Case study on assembling and assessing AOP information: Alkylation of DNA leading to heritable genetic effects

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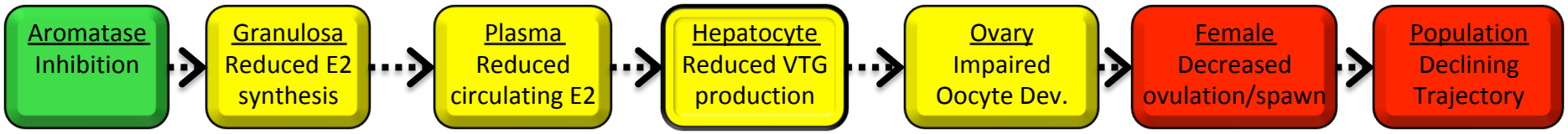
Outline

- Review - MIE, KE, AO, KERs and the AOP
- Introduction and background relevant to case study AOP
- Selection and summary of events
- Summary of weight of evidence for key event relationships
  - Biological plausibility, empirical support, uncertainties/ inconsistencies
- Assessment of overall weight of evidence
  - Biological plausibility, empirical support, essentiality
- Conclusions

What are AOPs?



An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment (Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)

- A way to organize information
- Based on biological plausibility and/or statistical inference
- A hypothesis and a set of measurements we can make to test that hypothesis
- Events span levels of biological organisation relevant to risk assessment (e.g., molecular, cellular, tissue/organ, organism and/or population).

Five Principles of AOP Development

1. AOPs are not chemical specific

2. AOPs are modular

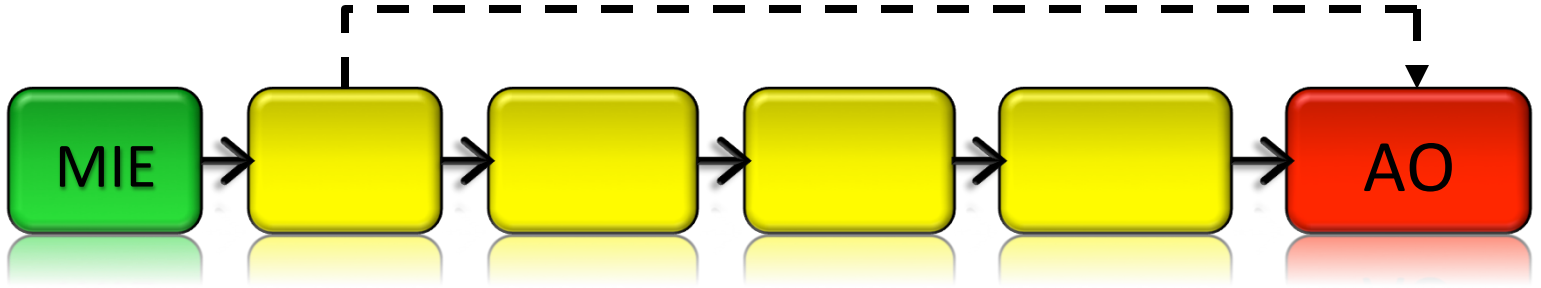
3. Individual AOPs are a pragmatic simplification of biology

4. AOP networks are the functional unit of prediction (in most cases)

5. AOPs are “living” documents

Principles of AOP Development

Two Primary Building Blocks



Key Events (KEs)

- *Functional unit of observation/ verification*
- Observable Δ biological state (measurable)
- Essential (but not necessarily sufficient)

Key Events Relationships (KERs)

- *Functional unit of inference/extrapolation*
- directed relationship
- State of KE_{up} provides some ability to predict or infer state of KE_{down}
- Supported by plausibility and evidence
- Quantitative understanding

Creating KERs

KER Pages

- Title
- Description
- Biological plausibility
- Empirical support
- Inconsistencies and uncertainties
- Quantitative understanding

WOE Assembly

Plausibility

- largely based on “normal biology”
- Understanding of structural and functional relationships between KEs.

Empirical Support

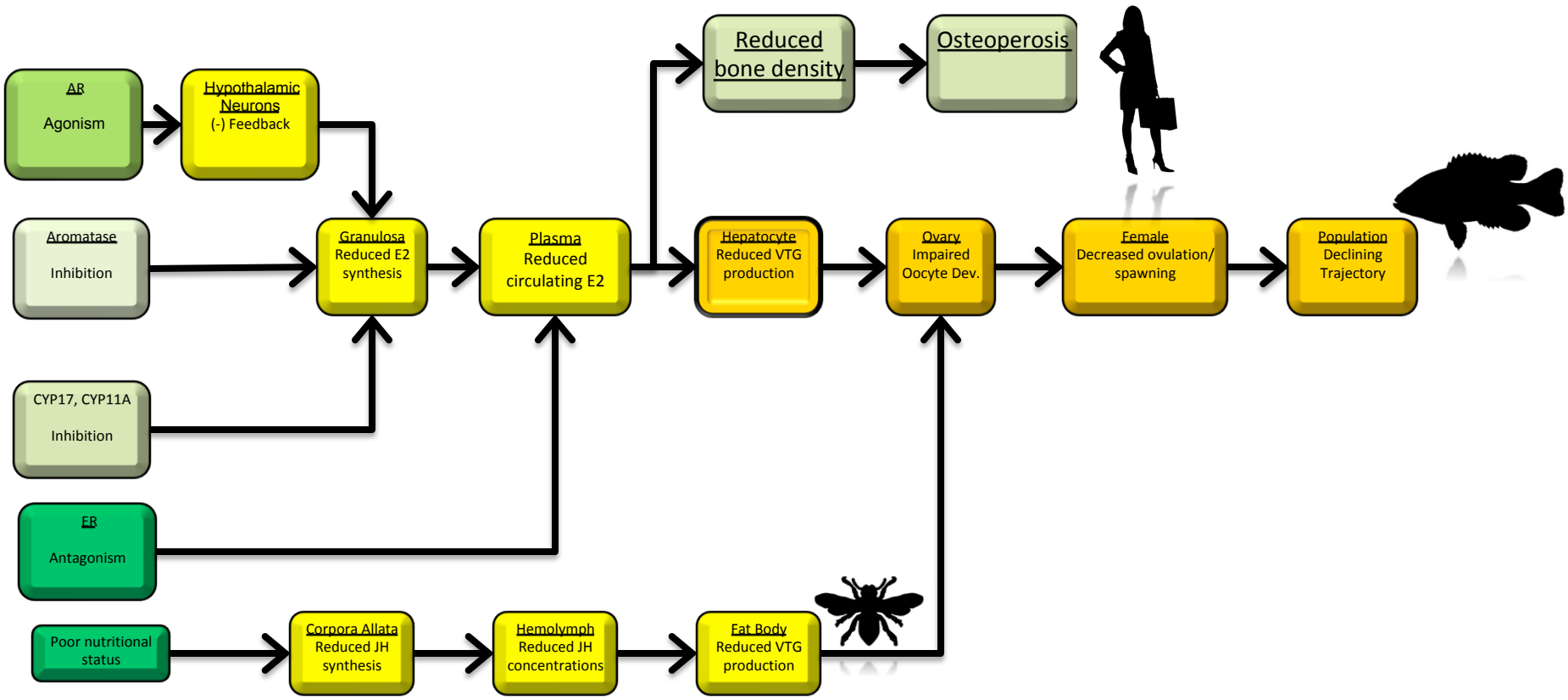
- Largely based on perturbation studies (e.g., toxicology studies)

Overall assessment of AOPs
Weight of evidence assessment for causality

Bradford Hill criteria: minimal conditions necessary to provide evidence of a causal relationship between events and the consequence forming the weight of evidence considerations for causality & level of confidence in an AOP.

1. Biological Plausibility		
Is there a mechanistic relationship between Key Event (KE_{up}) and KE_{down} consistent with established biological knowledge?		
High (strong) confidence Extensive understanding based on published literature with broad acceptance in the scientific community.	**Moderate confidence** Coherence with accepted biological relationships but scientific understanding not fully established.	**Low (weak) confidence** Empirical support, but the structural or functional relationship is not understood.
2. Essentiality		
Are downstream KEs and/or the adverse outcome prevented if an upstream KE is blocked?		
High (strong) confidence Direct experimental evidence (e.g., antagonism, knockout models, etc.)	**Moderate confidence** Indirect experimental evidence (e.g., increase in KE_{up} leads to an increase in KE_{down}).	**Low (weak) confidence** Lack of evidence, or contradictory experimental evidence.
3. Empirical Support		
Does the empirical evidence support that a change in KE_{up} leads to an appropriate change in KE_{down}? Does KE_{up} occur at lower doses, earlier time points, and higher in incidence than KE_{down}? Are there any uncertainties and inconsistencies?		
High (strong) confidence Extensive evidence to support KE dependent changes in downstream events, as well as temporal, dose-response and incidence concordance, with no or few critical data gaps or conflicting data.	**Moderate confidence** Demonstrated dependent change in KEs but some evidence inconsistent with an expected pattern that may be explained by factors such as experimental design, or technical considerations, etc.	**Low (weak) confidence** Limited or no studies reporting dependent change in KEs i.e. endpoints never measured in the same study or not at all, and/or significant inconsistencies.

For most real-world applications, AOP networks are the functional unit of prediction



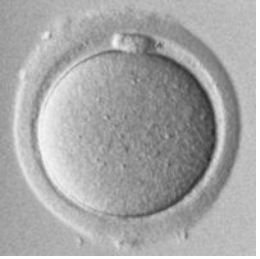
Recommended Workflow for AOP Creation

1. **Map** out your AOP ahead of time

2. **Search/browse** the wiki for related AOPs/KEs/KERs before entering new AOP.

3. **Identify existing content** your AOP can link to.

4. **Enter** your AOP, by linking to existing content and/or creating new pages as appropriate.



CASE STUDY: Alkylation of DNA leading to heritable genetic effects

Principle: Germline genetic damage occurs in sperm or egg and can be transmitted to an offspring

IMPORTANT BECAUSE:

Germline mutations are deleterious and can result in death of the developing embryo.

May result in any type of genetic disease in an offspring.

Can potentially contribute to the population gene pool.

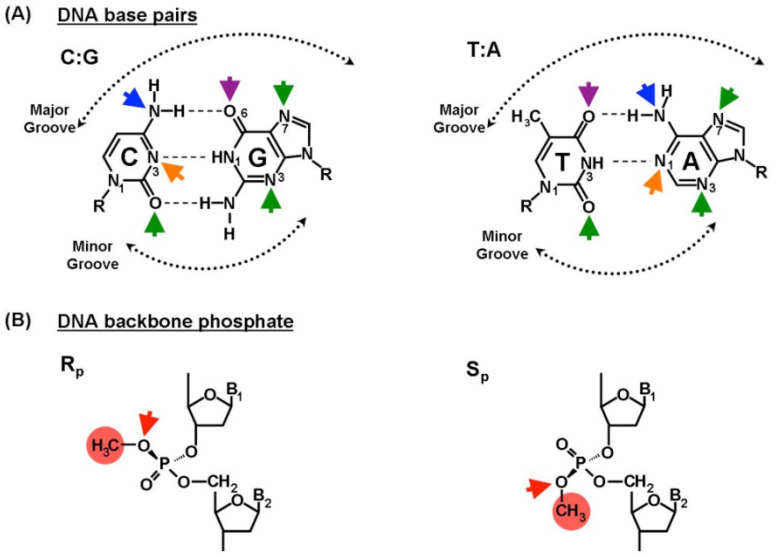
AOP: Alkylation of DNA leading to heritable mutations

Motivation:

- One of the best characterized modes of action in genetic toxicology.
- Provide context of use for new methods/technologies to detect somatic and germ cell mutations.
- Emphasize gaps in research in this field.
- ***De novo* mutations are increasingly recognized as contributing to a large array of human genetic diseases. Proposed changes in the way heritable hazards are assessed are being considered.**

Background on biology of this AOP

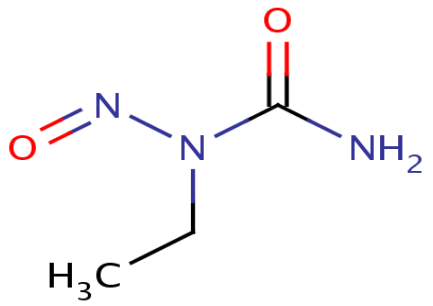
- Alkylating agents: chemicals that add alkyl groups (e.g., methyl, ethyl, propyl) to cellular molecules.
- Occurs at various sites in DNA.



Each site has a different degree of stability.

Prototype alkylating agent

- Although AOPs are not chemical-specific, the database on alkylation of DNA is heavily biased towards a few prototype agents.



- E.g., N-ethyl-N-nitrosourea (ENU)

Alkyl adducts can be repaired

- Primary repair protein is AGT: Alkyl guanine transferase.
- AGT irreversibly binds the alkyl group and is inactivated.
- Very efficient repair at LOW doses.
- AGT overwhelmed at HIGH doses = alkyl adducts retained.
- Not very good at repairing all adduct types.

Replication of alkylated DNA causes mutations

- Replication over an alkyl adduct can cause insertion of an incorrect base in the DNA duplex.
- Mutation becomes ‘fixed’ and can propagate to daughter cells.

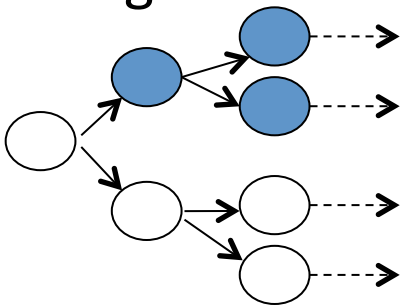
O^4 thymine alkylation = AT-GC transitions

O^6 guanine alkylation = GC-AT transitions

Note: some adducts not mutagenic. N-alkyl adducts tend to be bypassed error-free

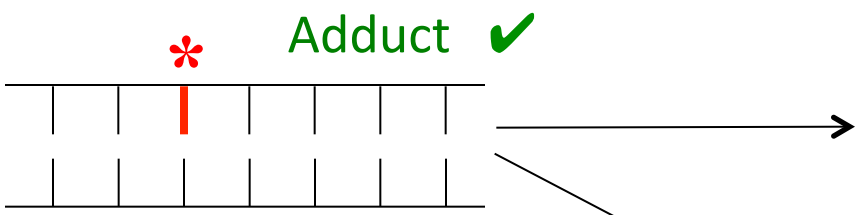
TARGET CELLS: Spermatogonial stem cells

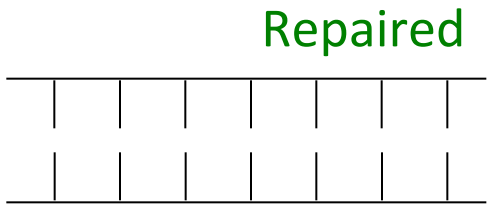
- Mutations in pre-meiotic, replicating, spermatogonial stem cells can persist in an organism and clonally expand.

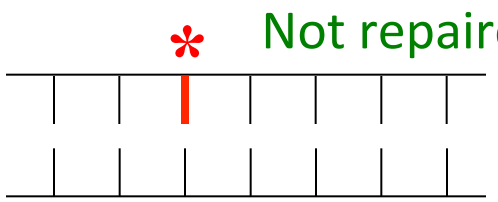


- Sperm derived from these stem cells will carry these mutations.
- **Fertilization of an egg with sperm carrying mutations can result in an offspring with these mutations.**

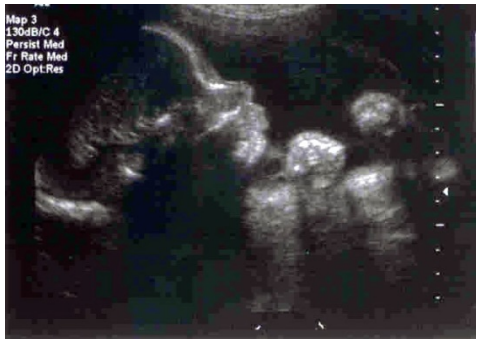
Selection of measurable endpoints





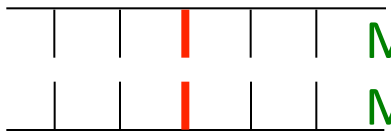


Offspring with mutations ✔

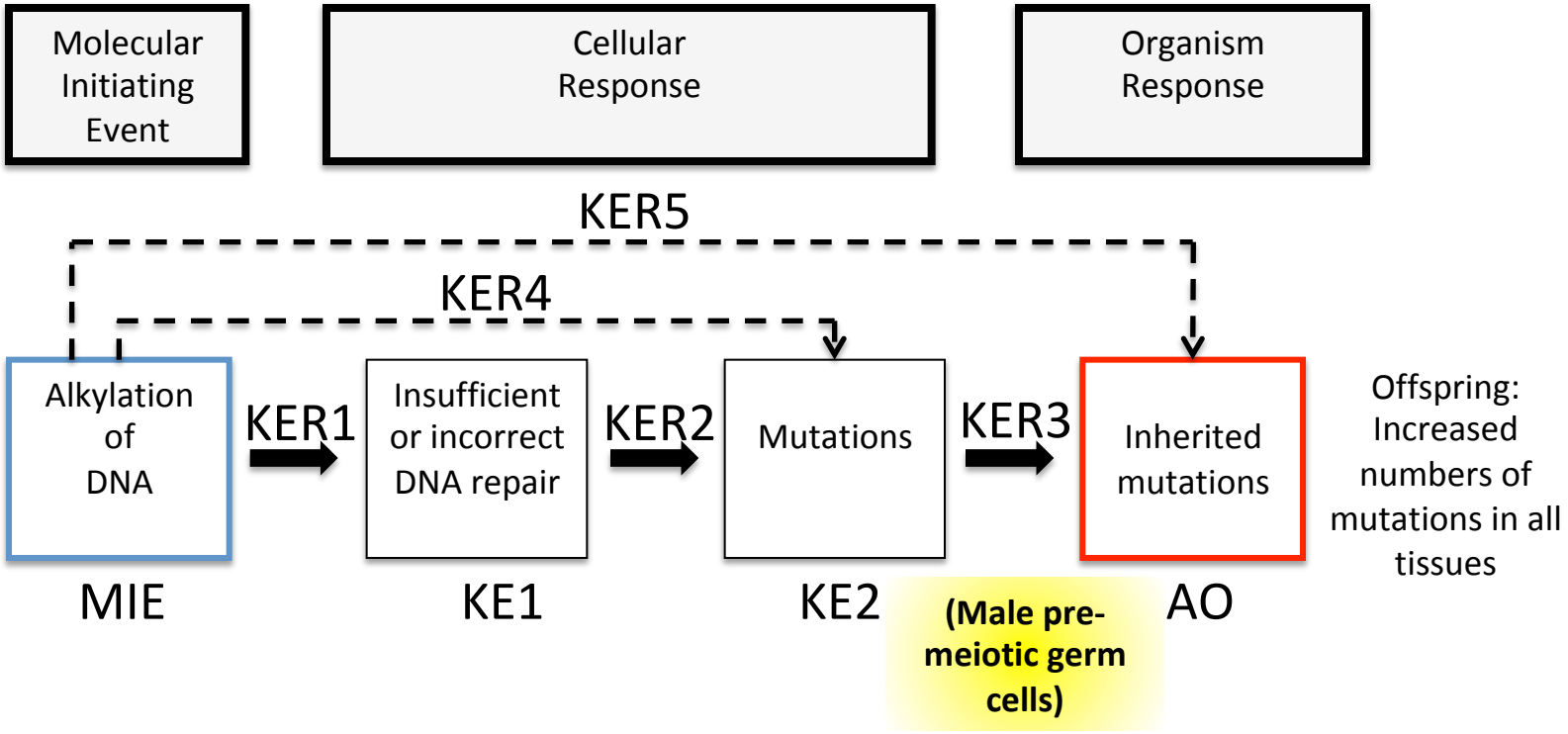


Sperm with mutation ✗ fertilizing egg

Replication ✗



AOP: Alkylation of DNA leading to heritable mutations



Entry of information into OECD knowledgebase

AOPWiki AOPs Key Events KE Relationships Stressors sign in sign up

Aop: 15

AOP Title

Alkylation of DNA in male pre-meiotic germ cells leading to heritable mutations

Short name:

Alkylation of DNA leading to heritable mutations

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Point of Contact

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AOPWiki AOPs Key Events KE Relationships Stressors sign in sign up

Molecular Initiating Event

Title	Short name
Alkylation, DNA	Alkylation, DNA

Key Events

Title	Short name
Increase, Mutations	Increase, Mutations
N/A, Insufficient or incorrect DNA repair	N/A, Insufficient or incorrect DNA repair

KEs

Adverse Outcome

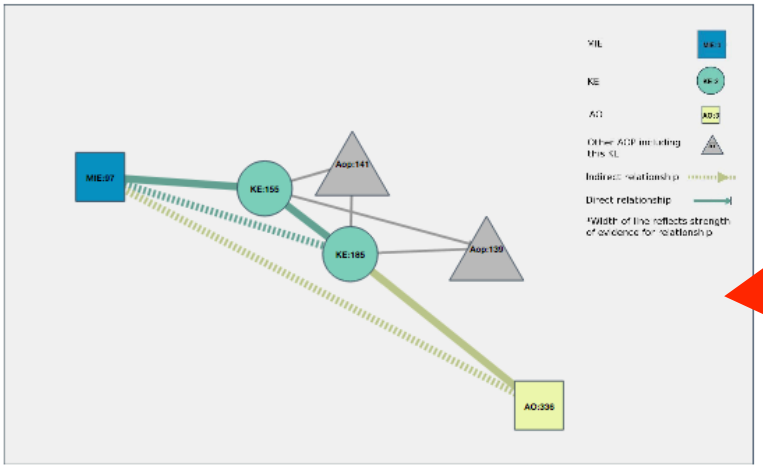
Title	Short name
Increase, Heritable mutations in offspring	Increase, Heritable mutations in offspring

Relationships Between Two Key Events (Including MIEs and AOs)

Title	Directness	Evidence	Quantitative Understanding
Alkylation, DNA leads to N/A, Insufficient or incorrect DNA repair	Directly leads to	Strong	Moderate
N/A, Insufficient or incorrect DNA repair leads to Increase, Mutations	Directly leads to	Strong	Moderate
Alkylation, DNA leads to Increase, Mutations	Indirectly leads to	Strong	Moderate
Alkylation, DNA leads to Increase, Heritable mutations in offspring	Indirectly leads to	Strong	Moderate
Increase, Mutations leads to Increase, Heritable mutations in offspring	Directly leads to	Strong	Moderate

KERs

Network View



Event:185 - Increase, Mutations

Network view

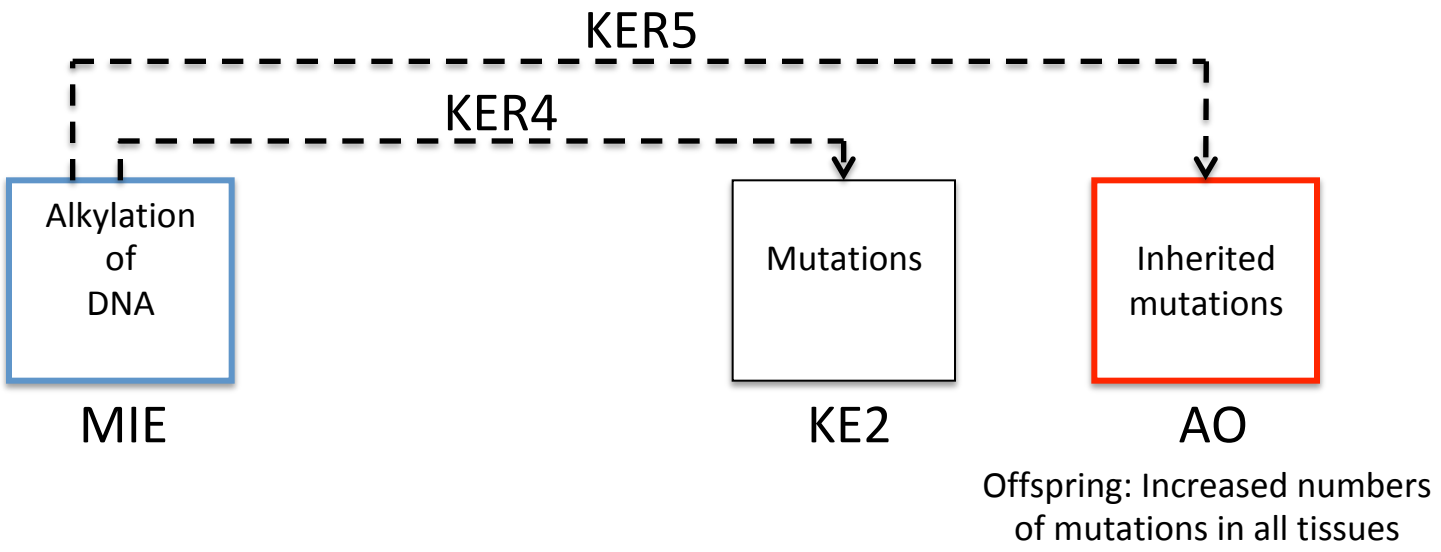
Entry of information into OECD knowledge base

Weight of Evidence for Key Event Relationships

1. **Biological plausibility**
   - Each event is well characterized and understood. Highly biologically plausible.

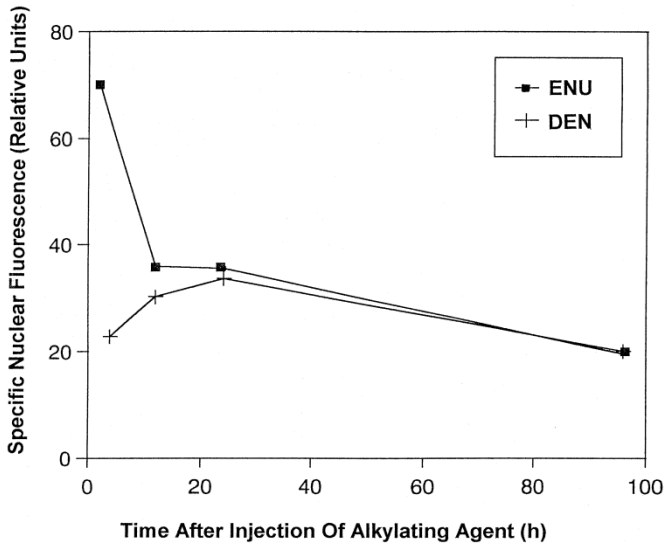
2. **Empirical evidence in support of relationships**
   - Find evidence to support that KE1 occurs before KE2.
   - Find evidence to support that incidence KE1 > KE2.
   - Examine concordance of dose- and response–response relationships

Key Event Relationships Evaluation



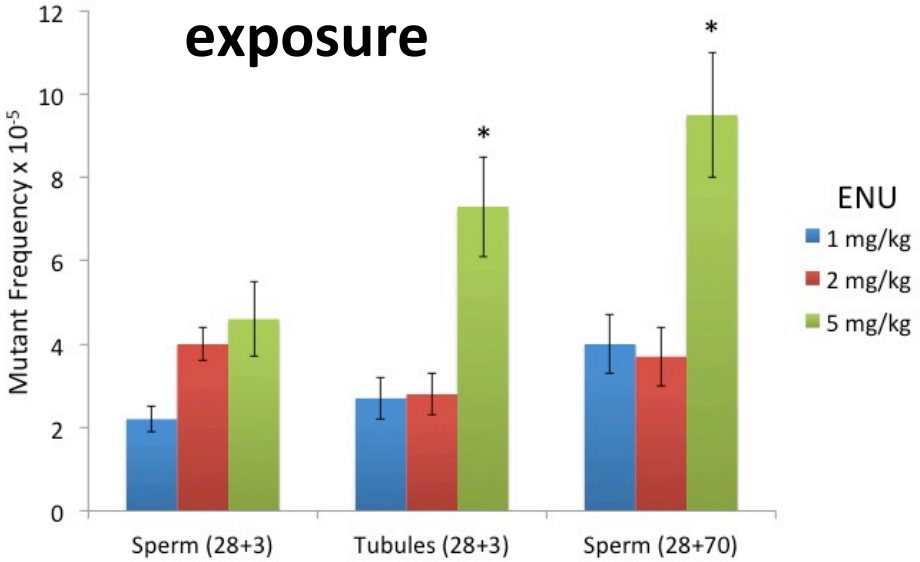
Temporal concordance

Adducts: Peak early, some persist to 6 d



Seiler et al. 1997. Mutation research 385(3):205-211.

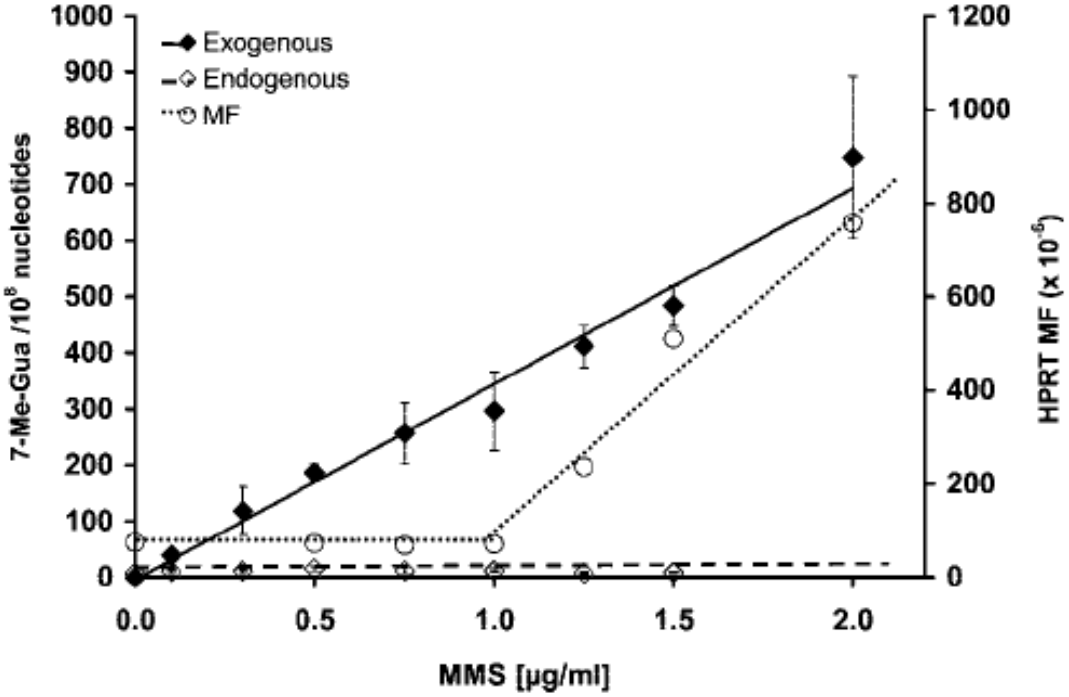
Mutations in sperm: Exposed 28 d, mutations occur > 49 days post-exposure



Data derived from O'Brien et al. 2015 Environ Mol. Mutagen. 56(4):347-55.

Incidence concordance

Adducts > Mutations



Swenberg et al. (2008) Chem Res Toxicol 21(1):253-265.

Incidence concordance: consolidated ENU data

ENU dose (mg/kg)	Alkyl adducts per nucleotide (x10E7) in male germ cells sampled 2 hrs post-exposure	Mutant frequency per nucleotide (x10E7) in sperm sampled > 42 days post-exposure	Mutant frequency per nucleotide (x10E7) in the offspring of males mated > 42 days post-exposure
0		1.48	0.37
10	7.6		
20	10.5		
25		2.5	
40	25.3		1.8
50		3.2	
75			11
80	39.7		10.5
100		19	16.5
150		22.1	19.5
160	79.4		25.3
200			24
250	189		33.2

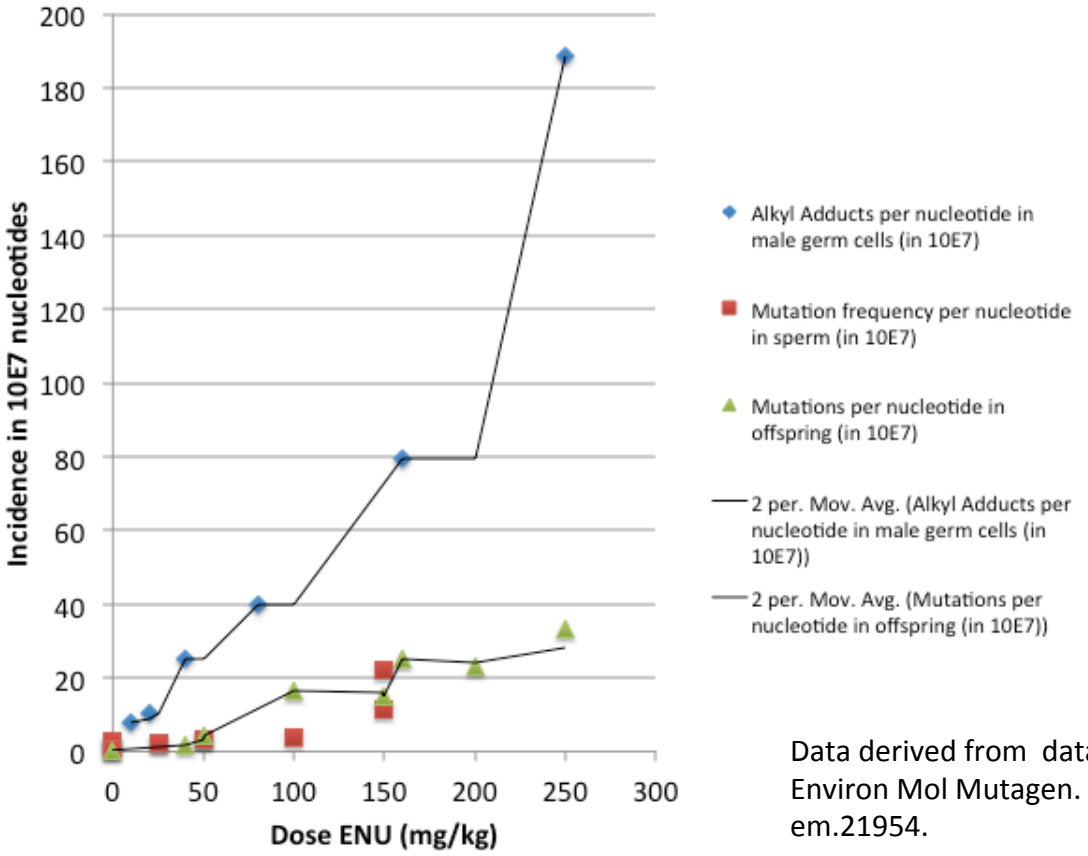
Adducts >>

Mutations in sperm ≥

Mutations in offspring

Data derived from Yauk et al. Environ Mol Mutagen. 2015. doi: 10.1002/em.21954.

Incidence concordance: graphical



Data derived from data in Yauk et al. Environ Mol Mutagen. 2015. doi: 10.1002/em.21954.

Consider uncertainties and inconsistencies

- Data gaps – empirical evidence comes almost entirely from the chemical ENU in germ cells.
- Some inconsistencies across experiments due to sub-standard protocols.
- Some inconsistencies in incidence concordance – not appropriate to quantitatively compare some endpoints.
- Differences across experiments in chemical delivery made comparisons difficult.

Assessment of overall weight of evidence

- AOP based on 32 high quality studies across three rodent species, flies and fish.
- No single study compared multiple events, but extrapolations could be made across studies.

Assessment of overall weight of evidence

Biological Plausibility: Is there a mechanistic relationship between KE_{up} and KE_{down} consistent with established biological knowledge?"

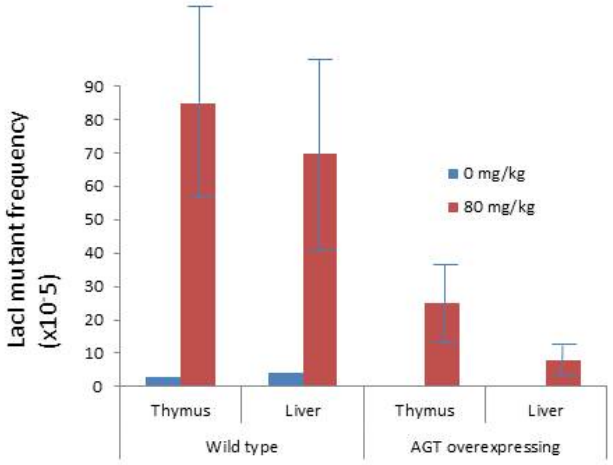
- Broad understanding and extensive knowledge for all of the key event relationships.
- Generally widely accepted.
- **Strong.**

Assessment of overall weight of evidence

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

- Evidence support that overcoming DNA repair is required for mutation to occur.
- Knock-down DNA repair, mutations increase; over-express DNA repair, mutations decrease.
- **Moderate.**

Figure derived from data in: Allay et al. (1999) Oncogene: 18(25):3783-3787.



Assessment of overall weight of evidence

Empirical support: Does empirical evidence support that a change in KE_{up} leads to an appropriate change in KE_{down}? Does KE_{up} occur at lower doses and earlier time points than KE_{down} and is the incidence of KE_{up} > than that for Ke_{down}?

- Strong primarily for the indirect (non-adjacent) KERs
- Use of OECD test guideline or 'gold standard' approaches in data collection.
- High quality studies.
- Overall: **moderate – strong.**

Conclusions

- Currently leveraging KEs and KERs to build other AOPs.
- https://aopkb.org/aopwiki/index.php/Aop:15
- See also: Yauk et al. Development of the adverse outcome pathway "alkylation of DNA in male premeiotic germ cells leading to heritable mutations" using the OECD's users' handbook supplement. Environ Mol Mutagen. 56(9):724–50. 2015 **AND** Yauk et al., Adverse Outcome Pathway on Alkylation of DNA in Male Pre-Meiotic Germ Cells Leading to Heritable Mutations. OECD Series on Adverse Outcome Pathways ISSN: 2415-170X. http://dx.doi.org/10.1787/2415170X