (KERs) that connect them. The framework has widely been adopted in the toxicology community and more recently projects have applied it to the radiation safety field. Presently, a collaborative effort aims to further expand the use of AOPs through creating a network linking exposure to the space exposome with resulting human health outcomes. The network contains four adverse non-cancer outcomes for which participants of future long-range space missions will be at risk. The work of this thesis has contributed to the construction of the space-health AOP network by accomplishing two main objectives. The first was the creation of a novel protocol for collecting a weight of evidence (WOE) that included the benefits of scoping review and artificial intelligence (AI) tools for literature screening. The scoping review WOE collection strategy was then deployed for collecting data across all four outcomes in the space-health network. The second objective was to identify KEs and KERs and summarize the WOE linking space exposure to one of the four AOs: vascular remodeling. In addition to summarizing the pathway, we have also highlighted important modulating factors and knowledge gaps in the WOE. This thesis work contributes to the future of the AOP framework

by formulating a new development protocol and employing it in a novel regulatory context. Using the new protocol, this thesis has furthered biological understanding of the effects of space exposure on the cardiovascular system by collating mechanistic information across scientific disciplines to identify KEs and KERs in occurrence of vascular remodeling.

Résume

Il peut être difficile d'organiser les preuves scientifiques nécessaires pour prendre des décisions politiques qui protègent la santé humaine. La recherche primaire est souvent cloisonnée entre différents dépôts d'agences, le rythme de publication est ininterrompu et une collaboration interdisciplinaire à grande échelle peut être difficile sur le plan logistique. Depuis 2012, le cadre de l'Organisation de Coopération et de Développement Économiques (OCDE) relatif aux chemins de l’effet néfaste (AOP en anglais) apporte des solutions à certains des problèmes liés à la fourniture de données scientifiques pertinentes et accessibles pour la prise de décisions fondées sur des données probantes. L'élaboration des AOP est guidée par une approche participative dans laquelle l'évolution des effets néfastes (AO en anglais) est distillée en voies ne contenant que les événements clés (EC) essentiels et les relations causales entre événements clés (KER en anglais) qui les relient. Ce cadre a été largement adopté par la communauté toxicologique et, plus récemment, des projets l'ont appliqué à l'espace de la radioprotection. Actuellement, un effort de collaboration vise à étendre l'utilisation des AOP en créant un réseau reliant l'exposome spatial aux conséquences qui en découlent pour la santé humaine. Le réseau contient quatre effets néfastes, non cancéreux, pour lesquels les participants aux futures missions spatiales de longue durée seront à risque. Le travail de cette thèse a contribué à la construction du réseau d'AOPs espace-santé en accomplissant deux objectifs principaux. Le premier était la création d'un nouveau protocole pour la collecte des éléments de preuve (WOE en anglais) qui comprenait les avantages de l'examen de la portée et des outils d'intelligence artificielle (IA) pour la sélection de la littérature. La stratégie de collecte des WOE a ensuite été déployée pour recueillir des données sur les quatre résultats du réseau espace-santé. Le deuxième objectif était d'identifier les KE et KER et de résumer les WOE reliant l'exposition spatiale à l'un des quatre

AO : le remodelage vasculaire. En plus de résumer la voie, nous avons également souligné les facteurs modulateurs importants et les lacunes dans les connaissances du WOE. Ce travail de thèse contribue à l'avenir du cadre des AOPs en formulant un nouveau protocole de développement et en l'employant dans un nouveau contexte réglementaire. En outre, il a permis d'approfondir la compréhension biologique des effets de l'exposition à l'espace sur le système cardiovasculaire en rassemblant les informations mécanistiques de plusieurs disciplines scientifiques afin d'identifier les KE et KER dans l'apparition du remodelage vasculaire.

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List of Abbreviations

AI - Artificial Intelligence

AOP – Adverse Outcome Pathway

AO – Adverse Outcome

BH – Bradford Hill

BH_4 - Tetrahydrobiopterin

CMVEC - Cerebral Microvascular Endothelial Cells

CVD – Cardiovascular Disease

CSA – Canadian Space Agency

DIASY - DistillerSR Artificial Intelligence System

DOX - Doxepin hydrochloride

eNOS – Endothelial Nitric Oxide Synthase

GCR – Galactic Cosmic Rays

Gy – Gray

HAoEC - Human Aortic Endothelial Cells

HUVEC - Human Umbilical Vein Endothelial Cells

HZE - High (H) Atomic number (Z) Energy (E) particles

IL-1 - Interleukin-1

ICAM-1 - Intercellular Adhesion Molecule 1

iNOS – Inducible Nitric Oxide Synthase

KE – Key Event

KER – Key Event Relationship

LEO – Low Earth Orbit

MIE – Molecular Initiating Event

mTOR - mammalian/mechanistic target of rapamycin

NASA - National Aeronautics and Space Administration

NADPH - Nicotinamide adenine dinucleotide phosphate

NO – Nitric Oxide

NOX - Nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase

OECD - Organisation for Economic Co-operation and Development

PI3K - Phosphoinositide 3-kinase

PECO – Population, exposure, comparator, and outcome

PEOE – Population, exposure, outcome and endpoint

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RICVD – Radiation Induced Cardiovascular Disease

ROS – Reactive Oxygen Species

RNS – Reactive Nitrogen Species

SEM – Systematic Evidence Map

SOD – Superoxide Dismutase

SWIFT - Sciome Workbench for Interactive computer-Facilitated Text-mining

TIME - Telomerase-immortalized Microvascular Endothelial cells

TICAE - Telomerase-immortalized Coronary Artery Endothelial cells

TNF-α – Tumor necrosis factors alpha

WOE – Weight of Evidence

Statement of Contributions

Chapter 2 – Scoping Reviews and AOPs (Kozbenko et al. 2022)

- **Manuscript preparation –** Tatiana Kozbenko
- **Preliminary pathway creation –** Vita Lai, Nadine Adam, Vinita Chauhan
- **Literature Screening –** Snehpal Sandhu, Jacqueline Kuan, Veronica Grybas, Danicia Flores, Tatiana Kozbenko, Marieke Groot, Papiha Joharapurkar
- **Scoping Review protocol creation** - Tatiana Kozbenko, Vinita Chauhan, Vita Lai, Snehpal Sandhu, Jacqueline Kuan, Danicia Flores
- **Literature Searches –** Robyn Hocking
- **SEM creation protocol –** Hannah Parker, Tatiana Kozbenko
- **Expert consultation –** Katya Tsaioun
- **Manuscript Review –** Vinita Chauhan, Carole Yauk, Benny Ling, Dave Stieb

Chapter 3 - CVD AOP Report

- **Manuscript preparation –** Tatiana Kozbenko
- **KER Description Writing –**Veronica Grybas, Dalya Alomar, Benjamin Smith; Janna Abdelaziz, Amanda Pace, Tatiana Kozbenko
- **KE Description Writing –** Veronica Grybas, Dalya Alomar, Benjamin Smith, Mitchell Keyworth, Tatiana Kozbenko
- **Expert consultation –** Marjan Boerma, Omid Azimzadeh, Steve Blattnig, Nobuyuki Hamada, Carole Yauk
- **Manuscript Review –** Vinita Chauhan, Carole Yauk

Chapter 1 – Introduction

1.1 Project context

This thesis work is a part of a larger partnership between Health Canada, the Canadian Space Agency (CSA), the National Aeronautics and Space Administration (NASA) and the University of Ottawa. The overall aim of this collaboration is to use the benefits of the adverse outcome pathway (AOP) framework to organize information about the adverse health effects of space travel. The principles of AOP development provide guidance for identifying the essential events in adverse outcome (AO) progression through considering data across all disciplines and levels of biological organization. The identification of key events (KEs) further streamlines evidence collection and evaluation and can also inform possible points of intervention for prevention of the AO. A space-health AOP network will provide a summary of key data that can be used to create policy as well as outline knowledge gaps that will direct future research work.

1.2 Why space flight health outcomes?

Space and space travel are unequivocally dangerous for humans; the numerous confounding stressors, such as microgravity, ionizing radiation and altered atmospheric gases, create a hostile environment for all those venturing off the blue marble of Earth (Mcphee & Charles, 2009; Patel et al., 2020; Vernice et al., 2020). Of all the stressors present in space, radiation is of particular interest to risk assessment due to its known potential to induce a variety of adverse health outcomes and the difficulty of shielding astronauts from its effects (Cucinotta et al., 2006). Space travelers are exposed to radiation from numerous sources, such as from the Van Allen radiation belt, galactic cosmic rays (GCRs) and solar particle events (Baker et al., 2011; Boerma et al., 2016; Durante & Cucinotta, 2008; Furukawa et al., 2020). In contrast to space travel, those on Earth are conferred protection from a large portion of space radiation by the earth's magnetosphere (Wei et al., 2012). However, future space missions aim to travel outside of this zone of protection, with NASA projecting manned space missions to Mars as early as the 2030s (https://www.nasa.gov/what-is-artemis). These missions will extend far past the current records for both time spent, and distance travelled in space, and will venture far outside the Earth's magnetosphere.

In order to assess associated risks and identify countermeasures to protect astronauts, an understanding of the current state of knowledge on molecular, cellular, tissue and system level effects needs to be better characterized. While space travel can affect many aspects of human physiology (Garrett-Bakelman et al., 2019), effects on the cardiovascular system are of high concern for long range travel (Patel et al., 2020) and are the focus of the second half of this thesis. An approach to support the endeavor for characterizing the current state of knowledge is the Organisation for Economic Co-operation and Development's (OECD) AOP knowledge framework.

1.3 Adverse Outcome Pathway Framework

Launched in 2012 by the OECD, the AOP knowledge framework is a method to organize research findings in a way that makes them accessible for risk assessors and regulatory decision makers (OECD, 2017). The results of primary research can often be silo-ed in knowledge repositories such as academic journals, government databases or in archives within academic institutions. The AOP framework aims to facilitate knowledge transfer between all available sources by acting as a single repository for information from diverse levels of biological organization across taxa, life stage and time. In addition, AOP development readily leads to the identification of knowledge gaps, which is useful for prioritizing further research and reducing duplicative efforts.

The introduction of comprehensive guidelines for the development and evaluation of AOPs fosters transparency and regulatory utility. Conventions and instructions for the construction of AOPs are available in the OECD Users' Handbook (OECD, 2018) as well as in the extensive training module found on the AOP-Wiki website (aopwiki.com). The following information about AOP creation has been adapted from these sources.

In the broadest terms, an AOP is an analytical construct that illustrates the chain of events occurring in a biological system due to exposure to a toxicant or stressor. It spans all levels of biological organization, starting from the smallest and progressing to whole-organism or population effects.

Each AOP consist of the following components (Figure 1.1).

a) Key Event (s) (KE) - KEs in each pathway represent a measurable change in a given biological state that is essential (but not necessarily sufficient) to trigger the subsequent KEs.
b) Key Event Relationship(s) (KER) - The KEs in a pathway are causally linked, and this interconnectivity is defined as KERs. KERs can link adjacent KEs in the pathways or can represent linkages of non-adjacent KEs. For example, double strand DNA breaks leading to chromosomal aberrations.
c) Molecular Initiation Event (MIE) - an MIE is a specialized KE at the lowest level of biological organization. The MIE is the molecular perturbation that initiates the pathway.
d) Adverse Outcome(s) (AO) - AOs are a specialized KE that are of regulatory interest. They can be at an individual organ (ex. lung cancer), individual (ex. decreased fertility), or a population level (ex. extinction) and represent the end of a given pathway.

There are also five principles for the development of AOPs

1 AOPs are not chemical (or stressor) specific; they do not describe the effect of a given toxicant, instead they describe the chain of events that any toxicant that perturbs the MIE is likely to cause.
2 AOPs are modular, consisting of KEs and KERs that can be shared between two or more pathways. Individual pathways can overlap through shared KEs and in turn create a combined AOP network.
3 An individual AOP is a pragmatic unit of development and evaluation. By convention, most individual AOPs describe a single possible path from MIE to AO; it is not a representation of all possible routes to a given outcome.
4 For most real-world applications, AOP networks - not individual pathways - are the functional unit of prediction. The complexity of a biological question cannot be captured in any single given pathway. As such, when using AOPs to answer regulatory questions, networks of intersecting pathways are the functional construct.

An important aspect to highlight is that AOPs constitute a purposefully simplified biological representation of the complex biology. Their purpose is not to describe all effects and perturbations resulting from a toxicant exposure. Instead, each pathway only contains the KEs that are both essential to the manifestation of the AO as well as measurable. It is this simplicity that makes them an efficient and pragmatic tool for risk assessment.

The causal relationship described in KERs is supported by a weight of evidence (WOE) analysis that applies modified Bradford-Hill criteria. First described in 1965, the Bradford-Hill criteria are a collection of principles used in epidemiology and toxicology to define a causal relationship between a presumed cause and observed effect (Hill, 1965). The Bradford-Hill criteria have since been modified and used in mode of action (MOA) analysis for single chemical toxicants (Becker et al., 2015). The modified criteria used to evaluate the WOE for AOPs is a subset of those used in MOA analysis (Table 1.1).

1.4 WOE collection for AOPs: Current Practices and Considerations

The OECD guidance document (OECD, 2017) and accompanying supplemental AOP handbook (OECD, 2018) describe the governing principles of AOP development and their practical application. Additionally, Villeneuve et al. (2014) outline best practices for AOP description writing while Becker et al. (2015) detail how to consider BH criteria when selecting supporting evidence (Becker et al., 2015; Villeneuve et al., 2014). Together these references arm developers with extensive guidance for what should be included in a completed AOP. Following the submission of an AOP, there is also a rigorous review process to ensure the pathway and its WOE adhere to all prerequisites of the framework. Yet, for all of the requirements of the final product, currently there is no standardization for methodology for WOE collection. This approach has its benefits; developers are able to use their expertise in a given research field to collate the data known to be relevant, potentially accelerating AOP development. Furthermore, ensuring that contributing to the knowledge base is straightforward and accessible to the broad scientific community is critical for the crowd-sourced framework, and too many restrictions could impede input. The flip side is a lack of transparency in selection of literature making up the WOE, which impacts the level of confidence assigned by the developers.

At its core, collecting an AOP WOE is reviewing and synthesizing literature, and there is growing interest in exploring how the framework could benefit from incorporating approaches from parallel work in the systematic literature review space (de Vries et al., 2021). Literature synthesis in the form it takes today, is commonly attributed to Dr. Archie Cochrane whose work in the late 20th century motivated the creation of the Cochrane collaboration (Chalmers, 1993; Cochrane, 1989). Originally applied in the healthcare field, the general principles of a structured literature review center around setting standardized requirements for incorporating methodology to reduce statistical imprecision and bias (Chalmers et al., 2002). Much work has been done since the inception of the Cochrane framework, and today a plethora of sophisticated tools exist to provide confidence and objectivity in literature synthesis results. These include tools like the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) that provides checklists for attributes to be included in all systematic review protocols (Page et al., 2021), and the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool that guides impartial evaluation of internal study validity (OHAT, 2019).

Approaches to literature review can be divided into several categories. Currently, most AOPs are created using what can be described as a narrative review, which have few methodological requirements and in which experts select relevant references based on their experience and opinion (Gasparyan et al., 2011). On the opposite end of the spectrum is full-scale systematic review (SR). SR centers around a clearly defined research objective and must meet exact specifications in search strategy, screening, data extraction, literature appraisal and synthesis, structured characterization of confidence, and assessment of study quality in order to be considered truly systematic (Higgins et al., 2019). The interest for including SR approaches (de Vries et al., 2021) stems from the transparency, comprehensiveness and reproducibility of the SR methodology. Additionally, structure in evidence collection methods could benefit new developers by laying out clear steps for literature review.

However, a WOE that supports an AOP network includes peer-reviewed research from multiple research fields and of various study designs. Due to this breadth, using a standard SR to collect a WOE is not a desirable approach; this is because there is no single question around which the work is organized. Fortunately, there exists a moderate option in the form of scoping reviews and their resulting systematic evidence maps (SEMs). First described by Arskey and O'Malley in 2005, scoping reviews are more flexible in approach and aim to broadly describe evidence within a given literature space (Arksey & O'Malley, 2005). While there is still guidance for their structure and methodological components for literature synthesis, they are far less prescriptive than their SR counterparts (Munn et al., 2018; Peters et al., 2015). Scoping reviews and SEMs are similar in that they are both methods for asking open-ended questions and identifying trends across a field of research (Wolffe et al., 2019). Where they differ, is in their manner of presenting their findings; scoping reviews use narrative or tabular format, while systematic evidence maps are used for cases when the information can be explored through data visualization (Miake-Lye et al., 2016; Wolffe et al., 2019). Figure 1.2 from (Wolffe et al., 2019) illustrates the differences in goals of a SEM versus a focused SR.

A growing body of research has begun to incorporate aspects of the SR framework for various contributions to the AOP framework. For example, Halappanavar et al. (2019) detail the process of systematically establishing KEs for nanomaterial relevant AOPs (Halappanavar et al., 2019). In this work, references are screened using clearly described inclusion/exclusion criteria, a quality evaluation tool was tested, and the flow of literature through the study was recorded and published (Halappanavar et al., 2019). Waspe and Beronius (2022) have used similar tools in the creation of an AOP linking intrahepatic cholestasis and preterm birth (Waspe & Beronius, 2022). Their work catalogues the literature search strategy, screening methodology, inclusion criteria, and resulting flow of literature (Waspe & Beronius, 2022). Lastly, Huliganga et al. (2022) have tested structured literature search and screening methods for the incorporation of a

KE into a pre-existing AOP (Huliganga et al., 2022). Together these studies reflect the interest of the AOP community in moving towards evidence-based approaches for WOE collection.

1.5 Thesis Objectives

With these considerations in mind, this thesis had the following main objectives:

a) **Create a protocol for AOP WOE collection that includes the benefits of scoping review and AI tools.**

   Specifically, create a workflow that incorporates structured literature searches, AI assisted literature prioritization, a multiple reviewer format, and predetermined inclusion criteria, amongst other benefits. Additionally, explore recommendations for a flexible fit-for-purpose approach for future AOP development teams looking to adapt the protocol for their unique needs. Finally, apply this workflow to collect evidence to support all four outcomes in the space-health network.

b) **Summarize the collected evidence linking exposure to the space environment with vascular remodeling in the form of an AOP report.**

   Provide an overview of the KEs in the cardiovascular AOP pathway and the empirical evidence supporting the KERs that connect them. Furthermore, summarize the impact of any modulating factors and highlight gaps in the knowledge base.

1.6 The Team

The construction of a space-health AOP network is an ambitious undertaking. The completion of this overarching project has been the work of a team consisting of members from multiple organizations. The core team consists of members from the Health Canada Consumer and Clinical Radiation Protection Bureau including Dr. Ruth Wilkins (Division Chief), Dr. Vinita Chauhan (Senior Scientist), Nadine Adams (Technician), Vita Lai (Technician), and Robyn Hocking (Librarian). Co-op, honours, and placement students have also joined for the duration of their work term requirements. As of September 2022, the student team has included Danicia Flores, Marieke Groot, Papiha Joharapurkar, Snehpal Sandhu, Jacqueline Kuan, Veronica Grybas, Meghan Appleby, Emma Carrothers, Hanna Parker, Janna Abdelaziz, Amanda Pace, Mitchell Keyworth, Saina Karimi-Jashni, Benjamin Smith, and Dalya Alomar. Guidance and advice has been provided by Dr. Carole Yauk as an AOP expert and a consultation committee comprised of scientists and clinicians with expertise spanning various areas of biology. Specific details of contributions are outlined in the statement of contribution.

1.7 Preliminary Work

Prior to the beginning of this thesis work, a preliminary space-health AOP network was created using a narrative review of ~100 key articles and consultation with subject matter experts (Supplementary Figure 1). Additionally, a preliminary protocol was prepared in accordance with the guidelines and principles outlined by the Environment International (ENVINT) Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) Systematic Map and Protocol report (available at www.elsevier.com/journals/environment-international/0160-4120/guidance-notes (Elsevier, 2017). The preliminary protocol outlined high-level approaches for the review of primary research from diverse areas with the aim to identify a body of evidence to support the proposed AOP network. The first component of my thesis work was to refine and finalize this protocol to include the benefits of scoping review tools as well as the potential application of artificial intelligence (AI) tools. The creation of this protocol is detailed in Chapter 2.

1.8 Figures and Tables

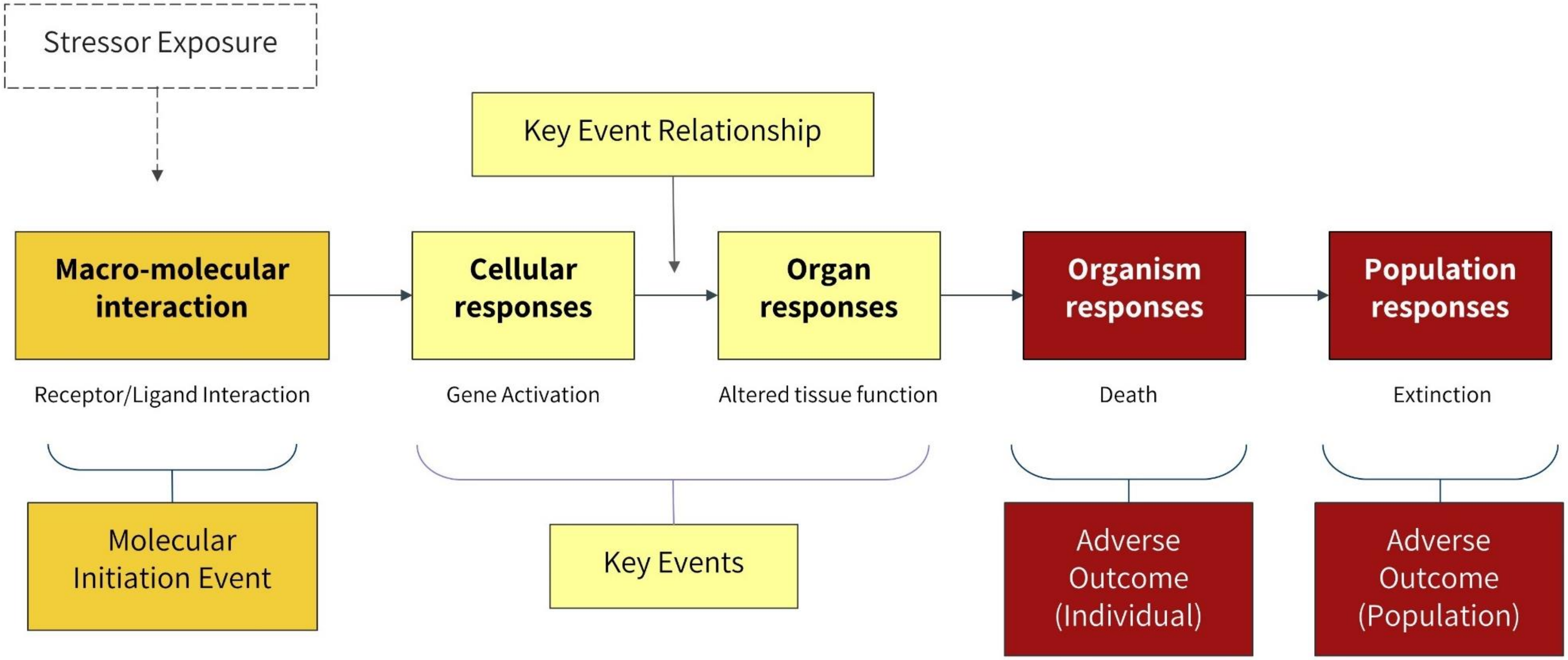

Figure 1.1 An abstract adverse outcome pathway (AOP) containing all essential components.

Table 1.1 Original and corresponding modified Bradford Hill criteria used in Mode Of Action (MOA) analysis and AOP construction (orange highlight) *.

Original 1965 Criteria	**Modified Criteria for MOA Evaluations**
Strength – Strength of association between suspected cause and observation.	**Strength** – N/A. Not considered as applicable to MOA data as specificity and consistency.
Consistency – Repeatability of an association by different persons, in different places, circumstances and times.	**Consistency** – Is the pattern of effects across species/strains/organs/test systems what would be expected?
Specificity – The association is limited to a specific population and to particular sites and types of disease.	**Essentiality of key events** – Is the sequence of events reversible if dosing is stopped or a key event prevented?
Temporality – The exposure occurs before the effect.	**Temporal concordance** – Are they key events observed in hypothesized order?
Biological Gradient - Risk of disease increases with increasing exposure.	**Dose-response concordance** – Are they key events observed at doses below or similar to those associated with the end (adverse) effect?
Plausibility – Biological knowledge supports suspected causation.	**Biological Plausibility** – How well established is the MOA in the biological database; does the proposed MOA conflict with biological knowledge?
Coherence – The association agrees with the generally known facts of the history and biology of the disease.	**Coherence** – N/A. Not considered as applicable to MOA data as consistency and plausibility.
Experiment – Experimental evidence alters frequency of associated events.	**Experiment** – N/A. Not considered applicable to MOA data.

*Table from the AOP Wiki training module (aopwiki.org)

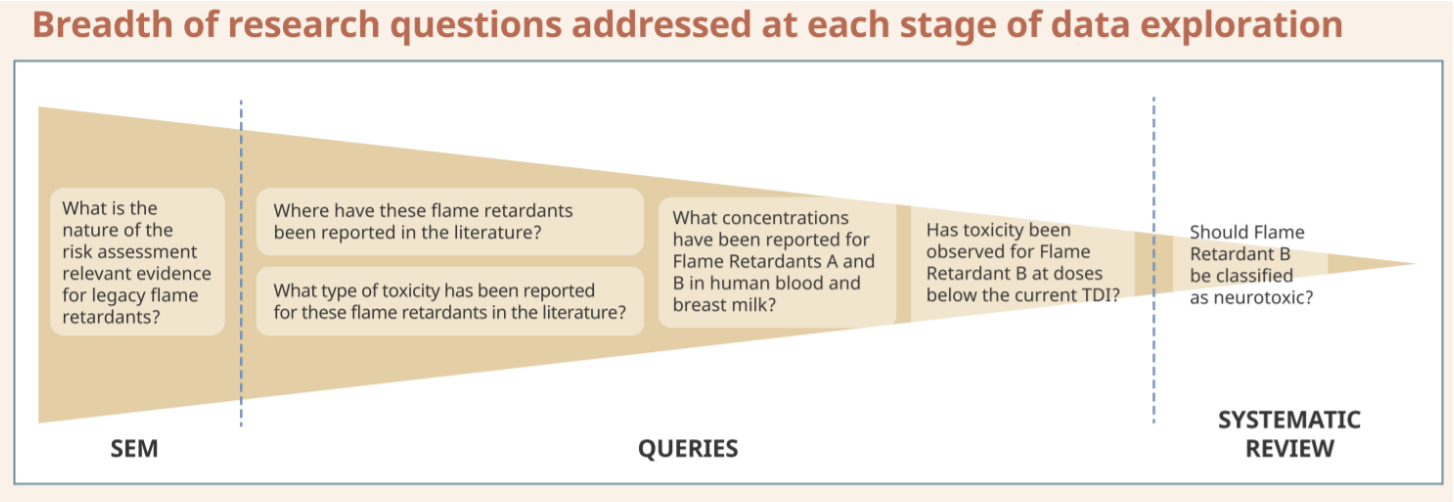

Figure 1.2 Figure from Wolffe et al. (2019) illustrating the difference between the goals of a scoping review, in which results have been visualised in a systematic evidence map (SEM) and a systematic review. (Wolffe et al., 2019)

Chapter 2 – Protocol for Scoping Review use in AOPs

Deploying elements of scoping review methods for Adverse Outcome Pathway development: A space travel case example

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2.1 Abstract

Purpose: Health protection agencies require scientific information for evidence-based decision-making and guideline development. However, vetting and collating large quantities of published research to identify relevant high-quality studies is a challenge. One approach to address this issue is the use of Adverse Outcome Pathways (AOPs) that provide a framework to assemble toxicological knowledge into causally linked chains of key events across levels of biological organization to culminate in an adverse health outcome of significance. Traditionally, AOPs have been constructed using a narrative review approach where the collection of evidence that supports each pathway is based on prior knowledge of influential studies that can also be supplemented by individually selecting and reviewing relevant references. **Objectives:** We aimed to create a protocol for AOP weight of evidence gathering that harnesses elements of both scoping review methods and artificial intelligence tools to increase transparency while reducing bias and workload of human screeners. **Methods:** To develop this protocol, an existing space-health AOP in the workplan of the Organisation for Economic Co-operation and Development (OECD) AOP program was used as a case example. To balance the benefits of both scoping review tools and narrative approaches, a study protocol outlining a screening and search strategy was developed, and three reference collection workflows were tested to identify the most efficient method to inform weight of evidence. The workflows differed in their literature search strategies, and combinations of software tools used. **Results:** Across the three tested workflows, over 59 literature searches were completed, retrieving over 34000 references of which over 3300 were human reviewed**.** The most effective of the three methods used a search strategy with searches across each component of the AOP network, SWIFT Review as a pre-filtering software, and DistillerSR to create structured screening and data extraction forms. This methodology effectively retrieved relevant studies while balancing efficiency in data retrieval without compromising transparency, leading to a well-synthesized evidence base to support the AOP. **Conclusions:** The workflow is still exploratory in the context of AOP development, and we anticipate adaptations to the protocol with further experience. To further the systematicity, future iterations of the workflow could include structured quality assessment and risk of bias analysis. Overall, the workflow provides a transparent and unbiased approach to support AOP development, which in turn will support the need for rigorous methods to identify relevant scientific evidence while being practical to allow uptake by the broader community.

2.2 Introduction

Regulatory-based decision-making requires evidence from high quality empirical studies. However, due to the sheer volume of research published daily and barriers to accessibility such as siloed information storage, collecting and organizing relevant studies and information that represent the current state of research can constitute a hurdle for timely policy development. The need to organize scientific information to understand toxicological effects is one of the catalysts of the Organisation for Economic Co-operation and Development (OECD) Program on the Adverse Outcome Pathway (AOP) knowledge framework. First conceptualized over a decade ago, the AOP framework (Ankley et al., 2010) is a structure for assembling knowledge using studies that can inform regulatory decision-making (OECD, 2018). Stored as living documents in the web-based and crowd-sourced AOP-Wiki (aopwiki.org), the pathways can be continuously updated to reflect the ever-changing state of scientific knowledge. AOPs represent a hypothetical chain of events occurring across levels of biological organization that lead from a molecular initiating event (MIE) to an adverse health outcome of regulatory significance (Ankley et al., 2010). Key Events (KEs) in each pathway are connected by causal Key Event Relationships (KERs) that are supported by a WOE in the form of the modified Bradford Hill criteria (BH criteria) (Becker et al., 2015). In the context of AOP development, the relevant BH criteria are biological plausibility, dose-response concordance, temporal concordance, essentiality of key events, and consistency (aopwiki.org). The process of collecting and reviewing literature to inform the WOE across KERs in a proposed AOP is at the discretion of the AOP developer and can be dependent on the application of the AOP. Case studies using different methodologies with lessons learned are needed to help standardize data collection in AOP development.

Originally, AOPs were used to organize data for chemical and ecological risk assessment. Recent work by Chauhan et al. (2020) & Preston et al., (2021) showed the benefits of expanding AOPs for use in the field of radiation, where AOPs are now being explored for research and regulation to support low dose and low dose-rate exposures. Using the present case study, we continue to expand AOP framework application by building a collection of pathways that explore the health effects of space travel. The human body is finely tuned for life on earth, and travel to space comes with exposure to multi-faceted physiological stressors including chronic low dose ionizing radiation exposure and microgravity. To assess associated risks and identify countermeasures to protect future space travelers, the current state of knowledge on molecular, cellular, tissue and system level effects need to be better characterized. Indeed, international radiation governing bodies (e.g., the International Commission on Radiological Protection (ICRP), National Council on Radiation Protection and Measurements (NCRP), United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR)) have directed efforts to understanding low dose and low dose-rate effects, and some are considering AOPs as a valuable tool to integrate knowledge from the molecular to population level (Laurier et al., 2021). For this reason, harmonization of approaches for evidence gathering that ensures transparency of data collection would benefit the process of AOP construction. This in turn will help facilitate the identification of the most relevant studies in the radiation field that could support the development of quantitative AOPs, whereby the empirical evidence is used for risk-model development.

Conventionally, AOP building is completed through a narrative review approach based on prior knowledge and expert opinion. Adopting systematic review (SR) tools for AOP WOE collection has been proposed in order to improve objectivity, comprehensiveness, reproducibility, and transparency of the process and to create a set of standardized best practices for AOP developers of the future (de Vries et al., 2021). This new structure could also help with those new to AOP development since it could serve as a step-by-step guide through the process.

Although there are a number of methodologies to explore for AOP development (de Vries et al., 2021), for data-rich areas like the broad preliminary AOP network of the current project, a scoping review methodology may be most suitable (Arksey & O'Malley, 2007; Munn et al., 2018). While full systematic reviews are considered the highest standard in evidence search, selection and synthesis, they are built around a focused PECO (Population, Exposure, Control and Outcomes) question (Morgan et al., 2018). Scoping reviews, on the other hand, have a wider scope with more expansive inclusion criteria. Scoping reviews frequently precede systematic reviews and allow the research team to identify knowledge gaps, that a PECO-focused systematic review would feasibly address. Scoping reviews do not aim to produce critically appraised and synthesized results and/or answer a particular question (Peters et al., 2015) instead they are used for mapping the evidence on a particular topic and identifying key concepts. Furthermore, the assessment of methodological limitations or risk of bias of the individual studies is not required (Peters et al., 2015). While scoping reviews are a methodology through which literature is screened, SR methodologies also contains systematic evidence maps (SEMs) that are used to visually represent the results of literature screening. SEMs are useful for at-a-glance identification of trends in broad collections of evidence and synthesizing review results in a user-friendly product (Miake-Lye et al., 2016; Wolffe et al., 2019). Our aim is to include the benefits of both scoping literature review and data summary using SEMs in AOP development.

We tested several workflows and considered steps that can be automated by exploring the inclusion of natural language processing (NLP) artificial intelligence (AI) software with the goal to accelerate literature review and alleviate the burden on human screeners. The data management aspects of the software tools have successfully been tested and validated by others (van der Mierden et al., 2019), but the automated screening features were not found to accurately identify relevant studies (Gartlehner et al., 2019; Gates et al., 2019). With these considerations, we opted to test the use of Distiller SR and SWIFT Review in AOP development with the following specific objectives:

1. Create a methodology that optimizes the inclusion of elements of scoping review tools and text mining software in the evidence collection process required for AOP construction
2. Demonstrate the process using a case study that is being developed related to the radiation field

Here, we outline a practical protocol, and present results from the screening process involved in the development of an AOP relevant to the radiation field that is comprised of four health outcomes and 20 (19 KEs + 1 MIE) KEs. Additionally, we present the workflows that have been tested and discuss their merits and drawbacks. A major effort of our group is to advance radiation risk assessment applications and engage the community in the use of AOPs for this purpose. Thus, the overarching objectives are both to build radiation AOPs as well as to evaluate the use of elements of SR tools in AOP development.

2.3 Methods

Resources:

Tools and resources used in collecting the WOE for the AOP were: SWIFT Review ((**S**ciome **W**orkbench for **I**nteractive computer-**F**acilitated **T**ext-mining) www.sciome.com/swift-review/ released 08.28.2019: Version 1.43) and DistillerSR (Evidence Partners. www.evidencepartners.com/products/distillersr-systematic-review-software released 12.06.2020 Version 2.34.0). The statistical text-mining and machine learning features of SWIFT review (such as the SWIFT tag browser feature) were used to prioritize the results of literature searches prior to human screening. Distiller was used to create structured screening forms for reviewers that facilitated reference evaluation while tracking reviewer responses and data-management. Additionally, three AOP-reference collection workflows (henceforth referred to as simply “workflow” or “flow”) were evaluated to determine the most efficient iteration of the protocol. Lastly, the machine learning based automated reviewer feature (DAISY) was tested for efficacy in assisting with literature screening.

2.3.1 High level project overview

The project is comprised of three phases, as outlined in Figure 2.1. In the completed first phase of the project, the work focused on the creation of a preliminary AOP network that is being used as a case-example and predominantly informed by studies relevant to the space environment. Phase II, which is the focus of the current work, is the creation and validation of a protocol to collect a WOE for preliminary AOP developed in Phase I. The detailed results of Phase III will be published in the future as a narrative that summarizes the WOE and will be input into the AOP-Wiki (www.aopwiki.org), as well as visualized in systematic evidence maps (SEMs).

2.3.2 Preliminary AOP network – Phase I

A case-study AOP network related to health outcomes relevant to space stressors was the basis for testing elements of scoping review methodologies and also developing appropriate filtering criteria to identify relevant studies that could support causal linkages to the AO. This network was initially built by screening ~100 expert-selected articles. Studies were retrieved manually by study authors using a variety of search engines (ex. Google Scholar, PubMed) and literature databases from the National Aeronautics and Space Administration (NASA), Canadian Space Agency (CSA), as well as from authoritative reports from these agencies. The retrieved studies and agency reports guided the development of an AOP network for the following adverse outcomes (AOs): cardiovascular disease, impaired learning and memory, bone loss, and cataracts. While cancer is another well-studied outcome of ionizing radiation, due to the chronic low dose nature of the space exposure scenario, the present work is focused on non-cancer health effects for which there is growing concern (Patel et al., 2020). The preliminary network contained a total of 40 adjacent Key Event Relationships (KERs) relationships and 20 non-adjacent KERs (Supplementary Figure 1).

2.3.3 Creation of Protocol – Phase II

Following the completion of Phase I and procurement of SR software (Distiller SR), a modified scoping review protocol was developed with the aim of identifying relevant evidence to support each KER in the AOP as well as to identifying any additional KEs. The study protocol was developed based on the guidelines and principles outlined in the ENVINT PRISMA-SM-P report (available at (Elsevier, 2017). The protocol has been registered at osf.io/t9amw.

2.3.4 Weight of Evidence Gathering – Phase III

The project then continued to Phase III step 1, with the outlined study protocol being tested to collect the appropriate AOP WOE. As practical experience was gained through the literature screening process, there was a return to Phase II step 2 with amendments being made to the protocol. All amendments were logged and are detailed in the results. Throughout the testing and amending of the protocol, three distinct workflows (Figure 2.3 of Results) were produced. The first test of the Phase II step 2 workflow (hereafter referred to as flow 1) was the first iteration process, the two subsequent versions (flow 2 and flow 3) were modified from flow 1 to address inefficiencies of the first approach.

ii. Information Retrieval

All literature searches to support Phase II of the AOP construction were developed by a Health Canada librarian (RH) on Ovid Medline, with no language or date restrictions. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009) study flow diagram (Supplementary Figure 2) was created to track the number and source of the references collected. Any additional papers sourced outside of the literature searches (e.g., passed along from subject matter experts or the reference sections of review articles) were marked as "Other Sources" on the study flow diagram.

To identify relevant studies, a series of keywords, assays/endpoints and applicable Medical Subject Headings (MeSH) terms were collected to describe each of the components (MIE, KE, AO) of the AOP network. The list of endpoints defined the measurement technique for each of the KEs, which ensured consistency in study identification across multiple screeners. The literature searches were conducted by a librarian and were divided into three sets: 1) MIE to AO searches and 2) independent KER searches 3) KER searches that were pooled together along with MIE to AO searches. MIE to AO searches were broad, overarching searches that captured any studies discussing each of the AOs in the context of relevant exposure conditions and allowed the identification of KEs related to the network. In contrast, the KER searches focused on studies that explored the relationships between specific KEs. To manage the number of studies and retrieve relevant articles of significance to the space field all searches were filtered using space exposure terms. If KER searches returned insufficient references, the scope was broadened to include references from chemical stressors.

Set 1 – MIE to AO searches (used for Workflow 1 only)

MIE terms + AO terms

Set 2 – KER searches (used for Workflow 2)

KE_X terms + KE_Y terms; KE_X terms + AO_Y terms

Set 3 – Pooled searches (used for Workflow 3)

(MIE terms + AO terms) + (KE_X terms + KE_Y terms) + (KE_X terms + AO_Y terms) + (KEz + ….)…

A full list of the completed search strategies is available in the supplementary material. The search strategy was validated using the ~100 articles manually curated for creation of the preliminary AOP network. When the pilot searches retrieved articles from the manually collected list, it provided confidence in the search workflow.

iii. Eligibility Criteria

To be deemed eligible for the workflow, studies had to be published, peer-reviewed articles written in English. Letters to the editor, opinion pieces, editorials, press releases, advertisements, books, book chapters, theses, conference abstracts or proceedings and posters were not considered. Additionally, study eligibility was the same for all three workflows and was determined by the PEOE criteria outlined in Table 2.1. Meaning, to be eligible, a study must have explored one type of evidence from the population, exposure, and outcomes ***or*** endpoints element (P and E and (O or E)). One type of evidence per element was sufficient; a study did not need to consider every type of evidence in each element (e.g., a study needed to only consider one population of interest in the listed items shown in Table 2.1). These PEOE criteria specify the defined space-relevant stressors in the context of the current work.

iv. Data Management

Although many tools are available for data management (van der Mierden et al., 2019), after consultation with experts in systematic methodologies, Distiller was selected as a reliable tool for the this project. Structured literature screening forms were created (Supplementary Figure 3) that allowed references to either continue through the workflow or be excluded. Metadata for each reference was tracked including publication information, inclusion/exclusion decisions, and responses to form questions. Distiller was also used to create PRISMA diagrams that tracked the progression of all the references through the workflow (Supplementary Figure 2).

v. Selection and data collection process

In selecting data, filtering criteria were employed that prioritized radiation studies of all qualities and stressors relevant to the space environment. Additionally, by prioritizing searches, it facilitated identifying relevant articles, and limiting the burden of review for human screeners.

Following the literature database search, the results were imported into SWIFT Review (SWIFT) where the SWIFT-generated tags were used to triage studies according to the following levels of priority:

Evidence stream: human > *in vivo* > *in vitro* > *in silico*

Dose: low > moderate > high

Exposure: space environment > ionizing radiation stressors > non-ionizing radiation stressors > chemical

Articles triaged using SWIFT to identify the highest priority references (e.g., were tagged by SWIFT with the greatest simultaneous number of high priority tags) were exported into Distiller where they were screened in a three-level process. The Distiller Artificial Intelligence System

(DAISY) re-rank setting was enabled for all include/exclude levels of Distiller screening; this feature learns from the decisions of human screeners and continuously ranks unscreened articles to bring more relevant articles to the top of the screening queue.

Level 1 – Title and Abstract were screened by two screeners (JK, SS, DF, VL, MA, NA, TK) who evaluated adherence to the PEOE criteria. Screener consensus was required for progression or exclusion; conflict resolution was completed by a discussion between the reviewers and resolved by a third reviewer (RW, VC) as necessary. The screening was piloted to ensure conflicts did on exceed 10% of the total number of articles screened.

Level 2 – Full text was screened by two screeners (JK, SS, DF, VL, NA, TK) to verify that the full text was a peer -reviewed published article, available in English, and adhered to the PEOE criteria. Screener consensus was required for progression or exclusion; conflict resolution was accomplished by a discussion between the reviewers and resolved by a third reviewer (RW, VC) as necessary.

Level 3 – Full text was used for data extraction.

vi. Data extraction

Data extraction was completed in Distiller at level 3 of the screening workflow using customized template forms. Key data points collected at this level were which KE/KER(s) were investigated, what element of the BH criteria is examined, as well as whether the study investigated any KE/KER(s) that were not currently in our proposed network, and if any confounding factors were studied. The full list of data extraction and coding questions is available in supplementary figure 3 (c-d).

vii. Testing Distiller Automated Screener

KER searches completed for the cardiovascular disease AOP were used to test the efficacy of the Distiller automated screener. Individual KER searches were triaged in SWIFT then imported to Distiller. Screening at the first two levels was completed by one human screener and the Distiller Automated Screener feature replaced the second screener (semi-automated screening). Any conflicts between the decisions of the human screener and the AI were logged.

2.4 Results

2.4.1 Phase I: Preliminary AOP development

Phase I of AOP construction began with a preliminary screening of the literature to identify the authoritative and relevant review articles for the stressor(s) and AO(s) of interest. This was achieved using via a non-systematic approach using search terms relevant to the stressor(s) and AO(s) as the anchoring points. In this stage, it was important to identify the relevant review articles in the field, including documents generated by the international radiation governing bodies. Assembly of these documents facilitated identification of the mechanistically well-defined KEs/KERs for the preliminary network (Supplementary Figure 1). This preliminary screen was managed without the use of specialized software.

2.4.2 Phase II: Study Protocol Development

The methods used for assembling knowledge to support the AOP network developed in Phase I were documented in the form of a study protocol. This protocol defined the study question, the SR software employed, information sources, databases, search strategy, and inclusion/exclusion criteria. These design features and the technical input (e.g., assay glossary Supplementary table 1) are an essential aspect for an effective and reliable process. The format of the protocol was developed using Pelch et al. (2019) as the example. In total, as of January 2022 over 34000 articles were retrieved using the protocols search strategy, with over 3300 over them being reviewed by human reviewers. A PRISMA flow diagram was generated to track the screening process (Supplementary Figure 2).

2.4.3 Phase III: Evidence Gathering

Phase III of the process involved identifying studies that met modified BH criteria through the rigorous evidence gathering protocol outlined in Phase II. This required the development of search logic; with the help of the librarian (RH), a database of studies was identified using search strategies for KEs, KERs, MIE, and AOs.

Each of the databases was then taken through level 1 (Figure 2.2) of screening to determine which best identified studies met the PEOE statement. In the process of testing the scoping review protocol, three iterations of the workflow evolved (Figure 2.3). Once studies passed level 2 screening in Distiller they went into full data extraction. This process extracted the relevant information from each study to help develop the causality statements for AOP development.

i) Flow 1 – Screening the MIE to AO search (Figure 2.3)

The first flow (Flow 1, Figure 2.3) used search terms specific to only the MIE and AO (MIE to AO search). Results from this search were uploaded to Distiller and screened at all three levels. This search strategy yielded thousands of references for each of the four pathways (Cognitive: 6645, Cardiovascular: 3923, Cataracts: 1671, Bone Loss: 3154). After the second level of screening, the majority were found to not support any BH criteria. A benefit of this method was the identification of a collection of literature that was later used to validate the inclusion of later proposed KEs and novel KERs (Figure 2.4).

ii) Flow 2 – Individual KER searches using SWIFT (Figure 2.3)

To address the challenges of flow 1, adjustments were made and flow 2 was generated in which relevant literature was retrieved for each of the KERs in the AOP. The results of the KER-specific literature searches were then uploaded to SWIFT review, where the statistical text mining and machine learning tools are used to prioritize references based on their association to the PEOE statement (Side panel – Figure 2.2). Following the AI prioritization, human screeners reviewed the list to select the top group of relevant references to then imported into Distiller. Inputting references through SWIFT allowed us to triage references based on tags such as: type of exposure, model tested and health outcome. This ensured human attention could be prioritized for references that hit the greatest number of categories of interest first. This flow greatly reduced the number of full text articles requiring review by human screeners, and thereby saved human resources for articles of greatest relevance. Furthermore, it was flexible in that it allowed reviewers to return to a specific KER search and broaden criteria in SWIFT if the original references identified were insufficient. Finally, this flow also simplified project management since screening could be completed in a methodical fashion, with one KER being taken from screening to data extraction at a time.

iii) Flow 3 – Pool of all KER searches using SWIFT (Figure 2.3)

The last third flow shared many similarities with the second flow; however, the results from literature searches for all KERs were combined and screened collectively. Individual literature searches were completed for each KER and then all resulting files were collectively uploaded to SWIFT. In SWIFT, references were prioritized based on exposure and model tags before being uploaded to Distiller. Overall, using the scoping review approach was found to have a number of advantages as highlighted through the example of the cardiovascular pathway. By using this approach, we have been able to transparently adapt the preliminary pathway to accurately represent the data informing the WOE. This is exemplified in the SEMs of Figure 2.5a-b that show the quantity as well as qualitative attributes of the WOE collected and subsequent changes reflected to the pathway (Figure 2.6). For example, KE23 “Decrease in NO” was added following screening (Figure 2.6). The addition of this KE was facilitated through screener responses to the question “Does the study support a KE or KER not currently included in the proposed AOP?” in the screening form (Supplementary Figure 2.3a-b), as well as through expert recommendation that was validated using the MIE to AO literature search database (Figure 2.4). Using these methods provided documentation and support for the additions made to the network, thereby increasing the transparency of the process. Furthermore, KE14 (protein modification/expression changes) and the original AO (cardiovascular disease) were removed from the network (Figure 2.6). Removal of a KE from the network does not negate biological relevance; instead, as the SEM of Figure 2.5a highlights, it shows that empirical evidence in the form of time, dose, incidence concordance (demonstrating the essential and causal connectivity) was not identified. Another hurdle was clearly defining the scope of each KE in terms of the endpoints and translating this to screeners through training and detailed protocols to ensure consistency in data retrieval with minimal conflicts.

iv) Results for Testing the Distiller Automated Screener

In using the Distiller automated screening feature to assist with the screening of cardiovascular KER searches, we noted that the feature performed better for the title and abstract level than the full text level. The first KER screened with the automated reviewer (KE16 (vascular remodeling) + AO) generated conflicts at level 1 (title and abstract screening) with the automated screener disagreeing with the human screener’s inclusion/exclusion choice 39% of the time (Table 2.2). However, for all other KERs screened, the automated screener and human screener agreed for all inclusion/exclusion decisions (Table 2.2). In level 2 (full text screening), there was a noticeable increase in conflicts between the human and automated reviewer (ranging all the way from 0 – 100%), with full agreement only occurring for one single KER.

2.5 Discussion

In this work, a suggested workflow is described for the development of AOPs using SR tools. This includes a clearly documented path to identify a strategy for assembling the appropriate evidence to support the KERs that ensures transparency, objectivity, and reproducibility in data retrieval. The fundamental principles for AOP development provided by the OECD Users' Handbook Supplement (OECD, 2018) were followed throughout the development process. With the addition of elements of SR tools, we produced a methodology that enables efficient and transparent study retrieval, harmonized reporting, and the eventual selection of relevant studies for the development of quantitative AOPs in the future.

Increasing the standardization of AOP development has been an ongoing discussion, from a development of best practices for AOP descriptions (Villeneuve et al., 2014), to suggestions of systematic collection of evidence as a next step for the framework (Leist et al., 2017; Svingen et al., 2021). Bridging the fields of SRs and AOP development and the inclusion of AI techniques to facilitate literature screening have also been identified as methods to increase certainty and confidence in the AOP framework (de Vries et al., 2021). To our knowledge, this is the first time that scoping review protocols have been tested for the development of a case study AOP network.

In selecting methods most efficient in retrieving relevant articles to support AOP development, three workflows evolved. The simplest workflow was built from search terms related to the MIE and AO (flow 1). It was envisioned that this workflow would identify new KEs that were not considered in the preliminary AOP as well as studies to support the modified BH criteria.

This workflow, however, retrieved the least number of relevant studies, despite having the greatest number of papers to review. The number of references retrieved was in the thousands, and many did not include empirical data to evaluate the BH criteria. A specific challenge was that references that explored KEs (but not KERs) would fulfill the PEOE statement at level 1 and pass forward for full-text review, overwhelming screeners with hundreds of non-AOP relevant full text articles to review. Modifications of the PEOE statement were discussed, but it was determined that any changes would inadvertently exclude relevant references. However, this method alongside expert consultation did identify a collection of literature that was used to propose new KEs that were not present in the preliminary network. The SWIFT tag browser feature was then used to identify the number of studies that, in their title and abstracts, referred to combinations of these alternative KEs. The representative example is provided in the heat map (Figure 2.4) that highlights the number of studies within the MIE to AO search that discuss novel KERs. Overall, the methodology of flow 1 is not recommended for identifying studies that support the causality of KERs, as more efficient workflows were identified. However, the search strategies used in this workflow are a viable option for identifying a body of literature that is useful for validating novel or alternative KEs for further exploration.

Flow 2 (Figure 2.3) involved independent KER searches, with SWIFT prioritization. This flow was both resource- and time-efficient for the reviewers. However, this method requires significantly more investment from information specialists enlisted in reference retrieval. No quantitative measures of efficiency improvements were taken; however, the number of references for human screening was reduced from thousands in flow 1 to 20-30 papers, representing a considerable decrease in human resource demand. Despite increased efficiency through inclusion of SWIFT, a drawback was the training requirements for use of this new software tool.

Additionally, there was reference redundancy; there were instances where articles that measured multiple KERs would appear in multiple literature searches and would need to be triaged in SWIFT multiple times. Nonetheless, this workflow is recommended for those working in a broad literature space as focused KER searches were efficient for identifying relevant articles, and SWIFT was an effective tool for prioritizing references for human review.

Flow 3 can be viewed as an alternative approach to Flow 2. It has all the same benefits of increased efficiency (for the screeners) and a high success of finding appropriate articles. Pooling the literature was beneficial, since many relevant references discuss multiple KERs or KEs in one article and articles were being retrieved by numerous searches. By pooling all the results together, duplicates were removed in one step rather running the Distiller de-duplicate feature for each search. Overall, flow 3 was a variation on flow 2 that marginally increased efficiency through removing redundant references earlier in the workflow. However, it does require more training for incoming screeners since it combines the use of Distiller as well as a more in-depth use of SWIFT. Additionally, like flow 2, flow 3 requires greater time investment from library or information specialists. Both flow 2 and 3 are equally efficient in the identification of appropriate studies.

Distiller's automated screener was qualitatively tested and found to have variable accuracy, especially outside of title and abstract screening levels (Table 2.2). This is in line with the work done by Gates et al., which found that semi-automated use of Distiller semi-automated had no improvement on a single reviewer (Gates et al., 2019). Overall, Distiller was found to be most useful for its unique literature review project management features. This does not negate the potential for AI inclusion to the AOP development workflow. Teams have had success incorporating a variety of AI approaches (approaches outside the scope of the present project) to their construction of AOP pathways (Carvaillo et al., 2019; Jeong et al., 2019; Rugard et al., 2020).

AOP developers working on pathways with a more focused question and defining the technical terminology, such as assay names that delineate the endpoints, may have better success in incorporating current tools in their process.

Our methods of evidence collection also lend themselves to the summary through SEMs that will enable exploration of the WOE that supports our AOP network (Figure 2.5). The structured screening forms (Supplementary Figure 2.3) contained questions that give important information about the nature of the studies screened. SEMs have been created (Figure 2.5 a-b) that demonstrate how the collected data collected can be visually represented and used to justify changes to the network as well as identify knowledge gaps.

Each of the tools and approaches discussed herein have the potential to contribute to AOP WOE. The use of these tools relies on training, personnel, and time. As such, we recommend a fit-for-purpose approach (Supplementary Figure 4) based on availability of resources. Our recommendation is for the base-level literature screen to contain the following: structured and recorded literature searches, pre-determined inclusion/exclusion criteria, creation of PRISMA diagrams, and SWIFT triage of references in data-rich areas. These tools are all free and would already vastly improve upon the narrative review approach. Resource permitting, we then recommend supplementing the base-level approach with the addition of structured screening/data extraction forms and project management tools of Distiller, as well as multiple reviewers at all inclusion/exclusion levels, and search strategies in more than one database. Future efforts may include validating our methodology further using a previously well-defined AOP. Teams with the resources to go beyond what was explored in our work could include an additional structured risk of bias analysis step to their workflow. This can be accomplished through using tools such as the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating framework (OHAT,

2019) to systematically evaluate WOE. Lastly, in all cases, we recommend considering literature retrieval logistics during the project planning phase as it is important for managing project timelines.

In the broader context, AOPs have been gaining momentum as a framework to support the radiation field, particularly in low dose and low dose-rate research. To continue advancing adoption from the community, the process of transparent data collection and documentation will be a critical step. Particularly, if AOP-based approaches are to be used by the international radiation governing bodies and relevant stakeholders, the approach needs to be recognized as a means to produce transparent and objective literature-based narratives, where data-retrieval can be verified. We propose that the standardization of AOP development, including the identification of the optimal AOP-reference collection workflow described in this work, is valuable to transparent data retrieval. It helped identify the approach which that yielded the most relevant studies within broad literature spaces, a process that is central to the development of quantitative AOPs and an area of great interest to the radiation field.

In conclusion, our work supports larger efforts to standardize AOP development. We believe that the use of elements of scoping review methodologies present value and can be incorporated in a number of ways for screening articles for AOP WOE. We outline three tested workflows and discuss the benefits and hurdles. Additionally, we have provided detailed recommendations for future AOP builders looking to define their methodology. Overall, the most flexible approach applied the use of filtering and screening software such as SWIFT and Distiller, as well as structured literature searches across each of the KERs in the AOP with a predetermined inclusion and exclusion criteria, multiple screening levels and documentation of reference progression through the screening. The addition of these systematic tools is an improvement to the traditional narrative approach of AOP WOE collection as it reduced bias, increased transparency, and a method for processing large volumes of primary research.

2.6 Figures and Tables

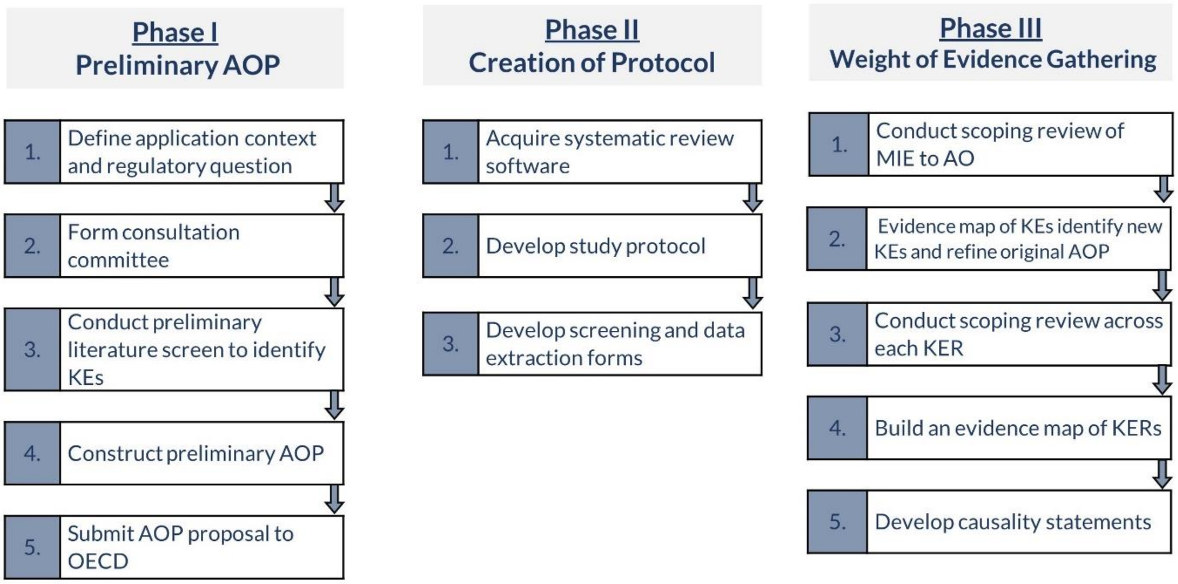


Figure 2.1 Overview of the three phases of the AOP project. The current publication focuses on the second phase; the process of developing and validating a weight of evidence collection protocol. After deploying the first iteration of the reference screening workflow, changes were made to the methodology resulting in versions 2 and 3 of the scoping review literature screening workflows. Differences between the workflows are detailed in results.

Table 2.1 The PEOE statement (**P**opulation, **E**xposure, **O**utcomes and **E**ndpoints) used to inform the inclusion and exclusion criteria for the scoping review.

PEOE element	Evidence
Populations	Human, *ex-vivo*, *in-vivo*, cell, cell-lines, epidemiological, cohort, population, organ, tissue, cellular, molecular, cellular components, *in-silico* models, animal models, biologically based models
Exposures	Radiation (ionizing and non-ionizing), hydrogen peroxide (radiation mimetic), microgravity, environmental CO_2, atmospheric gas, space environment/conditions
Outcomes	Cognitive disorders, cardiovascular disease, cataracts, bone loss
Endpoints	Any KEs in the preliminary network
Inclusion requirements: P and E and (O or E)	

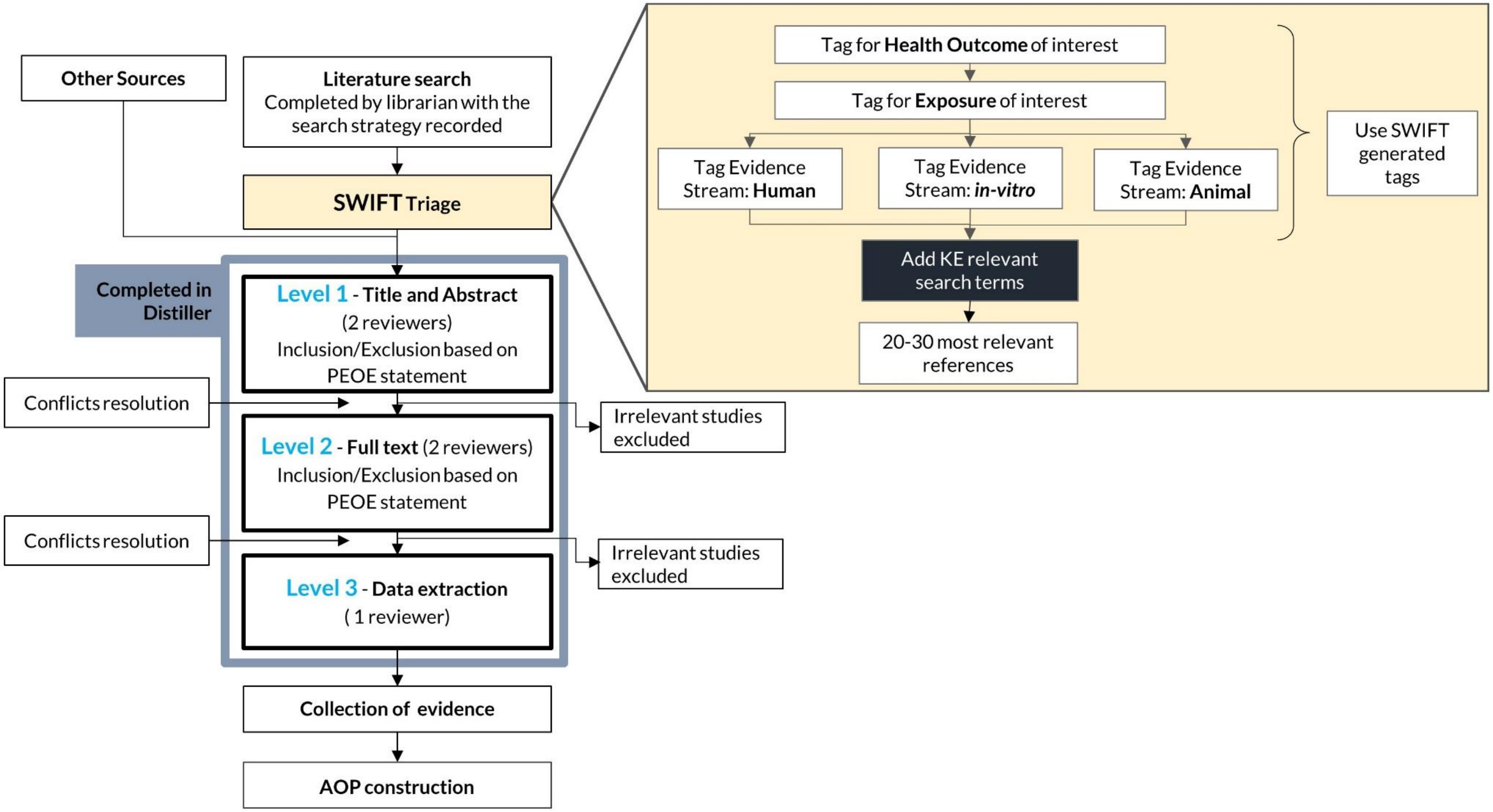


Figure 2.2. Overview of scoping review protocol. References retrieved by a literature search were prioritized in SWIFT (shown in side panel) to identify the most relevant references before being imported to Distiller for a three-level literature screen (outlined in blue). “Other Sources” refers to references acquired outside of the literature searches (e.g., passed along from subject matter experts or the reference sections of review articles).

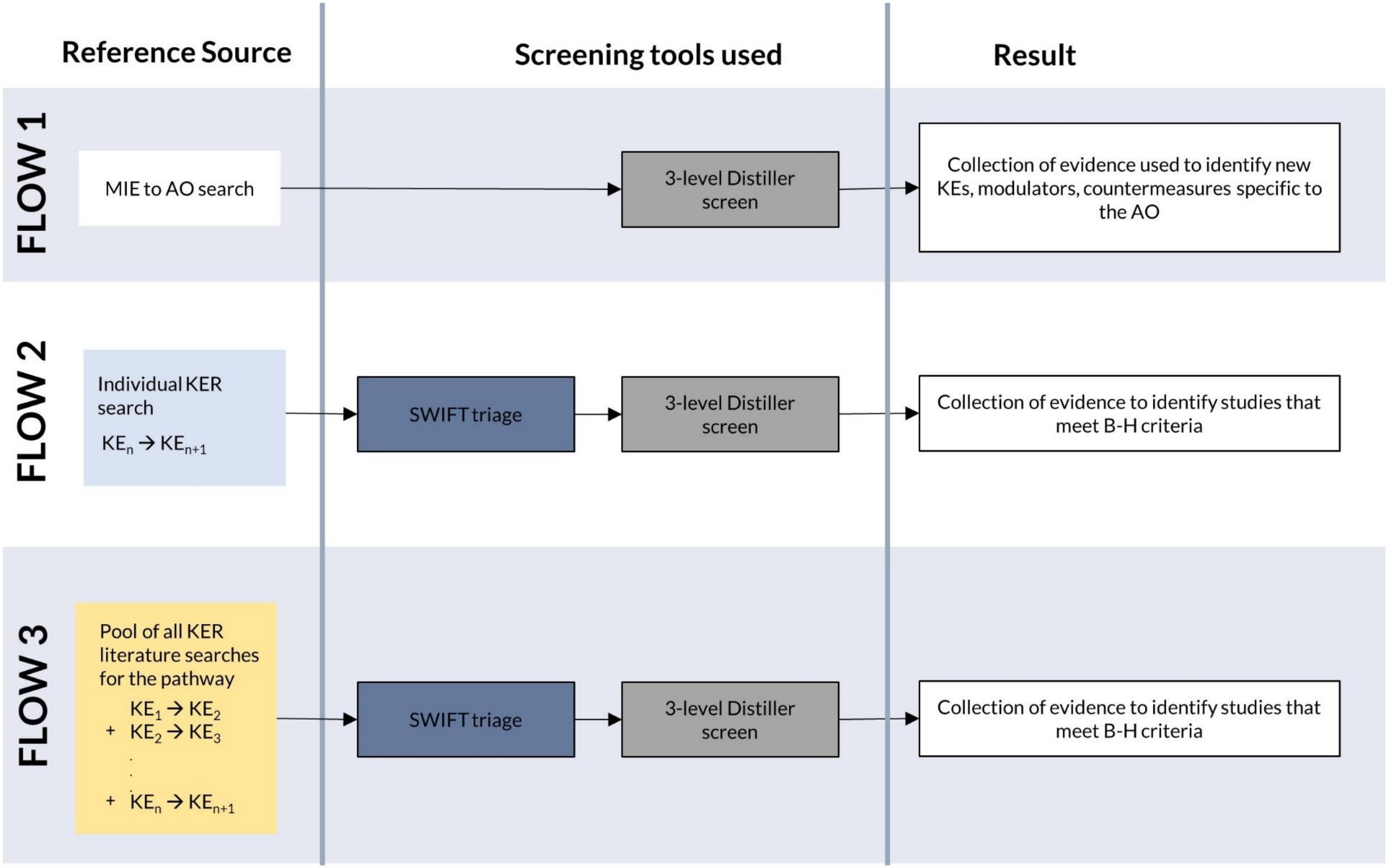
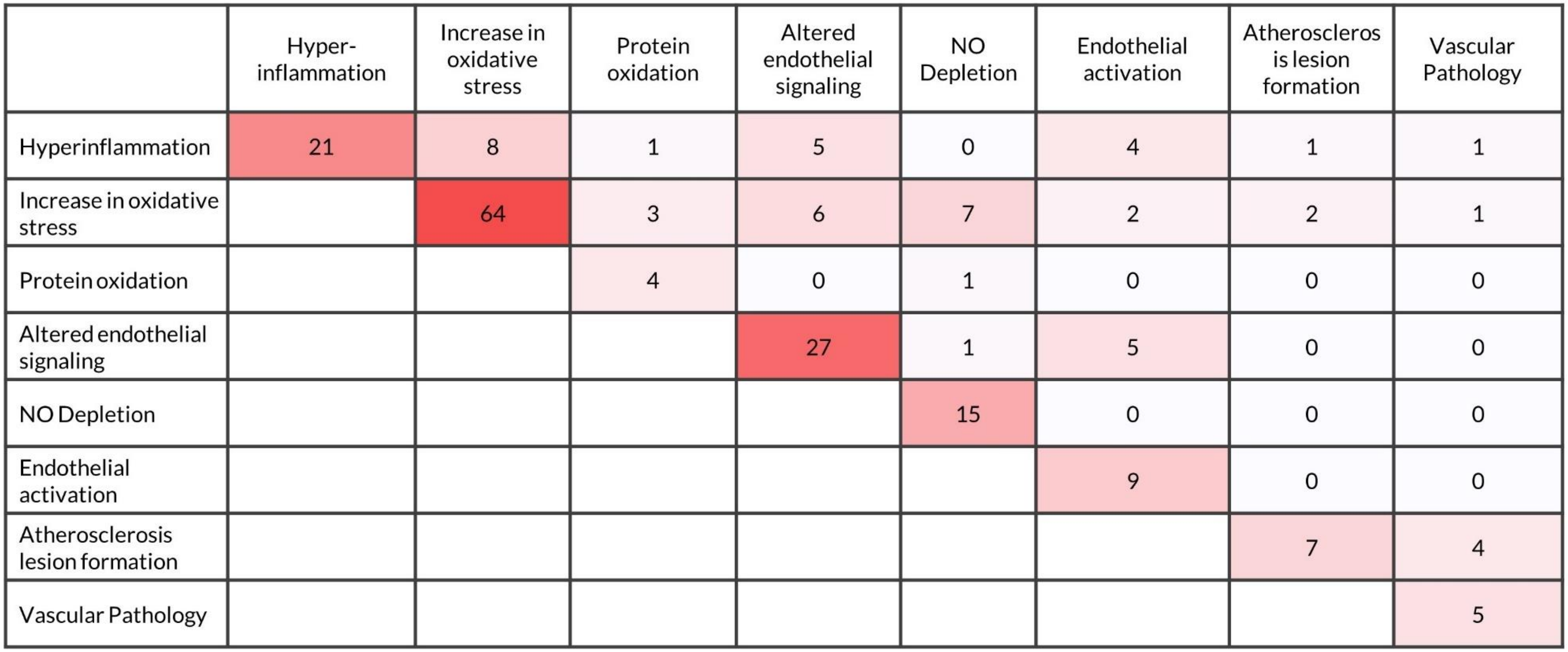


Figure 2.3. Summary of the three iterations of the scoping review protocol tested. Flow 1 was the initial created protocol, while flow 2 and 3 developed after flow 1 was tested, and inefficiencies in the methodology were addressed.

Table 2.2 - Percentage of conflicts in inclusion/exclusion decisions between human screener and Distiller Automated Screener in screening KER searches for the cardiovascular disease pathway. Level 1 refers to Title and Abstract screening, and Level 2 is the full text screening.

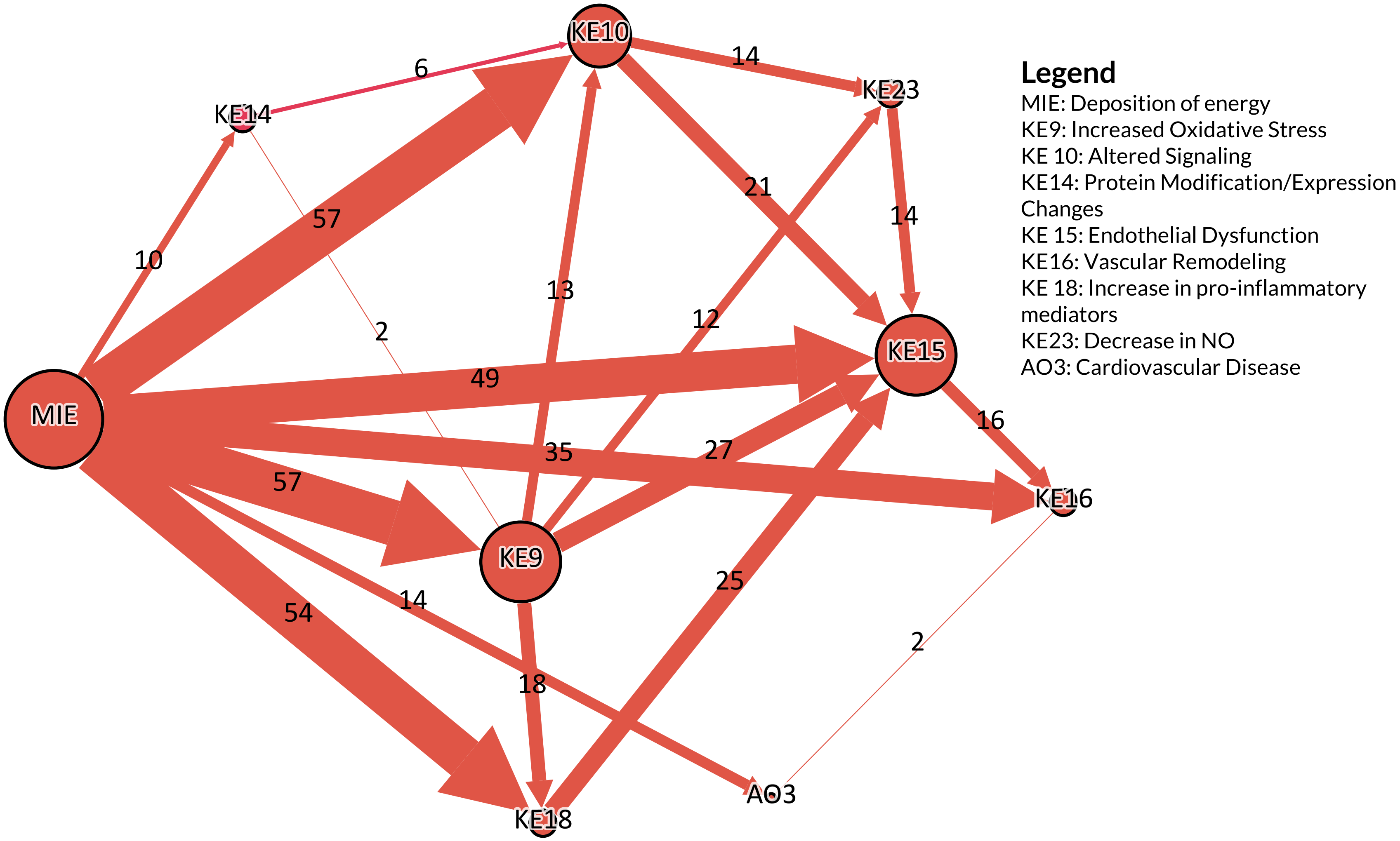
KER search screened	Percentage of conflict with AI decision **(Level 1)**	Percentage of conflict with AI decision **(Level 2)**
MIE + KE 9	0%	50%
MIE + KE 18	0%	100%
MIE + KE 10	0%	30%
MIE + AO	0%	78%
KE 9 + KE 14	0%	0%
KE 9 + KE 18	0%	67%
KE 9 + KE 10	0%	13%
KE 9 + KE 15	0%	40%
KE 18 + KE 15	0%	36%
KE 10 + KE 15	0%	14%
KE 16 + AO	39%	38%
KE9 + KE23	0%	100%
KE10 + KE23	0%	100%
KE15 + KE23	0%	50%

Legend
MIE: Deposition of Energy
KE9: Increase, Oxidative Stress
KE10: Altered Signaling
KE 14: Altered Proteins
KE18: Increase, Pro-inflammatory Mediators
KE15: Altered Nitric Oxide Levels
KE23: Endothelial Dysfunction
KE16: Vascular Remodeling
AO: Cardiovascular Disease



	Hyper-inflammation	Increase in oxidative stress	Protein oxidation	Altered endothelial signaling	NO Depletion	Endothelial activation	Atherosclerosis lesion formation	Vascular Pathology
Hyperinflammation	21	8	1	5	0	4	1	1
Increase in oxidative stress		64	3	6	7	2	2	1
Protein oxidation			4	0	1	0	0	0
Altered endothelial signaling				27	1	5	0	0
NO Depletion					15	0	0	0
Endothelial activation						9	0	0
Atherosclerosis lesion formation							7	4
Vascular Pathology								5

Figure 2.4. Validation of alternative KEs using the MIE to AO literature search. Following expert suggestion of alternative KEs for the cardiovascular disease pathway, SWIFT tag browser feature was used to explore the titles and abstracts of the cardiovascular MIE to AO literature search. Values represent the number of references returned when using combinations of given KEs, highlighting potential for novel KERs.



Legend

MIE: Deposition of energy
KE9: Increased Oxidative Stress
KE 10: Altered Signaling
KE14: Protein Modification/Expression Changes
KE 15: Endothelial Dysfunction
KE16: Vascular Remodeling
KE 18: Increase in pro-inflammatory mediators
KE23: Decrease in NO
AO3: Cardiovascular Disease

Figure 2.5 a) Systematic evidence map (SEM) illustrating quantity of evidence across all the KERs considered for the cardiovascular pathway. Arrow size indicates relative evidence weight, while the size of the KE circles represents relative degree of connectivity (as determined by number of connections up and downstream from the KE). Values represent the number of references supporting each KER; in order the support a KER a reference must demonstrate at least one of the Bradford-Hill (BH) criteria.

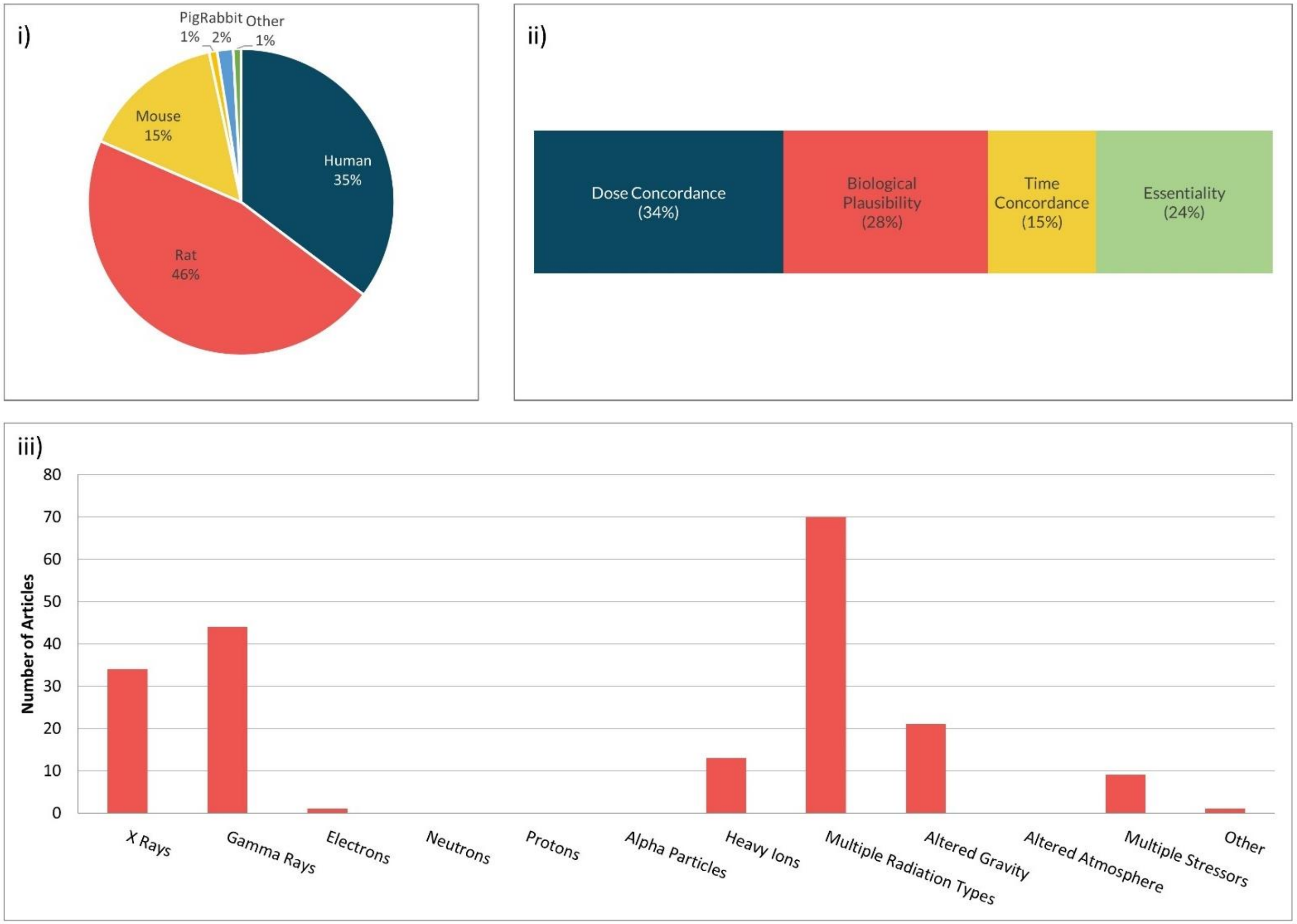


Figure 2.5 b) Systematic evidence map (SEM) illustrating qualitative aspects of the weight of evidence supporting the cardiovascular pathway. i) illustrates the taxonomic applicability breakdown, ii) the Bradford-Hill criteria supported and iii) the stressors considered by the studies. “Multiple stressors” encompasses studies that explored more than one stressor simultaneously, while “other” includes any other exposures from the PEOE table not otherwise listed (hydrogen peroxide (radiation mimetic), environmental CO_2, atmospheric gas, space environment/conditions).

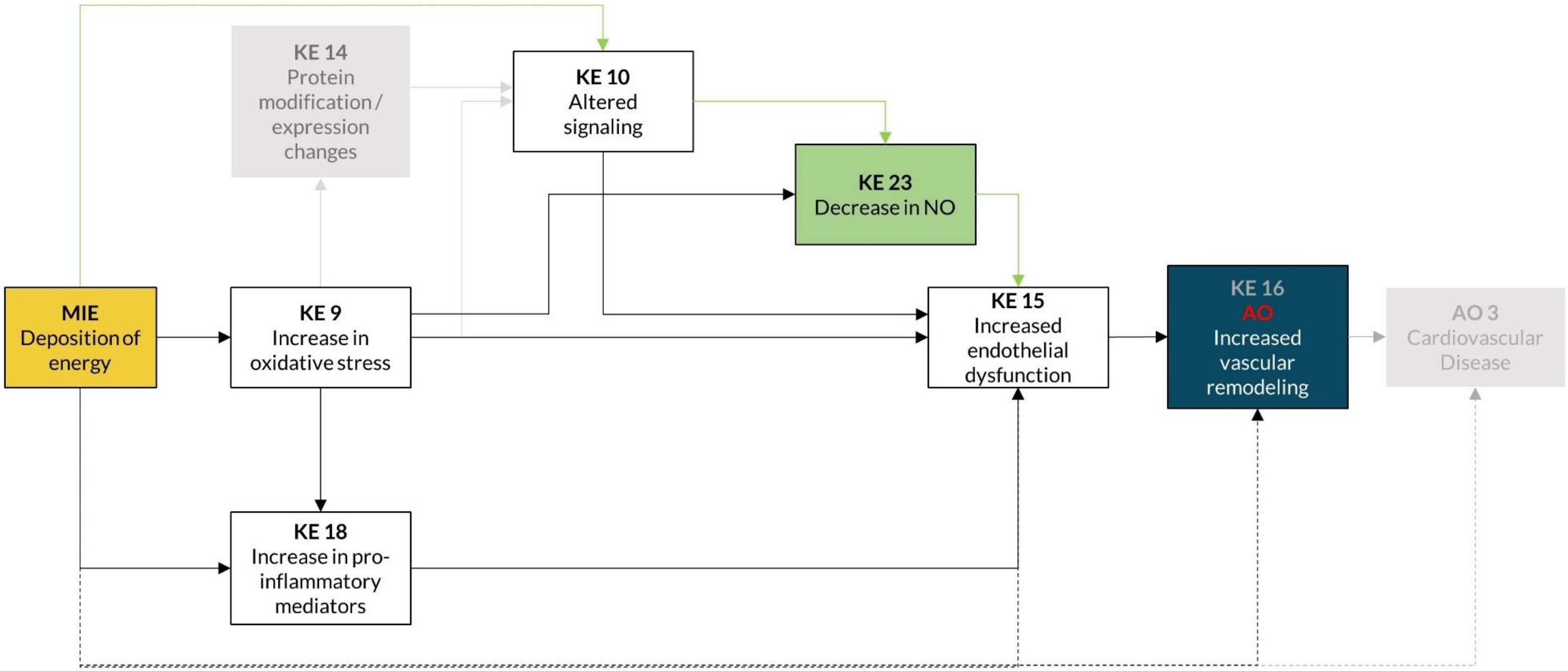


Figure 2.6. Updates to the preliminary cardiovascular AOP. Changes to the preliminary pathway were made following literature screening to reflect the contents of the weight of evidence. Any KEs/KERs with insufficient empirical evidence or no additional evidence from that already in the AOP Wiki (www.aopwiki.org) are greyed out, KE titles that have been updated are highlighted in red with original titles faded out, and any newly added KEs/KERs are represented in green. The MIE is in yellow, while the AO in dark blue.

Chapter 3 – AOPs, Radiation & Cardiovascular Outcomes

AOP Report: Development of an adverse outcome pathway for deposition of energy leading to vascular remodeling

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Foreword: The following chapter and its section headers, content and word count are formatted for submission to the journal of *Environmental and Molecular Mutagenesis*. The protocol for WOE collection was described in Chapter 2 and is extensively referenced in the "AOP development strategy" section. For journal submission, this information will be adapted as supplementary material.

3.1 Abstract

The Adverse Outcome Pathway (AOP) framework has primarily been used for organizing data on the mechanistic progression of health outcomes induced by chemical stressors. The present work employs the AOP framework for a novel use in space health risk assessment. Through expert consultation, an AOP network to four intersecting adverse outcomes (AOs) was constructed that summarizes the non-cancer health effects from space travel. An AOP begins with a molecular initiating event (MIE) and leads through a series of hypothetical key events (KEs) to an AO of regulatory significance. The KEs along an AOP are causally linked and described in key event relationships (KERs). Herein, the AOP framework was applied to develop and evaluate the weight of evidence (WOE) for an AOP to vascular remodeling. Following the creation of a preliminary pathway with the guidance of field experts and authoritative reviews, a scoping review was conducted. This informed final KE selection and facilitated WOE evaluation of the KERs and AOP. The AOP describes how the MIE, deposition of energy, leads to ionization events that increase oxidative stress, which concurrently causes the release of pro-inflammatory mediators and imbalance of cellular homeostasis. This can then alter signaling pathways to maintain cellular balance. These KEs cause altered nitric oxide levels leading to endothelial dysfunction and subsequent vascular remodeling (the AO). Through the review of ~3000 articles, distinct KEs were delineated, and their essentiality evaluated. Known modulating factors of the AOP were also described. Our work identifies evidence needed to strengthen understanding of the causal associations for the KERs, emphasizing where the knowledge gaps and uncertainties in both qualitative and quantitative evidence lie. We anticipate this AOP will direct future research to better understands the effects of space on the human body and eventually develop countermeasures to better protect future space travelers.

3.2 Introduction

The Organisation for Economic Co-operation and Development (OECD) Adverse Outcome Pathway (AOP) framework is used for regulatory applications to organize existing scientific knowledge in toxicology into a linear chain of causally related events (Ankley et al., 2010; OECD, 2018). Each pathway in this framework begins with a molecular initiating event (MIE) and proceeds through a varying number of key events (KEs) before culminating in an adverse outcome (AO) of regulatory interest. The pathways are also uni-directional, with inclusion of appropriate feedback loops, progressing through KEs at increasing levels of biological organization. KEs are connected by key event relationships (KERs) for which causality is evaluated according to the modified Bradford-Hill (BH) criteria (Becker et al., 2015). These criteria include biological plausibility, evidence for the essentiality of KEs, and empirical evidence in the form of dose-, temporal- and incidence-concordance of the KERs. These directional and causal relationships are supported by a weight of evidence (WOE) comprised of primary research data that justifies the strength of evidence for each KER (Villeneuve et al., 2014). The pathways are purposefully simplified, describing KEs that can be routinely measured and are essential to pathway progression, to facilitate regulatory utility and test strategy development (Ankley et al., 2010).

Conventionally, AOPs have been used to organize information on the effects of chemicals on human and ecological health. However, recently there has been interest to expand AOP use in the radiation field (Chauhan et al., 2019, 2022; Chauhan, Sherman, et al., 2021). To this end, through collaborative efforts with the Canadian Space Agency (CSA) and National Aeronautics and Space Administration (NASA) an AOP network is being built to space-relevant non-cancer AOs (Kozbenko et al., 2022). Space exposure presents unique challenges because of the multi-faceted nature of the exposome, which include stressors like ionizing radiation, microgravity and others. For this regulatory context, an AOP network of four pathways has been developed that summarizes the progression of bone loss, cataract formation, cognitive impairments, and vascular remodeling following space exposure (Supplemental Figure 1). We also note that it is well known that radiation increases the risk of cancer; while it is outside the scope of the current network, it is the subject of other AOPs (e.g., AOP #272 (Chauhan, Sherman, et al., 2021)).

In this AOP report, we summarize the evidence linking deposition of energy to vascular remodeling (Figure 3.1). The proposed pathway presented is intentionally simplified, demonstrates one possible mechanistic progression and includes select essential/measurable KEs across levels of biological organization. Though the principles of AOP development indicate that an AOP should be stressor-agnostic, we have used the collection of stressors encountered in space as this is a primary regulatory concern. In addition, our MIE (energy deposition) narrowed the literature search in this data-rich area to radiation stressors for the overall AOP assessment. However, we note that because AOPs are living documents, the WOE for this AOP can be supported in future work through the inclusion of empirical data from studies using other stressors as required. In the long-term, we foresee the expansion of this pathway as the evidence is strengthened by more directed research focused on knowledge gaps identified in the present work. This will inform the development of quantitative risk models and countermeasure strategies for protection of future space travelers.

3.3 Adverse Outcome Pathway (AOP #470) – Brief Summary

The present AOP (AOP #470) begins with deposition of energy (MIE) and ends with vascular remodeling (AO). Detailed descriptions can be found in the AOP-Wiki (aopwiki.com).

Several KEs of this pathway are shared with the broader space-health network, as well as with existing pathways in the AOP-Wiki. Table 3.1 presents a summary of the KEs in this AOP alongside the methods of detection and links to existing events where applicable. Tables 3.2 and 3.3 summarize the empirical evidence for adjacent and non-adjacent KERs. Throughout this thesis, KEs are numbered to be consistent with the numbers used in Chapter 2; however, these numbers differ from those assigned in the AOP-Wiki.

Pathway progression begins with the deposition of energy (MIE), which refers to the ionization events occurring after intersection of biological systems by sufficiently high energy subatomic particles or electromagnetic waves. This MIE was developed previously for the first AOP specifically tailored to radiation risk assessment (AOP #272) that was approved by the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST) in 2022. The deposition of energy leads to an increase in oxidative stress [KE9], which also already exists in the AOP-Wiki (Table 3.1). An increase in oxidative stress leads in turn to altered signaling pathways [KE10] and an increase in pro-inflammatory mediators [KE18], all at the macromolecular level. The WOE for deposition of energy leading to endothelial dysfunction [KE 15], altered nitric oxide (NO) levels [KE 23] and the AO is also supported in non-adjacent KERs to increase the overall confidence in the AOP. Changes to signaling pathways and the state of oxidative stress then lead to alterations in NO levels [KE 23]. Fluctuations of the PI3K/Akt, RhoA/ROCK and RAAS pathways all affect NO levels through altering the phosphorylation and associated activation of the endothelial nitric oxide synthase (eNOS) enzyme responsible for NO synthesis in vasculature. Simultaneously, the increased reactive oxygen species (ROS) and reactive nitrogen species (RNS) levels characteristic of a state of oxidative stress reduce NO levels through directly reacting with the NO, while also inhibiting the dimerization of and promoting the uncoupling of eNOS to affect its function. All of these upstream KEs work together to cause an increase in endothelial dysfunction [KE 15] at the tissue level, defined as a prolonged state of activation marked by premature endothelial cell senescence and reduced vasomotive response (Deanfield et al., 2007). Chronic endothelial dysfunction eventually leads to vascular remodeling [AO] as vessels compensate for dysregulation in blood flow and angiogenesis. Vascular remodeling is a broad umbrella term that encompasses a variety of changes to vessel structure including artery wall thickening, increased vessel stiffness and decreased capillary density. Remodeling of the vasculature can be considered the gateway for a number of cardiovascular pathologies including coronary artery disease, acceleration of age-related atherosclerosis and myocardial ischemia (EPRI, 2020).

3.4 Background

3.4.1 Cardiovascular Disease and Vascular Remodeling

Cardiovascular diseases (CVDs) are a leading health risk in North America and around the world. Globally, CVDs are the leading cause of death, accounting for over an estimated 17 million deaths in 2019 (World Health Organization, 2021) and ~19 million deaths in 2020 (Tsao et al., 2022). In the United States between 2015 and 2018, CVD was prevalent in 49.2% of adults over 20 (Tsao et al., 2022). This prevalence rate does not include peripheral artery disease, which in the US is estimated to develop in over 20% of individuals in their lifetime (Matsushita et al., 2019) and leads to an elevated risk for subsequent cardiovascular mortality (Heald et al., 2006).

The CVD category includes a broad range of pathologies affecting the blood vessels and the heart. These disorders can be grouped into various sub-categories dependent on the cells/tissues involved. For example, endothelial cells play a dominant role in the development of atherosclerosis, hypertension and coronary artery disease, while cardiomyocyte dysfunction is involved in hypertrophic cardiomyopathy and myocardial infarction (Chauhan, Hamada, et al., 2021). Alternatively, CVD can be classified according to which structure it affects (vessel, heart muscle, or valve), or the nature of dysfunction (arrythmia, structural disease, or heart failure). Nonetheless, changes to vessel structure through vascular remodeling is a shared KE across the different pathologies. Vascular remodeling encompasses a variety of structural changes to vessels that can include thickening of various vessel layers, increased stiffness, changes to vessel wall composition, and degradation of the extracellular matrix (Intengan & Schiffrin, 2001; Van Varik et al., 2012; Zieman et al., 2005). Altered blood flow can also lead to microvascular remodeling, with a decrease of capillary density in cases of low perfusion, or increase in vascularization in high flow scenario (Santamaría et al., 2020; Slezak et al., 2017).

Modification to vascular structure is not inherently detrimental; in fact, continual restructuring is crucial to promote cardiovascular health (Pries et al., 2001; Santamaría et al., 2020; Zakrzewicz et al., 2002). However, some forms of remodeling are important markers for future adverse cardiovascular events (Cohn et al., 2004; Van Varik et al., 2012). Measures like increased carotid-wall intima–media, elevated arterial stiffness or decreased lumen diameter in the case of peripheral artery disease, are all markers for the development and potential mortality and morbidity from CVD (Heald et al., 2006; Hodis et al., 1998; Polak et al., 2011; Zieman et al., 2005). With vascular remodeling being present in many CVDs and its power as a predictive marker of cardiac outcomes, it is an important endpoint of regulatory interest.

3.4.2 Radiation Stressors and CVD

The etiology of CVD and vascular remodeling are as varied as the diseases themselves. Their progression is an interplay of risk factors such as age, sex, genetics and lifestyle components. These modulating factors are described in further detail in the AOP-Wiki entry for the present AOP (AOP #470) as well as summarized later in this thesis. In addition to risk factors, it is known that exposure to radiation can lead to what is often referred to as radiation induced CVD (Belzile-Dugas & Eisenberg, 2021; Boerma et al., 2016; Sylvester et al., 2018; Tapio, 2016; Heru Wang et al., 2019)

When high energy particles or waves interact with biological matter, the energy they carry deposits onto cellular components leading to direct and indirect effects (Desouky et al., 2015). Radiation type, dose and dose rate all play a role in the downstream outcomes of radiation exposure (Desouky et al., 2015). The effects of energy deposition can be far reaching, potentially affecting all systems in the body; in the cardiovascular system, pathology can manifest years or decades after exposure (Boerma et al., 2016; Dörr, 2015; EPRI, 2020; Menezes et al., 2018). The delay in the development of CVD leads to a need for identifying endpoints that can be monitored for early signs of disease progression. Endpoints like vascular remodeling that occur prior to the development of more severe outcomes, can serve as important indicators for intervention necessity.

High-dose radiation exposure has also been strongly linked to radiation induced CVD. Growing evidence suggests that lower doses than previously considered contribute to cardiovascular risk (Boerma et al., 2016; EPRI, 2020; Little et al., 2021; UNSCEAR, 2008). Multiple reviews and technical reports have examined the correlation between radiation exposure and CVD (Baker et al., 2011; Boerma et al., 2016; Darby et al., 2010; EPRI, 2020; Little et al., 2021; UNSCEAR, 2008). We highlight some key studies used support this relationship below.

A literature review of studies of cancer patients that had received radiotherapy suggests an association of radiation exposure with elevated risk for later adverse cardiovascular effects (Zou et al., 2019). In addition to clinical exposure scenarios, epidemiological studies of workplace exposures and Japanese atomic bomb survivors have also been conducted. For example, a number of studies have examined cardiovascular outcomes within the Life Span Study cohort (a cohort study of atomic bomb survivors) throughout the decades, detailing the ways CVD risk was affected by exposure (Little et al., 2021; Ozasa et al., 2012; Preston et al., 2003; Shimizu et al., 2010; Takahashi et al., 2017). Long-term follow up of individuals exposed in the Chernobyl disaster region also identified statistically significant elevation in CVD risk (Ivanov et al., 2006; Kashcheev et al., 2017). International workplace exposure studies have also been conducted in an effort to understand the relationship between low-dose chronic exposure and cardiovascular health of nuclear workers (Azizova et al., 2018; Gillies et al., 2017; Zielinski et al., 2009). The workplace exposure studies suggest positive associations between received dose and excessive relative risk of circulatory diseases (Little et al., 2021; Zielinski et al., 2009), CVD mortality (Gillies et al., 2017) and occurrence of ischemic and cerebrovascular disease (Azizova et al., 2018).

3.4.3 Motivation for a regulatory framework for health protection of the unique space environment

Deep space travel represents a unique exposure scenario as the nature, dose and dose-rate of radiation encountered in space differs from earth. On earth and in low earth orbit , exposure to radiation is mostly comprised of low linear energy transfer (low-LET) background, medical and workplace radiation (Baker et al., 2011; Durante & Cucinotta, 2008). This is in contrast to the space environment, where solar particle events and galactic cosmic rays additionally contribute high-LET particles and high energy ions (HZEs) (Baker et al., 2011; Boerma et al., 2016; Durante & Cucinotta, 2008). Solar particle events contribute intermittently with predominantly lower-energy protons, while galactic cosmic rays are perpetually present with low-doses of heavy ions and energetic protons (Baker et al., 2011). Heavy ions present a particular challenge, since their high ionization potential makes them simultaneously proportionally greater contributors to total equivalent physical dose and capable of creating secondary particles as they penetrate shielding (Cucinotta et al., 2006; Simonsen et al., 2020). Cosmic radiation exposure is also different because unlike a clinical scenario in which there is acute exposure for a target site, space exposure indiscriminately impacts the whole body at a low fluence rate (Norbury et al., 2016).

Some cosmic radiation does reach earth and its inhabitants; however, on earth and in low earth orbit the earth's magnetosphere provides a natural shield from the brunt of exposure. With the exception of the Apollo lunar missions, human space exploration has all happened within this protected zone, as the international space station orbits mostly within the low earth orbit. However, future missions are planned beyond the protective space of the earth's magnetosphere, including plans for a lunar base and manned Mars mission (https://www.nasa.gov/what-is-artemis). These missions will push the previous limits of time spent and distance traversed in space and require rigorous protection of participants. While radiation is of large concern, in the space exposome (i.e., the combination of all stressor encountered by space travelers) its effects are combined with microgravity that is expected to also affect the gravity-sensitive cardiovascular system (Baran et al., 2021; Hughson et al., 2017). With these considerations in mind, CVD risk for astronauts on long-range missions has been identified as being of highest priority due to its combined high likelihood of occurrence and potential for significant impact on crew health both during missions and upon return to earth (Patel et al., 2020). Reviews have explored the unique effect of space environment on CVD progression and what to consider as missions are planned (Huff et al., 2022; Hughson et al., 2018; Mitchell et al., 2019)

The present work harnesses the strengths of the AOP framework to contribute to the organization of research on the topic of CVD risk in space. Through collecting interdisciplinary research at all levels of biological organization and systematically evaluating the WOE in the KE(R)s to disease progression, we summarize current knowledge and prioritize key areas of future research focus.

3.5 AOP Development Strategy

KERs are the directional and causal links between KEs in the AOP. Each KER is supported by a collection of primary research used to infer causality through modified BH criteria (Becker et al., 2015), and considers modulating factors and uncertainties. In creating the present AOP (Figure 3.1), a preliminary pathway (Supplementary Figure 1) was first built based on biological plausibility identified through a narrative review of ~100 key articles and input from subject matter expert consultants (Chauhan, Hamada, et al., 2021). While many potential KEs were considered, attention was focused on those considered the most essential to disease progression and with accompanying evidence to support their causal connection to the pathway. As mentioned previously, the space exposome is a combination of various stressors; as such, MIEs encapsulating changes resulting from stressors like microgravity were also considered. However, no distinct molecular event related to microgravity exposure could be determined and therefore deposition of energy was used as the MIE. While the MIE is primarily related to radiation exposure, the pathway is informed by studies using a variety of space-related stressors as described in the inclusion criteria (Table 2.1) and is not dose or dose-rate specific. The preliminary pathway was then used as a basis for a scoping review (protocol described in Chapter 2, Figure 2.2) (Kozbenko et al., 2022). Results from this scoping review form the database for the WOE evaluation in the present AOP report and are summarized in several systematic evidence maps (SEMs) (Figures 2.4, 2.5 a, b, c and Figure 3.2).

Through the process of reviewing the literature, changes were made to the preliminary pathway to better reflect the available empirical data (Figure 2.6). A systematic evidence map (Figure 2.4) justified the creation of a KE for altered NO levels [KE23] that is separate from other changes to signaling pathways [KE10] (Kozbenko et al., 2022). While nitric oxide (NO) is a signaling molecule, the review identified sufficient literature focusing on its unique role in the progression of endothelial dysfunction to warrant it as a separate KE. Following literature screening, two KEs were also removed from the preliminary pathway: protein modification and expression changes [KE14], and cardiovascular disease [AO3]. There was significant overlap between the protein modification and altered signaling KEs; modified proteins were generally part of signaling pathways and therefore, downstream effects were better described as being due to signaling changes. It was recognized that protein oxidation could be downstream of altered signaling pathways; however, the current literature assessment did not provide enough empirical evidence to warrant inclusion. As such, these two KEs (protein modification and altered signaling) were combined under the altered signaling pathway KE. Finally, while cardiovascular disease was the original AO, we found that this was too broad because it encompasses many pathologies each with their own progression. This led to difficulties in identifying empirical data to support causal relationships with the KEs. Instead, the pathway now terminates with vascular remodeling, which as discussed previously is an important regulatory endpoint. It is essential to note that removal of KEs from the current pathway does not indicate a lack of biological importance. Rather, these decisions were based on a lack of data availability to support the linkage of these KEs to the current pathway. There is opportunity for future work to strengthen the understanding of their role; indeed, this is one of the strengths of the modular format of AOPs and publication in the AOP-Wiki.

3.6 Summary of Scientific Evidence

3.6.1 Description and evaluation of biological plausibility and empirical evidence

Biological plausibility is considered the most important form of evidence of the BH criteria (Becker et al., 2015) and the strongest criteria for the WOE in the present AOP. Below we describe the well-established understanding of the mechanisms underlying this AOP and highlight the supporting literature. More detailed examples of the empirical data supporting each KER, and the WOE calls based on those data, are found in Tables 3.2 and 3.3, as well as in the AOP-Wiki.

The deposition of energy [MIE] is linked via an adjacent relationship to an increase in oxidative stress [KE9] as well as to altered nitric oxide levels [KE23], endothelial dysfunction [KE15] and vascular remodeling [AO] through non-adjacent relationships (Figure 3.1). It is very broadly understood that when energy is deposited in the cell, there is direct damage to cellular structures and indirect damage through radiolysis of water forming damaging ROS (Desouky et al., 2015). In addition to radiolysis, deposition of energy can elevate intracellular ROS through activating the ROS-producing NADPH oxidase (NOX) enzyme or damaging mitochondrial structures and causing leaked electrons to ionize surrounding molecules (Mittal et al., 2014; Soloviev & Kizub, 2019). Cellular defense mechanisms, like antioxidant enzymes, alongside various vitamins and minerals serve to attenuate ROS damage (Fletcher, 2010). As antioxidants are consumed during ROS neutralization, the pace of ROS and RNS production can outpace the rate of antioxidant replenishment and the cell enters the well-studied state of oxidative stress (Slezak et al., 2017; Tahimic & Globus, 2017; Heru Wang et al., 2019).

Together, damage to cellular structures, either directly or via excessive ROS and RNS, disrupts cellular balance and enlists the immune system in the same way damage from a pathogen would (Lumniczky et al., 2021; Schaue et al., 2015). Activation of the immune system promotes many repair mechanisms, some of which involve rapid release of pro-inflammatory cytokines by macrophages (Stanojković et al., 2020). The cytokine profile varies between tissue types and is affected by radiation parameters (Di Maggio et al., 2015), but TNF-α and IL-1 are of note as they play a critical role by triggering a cytokine cascade that initiates an inflammatory response integral to healing (Slezak et al., 2017; Srinivasan et al., 2017). An immune reaction can in itself contribute to oxidative stress, with cells involved in host-defense producing ROS and leading to a cycle of inflammation and oxidative stress (Mittal et al., 2014).

In addition to recruiting repair mechanisms, there is general acceptance that ROS/RNS damage can also modulate signaling pathways. The effects of oxidative stress on signaling pathways often takes the form of protein oxidation of signaling components (Ping et al., 2020; Schmidt-Ullrich et al., 2000; Valerie et al., 2007). Cysteine and methionine residues are particularly sensitive, and their oxidation can result in structural and functional detriments (Ping et al., 2020). The effect of oxidative stress has been observed in various pathways including the Akt/PI3K/mTOR pathway, where impaired cell survival signaling can lead to the initiation of cellular senescence (Hassan et al., 2013; Ping et al., 2020). Additionally, inhibition of tyrosine phosphatases during oxidative stress can lead to elevated levels of phosphorylation of pathway components (Schmidt-Ullrich et al., 2000; Valerie et al., 2007) like p53 from the p53-p21 pathway (Ashcroft et al., 1999; Gen, 2004). A phosphorylated p53 serves to activate mitogen-activated protein kinase (MAPK) and extracellular-signal-regulated kinase (ERK) signaling molecules and initiates a cascade ending in apoptosis (Gen, 2004). Furthermore, oxidative DNA damage can cause mutations or changes in transcription of proteins in signaling pathways (Ping et al., 2020; Schmidt-Ullrich et al., 2000). Through affecting cell signaling pathways, damage caused by the deposition of energy and subsequent elevated ROS/RNS affects cells beyond those directly irradiated (Ramadan et al., 2021).

Changes to signaling pathways and a prolonged state of oxidative stress together contribute to changes in NO levels. While a state of oxidative stress encompasses an overabundance of both ROS and RNS, most mechanistic studies have focused on the effects of ROS (Nagane et al., 2021). In the endothelial cells of the cardiovascular system, NO is synthesized from L-arginine by the eNOS enzyme; ROS can interrupt this process in several well characterized ways (Förstermann & Münzel, 2006; Mitchell et al., 2019). Superoxide can react with NO to form peroxynitrite ($ONOO^-$), directly reducing the amount of bioavailable NO. Peroxynitrite oxidizes BH_4, the eNOS cofactor, which in turn inhibits eNOS dimerization in a process often referred to as eNOS uncoupling (Deanfield et al., 2007). When eNOS is uncoupled, it begins producing ROS in the form of superoxide anion ($O_2{\cdot}^-$) instead of NO. The switch from NO to ROS production means less NO is created and more ROS is present to react with remaining NO further decreasing its bioavailability (Förstermann, 2010; Förstermann & Münzel, 2006; Mitchell et al., 2019; Nagane et al., 2021; Soloviev & Kizub, 2019). Studies exploring NO production often use endothelial and inducible NOS (eNOS and iNOS) levels as a proxy measure for NO. Generally, increases in NOS expression correspond with increases of bioavailable NO, and vice versa, but there are some important exceptions. In the presence of excessive ROS, the additional NO produced by high levels of NOS serves as excess reagent for additional production of peroxynitrite. Since peroxynitrite is a ROS, in this case elevated NOS actually leads to a decrease in bioavailable NO that is instead replaced with ROS (Förstermann, 2010; Förstermann & Münzel, 2006; Soloviev & Kizub, 2019).

eNOS activity can be modulated by phosphorylation that is controlled by various signaling pathways; phosphorylation at the Ser1177 position leads to activation while Thr495 phosphorylation decreases enzymatic activity (Förstermann, 2010; Nagane et al., 2021). When Akt from the Akt/PI3K/mTOR pathway is activated, it phosphorylates eNOS at Ser1177 and upregulates NO production (Karar & Maity, 2011). Conversely, activation of the RhoA/ROCK pathway destabilizes eNOS mRNA and prevents Ser1177 phosphorylation by Akt, thereby decreasing NO bioavailability (Yao et al., 2010). Finally, angiotensin II (AngII), the end product of the RAAS pathway, is involved in both downregulating Ser1177 phosphorylation to prevent NO creation (Ding et al., 2020) and activating eNOS as a corrective measure (Millatt et al., 1999). While the involvement of various pathways in changes to NO levels and endothelial dysfunction is generally accepted, further mechanistic understanding would strengthen the causality of the linkages.

All branches of the pathway converge at endothelial dysfunction. A single layer of endothelial cells lines all vessels in the human body; these cells are crucial for maintaining vascular homeostasis and are the most vulnerable part of the cardiovascular system to ROS damage (Bonetti et al., 2003; Deanfield et al., 2007). Most of the time endothelial cells are quiescent with high levels of vaso-protective NO (Carmeliet & Jain, 2011). Activation of the endothelium is part of a normal host-defense response; however, prolonged activation is the pathological state of endothelial dysfunction (Deanfield et al., 2007). It is widely accepted that endothelial dysfunction is characterized by several key traits including the prolonged lack of bioavailable NO, lack of endothelium-dependent vasodilation and chronic pro-thrombotic and inflammatory state (Baran et al., 2021; Bonetti et al., 2003; Deanfield et al., 2007; Krüger-Genge et al., 2019). Mechanisms described previously all contribute to the decrease of bioavailable NO. Prolonged lack of NO has the well-known consequence of increased leukocyte adhesion and fibrous plaque formation contributing to the pro-thrombotic dysfunctional environment (Schiffrin, 2008; Senoner & Dichtl, 2019; Venkatesulu et al., 2018). Repeated or prolonged insult like oxidative stress, chronic inflammation or modulation of the Akt/PI3K/mTOR pathway can all cause premature endothelial cell senescence (Borghini et al., 2013; Hughson et al., 2018; Schiffrin, 2008; Senoner & Dichtl, 2019). Senescent cells have decreased levels of NO production and have a pro-inflammatory secretory phenotype all of which feeds back to a state of endothelial dysfunction (Ungvari et al., 2013; Y. Wang et al., 2016). Furthermore, p53 phosphorylation and ceramide production in the sphingomyelin ceramide pathway can both contribute to endothelial cell apoptosis, elevated levels of which are a marker for endothelial dysfunction (Soloviev & Kizub, 2019; Venkatesulu et al., 2018).

In a dysfunctional state, vascular remodeling can include changes to the endothelial layer like changes to membrane permeability or structural changes that compensate for dysfunction. For example, chronic inflammation combined with impaired healing and lack of endothelium-dependent vasodilation leaves the vessels vulnerable to damage from non-laminar flow and maladaptive repair (Sylvester et al., 2018). To compensate for non-laminar flow and chronic inflammation, there can be thickening of vessel walls and increased risk for down-stream atherosclerosis (Hughson et al., 2018; Slezak et al., 2017; Sylvester et al., 2018). In cases of maladaptive repair of vessels, vascular remodeling can look like an increase in fibrosis (Hsu et al., 2019). Endothelial cell senescence can cause disruptions in endothelial integrity and lead to increased lymphocyte adhesion and thrombus formation. In this environment, vessel occlusion becomes more likely, decreasing vascular density and corresponding increase in vascular resistance can triggers compensatory vascular remodeling (Slezak et al., 2017). This is in part why increased leukocyte adhesion during dysfunction is an early event in the development of atherosclerosis (Senoner & Dichtl, 2019). Lack of NO has also been linked to increases in arterial stiffness (Boerma et al., 2015, 2016; Patel et al., 2020), which is observed through increased collagen and smooth muscle content paired with decreased elastin and degradation of the extracellular matrix (Zieman et al., 2005). Many of the vascular structural changes stemming from the deposition of energy have been compared to a form of accelerated age-related atherosclerosis (Boerma et al., 2016; Sylvester et al., 2018; Vernice et al., 2020).

3.6.2 Essentiality of the KEs

Since there are no chemical analogues to deposition of energy, essentiality of the MIE is assessed by comparing endpoints between an irradiated and non-irradiated control group or observation of endpoints following the removal of the radiation stressor (i.e., start-stop experiments). Events in the pathway are triggered by initiating events outside of the deposition of energy; for example, vascular remodeling is part of the natural aging process (Zieman et al., 2005), oxidative stress can occur from any number of injuries and changes in NO levels are part of normal endothelial activation. However, in comparing irradiated and non-irradiated groups the deposition of energy has been shown to accelerate or worsen these events (Table 3.3 row 3).

Essentiality of oxidative stress is often established through the administration of various antioxidants. Antioxidant treatments that can decrease ROS production, scavenge existing ROS or bolster the activity of the antioxidant defense system have been shown to both decrease markers of oxidative stress and simultaneously inhibit downstream KEs. An example is endothelium-dependent vasodilation decreased by radiation exposure being improved by decreasing peroxide and superoxide levels through administration of SOD mimetics (Hatoum et al., 2006). The inhibition of the cardiac ROS contributing enzyme xanthine oxidase (XO) via treatment with oxipurinol was found to improve in recovery of endothelium-dependent vasodilation and return of vascular stiffness to control levels (Soucy et al., 2007, 2010, 2011). By simultaneously measuring multiple endpoints, this trio of studies demonstrates the essentiality in relationships between oxidative stress, endothelial dysfunction, and vascular stiffness. Similarly, human bone marrow mesenchymal stem cells (hBMSCs) were found to be effective in increasing antioxidant enzyme activity, decreasing apoptosis and returning wall thickness to control levels in the aorta (Shen et al., 2018).

Essentiality for changes in signaling pathways can be challenging to determine due to the flexibility of signaling networks. Studies have explored the use of signaling molecule inhibitors like Y27632 and DPM to suppress signaling pathways and shown decreases in apoptosis and recovery of endothelium-dependent vasodilation (Soloviev & Kizub, 2019; Venkatesulu et al., 2018; Y. Wang et al., 2016). Other groups have incubated endothelial cells in mesenchymal stem cell conditioned media (MSC-CM) that increase both Akt and p-Akt cell signaling components and similarly observed a decrease in apoptosis (Cheng et al., 2017). In studying changes to NO levels, signaling modulators like LY294002 and wortmanin (PI3K inhibitors), and BPF (angiotensin-converting enzyme inhibitor) have been tested. Inhibition of P13K reversed increases in p-Akt and subsequent eNOS, p-eNOS and NO levels (Shi et al., 2012; Siamwala et al., 2010). Similarly, BPF treatment following irradiation returned AngII and iNOS levels to control (Hasan et al., 2020). Overall, further mechanistic studies are required to more specifically describe the how changes in signaling pathways affect changes in NO levels and endothelial dysfunction.

Pro-inflammatory mediators are a component of the immune response to many stressors. A few studies have evaluated the essentiality of pro-inflammatory mediators by suppressing their expression and observing changes in endothelial dysfunction endpoints (Chang et al., 2017; Ramadan et al., 2020). When TAT-Gap19 was used to block connexin43 hemichannels there was a decrease in pro-inflammatory mediators and associated decrease in endothelial cell senescence (Ramadan et al., 2020). In the same study, Cheng et al. demonstrated that incubation in mesenchymal stem cell conditioned media (MSC-CM) caused a decline in key cytokines IL-1α, IL-6 and TNF-α and a decrease in endothelial apoptosis (Chang et al., 2017).

As described above, several studies have explored changes to vascular structure as one of several measured endpoints. An additional set of studies explored the role of the ASM/Cer pathway in structural changes related to microgravity. The ASM/Cer pathway balances apoptosis and cell proliferation of vessel walls mediating thickness. Following hindlimb unloading, mice showed a decrease in apoptosis and a corresponding increase in intima-media thickness (IMT) in vessels above the heart, with the opposite trend of elevated apoptosis and decrease in IMT below the heart (Cheng et al., 2017; Su et al., 2020). Inhibition of ASM or treatment with doxepin hydrochloride (DOX) led to decreases in apoptosis and increases in cell proliferation and vessel thickness respectively (Su et al., 2020). Various studies comparing vascular structure between irradiated and sham or non-irradiated control groups have also shown the differences in vascular structure between the groups (Hamada et al., 2020, 2021; Sárközy et al., 2019; Shen et al., 2018; Sridharan et al., 2020; Yu et al., 2011).

3.6.3 Modulating factors

Vascular remodeling and associated risk of CVD is modulated by a complex interplay of various factors. The first of these factors is sex. The different actions of sex hormones play important roles in remodeling mechanisms like hypertrophy, inflammation, fibrosis and apoptosis. In part, this is due to sex-specific genetic components involved in remodeling (Kessler et al., 2019; Winham et al., 2014). Historically, men have been considered to be at higher risk for cardiovascular pathology with higher age-adjusted mortality rates for both CVD and coronary heart disease (CHD) (Mosca et al., 2011). However, rates of radiation-induced coronary artery disease (R-CAD) following radiation treatment for certain cancers are reported as 4-times higher in women than men (Khalid et al., 2020) and the absolute number of women living with and dying of CVD and stroke is greater than males (Mosca et al., 2011). With respect to sex, it is also important to mention the historic bias towards using male models in biomedical research (Karp & Reavey, 2019), leading to knowledge gaps in mechanistic understanding for the female system. Overall, sex is considered to be an established risk factor in disease progression and an important consideration for this AOP (Mozaffarian et al., 2008).

Another well-established risk factor is age (Harvey et al., 2015; Mozaffarian et al., 2008; North & Sinclair, 2012; Rodgers et al., 2019; Ungvari et al., 2018). Increased age leads to the cardiovascular system undergoing natural structural changes like increases in arterial stiffness, arterial wall thickening and decreased endothelial function (North & Sinclair, 2012; Zieman et al., 2005). For RICVD, the age at which there is radiation exposure is thought to play a role, with a younger age of exposure related to elevated risk of eventual RICVD (Belzile-Dugas & Eisenberg, 2021; Yang et al., 2021). Age and sex risk factors intersect - age-related changes to sex hormone levels can modulate risk for various CVDs (Kessler et al., 2019; Mosca et al., 2011; Rodgers et al., 2019; Winham et al., 2014).

Predisposition for adverse vascular remodeling includes a large genetic component. Baseline measures of carotid intima-medial thickness (CIMT), vascular stiffness and prevalence of coronary calcification are in part hereditary and vary between ethnicities (Berk & Korshunov, 2006; Winham et al., 2014). Sex specific genetic risk factors include polymorphisms in genes related to adverse cardiac remodeling on the Y chromosome, with X chromosome inactivation being implicated in remodeling (Berk & Korshunov, 2006). A number of large scale genome wide association studies have identified loci associated with ischemic heart disease (Nelson et al., 2017; Nikpay et al., 2015), stroke (Malik et al., 2018) and atherosclerosis (Klarin et al., 2019).

Lastly, lifestyle factors contribute to the modulation of vascular remodeling in CVD (Mozaffarian et al., 2008; Tsao et al., 2022). Poor diet, inadequate physical activity, smoking and adiposity are linked with downstream risk factors like high blood pressure, elevated cholesterol and perturbations of glucose-insulin homeostasis (Mozaffarian et al., 2008). While encouraging lifestyle changes can be a policy and societal challenge, modification of lifestyle factors has been suggested to be more effective in mitigating cardiovascular risk than pharmacological intervention for symptoms (Chiuve et al., 2006). Socioeconomic status is also intertwined with lifestyle factors and measures such as income level, educational attainment, employment status and neighborhood factors can be predictive of CVD risk (Schultz et al., 2018). In the context of space-health, fitness and health requirements for professional astronauts creates a study cohort that when compared to the general population is subject to the healthy worker effect (Chowdhury et al., 2017). Since the astronaut population is still relatively small, ability to complete internal cohort comparisons is still lacking, reinforcing the need for determining an appropriate control group to account for unique astronaut lifestyle factors.

3.6.4 Uncertainties and Knowledge Gaps

While RICVD and related vascular remodeling is a much-discussed topic, here we seek to outline several knowledge gaps we identified through our WOE collection. Details of specific inconsistencies can be found in the KER descriptions on the AOP-Wiki. Below we highlight broad trends in the WOE.

The first of the knowledge gaps center around inconsistent findings when evaluating NO levels. According to the biological plausibility, deposition of energy and subsequent KEs would lead to a decrease in NO that contributes to endothelial dysfunction. However, primary research concludes that NO can both increase (Abdel-Magied & Shedid, 2020; Hirakawa et al., 2002; Sakata et al., 2015; Sonveaux et al., 2003) or decrease (Baker et al., 2009; Fuji et al., 2016; Hamada et al., 2020) following irradiation. In part, this could be due to the variety of proxy measures used to measure NO levels. NO is challenging to measure directly (Luiking et al., 2010) and the best method (e.g., measurement in cells vs cell medium vs serum or tissue alone) is subject to debate. Proxy measures can include iNOS and eNOS levels, nitrite/nitrate ratios and substrate levels, but all proxy measures leave room for interpretation of bioavailability of related NO levels. Context is important to consider; for example, an increase in eNOS implies higher levels of NO produced. However, if the NO is produced in an environment with excessive ROS, the NO will serve as abundant reagent for the formation of peroxynitrate; high levels of NO production do not guarantee high levels of bioavailable NO.

Additionally, it is important to consider that much of the research surrounding radiation and endothelial function is based on cancer. It is well documented that endothelial cells from a cancer niche have different properties than other endothelial cells (Baghban et al., 2020; Hida & Maishi, 2018). Studies addressing NO levels in irradiated cells outside of this context would benefit the current WOE. Overall, it is the depletion of NO that is known to be a critical component of downstream endothelial dysfunction (Deanfield et al., 2007; Krüger-Genge et al., 2019). However, upstream, there are uncertainties in how deposition of energy, altered signaling and oxidative stress affect NO levels. Further standardization in interpretation and measurement of NO could lead to refining this AOP to specify that the KE is a depletion of NO.

Another large knowledge gap is the lack of female-specific data. As discussed in the modulators section of this report, sex plays a crucial modulating role in the progression of CVD and vascular remodeling. Studies also suggest that vascular remodeling responses of astronauts varies by sex (Hughson et al., 2016). In the 89 unique references making up the final WOE for cardiovascular outcomes (Figure 3.3), only four considered female models (Chen et al., 2005; S. M. C. Lee et al., 2020; On et al., 2001; Russell et al., 2009). Of these four studies, one used exclusively female rats (On et al., 2001), while the remaining three reported the sex of participants but did not separate male from female results (Chen et al., 2005; S. M. C. Lee et al., 2020; Russell et al., 2009). The consequence of so few studies is the very large knowledge gaps in mechanistic data for the female body. Filling these knowledge gaps at all levels of biological organization will be an important step in solidifying the AOP.

Furthermore, this AOP has the potential for application in developing strategies to protect astronaut health in future long-range missions. To be more useful for this purpose, several knowledge gaps must be addressed. The first is the relative lack of chronic and low-dose exposure data. Estimating astronaut risk for radiation exposure is a difficult task (Chancellor et al., 2018) with variables like solar particle events having a large effect on total dose. Our own WOE (Figure 3.4) reflects what has been observed by Boerma et al. – most studies use high dose rate and single high dose radiation (Boerma et al., 2015, 2016), while studies exploring low doses (<0.5 Gy (EPRI, 2020)) are limited. Additionally, there were discrepancies in dose-concordance data between oxidative stress and pro-inflammatory mediators; this could be in part due to inflammation being a cause and effect of oxidative stress (Venkatesulu et al., 2018). Efforts are underway to create a more space-like radiation exposure scenario (Norbury et al., 2016); the results from such studies will be important future additions to the AOP. Additionally, more studies are required to demonstrate how the various stressors in the space exposome interact to cause their effects on the cardiovascular system. Existing data from mice (Sofronova et al., 2015) and humans (S. M. C. Lee et al., 2020) having experienced LEO exposure produced inconsistent or non-significant changes in vascular remodeling endpoints.

Lastly, the present AOP is qualitative in nature, and the WOE inclusion criteria (Table 2.1) encompassed all forms of radiation, irrespective of dose, dose-rate, and radiation nature. Our work has collated information on the occurrence and causality of relationships between KEs across multiple radiation types and wide dose ranges (Tables 3.1, 3.2, 3.3). However, the characteristics of radiation differentially affect biological response (Ogata et al., 2005; Rühm et al., 2015) and more work is required to develop a quantitative understanding of dose and dose-rate effects on KEs and KERs. Overall, a prioritization for harmonized experiments with consistent data reporting would help validate quantitative aspects of the relationships. Harmonized experiments could include testing endpoints for several KEs across a range of environmentally relevant doses and time-points in a single set of experiments. In the space-health context, deeper quantitative understanding would help determine threshold doses for cardiovascular effects that would inform occupational safety policy. Additionally, as astronauts on future long-range missions will encounter more space stressor exposure than could previously be studied, being able to use predictive modeling to anticipate effects will be vital. Together known threshold doses and predictive modeling could inform mission criteria such as the amount and nature of shielding required, as well as dosage of countermeasure drugs and therapies. The present qualitative AOP with its delineated KEs and endpoints can serve to direct future primary research that will fill the gaps in quantitative understanding.

3.6.5 Overall weight of Evidence Evaluation

- Biological plausibility: Strong

  The relationships described in the present AOP are well supported in literature by a broad collection of in-depth review articles.

- Essentiality: Low

  Many of the KEs in the network are shared with other pathways. Furthermore, downstream KEs like endothelial dysfunction are simultaneously caused by several upstream KEs. Interconnected pathways and converging KERs make it difficult to conduct experiments that isolate the essentiality of single KEs and their downstream effects. Although essentiality is assumed for each of the KEs, with the exception of oxidative stress, the actual WOE supporting essentiality is generally low.

- Empirical evidence: Moderate

  KERs in this pathway vary in strength of empirical evidence. While some relationships like deposition of energy [MIE] causing oxidative stress [KE9] are extremely well studied, others like altered signaling pathways [KE10] to changes in NO levels [KE23] require further concordance studies to strengthen the WOE.

- Quantitative understanding: Weak

  There are many kinds of studies included in this AOP that span a large variety of stressor types, doses, study designs, and model systems. There were very few individual studies wherein a single study design/model was used to establish the effects of dose and exposure duration on multiple endpoints. Overall, the database to build quantitative understanding is incomplete. In addition, the nature of radiation has a large effect on biological outcomes,; standardization would be required to make quantitative conclusions.

3.7 Concluding Remarks

CVD is an outcome affecting a large portion of the earth's population; with increasing space exploration and tourism it has the potential to become an interplanetary concern. Vascular remodeling is an important predictor of future CVD development and can serve as an important regulatory endpoint. There are a variety of risk factors for adverse vascular remodeling; the present AOP summarizes how the deposition of energy leads to its development. While this AOP is focused on the cardiovascular system, it is part of a larger AOP network that also considers ocular, bone and cognitive outcomes. The pathways in this network are interconnected through various shared early KEs including increases in oxidative stress [KE9] and pro-inflammatory mediators [KE18]. Although supported by strong biological plausibility, the WOE supporting the pathway lacks studies demonstrating essentiality and time-concordance. Dose-concordance is moderately demonstrated; however, the wide variety of experimental designs included in the WOE create a barrier for description of quantitative response-response relationships. Once quantitative understanding of the AOP is strengthened through additional harmonized experiments, the information can be used to support building quantitative risk models. The development of the AOP led to the identification of priority knowledge gaps including needs to: refine NO measurement methodologies to resolve discrepancies surrounding the qualitative and quantitative relationships with this KE, generate more female-specific data, and conduct experiments using space-relevant radiation dosage. Additionally, future iterations of the pathway might consider the role of vascular remodeling in other outcomes (e.g., cerebrovascular diseases and associated cognitive impacts). As new data are added, this AOP can be adapted to develop countermeasures that will protect the cardiovascular systems of those on earth as well as outside of LEO.

3.8 Figures and Tables

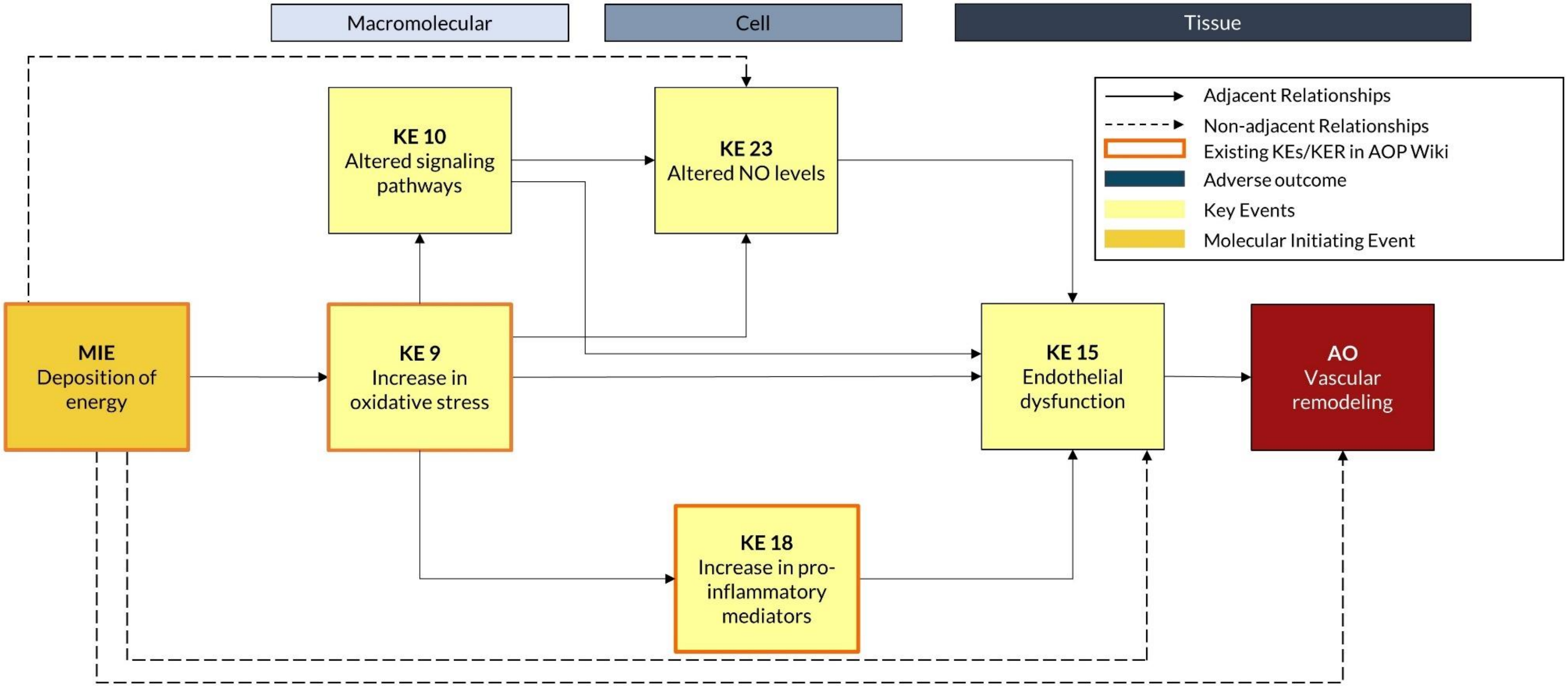

Figure 3.1. The visual representation of AOP #470 linking deposition of energy to vascular remodeling. Figure has been updated from Kozbenko et al. 2022 with the removal of relationships between MIE to KE 10 and MIE to KE18, and addition of a relationship between KE9 and KE10 following additional expert consultation.

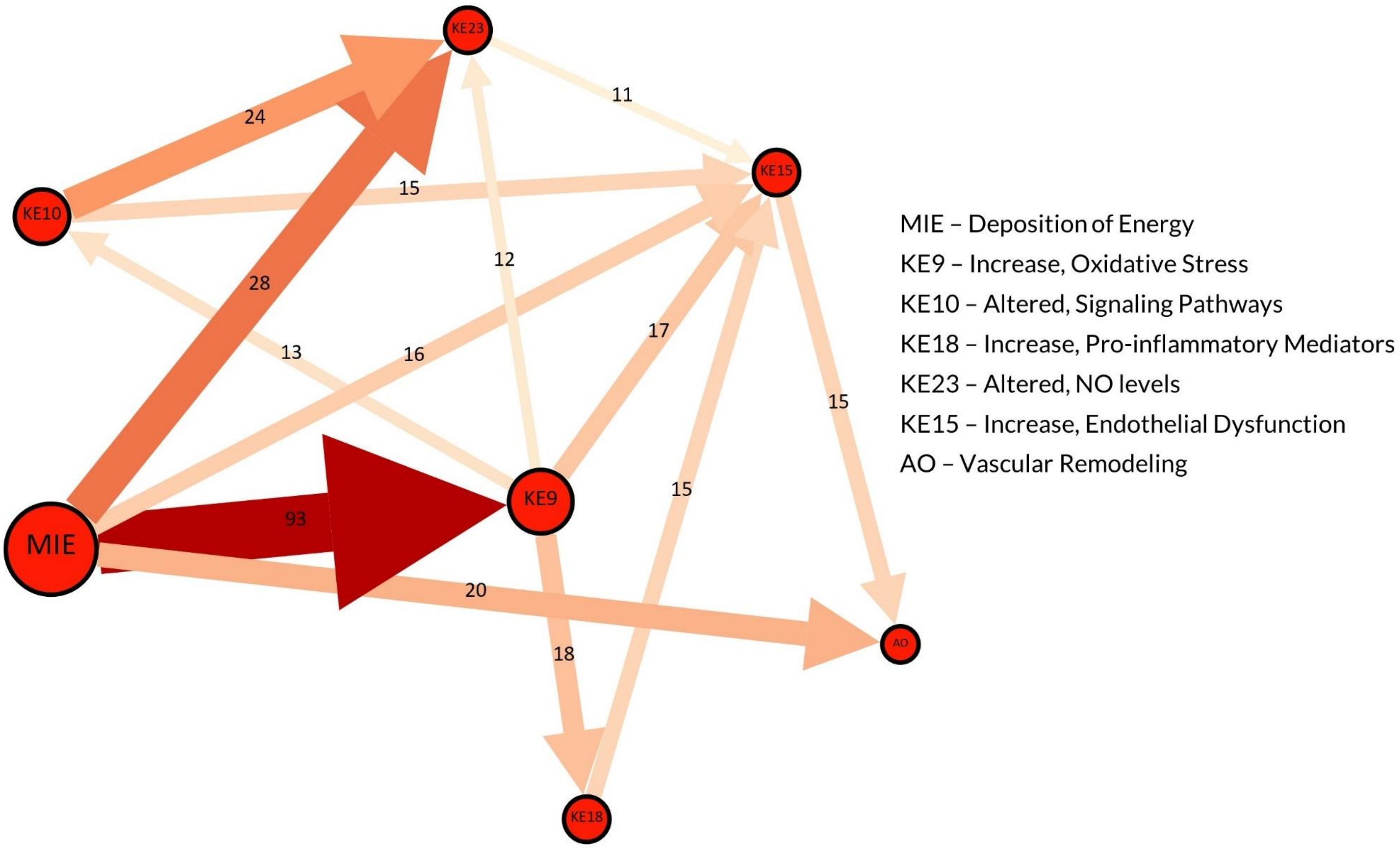

Figure 3.2. Systematic evidence map representing the quantity of evidence supporting all relationships in the AOP #470 pathway. Quantity of support for each KER is demonstrated visually with relative arrow weight and shade, and KE degree of connectivity (as determined by the number of downstream connections) is represented by circle size.

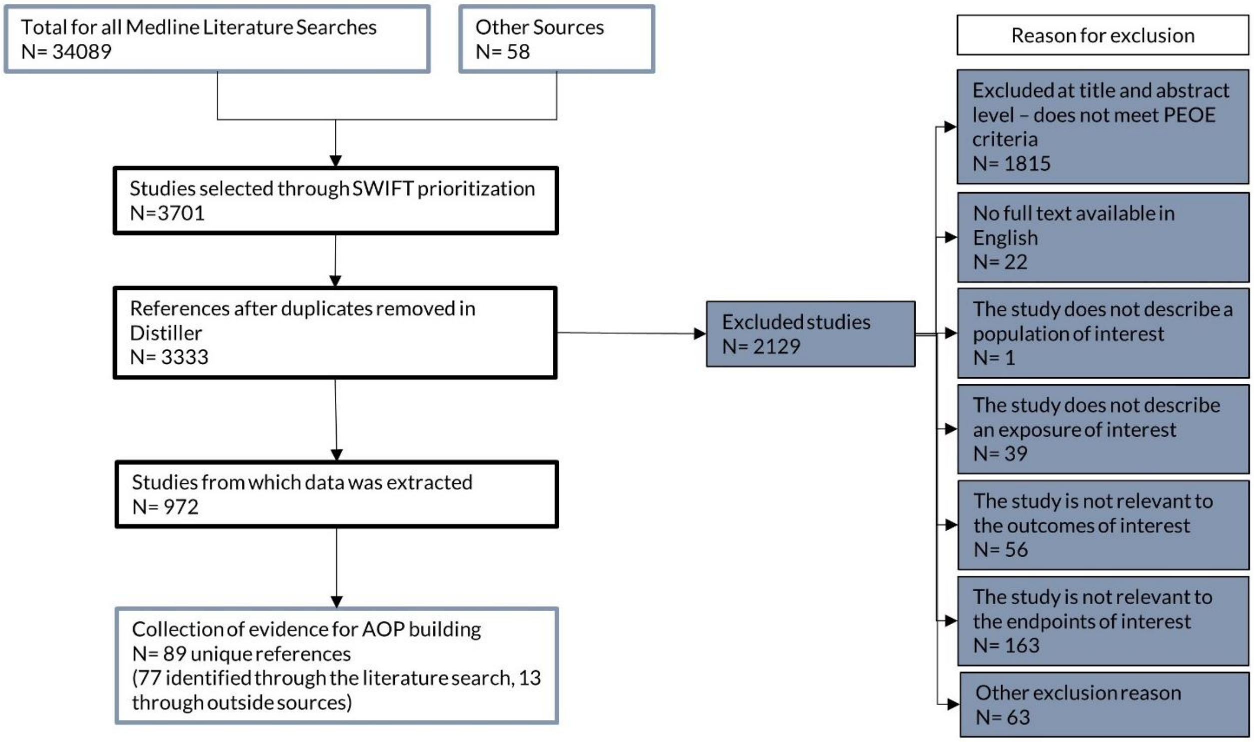

Figure 3.3. PRISMA diagram showing the progression of references through the scoping review described in Kozbenko et al. 2022. “Other sources” refers to papers retrieved outside of literature searches (e.g., from subject matter experts and reference sections of review articles). References could be excluded for multiple exclusion reasons simultaneously. Figure updated from Kozbenko et al. 2022 to include number of references in final AOP WOE.

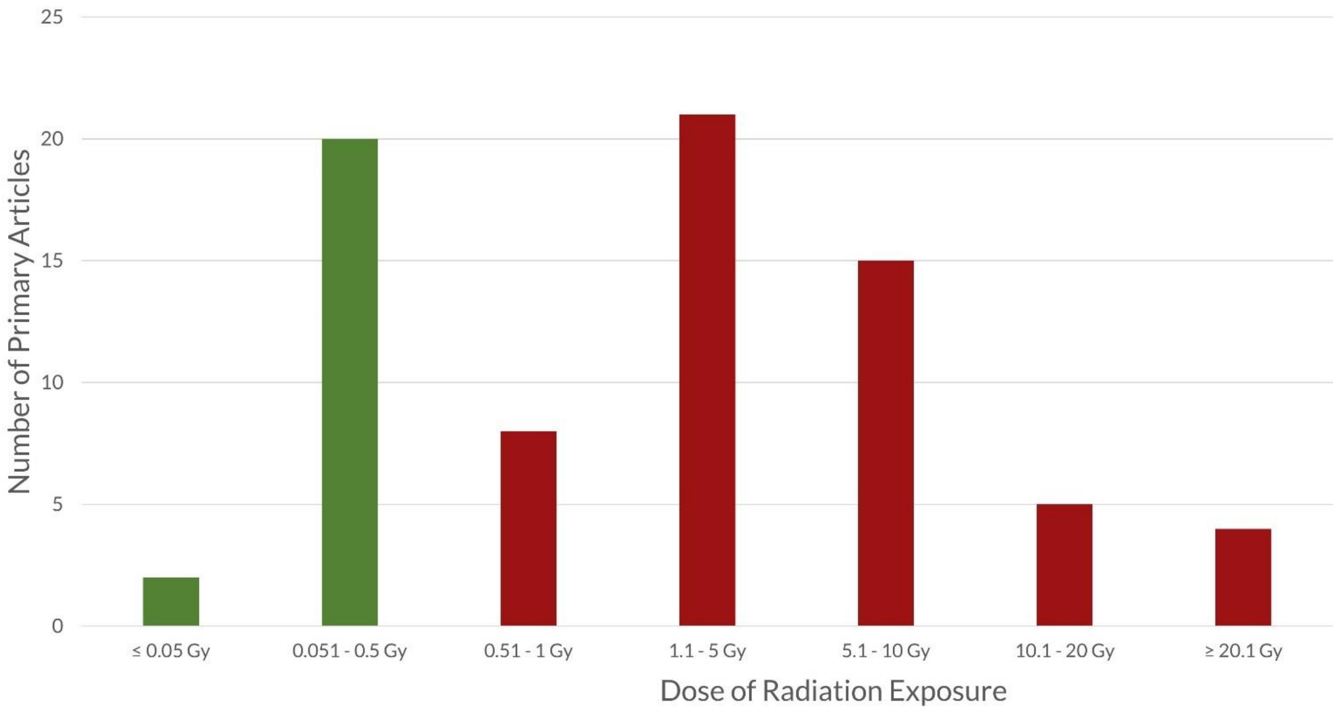

Figure 3.4. Dosages used in studies included in the WOE. Studies recording changes to endpoints at multiple doses are included more than once. Low doses (<0.5 Gy (EPRI, 2020)) are shown in green, while moderate and high doses are shown in red.

Table 3.1 – Summary of Key Events in AOP #470 and methods of measurement.

Type	Event title	Description	Measurement methods
Molecular Initiation Event (MIE)	Deposition of Energy Existing KE in AOP Wiki KE #1686	- Ionization of biological media following its traversal by high energy subatomic particles or electromagnetic waves. - Particles and waves can ionize cell structures or cause water radiolysis.	- Monte Carlo Simulations (Douglass et al., 2012, 2013) - Fluorescent Nuclear Track Detector (FNTD) (Kodaira et al., 2015; Niklas et al., 2013; Sawakuchi et al., 2016) - Tissue equivalent proportional counter (TEPC) (Straume et al., 2015)
Key Event [KE 10]	Increase, Oxidative Stress Several versions of this KE exist in the AOP-Wiki, we have added to the KE titled: KE #1392: Oxidative Stress	- Elevated production of reactive oxygen and nitrogen species (ROS and RNS) leads to imbalance between ROS/RNS and cellular antioxidant defenses. - Excessive ROS/RNS levels defines the damaging state of oxidative stress.	Direct measurement of ROS can be challenging because of its instability. Many forms of indirect measurements exist, some examples include: - Chemiluminescence of ROS induced electron transition (Griendling et al., 2016; Lu et al., 2006). - Spectrophotometry of ROS reduced nitrite (Griendling et al., 2016). - Cytochrome C Reduction Assay evaluates O_2^- as O_2^- oxidizes ferricytochrome c and resulting change in absorbance is measured (Griendling et al., 2016). - Dichlorodihydrofluorescein Diacetate (DCFH-DA) to indirectly measure H_2O_2 as it oxidizes the fluorescent product. (Griendling et al., 2016)
Key Event [KE 10]	Altered signaling pathways	- Changes to signaling pathways can include up or down regulation,	Measurement methods can be diverse across diverse signaling pathways. Methods usually center around evaluating quantity of

	New KE created for this project: KE #2066	initiation/activation, and suppression/silencing. - Changes can happen through transcriptional up/downregulation, modification of component proteins, elevated production of substrate and many others.	modification of pathway components. Some examples include: - Bradford protein assay to evaluate protein expression levels (Sapan et al., 1999). - Kinase assay inhibiting kinase activity and monitoring activity of kinase of interest (Svoboda & Reenstra, 2002). - Fluorescence microscopy/ spectroscopy to evaluate protein interaction or location (Oksvold et al., 2002). - Fluorescence Resonance Energy Transfer (FRET) to evaluate protein-protein interactions (Bunt & Wouters, 2017). - Fluorescence recovery after photobleaching (FRAP) determines mobility of small molecules (Svoboda & Reenstra, 2002).
Key Event [KE 18]	Increase, Pro-inflammatory mediators Existing KE in AOP wiki KE #1493	- Increased levels and activation of inflammatory response mediators. - Inflammatory profile depends on the tissue and nature of stressor, common groups include pro-inflammatory cytokines, prostaglandins, bioactive peptides, vasoactive amines, ROS and RNS.	- RT-qPCR and Q-PCR evaluating mRNA expression of inflammatory markers (Alwine et al., 1977; Forlenza et al., 2012; Veremeyko et al., 2012). - Immunoblotting to detect mediator presence (J. W. Lee et al., 2008). - Whole blood simulation assay to detect cytokines in blood (Thurm & Halsey, 2005). - Imaging tests like immunofluorescence confocal microscopy using cytokine specific antibodies to visualize expression and location of cytokines (Rollins & Miskolci, 2014). - Immunoassays like ELISA, inflammatory cytokine arrays or flow cytometry using antibodies to detect cytokine presence in a

			liquid sample (Amsen et al., 2009; Engvall & Perlmann, 1972; Karanikas et al., 2000). - Immunohistochemistry using antibodies to visualize location of mediators within a fixed tissue or cell culture sample (Amsen et al., 2009; Coons et al., 1942).
Key Event [KE 23]	Altered, Nitric Oxide Levels New KE created for this project: KE #2067	- Nitric oxide is a diffusible molecule and an important mediator in vasodilation and endothelial function. - Bioavailable NO levels can be affected through changes in production by NOS or through their depletion by ROS.	Direct measure of NO is challenging due to its transient nature, many studies use proxy measures such as: - Determining eNOS and iNOS levels through immunoblotting (Azimzadeh et al., 2015, 2017; Hong et al., 2013; Yan et al., 2020), immunohistochemical staining (Fuji et al., 2016), immunofluorescence (Hamada et al., 2020) or ELISA (Azimzadeh et al., 2015; Hasan et al., 2020). - Evaluating levels of nitrite/nitrate (NOx) (Azimzadeh et al., 2017; Cervelli et al., 2017; Siamwala et al., 2010; Yan et al., 2020). Some direct measurement methods do exist such as: - 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM) fluorescent probe that reacts with and thus visualizes NO (Soucy et al., 2010, 2011).
Key Event [KE 15]	Increased, Endothelial Dysfunction New KE created for this project:	- When exposed to stressors, the vascular endothelial layer becomes activated as part of a healthy immune response. - Prolonged activation leads to a state of endothelial dysfunction that includes	- Senescence-associated beta-galactosidase staining (SA-beta-gal) to detect premature senescence (Farhat et al., 2008; Noren Hooten & Evans, 2017).

	KE #2068	impaired vasodilation, prolonged lack of bioavailable NO, premature endothelial cell senescence and apoptosis, chronic inflammation, and a pro-thrombotic environment.	- Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay to detect elevated apoptosis levels (Kressel & Groscurth, 1994). - Concentration response curves to vasodilators/vasoconstrictors to detect impaired endothelial-dependent vasodilation (Deanfield et al., 2007; Verma et al., 2003).
Adverse Outcome (AO)	Vascular Remodeling Existing KE in AOP wiki KE #2003	- Changes to vascular structure including increased vascular stiffness, wall shear stress, intima-media thickening (IMT), increased intima-media section area, increased vessel diameter, changes to endothelial permeability, fibrosis, accelerated age-related atherosclerosis and decreased capillary density.	With a wide variety of changes possible, there are many potential methods of measurement some examples include: - Pulse wave velocity as a measure of vessel stiffness (Soucy et al., 2007, 2010, 2011). - NIS-Elements image analysis software (Nikon) which measures intraluminal perimeter and vessel wall thickness (Soucy et al., 2011). - Hematoxylin-eosin (HE) staining used to measure aortic wall thickness, IMT, outer media perimeter, and media cross section area (CSA)of fixed tissue samples (Cheng et al., 2017; Delp et al., 2000; Shen et al., 2018; Su et al., 2020) - Wire myography which estimates relaxed inner diameter (Tarasova et al., 2020). - Verhoeff-van Gieson staining determining vessel wall elastin-collagen content (Sofronova et al., 2015) - Sonography to evaluate IMT and intima-media area (S. M. C. Lee et al., 2020; Sárközy et al., 2019; Sridharan et al., 2020)

Table 3.2 – Summary of Adjacent KERs in AOP #470 and examples of empirical evidence.

Key event relationship (KER) title	**Description**	**Example of empirical evidence**
Deposition of energy leads to increases in oxidative stress KER #2769	When energy is deposited on an aqueous solution, ionization of water causes breaking of bonds and results in the formation of reactive oxygen species (ROS). When the levels of ROS and reactive nitrogen species (RNS) are high enough to overwhelm the natural antioxidant defense system a cell enters a state of oxidative stress.	Empirical evidence: High There is a large body of evidence supporting dose- and time- response of the relationship. Some examples include: - Human Umbilical Vein Endothelial Cells (HUVECs) irradiated with 0.2, 0.5, 1, 2, and 5 Gy of ^{137}Cs gamma rays showed linear increase in ROS production across doses (H. Wang et al., 2019). - Human telomerase-immortalized human Coronary Artery/ Microvascular Endothelial cells (TICAE/TIME) were irradiated with 0.1 and 5 Gy of X-rays at a rate of 0.5 Gy/minute. Following completion of irradiation intracellular ROS was elevated at the 45 minute time point before decreasing in a time-dependent manner, returning to baseline levels by 3 hours (Ramadan et al., 2020).
Altered signaling pathways leads to altered NO levels KER #2773	NO creation by eNOS is modulated through phosphorylation controlled by various signaling pathways. Akt/PI3K/mTOR and PPARα are involved in upregulation, while RhoA/ROCK destabilize eNOS mRNA. Lastly, AngII from the RAS pathways is involved in both up and down regulation of eNOS depending on the mechanism.	Empirical evidence: Moderate There is a moderate number of studies demonstrating a dose- and time- dependent relationship. As discussed in the inconsistencies section there is variability in the conclusions drawn from NO proxy measures. - Following exposure to microgravity via a clinostat, HUVECs showed an increase in p-Akt as well as an increase in eNOS and p-eNOS expression (Shi et al., 2012).

		- Mice exposed to heart X-ray radiation of 8 or 16 Gy showed significant changes in abundance and phosphorylation of PI3K/Akt pathway components at both doses. p-eNOS and NO showed a decrease at both doses, with a higher decrease at a higher dose (Azimzadeh et al., 2015).
Altered signaling pathways leads to endothelial dysfunction KER #2775	Inhibition of cell survival signaling from the Akt/PI3K/mTOR leads to premature endothelial cell senescence, while p53 phosphorylation and ceramide production in the sphingomyelin ceramide pathway can both contribute to endothelial cell apoptosis. Premature cell senescence and excessive apoptosis contribute to endothelial dysfunction.	Empirical evidence: Low There is a lack of studies exploring this relationship and therefore there is difficulty determining dose- and time-concordance. Some examples of studies that do explore the relationship include: - HUVECs irradiated with 10 Gy of X-rays showed an increase in the cleaved caspace-3 component of the apoptosis cascade and a correlated increase of apoptotic cells that were used as a marker of endothelial dysfunction (Chang et al., 2017). - HUVECs irradiated with ^{137}C gamma radiation at 1.4 mGy/h or 2.4 mGy/h dose rates over a period of 10 weeks. The 1.4 mGy/h dose rate led to changes in the levels of Akt/PI3K/mTOR pathway components but no changes in endothelial cell senescence. In contrast, the 2.4 mGy/h dose rate showed changes in both the Akt/PI3K/mTOR pathway and increased rates of endothelial cell senescence (Yentrapalli et al., 2013). - In hind-limb unloaded rats, the RhoA component of the RhoA-Rho-kinase pathway decreased and was accompanied by an inhibited vaso-motive response (Summers et al., 2008).

Increases in oxidative stress leads to altered NO levels KER #2773	Excessive ROS inhibits the dimerization of eNOS resulting in the production of ROS instead of NO. Furthermore, ROS can react with NO forming peroxynitrite, forming additional ROS exacerbating NO decrease.	Empirical evidence: Low There is a lack of studies exploring this relationship and therefore there is difficulty determining dose- and time-concordance. Some examples of studies that do explore the relationship include: - Studies using 4 Gy and 10 Gy of X-ray, and 1 Gy and 5 Gy of heavy ions and gamma rays respectively, both showed dose dependent increase in ROS production accompanied by decreases in NO levels (Soucy et al., 2010, 2011; Yan et al., 2020). Conclusions drawn from proxy measures of NO can be varied and are further described in the inconsistencies section.
Increases in oxidative stress leads to endothelial dysfunction KER #2776	Excess ROS during oxidative stress can go on to damage endothelial cells. Additionally, ROS can react with NO forming peroxynitrite, replacing the vasodilator with additional ROS. Prolonged lack of bioavailable NO is a key characteristic of endothelial dysfunction as it decreases vasorelaxation.	Empirical evidence: Moderate Evidence supporting a dose- and time- dependent relationship comes from a variety of models and use a variety of endpoints to evaluate endothelial dysfunction. For example: - Rats were exposed to fractionated radiation culminating in a total dose of 22.50 Gy across 19 days. Gut microvessels showed an increase in superoxide and peroxides starting after the 5th day and continuing through the rest of the experiment. Vasodilation also became significantly decreased starting from the 5th day and continued through until day 19 (Hatoum et al., 2006). - Rat cerebral microvascular endothelial cells (CMVECs) were exposed to ^{137}Cs gamma radiation in

		doses between 2-8 Gy. ROS levels as well as endothelial cell senescence and apoptosis increased in a dose-dependent manner (Ungvari et al., 2013).
Increases in oxidative stress leads to increase in pro-inflammatory mediators [KER #2772]()	Cellular damage caused by excessive ROS elicits a response from the immune system that involves an increase in release of pro-inflammatory mediators.	Empirical Evidence: Low While there is some primary research supporting a relationship, dose- and time- concordant data is lacking. This could be in part due to inflammation being both a cause and effect of oxidative stress (Venkatesulu et al., 2018). - HUVECs irradiated with 0.2, 0.5, 1, 2, and 5 Gy of gamma radiation showed a dose-dependent increase of ROS. IL-6 levels increased from 0 – 0.2 Gy, decreased at 0.2-0.5 Gy and increased again between 0.5 to 5 Gy. TNF-α levels did not change until an increase between 2 and 5Gy doses (H. Wang et al., 2019). - Human TICAE cells irradiated with 0.25, 0.5, 2, and 10 Gy of ^{137}Cs gamma rays. The antioxidant enzyme SOD1 did not demonstrate radiation dose-dependent changes but were increased above control level at all doses. Simultaneously, the pro-inflammatory mediator ICAM1 increased in a dose-dependent manner (Philipp et al., 2020).
Increase in oxidative stress, leads to altered signaling pathways [KER #2771]()	Increase in ROS/RNS has been shown modulate a number of different pathways, including inhibiting Akt/PI3K/mTOR cell survival signaling, phosphorylation of p53 from the p53-p21 pathway, which in turn kicks off the MAPK/ERK cascade resulting in apoptosis.	Empirical evidence: Moderate Evidence for this relationship has been shown at doses between 0.5 to 16 Gy, with effects being measured between 5 minutes to 16 weeks post irradiation. - Ventricular cardiac tissue of mice irradiated with 3Gy/day for 5 days showed an increase in ROS

		production accompanied by p-p38/p38 and p-Nrf2/Nrf2 decrease (Fan et al., 2017) - Human endothelial cells irradiated with 10 Gy of X ray showed an increase in ROS generation at both 24- and 72h time points. ERK1/2 initially decreased at 5 minutes before increasing in the following 24h; additionally, there was a decrease in p-Akt and no significant changes in p38 and JNK (Sakata et al., 2015).
Increases in pro-inflammatory mediators leads to endothelial dysfunction KER #2777	While inflammation and endothelial activation is part of a healthy host-defense immune response, prolonged or chronic inflammation leads to a decrease in endothelial function.	Empirical Support: Low There are few studies exploring the dose- and time-concordant nature of this relationship. Limited studies have found some evidence of the relationship: - Mouse aortas irradiated with 18 Gy X ray showed an increase in TNF-α and ICAM-1 pro-inflammatory mediators becoming significant after 3 days and peaking at 7 days post-exposure. Apoptosis, used as marker of endothelial dysfunction, was also increased, peaking at 7 days post-exposure (Shen et al., 2018) - Rat endothelial cells irradiated with 6 Gy of gamma rays showed elevated IL-6, IL-1α, MCP-1 and IL-1β. At the same time, endothelial cell senescence was elevated ~30% above control levels (Ungvari et al., 2013).
Endothelial dysfunction leads to vascular remodeling KER #2778	The chronic inflammatory and prothrombotic state of endothelial dysfunction paired with the decrease of vasodilation and changes to membrane composition cause compensatory vascular remodeling.	Empirical support: Moderate The relationship has been explored across 0.031 to 18 Gy doses of radiation as well as 3-to-340-day exposure to microgravity.

- Rats irradiated with ^{137}C gamma radiation at 0.5, 1.6 and 5 Gy showed no changes in endothelial-dependent vasodilation at 0.5 and 1.6 Gy doses. The 5 Gy dose showed a decrease in vasomotion, and increase in vascular stiffness (Soucy et al., 2007, 2010).
- Mice irradiated with 18 Gy X-ray showed an increase in apoptosis indicating decrease in endothelial function and increase in aortic thickness and collagen content compared to control. A significant increase in apoptosis was observed following day 3, while increase in aortic thickness was significant after day 7, and collagen became significant after day 14 (Shen et al., 2018).

Table 3.3 – Summary of non-adjacent KERs in AOP #470 and examples of empirical evidence

Key event relationship (KER) title	Description	Example of empirical evidence
Deposition of energy leads to altered NO levels KER #2779	Deposition of energy can cause changes in NO levels through various biologically plausible mechanisms such as affecting changes in signaling pathways and increasing levels of oxidative stress.	Empirical evidence: Moderate Empirical evidence for this relationship has been shown in doses ranging from 0.05 to 60 Gy, and across time scales ranging from 1 day to 6 months. Some examples include: - X-ray irradiation of mouse cardiovascular cells at doses of 8 Gy and 16 Gy showed a dose-dependent decrease in p-eNOS (Azimzadeh et al., 2015). - HUVEC irradiated with fractionated doses of gamma radiation demonstrated changes in eNOS levels. An increase in fractionated and total dose led to a relative decrease in eNOS (Sadhukhan et al., 2020). - Mice irradiated with 5 Gy gamma rays showed a decrease in eNOS levels. The largest decrease was observed at the 1-month time point before gradually improving at the 3 and 6-month points (Hamada et al., 2020).
Deposition of energy leads to endothelial dysfunction KER #2780	Damage caused by the deposition of energy has been shown to affect endothelial function at the tissue level.	Empirical evidence: Moderate Evidence for this relationship has been demonstrated at doses between 0.05 and 18 Gy and across time scales ranging from 3 days to 4 months post irradiation. - Human endothelial cells irradiated at a 0.5 Gy/min dose rate for a total dose of 2 Gy showed an increase in the senescence-associated beta-galactosidase endothelial cell senescence marker (Baselet et al., 2017). - Mouse aortas irradiated with 18 Gy of X-ray showed a significant increase in apoptosis as soon as 3 days

		following radiation and peaking 7 days post exposure. Following the peak, apoptosis levels gradually decreased but remained elevated above control until the last time point tested of 84 days (Shen et al., 2018).
Deposition of energy leads to increases in vascular remodeling KER #2770	Damage caused by the deposition of energy has been shown to affect vasculature at a structural level.	Empirical evidence: Moderate Evidence for this relationship has been shown at doses between 0.1 to 50 Gy, and across time scales ranging from 1 day to 6 months post irradiation. - Vessel stiffness in irradiated rats was shown to increase with an increase of radiation dose across three studies using 0.5, 1, 1.6 and 5 Gy doses (Soucy et al., 2007, 2010, 2011). - 13 weeks after irradiation, mice receiving 2 and 5 Gy doses showed an increase in intima thickness that returned to control levels after 40 weeks (Yu et al., 2011). - Mice irradiated with a 5 Gy dose showed increase in vascular permeability that was evaluated at the 1-, 3- and 6-month time points. Permeability was elevated at all points, peaking at 3-months post irradiation (Hamada et al., 2020).

Chapter 4 – Discussion and Conclusions

4.1. Research Summary and Conclusions

The first objective of this thesis was to create an AOP WOE collection protocol using elements of systematic review methodologies and AI tools. This protocol was then deployed to collect evidence to support four AOPs concerning the adverse health consequences of interaction with the space exposome. The second objective of this thesis was to develop the vascular remodeling AOP and summarize the WOE gathered in the format of an AOP report. With both objectives accomplished, the outcomes and impacts of the work are briefly summarized below.

4.2. Protocol for Scoping Review Use in AOPs

4.2.1 Scoping Review Protocol Creation

A major contribution of this thesis is the expansion of the AOP framework from its most prominent application in chemical risk assessment and into the field of radiation biology. As the international radiation research community is recognizing the utility of the framework, there was an initial investment in the development of radiation AOPs (Chauhan et al., 2019, 2020, 2022). However, to effectively support the regulatory requirements of the radiation field, there is a need for a more transparent and documented approach for WOE gathering. For this purpose, the integration of various evidence-based methods were proposed as a way to address the challenges of evidence collection by providing structured guidance for AOP developers, thereby ensuring a quality end product (de Vries et al., 2021; Leist et al., 2017). Based on these recommendations, Chapter 1 of this thesis is the first publication of its kind to test and develop a full scoping review protocol specifically for AOP WOE evidence collection (Kozbenko et al., 2022). Working in consultation with a team of experts, and in an iterative fashion, we developed a protocol that harnesses structured literature searches, pre-determined inclusion/exclusion criteria, detailed screening forms, and a multiple reviewer format to provide an efficient and transparent approach to evidence gathering and summary. We tested this protocol for a space-health AOP network and documented our experience with the AI tools tested. Finally, we provided a set of recommendations in the form of a fit-for-purpose approach (Supplementary Figure 4) to allow the adaptation of the protocol for application of AOP development in any area of biology and risk assessment.

4.2.2 Systematic Evidence Maps

In addition to a scoping review approach in the WOE gathering process, the present thesis integrated the use of SEMs to summarize the final WOE. While all AOPs are represented with a flow diagram in the AOP-Wiki, our work was novel because it also visually summarized quantitative and qualitative aspects of the WOE, including the evidence streams from the BH criteria, taxonomic applicability, and type of stressors informing the AOP. This facilitated a deeper understanding on the type and extent of data supporting the KERs and helped to highlight the knowledge gaps. SEMs were created to visualize the strength of evidence across the entire pathway, this representation is reminiscent of directed acyclic graphs (DAGs), which are sometimes used in epidemiology for visualization of directional causal assumptions (Suttorp et al., 2015). DAGs differ from the SEMs created in the second chapter in that they focus on representing the implications of confounding factors such as sex on causal relationships. Future iterations of AOP SEMs could emulate confounding factor representation, creating an AOP specific version of DAGs. Perhaps also in the future, standardization of AOP creation will extend to the final data visualization using DAG-inspired SEMs and lending Wiki users the ability to compare the nature of the WOE across pathways at a glance.

4.2.3 Benefits of our approach

The general benefits of including evidence-based methods in AOP development have been previously described both in the first chapter of this thesis and other publications on the topic (Audouze et al., 2021; de Vries et al., 2021). Presently we describe some notable benefits in developer experience. First, our evidence collection process provided confidence in the selection of our network KEs and KERs, as the supporting evidence was readily available from a vetted literature database. Second, the structured workflow allowed onboarding for new team members to be seamless. Having created documentation prior to screening that clearly defined inclusion/exclusion criteria, endpoints of interest and measurement methods, ensured smooth transitions between co-op students. A structured methodology also ensured consistency in screening decisions across reviewers. Lastly, a scoping review allowed our team to create a database of screened and catalogued studies from which data had already been extracted. This database was then used for considering the addition of new KEs or KERs and in discussion with consultants.

4.2.4 Challenges

Broadening AOP application to novel stressors has come with challenges. Challenges were faced in defining the scope of KEs and accurately naming them to best reflect the biology of disease and the associated endpoints of measurement. As well, there was also the deliberations on identifying the best MIE for the AOP. For example, alongside radiation, prolonged altered gravity is also known to adversely affect the human body, especially upon return to earth (Baran et al., 2021; Iwase et al., 2020; Man et al., 2022); however, it was difficult to identify an accurate MIE with a causal progression to the AOs of interest. The challenges faced with stressors such as altered gravity raises broader questions as AOP use continues to expand outside of chemical exposures; what is the path forward for incorporating these novel stressors that might not act at a molecular level? Is there a place for these stressors in the framework? Determining the process for inclusion of these non-traditional stressors or alternatively outlining the bounds of AOP application could direct the focus of future work.

As with other projects working at the intersection of AOPs and systematic reviews (Halappanavar et al., 2019; Huliganga et al., 2022; Waspe & Beronius, 2022), our workflow faced challenges in balancing thoroughness and resource management. Determining the boundaries of literature searches was a subject of much discussion as the practical considerations of team resources limited the number of references that could be reviewed. Our work tested a protocol whose rigor went beyond what is currently required for AOP development. The rigor had the previously discussed benefits of transparency and structure; however, the utility of the AOP knowledge base centers around encouraging crowd-sourced contribution. Adding stringent requirements for WOE collection could hinder the framework by creating a barrier to entry for teams with insufficient time, resources, or interest in complete full scoping/systematic reviews. A potential alternative has been presented by Svingen et al., who proposed a transition towards using KERs instead of entire pathways as the base unit of AOP development (Svingen et al., 2021). In the case of including evidence-based methods in pathway building, this could imply a team focus resources on smaller projects of a more rigorous and systematic nature. This approach does have some drawbacks, as there are concerns about holding up the endorsement of full AOPs with review and approval processes for individual KERs. As with many dilemmas, the solution most likely lies somewhere in the middle. Flexibility in approach will be vital in accommodating a variety of AOP development teams and disseminating the use of the framework. The fit-for-purpose recommendations presented in this thesis (Supplementary Figure 4) offer additional options for including scoping review benefits as resources permit.

Lastly, while initially promising, the AI features of systematic review software explored by our team had mixed results in aiding with efficiency. However, there are groups that have had success using AI tools outside of our scope in some aspects of AOP development (Carvaillo et al., 2019; Jeong et al., 2019; Rugard et al., 2020). Overall, application of existing tools depends heavily on the nature of the literature screened. Literature spaces with highly unique terminology (e.g., drug or chemical names) are better suited for review assisted by text-mining, while KERs with more nebulous endpoints pose a challenge. As AI tools continue to rapidly improve and the understanding of their effective implementation in AOP development continues to grow, we anticipate they will be increasingly useful for AOP building.

4.3. AOPs, Radiation & Cardiovascular Outcomes

4.3.1. Overview

In the second chapter of the thesis, the evidence supporting the cardiovascular AOP was collected in the form of an AOP report. Key scientific evidence was summarized according to modified BH criteria and knowledge gaps in the research were highlighted. Through the literature review, measurable and essential events leading to vascular remodeling progression and a WOE supporting their casual relationships emerged. Importantly, we propose a pathway that delineates overlapping biological events into distinct KEs. Chapter 2 contributes to ongoing research to understand the risks from space travel on the cardiovascular system with the aim to better protect long term space travelers of the future. The findings of this chapter also shed light on some important knowledge gaps and highlight areas for future study. Throughout the evidence collection process, the preliminary pathway created at the beginning of the AOP project evolved and SEMs were created to explain and justify any changes made to the pathway (Kozbenko et al., 2022). Being able to support the changes with evidence was in large part due to the application of the structured approach created in the first half of this thesis work.

4.3.2. Contribution to Biological Understanding Vascular Remodeling

Through compiling evidence in the form of an AOP report, we provide the state of science in our current understanding of vascular remodeling. The evidence used to support the AOP can continually evolve within framework, thereby helping to prioritize research needs.

The macromolecular level events in the AOP have been well studied and their linkages are well characterized. The intertwined relationships between oxidative stress, pro-inflammatory mediators and altered signaling pathways have been studied extensively following exposure to many stressors. Our work adds to the pre-existing events in the AOP-Wiki with data that links the deposition of energy with the common stressor response. As per AOP development principles, our pathway is a simplified progression towards the AO and therefore the trio of macromolecular events is represented once at the beginning. However, *in vivo* elevated oxidative stress, inflammation and altered signaling occur at all levels of biological organization. These KEs are regulated through flexible mechanisms and therefore fully suppressing a KE in order to evaluate its essentiality is challenging. Nevertheless, their combined role in the KEs that follow is well supported through biological plausibility and empirical evidence.

While the macromolecular KEs in the pathway have relatively standardized definitions in the literature, the downstream KEs of altered NO levels and endothelial dysfunction are more ambiguous. The literature reviewed was clear on the importance of NO bioavailability in endothelial function and endothelium dependent vasodilation. However, quantification of NO is non-standardized and proxy measures can be subject to interpretation. Likewise, there is no one metric that distinguishes the state of endothelial activation from that of endothelial dysfunction. Our work has reviewed the various definitions and has summarized the elements common between them. While perhaps ambiguous, endothelial dysfunction is also undoubtedly important as growing research suggests that changes to the endothelial layer's structure and function go on to result in vascular remodeling.

4.3.3. Recommendations for future work

The summary of the data highlighted several potential next steps.

Cancer is also a key concern of NASA Human Research Program (Patel et al., 2020). While cancer was outside the scope of the current network, there are other AOP development teams working to characterize the diseases' progression. There is leukemia (AOP #432), lung cancer (AOP #272) and various solid cancer (for example AOP #298 and AOP#443) AOPs both in progress and already existing in the Wiki. In the future, the present space-health network could be connected with these cancer pathways to form an even more comprehensive network of space health risk. Many KEs in the present space-health network are already included in these pathways and can serve as connection nodes for network expansion. Similarly, there is potential for cross-connectivity of AOs within the space-health network. In case of vascular remodeling, its effects extend beyond the cardiovascular system. For example, future KERs could link vascular remodeling with impaired learning and memory in the form of cerebrovascular diseases (Parfenov et al., 2019). Additional connectivity would allow for new hypotheses about the health effects of space travel to emerge and sets the stage for future research.

Lastly, as discussed in depth in chapter 3, additional primary research is needed to fill important knowledge gaps. These gaps include a lack of studies that consider biological differences between the sexes and lack of studies that include environmentally relevant dose groups. Furthermore, the AOP that was created is qualitative and thus included evidence for the occurrence of KEs across a broad range of doses, dose-rates, stressors, and radiation types. The variety in experimental design and measurement methods of endpoints means pooling data across studies is not possible and therefore dose relationships between KEs cannot be determined. The present qualitative AOP provides the structure of delineated KEs and KERs with collated measurement methods that can direct future standardized research that would contribute to important quantitative understanding. Additional quantitative data could help determine time and dose thresholds for cardiovascular effects for space travelers. Filling these knowledge gaps would provide vital important information in risk assessment decisions for future space travelers.

4.4. Final Statement

Since the launch of the AOP program in 2012 (OECD, 2017), the AOP framework has been evolving to provide an effective tool for combating information silo-ing and acting as a source of vital mechanistic information that can inform evidence-based decision making. Extensive work in toxicology and the push for adoption in radiation research have laid a strong foundation for broadening the application of AOPs. The key principles of AOP development give guidance for teasing out the most relevant aspects of complex biological data sets that can be used to inform testing strategies, MOA development and decision-making. These guiding principles are beneficial far beyond the toxicology, radiation safety and space-health arenas in which they have so far been applied. Looking to the future, the framework has the potential to be adopted by an ever-growing number of fields for answering an expanding range of regulatory questions. Directions for the evolution of the framework are the subject of much discussion; however, there are increasing efforts focused on developing recommendations or guidance on

AOP development to ensure that transparency and thoroughness are essential components. The present thesis has contributed to the expansion of the AOP framework by testing a novel strategy for AOP development that harnesses and adapts tools used for systematic review. It has applied the framework in a novel regulatory context and summarized the findings for adverse cardiovascular outcomes. The work completed will serve as a template for future AOP developers looking to add structure to their WOE collection and to break new ground in AOP application.

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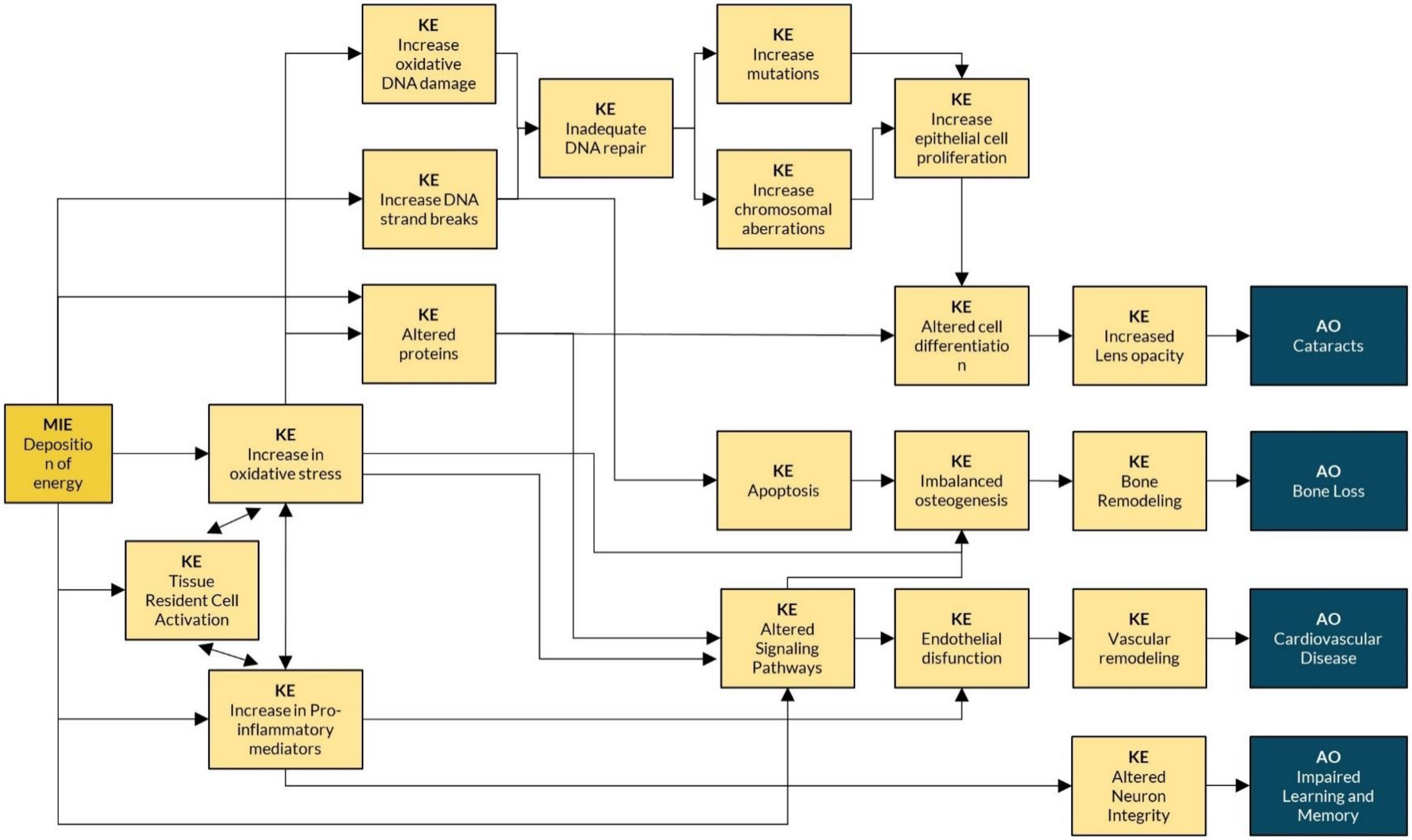
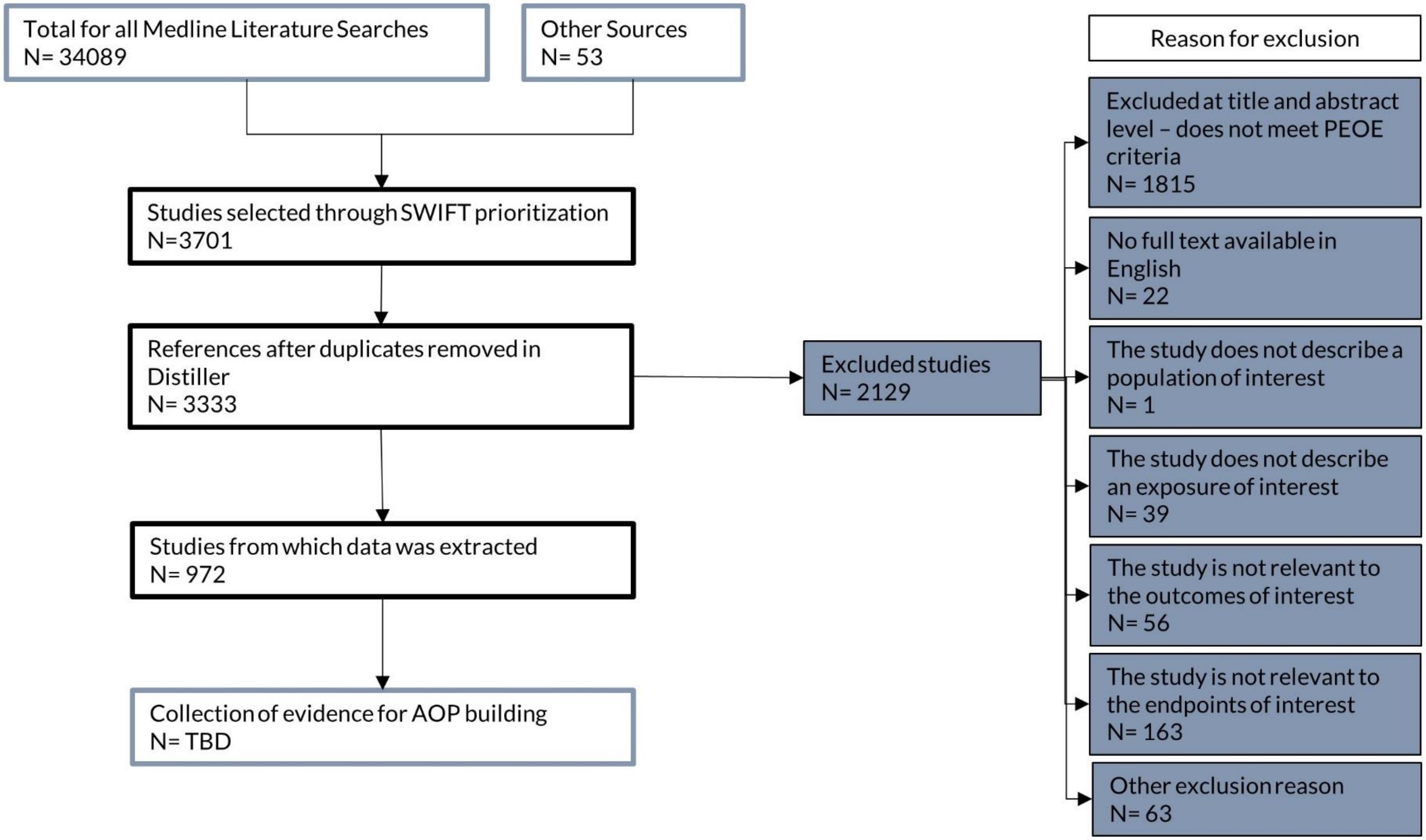
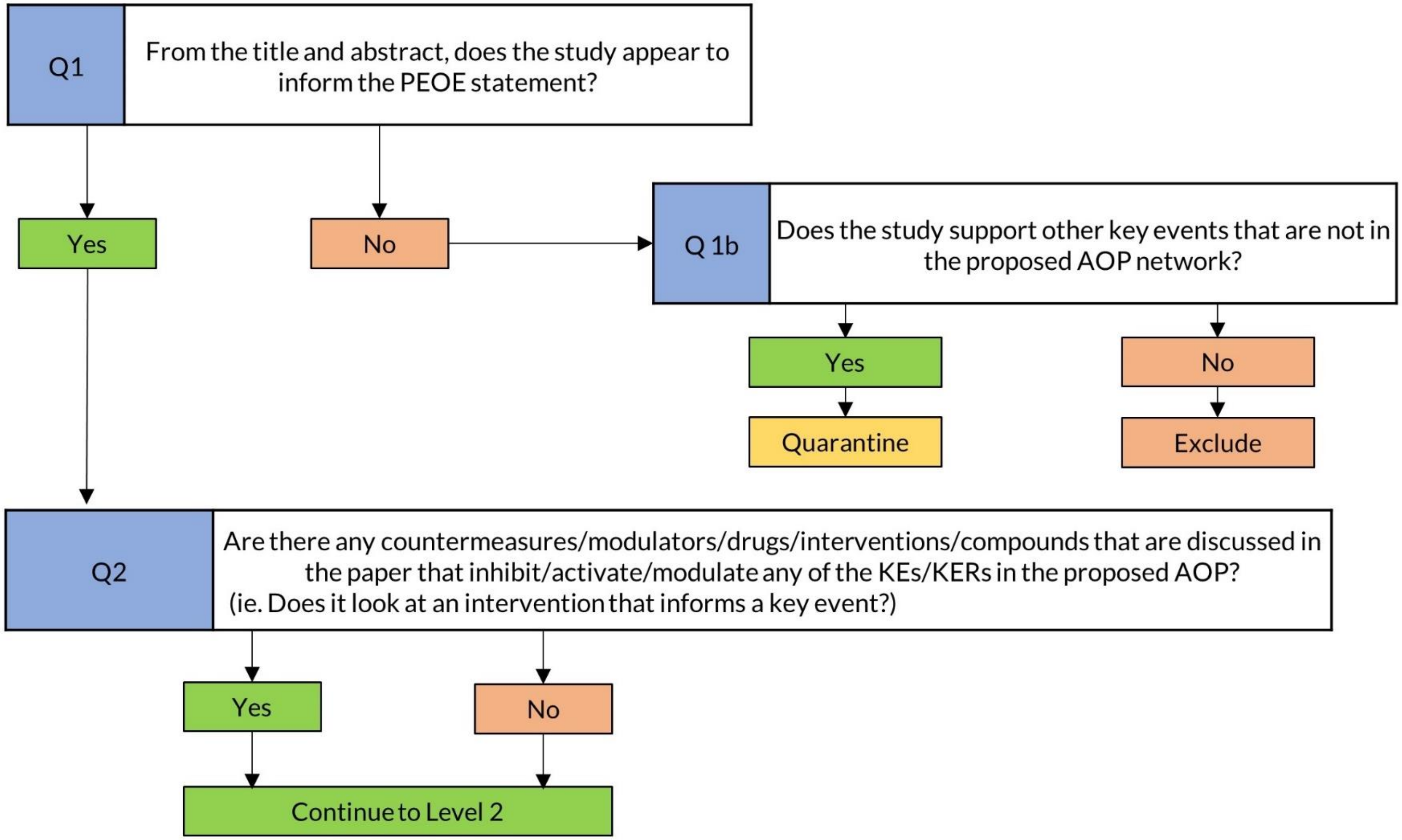
Supplementary Figures



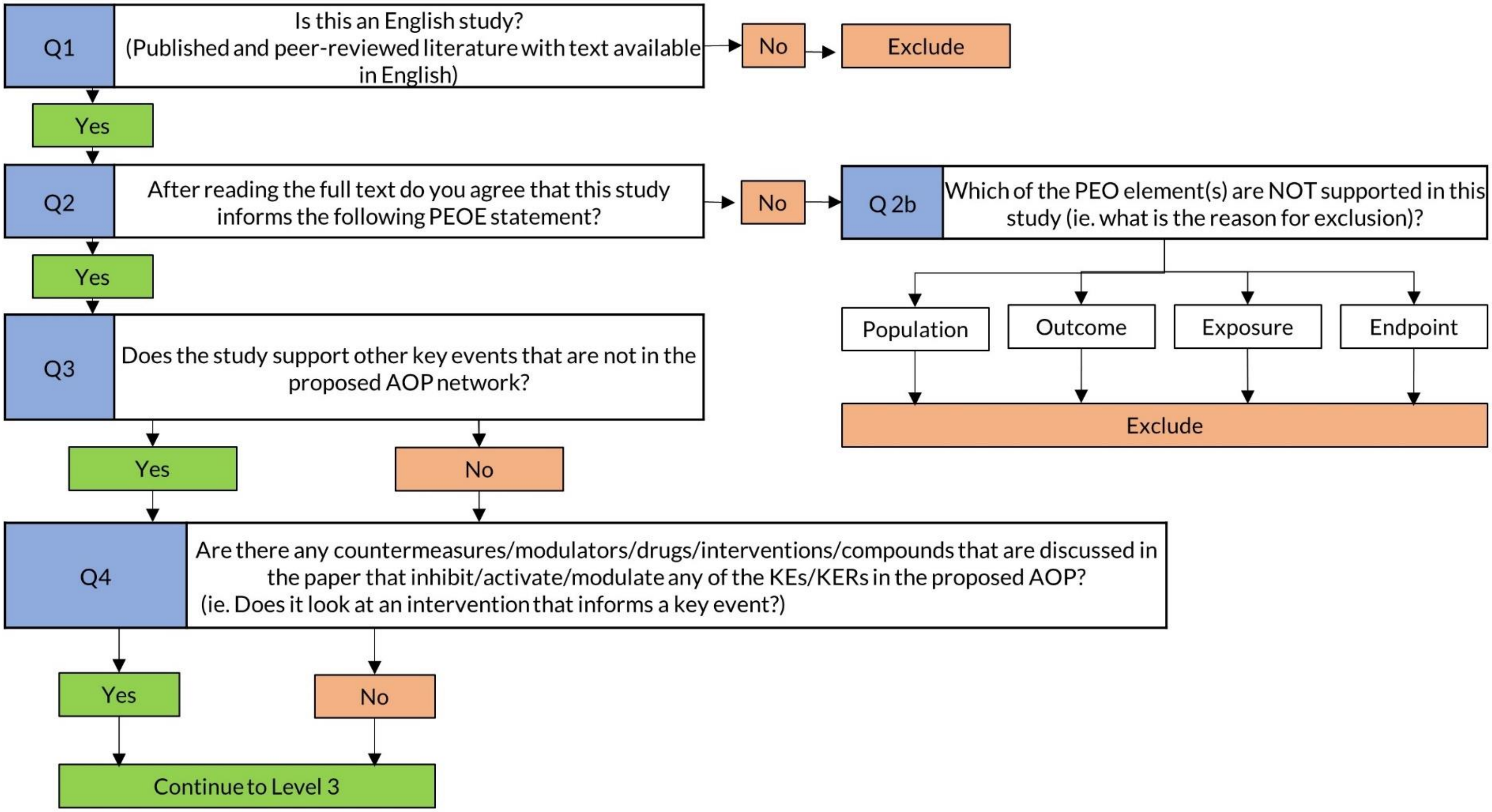
Supplementary Figure 1 – Preliminary AOP network created after reviewing ~100 review articles using a non-systematic approach.



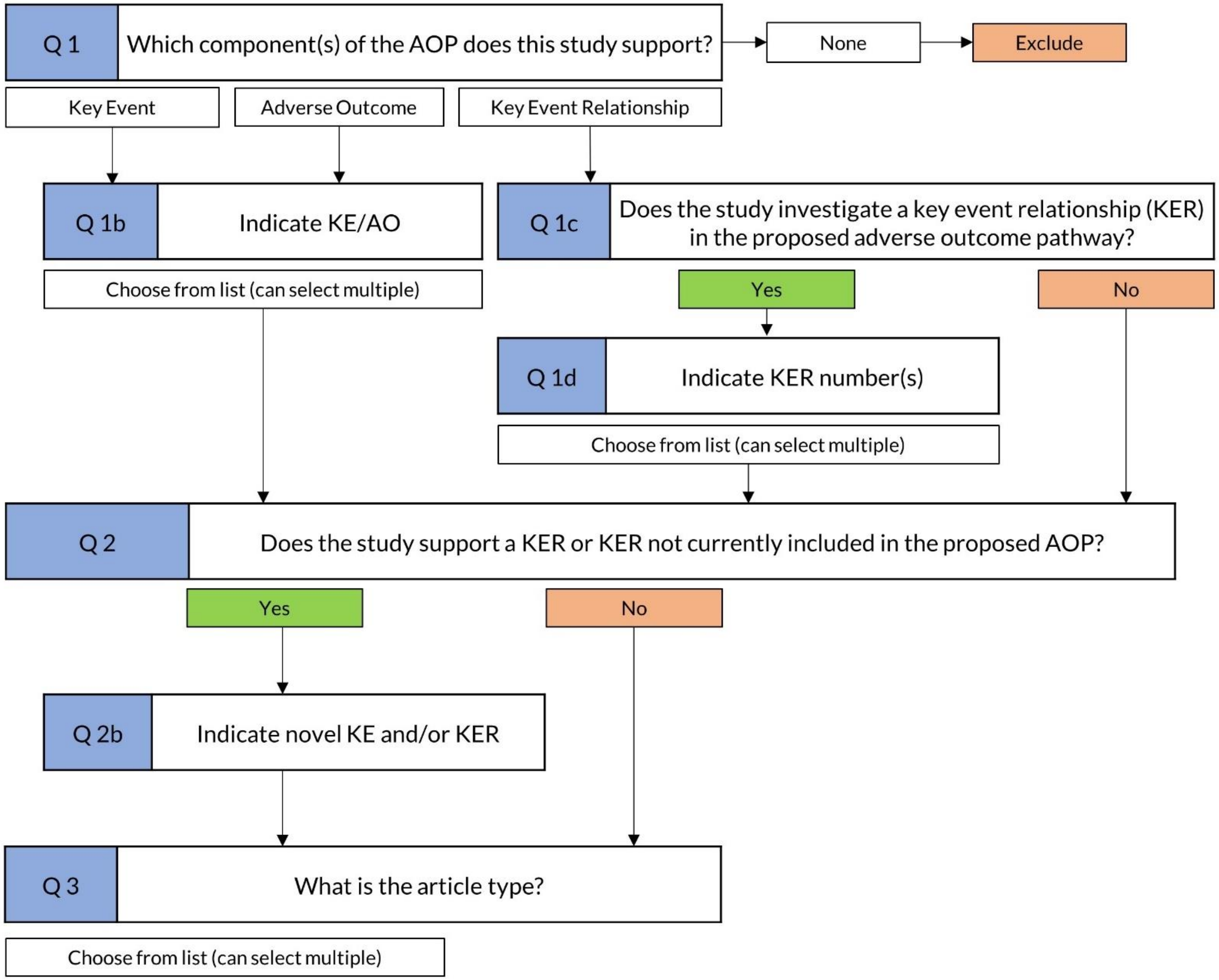
Supplementary Figure 2 – PRISMA diagram (as of January 2022) showing the progression of references through the study. "Other sources" refers to papers retrieved outside of literature searches (e.g., from subject matter experts and reference sections of review articles). References could be excluded for multiple exclusion reasons simultaneously. Numbers are subject to change upon the completion of AOP development.



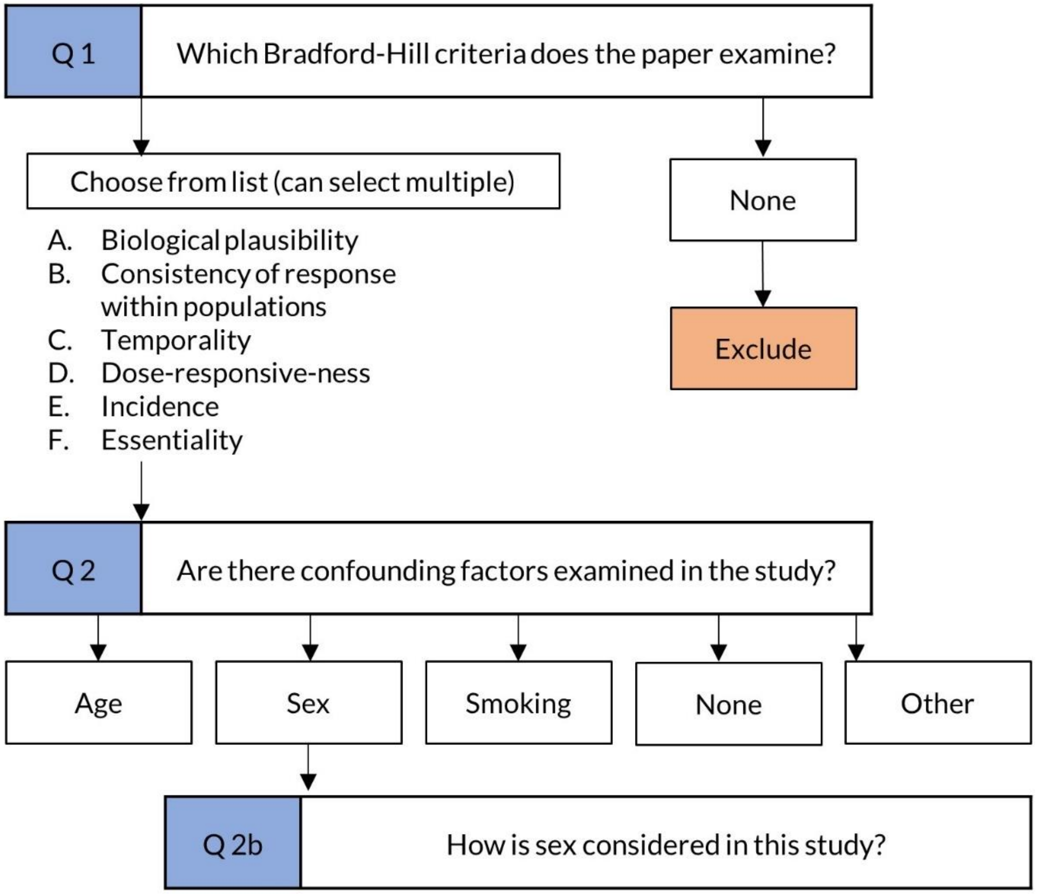
Supplementary Figure 3a. – Structured screening form for - Level 1 – Title and Abstract Screen



Supplementary Figure 3b. – Structured screening form for - Level 2 – Full Text Screen. Questions 1b and 2 are used to categorize studies for easier retrieval and are not used for inclusion/exclusion purposes.

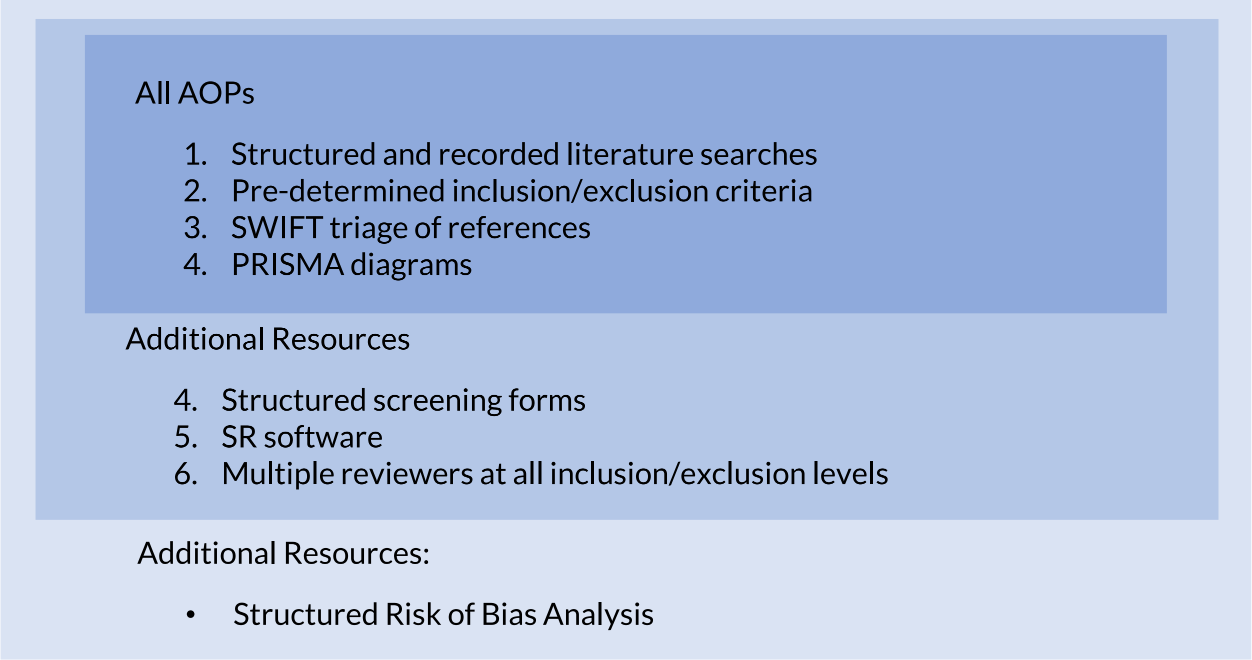


Supplementary Figure 3c. – Structured screening form for - Level 3 – Data Extraction, questions regarding AOP components



Supplementary Figure 3d. – Structured screening form for - Level 3 – Data Extraction, questions regarding BH criteria

Supplementary Figure 3d. – Structured screening form for - Level 3 – Data Extraction, questions regarding BH criteria



All AOPs

1. Structured and recorded literature searches
2. Pre-determined inclusion/exclusion criteria
3. SWIFT triage of references
4. PRISMA diagrams

Additional Resources

4. Structured screening forms
5. SR software
6. Multiple reviewers at all inclusion/exclusion levels

Additional Resources:

- Structured Risk of Bias Analysis

Supplementary Figure 4. Summary of recommendations for a fit for purpose approach to AOP weight of evidence collection.

Supplementary Tables

Supplementary Table 1 – Sample assay glossary for KE9 – Increase in Oxidative stress. Such assay lists were created for each KE prior to screening to facilitate reviewers' identification of studies with methodologies that appropriately observe a given KE.

Levels of ROS (local and systemic)	• Direct Chemiluminescent Assay • Spectrophotometry • Direct or Spin-Trapping Based EPR Spectroscopy • Nitroblue Tetrazolium Assay • Fluorescence Analysis of DHE of Hydrocyans • Amplex Red Assay • DCFH-DA (fluorescent dye dichlorofluorescin diacetate) • HyPer Probe • Cytochrome C Reduction Assay • PEDRI

Activity of ROS-clearing enzymes	• Magnetic Resonance Spectroscopy (MRS)
	• Magnetic Resonance Imaging (MRI)
	• Microdialysis
	• MALDI-MS
	• Förster Resonance Energy Transfer (FRET)
	• Chemical Exchange Saturation Transfer (CEST)
	• Electroosmotic Push-Pull Perfusion (EOPPP)
	• Examples of enzymes:
	    • Cytoplasm
	        • G6PD
	    • Mitochondria
	        • Glutathione
	        • Glutaredoxin (Grx2 and Grx5)
	        • Thioredoxin (Trx2 and TrxR2)
	    • Peroxisome
	        • GPX
	        • Catalase
	        • Prdx1 and Prdx5
	        • SOD1 and SOD2
	    • Endoplasmic Reticulum
	        • Prdx4
	        • GPX7 and GPX8

Activity of ROS-generating enzymes	• Magnetic Resonance Spectroscopy (MRS) • Magnetic Resonance Imaging (MRI) • Microdialysis

<table>
<tr><td></td><td>

MALDI-MS
Förster Resonance Energy Transfer (FRET)
Chemical Exchange Saturation Transfer (CEST)
Electroosmotic Push-Pull Perfusion (EOPPP)
Examples of enzymes:

Cytoplasm

NOX enzymes (NADPH oxidase)
iNOS

Mitochondria

Complexes I to III
α-ketoglutarate dehydrogenase (KGDHC)
Pyruvate Dehydrogenase (PDC)
Branched-chain α-keto acid dehydrogenase complex

Peroxisome

ACOX
d-amino acid oxidase
d-aspartate oxidase
polyamine oxidase
xanthine oxidase
L-α-hydroxyacid oxidase
L-pipecolic oxidase

Endoplasmic Reticulum

cytochrome p450
cytochrome b5 reductase

</td></tr>
<tr><td>Oxidative damage to lipids</td><td>

TBARS to measure lipid peroxidation
MDA assay to measure lipid peroxidation

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