August 9, 2024

To whom it may concern,

I declare that the review process for the AOP report titled "Development of an adverse outcome pathway for deposition of energy leading to cataracts" and its accompanying AOPWiki entry (https://aopwiki.org/aops/478) 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:
Jason O'Brien

Reviewers:
Gayle Woloschak
Norman Kleiman
Stephen Barnard

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: 478, Deposition of energy leading to occurrence of cataracts

Author: Emma Carrothers, Meghan Appleby, Vita Lai, Tatiana Kozbenko, Dalya Alomar, Benjamin Smith, Robyn Hocking, Carole Yauk, Ruth Wilkins, Vinita Chauhan

Associated wiki page: *https://aopwiki.org/aops/478*

Compliance Reviewer Information

Name: Jason O'Brien

Organisation: Environmental and Molecular Mutagenesis

E-mail: jason.obrien@ec.gc.ca

Review Information

Date this checklist has been filled: 2023-05-26

Date of final draft PDF snapshot proposed for external review: 2023-05-26

General Observations and Recommendations of the Reviewer

- This a miniature AOP network
- Many of the KEs and KERs are part of previously reviewed AOPs

KE ID	KE Title	Previously Reviewed	In which AOP(s)?
1686	Deposition of Energy	YES	272
2081	Increased Modified Proteins	NO	
1634	Increase, Oxidative damage to DNA	YES	296
1635	Increase, DNA strand breaks	YES	272, 296
155	Inadequate DNA repair	YES	15, 272, 296
185	Increase, Mutations	YES	15, 272, 296
1636	Increase, Chromosomal aberrations	YES	272, 296
870	Increase, Cell Proliferation	YES	272
1392	Oxidative Stress	YES	17, 220
2083	Occurrence of Cataracts	NO	

KER ID	KER title	adjacency	Previously reviewed?	In which AOPs?
1977	Energy Deposition leads to Increase, DNA strand breaks	adjacent	YES	272
2769	Energy Deposition leads to Oxidative Stress	adjacent	NO	
2809	Energy Deposition leads to Modified Proteins	adjacent	NO	
2810	Oxidative Stress leads to Increase, Oxidative DNA damage	adjacent	NO	
2811	Oxidative Stress leads to Increase, DNA strand breaks	adjacent	NO	
2812	Oxidative Stress leads to Modified Proteins	adjacent	NO	
1909	Increase, Oxidative DNA damage leads to Inadequate DNA repair	adjacent	YES	296
1911	Increase, DNA strand breaks leads to Inadequate DNA repair	adjacent	YES	272, 296
164	Inadequate DNA repair leads to Increase, Mutations	adjacent	YES	15, 272, 296
1912	Inadequate DNA repair leads to Increase, Chromosomal aberrations	adjacent	YES	272, 296
1978	Increase, Mutations leads to Increase, Cell Proliferation	adjacent	YES	272
1979	Increase, Chromosomal aberrations leads to Increase, Cell Proliferation	adjacent	YES	272
2816	Modified Proteins leads to Cataracts	adjacent	NO	
2819	Increase, Cell Proliferation leads to Cataracts	adjacent	NO	
1913	Increase, Oxidative DNA damage leads to Increase, DNA strand breaks	adjacent	YES	296
2813	Energy Deposition leads to Increase, Oxidative DNA damage	Non-adj	NO	
1981	Energy Deposition leads	Non-adj	YES	272

	to Increase, Mutations			
1982	Energy Deposition leads to Increase, Chromosomal aberrations	Non-adj	YES	272
2814	Energy Deposition leads to Increase, Cell Proliferation	Non-adj	NO	
2815	Energy Deposition leads to Cataracts	Non-adj	NO	
2817	Inadequate DNA repair leads to Cataracts	Non-adj	NO	
2818	Oxidative Stress leads to Cataracts	Non-adj	NO	

AOP Coach Checklist and Final Review Report

Checklist

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

KEY EVENTS

KE number, title: *1686 Deposition of Enery* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	***YES 272***			
Has the KE been reviewed by EAGMST?	*YES*			
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)?	✓			
Is the biological context (level of organization, terms) specified?	✓			
Are KE components defined using structured ontology terms (Process, Object, Action)?		*NO*		
Is the KE description clear?	✓			
Are measurement methods specified, described and referenced?	✓			
Is the domain of applicability described?	✓			
Specific Comments: *Will as authors about KE components during scientific review*				

AOP Coach Checklist and Final Review Report

KE number, title: *2081 Increased modified Proteins* ***(including MIE and AO; copy this table for each KE)***	*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?				
Is the domain of applicability described?	*YES*			
Specific Comments: • *Will as authors about KE components during scientific review*				

AOP Coach Checklist and Final Review Report

KE number, title: *1634, Increase, Oxidative damage to DNA* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 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)?	*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* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 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 KE components during scientific review*				

AOP Coach Checklist and Final Review Report

KE number, title: *155, Inadequate DNA Repair* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 15 272 296***			
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?				
Are KE components defined using structured ontology terms (Process, Object, Action)?		*minor*		
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments: • *Please review if "functional change" is the most appropriate "action" for the components*				

AOP Coach Checklist and Final Review Report

KE number, title: *185, increase mutations* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 15 272 296***			
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)?	*YES*			
Is the KE description clear?	*YES*			
Are measurement methods specified, described and referenced?	*YES*			
Is the domain of applicability described?	*YES*			
Specific Comments: *none*				

AOP Coach Checklist and Final Review Report

KE number, title: *1636, increase, chromosomal aberrations* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 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 ask authors to define KE components during scientific review*				

AOP Coach Checklist and Final Review Report

KE number, title: *870, increase, cell proliferation* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 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)?	*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: *1396, oxidative stress* ***(including MIE and AO; copy this table for each KE)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KE already been used in other AOPs?	*YES*			
Has the KE been reviewed by EAGMST?	***YES 14 220***			
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: *none*				

AOP Coach Checklist and Final Review Report

KE number, title: *2083, occurrence of cataracts* ***(including MIE and AO; copy this table for each KE)***	*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: *NONE*				

KEY EVENT RELATIONSHIPS

KER number, title: *1977 Energy deposition LEADS TO increase DNA strand breaks* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *2769, Energy deposition LEADS TO oxidative stress* ***(copy this table for each KER)***	*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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *2809, Energy deposition LEADS TO modified proteins* ***(copy this table for each KER)***	*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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *2810, oxidative stress LEADS TO increase oxidative stress* ***(copy this table for each KER)***	*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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *2811, Oxidative stress LEADS TO DNA strand breaks* ***(copy this table for each KER)***	*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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *2812, Oxidative stress LEADS TO Modified proteins* ***(copy this table for each KER)***	*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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *1909, oxidative DNA damage LEADS TO inadequate DNA repair* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES 296*			
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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *1911, increase DN strand breaks LEADS TO inadequate DNA repair* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***272*** ***296***			
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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *164, inadequate DNA repair LEADS TO increase mutations* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***15 272 296***			
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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *1912, inadequate DNA repair LEADS RO increase, chromosomal aberrations* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***272*** ***296***			
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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *1978, increase mutations LEADS TO increase cell proliferation* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *1979, increased chromosomal aberrations LEADS TO increase cell proliferation* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***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: none				

AOP Coach Checklist and Final Review Report

KER number, title: *2819, Modified Proteins LEADS TO Cataracts* ***(copy this table for each KER)***	*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?		*NO*		
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: *2819, Increase cell proliferation LEADS TO Cataracts* ***(copy this table for each KER)***	*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?		*NO*		
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: *1913, Increase oxidative DNA damage LEADS TO increase DNA strand breaks* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***296***			
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: *2813, Energy Deposition leads to Increase, Oxidative DNA damage*	*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: *1981, Energy Depostion LEADS TO increase Mutations* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***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: *1982, Energy Deposition leads to Increase, Chromosomal aberrations* ***(copy this table for each KER)***	*Yes*	*For revision*	*Revision agreed*	*Not applicable*
Has the KER already been used in other AOPs?	*YES*			
Has the KER been reviewed by EAGMST?	*YES* ***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: *2814, Energy Deposition leads to Increase, Cell Proliferation* ***(copy this table for each KER)***	*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: *2815,* Energy Deposition leads to Cataracts ***(copy this table for each KER)***	*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: *2817,* Inadequate DNA repair leads to Cataracts ***(copy this table for each KER)***	*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: *2818, Oxidative Stress leads to Cataracts* ***(copy this table for each KER)***	*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: One of the most well-organized AOPs I have seen to date!				

REVIEWER COMMENTS

KEY EVENTS

Key Event 1 (Molecular Initiating Event, MIE): 1686, Deposition of Energy
Note: Shared KE, Previously reviewed in AOP 272

Comments:
REVIEWER#1:
Clearly reviewed extensively under previous AOPs, a huge amount of epidemiology data to support deposition of energy leading to cataract

REVIEWER#2:
For overview, consider changing "energy deposition on the atoms and molecules" to "on the atoms that make up molecules".
Second sentence: "higher energy deposits", higher than what? Higher than Low LET is assumed but should be stated. LET is defined as deposition of energy per unit distance, and this should be stated in the second and third sentences.
Parameters in third sentence should specify that the radiation track is still stochastic and not predictable even if influenced by many parameters.
For Energy can be deposited into any substrate both living and non-living…and then "it is independent of age…etc". Energy is independent of those things, but I think it means energy deposition. This should be stated.

Key Description:
"Deposition of energy depends…" should state that ionizing radiation is some cases can cause excitation without ionization. Ionizing radiation does not always lead to an ionization.

In the paragraph "Ionizing radiation can cause", second to the last line I would add "ingestion" as another example.

In the paragraph about direct ionization there is confusion. Photons are not charged but rather create electrons which cause ionizations. This paragraph says only charged particles can cause direct ionizations but uses photons as an example.

The paragraph on spatial structure should be changed:
Second line should say High LET refers to energy…..which OFTEN produces more complex…"
It state low LET travels farther into tissues, but neutrons often travel as far as X-rays and gamma-rays, and C ions have a Bragg peak within the tissue.

Key Event 2: 1392, Oxidative Stress
Note: Shared KE, Previously reviewed in AOP 17 and 220

Comments:
REVIEWER#1:
Well documented effects and reviewed in other AOPs quite well, although the key event description is not well referenced

REVIEWER#2: no comment

Key Event 3: 2081, Increased Modified Proteins

Comments:
REVIEWER#1:
Well documented effects, although the key event description is not well referenced.
Specific to the lens, no mention of heat shock protein modifications that have been reported, or changes to crystallin proteins, aquaporins etc.

REVIEWER#2:
This topic is confusing. It seems to consider only direct changes in the protein that come about from radiation, but what about changes that occur as a result of radiation-induced signal transduction…for example phosphorylation. This governs responses in the cells throughout all organisms.
Many of these responses are dose-dependent. For example, protein denaturation (noted here as unfolding) occurs predominantly at high doses, not low doses. This would be particularly relevant for cataract formation, as is noted.
Assays for protein phosphorylation, transcription, and others are not mentioned.

Key Event 4: 1634, Increase, Oxidative damage to DNA
Note: Shared KE, Previously reviewed in AOP 296

Comments:
REVIEWER#1: no comment

REVIEWER#2:
The reference included for the overview is not clear…why use this paper?
I believe that there is evidence that oxidative damage is created by Cs-generated gamma-rays as well and also by a variety of radionuclides not mentioned here. This is not very comprehensive and is too limiting.
It may also be worth noting that radiation may generate a different spectrum of oxidative species than other oxidizing agents. The most powerful oxidizing agent induced by IR is .OH (hydroxyl radical).

Perhaps it is also worth mentioning that the ROS must be generated in close proximity to the DNA (2-4nm) and if they are more distant they will not lead to DNA damage as they will be repaired by the cell.
It may also be worth noting that ROS generation increases with increasing dose. It is also affected by dose-rate.
Almost none of the assays are specific for ROS…most will occur as the result of direct DNA damage (comet assay, etc.). ROS also causes SSB and DSB, although DSB are less common being two SSB that are close to each other. These too are from oxidative damage. It is hard to separate this from the next category.

Key Event 5: 1635, Increase, DNA strand breaks
Note: Shared KE, Previously reviewed in AOP 272 and 296

Comments:
REVIEWER#1:
Well documented, lots of literature supporting this

REVIEWER#2:
It might be worth mentioning that there are error-prone and error-free forms of DSB repair and that the pathways for SSBR and DSBR are distinct.
Complex lesions can also lead to mutations not only inappropriate recombination.
The excision repair should be specified as BASE excision repair, a form of which is considered to repair SSBs. Most BER repairs base damage which is included above.
Beir 199 reference?
There is only one nearly recent paper here.
Alternative NHEJ is also not mentioned.
Assays: 53BP1 should be added

Key Event 6: 155, Inadequate DNA repair
Note: Shared KE, Previously reviewed in AOP 15, 296, and 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
This is on the damage reversal section: It is not clear why the photolyases are mentioned here. They are for UV damage in bacteria. They do not act in mammalian cells for ionizing radiation. I don't think bacteria get cataracts. So much is known about mammalian DNA repair, it is not certain

BER should mention the polymerase and ligation by a ligase since as it is discussed it only includes the removal of the base and not the actual repair.

NER is the same…only the excision (which is actually about 29-30bp) is mentioned and not the repair polymerase or ligation reaction.

SSBR does not mention the fact that a common intermediate is generated and that this is the same for BER and SSBR. End processing is generally by Polynucleotide Kinase.

DDSBR: HRR occurs in late S and G2. It might be worth noting that there is no repair in M phase.

NHEJ; AGAIN, there is no repair in M chase. DNA PKcs is the catalytic subunit, not the complex as is mentioned in the text.

Perhaps something should be mentioned about complex lesions and the difficulty in repairing them?

Dose response curve for alkyl addutcts/mutations—This section is unclear.
Alt-NHEJ is a low fidelity mechanism, requires microhomology repeats, uses polymeras Q (or theta). This should be mentioned.

Fidelity of DNA Repair: The issue is made that NER may be low in testes. This may be irrelevant. How often are sperm exposed to UV/?

While NHEJ is error-prone, HRR is not. This is not coming out clearly.

How isit measured or detected? This first paragraph is not clear. Why is it talking about repair of plasmid DNA in vitro?

This entire section about alkylated DNA talks about chemical exposure, not ionizing radiation exposure. It is not clear how this is relevant at all.

For the direct measurement table, it might be worth mentioning which of these methods are good for low dose vs high dose. It is unclear why alkylation exposure is so important here when it is not so important for ionizing radiation. The Comet assay should mention the SSB measurements that are used with alkaline solution coment.

Key Event 7: 185, Increase, Mutations
Note: Shared KE, Previously reviewed in AOP 15, 296 and 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
The section on How it is measured or detected is mostly irrelevant to radiation. The Ames test is not used in radiation, these assays are chemical-specific and not related to radiation.The issues

about the different spectrum of lesions is also irrelevant to radiation since radiation causes base damage, SSBs, DSBs and there are no known signature lesions.
A few of the comments are relevant…the HPRT assay, TK assays, and others. Nevertheless the information is still very oriented toward chemicals and not radiation.
This information is all generic and not relevant to radiation.s

Key Event 8: 1636, Increase, Chromosomal aberrations
Note: Shared KE, Previously reviewed in AOP 296 and 272

Comments:
REVIEWER#1: No further comments – well established and the assays in the table is thorough

REVIEWER#2:
Are chromatid aberrations types of chromosome aberrations? This sentence on two types of chromosome injuries is unclear. Later there is mention of chromosomal aberrations and chromatid do not seem to be included.

The interstitial deletion is not correct, what is described is an interstitial inversion.

There is no distinction between lethal and non-lethal aberrations in this discussion. This should be included It should be noted that most of the deletions (whether terminal or interstitial should be small. If they are too large, too much genetic material is lost and it will be lethal..

MN are not always just in bi-nucleated cells. Most people relate MN to dicentrics.

Key Event 9: 870, Increase, Cell Proliferation
Note: Shared KE, Previously reviewed in AOP 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
Flow cytometry is a common way of measuring percentage of cells in and out of the cell cycle, and this was not mentioned at all.

It is not certainly why the olfactory epithelium is relevant here.
It is not certain why nasal tissue is mentioned here.
It is noted that IR can induce proliferation, but it can also lead to cell cycle blocking, which is not mentioned.
For most mammalian cells, it is not just nutrients, but also oxygen that is required for cell proliferation. Low oxygen inhibits cell proliferation. This should be mentioned.
For how to measure it should state that BrdUDR or I-UdR are used, since both are frequently used.
Flow cytometry is not really mentioned adequately.

Key Event 10 (Adverse Outcome, AO): 2083, Occurrence of Cataracts

Comments:
REVIEWER#1:
sex applicability – is it worth including studies where no sex effect is reported to balance this sentence out? I feel the key event description of 'cataract' should be consistent across all relevant KERs subsequently, but this seems to change.

"Therefore, opacities are not removed and accumulate with time" – I think this sentence could be worded better, this sounds like multiple opacities develop over time, whereas 'opacity' is a measurable change in the lens as a whole leading to vision impairment.

"These include proper organization of proteins such as crystallins" – not just organisation, but also proper development and balance of crystallins.

"no organelles within the lens fiber cells" – no organelles in mature lens fiber cells, the organelle loss is a gradual process and therefore early lens fiber cells can still contain some organelles until they migrate towards the centre of the lens, the organelle free zone. This occurs alongside the gradual increase in crystallin proteins (Hejtmancik and Shiels 2015).

"which increases lens opacity, contributing to cataract formation and intensity" – I would say density instead of intensity, to be consistent with literature terminology.

"They classify cataracts on a scale of severity, which is often subjective, relying upon the examiner's judgement" – no mention of Scheimpflug imaging (McCarron et al 2022, Fuchs et al 2011, Puk, de Angelis and Graw 2013, Dalke et al 2018) which can be used to measure lens density, taking away some subjectivity. Although Scheimpflug imaging has been mentioned int eh table below. Worth mentioning I feel as the technology has been incorporated in specific studies of radiation-induced cataract in animals relevant to this AOP as a whole.

REVIEWER#2: no comment

KEY EVENT RELATIONSHIPS

Adjacent KERs

1977, Energy Deposition leads to Increase, DNA strand breaks
Note: Shared KER, Previously reviewed in AOP 272

Comments:
REVIEWER#1:
Biological plausibility – Ainsbury et al 2026, not Ainsbury 2016
Quantitative understanding – Dose concordance - Barnard et al 2019 study has an incorrect result reported in the table? An inverse dose-rate response was reported in the study whereby the lower dose rate produced the higher frequency of foci. Check wording and clarify?

"Furthermore, primary normal human fibroblasts exposed to 1.2 – 5 mGy X-rays at 5.67 mGy/min showed a supralinear relationship, indicating at low doses, the DSBs are mostly due to radiation-induced bystander effects" – reference this sentence please.

REVIEWER#2:
There are some data in UNSCEAR 2020/2021 report on low dose radiation that indicate that rarely low LET radiations and low doses can produce complex lesions. This would be important as possibly having an impact on DNA damage. This is not necessarily in lens cells but if it can occur in other cell types it might also occur in lens cells.

2769, Energy Deposition leads to Oxidative Stress
Note: Shared KER

Comments:
REVIEWER#1: no comment

REVIEWER#2:
Perhaps the OH. Should be mentioned as being among the most long-lasting.
I am not certain how all the references were chosen as being relevant. They do not all involve lens cells. Why were cardiac and neuronal cells included?

2809, Energy Deposition leads to Modified Proteins
Note: Shared KER

Comments:
REVIEWER#1:
Dose concordance – "Strong evidence is available in lens cells to support a dose response relationship between energy deposition and protein modification. A study showed, that lens

crystallin proteins continuously irradiated in vitro for 24 hrs using UV contained high molecular weight proteins relative to controls (Zigler & Goosey, 1981)" – specify what UV (A, B, broad spectrum, solar simluated) and at what energy/dose. In vitro exposures using UV can generate heat which could affected protein modifications, to note. This information has been presented by the authors in other KERs and is very useful to the reader.

KER states most studies are from male animals, but this is specified in the tables, perhaps could include which sex of animal was used in each study. This KER feels quite weak in general compared to others,

REVIEWER#2:
It may be worth noting that protein modifications can be short-lived within cells as the accumulation would depend upon the turnover of the protein and the turnover of the cells themselves. Not all protein changes are equally damaging.
Often UV and ionizing radiation are compared, yet these are not really acting via the same mechanisms. This should be noted.

2810, Oxidative Stress leads to Increase, Oxidative DNA damage

Comments:
REVIEWER#1:
A few studies have also found that single stranded DNA (ssDNA) is more likely to be oxidized than double stranded DNA (dsDNA). This indicates that persistent ssDNA sites, such as Z-DNA, stable R-loops, cruciforms, quadruplexes, or intramolecular triplexes might have higher incidences of oxidative damage (Amente et al., 2019) – 'a few studies' but you have only referenced one?

REVIEWER#2:
For the two BER pathways, one is for SSBR, which is not really mentioned here. SSB are dangerous because when two are in juxtaposition, they become DSB, which as noted throughout this document are damaging.
The comment about the amount of oxidative damage accumulating over months is confusing. Sometimes it occurs very rapidly following oxidative stress, and often it can be repaired rapidly.

2811, Oxidative Stress leads to Increase, DNA strand breaks
Note: Shared KER

Comments:
REVIEWER#1: no comment

REVIEWER#2:
This section does not really talk that much about SSBs. While they are repaired rapidly, they can contribute to complex lesions and to DSBs.

The SOD that is part of the defense needs to be specified—SOD1, 2 or 3 or all?
Again, it is confusing because UV and ionizing radiation are used interchangeably in places, and they are not at all the same. This becomes very confusing later in the document when radiation is mentioned and only IR is considered.
Increased XRCC2 and 3 are not just associated with BER but also SSBR.

2812, Oxidative Stress leads to Modified Proteins

Comments:
REVIEWER#1: no comment

REVIEWER#2: no comment

1909, Increase, Oxidative DNA damage leads to Inadequate DNA repair
Note: Shared KER, Previously reviewed in AOP 296

Comments:
REVIEWER#1:
The whole key event relationship description is supported by a single review paper, which is fine but it would be nicer to cite some of the key statements individually. In general, some key event relationships descriptions are heavily referenced, and others, such as this, less so.

REVIEWER#2:
SSBs can also be the result of oxidative lesions directly, this is not realy stated clearly.

1911, Increase, DNA strand breaks leads to Inadequate DNA repair
Note: Shared KER, Previously reviewed in AOP 296 and 272

Comments:
REVIEWER#1: Very well supported by literature, I have little further to add.
Empirical evidence (and throughout) - Rothkamm & Lo, 2003 should be Rothkamm & Lobrich.
Throughout – reference should be McMahon et al 2016, not Mcmahon
Quantitative understanding – "all data is significantly significant" – you mean statistically significant

REVIEWER#2:
Cells that are damaged in G0 also appear to use NHEJ repair, this was not mentioned.
Defects in NHEJ and HRR both lead to extreme radiosensitivity, this should be mentioned.
NHEJ is favored in mammalian cells because MOST cells are not in S or G2 phase of the cell cycle, when HRR occurs.

Perhaps it is worth mentioning that HRR can be problematic if there is a mutant copy of a gene that is copied leading to LOH.
Is it worth mentioning SSA here since that appears to be a low fidelity menchanism that functions when other repair pathways are compromised.?
Another point that has been made in UNSCEAR 2020/2021 is that HRR predominates in stem cells because they are frequently in the cell cycle. Perhaps that is relevant for the lens?

164, Inadequate DNA repair leads to Increase, Mutations
Note: Shared KER, Previously reviewed in AOP 15, 296 and 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
Again, perhaps SSA should be mentioned here since it seems to be more important when there are cells that have inadequate NHEJ and especially HRR.
It is not clear why the germ cells are mentioned here. How are they relevant to the cataract endpoints?
The justification for inclusion of XP data and not MMR deficiencies is not clear.
Mention is made in the Essentiality about Ku80 protein deficiency, but not SCID which is the disorder for DSB repair deficiency for NHEJ or AT which is missing DSB repair as well.
The references chosen seem to be few..
Discussion here is strong on NER, which is usually involved in UV damage and not much for IR damage.
DNAPK is mentioned, but ATM is not really discussed along with its role as a signaling agent for DSB repair…which is involved in CA.

1912, Inadequate DNA repair leads to Increase, Chromosomal aberrations
Note: Shared KER, Previously reviewed in AOP in 296 and 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
It is mentioned that when NHEJ and HRR are compromised, alt-NHEJ predominates. Recent papers looking at BRCA2 deficient cells (BRCA2 is involved in both types of DSB) suggested that SSA takes over. This should be mentioned.

Also, there is nothing about DNA replication stops that occur with DNA damage. The retarting of replication can be problematic for repair.

1978, Increase, Mutations leads to Increase, Cell Proliferation
Note: Shared KER, Previously reviewed in AOP 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
"For a mutation to occur, damaged DNA must be passed on to the next generation" is not accurate statement. A mutation can occur if it is not passed on to the offspring, it can lead to cell death. Damaged DNA is not passed on , the mutation is.
There is much information that also suggests that DNA damage STOPS proliferation for awhile after irradiation; this needs to be mentioned. If a cell fails to stop DNA synthesis (in ATM mutant cells e.g.) then errors accumulate. This is not mentioned.

1979, Increase, Chromosomal aberrations leads to Increase, Cell Proliferation
Note: Shared KER, Previously reviewed in AOP 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
There are situations when increased chromosomal aberrations lead to a stalling of replication because the cell can't survive well with the abnormality.
It is also not clear whether the increase in proliferation from chromosome aberrations is from cellular transformation or not.

2816, Modified Proteins leads to Cataracts

Comments:
REVIEWER#1: as stated, low body of evidence but some empirical evidence, although is weak. Studies have used very high doses relatively speaking,

REVIEWER#2:
There is a literature that the PSC cataracts are IR-induced, but this is not really discussed in the document. There is likely to be something different about the different mechanisms by which cataracts are induced by the different cataract-inducing agents.

2819, Increase, Cell Proliferation leads to Cataracts

Comments:
REVIEWER#1: The McCarron et al reference doesn't seem particularly relevant in the KER relationship description?. Although quite old studies, the work by Von Sallman et al in the 1960s

are quite relevant but have been omitted from this KER. The grammar needs checking in this wiki page in general, some sentences do not make senses or words are missing.

REVIEWER#2:
Not all cells of the lens are capable of proliferation. This might be worthy of mention.

1913, Increase, Oxidative DNA damage leads to Increase, DNA strand breaks
Note: Shared KER, Previously reviewed in AOP 296

Comments:
REVIEWER#1: no comment

REVIEWER#2:
The problem throughout is that there are inconsistencies. Here finally SSBs are mentioned, but they are not mentioned in other sections that deal with oxidative DNA damage and strand breaks.

Non-adjacent KERs

2813, Energy Deposition leads to Increase, Oxidative DNA damage

Comments:
REVIEWER#1: no comment

REVIEWER#2:
Again, there is a misunderstanding of UV damage. This section notes that UV causes ionization, it does not. Ionizing radiation causes ionization.

1981, Energy Deposition leads to Increase, Mutations
Note: Shared KER, Previously reviewed in AOP 296

Comments:
REVIEWER#1: no comment

REVIEWER#2:
It states “The DNA is particularly susceptible to damage which can be in the form of mutations”. DNA damage and mutations are different. DNA damage includes DSB, base damage, etc…the mutations are the changes that occur as repair proceeds. This confusion is misleading in the document.

Damage can be by direct and indirect mechanisms in the nucleus….indirect involves oxidative damage of water that damages the DNA and direct involves action on the DNA directly. This is not clear in this section.
Again, why is radiation exposure of germ cells discussed here?
A point is made about dose rate, but this is not really described well in the write-up.
Variation for uncertainties should also not differences in endpoints that affect the interpretation of the data.

1982, Energy Deposition leads to Increase, Chromosomal aberrations
Note: Shared KER, Previously reviewed in AOP 272

Comments:
REVIEWER#1: no comment

REVIEWER#2:
It is noted that chromosomal injury comes about from the DIRECT damage of DNA. How can we be sure it is not indirect?
For known modulating factors, it is stated that females are more sensitive. Is this in humans or all species?

2814, Energy Deposition leads to Increase, Cell Proliferation

Comments:
REVIEWER#1: no comment

REVIEWER#2:
As in the notes above, it is not clear that the group has considered the block in DNA replication that takes place following radiation exposure. In addition, is the proliferation just about cancer induction or is it something else?
Obviously the time-frame matters and that is not discussed in this section.

2815, Energy Deposition leads to Cataracts

Comments:
REVIEWER#1:
the key event relationship description is very poorly cited. Once you've used and introduced the 'LEC' abbreviation, you do not need to continue to write 'lens epithelial cell'.
"When the rate of division in mitotically-active lens epithelial cells becomes too high, they become incapable of transforming into typical elongated, organelle-free lens fiber cells" – change transforming to 'differentiating'.

"The overall consensus is that cataract risk increases with radiation dose, as measured based on various forms of cataracts" – maybe mention the stochastic versus deterministic argument here for context, as the literature is still not super clear on which applied to cataract.
No mention of the studies by Little et al in the main text and the radiologic technologist cohort, which provide some good evidence and data for a very large occupationally exposed cohort to support low dose exposures.
"Studies using visual acuity to measure cataracts pose challenges as the test is not specific for cataracts, even though the measurement is an indicative test for the ultimate function of the lens" – reference this sentence.
Latency effect – many biological studies are performed in rodents with limited lifespans, to note, and so a reason for lack of cataract development in studies with 'controlled' radiation dose exposures as opposed to human occupational or accident cohorts.
Known modulating factors – Genetics – McCarron study referenced twice with different years? There is only one McCarron paper and it should be cited as 2022.
Race – 'White people' might be better written as 'white skin tone'

REVIEWER#2:
Information on neutron exposures is needed. What is the energy of the neutrons? Radiation weighting factor is dependent on energy of neutron. It is noted that dose-rate plays a role, but dose-rate is not listed.

2817, Inadequate DNA repair leads to Cataracts

Comments:
REVIEWER#1:
McCarron et al 2021? It is 2022. This needs to be made consistent throughout the AOP

"estrogen-implicated increase in speed of cataract progression" – some mentioned of the studies by Dynlacht et al might be worth including here, albeit using rats exposed to high-LET rather than mice. In KE 2083, sex applicability subsection includes studies and references not included in the KER which are relevant to understanding the KER.

REVIEWER#2:
A list of human datasets that contribute to our understanding of cataracts is given, but nothing is mentioned about RT patients, which influenced much about what is known about cataracts.

2818, Oxidative Stress leads to Cataracts

Comments:
REVIEWER#1: in the key event relationship description, you say that cataracts have occurred once 5% of the lens is opaque, but in other sections you've highlighted the subjectivity of cataract

classification and the different methods used to score them. Can this paragraph be better cited and clarified? This paragraph feels quite clumsy in general.

Identification and quantification of ionising radiation-induced oxysterol formation in membranes of lens fibre cells - ScienceDirect – may be a useful reference for you in this KER

Uncertainties and inconsistencies section – Spector 1995 is not properly cited.

"unless otherwise indicated, all data is significantly significant" – do you mean 'statistically significant'?

REVIEWER#2: no comments

OVERALL AOP PAGE

Comments:
REVIEWER#1:
In general, the pages feel disjointed, the way cataract are described changes quite a bit page to page. Referencing seems inconsistent throughout. Some 'Evidence supporting this KER' state the weight of evidence (low, moderate etc.) but others don't – but this is useful information to state.

REVIEWER#2:
Overall, this is important and contributes new information to the literature. The vast collection of information is incredible and the authors deserve much credit of it.

The major limitations of the work is that it is written by several authors (or so it would seem) and there are many inconsistencies about what is included and what is not. The sections on DNA repair are perhaps among the most confusing. Questions about what is to be included and why is unclear. It is not justified why mutations in germ line are mentioned. There are also some statements where mutations and DNA damage are confused.

AOP REPORT MANUSCRIPT

Comments:
REVIEWER#1: no comment

REVIEWER#2:
The comments are much the same as those listed above for the AOP itself.

AUTHORS' RESPONSE LETTER

The authors are grateful for the reviewers time and for their helpful feedback on the AOP package. All comments have been adequately addressed and responses are provided below. Note page numbers listed in the "reply" section correspond to the *marked* version of the snapshot and the AOP report. Also understand that the page numbers reported for the snapshot may be off by 1-2 pages due to the formatting issue of the snapshot as a result of track changes, which is generated from the AOP wiki.

KEY EVENTS RESPONSES

Key Event 1 (Molecular Initiating Event, MIE): 1686, Deposition of Energy
Note: Shared KE, Previously reviewed in AOP 272
REVIEWER#1:
Comment: Clearly reviewed extensively under previous AOPs, a huge amount of epidemiology data to support deposition of energy leading to cataract
Reply: Thank you for the comment, the authors agree that there is extensive data to support energy deposition leading to cataracts. No change was made for this comment.

REVIEWER#2:
Comment: For overview, consider changing "energy deposition on the atoms and molecules" to "on the atoms that make up molecules".
Reply: The overview has been revised considerably. This sentence has been removed, and it is now reads as follows: " The energy may either be sufficient (e.g. ionizing radiation) or insufficient (e.g. non-ionizing radiation) to ionize atoms or molecules (Beir et al.,1999)", it can be found on page 28 of snapshot

Comment: Second sentence: "higher energy deposits", higher than what? Higher than Low LET is assumed but should be stated. LET is defined as deposition of energy per unit distance, and this should be stated in the second and third sentences.
Reply: The paragraph has been modified as follows: "Energy deposition differs with the linear energy transfer (LET) defined as deposition of energy per unit distance (Hall and Giaccia, 2018 UNSCEAR, 2020). High LET radiation refers to energy above 10 keV µm-1 which often produces more complex, dense structural damage than low LET radiation (below 10 keV µm-1). High LET radiation includes heavy ions, alpha particles and high-energy neutrons. Low-LET radiation such as photons (X- and gamma rays), electrons as well as high-energy protons produces sparse ionization events. Low LET radiation travels farther into tissue but deposits smaller amounts of energy, whereas high LET radiation does not travel as far but deposits larger amounts of energy into tissue at the same absorbed dose." on page 28-29 of snapshot

Comment: Parameters in third sentence should specify that the radiation track is still stochastic and not predictable even if influenced by many parameters.

Reply: This section has been modified as follows: "Ionizing radiation can cause the ejection of electrons from atoms and molecules, thereby resulting in their ionization and the breakage of chemical bonds. The excitation of molecules can also occur without ionization. These events are stochastic and unpredictable." on page 28 of snapshot

Comment: For Energy can be deposited into any substrate both living and non-living…and then "it is independent of age…etc". Energy is independent of those things, but I think it means energy deposition. This should be stated.
Reply: A sentence was modified to "Energy deposition is independent of age, sex, or life-stage." on page 28 of snapshot

Key Description:
Comment: "Deposition of energy depends…" should state that ionizing radiation is some cases can cause excitation without ionization. Ionizing radiation does not always lead to an ionization.
Reply: A sentence was phrased to "Ionizing radiation can cause the ejection of electrons from atoms and molecules, thereby resulting in their ionization and the breakage of chemical bonds. The excitation of molecules can also occur without ionization." on page 28 of snapshot

Comment: In the paragraph "Ionizing radiation can cause", second to the last line I would add "ingestion" as another example.
Reply: A sentence was rephrased to "The energy deposited can induce direct and indirect ionization events and can result from internal (injections, inhalation, ingestion) or external exposure." on page 27-28 of snapshot

Comment: In the paragraph about direct ionization there is confusion. Photons are not charged but rather create electrons which cause ionizations. This paragraph says only charged particles can cause direct ionizations but uses photons as an example.
Reply: Sentence was clarified to say "Photons, which are electromagnetic waves, can also create elections that can cause direct ionization." on page 27-28 of snapshot

Comment: The paragraph on spatial structure should be changed:
Second line should say High LET refers to energy…..which OFTEN produces more complex…"
Reply: "Often" was added to read "High LET radiation refers to energy above 10 keV µm-1 which often produces more complex" on page 27-28 of snapshot

Comment: It state low LET travels farther into tissues, but neutrons often travel as far as X-rays and gamma-rays, and C ions have a Bragg peak within the tissue.
Reply: A sentence was modified to "whereas, typically, high LET particles do not travel as far..." on page 28-29 of snapshot

<u>Key Event 2: 1392, Oxidative Stress</u>
Note: Shared KE, Previously reviewed in AOP 17 and 220

REVIEWER#1:
Comment: Well documented effects and reviewed in other AOPs quite well, although the key event description is not well referenced
Reply: A few more references were added to the key event description: Pizzino et al., 2017; Sharifi-Rad et al., 2020; Snezhkina et al., 2019; Jena et al., 2023 – page 82 of snapshot

REVIEWER#2:

Key Event 3: 2081, Increased Modified Proteins

REVIEWER#1:
Comment: Well documented effects, although the key event description is not well referenced.
Reply: The following additional references have been added to the key event description: Alberts et al., 2002, Dalle-Donne et al., 2006; Krisko & Radman, 2019; Reisz et al., 2014; Hamada et al., 2014 . - page 30 of snapshot

Comment: Specific to the lens, no mention of heat shock protein modifications that have been reported, or changes to crystallin proteins, aquaporins etc.
Reply: Modified proteins are applicable to a wide range of diseases and the description is meant to be generic, KEs are intended to be reused by other AOP developers, therefore descriptions are intended to be brief outlines of the event and how it is measured, some illustrative examples can be provided. Crystallin is mentioned as an example (first paragraph, last sentence). A comprehensive list of specific targets for different cell types does not need to be detailed. This type of information is discussed within the relevant KERs.

REVIEWER#2:
Comment: This topic is confusing. It seems to consider only direct changes in the protein that come about from radiation, but what about changes that occur as a result of radiation-induced signal transduction…for example phosphorylation. This governs responses in the cells throughout all organisms.
Reply: A sentence was rephrased to "Protein modifications can include post-translational modifications such as deamidation, oxidation, phosphorylation and carbonylation." on page 30 of snapshot

Additionally, in the KER describing modified proteins leading to cataracts, the following sentence was added: "Ample evidence has shown that protein modifications, particularly phosphorylation, may be associated with cataracts. These studies used human and animal models with pre-existing cataracts, and show the presence of phosphorylated crystallin, MDM2 and tyrosine proteins (Wang et al. 2020; Hui-Ju et al. 2013; Chandrasekher et al. 2004)" on pg. 212 of snapshot

Comment: Many of these responses are dose-dependent. For example, protein denaturation (noted here as unfolding) occurs predominantly at high doses, not low doses. This would be particularly relevant for cataract formation, as is noted.
Reply: Yes, agreed. This aspect is detailed within the KER descriptions associated with altered proteins and cataracts, dose-dependence of the relationships.

Comment: Assays for protein phosphorylation, transcription, and others are not mentioned.
Reply: A few of the assays have been removed and those most relevant to detecting post-translational modifications (mass spec, western blot, ELISA) are now described. Transcriptional assays are not discussed as they are more relevant to genes. Pg. 28 of the snapshot

Key Event 4: 1634, Increase, Oxidative damage to DNA
Note: Shared KE, Previously reviewed in AOP 296

REVIEWER#1: no comment

REVIEWER#2:
Comment: The reference included for the overview is not clear…why use this paper?
Reply: Unsure which reference the reviewer is referring to as many were cited in the key event description (page 31 of snapshot): Swenberg et al., 2011; Markkanen, 2017; Turner et al., 2002; Schoenfeld et al., 2012; Tangvarasittichai and Tangvarasittichai, 2019; Nilsson & Liu, 2020; Poetsch, 2020a. This KE is shared across multiple AOPs thus the references relate to both chemical and radiation field.

Comment: I believe that there is evidence that oxidative damage is created by Cs-generated gamma-rays as well and also by a variety of radionuclides not mentioned here. This is not very comprehensive and is too limiting.
Reply: To include all types of radiation, the statement has been revised to "However, direct chemical insult from specific compounds, exposure to various forms of radiation..." on page 31 of the snapshot

Comment: It may also be worth noting that radiation may generate a different spectrum of oxidative species than other oxidizing agents. The most powerful oxidizing agent induced by IR is .OH (hydroxyl radical).
Reply: The comment is better suited to be included in the KER describing deposition of energy to oxidative DNA damage. Within the KER we have incorporated OH radicals as a powerful oxidizing agent.

Comment: Perhaps it is also worth mentioning that the ROS must be generated in close proximity to the DNA (2-4nm) and if they are more distant they will not lead to DNA damage as they will be repaired by the cell.

Reply: A sentence "It is worth noting that ROS must be generated near the DNA to cause damage, otherwise, if ROS was produced more distantly, then it can be removed by the cell (Nilsson & Liu, 2020)." was added on page 36 of the snapshot

"This KE describes an increase in oxidative lesions of a broad spectrum i.e superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (OH), peroxyl radical (RO_2), single oxygen ($^1O_2$) in the nuclear DNA above the steady-state level. Oxidative DNA damage can occur in any cell type with nuclear DNA under oxidative stress." was added on page 34 of the snapshot.

Comment: It may also be worth noting that ROS generation increases with increasing dose. It is also affected by dose-rate.
Reply: This information is more relevant to the key event relationship of deposition of energy to oxidative stress on page 118 of the snapshot, where this type of information is detailed.

Comment: Almost none of the assays are specific for ROS…most will occur as the result of direct DNA damage (comet assay, etc.). ROS also causes SSB and DSB, although DSB are less common being two SSB that are close to each other. These too are from oxidative damage. It is hard to separate this from the next category.
Reply: Measuring oxidative DNA damage typically involves assessing the extent of DNA lesions or modifications caused by reactive oxygen species (ROS) or other oxidative stressors. The techniques that are listed are the commonly employed methods. The most relevant is 8-OHdG assay which can be detected using ELISA. Within the table, the description provides more specific details on the assay related to oxidative adducts/nucleotide oxidation on pages 36 of the snapshot

<u>Key Event 5: 1635, Increase, DNA strand breaks</u>
Note: Shared KE, Previously reviewed in AOP 272 and 296

REVIEWER#1:
Well documented, lots of literature supporting this

REVIEWER#2:
Comment: It might be worth mentioning that there are error-prone and error-free forms of DSB repair and that the pathways for SSBR and DSBR are distinct.
Reply: A sentence "It is also worth noting that there are error-prone and error-free forms of DSB repair and that the SSB repair pathways are distinct from the DSB repair pathways" was added on page 40 of the snapshot

Comment: Complex lesions can also lead to mutations not only inappropriate recombination.

Reply: A sentence was modified to "the spectrum of damage can be intricate, resulting in complex clustered damage defined as two or more oxidized bases that lead to inappropriate recombination and mutations" on page 40 of the snapshot

Comment: The excision repair should be specified as BASE excision repair, a form of which is considered to repair SSBs. Most BER repairs base damage which is included above.
Reply: A sentence was modified to "Strand breaks are intermediates in various biological events, including DNA repair (e.g., base excision repair)" on page 42 of the snapshot

Comment: Beir 199 reference?
Reply: This reference was added. On page 44 of the snapshot

Comment: There is only one nearly recent paper here.
Reply: Some more recent papers (Cannan and Pederson, 2016; Barbieri et al., 2019; Tamanoi and Yoshikawa, 2022; Tripathy et al., 2021) published after 2016 were added that were relevant to the key event description – page 42 of snapshot.

Comment: Alternative NHEJ is also not mentioned.
Reply: This key event is intended to describe DNA strand breaks and how they are formed including the different types of lesions. The discussion of repair mechanisms is better suited in the Inadequate DNA Repair key event. The following sentences have been added to the Inadequate DNA Repair Key Event Description and the Assays sections:

"Alternative NHEJ, or alt NHEJ, uses small similar sequences in two broken DNA ends to join them together. Unlike the usual repair method (cNHEJ), alt NHEJ does not need specific proteins like LIG4 and KU. Instead, it relies on the MRN complex to process the breaks. However, alt NHEJ tends to cause mutations by adding or removing bits of DNA during the repair (Chaudhuri and Nussenzweig, 2017)."

Chaudhuri, R.A. and Nussenzweig, A. (2017), "The multifaceted roles of PARP1 in DNA repair and chromatin remodelling". Nat Rev Mol Cell Biol 18, 610–621. https://doi.org/10.1038/nrm.2017.53

Assay Name	References	Description	DNA Damage/Repair Being Measured	OECD Approved Assay
Flow Cytometry	Corneo et al., 2007	The alt-NHEJ flow cytometer method involves utilizing an extrachromosomal	Alt NHEJ	No

		substrate. Green fluorescent protein (GFP) expression is indicative of successful alt-NHEJ activity, contingent on the removal of 10 nucleotides from each end of the DNA and subsequent rejoining within a 9-nucleotide microhomology region. This approach provides a quantitative and visual means to measure the efficiency of alternative non-homologous end joining in cellular processes.		

Corneo, B. et al., 2007, "Rag mutations reveal robust alternative end joining". *Nature* 449, 483–486 (2007). https://doi.org/10.1038/nature06168

Comment: Assays: 53BP1 should be added
Reply: 53BP1 was specified in a few assays under methods of measurement on pages 42-43 of the snapshot

Key Event 6: 155, Inadequate DNA repair
Note: Shared KE, Previously reviewed in AOP 15, 296, and 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: This is on the damage reversal section: It is not clear why the photolyases are mentioned here. They are for UV damage in bacteria. They do not act in mammalian cells for ionizing radiation. I don't think bacteria get cataracts. So much is known about mammalian DNA repair, it is not certain
Reply: In line with AOP principles, KEs can are used by multiple AOPs and therefore evidence from any model system relevant to the KE could be described. This particular KE is used in eight other AOPs.

Comment: BER should mention the polymerase and ligation by a ligase since as it is discussed it only includes the removal of the base and not the actual repair.

Reply: A sentence was added "This leads to an intermediate that contains a DNA strand break, whereby DNA ligase is recruited to seal the ends of the DNA." on page 49 of snapshot.

Comment: NER is the same…only the excision (which is actually about 29-30bp) is mentioned and not the repair polymerase or ligation reaction.
Reply: A sentence was rephrased to "... the damaged nucleotide is removed prior to DNA resynthesis within the resultant gap and sealing of the ends by DNA ligase." on page 49 of snapshot.

Comment: SSBR does not mention the fact that a common intermediate is generated and that this is the same for BER and SSBR. End processing is generally by Polynucleotide Kinase.
Reply: A sentence was rephrased to "...or Pol δ/ε (long patch repair) can bind to synthesize over the gap, although end processing is generally done by polynucleotide kinase." on page 49 of snapshot.

Comment: DDSBR: HRR occurs in late S and G2. It might be worth noting that there is no repair in M phase.
Reply: HR occurs in dividing cell types, and NHEJ occurs in both types. Revised phrase as follows: "...which operates primarily during the S phase of dividing cells types, and nonhomologous end joining (NHEJ), which can function in both dividing and non-dividing cell types (Teruaki Iyama and David M. Wilson III, 2013). DNA repair in mitosis is controversial (Mladenov et al., 2023)." on page 49 of snapshot

Comment: NHEJ; AGAIN, there is no repair in M chase. DNA PKcs is the catalytic subunit, not the complex as is mentioned in the text.
Reply: A sentence was rephrase to "This protects the DNA from exonucleolytic attack and acts to recruit DNA-PKcs, the catalytic subunit" on page 50 of snapshot,

Comment: Perhaps something should be mentioned about complex lesions and the difficulty in repairing them?
Reply: A sentence was added "Complex lesions can be created by a single mutagen and can be more difficult to repair, as the mechanism behind how different repair pathways cooperate to address this is still unclear" on page 49 of snapshot

Comment: Dose response curve for alkyl adducts/mutations—This section is unclear.
Reply: The section was rephrased to "It is important to consider that some adducts are not mutagenic at all because they are very effectively repaired. Others are effectively repaired, but if these repair processes become overwhelmed mutations begin to occur. The relationship (shape of dose-response curve) between exposure to mutagenic agents and mutations provide an indication of whether the removal of adducts occurs, and whether it is more efficient at low doses. A sub-linear dose-response curves (hockey stick or j-shape curves) for mutation induction indicates that adducts are not converted to mutations at low doses. This suggests the effective repair of adducts at low doses, followed by saturation of repair at higher doses (Clewell et al., 2019). Thus, measurement of a clear point of inflection in the dose-response curve for mutations

suggests that repair does occur, at least to some extent, at low doses but that reduced repair efficiency arises above the inflection point. A lack of increase in mutation frequencies (i.e., flat line for dose-response) for a compound showing a dose-dependent increase in adducts would imply that the adducts formed are either not mutagenic or are effectively repaired." on pg 52 of the snapshot

Comment: Alt-NHEJ is a low fidelity mechanism, requires microhomology repeats, uses polymeras Q (or theta). This should be mentioned.
Reply: A sentence was rephrase to "The process of alt-NHEJ is less well understood than C-NHEJ and is a lower fidelity mechanism. Alt-NHEJ is known to involve slightly different core proteins than C-NHEJ and requires microhomology repeats,..." on page 51 of snapshot.

Comment: Fidelity of DNA Repair: The issue is made that NER may be low in testes. This may be irrelevant. How often are sperm exposed to UV/?
Reply: This KE exists in multiple AOPs and is relevant to multiple stressors.

Comment: While NHEJ is error-prone, HRR is not. This is not coming out clearly.
Reply: A sentence was rephrase to "In contrast to NHEJ, HR takes advantage of similar or identical DNA sequences to repair DSBs and is not error-prone" on page 51 of snapshot.

Comment: How is it measured or detected? This first paragraph is not clear. Why is it talking about repair of plasmid DNA in vitro?
Reply: This KE was adapted from an existing one, therefore it is present in multiple AOPs.

Comment: This entire section about alkylated DNA talks about chemical exposure, not ionizing radiation exposure. It is not clear how this is relevant at all.
Reply: Key events are shared across other AOP and can be relevant to multiple stressors.

Comment: For the direct measurement table, it might be worth mentioning which of these methods are good for low dose vs high dose. It is unclear why alkylation exposure is so important here when it is not so important for ionizing radiation.
Reply: The key event description is a biological description and meant to be stressor agnostic, thus specific exposure parameters are not required here per the AOP development guidance document.

Comment: The Comet assay should mention the SSB measurements that are used with alkaline solution coment.
Reply: A sentence was added to "Comet assay is performed with a time-course under alkaline conditions to detect SSBs and under neutral conditions to detect DSBs..." on page 53 of snapshot.

<u>Key Event 7: 185, Increase, Mutations</u>
Note: Shared KE, Previously reviewed in AOP 15, 296 and 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: The section on How it is measured or detected is mostly irrelevant to radiation. The Ames test is not used in radiation, these assays are chemical-specific and not related to radiation. The issues about the different spectrum of lesions is also irrelevant to radiation since radiation causes base damage, SSBs, DSBs and there are no known signature lesions.
A few of the comments are relevant…the HPRT assay, TK assays, and others. Nevertheless the information is still very oriented toward chemicals and not radiation.
This information is all generic and not relevant to radiation.
Reply: The key event descriptions and associated measurements are intended to be relevant to any stressor. Assays relevant to radiation are also included such as HPRT, Ames and TK.

Key Event 8: 1636, Increase, Chromosomal aberrations
Note: Shared KE, Previously reviewed in AOP 296 and 272

REVIEWER#1: No further comments – well established and the assays in the table is thorough
Reply: Thank you

REVIEWER#2:
Comment: Are chromatid aberrations types of chromosome aberrations? This sentence on two types of chromosome injuries is unclear. Later there is mention of chromosomal aberrations and chromatid do not seem to be included. -
Reply: The last paragraph above the measurement methods section classifies CAs depending on if the chromosome or chromatid is affected page 67-69 of snapshot

Comment: The interstitial deletion is not correct, what is described is an interstitial inversion.
Reply: This was corrected to interstitial inversion on page 67 of snapshot

Comment: There is no distinction between lethal and non-lethal aberrations in this discussion. This should be included. It should be noted that most of the deletions (whether terminal or interstitial should be small. If they are too large, too much genetic material is lost and it will be lethal.
Reply: A sentence was modified to "Deletions happen when a portion of the genetic material from a chromosome is lost and can be lethal if an excessive amount of genetic material is lost." on page 67 of snapshot

Comment: MN are not always just in bi-nucleated cells. Most people relate MN to dicentrics.
Reply: When cells are blocked at the cytokinesis step, micronuclei can appear in the cytoplasm of binucleated cells. These micronuclei are an indication of CAs and are often related to dicentric chromosomes. Dicentric chromosomes can also cause nucleoplamic bridges...

Key Event 9: 870, Increase, Cell Proliferation
Note: Shared KE, Previously reviewed in AOP 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: Flow cytometry is a common way of measuring percentage of cells in and out of the cell cycle, and this was not mentioned at all.
Reply: Another row was added to the method of measurement table to describe flow cytometry on page 74 of snapshot

Comment: It is not certainly why the olfactory epithelium is relevant here.
Reply: In line with AOP principles, KEs can exist in multiple AOPs and therefore evidence from other cell types can be used to understand the structural and functional aspects of the relationships, helping to strengthen the relationship.

Comment: It is not certain why nasal tissue is mentioned here.
Reply: In line with AOP principles, KEs can exist in multiple AOPs and therefore evidence from other cell types can be used to understand the structural and functional aspects of the relationships, helping to strengthen the relationship

Comment: It is noted that IR can induce proliferation, but it can also lead to cell cycle blocking, which is not mentioned.
Reply: A sentence was modified to "There is a large body of evidence supporting the effectiveness of ionizing radiation, UV, and mechanical wounding as stressors for increased cell proliferation, although it can also cause cell cycle blocking." on page 73 of snapshot

Comment: For most mammalian cells, it is not just nutrients, but also oxygen that is required for cell proliferation. Low oxygen inhibits cell proliferation. This should be mentioned.
Reply: A sentence was modified to "Progression through the cycle is dependent on sufficient nutrient and oxygen availability..." on page 74 of snapshot

Comment: For how to measure it should state that BrdUDR or I-UdR are used, since both are frequently used.
Reply: A sentence was added "Similarly, 5-iodo-2'-deoxyuridine (IdU) is another analogue of thymidine used to measure cell proliferation as it is also incorporated into DNA during its synthesis" on page 73 of snapshot

Comment: Flow cytometry is not really mentioned adequately.
Reply: Another row was added to the method of measurement table to describe flow cytometry on page 74 of snapshot

Key Event 10 (Adverse Outcome, AO): 2083, Occurrence of Cataracts

REVIEWER#1:

Comment: sex applicability – is it worth including studies where no sex effect is reported to balance this sentence out? I feel the key event description of 'cataract' should be consistent across all relevant KERs subsequently, but this seems to change.
Reply: Sex applicability highlights what sex the AOP is applicable to. Cataract development is applicable to both sexes. A sentence was revised as follows: "This adverse outcome can develop in both sexes; however, females have a small increased background risk of cataracts" on page 94 of snapshot

Comment: I feel the key event description of 'cataract' should be consistent across all relevant KERs subsequently, but this seems to change.
Reply: We have revised all relevant KERs related to cataracts to have the same definition as follows "Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute, 2022). For the purposes of this AOP, cataracts are defined as over 5% of cells in the lens exhibiting opacities." on page 91 of the snapshot

Comment: "Therefore, opacities are not removed and accumulate with time" – I think this sentence could be worded better, this sounds like multiple opacities develop over time, whereas 'opacity' is a measurable change in the lens as a whole leading to vision impairment.
Reply: A sentence was rephrased to "Therefore, damaged proteins that are not removed can accumulate over time contributing to opacities and the formation of cataracts (Hamada, 2017)." on page 93 of snapshot

Comment: "These include proper organization of proteins such as crystallins" – not just organisation, but also proper development and balance of crystallins.
Reply: A sentence was rephrased to "These include proper organization, development and balance of proteins such as crystallins..." on page 93 of snapshot

Comment: "no organelles within the lens fiber cells" – no organelles in mature lens fiber cells, the organelle loss is a gradual process and therefore early lens fiber cells can still contain some organelles until they migrate towards the centre of the lens, the organelle free zone. This occurs alongside the gradual increase in crystallin proteins (Hejtmancik and Shiels 2015).
Reply: A sentence was rephrased to "...no organelles within mature lens fiber cells..." and the suggested reference was included on page 93 of snapshot

Comment: "which increases lens opacity, contributing to cataract formation and intensity" – I would say density instead of intensity, to be consistent with literature terminology.
Reply: A sentence was rephrased to "...which increases lens opacity, contributing to cataract formation and density." on page 93 of snapshot

Comment: "They classify cataracts on a scale of severity, which is often subjective, relying upon the examiner's judgement" – no mention of Scheimpflug imaging (McCarron et al 2022, Fuchs et al 2011, Puk, de Angelis and Graw 2013, Dalke et al 2018) which can be used to measure lens density, taking away some subjectivity. Although Scheimpflug imaging has been mentioned int

eh table below. Worth mentioning I feel as the technology has been incorporated in specific studies of radiation-induced cataract in animals relevant to this AOP as a whole.
Reply: A sentence was rephrased to **"**They classify cataracts on a scale of severity, which is often subjective, relying upon the examiner's judgement, although Scheimpflug imaging is less subjective by measuring lens density" on page 93 of snapshot

REVIEWER#2: no comment

KEY EVENT RELATIONSHIPS RESPONSES

Adjacent KERs

1977, Energy Deposition leads to Increase, DNA strand breaks
Note: Shared KER, Previously reviewed in AOP 272

REVIEWER#1:
Comment: Biological plausibility – Ainsbury et al 2026, not Ainsbury 2016
Reply: Text was modified to Ainsbury et al., 2016, page 99 of snapshot.

Comment: Quantitative understanding – Dose concordance - Barnard et al 2019 study has an incorrect result reported in the table? An inverse dose-rate response was reported in the study whereby the lower dose rate produced the higher frequency of foci. Check wording and clarify?
Reply: The following phrase has been added "Although an increase in dose-response was observed, an inverse-dose rate response was reported, with higher 53BP1 foci persisting at lower dose rates." page 106 of snapshot

Comment: "Furthermore, primary normal human fibroblasts exposed to 1.2 – 5 mGy X-rays at 5.67 mGy/min showed a supralinear relationship, indicating at low doses, the DSBs are mostly due to radiation-induced bystander effects" – reference this sentence please.
Reply: The following reference has been added: (Ojima et al., 2008) page 107 of snapshot.

REVIEWER#2:
Comment: There are some data in UNSCEAR 2020/2021 report on low dose radiation that indicate that rarely low LET radiations and low doses can produce complex lesions. This would be important as possibly having an impact on DNA damage. This is not necessarily in lens cells but if it can occur in other cell types it might also occur in lens cells.
Reply: We have included the following sentence in the KER "Some data reports that low dose of low LET radiation can lead to complex lesions, which can cause unrepairable DNA damage. However, determining the actual frequency of the complexity of these lesions has proven challenging (Wilkinson et al., 2023)." page 99 of AOP snapshot.
We have also incorporated within the AOP report that an "including the understanding of low dose radiation and complex lesions in the context of DNA damage is an area to explore" page 20 of AOP report

2769, Energy Deposition leads to Oxidative Stress
Note: Shared KER

REVIEWER#1: no comment

REVIEWER#2:
Comment: Perhaps the OH. Should be mentioned as being among the most long-lasting.
Reply: The half-life of various ROS species has been reported and OH radicals are among the many with shorter half-lives (hydroxy 10^{-9} sec, alkoxyl 10^{-6} sec, peroxyl radical 7 sec) as summarized in tables in the references listed below (SIES, 1993; Rubio & Cerón, 2021).

SIES, H. (1993), Strategies of antioxidant defense. European Journal of Biochemistry, 215: 213-219. https://doi.org/10.1111/j.1432-1033.1993.tb18025.x

Rubio, Camila & Cerón, José. (2021). Spectrophotometric assays for evaluation of Reactive Oxygen Species (ROS) in serum: general concepts and applications in dogs and humans. BMC Veterinary Research. 17. 10.1186/s12917-021-02924-8.

Comment: I am not certain how all the references were chosen as being relevant. They do not all involve lens cells. Why were cardiac and neuronal cells included?
Reply: In line with AOP principles, KERs are living documents, that can exist in multiple AOPs and therefore evidence from any cell types and models can be used to understand the structural and functional aspects of the relationships.

2809, Energy Deposition leads to Modified Proteins
Note: Shared KER

REVIEWER#1:
Comment: Dose concordance – "Strong evidence is available in lens cells to support a dose response relationship between energy deposition and protein modification. A study showed, that lens crystallin proteins continuously irradiated in vitro for 24 hrs using UV contained high molecular weight proteins relative to controls (Zigler & Goosey, 1981)" – specify what UV (A, B, broad spectrum, solar simluated) and at what energy/dose.
Reply: The following was added "from white light daylight fluorescent lamps with a measurement of 500 ft-c (foot-candles which equals 1 lumen/ft^2) (1.7 mW/cm2)" on page 140 of snapshot.

Comment: In vitro exposures using UV can generate heat which could affected protein modifications, to note. This information has been presented by the authors in other KERs and is very useful to the reader.
Reply: In the biological plausibility section of the snapshot we now indicate the following on page 133: "It is important to note that ionizing and non-ionizing radiation work by different mechanisms; ionizing radiation has enough energy to remove tightly bound electrons from atoms, leading to the formation of ions (charged particles), and can also cause excitation without

ionization. The absorption of non-ionizing radiation leads to molecular vibrations and rotations, resulting in heat generation (Alcócer et al., 2020)."

Comment: KER states most studies are from male animals, but this is not specified in the tables, perhaps could include which sex of animal was used in each study.
Reply: The sex of the animals has now been specified (where information available) across all the tables in the Empirical evidence section of the snapshot

REVIEWER#2:
Comment: It may be worth noting that protein modifications can be short-lived within cells as the accumulation would depend upon the turnover of the protein and the turnover of the cells themselves. Not all protein changes are equally damaging.
Reply: The following sentence "However, the extent of damage from different types of protein modifications could vary as these protein changes may be short-lived due to the cell life cycle and the associated regulation of the protein (Basisty et al., 2018)" was added to page 133 of snapshot under the Key Event Relationship Description section with the added reference "Basisty, Nathan et al. (2018) "Protein Turnover in Aging and Longevity", *Proteomics*, Vol.18, https://doi.org/10.1002/pmic.201700108"

Comment: Often UV and ionizing radiation are compared, yet these are not really acting via the same mechanisms. This should be noted.
Reply: See reply above, a sentence "It is important to note that ionizing and non-ionizing radiation work by different mechanisms; ionizing radiation has enough energy to remove tightly bound electrons from atoms, leading to the formation of ions (charged particles), and can also cause excitation without ionization. The absorption of non-ionizing radiation leads to molecular vibrations and rotations, resulting in heat generation (Alcócer et al., 2020)." was added to page 133 of snapshot and also AOP report

2810, Oxidative Stress leads to Increase, Oxidative DNA damage

REVIEWER#1:
Comment: A few studies have also found that single stranded DNA (ssDNA) is more likely to be oxidized than double stranded DNA (dsDNA). This indicates that persistent ssDNA sites, such as Z-DNA, stable R-loops, cruciforms, quadruplexes, or intramolecular triplexes might have higher incidences of oxidative damage (Amente et al., 2019) – 'a few studies' but you have only referenced one?
Reply: "a few studies have also found that" was removed from the sentence and it was revised to "Single stranded DNA (ssDNA) is more likely to be oxidized than double stranded DNA (dsDNA). This indicates that persistent ssDNA sites, such as Z-DNA, stable R-loops, cruciforms, quadruplexes, or intramolecular triplexes might have higher incidences of oxidative damage (Amente et al., 2019)." on page 148 of snapshot in the Biological Plausibility section

REVIEWER#2:

Comment: For the two BER pathways, one is for SSBR, which is not really mentioned here. SSB are dangerous because when two are in juxtaposition, they become DSBs, which as noted throughout this document are damaging.
Reply: A sentence "Another kind of BER pathway is SSBR (single strand break repair). When two SSBs are in juxtaposition, detrimental because when two are in juxtaposition, they can form DSBs, which can be damaging (Caldecott, 2024: Pfeiffer et al. 2000)" was added on page 148 of snapshot.

Comment: The comment about the amount of oxidative damage accumulating over months is confusing. Sometimes it occurs very rapidly following oxidative stress, and often it can be repaired rapidly.
Reply: The sentence has been added as "Although DNA damage by oxidative stress can be repaired rapidly, the accumulation of oxidative stress typically causes oxidative DNA damage after several months (Cadet et al., 2017)." in page 148 of snapshot and a reference is added to support his statement

2811, Oxidative Stress leads to Increase, DNA strand breaks
Note: Shared KER

REVIEWER#1: no comment

REVIEWER#2:
Comment: This section does not really talk that much about SSBs. While they are repaired rapidly, they can contribute to complex lesions and to DSBs.
Reply:
The following sentences have been revised and added to the KER with references:
"Hydroxyl radicals, in addition to being highly reactive, are capable of causing DNA damage leading to single stranded breaks (SSBs), double stranded breaks (DSBs) and complex lesions (Cadet and Davies, 2017; Halliwell et al., 2021; Engwa et al., 2020; Wilkinson et al., 2023)." on page 154 of the snapshot;
"The biological plausibility of the relationship between increased oxidative stress leading to increased DNA damage (e.g. SSBs, DSBs, complex lesions, abasic sites, and oxidized bases) (Cadet et al., 2012; Cadet and Davies, 2017) is highly supported by the literature." on page 154 of the snapshot; and
"Indirect SSB formation could also occur during the repair process by the BER pathway and thus unrepaired SSB could stall the replication mechanism and may lead to a potential DSB (Cadet and Davies, 2017)". on page 154 of the snapshot.

The following sentence in regard to complex lesions from the KER description has been added to the AOP report on page 137 of snapshot:
"The production of radicals from oxidative stress can cause DNA damage leading to complex lesions which include single stranded breaks, double stranded breaks, abasic sites and oxidized bases (Cadet et al., 2012; Cadet and Davies, 2017)."

Comment: The SOD that is part of the defense needs to be specified—SOD1, 2 or 3 or all?
Reply: SODs control ROS by catalyzing the conversion of superoxide anion radical into hydrogen peroxides. The three isotypes involved in the defense are copper-zinc SOD (CuZn-SOD), manganese SOD (MnSOD) and extracellular SOD (ECSOD) (Engwa et al., 2020).

The following sentence was revised in KER to be on page 154 of the snapshot: The ADR is recruited to manage RONS levels, with antioxidants such as superoxide dismutase (SOD) functioning as the first line of defense, this includes the three isotypes (copper-zinc SOD (CuZn-SOD), manganese SOD (MnSOD) and extracellular SOD (ECSOD)) (Engwa et al., 2020).

Comment: Again, it is confusing because UV and ionizing radiation are used interchangeably in places, and they are not at all the same. This becomes very confusing later in the document when radiation is mentioned and only IR is considered.
Reply: The following sentence has been removed to avoid confusion on UV vs ionizing radiation: "As energy is deposited in an aqueous solution, water molecules undergo radiolysis, breaking bonds to produce ROS (Ahmadi et al., 2021; Karimi et al., 2017)." Page 146 of snapshot.

Comment: Increased XRCC2 and 3 are not just associated with BER but also SSBR.
Reply: Limited data was retrieved to support XRCC2 and 3 being associated with SSBR, therefore we have not included any additional information related to XRCC2 and 3 in this KER. However, in Table 21 - KE1 Increase Oxidative Damage to DNA to KE3 Inadequate DNA Repair, the following sentence was added on page 168 of snapshot: "Nucleotide excision repair (NER) and single-strand base repair is also involved in repairing oxidized bases to a lesser extent (Shafirovich et al., 2016) (Hegde et al., 2012)." This statement highlights that SSBR is also involved in repairing oxidative lesions.

2812, Oxidative Stress leads to Modified Proteins

REVIEWER#1: no comment

REVIEWER#2: no comment

1909, Increase, Oxidative DNA damage leads to Inadequate DNA repair
Note: Shared KER, Previously reviewed in AOP 296

REVIEWER#1:
Comment: The whole key event relationship description is supported by a single review paper, which is fine but it would be nicer to cite some of the key statements individually. In general, some key event relationships descriptions are heavily referenced, and others, such as this, less so.
Reply: The KER is supported by a few references (Swenberg et al., 2011), (Hedge et al. 2012) (Shafirovich et al., 2016) and (Markkanen, 2017) on page 162 of snapshot.

REVIEWER#2:

Comment: SSBs can also be the result of oxidative lesions directly, this is not realy stated clearly.
Reply: The role in SSB and oxidative lesions is described in more detail in the KER related to oxidative stress leading to DNA damage. This KER is more on the repair mechanisms involved in already existing oxidative DNA damage.

1911, Increase, DNA strand breaks leads to Inadequate DNA repair
Note: Shared KER, Previously reviewed in AOP 296 and 272

REVIEWER#1: Very well supported by literature, I have little further to add.
Comment: Empirical evidence (and throughout) - Rothkamm & Lo, 2003 should be Rothkamm & Lobrich.
Reply: It has been revised to "Rothkamm & Löbrich, 2003" throughout the document.

Comment: Throughout – reference should be McMahon et al 2016, not Mcmahon
Quantitative understanding – "all data is significantly significant" – you mean statistically significant
Reply: It has been revised to "McMahon" throughout the document.
Sentences were revised to "The following tables provide representative examples of the relationship. Unless otherwise indicated, all data is statistically significant." under the Quantitative Understanding of the Linkage sections.

REVIEWER#2:
Comment: Cells that are damaged in G0 also appear to use NHEJ repair, this was not mentioned.
Reply: The sentence has been revised to " Cells in other phases of the cell cycle (S or G2) use HR (Ceccaldi et al., 2016) and damaged cells in G0 also appear to use NHEJ repair (Frock et al., 2021)." to refer to then Biological Plausibility section page 171 of snapshot and a reference to support this statement has been added.

Comment: Defects in NHEJ and HRR both lead to extreme radiosensitivity, this should be mentioned.
Reply: Specific information related to a stressor that is not informing B-H criteria is not relevant to include in AOPs. This statement does not support an understanding of the relationship between increase DNA strand breaks to inadequate repair.

Comment: NHEJ is favored in mammalian cells because MOST cells are not in S or G2 phase of the cell cycle, when HRR occurs.
Reply: The following is stated in the KER page 171 of snapshot- "NHEJ is most active in the following order of the cell cycle: G1 > S > G2/M (Mao et al., 2008). Since most somatic mammalian cells are in the G1 pre-replicative phase, DSBs also usually appear in this phase and thus are often repaired using the error-prone NHEJ (Jeggo et al., 1995). Cells in other phases of the cell cycle (S or G2) use HR (Ceccaldi et al., 2016) and damaged cells in G0 also appear to use NHEJ repair (Frock et al., 2021)".

Additionally, we also indicate: “HR is operative during late S and G2 phases where the sister chromatid can be used as template for error-free repair (Van Gent et al., 2001).” - page 171 of snapshot

Comment: Perhaps it is worth mentioning that HRR can be problematic if there is a mutant copy of a gene that is copied leading to LOH.
Reply: We have added the following: “Loss of heterozygosity (LOH) is an example of how during the repair of incorrect DNA that uses HR, there may be a loss of an allele during repair (Smukowski et al. 2023).” on page 169 of snapshot.

Comment: Is it worth mentioning SSA here since that appears to be a low fidelity menchanism that functions when other repair pathways are compromised.?
Reply: The following has been added: “In higher-order eukaryotes such as humans, NHEJ is the favored DNA repair mechanism because of the large non-coding regions within the genome. However, when other repair mechanisms (eg., NHEJ, HR) are compromised, single strand annealing, which is a low fidelity mechanism may be involved (Chang et al., 2017). All repair mechanisms are error-prone, meaning that insertions and deletions are sometimes formed due to the DSBs being repaired imperfectly (Thurtle-Schmidt and Lo, 2018).” on page 171 of snapshot.

Comment: Another point that has been made in UNSCEAR 2020/2021 is that HRR predominates in stem cells because they are frequently in the cell cycle. Perhaps that is relevant for the lens?
Reply: The following has been added “The two most common DSB repair mechanisms are non-homologous end joining (NHEJ) and homologous recombination (HR). The latter predominates in stem cells as they are frequently in the replicative phase of the cell cycle (Choi et al. 2021).” on page 171 of snapshot.

164, Inadequate DNA repair leads to Increase, Mutations
Note: Shared KER, Previously reviewed in AOP 15, 296 and 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: Again, perhaps SSA should be mentioned here since it seems to be more important when there are cells that have inadequate NHEJ and especially HRR.
Reply: The following sentence is added: “Although NHEJ is predominantly the preferred repair mechanism throughout the cell cycle, homologous recombination (HR) and single-stranded annealing (SSA) are favored during the S and G2 phases in scenarios where the NHEJ repair pathway is inhibited. The absence of HR leading to an increase in SSA activity is still a matter to debate (Ceccaldi et al., 2016)”. on page 186 of snapshot.

Comment: It is not clear why the germ cells are mentioned here. How are they relevant to the cataract endpoints?
Reply: As this is a shared KER, multiple cell types will be mentioned that are relevant to a variety of AOPs. It is not specific to the cataract endpoint only.

Comment: The justification for inclusion of XP data and not MMR deficiencies is not clear.
Reply: The following sentence was added on page 182 of snapshot: "Furthermore, other repair mechanisms such as a loss in the mismatch repair (MMR) system can lead to a buildup of errors such as base-base mismatches and insertion-deletion errors in repetitive DNA sequences which are known as microsatellites. This could occur if an MMR gene (e.g. MLH1, PMS2) is inactivated through mutations or epigenetic silencing (Wang et al., 2022)."

Comment: Mention is made in the Essentiality about Ku80 protein deficiency, but not SCID which is the disorder for DSB repair deficiency for NHEJ or AT which is missing DSB repair as well.
Reply: No studies were retrieved that show SCID models lead to increased mutation frequencies. Hence, SCID was not used to support the KER.

Comment: Discussion here is strong on NER, which is usually involved in UV damage and not much for IR damage.
Reply: The KERs can be support by any type of stressor provided that data has to meet the dose-, temporal and incidence concordance aspect. Most UV related studies supported this criterion. Limited IR studies in the context of lens cells were found.

Comment: DNAPK is mentioned, but ATM is not really discussed along with its role as a signaling agent for DSB repair…which is involved in CA.
Reply: This information has been provided in the KER related to inadequate DNA repair leading to chromosomal aberrations. Studies (prior to 2022) that use ATM knockout assess CAs and not mutational frequencies.

1912, Inadequate DNA repair leads to Increase, Chromosomal aberrations
Note: Shared KER, Previously reviewed in AOP in 296 and 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: It is mentioned that when NHEJ and HRR are compromised, alt-NHEJ predominates. Recent papers looking at BRCA2 deficient cells (BRCA2 is involved in both types of DSB) suggested that SSA takes over. This should be mentioned.
Reply: The sentence has been added to the snapshot on page 190 as " As BRCA2 is involved in both the NHEJ and HR repair pathways, it has recently been observed in BRCA2 deficient cells that single-strand annealing (SSA) may be triggered (Han et al. 2017) ." and a reference was added to support the sentence.

Comment: Also, there is nothing about DNA replication stops that occur with DNA damage. The restarting of replication can be problematic for repair.
Reply: The following sentence was added: "DNA replication stops can also be problematic for repair." on page 192 of snapshot.

1978, Increase, Mutations leads to Increase, Cell Proliferation
Note: Shared KER, Previously reviewed in AOP 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: "For a mutation to occur, damaged DNA must be passed on to the next generation" is not accurate statement. A mutation can occur if it is not passed on to the offspring, it can lead to cell death. Damaged DNA is not passed on , the mutation is.
Reply: "For a mutation to occur, damaged DNA must be passed on to the next generation" was removed from the document in page 198 of snapshot.

Comment: There is much information that also suggests that DNA damage STOPS proliferation for awhile after irradiation; this needs to be mentioned. If a cell fails to stop DNA synthesis (in ATM mutant cells e.g.) then errors accumulate. This is not mentioned.
Reply: The following sentence was added on page 199 of snapshot:
Although the majority of DNA damage is addressed through the activation of repair mechanisms, if the cells fail to prevent DNA synthesis prior to repairing the DNA damage (eg. ATM mutant cells), erroneous repair accumulates which could lead to the activation of cell proliferation or cell death (Levine and Holland, 2018).

1979, Increase, Chromosomal aberrations leads to Increase, Cell Proliferation
Note: Shared KER, Previously reviewed in AOP 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: There are situations when increased chromosomal aberrations lead to a stalling of replication because the cell can't survive well with the abnormality.
It is also not clear whether the increase in proliferation from chromosome aberrations is from cellular transformation or not.
Reply: The following sentence has been revised and added to the KER description on page 206: "CAs arising from cell transformation can lead to stalling in cell replication to initiate repair (Jackson et al., 2009). CAs can also cause a loss of cell cycle checkpoints resulting in cell proliferation due to the entry into S-phase of the cell cycle (Jackson et al., 2009; Hanahan & Weinburg, 2011).

2816, Modified Proteins leads to Cataracts

REVIEWER#1: as stated, low body of evidence but some empirical evidence, although is weak. Studies have used very high doses relatively speaking,

Reply: Thank you for the summary, no changes have been made to the document with respect to this comment. Most studies that support B-H criteria use high doses.

REVIEWER#2:
Comment: There is a literature that the PSC cataracts are IR-induced, but this is not really discussed in the document. There is likely to be something different about the different mechanisms by which cataracts are induced by the different cataract-inducing agents.
Reply:
This information is better suited for the cataract KE. The following has been added and appropriately referenced in that key event relationship in the description section on page 88 of the snapshot: "Research has shown that posterior subcapsular (PSC) cataracts are a subtype of cataract that are most often found with IR exposure. This may be due to radiation exposure causing the improperly differentiated lens epithelial cells (LECs) to leave the germinative zone (GZ) and migrate along the posterior capsule towards the center of the lens. As atypical lens fiber cells (LFCs), and atypical LECs accumulate in this area, they may cause the development of a PSC cataract."

2819, Increase, Cell Proliferation leads to Cataracts

REVIEWER#1:
Comment: The McCarron et al reference doesn't seem particularly relevant in the KER relationship description?. Although quite old studies, the work by Von Sallman et al in the 1960s are quite relevant but have been omitted from this KER.
Reply: The McCarron reference has been replaced with Wride 2011 on page 258 of the snapshot. Von Sallmann (1952) was not included in the KER description as the authors do not provide data to support the measurements of lens opacity. However, this paper is already cited within the KER of Deposition of Energy to Cell Proliferation.

The grammar needs checking in this wiki page in general, some sentences do not make senses or words are missing.
Reply: The description has been improved for readability, changes have been tracked in the KER description.

REVIEWER#2:
Not all cells of the lens are capable of proliferation. This might be worthy of mention.
Reply: The sentence "If this occurs in lens epithelial cells, then cataracts can develop. Of note, not all cells of the lens are capable of proliferation (West-Mays et al, 2009)." was added under key event relationship description on page 218 of the snapshot, with the supporting reference cited.

1913, Increase, Oxidative DNA damage leads to Increase, DNA strand breaks
Note: Shared KER, Previously reviewed in AOP 296

REVIEWER#1: no comment

REVIEWER#2:

Comment: The problem throughout is that there are inconsistencies. Here finally SSBs are mentioned, but they are not mentioned in other sections that deal with oxidative DNA damage and strand breaks.

Reply: The following KERs that deal with oxidative DNA damage, DNA strand breaks and inadequate DNA repair have been revised to include mention of SSBs, DSB and complex lesions:

The following sentences was added to the Biological Plausibility and KER description of "Increase Oxidative Stress to Increase Oxidative Damage to DNA":

"The first scenario initiates DNA-protein cross-links, inter and intra-strand links, and tandem base lesions, while the second scenario produces more complicated lesions, known as oxidatively generated clustered lesions (ODCLs). These can include single strand breaks (SSBs), double strand breaks (DSBs), abasic sites, and oxidized bases (Cadet et al., 2012) which can cause chromosomal aberrations, cytotoxicity, and oncogenic transformations (Stohs, 1995) as well as structural changes to the DNA, such as blocking polymerases (Zhang et al., 2010)." on page 141 of the snapshot.

"Another kind of BER pathway is SSBR (single strand break repair). When two SSBs are in juxtaposition, they can form DSBs, which are detrimental (Caldecott, 2024; Pfeiffer et al., 2000)." on page 141 of the snapshot.

The following sentence was added to the KER description of "Increase Oxidative Damage to DNA leads to Increase DNA strand breaks":

"Additionally, SSBs in close proximity can become complex lesions to form DSBs (Caldecott, 2024)." On page 222 of the snapshot

The following sentence was added to the KER description of "Deposition of Energy (MIE) to Increase DNA strand breaks":

"Some data reports that low dose of low LET radiation can lead to complex lesions, which can lead to unrepairable DNA damage. However, determining the actual frequency of the complexity of these lesions has proven challenging (Wilkinson et al., 2023)" on page 82 of the snapshot. On page 98 of the snapshot.

The following sentence was added to the KER description of "Increase DNA strand breaks to Inadequate DNA repair":

"For example, with multiple single strand breaks (SSBs) in close proximity that can lead to DSBs (Caldecott, 2024)" on page 169 of the snapshot.

The following sentence was added to the Biological Plausibility of "MIE (Deposition of Energy) to Increase Oxidative Damage to DNA":

"For example, SSBs are usually repaired quickly (Collins, 2014), while DSBs are more complex and are therefore less likely to be repaired correctly (Schoenfeld et al., 2012; Markkanen 2017).

However, some SSBs can lead to complex lesions resulting in DSBs (Caldecott, 2024)." on page 227 of the snapshot.

Non-adjacent KERs

2813, Energy Deposition leads to Increase, Oxidative DNA damage

REVIEWER#1: no comment

REVIEWER#2:
Comment: Again, there is a misunderstanding of UV damage. This section notes that UV causes ionization, it does not. Ionizing radiation causes ionization.
Reply:UVC causes ionization events. Nonetheless the sentence has been modified to "It is widely accepted that the deposition of energy results in immediate ionization or non-ionization events, leading to oxidative stress and damage to DNA molecules." Page 228 of snapshot

1981, Energy Deposition leads to Increase, Mutations
Note: Shared KER, Previously reviewed in AOP 296

REVIEWER#1: no comment

REVIEWER#2:
Comment: It states "The DNA is particularly susceptible to damage which can be in the form of mutations". DNA damage and mutations are different. DNA damage includes DSB, base damage, etc…the mutations are the changes that occur as repair proceeds. This confusion is misleading in the document.
Reply: A sentence was revised to "The DNA is particularly susceptible to damage in the form of DNA strand breaks and the inadequate repair of these lesions can lead to mutations" on page 232 of the snapshot.

Comment: Damage can be by direct and indirect mechanisms in the nucleus….indirect involves oxidative damage of water that damages the DNA and direct involves action on the DNA directly. This is not clear in this section.
Reply: The following was added: **"**The DNA is particularly susceptible to damage in the form of DNA strand breaks and the inadequate repair of these lesions can lead to mutations. DNA damage can be caused by direct and indirect mechanisms. Indirect involves formation of free radicals from the breakage of water molecules that can oxidize DNA and direct involves action on the DNA leading to strand breaks and complex lesions (Cannan & Pederson, 2016)." on page 232 of the snapshot.

Comment: Again, why is radiation exposure of germ cells discussed here?
Reply: As the KER is shared in more than one AOP, it is meant to be relevant to any cell type.

Comment: A point is made about dose rate, but this is not really described well in the write-up.

Variation for uncertainties should also not differences in endpoints that affect the interpretation of the data.
Reply: The following sentence was added: "It has been shown that various dose rates of radiation exposure can lead to distinct types of damage. High dose-rate radiation has been observed to generate a higher number of DNA strand breaks, resulting in a variety of mutations, including small base changes and deletions. Moreover, the likelihood of insufficient repair is elevated, contributing to an overall increase in mutation frequency. In contrast, low dose-rate radiation has been found to have a significantly lower mutation frequency, particularly in deletions and rearrangements (Brooks et al., 2016; Sankaranarayanan & Nikjoo, 2015)"on page 232 of the snapshot.

Under the uncertainty section, we now indicate that "4) Difference in measurements of mutational frequency can affect the interpretation of the data" on page 242 of the snapshot.

1982, Energy Deposition leads to Increase, Chromosomal aberrations
Note: Shared KER, Previously reviewed in AOP 272

REVIEWER#1: no comment

REVIEWER#2:
Comment: It is noted that chromosomal injury comes about from the DIRECT damage of DNA. How can we be sure it is not indirect?
Reply: Indication was added that it can be both direct and indirect in the biological plausibility section of the KER on page 4 (snapshot document). The following statement was added to the biological plausibility section and appropriately referenced "Direct damage to DNA occurs when radiation directly interacts with the DNA molecule, causing structural alterations such as breaks or cross-links. In contrast, indirect damage results from radiation interacting with nearby molecules, producing reactive species like free radicals, which can then indirectly affect the DNA by causing chemical modifications and impairing its integrity (Chatterjee et al, 2017)" on page 245 on snapshot

Comment: For known modulating factors, it is stated that females are more sensitive. Is this in humans or all species?
Reply: The following sentence in the Known Modulating Factors section has been revised to "Iuman females were found to have increased aberrant cells and chromosome breaks relative to males." on page 23 on snapshot.

2814, Energy Deposition leads to Increase, Cell Proliferation

REVIEWER#1: no comment

REVIEWER#2:

Comment: As in the notes above, it is not clear that the group has considered the block in DNA replication that takes place following radiation exposure. In addition, is the proliferation just about cancer induction or is it something else?
Reply: It is noted in the Uncertainties and Inconsistencies section that radiation can cause DNA damage which can trigger cell cycle checkpoint arrest and repair mechanisms, but persistent damage may contribute to aberrant proliferation. However, there are inconsistencies in the responses observed.
Additionally, cancer-related articles were used to explain the mechanism of how a deposition of energy can induce increased cell proliferation, because the mechanisms behind increased lens cell proliferation remain incompletely understood. and is an active area of research. It should be noted that empirical evidence was presented to support observations of cell proliferation in the context of cataractogenesis.

Comment: Obviously the time-frame matters and that is not discussed in this section.
Reply: In the uncertainty and inconsistencies section, the time frame of cell proliferation was discussed as the studies found that proliferation was either increased or decreased based on the time since irradiation. The following sentence was added to the section: "In using different model systems (rats, mice, rabbits) the proliferation rate could vary anywhere from 17 to 20 days in LECs. This was also shown to be dependent on genetic, environmental factors and individual lifestyle (Ainsbury et al., 2016; Barnard et al., 2022)." - page 252 on snapshot

2815, Energy Deposition leads to Cataracts

REVIEWER#1:
Comment: the key event relationship description is very poorly cited. Once you've used and introduced the 'LEC' abbreviation, you do not need to continue to write 'lens epithelial cell'.
Reply: The authors believe the KER is well-cited, as we have retrieved an adequate number of primary research articles and review articles to understand the structural and functional aspects of the KER. We have provided sufficient depth of information to support elements of the modified Bradford-Hill criteria. The overall rating of this KER is high as seen in the overall assessment document. Any further support for this KER would not improve the understanding of the KER or the overall weight of evidence call.

In the Biological Plausibility Section of the KER, we have cited many review articles that describe epidemiological studies in the context of cataract formation. Not all studies are listed in the table, as we prioritize space-relevant studies. However, we have added a few studies related to radiation therapy and cataract formation as per Reviewer 2's suggestions. The following studies were added to the Dose and Time Concordance Description and Table:
Arefpour, A. M., Bahrami, M., Haghparast, A., Khoshgard, K., Aryaei Tabar, H., & Farshchian, N. (2021). Evaluating Dose-response of Cataract Induction in Radiotherapy of Head and Neck Cancers Patients. Journal of biomedical physics & engineering, 11(1), 9–16. https://doi.org/10.31661/jbpe.v0i0.834
McCarron, R. A., Barnard, S. G. R., Babini, G., Dalke, C., Graw, J., Leonardi, S., Mancuso, M., Moquet, J. E., Pawliczek, D., Pazzaglia, S., De Stefano, I., & Ainsbury, E. A. (2022). Radiation-

Induced Lens Opacity and Cataractogenesis: A Lifetime Study Using Mice of Varying Genetic Backgrounds. Radiation research, 197(1), 57–66. https://doi.org/10.1667/RADE-20-00266.1

The following text was added to Dose Concordance Description on page 264-263:
Patients with head and neck cancer showed a rise in the percentage of lens opacity three and six months following radiotherapy (Arefpour et al., 2021).

In another study, various mouse species exposed to ^{60}Co γ-irradiation at multiple doses (0.5, 1, and 2 Gy) using two dose rates (0.063 and 0.3 Gy min-1), revealed that the average lens density was elevated with dose and dose rate when Ercc2 and Ptch1 mutations were present (McCarron et al., 2022)

Dose Concordance Table

Arefpour et al., 2021	Humans (both sexes) with head and neck cancer were exposed to radiation therapy ranging from 0-22 Gy) for treatment. Lens opacity was measured in 3 and 6 months after radiation therapy.	The analysis of the data derived from radiotherapy patients exposed to doses of radiation using a linear accelerator ranging from 0-22 Gy showed an exponential dose response relationship with maximum lens opacity observed after 3 months post-exposure.
McCarron et al., 2022	*In vivo*, mixed sex mouse models of lenses were exposed to 0.5, 1, 2 Gy of ^{60}Co γ-irradiation with a dose-rate of 0.063 and 0.3 Gy min^{-1} and the maximum opacification were measured 1-18	Mice irradiated to 0.5, 1, 2 Gy ^{60}Co γ-rays at a dose-rate of 0.063 and 0.3 Gy min^{-1} resulted in an increased incidence of lens opacity in a dose response manner.

	months post-irradiation.	

Time Concordance Description on page 264:
McCarron et al. exposed different mouse species to ^{60}Co γ-irradiation at doses of 0.5, 1, and 2 Gy, employing two dose rates (0.063 and 0.3 Gy min-1). Lens opacity was evaluated from 0-18 months post irradiation. The results indicate that with the passage of time, there is a gradual rise in lens opacity (McCarron et al., 2022).

Time Concordance Table

Arefpour et al., 2021	Humans (both sexes) with head and neck cancer were exposed to radiation therapy ranging from 0-22 Gy) for treatment. Lens opacity was measured in 3 and 6 months after radiation therapy.	The analysis of the data derived from radiotherapy patients exposed to doses of radiation using a linear accelerator ranging from 0-22 Gy showed a time response relationship with maximum lens opacity observed after 3 months post-exposure.
McCarron et al., 2022	*In vivo*, mixed sex mouse models of lenses were exposed to 0.5, 1, 2 Gy of ^{60}Co γ-irradiation with a dose-rate of 0.063 and 0.3 Gy min^{-1} and the maximum opacification were measured 1-18	Mice irradiated to 0.5, 1, 2 Gy ^{60}Co γ-rays at a dose-rate of 0.063 and 0.3 Gy min^{-1} resulted in an increased incidence of lens opacity in a time response manner.

	months post-irradiation.	

Comment: "When the rate of division in mitotically-active lens epithelial cells becomes too high, they become incapable of transforming into typical elongated, organelle-free lens fiber cells" – change transforming to 'differentiating'.
Reply: The sentence was revised to "When the rate of division in mitotically-active lens epithelial cells becomes too high, they become incapable of differentiating into typical elongated, organelle-free lens fiber cells" on page 257 in the Biological Plausibility section.

Comment: "The overall consensus is that cataract risk increases with radiation dose, as measured based on various forms of cataracts" – maybe mention the stochastic versus deterministic argument here for context, as the literature is still not super clear on which applied to cataract.
Reply: The following has been added to the Uncertainties Section "The overall consensus is that cataract risk increases with radiation dose as a stochastic effect due to the linkage of cataracts to genotoxic effects (Seals et al., 2016). This is reinforced through cataract occurrence in animals with genetic mutations relating to DNA repair and cell division; the stochasticity is apparent because damage to singular cells is transmitted to successive cells, resulting in cataract formation (Seals et al., 2016). However, there is also controversy on whether there is a threshold dose below which tissue reactions (deterministic effects) do not occur (Thome et al., 2018; Hamada 2023)" on page 261.

Comment: No mention of the studies by Little et al in the main text and the radiologic technologist cohort, which provide some good evidence and data for a very large occupationally exposed cohort to support low dose exposures.
Reply: Among the studies published by Little et al, the following two have been referenced in text under Biological Plausibility as it strongly provides empirical evidence to support the KER: "It has been reported there is a significantly increased risk of cataracts below 100 mGy (but not below 50 mGy) in occupational technologists exposed to radiation (Little et al., 2018; Little et al., 2020)." to page 258 of snapshot.

Comment: "Studies using visual acuity to measure cataracts pose challenges as the test is not specific for cataracts, even though the measurement is an indicative test for the ultimate function of the lens" – reference this sentence.
Reply: The reference (Elliot et al, 1990) has been added on page 261 of the snapshot.

Comment: Latency effect – many biological studies are performed in rodents with limited lifespans, to note, and so a reason for lack of cataract development in studies with 'controlled' radiation dose exposures as opposed to human occupational or accident cohorts.
Reply: A sentence was modified to "The risk for cataracts caused by low doses of high energy particles may be underestimated in many studies due to length of the observation period used or the limited lifespans of some models." on page 261 of the snapshot.

Comment: Known modulating factors – Genetics – McCarron study referenced twice with different years? There is only one McCarron paper and it should be cited as 2022.
Reply: The following reference: "McCarron et al., 2021" has been removed from page 269 in the table of the Known modulating factors section.

Comment: Race – 'White people' might be better written as 'white skin tone'
Reply: It was revised to "White skin tone" on page 269 in the table of the Known modulating factors section of the snapshot.

REVIEWER#2:
Comment: Information on neutron exposures is needed. What is the energy of the neutrons? Radiation weighting factor is dependent on energy of neutron. It is noted that dose-rate plays a role, but dose-rate is not listed.
Reply: The following information has been included in the Dose Concordance table On page 269 of the snapshot:
Upton et al., 1956 - The cyclotron fast neutrons had dose rate of 60-125 rep/min. Fast neutrons from a Po-B source had energies of 2-3 MeV and a dose rate of 1-4 rep/h.
Worgul et al., 1996 - The irradiating neutrons had an energy of 440 keV and a dose rate of 8 mGy/min.
Bateman et al., 1963 - Neutrons of 0.43 Mev (no mention of dose rate for neutrons used)

2817, Inadequate DNA repair leads to Cataracts

REVIEWER#1:
Comment:
McCarron et al 2021? It is 2022. This needs to be made consistent throughout the AOP
Reply: The reference was revised to "McCarron et al., 2022" and the year was modified in the reference section to "2022" on page 282 of the snapshot

Comment: "estrogen-implicated increase in speed of cataract progression" – some mentioned of the studies by Dynlacht et al might be worth including here, albeit using rats exposed to high-LET rather than mice.
Reply: Dynlacht et al. (2006; 2008) studies were added under Uncertainties and Inconsistencies section of the KER description on page 282 of the snapshot

Comment: In KE 2083, sex applicability subsection includes studies and references not included in the KER which are relevant to understanding the KER.

Reply: We reviewed the references in the sex applicability of the Key Event 2083 among 8 of references listed, two of the references (Ainsbury et al., 2016; Garrett et al., 2020) have now been included in the document under Biological Plausibility and Essentiality sections respectively as follows:

Biological Plausibility on page 287 of the snapshot:
The maintenance of lens transparency involves the participation of DNA repair pathways (NER, BER, repair of DNA strand breaks, and direct reversal of DNA damage) and changes in the activity of DNA repair genes have been linked to age-related cataracts. The lens epithelium expresses a minimum of 92 genes associated with DNA repair, crucial for safeguarding the integrity of the cellular genome (Ainsbury et al., 2016).

Essentiality on page 287 of the snapshot:
It has been observed that E2, a form of estrogen, may disrupt the pathways responsible for repairing direct DNA damage. This disruption could lead to increased occurrence and faster progression of cataracts in groups exposed to E2, whether it is produced within the body or comes from external sources (Garrett et al., 2020).

REVIEWER#2:
Comment: A list of human datasets that contribute to our understanding of cataracts is given, but nothing is mentioned about RT (radiotherapy) patients, which influenced much about what is known about cataracts.
Reply: In terms of radiotherapy studies, we were unable to find any that support inadequate DNA repair to cataracts. However, we have added a study by Chodick et al. (2016) pertaining to radiotherapy which supports the MIE to cataracts KER. The following information was added: "Reports from radiotherapy patients and cancer survivors also serve as evidence for radiation induced cataracts. In a study by Chodick et al. (2016) 3.5% of subjects experienced a case of cataracts during the first 5 years after cancer diagnosis, with prevalence increasing as the dose of radiotherapy increased." on page 257 under Dose concordance section.

2818, Oxidative Stress leads to Cataracts

REVIEWER#1:
Comment: in the key event relationship description, you say that cataracts have occurred once 5% of the lens is opaque, but in other sections you've highlighted the subjectivity of cataract classification and the different methods used to score them. Can this paragraph be better cited and clarified? This paragraph feels quite clumsy in general.
Reply: The key event relationship description has been revised on page 288 to include a statement clarifying the increased ROS levels from pathways of oxidative stress can lead to cataracts (as defined in this KER) which is defined as having 5% of the lens becoming opaque.

The paragraph has been revised to "Increased ROS levels from different pathways of oxidative stress can damage proteins, lipids, and important cellular processes. If this occurs in the lens of the eye and damage accumulates over time, eventually the increased opacity of the lens prevents light from passing freely, leading to cataracts (Tangvarasittichai and Tangvarasittichai, 2019). Cataracts are a progressive condition in which the lens of the eye develops opacities and becomes cloudy, resulting in blurred vision as well as glare and haloes around lights (National Eye Institute,

2022). For purpose of this AOP, cataracts are defined as over 5% of cells in the lens exhibiting opacities."
The methods to measure cataracts in this KER have been generalized to the opacity of the lens which is inclusive of how cataracts were defined.

Comment:
Identification and quantification of ionising radiation-induced oxysterol formation in membranes of lens fibre cells - ScienceDirect – may be a useful reference for you in this KER
Reply: This study does not support a linkage between oxidative stress and cataracts as there was no endpoint measured for lens opacity. Therefore we did not cite the suggested reference.

Comment: Uncertainties and inconsistencies section – Spector 1995 is not properly cited.
Reply: The "Spector (1995)" reference has been revised on page 260 in the Uncertainties and inconsistencies section.

Comment: "unless otherwise indicated, all data is significantly significant" – do you mean 'statistically significant'?
Reply: It was corrected to "statistically significant" on page 260 under the Quantitative Understanding of the Linkage section.

REVIEWER#2: no comments

OVERALL ASSESSMENT AND AOP REPORT RESPONSES
Comments:
REVIEWER#1:
Comment: In general, the pages feel disjointed, the way cataract are described changes quite a bit page to page. Referencing seems inconsistent throughout. Some 'Evidence supporting this KER' state the weight of evidence (low, moderate etc.) but others don't – but this is useful information to state.
Reply: The references have been checked and formatted consistently for those relevant to the Cataract AOP.
The definition of cataract has been made consistent throughout the document.
A weight of evidence call (low, moderate, high) has been added across each KER description

REVIEWER#2:
Comment: The major limitations of the work is that it is written by several authors (or so it would seem) and there are many inconsistencies about what is included and what is not.
Reply: Several KERs in this AOP are shared and reused from those built by other AOP developers. At the time the AOP was developed, there were no clear guidelines on a specific format to present the material and the depth of information needed to support each KER. This is an area being

discussed within the OECD AOP subgroups as a challenge with solutions being proposed for next generation AOP development.

Comment: The sections on DNA repair are perhaps among the most confusing. Questions about what is to be included and why is unclear. It is not justified why mutations in germ line are mentioned. There are also some statements where mutations and DNA damage are confused.
Reply: Since the KERs/KEs in the cataract AOP are shared with multiple AOPs (e.g AOP to heritable mutations), different models are used to support the relationship. As per OECD guidelines, KERs are independent units from the rest of the AOP, therefore they can be supported by different types of stressors, models, exposure parameters.

Comment: The comments on the AOP report are much the same as those listed above for the AOP itself.

Reply: The following revisions have been made to the AOP report and overall assessment documents:

Addition of the concept of complex lesions from SSB and its contribution to oxidative DNA damage and inadequate repair

Pg. 6 of the overall assessment

Furthermore, these strand breaks and a combination of various DNA abnormalities occurring in close proximity can create complex lesions that are more difficult to repair (Nickoloff et al., 2020).

Pg. 12-13 of the report

Oxidative stress is a consequence of increased ROS, and the production of these radicals can cause DNA damage leading to complex lesions that include SSBs, DSBs, abasic sites, and oxidized bases (Cadet et al., 2012; Cadet and Davies, 2017).

The concept of different repair processes contributing to inadequate repair, although DSBs are predominantly handled by NHEJ, if this is compromised other repair processes can help out including SSA

Pg. 6 of the overall assessment

Although NHEJ is predominantly the preferred repair mechanism throughout the cell cycle, homologous recombination (HR) and single-stranded annealing (SSA) are favored during the S and G2 phases in scenarios where the NHEJ repair pathway is inhibited. The absence of HR leading to an increase in SSA activity is still a matter to debate (Ceccaldi et al., 2016).

Pg. 7 of the report

Homologous recombination and single-stranded annealing are favored during the S and G2 phases of the cell cycle (Ceccaldi et al., 2016).

Discussion on indirect (through oxidative stress) vs direct damage to the DNA
The following is stated in the overall assessment-first paragraph, biological plausibility, pg. 4 (no changes were made)
Indirect damage can also occur when water molecules dissociate producing reactive oxygen species (ROS) that induce DNA breaks (Ahmadi et al., 2022).

The following is stated in the report on pg. 10-11
In a well-accepted sequence of events, direct damage occurs when ionization events from deposited energy onto a cell interact directly with the DNA, while indirect damage can occur when deposited energy dissociates water molecules located near DNA, producing ROS that are capable of inducing DNA breaks (Ahmadi et al., 2022).

The need for additional references related to human studies to support cataract formation from radiation
Pg. 19 and 24 of the overall assessment
Wang et al. 2020; Chandrasekher et al., 2004

The need for more human studies was already mentioned in Table 4 on pg. 52 of the report.
Little et al reference was already included in Table 2 on pg. 36

Statement that there are differences in mechanisms of cataracts between UV vs ionizing

Pg. 9 of the report, Page 4 of the overall assessment
Many studies use ultraviolet radiation as a stressor, and it is important to note that ionizing and non-ionizing radiation work with different mechanisms when inducing cataracts. Ionizing radiation can remove tightly bound electrons from atoms to create charged particles, whereas the absorption of non-ionizing radiation results in heat generation from molecular vibrations (Alcócer et al., 2020).

Information on how the delivery of radiation (different dose-rates) can affect the progression of cataract
Pg. 3 and 8 of the overall assessment
It is believed the progression of cataracts at high dose at higher dose-rates generally induce more damage than lower dose-rates (Brooks et al., 2016).

Pg. 19-20 of the report

The rate of dose delivery may also affect the progression of cataracts as higher dose-rates generally induce more damage to DNA than lower dose-rates (Brooks et al., 2016).

The addition of data interpretation as an uncertainty depending on end points assessed.

Pg. 8 of the overall assessment and pg 10 of AOP report

The use of different assays to assess KEs can result in diverse quantitative interpretations of data.

The addition of information on phosphorylation of proteins was added to the overall assessment with the appropriate references

Pg. 11 of Report

Concurrently deposited energy can lead to protein modifications in the form of deamidation, phosphorylation, oxidation, disulfide bonds (Hanson et al., 2000; Wang et al., 2020; Chandrasekher et al., 2004), increased cross-linking, altered water-solubility, and increased protein aggregation (Fochler & Durchschlag, 1997).

Pg. 6 already in the overall assessment section

Alongside DNA as a target to energy deposition, other macromolecules can be damaged. In terms of cataracts, there is much evidence to show that protein modifications such as phosphorylation, deamidation, oxidation, disulfide bonds (Hanson et al., 2000), increased cross-linking, altered water-solubility, and increased protein aggregation are critical to disease progression (Fochler & Durchschlag, 1997; Reisz et al., 2014; Wang et al., 2020; Chandrasekher et al., 2004).