Adversary Outcome Pathway (AOP) for a Mutagenic Mode of Action for Cancer: AFB_1 and Hepatocellular Carcinoma (HCC)

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Abstract

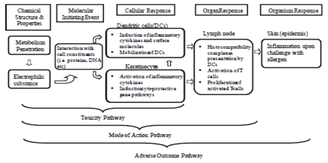
AOPs provide a framework to describe a sequence of measureable key events (KEs), beginning with a molecular initiating event (MIE), followed by a series of identified KEs linked to one another by KE Relationships (KERs), all anchored by a specific adverse outcome (AO). Each KE/KER is supported by data and evaluated against criteria to assess biological plausibility, weight/strength of evidence, specificity, and confidence. AOPs offer an approach to using toxicological data and predictive modeling to actualize use of mode-of-action (MOA) for such purposes as read-across, integrated approaches to testing & assessment, and risk assessment. Different applications will depend partly on the scientific confidence underpinning each KE/KER and the overall AOP. An OECD program encourages development of AOPs, with a wiki that allows for public review & comment to foster collaborations and broaden understanding & application of AOPs. Developing an AOP for a mutagenic MOA for cancer as a case study in the OECD program lays a path towards determination of such an MOA and its use in chemical assessment programs. Aflatoxin B1 (AFB1), with ubiquitous exposure and a rich database, was selected for this case study. AFB1 has been determined to induce hepatocellular carcinoma (HCC) *via* a DNA-reactive MOA in many species, including humans. The sequential KEs identified for AFB1 are as follows: pre-MIE: Hepatic metabolic activation; MIE: Formation of a pro-mutagenic DNA adduct (N7-AFB1-guanine or AFB1-FAPy); KE#1: Inadequate or mis-repair of the pro-mutagenic DNA adducts; KE#2: Induced mutation in critical gene(s); KE#3: Cellular proliferation and clonal expansion of mutant cells (pre-neoplastic lesions); AO: HCC. These KEs and the various KERs—both direct and indirect—are mapped out with supporting data for each. Assessment of quantitative aspects of the dose-response relationships for the KEs and KERs will support its use in quantitative risk assessment.

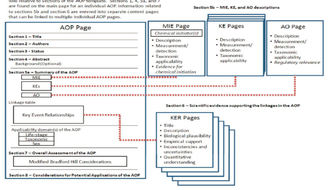
Background: OECD AOPs

Adverse Outcome Pathways (AOP) offers a way of organizing information for routine integration of mode of action (MOA) information into risk assessment.

- OECD initiated an AOP programme, published guidance & a handbook, opened-a public wiki.
- An AOP = sequence of key events from the exposure of an individual or population to a chemical substance through a final adverse (toxic) effect (Adverse Outcome [AO]) at the individual level (for human health) or population level (for ecotoxicological endpoints).
- Key events in an AOP are definable and make sense from a physiological and biochemical perspective.
- AOPs incorporate concepts of toxicity pathways and MOA for an adverse effect.
- AOPs may be related to other mechanisms and pathways as well as to detoxification routes and span multiple levels of biological organization; AOPs often start out being depicted as sequential processes.
- The detail and linearity characterizing the pathway between a molecular initiating event (MIE) and an AO within an AOP can vary substantially, both as a function of existing knowledge and assessment needs
- AOPs are modular and not ~~necessarily~~ tied to a particular chemical; they can branch, intersect, and converge with other AOPs, relying on the same KEs/KERs or arrive at the same AO *via* different paths.

AOP / MOA/Toxicity Pathways:
Comparison across scopes





OECD AOP Wiki:

Public, structured forum to capture and present AOPs for review, comment, and use, with Guidance and Handbook to advise on entry process and review. Available at aopkb.org

Background: AOP Projects

- USEPA proposed development of an AOP on mutagenic MOA for cancer to OECD
- Proposed to ACC ARASP as a dual project to develop two AOPs under OECD
  - Mutagenic MOA for cancer (USEPA & ex-NCTR scientists on the team);
  - 'Non-mutagenic' MOA for cancer from genotoxic chemicals (VAM & PO)
- Dual project ARASP sponsorship agreed in early 2014.
- Both AOP proposals accepted into OECD AOP programme.
- Planned completion/OECD wiki entry for these qualitative AOPs by 1Q2015
- Proposed AOP for Mutagenic MOA for AFB1 HCC presented here; available at https://aopkb.org/aopwiki/index.php/Aop:46

Objectives: AOP for Mutagenic MOA

1. OECD-agreed, quantitative AOP developed using AFB1 as example, which incorporates aspects of dose-response for different key events based on available data
2. Stepwise approach: qualitative AOP, then develop further with quantitative aspects

Key Considerations for Mutagenic MOA

- Mutagenic MOAs are distinguished from other cancer MOAs in that the chemical induces mutations in genes that are involved in the etiology of the cancer. Non-mutagenic MOAs are those where the chemical causes proliferation of cells with existing mutations, or in some other way promotes the growth of cancer gene mutant cells, to result in tumors.
- It is important to note that all cancers involve both an increase in cells containing mutations in cancer critical gene(s) and cell proliferation. While mutation plays a key role in both MOAs, it is an early, driver event in a mutagenic MOA, while it may be a later event in a non-mutagenic MOA.
- To establish a mutagenic MOA, it is necessary to determine the (the key events both in terms of temporality and dose-response concordance between the increase in the number of mutant cells, cell proliferation, the appearance of any pre-neoplastic lesions, and ultimately tumor occurrence.
- Useful MOA data include the chemical's ability to cause mutations, the temporality of those induced mutations, and the type of mutations that the chemical induces. The ability of the chemical to induce the type(s) of mutations seen in the majority of the specific tumors adds greatly to the weight of evidence.
- Positive results in any one of a number of standard gene mutation assays is not sufficient. Furthermore, the presence of mutations in the tumor tissue does not provide definitive information on MOA.
- A high frequency of tumors with specific mutations (*e.g.*, AFB_1) provides a hypothesis for further evaluation.
- The most definitive level of proof that a chemical acts *via* a mutagenic MOA is the demonstration that the chemical can induce the specific cancer gene mutation(s) observed in a majority of the specific tumors, and that the formation of this mutation is an early event in the sequence of key events. . Such information on specific chemical-induced mutations in cancer critical genes is uncommon, and currently, no such information is available for AFB_1.

Key Considerations for AOP on AFB_1 Mutagenic MOA

Starting Point: HESI DNA Adduct Committee Case Study on AFB_1

Pottenger *et al.*, *Crit. Rev. Toxicol.*, 2014

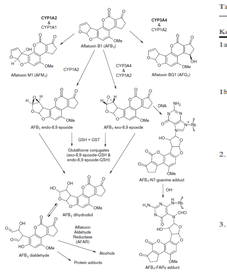


Table 7. Summary of the MOA key events tables for aflatoxin B_1, vinyl chloride, and tamoxifen.

Key events	Biomarker or bioindicator of key event	Aflatoxin B_1
1a. Exposure of target tissue.	Analytical measure of parent chemical, active metabolites or active radicals, or biomarkers of their presence in target tissue.	CYP3A4 metabolizes to AFB_1-exo-8,9-epoxide that covalently binds DNA at N7-guanine. Measured in liver.
1b. Reactions with other non-DNA cellular targets that have impact on adduct fate.	Cytoplasmic and/or nucleophilic binding of electrophilic species.	Hydrolysis of AFB_1-8,9-epoxide leads to diol; rearranges to dialdehyde; then reacts with protein lysine residues.
2. Reaction with DNA in target cells to produce promutagenic damage.	[Increase in] Chemical-specific promutagenic DNA adducts in target tissue.	AFB_1-N7 adduct and AFB_1-FAPy produced in livers of humans and rats exposed to AFB_1.
3. Misreplication on damaged DNA template or misrepair of DNA damage.	Chromosomal alterations and gene mutations.	Frequency of somatic mutations increased in *HPRT* gene of rats and humans exposed to AFB_1.
4. Mutations in critical genes in replicating target cell.	Mutations in oncogenes or tumor suppressor genes	*p53* mutations in liver.
5. Mutations in critical genes result in enhanced DNA/cell replication.	Mitotic figures, ^{3}H-thymidine, BrdU or proliferating cell nuclear antigen [PCNA], Ki67IHC	No AFB_1-specific data available; p53 mutations associated with inhibition of apoptosis.
6. New cell replication leads to clonal expansion of mutant cells.	Histological identification of growth of abnormal cells of similar morphology/type	Appearance of GSTP-positive foci in the livers of rats and other species

It is clear that (1) AFB_1 can induce mutations in gene mutation assays; (2) AFB_1 induces HCC in a variety of species, including humans; (3) there is a high frequency of a specific cancer gene mutation (codon 249 of p53) in the human HCCs found in people in regions with high AFB_1 exposure; and (4) the type of mutation seen in the human tumor (codon 249 of p53) is the same type of mutation that is seen in the surrogate gene mutation assay. Thus there is a high level of confidence that, AFB_1 has a mutagenic MOA for HCC in humans

DRAFT AOP Key Events (1/2015)

- **Pre-MIE**: Activation to exo-epoxide by hepatic metabolism
- **MIE:** Formation of pro-mutagenic DNA adducts
- **KE#1**: Insufficient repair or mis-repair of pro-mutagenic DNA adducts
- **KE#2**: Induction of mutation in critical gene(s)
- **KE#3**: Proliferation/clonal expansion of mutant cells (pre-neoplastic lesions/altered hepatic foci (AHF))
- **AO**: Hepatocellular carcinoma (HCC)

DRAFT AOP Key Event Relationships (KERs) (1/2015)

Direct KERs:

Pre-MIE → MIE

MIE → KE#1

KE#1 → KE#2

KE#2 → KE#3

KE#3 → AO

Indirect KERs:

MIE → KE#3

MIE → AO

KE#2 → AO

AOP Draft Concordance Tables: Temporality and Dose-Response

Hypothetical Ideal Dose- and Temporal-Concordance Table

Dose ↓ / Increasing Time →

	KE (ppb in diet)	Metabolic Activation	Pre-mutagenic DNA Adducts	Insufficient/Mis-repair of pro-mutagenic DNA adducts	Induced mutation in critical gene(s)	Clonal expansion of mutant cells (pre-neoplastic lesions)	Hepatocellular Carcinomas
	0	-	-	-	-	-/+ (0.06)	--- (0)
	1	+	++	+	+	+ (0.32)	-/+ (0.09)
	5	++	+++	++	++	+ (0.23)	-/+ (0.05)
	15	+++	++++	+++	+++	++(0.62)	+ (0.19)
	50	++++	+++++	+++++	+++++	++ (0.60)	+++ (0.8)
	100	+++++	++++++	+++++	+++++	++ (0.43)	++++ (1.0)
	Reversibility:						
	**AFB_1 w/oOltipraz	+++	+++	++	++	-/+	-/+
	**AFB_1 w/Oltipraz	+++	+	+	+	-/+	-/+
	***AFB_1 w/o CDDO-Im		+++			+++++	++++
	***AFB_1 w/ CDDO-Im		+			+	---

DRAFT AFB_1 Data-based Reversibility Concordance Table

EXP Details	KE / Dose	Met. Activ'n	Pro-mutagenic DNA Adducts	Insufficient /Mis-repair pro-mutagenic DNA adducts	Induced mutation in critical gene(s)	Clonal expan-sion of mutant cells (pre-neo-plastic lesions)	Hepatocellular Carcinomas
AFB_1 w/o Oltipraz^	0.25 mg AFB_1/kg		+++			-/+ 13%	-/+ 11%
AFB_1 w/ Oltipraz^	0.25 mg AFB1/kg + 0.075% Oltz in diet		+ (75% reduction)			-/+ 4%	-/+ 0%
AFB_1 w/o CDDO-Im^^	200 mg/kg		+++			+++++ (23/23)	++++ (96%;22/23)
AFB_1 w/ CDDO-Im^^	200 mg/kg + 16.2 CDDO-IM mg/kg		+ (N7: 50-80% reduction FAPy: 50-70% reduction)			+ (3/20)	--- (0/20)

DRAFT AFB_1 Data-based Dose- and Temporal-Concordance Table

Dose ↓ / Increasing Time →

	KE (ppb in diet)	Met. Activ'n	Pro-mutagenic DNA Adducts	Insufficient/Mis-repair of pro-mutagenic DNA adducts	Induced mutation in critical gene(s)	Clonal expansion of mutant cells	Hepatocellular Carcinomas
	0					-/+ (0.06)*	--- (0)
	1					+ (0.32)	-/+ (0.09)
	5					+ (0.23)	-/+ (0.05)
	15					++(0.62)	+ (0.19)
	50					++ (0.60)	+++ (0.8)
	100					++ (0.43)	++++ (1.0)
Rat liver m/k ip	10		0.37 pmol adduct/mg DNA				
Single doses	25		0.48 pmol adduct/mg DNA				
	65		1.47 pmol adduct/mg DNA				
	160		3.93 pmol adduct/mg DNA				
	390		8.54 pmol adduct/mg DNA				
	1000		16.48 pmol adduct/mg DNA				
Lac/iBig Blue Rat ip	0.25 mg/kg				500 mutants/ 10^6 (in surrogate genes)		
cII/Big Blue mouse i..p.	6 mg/kg (neonate)				900 mutants/ 10^6 (in surrogate genes)		
	6 mg/kg (adult)				No increase		
	60 mg/kg (adult)				No increase		

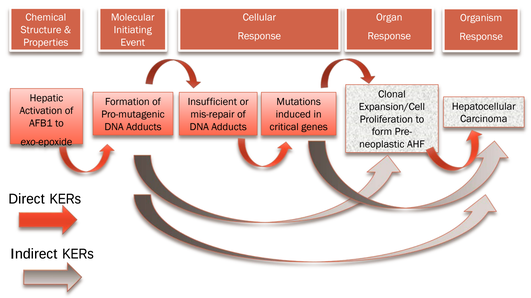
- Chemical-specific data to support all key events are not available for AFB_1, one of the most studied human carcinogens.
- In particular, additional dose-response data on adduct levels and induction of critical gene mutation(s) would strengthen the AOP on a mutagenic MOA
- However, a preponderance of less direct data and the biological plausibility of steps, coupled with the chemoprevention data, support a high level of confidence in a mutagenic MOA for AFB_1-induced Hepatocellular Carcinoma

Draft Evidence Tables

DRAFT AFB1 ~~Evidence~~ Evaluation Table for KERs: Initial Section

1. Support for Biological Plausibility of KERs	Defining Question	High (Strong),	Moderate	Low (Weak)
	a) Is there a mechanistic (i.e., structural or functional) relationship btwn KE_{up} and KE_{down} consistent with established biological knowledge?	Extensive understanding of the KER based on extensive previous docu-mentation and broad acceptance (e.g., mutation leading to tumors) -Established mechanistic basis	The KER is plausible but scientific understanding is not completely established.	Only limited or indirect evi-dence for KER (i.e., based on empirical support, only (See 3.)
Hepatic metabolic activation directly to formation of pro-mutagenic DNA adducts	Biological Plausibility of the pre-MIE => MIE is Strong Rationale: Long-established knowledge of the metabolism of AFB1 to specific reactive electrophiles that form pro-mutagenic DNA adducts.			
Pro-mutagenic adduct formation directly to Insufficient / Mis-repair of pro-mutagenic adducts	Biological Plausibility of MIE => KE1 is Strong. Rationale: not much direct empirical support but strongly accepted.			
Insufficient/Mis-repair directly to Induced mutations in critical gene	Biological Plausibility of KE1 => KE2 is Strong Rationale: Long established knowledge : Empirical data from yeast with defective repair systems leads to increased mutations—infer increased mutations in critical genes			
Induced mutations directly to clonal expansion of mutant cells	Biological Plausibility of KE2 => KE3 is Strong Rationale: Necessary. Based on chemoprevention studies, HBV, and the plethora of initiation-promotion studies.			
Clonal Expansion directly to HCC	Biological Plausibility of KE3 => KE4 is Strong Rationale: Long established knowledge; the plethora of longer term initiation-promotion studies provide much evidence of the link from clonal expansion of foci to HCC.			
Indirect: Pro-mutagenic adduct to AHF	Biological Plausibility of MIE => KE3 is Strong Rationale: Based on the relationship of adducts to AHF, data on chemopreventive agents that specifically decrease adduct formation also decrease the occurrence of AHF.			
Indirect: Pro-mutagenic adduct to HCC	Biological Plausibility of MIE => AO is Strong Rationale: The relationship of adducts to HCC depends of two well-established relationships between adducts and AHF and between AHF &HCC. Because of these well-established relationships, the biological plausibility is judged to be strong.			
Indirect: Mutations to HCC	Biological Plausibility of KE2 => AO is Strong Rationale: The relationship of mutations to cancer is well-established. However, what is not clear is whether mutations observed early in the cancer process are the same as those observed in tumors. However, the relationship of adducts to AHF and AHF to tumors are both strong , thus this indirect KER is also strong.			

AOP Flow Scheme (1/2015)



Sponsored by ACC Center for Advancing Risk Assessment Science and Policy (ARASP)

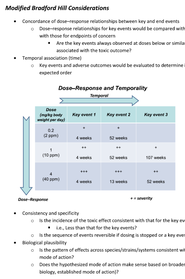
Considerations/Challenges

Planned Uses of AOPs:

- Predictions based on HTP data present considerable challenges
- Screening/Prioritization: good degree of acceptance
- Hazard identification: likely good use but not an end in itself (RISK!)
- Dose-response: opportunity to model individual KERs
- IATA Development: good use in identification of gaps/useful research
- ***Quantitative Risk Assessment: application of AOPs/MOAs in chemical assessments supporting regulatory decision-making…***

➢ Quantitative AOPs: Introduction of Dose-Response *via* KERs

➢ Hill Criteria: Biological Plausibility is a Critical Aspect



Modified & proposed by B. Meek *et al.*, 2014

➢ **Determination of Scientific Confidence: KEY ISSUE**

Scientific Confidence Framework for AOPs
(adapted from Cox et al. 2014 Reg Tox Pharm)

1	**Develop the AOP**
2	**Develop new (or map existing) specific assays to key events within the AOP**
3	Conduct (or document) **Analytical Validation** of each assay
4	Develop new (or map existing) models that predict a specific key event from one or more pre-cursor key events. (The input data for the prediction models comes from the assays described in Steps 2 and 3 above.)
5	Conduct (or document) **Qualification** of the prediction models
6	**Utilization**: defining and documenting where there is sufficient scientific confidence to use one or more AOP-based prediction models **for a specific purpose** (e.g., priority setting, chemical category formation, integrated testing, predicting *in vivo* responses, etc.)
7	Dissemination of all necessary datasets, model parameters, algorithms, etc. to enable fully independent verification and peer review. This will also enable other investigators to more readily add datasets and improve the AOP.

This framework was presented at 2014 SOT : "Improving the Development of Adverse Outcome Pathways: Lessons Learned from the AhR Rodent Liver Tumor and AhR Avian Teratogenicity/ Embryolethality AOPs" The Toxicologist, Abstract 2253, page 602.

Proposal from BIAC (R. Becker/ACC)

➢ **Application of OECD Approval Process: still under development!**

➢ **Address/Incorporate public comment from public Wiki: TBD**