September 24, 2024

To whom it may concern,

I declare that the review process for the AOP report titled “AOP Report: Development of an adverse outcome pathway for deposition of energy leading to abnormal vascular remodeling” and its accompanying AOPWiki entry (https://aopwiki.org/aops/470) was carried out according to OECD guidance for the scientific review of AOPs (OECD, 20211). All related documents are appended below. The review was conducted by the following committee members:

Review Manager:
Rex Fitzgerald

Reviewers:
Simone Mörtl
Jan Christian Kaiser

Sincerely,

Jason O’Brien, PhD
Handling Editor, Environmental and Molecular Mutagenesis

OECD (2021). Series on Testing and Assessment No. 344: Guidance Document for the scientific review of Adverse Outcome Pathways. Organisation for Economic Cooperation and Development, Paris. Available at: https://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm.

COACHES CHECKLIST AND REVIEW REPORT

ver. 2022-04-27

AOP Information

AOP number/title: 470

Author: Tatiana Kozbenko, Nadine Adam, Veronica Grybas, Benjamin Smith, Dalya Alomar, Robyn Hocking, Janna Abdelaziz, Amanda Pace, Carole Yauk, Ruth Wilkins, Vinita Chauhan

Associated wiki page: *https://aopwiki.org/aops/470*

Compliance Reviewer Information

Name: Jason O'Brien

Organisation: Environmental and Molecular Mutagenesis

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Review Information

Date this checklist has been filled: 2023-05-31

Date of final draft PDF snapshot proposed for external review: 2023-05-31

General Observations and Recommendations of the Reviewer

- It is actually a small AOP network
- NOTE: KE 1493 is part of an ongoing review for AOP 144

Several of the KEs and KER hav already ben reviewed

KE ID	KE Title	Previously reviewed?	Which AOP?
1686	Deposition of Energy	YES	272
1392	*Oxidative Stress*	YES	17, 220
1635	Increase, DNA strand breaks	YES	272, 296
1493	*Increased Pro-inflammatory mediators*	YES	17, 38, 144
2066	*Altered Signaling Pathways*	NO	

2067	Altered, Nitric Oxide Levels	NO	
2068	*Increase, Endothelial Dysfunction*	NO	
2069	*Occurrence, Vascular Remodeling*	NO	

KER ID	TITLE	ADJACENCY	Reviewed?	Which AOPs?
2769	Energy Deposition leads to Oxidative Stress	adjacent	NO	
1977	Energy Deposition leads to Increase, DNA strand breaks	adjacent	YES	272
2811	Oxidative Stress leads to Increase, DNA strand breaks	adjacent	NO	
2856	Increase, DNA strand breaks leads to Altered Signaling	adjacent	NO	
2711	Oxidative Stress leads to Altered Signaling	adjacent	NO	
2772	Oxidative Stress leads to Increased pro-inflammatory mediators	adjacent	NO	
2773	Altered Signaling leads to Altered, Nitric Oxide Levels	adjacent	NO	
2774	Oxidative Stress leads to Altered, Nitric Oxide Levels	adjacent	NO	
2775	Altered Signaling leads to Increase, Endothelial Dysfunction	adjacent	NO	
2777	Increased pro-inflammatory mediators leads to Increase, Endothelial Dysfunction	adjacent	NO	
2784	Increase, Endothelial Dysfunction leads to Occurrence, Vascular Remodeling	adjacent	NO	
2789	Altered, Nitric Oxide Levels leads to Increase, Endothelial Dysfunction	adjacent	NO	
2779	Energy Deposition leads to Altered, Nitric Oxide Levels	non-adjacent	NO	
2780	Energy Deposition leads to Increase, Endothelial Dysfunction	non-adjacent	NO	
2785	Energy Deposition leads to Occurrence, Vascular Remodeling	non-adjacent	NO	
2776	Oxidative Stress leads to Increase, Endothelial Dysfunction	non-adjacent	NO	

AOP Coach Checklist and Final Review Report

Checklist

The following tables are checklists for the individual KEs and KERs and overal AOP

KE number, title: *1686, Deposition of Energy*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***272***			
If an existing KE is being adapted, have the previous authors been informed?	*YES*			
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?		*NO*		
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments: • *Will ask authors to define components during scientific review*				

AOP Coach Checklist and Final Review Report

KE number, title: *1392, Oxidative Stress*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***17 220***			
If an existing KE is being adapted, have the previous authors been informed?	*YES*			
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?	*YES*			
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KE number, title: *1635, Increase, DNA strand breaks*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***272 296***			
If an existing KE is being adapted, have the previous authors been informed?	*YES*			
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?		*NO*		
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments: • *Will as authors to define components during scientifi review*				

AOP Coach Checklist and Final Review Report

KE number, title: *1493, Increased Pro-inflammatory mediators*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***17 38 144****			
If an existing KE is being adapted, have the previous authors been informed?	*YES*			
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?	*YES*			
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments: • *NOTE: part of an ongoing review for AOP 144*				

AOP Coach Checklist and Final Review Report

KE number, title: *2066, Altered Signaling Pathways*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?				*NO*
If an existing KE is being adapted, have the previous authors been informed?	*YES*			
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?		*NO*		
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			

Specific Comments:

- *Will ask authors to define components during scientific review*

AOP Coach Checklist and Final Review Report

KE number, title: *2067, Altered, Nitric Oxide Levels*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?				*NO*
Has the KE been reviewed by EAGMST?				*NO*
If an existing KE is being adapted, have the previous authors been informed?				*X*
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?	*YES*			
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KE number, title: *2068, Increase, Endothelial Dysfunction*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?				*NO*
Has the KE been reviewed by EAGMST?				*NO*
If an existing KE is being adapted, have the previous authors been informed?				*X*
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?		*NO*		
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			

Specific Comments:

- *Will ask authors to define components during scientific review*

AOP Coach Checklist and Final Review Report

KE number, title: *2069, Occurrence, Vascular Remodeling*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?				*NO*
Has the KE been reviewed by EAGMST?				*NO*
If an existing KE is being adapted, have the previous authors been informed?				*X*
Is the KE described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is the biological context (level of organization, terms) specified?	*YES*			
Are KE components defined using structured ontology terms (Process, Object, Action)?	*YES*			
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments: • *MINOR: "regulatory signifcance" is empty*				

KEY EVENT RELATIONSHIPS

KER number, title: *2769, Energy Deposition leads to Oxidative Stress*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?	*YES*			
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *1977, Energy Deposition leads to Increase, DNA strand breaks*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	***272***			
If an existing KER is being adapted, have the previous authors been informed?	*YES*			
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2811, Oxidative Stress leads to Increase, DNA strand breaks*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?	*YES*			
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2856, Increase, DNA strand breaks leads to Altered Signaling*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?	*YES*			
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2711, Oxidative Stress leads to Altered Signaling*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?	*YES*			
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2772, Oxidative Stress leads to Increased pro-inflammatory mediators*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2773, Altered Signaling leads to Altered, Nitric Oxide Levels*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2774, Oxidative Stress leads to Altered, Nitric Oxide Levels*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2775, Altered Signaling leads to Increase, Endothelial Dysfunction*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2777, Increased pro-inflammatory mediators leads to Increase, Endothelial Dysfunction*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*Yes*			
Is biological plausibility described/discussed?	*Yes*			
Is empirical evidence presented, referenced and discussed?	*Yes*			
Are uncertainties and inconsistencies described?	*Yes*			
Is Quantitative Understanding of the Linkage described?	*Yes*			
Is Domain of Applicability described?	*Yes*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2784, Increase, Endothelial Dysfunction leads to Occurrence, Vascular Remodeling*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2789, Altered, Nitric Oxide Levels leads to Increase, Endothelial Dysfunction*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2779, Energy Deposition leads to Altered, Nitric Oxide Levels*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2780, Energy Deposition leads to Increase, Endothelial Dysfunction*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2785, Energy Deposition leads to Occurrence, Vascular Remodeling*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

KER number, title: *2776, Oxidative Stress leads to Increase, Endothelial Dysfunction*	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?				*NO*
Has the KER been reviewed by EAGMST?				*NO*
If an existing KER is being adapted, have the previous authors been informed?				*X*
Is the KER described in a way that allows its reuse in other AOPs (i.e. independent of this AOP)?	*YES*			
Is biological plausibility described/discussed?	*YES*			
Is empirical evidence presented, referenced and discussed?	*YES*			
Are uncertainties and inconsistencies described?	*YES*			
Is Quantitative Understanding of the Linkage described?	*YES*			
Is Domain of Applicability described?	*YES*			
Specific Comments:				

AOP Coach Checklist and Final Review Report

OVERALL AOP

Overall AOP	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Does the title of the AOP follow the correct convention (MIE or first KE leading to AO)?	*YES*			
Does the title of the AOP reflect its content/domain?	*YES*			
Is a graphical representation included?	*YES*			
Is it clear who the authors/developers of the AOP are? Contact information for one or more corresponding author(s) should be included.	*YES*			
Is the status of the AOP described?	*YES*			
Does the abstract concisely describe the main content of the AOP in a standalone manner?	*YES*			
Have prototypical stressors been identified for the MIE?	*YES*			
Has the regulatory relevance of the AO been described?	*YES*			
Is the domain of applicability of the AOP defined in accordance with the OECD AOP Handbook?	*YES*			
Is the level of support for essentiality of the KEs described and assessed in accordance with the OECD AOP Handbook?	*YES*			
Has consideration been given to the level of support for the calls on the Overall WoE and the Quantitative Understanding?	*YES*			
Specific Comments:				

REVIEWER #1

general comments:

In summary, this AOP is very informative and useful to further understand the effects of radiation on the cardiovascular system. Also the weight of evidence is sufficient for the majority of KE and KERs. Suggestions for specific KE/KERs are listed below.

Scientific quality

- **Does the AOP incorporate all appropriate scientific literature and evidence?**

It is clear that not all relevant literature is included, which is not to be expected given the abundance of works on this topic. However, in all KE and KERs I miss an attempt to include non-linearity and adaptive response phenomena, which are often described in the radiation response of endothelial cells. Below are some specific points and suggestions:

KE DNA strand breaks:

ATM activation is reported as an indicator of DNA DSBs, however there are also reports showing ATM activation by ROS independent of double strand breaks (Guo et al. 2010, Science). Therefore the weight of evidence for this KE should be reconsidered. Maybe it is worth to include data on DNA repair mutants as way of manipulation to support the essentiality of this KE.

KE altered signalling:

this KE is an unspecific summary of observed molecular changes with no information on activation or suppression (which is provided for the other KEs). The KE is also incomplete and selection of pathways is unclear. For example, NFkappaB alterations in endothelial cells are an established radiation response mechanism in endothelial cells, but not mentioned in the KE (examples: DOI: 10.1007/s00066-004-1237-y), http://www.jstor.org/stable/24545503, https://doi.org/10.1667/RR14905.1, Murley et al. 2004, Rad.Res.). I suggest to reorganize the complete KE, maybe it is better to focus on one really established signalling pathway. Also the methods provided for measurement are incomplete. What is meant with "GFP for measurement of altered signalling"? Is GFP the only possible fluorescent protein? I this approach in the earlier mentioned "Fluorescence methods"? Why is only kinase measurement mentioned? There are a lot more measurable PTMs in endothelial cells in response to irradiation.

KE endothelial dysfunction: effects on barrier function are missing

Studies have provided evidence that radiation exposure destabilizes the endothelial barrier, increasing vascular permeability and altering vesicular trafficking. These effects have been studied in vivo in a wide variety of animal models and in vitro using cultured primary or immortalized endothelial cells EC. Also methods to measure permeability should be included. (examples: Bouten R, 2021, https://doi.org/10.1016/B978-0-12-818561-2.00007-2) Kabacik S. Oncotarget, 8 (47) (2017), pp. 82049-82063, Guipaud O., doi: 10.1259/bjr.20170762).

As an important assay to measure endothelial dysfunction the ability form vascular networks in matrigel is missing in the methods for detection.

KE proinflammatory mediators

the description of dose rate and adaptive response effects should be included (e.g. Ebrahimian (2015), Gueguen (2019))

- Does the scientific content of the AOP reflect current scientific knowledge on this specific topic?

Not all methods suggested for the detection of KEs reflect current knowledge, especially updates are necessary for DNA strand break detection, measurement of cytokines and signalling pathways (detection of strand breaks e.g. sBLISS by Bouwmann, assays for single strand breaks (Zilio and Ulrich, 2020), OLINK technology for in #1493, #2066 omics methods and pathway analysis, detection of PTMs beside phosphorylation).

existing KEs:

Maybe it is characteristic for the whole AOP approach, however, the complete takeover of already existing KE and KERs poses the risk of unspecific (sometimes irrelevant to the topic) information, which overwhelms the reader interested in the topic of AOP470.

In the description of KEs the focus should be on ionizing radiation as MIE and the adverse outcome of vascular remodelling. Information on stressors such as metals, UV radiation or effects in bone cells are less relevant the topic of AOP470. Also literature neither related to ionizing radiation nor to endothelial cells should be reduced.

At least the up-to-dateness of the described methods has to carefully checked (e.g. analytical methods of DNA strand breaks, cytokine measurement).

Weight of evidence

- Consider weight-of-evidence for each “Key Event Relationship (KER)” and the AOP as a whole.

weight of evidence for KER DNA strand breaks to altered signalling pathways is limited

- In your opinion, is the weight-of-evidence judgement/scoring well described and justified based on the evidence presented?

it would be good to describe the weight of evidence judgement in a more transparent way

Review of AOP Report Article

The AOP report is an adequate summary of the AOP and it is interesting to learn about the underlying considerations. However, the focus on space radiation (for example see page 4) in the introduction is not reflected by the provided (available) data in the AOP. Therefore the potential aims and applications should be presented in a more balanced way. Maybe limitations of the AOP can be discussed in more detail (for example non-linearity, adaptive response, chronic exposure, …..).

What are the criteria for the examples showed in Table II?

REVIEWER #2

Review of AOP report EMM-23-0055 "Development of an adverse outcome pathway for deposition
of energy leading to vascular remodelling" by Kozbenko et al. and the related AOP wiki content

Major comments:
The present article represents a new publication format for EMM in the form of an AOP report article which is a summary of the AOP wiki content. Both the AOP report and the AOP wiki content should be reviewed with different focus. The report should give an accurate summary of the wiki content. For the wiki content specific charge questions on scientific quality and weight of evidence should be asked based on the reviewer's expertise.

Against the backdrop of the large number of biological processes and adverse outcomes (AOs) in the field of cardiovascular diseases (CVDs) the authors have decided to reduce the complexity in the design of an AOP. They have chosen vascular remodelling (VR) as an early subclinical AO which is a milestone on the path to more severe clinical AOs. On the other hand, VR is sometimes differently defined in the literature and is not specifically ICD coded. I suggest that the authors present their understanding of VR more clearly in the introduction i.e. by inserting a table highlighting the criteria for VR.

Another concern is the notion that VR is not entirely detrimental but sometimes necessary to maintain regulated blood circulation. One might think that this property goes against the definition of an AO. In any case, it will make it more difficult to separate detrimental from possibly beneficial KERs. On the other hand, atherosclerosis (AT) is a preclinical endpoint closely related to VR with entirely detrimental effects and a thorough ICD codification.

In summary, I would consider the development of AOP470 a initiating endeavour, which well reflects the state of knowledge on the subject, but also highlights gaps and future research needs.

Minor comments:
AOP report

p 2, line 31: Should anti-inflammatory effects also considered? The are known to mitigate the generation of AT (see e.g. Ebrahimian et al. PMID: 29227739).

p 3, line 31: The concept of feedback loops is not easy to understand. Could you give an example?

p 4, line 13: Please explain in more detail your notion or VR as the focus of AOP 470.

p 5, line 8-10: These lines might also relate to AT. What is the difference between VR and AT?

p 5, line 31: CIMT is a weak predictor of stroke and myocardial infarction (MI) (see e.g. Lorenz et al. 2007 PMID: 17242284) and considered as a biomarker of subclinical atherosclerosis (Bauer et al. Swiss Med. Wkly. 2012; 142 (w13705)). Bots et al.1997 (PMID: 9412629) note that increased CIMT reflects a non-atherosclerotic adaptive response to changes in shear stress and tensile stress, possibly related to VR. Only beyond a certain level, CIMT more likely may represent AT. Simonetto et al. 2020 (PMID: 32005003) observed that deviations from a normal age-related CIMT level are a better risk predictor that CIMT alone. In summary, these findings suggest that VR is needed to maintain a regular blood flow and the risk for stroke and MI is more related to AT. In this case VR cannot be considered as an AO.

p 6-7, The key epidemiological studies for radiation-induced CVD risk have been cited, but I would suggest to mention that the evidence is more heterogeneous compared to cancer risk. For example, in the LSS a different spectrum of CVD is related to radiation exposure which makes a risk transfer to Western populations difficult (Ozasa et al. 2017, PMID: 28151038). Forthcoming results from the Million Person Study (MPS) show no risk for protracted exposure at low doses and dose rates for ischemic heart disease (Schöllnberger et al. 2023, PMID: 35930470).

p 8: One could be a bit more specific in the characterisation of radiation exposure during space flight. How long is a typical space mission and what are the expected doses? Can the dose estimates for cancer risk be applied to estimate CVD risk (Walsh et al. 2024 PMID: 37932191)?

p 10, line 43: Please explain the nature of the possible bias.

p 12, paragraph starting with line 13: This paragraph could be a motivation for developing AOP 470 and could go to the Introduction

p 12, line 27: A short characterisation of the missing elements for a more comprehensive CVD AOP would be helpful.

p 21, line 11: Are there epidemiological risk studies available which directly associate VR with CVD?

p 23, line 5: Which radiation qualities and doses are relevant for space flight?

p 23, line 52: Dosimetric radiation transport calculations could reduce the knowledge gap as is the case for cancer risk (Walsh et al. 2024 PMID: 37932191).

p 26, line 22: For lifetime risk calculations established dose responses are required which include consistent age dependencies. In my opinion based on the large heterogeneity of epidemiological

risk estimates the uncertainties for dose responses are still too large to allow reliable risk projections

p 30. Figure 1 caption: The last sentence is confusing. What is the point of the KER from MIE to AO curtailing all other KER? I thought the idea of an AOP is to dissect pathogenesis in small quantifiable units?

p 82, Figure 2: a colour code is missing?

AOP wiki content

Major comments
The wiki content is subdivided in an Abstract, Overall Assessment, Potential Applications (optional) and Appendices containing a list of MIEs and a list of KERs.

Essentiality of key events provides a heterogeneous picture, but the Weight of Evidence (WOE) is high for all KERs considered. However, Quantitative Understanding is low for many KERs.

The summarizing Tables on pages 6-11/168 provide a comprehensive overview on the topic couched in AOP language. This structured approach demonstrates the added value gained by applying the APO framework. It demonstrates that the general understanding of radiation-induced VR is not yet incomplete. Of special concern is the unclear essentiality of some key events such as KE#1493. If inflammation is not an important milestone on the path to VR, could the KE be dropped?

Although the focus of the AOP is by definition on adverse outcomes there are also beneficial effects of VR which might deserve a broader discussion in the AOP wiki.

Minor comments

p 5/168 2nd paragraph: “deposition of energy occurs immediately following irradiation” is inaccurate, better use “deposition of energy by irradiation”

p 11/168 Given the mostly low quantitative understanding of many KERs, parameter transfer to biologically based risk models might not be easily feasible.

AOP 470 Reviewers Response Document

Thank you for your thoughtful review of our adverse outcome pathway (AOP) manuscript and associated documents. We appreciate the time and effort you have dedicated to providing very constructive feedback. We have reviewed the comments and where appropriate have addressed them as outlined below. Please note the page and line numbers correspond to the "marked" version of the AOP report and the "marked" version of the snapshot**.**

REVIEWER #1

KE DNA strand breaks:

ATM activation is reported as an indicator of DNA DSBs, however there are also reports showing ATM activation by ROS independent of double strand breaks (Guo et al. 2010, Science). Therefore the weight of evidence for this KE should be reconsidered. Maybe it is worth to include data on DNA repair mutants as way of manipulation to support the essentiality of this KE.

Agreed. We have added oxidative stress as an activator of ATM in the Overall assessment-biological plausibility section of the snapshot page 6 as follows: "DNA strand breaks in endothelial cells can be induced either directly through energy deposition or indirectly through oxidative stress. Damaged DNA or increased production of free radicals can recruit and activate the protein kinases ataxia telangiectasia mutated (ATM) and ATM/RAD3-related (ATR) (Nagane et al., 2021; Guo et al., 2010)."

DNA repair mutants do not support the essentiality of the KE of "DNA strand breaks", as these relate to repair processes and would be better suited to support the essentiality of a KE to "Inadequate repair". This is a KE which is currently networked though an AOP developed for Cataracts. The essentiality of the KE to "inadequate repair" is indeed supported by evidence from DNA repair protein mutant or knock-out studies.

Although our scoping review retrieved limited studies that modulate DNA strand breaks directly to a downstream KE of "altered signaling", there are sufficient data from the other evidence streams (time-, dose-, incidence-concordance and biological plausibility) to justify the essentiality of the KE "DNA strand breaks" in our AOP. For this reason, our empirical evidence call is "High", despite essentiality being rated "Low".

KE altered signalling:

this KE is an unspecific summary of observed molecular changes with no information on activation or suppression (which is provided for the other KEs). The KE is also incomplete and selection of pathways is unclear. For example, NFkappaB alterations in endothelial cells are an established radiation response mechanism in endothelial cells, but not mentioned in the KE (examples: DOI: 10.1007/s00066-004-1237-y), http://www.jstor.org/stable/24545503, https://doi.org/10.1667/RR14905.1, Murley et al.

2004, Rad.Res.). I suggest to reorganize the complete KE, maybe it is better to focus on one really established signalling pathway. Also the methods provided for measurement are incomplete. What is meant with "GFP for measurement of altered signalling"? Is GFP the only possible fluorescent protein? I this approach in the earlier mentioned "Fluorescence methods"? Why is only kinase measurement mentioned? There are a lot more measurable PTMs in endothelial cells in response to irradiation.

Agreed. We can appreciate the criticism of the broad name assigned to this KE at present. To address this, we have renamed the KE to "altered stress response signalling". In line with AOP principles since the KE is shared to other AOs the description is not specific to vascular remodeling. We do however, elaborate on specific pathways, such as the cAMP-PKA (cyclic adenosine monophosphate-protein kinase A) pathway, the MAPK (mitogen-activated protein kinase) pathway, the PI3K-Akt (phosphoinositide 3-kinase-protein kinase B) and NFkB in more detail, including methods for their measurement. We hope this satisfies the reviewer's concerns for this KE.

In terms of the four articles suggested by the reviewer related to NFkB signaling, those articles examining NFkB in the context of an upstream or downstream KE in our AOP have been considered and were added to the appropriate KER, provided the studies support the Bradford-Hill criteria using measurable endpoints that define our KEs. Details are provided below:

- Chishti et al. 2018 examines NFkB in the context of target genes related to cell survival (after irradiation). Since these genes are not further assessed at the protein level the essentiality of these responses is an uncertainty and therefore, the paper is excluded.
- Rödel et al. 2004 examines the correlation between the anti-inflammatory transforming growth factor beta 1 (TGF-B1) expression and NF-kB activation in the context of endothelial adhesion, which is not an endpoint that is included in our AOP and therefore, the paper is excluded.
- Murley et al. 2004 examines NFkB and the effects of SOD2 induction with thiol-containing drugs in a tumor cell treatment context. No clear dose or time concordance data are presented in the context of an upstream or downstream KE in our AOP and therefore, the paper is excluded.
- Dong et al. 2015 has been added to the KER of "altered stress response signaling" to "increase endothelial dysfunction":
    - ✓ The following statement is added to page 144 of the snapshot: "The NFkB inhibitor, PS1145, reduced senescence-like cells by almost 2-fold after 8 Gy of irradiation (Dong et al., 2015)."

<u>KE endothelial dysfunction: effects on barrier function are missing</u>

Studies have provided evidence that radiation exposure destabilizes the endothelial barrier, increasing vascular permeability and altering vesicular trafficking. These effects have been studied in vivo in a wide variety of animal models and in vitro using cultured primary or immortalized endothelial cells EC. Also methods to measure permeability should be

included. (examples: Bouten R, 2021, https://doi.org/10.1016/B978-0-12-818561-2.00007-2) Kabacik S. Oncotarget, 8 (47) (2017), pp. 82049-82063, Guipaud O., doi: 10.1259/bjr.20170762).

Agreed, measurements of endothelial barrier permeability are added to the endothelial dysfunction KE. Specifically, we have added to the measurement table in the snapshot on page 68, "Permeability Assays" and "Electric Cell Substrate Impedance Sensing (ECIS)". Furthermore, permeability assays are also in the measurement table of the vascular remodeling KE within the snapshot on page 73.

Among the articles suggested by the reviewer, those that support the Bradford-Hill criteria and examine upstream KEs to vascular remodeling or endothelial dysfunction endpoints have been added as follows:

Bouten et al. 2021 was added into the abnormal vascular remodeling KE and the MIE to endothelial dysfunction KER with the following sentences on page 72 and 176 of the snapshot: "Vascular remodeling is a term for many histological changes, including increased vascular stiffness, wall shear stress, intima-media thickening (IMT), increased intima-media section area, altered vascular permeability and increased vessel diameter (Bouten et al., 2021; Herity et al., 1999)" and "In vitro studies have shown that radiation increases endothelial permeability through both reducing levels of cell-cell contact proteins and increasing contractility of endothelial cells (Bouten et al. 2021)"

Guipaud et al. 2018 was added to the MIE to Endothelial dysfunction KER with the following statement on page 176 of the snapshot: "Following irradiation, endothelial cells may lose their integrity and become senescent or apoptotic via alterations to signaling pathways related to cell survival, leading to endothelial dysfunction (Deanfield et al., 2007; Bonetti et al., 2003; Guipaud et al. 2018)."

Kabacik and Raj, 2017 examines the endothelial barrier by assessing the permeability of various sized macromolecules after radiation. The following statement was added to the snapshot on page 154 within the endothelial dysfunction to vascular remodeling KER: "Kabacik and Raj (2017) showed that the endothelial permeability increased as the measurement of macromolecules of various sizes increased in a dose-dependent manner after irradiation and VE-cadherin levels also decreased with increasing doses of radiation (0, 0.5, 2, 10 Gy)."

We added other papers measuring endothelial permeability to the "endothelial dysfunction to vascular remodeling" KER, by Kouman et al., 2019 and Narayanan et al., 2020 on page 154 of the snapshot: "Ionizing radiation increased the permeability of endothelial monolayers by 25-35% and decreased VE-cadherin protein expression approximately 5-10% from the control with each increasing dose (0, 2, 4 Gy) (Kouman et al., 2019). Narayanan et al. (2020) demonstrated that lymphatic endothelial cell (LEC) permeability significantly increased after exposure to 0.5, 1 and 2 Gy of X-ray radiation, when compared to 0 Gy controls. At the same doses, the expression of VE-cadherin significantly reduced."

Additionally, other papers to support permeability assays by Young, 2012 and Young & Smilenov, 2011 have been added to the MIE to endothelial dysfunction KER, on page 178 of the snapshot: "Young (2012) observed a transient, significant decrease in the monolayer resistance 3 hours after irradiation with 5 Gy of γ-rays. In another study, a significant 6-fold transient decrease was observed in transmonolayer resistance at 3 hours post irradiation with 5 Gy of γ-rays (Young & Smilenov, 2011)."

As an important assay to measure endothelial dysfunction the ability form vascular networks in matrigel is missing in the methods for detection.

Vascular networks are included in the measurement section of the abnormal vascular remodeling KE, with the following references: (Le et al., 2022; Ebrahimian et al., 2015; Guo et al. 2010; Cardus et al., 2013) on page 73 of the snapshot:

- *Passaniti A. (1992). "Extracellular matrix-cell interactions: Matrigel and complex cellular pattern formation." Laboratory investigation; a journal of technical methods and pathology, Vol. 67/6*
- *Guo, S., Cheng, Y., Ma, Y., & Yang, X. (2010). "Endothelial progenitor cells derived from CD34+ cells form cooperative vascular networks." Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology, Vol. 26 https://doi.org/10.1159/000322335*
- *Ebrahimian, T. et al. (2015). "Chronic Gamma-Irradiation Induces a Dose-Rate-Dependent Pro-inflammatory Response and Associated Loss of Function in Human Umbilical Vein Endothelial Cells." Radiation research, Vol. 183/4. https://doi.org/10.1667/RR13732.1*
- *Cardus, A., Uryga, A. K., Walters, G., & Erusalimsky, J. D. (2013). "SIRT6 protects human endothelial cells from DNA damage, telomere dysfunction, and senescence." Cardiovascular research, Vol. 97/3. https://doi.org/10.1093/cvr/cvs352*

KE proinflammatory mediators

the description of dose rate and adaptive response effects should be included (e.g. Ebrahimian (2015), Gueguen (2019))

Agreed. This type of information is appropriate to include in the uncertainty section of the KER of deposition of energy leading to vascular remodeling, page 186 of snapshot as follows:

"Low doses of radiation administered at a low dose rate have been shown to be anti-inflammatory leading to improved vascular function (reviewed in Guéguen et al., 2019)."

"Ebrahimian et al. (2015) highlights the importance of dose-rate effects, demonstrating that lower dose rates of radiation exposure in vitro can lead to a more subdued inflammatory response and reduced changes in vascular networks compared to high dose rates."

Not all methods suggested for the detection of KEs reflect current knowledge, especially updates are necessary for DNA strand break detection, measurement of cytokines and signalling pathways (detection of strand breaks e.g. sBLISS by Bouwmann, assays for single strand breaks (Zilio and Ulrich, 2020), OLINK technology for in #1493, #2066 omics methods and pathway analysis, detection of PTMs beside phosphorylation).

Thank-you for highlighting these assays, which we have added to the measurement section of the the KEs of increase, DNA strand breaks, altered signaling pathways and proinflammatory mediators.

DNA strand breaks KE: The following have been added to the table, page 40 of snapshot:

- ✓ STRIDE assay Zilio and Ulrich, 2020 STRIDE (SensiTive Recognition of Individual DNA Ends) combines in situ nick translation with the proximity ligation assay (PLA) to detect single-strand breaks (sSTRIDE) or double-strand breaks (dSTRIDE). In this process, lesions labeled through nick translation with biotinylated nucleotides are identified by a PLA signal, which arises from the interaction of two anti-biotin antibodies from different species.

- ✓ sBLISS Bouwmann et al. 2020 sBLISS (in-suspension breaks labeling in situ and sequencing) labels double-strand breaks (DSBs) in cells immobilized on glass coverslips, using double-stranded oligonucleotide adaptors that facilitate selective linear amplification through T7-mediated in vitro transcription (IVT), followed by next-generation sequencing (NGS) library preparation.

Pro-inflammatory mediator KE, page 49 of snapshot:

- ✓ Olink Wang et al. 2022 Highly specific and sensitive proximity extension assay technology which uses an inflammation panel to categorize pro-inflammatory markers.

Altered Stress-Response Signaling KE, page 55 of snapshot:

- ✓ Omics technologies (Dai and Shen. 2022) involve comprehensive, high-throughput analysis of DNA, RNA, proteins, and metabolites to understand cellular functions and dynamics, offering a systems-level view of biological processes. Pathway analysis can then be used to gain insights from large amounts of omics data (Palli et al. 2019). Transcriptomics RNA sequence libraries are generated, clustering analysis is done, then sequencing for gene analysis (Qin et al. 2023). Proteins have been analyzed with proteomic analysis through LC-MS/MS analysis, bioinformatic analysis, western blot, qRT-PCR analysis or molecular docking. Metabolites are mass analyzed using the Thermo Q EXACTIVE, and then the edited data matrix is imported to Metabo Analyst for analysis (Hu et al. 2022).

- ✓ Post-translational modifications (PTMs) can also be measured using techniques such as mass spectrometry, which identifies and quantifies modifications like ubiquitination, glycosylation, and phosphorylation. Western blotting and immunoassays detect specific PTMs using antibodies tailored to particular modifications, while labeling methods can highlight modifications like acetylation and methylation. These measurements help elucidate protein function, stability, and interactions within cellular processes.

Maybe it is characteristic for the whole AOP approach, however, the complete takeover of already existing KE and KERs poses the risk of unspecific (sometimes irrelevant to the topic) information, which overwhelms the reader interested in the topic of AOP470. In the description of KEs the focus should be on ionizing radiation as MIE and the adverse outcome of vascular remodelling. Information on stressors such as metals, UV radiation or effects in bone cells are less relevant the topic of AOP470. Also literature neither related to ionizing radiation nor to endothelial cells should be reduced.

We appreciate your comment and recognize that the approach to building an AOP is new for most in the radiation field. In terms of KEs these are intended to be described in a way that they can be reusable. KERs are modular units and independent from the rest of the AOP; therefore, they are supported by data derived from different cell types and organs as they may be relevant to multiple AOs and stressors. Thus, some KERs are supported by data from non-vascular cells and non-ionizing radiation, this is intended to be a strength of AOPs. The interest is on understanding structurally and functionally the upstream biological perturbations in the context of the downstream KEs. To help achieve this, qualitative AOPs can be supported by a wide range of radiation stressors and exposure parameters. We have made some clarifications to section 3 (Adverse Outcome Pathway (AOP #470) – Brief Summary) of the AOP report.

At least the up-to-dateness of the described methods has to carefully checked (e.g. analytical methods of DNA strand breaks, cytokine measurement).

Agreed. We have added STRIDE, Olink, sBLISS and vascular network matrigel within the measurements of pro-inflammatory/signaling, DNA strand break and vascular remodeling KEs respectively. See comments above.

Weight of evidence for KER DNA strand breaks to altered signalling pathways is limited. it would be good to describe the weight of evidence judgement in a more transparent way.

Agreed, it may not be readily apparent how the weight of evidence call is judged. We have added to the AOP report, the following on page 11 ln 245-248 “Guiding questions to assess the weight of evidence call for each KER is provided in the OECD AOP development handbook (OECD, 2021) and are summarized within each table presented in the overall assessment of AOP #470.”

To summarize each of the KERs is assessed as follows:

Biological Plausibility

Is there a mechanistic (structural or functional) relationship between the upstream KE and downstream KE consistent with established biological knowledge

High: The relationship is well understood based on extensive previous documentation and has an established mechanistic basis and broad acceptance

Medium: The KER is plausible based on an analogy to accepted biological relationships, but scientific understanding is not completely established

Low: There is empirical support for a statistical association between KEs but structural or functional relationship between them is not understood

Essentiality:

Defining Question: Are downstream KEs and/or the AO prevented if an upstream KE is blocked?

High: Direct evidence from specifically designed experimental studies illustrating essentiality for at least one of the important KEs

Moderate: Indirect evidence that sufficient modification of an expected modulating factor attenuates or augments a KE

Low: No or contradictory experimental evidence of the essentiality of any of the KEs

Empirical Evidence:

Defining Question: Does empirical evidence support that a change in KEup leads to an appropriate change in KEdown? Does KEup occur at lower doses and earlier time points than KEdown and is the incidence of KEup > than that for KEdown? Inconsistencies?

High: Multiple studies showing dependent change in both events following exposure to a wide range of specific stressors (Extensive evidence for temporal, dose-response and incidence concordance); No or few critical data gaps or conflicting data.

Medium: Demonstrated dependent change in both events following exposure to a small number of specific stressors; Some evidence inconsistent with expected pattern that can be explained by factors such as the experimental design, technical considerations, differences between laboratories, etc.

Low: Limited or no studies reporting dependent change in both events following exposure to a specific stressor (i.e. endpoints never measured in the same study or not at all); And/or significant inconsistencies in empirical support across taxa and species that do not align with expected pattern for hypothesized AOP.

Quantitative Understanding of KERs

Defining Question: What is the extent to which a change in KEdown can be predicted from KEup? What is the precision with which uncertainty in the prediction of KEdown can be quantified? What is the extent to which known modulating factors or feedback mechanisms can be accounted for? What is the extent to which the relationships can be reliably generalized across the applicability domain of the KER?

High: Change in KEdown can be precisely predicted based on a relevant measure of KEup; Uncertainty in the quantitative prediction can be precisely estimated from the variability in the relevant KEup measure; Known modulating factors and feedback/ feedforward mechanisms are accounted for in the quantitative description; Evidence for the quantitative relationship between the KEs generalizes across the relevant applicability domain of the KER.

Moderate: Change in KEdown can be precisely predicted based on relevant measure of KEup; Uncertainty in the quantitative prediction is influenced by factors other than the variability in the relevant KEup measure; Quantitative description does not account for all known modulating factors and/or known feedback/ feedforward mechanisms; Quantitative relationship has only been demonstrated for a subset of the overall applicability domain of the KER.

Low: Only a qualitative or semi-quantitative prediction of the change in KEdown can be determined from a measure of KEup; Known modulating factors and feedback/ feedforward mechanisms are not accounted for; Quantitative relationship has only been demonstrated for a narrow subset of the overall applicability domain of the KER.

For the KER of DNA strand breaks to altered signalling pathways pg 110, we retrieved multiple studies showing dependent change in both events following exposure to a wide range of specific stressors (Extensive evidence for temporal, dose-response and incidence concordance); DNA strand breaks can lead to altered signaling of various pathways through the DNA damage response. DNA strand breaks, which are a form of DNA damage, can induce ataxia telangiectasia mutated (ATM) and ATM/RAD3-related (ATR), two phosphoinositide 3- kinase (PI3K)-related serine/threonine kinases (PIKKs) (Abner and McKinnon, 2004; Lee and McKinnon, 2007; Nagane et al., 2021; Sylvester et al., 2018; Thadathil et al., 2019; Wang et al., 2020; Wang et al., 2017). Following DNA strand breaks, cellular DNA damage response signaling can phosphorylate downstream proteins and activate several transcription factors and pathways (Wang et al., 2017). Spontaneous DNA strand breaks from endogenous sources will induce signaling as a normal response to facilitate DNA repair. However, excessive DNA damage induced by a stressor will result in increased activation of these pathways and subsequent harmful downstream effects. Signaling pathways induced by DNA strand breaks include p53/p21 (Abner and McKinnon, 2004; Baselet et al., 2018; Lee and McKinnon, 2007; Nagane et al., 2021; Sylvester et al., 2018; Thadathil et al., 2019; Wang et al., 2020; Wang et al., 2017), caspase (Abner and McKinnon, 2004; Baselet et al., 2019; Wang et al., 2020; Wang et al., 2016) and mitogen-activated protein kinase (MAPK) family pathways (Ghahremani et al., 2002; Nagane et al., 2021).

In terms of quantitative understanding, the KER was rated moderate, because the quantitative description does not account for all known modulating factors and/or known feedback/ feedforward mechanisms; quantitative relationship has only been demonstrated for a subset of the overall applicability domain of the KER.

Review of AOP Report Article

The AOP report is an adequate summary of the AOP and it is interesting to learn about the underlying considerations. However, the focus on space radiation (for example see page 4)

in the introduction is not reflected by the provided (available) data in the AOP. Therefore the potential aims and applications should be presented in a more balanced way.

We appreciate this comment. Across the 17 KERs in our AOP, over 25% of the data are supported by stressors relevant to the space environment, either from hind limb unloading and/or heavy ions. Since this project was partially funded by the space agency and has involved NASA and Canadian Space Agency scientists, we feel it is appropriate to discuss space radiation. Where appropriate we have removed specific indication of the space context page 4, ln76 and ln 80. In this way the AOP is applicable to other types of radiation exposures (e.g. radiotherapy). As well, in the background section page 7-8, ln 154-172, we present clinical exposures, and epidemiological studies relevant to CVD before discussing the space scene and the rationale for focus on the space environment.

Maybe limitations of the AOP can be discussed in more detail (for example non-linearity, adaptive response, chronic exposure, …..).

The limitations of the AOP are discussed within each KER and also in the report from page 24-27, summarized in Table IV. In Uncertainties and Knowledge Gaps (page 48) we now also include adaptive responses as follows:

Studies show that lower dose rates of radiation exposure can lead to a more subdued inflammatory response compared to high dose rates (Ebrahimian et al, 2015). This can impact the level of vascular damage. Adaptive responses have also been observed in animal and in vitro models, where low doses of radiation can induce a protective effect, reducing the release of pro-inflammatory cytokines like IL-6, TNF, and IL-8 in subsequent higher radiation exposures (reviewed in Guéguen et al, 2019). However, regarding the role of adaptive responses in cardiovascular risk from radiation, specifically for cancer, UNSCEAR has recently concluded that the impact of beneficial effects posed by adaptive responses would not affect the magnitude of the radiation cancer risk (UNSCEAR, 2021).

In terms of the limitations, these are discussed in the "Uncertainties and knowledge gap" section of the AOP report, below we have provided a point-form summary of the content in the report for the reviewers reference.

Inconsistent Findings on NO Levels:

NO levels show both increases and decreases post-irradiation, which may result from different proxy measures and biological contexts.

Difficulty in direct NO measurement and varying proxy measures lead to challenges in interpreting bioavailable NO levels.

Impact of Energy Deposition on NO:

Uncertainties on how energy deposition and oxidative stress influence NO levels, with conflicting evidence on the mechanisms leading to endothelial dysfunction.

Lack of Female-Specific Data:

Limited studies on female models, resulting in significant knowledge gaps in mechanistic data for the female body in the context of CVD and vascular remodeling.

Age-related Effects:

Few studies investigate the impact of age on cardiovascular health after radiation exposure, despite age being a known risk factor for CVD.

Chronic and Low-Dose Exposure Data:

Scarcity of data on chronic and low-dose radiation exposure, crucial for assessing long-term health risks for astronauts and other populations chronically exposed to radiation.

Dose-Concordance Discrepancies:

Inconsistencies in dose-concordance data between oxidative stress and pro-inflammatory mediators, complicating the understanding of their interplay.

Interaction of Space Stressors:

Insufficient studies examining the combined effects of multiple space stressors on the cardiovascular system.

Qualitative Nature of the AOP:

The AOP is qualitative and includes various radiation types and doses, necessitating a quantitative understanding of dose and dose-rate effects on KEs and KERs.

Standardization of Measurement:

Need for standardized methods to measure and interpret NO levels to refine the AOP and specify the KE of NO depletion.

Consistent Data Reporting:

Prioritization of harmonized experiments with consistent data reporting to validate quantitative aspects of the relationships between KEs and KERs.

What are the criteria for the examples showed in Table II?

Table II is only to provide a few representative examples of studies from the Wiki content; we have revised the title of the table to indicate these are a few examples of studies that support each KER and the complete list can be found within the AOP Wiki.

REVIEWER #2

Against the backdrop of the large number of biological processes and adverse outcomes (AOs) in the field of cardiovascular diseases (CVDs) the authors have decided to reduce the complexity in the design of an AOP. They have chosen vascular remodelling (VR) as an early subclinical AO which is a milestone on the path to more severe clinical AOs. On the other

hand, VR is sometimes differently defined in the literature and is not specifically ICD coded. I suggest that the authors present their understanding of VR more clearly in the introduction i.e. by inserting a table highlighting the criteria for VR.

Agreed, we have added a paragraph describing the measurable events related to vascular remodeling. Please note that vascular remodeling is defined in Figure 2 and Table 1 and within the AOP Wiki KE 2069.

We have added the following to page 4 ln 86-95 of the report:

"Vascular remodeling encompasses endpoints related to abnormal structural changes in the blood vessel walls that can arise from endothelial dysfunction. These include alterations in vasculature functional properties as measured by pulse wave velocity, modifications in vessel wall thickness and increase in vascular permeability. Cellular level changes can also lead to vascular remodeling as characterized by processes of growth, death, migration and production or degradation of the extracellular matrix (ECM) resulting in inflammation (increase in VCAM, ICAM, cytokines, chemokines) and calcification (changes in ratios of collagen). These changes contribute to the development and progression of cardiovascular diseases such as hypertension (ICD-10 code I10), atherosclerosis (ICD-10 code I70), and heart failure (ICD-10 code I50).

Another concern is the notion that VR is not entirely detrimental but sometimes necessary to maintain regulated blood circulation. One might think that this property goes against the definition of an AO. In any case, it will make it more difficult to separate detrimental from possibly beneficial KERs. On the other hand, atherosclerosis (AT) is a preclinical endpoint closely related to VR with entirely detrimental effects and a thorough ICD codification.

Agreed, an excellent point. We highlight this as a point in the introduction page 6 ln 121-129:
"Modification to vascular structure is not inherently detrimental; in fact, continual restructuring is crucial in promoting the health of the cardiovascular system (Pries et al., 2001; Santamaría et al., 2020; Zakrzewicz et al., 2002). However, some forms of remodeling are pathological and important markers and risk factors for future adverse cardiovascular events (Cohn et al., 2004; van Varik et al., 2012). Measures like 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, it may potentially serve as a predictive marker of cardiac outcomes"

We have changed the KE name to "abnormal vascular remodeling" - this is to highlight the studies show maladaptive changes in vessels that cause sub-optimal functioning which can lead to downstream detriments such as atherosclerosis and fibrosis.

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p 2, line 31: Should anti-inflammatory effects also considered? The are known to mitigate the generation of AT (see e.g. Ebrahimian et al. PMID: 29227739).

Agreed, we have revised the sentence page 2, ln 41-42: "…which when persistent concurrently causes the release of pro-inflammatory mediators, suppresses anti-inflammatory mechanisms and alters stress response signaling pathways."

p 3, line 31: The concept of feedback loops is not easy to understand. Could you give an example?

Agreed, we have added the following page 3, ln 63-65: "The pathways are unidirectional, but with inclusion of appropriate feedback loops (e.g., oxidative stress activates production of pro-inflammatory cytokines that generate additional reactive oxygen species (ROS)), progressing through KEs at increasing levels of biological organization."

The feedback loops are also described within appropriate KERs- oxidative stress to increased pro-inflammatory mediators
Deposition of energy can also induce feedback loops of ROS production where structures and molecules damaged by ROS including the mitochondria and NADPH oxidase (NOX) further produce ROS (Mittal et al., 2014; Soloviev & Kizub, 2019).

Positive feedback loop: oxidative stress upregulates production of pro-inflammatory cytokines, which in turn upregulate ROS production. The macrophages that are recruited in an oxidative stress-induced inflammatory response can also produce ROS and activate the pro-inflammatory mediator, TGF-β (Venkatesulu et al., 2018).

Another positive feedback loop is formed by ROS and NF-κB, as ROS activates NF-κB, resulting in expression of the genes, COX-2 and 5-LPO, which are responsible for ROS production (Ping et al., 2020).

p 4, line 13: Please explain in more detail your notion or VR as the focus of AOP 470.

Agreed. Added the following page 4 ln 86-95:

"Vascular remodeling encompasses endpoints related to abnormal structural changes in the blood vessel walls that can arise from endothelial dysfunction. These include alterations in vasculature functional properties as measured by pulse wave velocity, modifications in vessel wall thickness and increase in vascular permeability. Cellular level changes can also lead to vascular remodeling as characterized by processes of growth, death, migration and production or degradation of the extracellular matrix (ECM) resulting in inflammation (increase in VCAM, ICAM, cytokines, chemokines) and calcification (changes in ratios of collagen). These changes contribute to the development and progression of cardiovascular diseases such as hypertension (ICD-10 code I10), atherosclerosis (ICD-10 code I70), and heart failure (ICD-10 code I50)."

p 5, line 8-10: These lines might also relate to AT. What is the difference between VR and AT?

Great question, the two can be delineated by the measurements. While both vascular remodeling and atherosclerosis involve changes to the vessel walls, atherosclerosis specifically refers to the

pathological accumulation of plaques leading to arterial narrowing and potential obstruction. In the context of our AOP we define vascular remodeling (refer to KE 2069 in the snapshot page 70; and table 1 of AOP report) as the structural changes in the blood vessel walls that result from various stimuli, including mechanical stress, oxidative stress, and inflammatory responses. These include changes in vasculature properties as measured by pulse wave velocity, alterations in vessel wall thickness and increase in vascular permeability. Cellular level changes can also lead to vascular remodeling as characterized by processes of growth, death, migration and production or degradation of the extracellular matrix (ECM) resulting in inflammation (increase in VCAM, ICAM, cytokines, chemokines) and calcification (changes in ratios of collagen and elastin). This initial tissue injury and resulting remodeling can be measured as it leads to turbulent blood flow causing further structural changes like increased vessel fibrosis.

Increased vascular remodeling can also lead to atherosclerosis which could be a future downstream KE to our AOP. Increased vascular remodelling is often associated with a build-up of plaque in the arteries due to impaired healing, which forces the vessel walls to attempt to remodel to maintain blood flow. The measurable endpoints for atherosclerosis include the presence and extent of atherosclerotic plaques, plaque composition, and the degree of luminal stenosis.

We have added the following to highlight that atherosclerosis and also fibrosis can be a downstream outcomes of vascular remodeling page 5-6 ln 117-120:“Initial tissue injury and resulting remodeling can also lead to turbulent blood flow causing further structural changes like increased vessel fibrosis and the build-up of plaques (atherosclerosis) due to impaired healing, which forces the vessel walls to attempt to remodel to maintain blood flow (Sylvester et al., 2018).”

p 5, line 31: CIMT is a weak predictor of stroke and myocardial infarction (MI) (see e.g. Lorenz et al. 2007 PMID: 17242284) and considered as a biomarker of subclinical atherosclerosis (Bauer et al. Swiss Med. Wkly. 2012; 142 (w13705)). Bots et al.1997 (PMID: 9412629) note that increased CIMT reflects a non-atherosclerotic adaptive response to changes in shear stress and tensile stress, possibly related to VR. Only beyond a certain level, CIMT more likely may represent AT. Simonetto et al. 2020 (PMID: 32005003) observed that deviations from a normal age-related CIMT level are a better risk predictor that CIMT alone. In summary, these findings suggest that VR is needed to maintain a regular blood flow and the risk for stroke and MI is more related to AT. In this case VR cannot be considered as an AO.

Agreed, CIMT has been removed from the list of predictors as we have not provided evidence of studies in our AOP meeting Bradford Hill criteria that show our downstream KEs leading to vascular remodeling though CIMT.

p 6-7, The key epidemiological studies for radiation-induced CVD risk have been cited, but I would suggest to mention that the evidence is more heterogeneous compared to cancer risk. For example, in the LSS a different spectrum of CVD is related to radiation exposure which makes a risk transfer to Western populations difficult (Ozasa et al. 2017, PMID: 28151038). Forthcoming results from the Million Person Study (MPS) show no risk for protracted exposure at low doses and dose rates for ischemic heart disease (Schöllnberger et al. 2023, PMID: 35930470).

Agreed. We have added the following page 7, ln 160-162: “However, the evidence is more heterogenous compared to radiation and cancer risk, particularly for the cardiovascular disease profile in Western countries relative to Japan (Ozasa et al., 2017).”

The MPS study is beyond the scoping review timeframe and therefore is not cited.

p 8: One could be a bit more specific in the characterisation of radiation exposure during space flight. How long is a typical space mission and what are the expected doses? Can the dose estimates for cancer risk be applied to estimate CVD risk (Walsh et al. 2024 PMID: 37932191)?

Agreed. The following has been added on page 8-9, ln 184-190 of the AOP report: “The average dose absorbed by spaceflight crewmembers traveling to the International Space Station are 0.3-0.4 mGy/day (Shavers et al. 2024), and total radiation dose depends on the length of stay in the space environment. While length of space flight has varied among missions, the majority have lasted 6 months (Cucinotta, 2024). Risk projections and exposure estimates fluctuate based on variables such as mission length and destination, vehicle design, and heliosphere conditions (e.g., solar activity) (Simonson and Slaba. 2021).”

Yes, dose estimated for cancer risk can be applied to the estimate of CVD for galactic cosmic rays but for solar particle exposures this is more complicated. For the purpose of this paper, we therefore have not discussed risk modelling. This may be better suited for future work towards quantitative AOP development.

p 10, line 43: Please explain the nature of the possible bias.

Agreed. We have added the following page 11-12 ln 255-261: “Although a risk-of-bias evaluation was not undertaken due to the broad scope of the work and as it is not required by current AOP guidance, this could be a future consideration for the AOP. Potential biases may include selection bias, where the studies included may not represent the full spectrum of available data; publication bias, where positive findings are more likely to be published than negative or null results; and information bias, where inconsistencies in study methodologies and data reporting could affect the interpretation of the results.”

p 12, paragraph starting with line 13: This paragraph could be a motivation for developing AOP 470 and could go to the Introduction

Agreed, the following is included to highlight the motivation for developing the AOP:

Page 3 ln 52-59: “Cardiovascular diseases (CVDs) are a major health risk in North America and around the world. Globally, CVDs are the leading cause of death, accounting for over 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, CVDs were prevalent in 49.2% of adults over 20 years of age (Tsao et al., 2022). The present work harnesses the strengths of the adverse outcome

pathway (AOP) framework to contribute to the organization of research about the role of stressors from space travel and radiotherapy on CVD."

Page 4 ln 84-97: "Abnormal vascular remodeling was chosen as the AO to classify CVD as it is present in many pathologies and can be used as a marker of cardiac and vascular outcomes. Vascular remodeling encompasses endpoints related to abnormal structural changes in the blood vessel walls that can arise from endothelial dysfunction. These include alterations in vasculature functional properties as measured by pulse wave velocity, modifications in vessel wall thickness and increase in vascular permeability. Cellular level changes can also lead to vascular remodeling as characterized by processes of growth, death, migration and production or degradation of the extracellular matrix (ECM) resulting in inflammation (increase in VCAM, ICAM, cytokines, chemokines) and calcification (changes in ratios of collagen). These changes contribute to the development and progression of cardiovascular diseases such as hypertension (ICD-10 code I10), atherosclerosis (ICD-10 code I70), and heart failure (ICD-10 code I50). Furthermore, vascular remodeling under certain forms has been considered as a predictive marker for disease risk (Cohn et al., 2004; van Varik et al., 2012)."

p 12, line 27: A short characterisation of the missing elements for a more comprehensive CVD AOP would be helpful.

Agreed, we have added a list of KEs that were considered page 13, ln 298-299 "Examples of KEs include: mitochondrial dysfunction, thrombosis, angiogenesis, and extracellular matrix remodeling."

p 21, line 11: Are there epidemiological risk studies available which directly associate VR with CVD?

No studies were found that met the Bradford-Hill criteria in the context of measurable CVD endpoints. We would be seeking studies that show a causal relationship between vascular wall thickness, vascular stiffness, collagen deposition to CVD markers such as elevated levels of troponin, creatine kinase-MB, ECG changes, consistently elevated systolic and/or diastolic blood pressure, quantification of atherosclerotic plaque, increased platelet aggregation activity.

p 23, line 5: Which radiation qualities and doses are relevant for space flight?

This is described earlier in the paper, page 8-9, ln 175-196.

p 23, line 52: Dosimetric radiation transport calculations could reduce the knowledge gap as is the case for cancer risk (Walsh et al. 2024 PMID: 37932191).

Agreed, but since the paper is beyond our scoping review timeframe, it is not included.

p 26, line 22: For lifetime risk calculations established dose responses are required which include consistent age dependencies. In my opinion based on the large heterogeneity of epidemiological risk estimates the uncertainties for dose responses are still too large to allow reliable risk projections

Agreed, we have removed the statement as it is not within the scope of this paper.

p 30. Figure 1 caption: The last sentence is confusing. What is the point of the KER from MIE to AO curtailing all other KER? I thought the idea of an AOP is to dissect pathogenesis in small quantifiable units?

Agreed. We have modified the sentence to make it clearer as follows: Non-adjacent KERs can be used to support the weight of evidence (WOE) of the whole AOP by bypassing KERs with less empirical evidence but that are still biologically plausible.

p 82, Figure 2: a colour code is missing?

The following is indicated in the figure legend, second sentence: Green arrows indicate the endpoint increases in the KE, and red arrows indicate the endpoint decreases in the KE.

AOP wiki content

Major comments

The summarizing Tables on pages 6-11/168 provide a comprehensive overview on the topic couched in AOP language. This structured approach demonstrates the added value gained by applying the APO framework. It demonstrates that the general understanding of radiation-induced VR is not yet incomplete. Of special concern is the unclear essentiality of some key events such as KE#1493. If inflammation is not an important milestone on the path to VR, could the KE be dropped?

We appreciate the reviewer's concern regarding the essentiality of the key event (KE#1493) related to pro-inflammatory mediators in the AOP for vascular remodeling (VR). While we acknowledge that the essentiality of this KE has been rated as low according to the Bradford Hill criteria due to the lack of studies specifically *knocking out* or *modulating inflammatory* markers and examining subsequent effects on endothelial dysfunction, we believe that inflammation remains a critical component of the AOP. Inflammation is widely recognized as a fundamental response to tissue injury and stress, including radiation exposure. Pro-inflammatory mediators such as IL-6, TNF-α, and IL-8 play significant roles in endothelial cell activation, leukocyte recruitment, and the promotion of a pro-thrombotic state, which are key processes in the progression to vascular remodeling. While direct knockout or modulation studies focusing on inflammation and subsequent VR are limited, numerous studies in other contexts support the role of inflammation in vascular pathology. Inflammation interacts with other key events in the AOP, such as oxidative stress and endothelial dysfunction. These interactions contribute to a feedback loop that exacerbates vascular damage. We recognize the current gap in direct experimental evidence where inflammation markers are specifically knocked out or modulated to observe effects on endothelial dysfunction or VR. This highlights an important area for future research.

Although the focus of the AOP is by definition on adverse outcomes there are also beneficial effects of VR which might deserve a broader discussion in the AOP wiki.

Agreed, we cite a review on the topic. We have highlighted adaptive responses in the uncertainty/Knowledge gap section on page 25 ln 568-571 of the AOP report: "Adaptive responses have also been observed in animal and in vitro models, where low doses of radiation can induce a protective effect, reducing the release of pro-inflammatory cytokines like IL-6, TNF, and IL-8 in subsequent higher radiation exposures (reviewed in Guéguen et al, 2019)."

Also, we have added in the KER of deposition of energy leading to vascular remodeling page 186 of snapshot as follows: "Low doses of radiation administered at a low dose rate have been shown to be anti-inflammatory leading to improved vascular function (reviewed in Guéguen et al., 2019)."

Minor comments

p 5/168 2nd paragraph: "deposition of energy occurs immediately following irradiation" is inaccurate, better use "deposition of energy by irradiation"

Agreed, revised as suggested.

p 11/168 Given the mostly low quantitative understanding of many KERs, parameter transfer to biologically based risk models might not be easily feasible.

Agreed, the sentence has been removed.