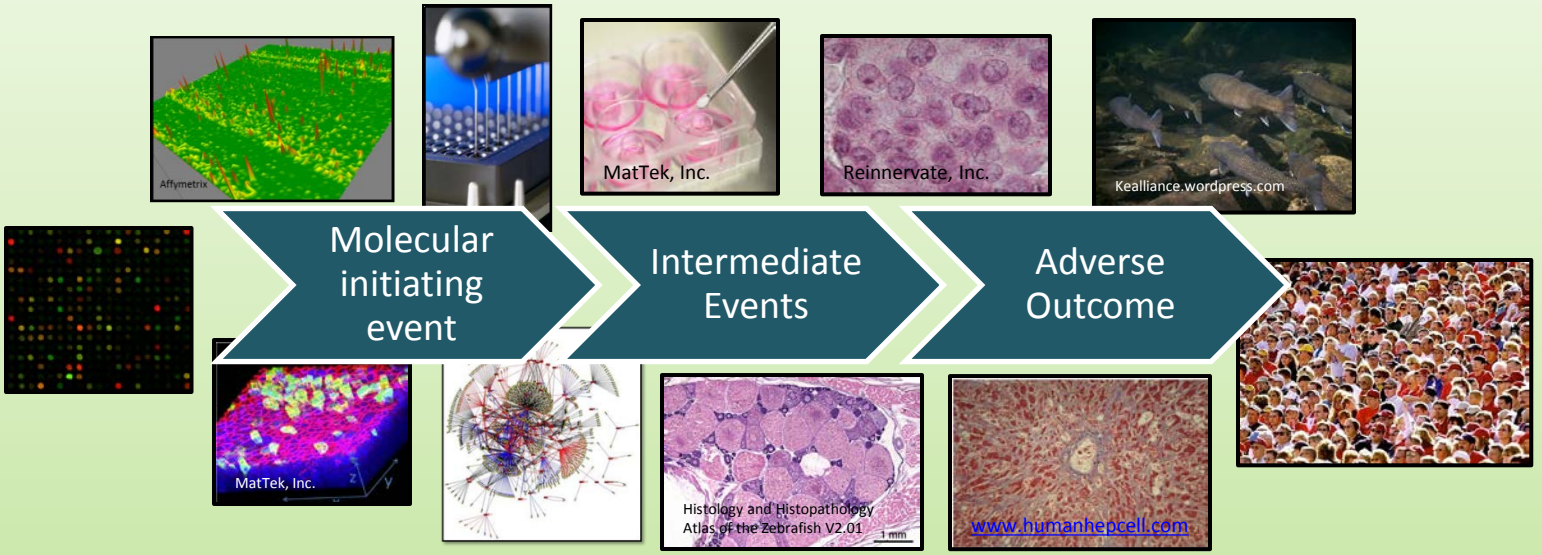
Building shared experience to advance practical application of pathway-based toxicology: repeat-dose liver toxicity



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HUMAN TOXICOLOGY PROJECT **CONSORTIUM**

Workshop: Building Shared Experience to Advance Practical Application of Pathway-Based Toxicity: Liver Toxicity Mode-of-Action

January, 2013

23 participants:

- academia, industry, regulatory, research institutes
- EU, US, India

Goals:

- inform the process of pathway development
- make recommendations for the use of pathways in safety decisions
- using two prototype hepatotoxicity pathways (from the JRC)

Publications

- Willett, C. et al. 2014. **Pathway-based toxicity: history, current approaches and liver fibrosis and steatosis as prototypes**. ALTEX. 2014 Jun 23. doi: http://dx.doi.org/10.14573/altex.
- Willett, C. et al., 2014**. Building Shared Experience to Advance Practical Application of Pathway – Based Toxicology: Liver Toxicity Mode-of-Action**. ALTEX. 2014 Feb 17. doi: http://dx.doi.org/10.14573/altex.

“Adverse Outcome Pathways”

- concept from ecotoxicology as a way of addressing uncertainty in risk assessment required by new legislation for an increasing number of chemicals and endpoints
- builds on the MoA concepts and includes NRC “toxicity pathways”
- allows for the integration of all types of information at different levels of biological organization, from molecular to population level
- provides a rational, biologically based argument (or series of hypotheses) to predict the outcome of an initiating event
- the level of detail and certainty of the relationships is related to the AOP’s applicability to hazard and risk assessments

Ankley G.T., Bennett R.S., Erickson, R.J. et al. (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29 (3), 730–741.

Organization for Economic Cooperation and Development (OECD) (2011). Report of the Workshop on Using Mechanistic Information on Forming Chemical Categories ENV/JM/MONO(2011)8. 18-May-2011 176 pp.

OECD AOP Guidance

Three basic elements:

1) Molecular Initiating Event →2) Intermediate Event(s)→3) Adverse Outcome

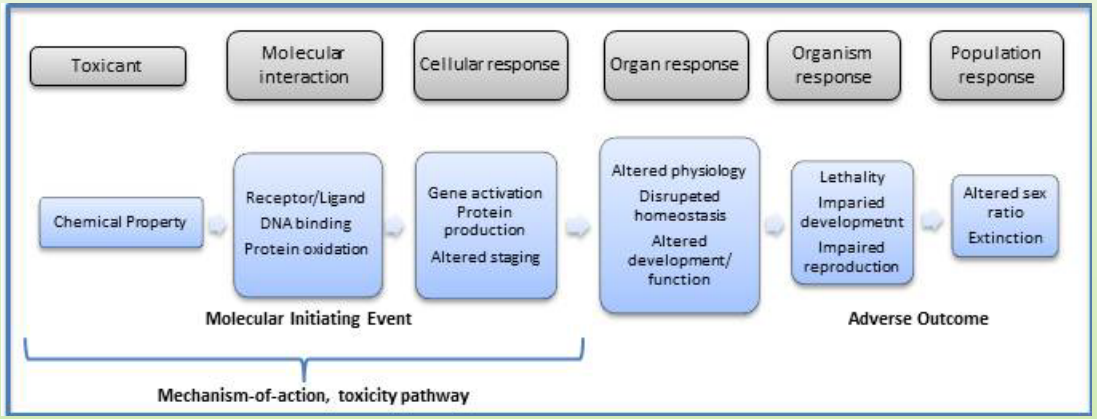


Figure 1 from Willett, C., 2014. Adverse Outcome Pathways: Development and Use in Toxicology. In: Wexler, P. (Ed.), Encyclopedia of Toxicology, 3rd edition vol 1. Elsevier Inc., Academic Press, pp. 95–99.

Guidance and Template published in 2013

AOP Handbook is under development

- Emphasis on Key Events and Key Event Relationships

Organization for Economic Cooperation and Development (OECD)(2013). Guidance Document on Developing and Assessing Adverse Outcome Pathways. Series on Testing and Assessment No. 184.

Complementary Research in Pathway Development

1. Johns Hopkins Center for Alternative to Animal Testing
   - Pathways of Toxicity
   - Evidence Based Toxicology
2. The Hamner Institutes
   - PPARα and Estrogen Receptor signaling pathways as prototypes
   - Including dose-response extrapolation modeling
3. SEURAT-I: EUR 50 million FP7 joint EC – Cosmetics Europe project to address "Safety Evaluation Ultimately Replacing Animal Testing"
   - Repeat-dose, liver toxicity
   - 6 research projects including:
     - COSMOS – integrated *in silico* models
     - ToxBank - database to support SEURAT projects

Current Uses of MOA in Regulatory Decisions

Examples from US EPA

- Chemical prioritization
  - disinfection byproducts → carcinogenicity
  - estrogen receptor expert system for antimicrobial pesticide and pesticide inert ingredients
- Use in read-across for industrial chemicals
- Development of targeted testing strategies
  - thyroid and developmental neurotoxicity
- Development of predictive tools
  - oncologic expert system → carcinogenic potential
- Use in cumulative risk assessment:
  - common mode-of-action for large chemical categories
  - exposure scenarios
  - selection of common endpoints
  - toxic potencies and points of departure (PODs) for chemicals of interest, etc.

Current Applications (example 1)

Skin sensitization – human health

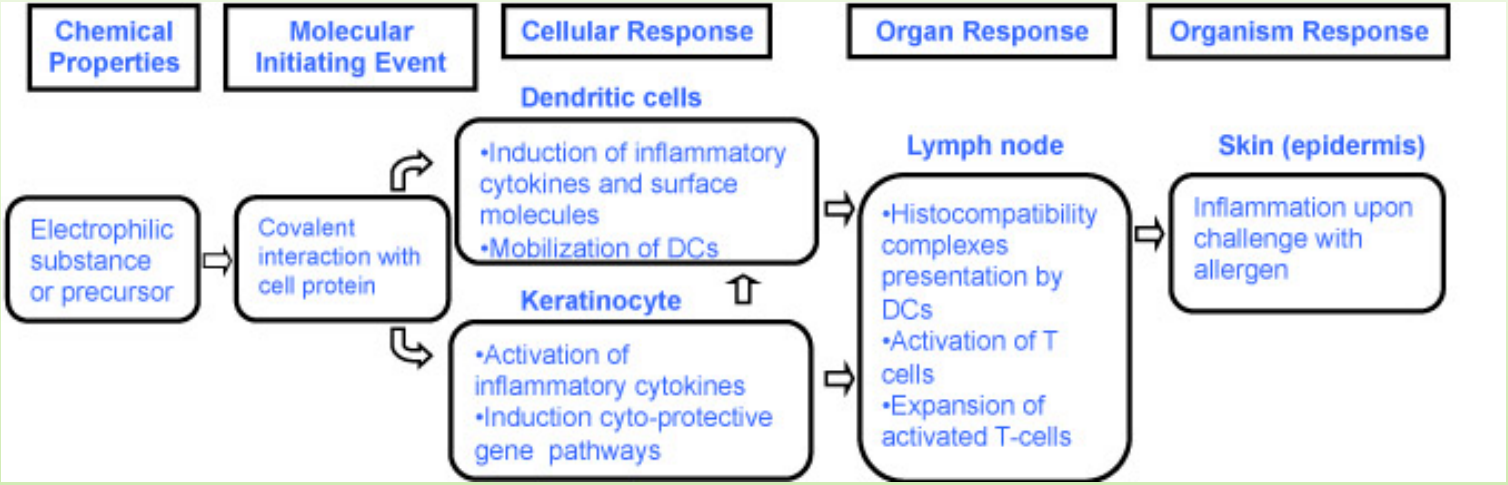
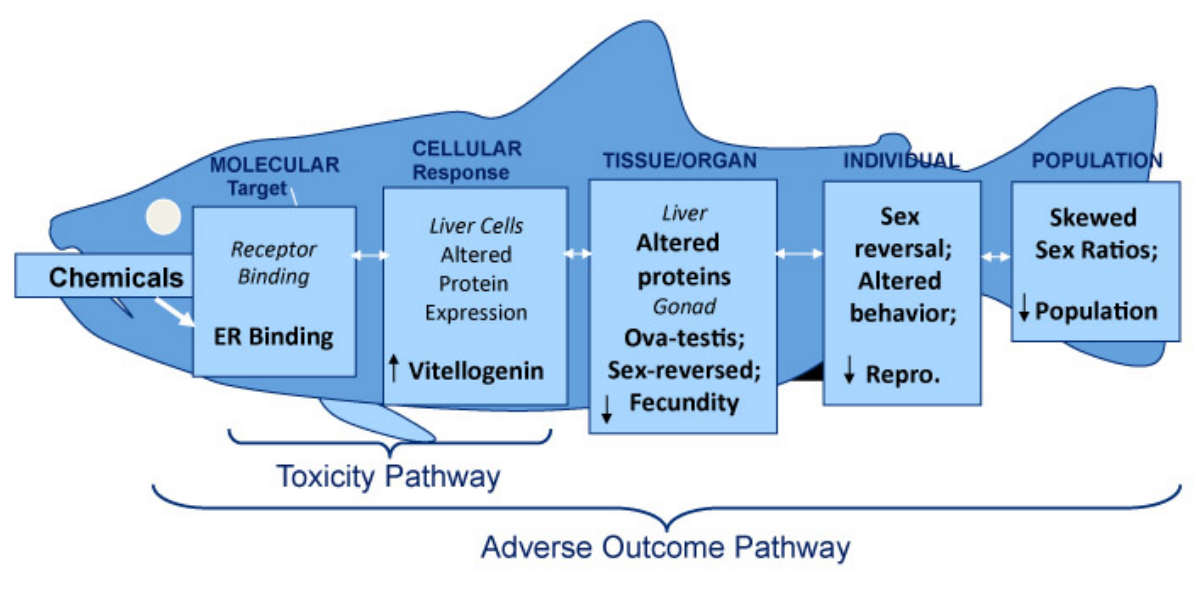


Figure 3 from: OECD. 2012. The Adverse Outcome Pathway for Skin Sensitization Initiated by Covalent Binding to Proteins.

1. Within the context of exposure
2. Focus is on human biology
3. In this case, using a small number of key events to predict AO
4. Industry emphasis on use in risk assessment decisions for consumer safety

Current Applications (example 2)

Estrogen receptor-mediated reproductive impairment: Human and ecological health



OECD (2009). Report of the Expert Consultation to Evaluate an Estrogen Receptor Binding Affinity Model for Hazard Identification. Series on Testing and Assessment, No. 111.

1. Well described MIE
2. Assays throughout the pathway from MIE to AO at population level
3. Being used to screen large chemical inventories for priority chemicals

Liver Toxicity as Case Study

1. Two main types: direct (apoptosis, necrosis) or indirect (e.g. cholestasis) cytotoxicity
2. Can involve inflammation and the immune system
3. Chronic liver injury can lead to:
    - fatty acid accumulation (steatosis)
    - ***cholestasis***
    - hepatitis (including granuloma formation)
    - accumulation of phospholipids (phospholipidosis)
    - ***fibrosis***
    - cirrhosis
    - neoplasia

Drug-Induced Liver Injury (DILI)

- Accounts for 50% of acute liver failure
- Is rare: 1 in 10,000 to 100,000 treated patients
- Would require clinical trials of 30,000 patients vs. 2 – 5K
- Is not well predicted by animal models
- Is due to unforeseen off-target activity or individual susceptibility
- Human health risk assessments for 20% of 544 environmental chemicals in EPA's Integrated Risk Assessment System (IRIS) database are based on liver toxicity

→ need for a better understanding of underlying mechanisms of liver toxicity in order to better predict potential occurrence

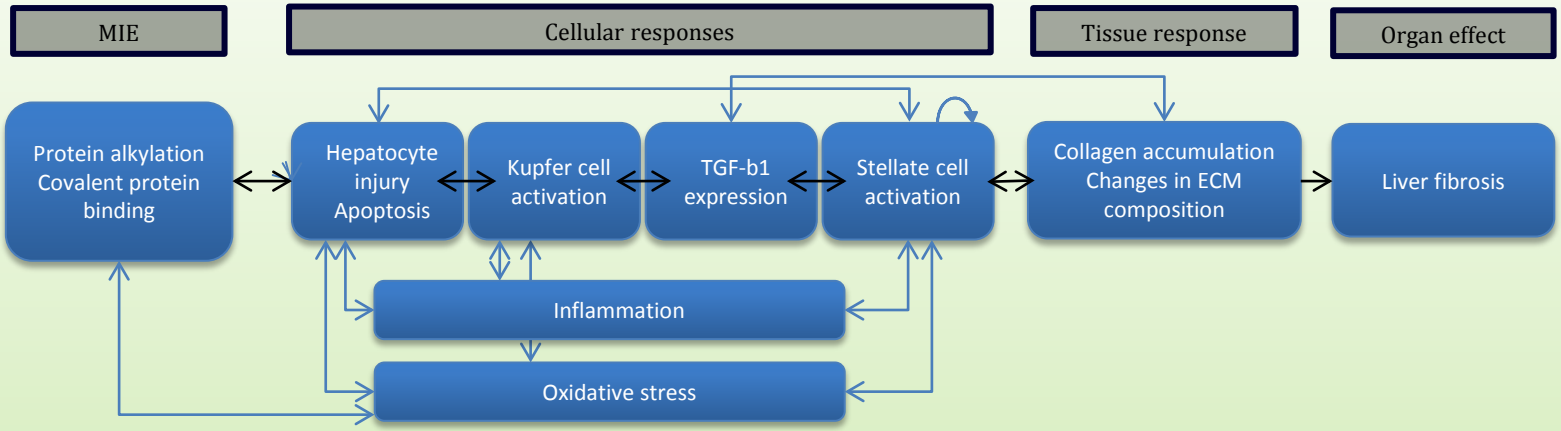
Liver Toxicity: Prototype Pathways

SEURAT-1: fibrosis and steatosis

- Common adverse outcomes associated with chronic liver injury
- Protein alkylation as common MIE for two reference chemicals for liver ***fibrosis***
  - allyl alcohol
  - carbon tetrachloride
- Nuclear receptor binding as MIE for ***steatosis***
  - LXR chosen from six relevant NRs
  - LXR agonist T0901317 reference chemical for liver steatosis
- Pathway development followed OECD guidance
  - systematic literature review
  - template
- OECD evaluation guidance

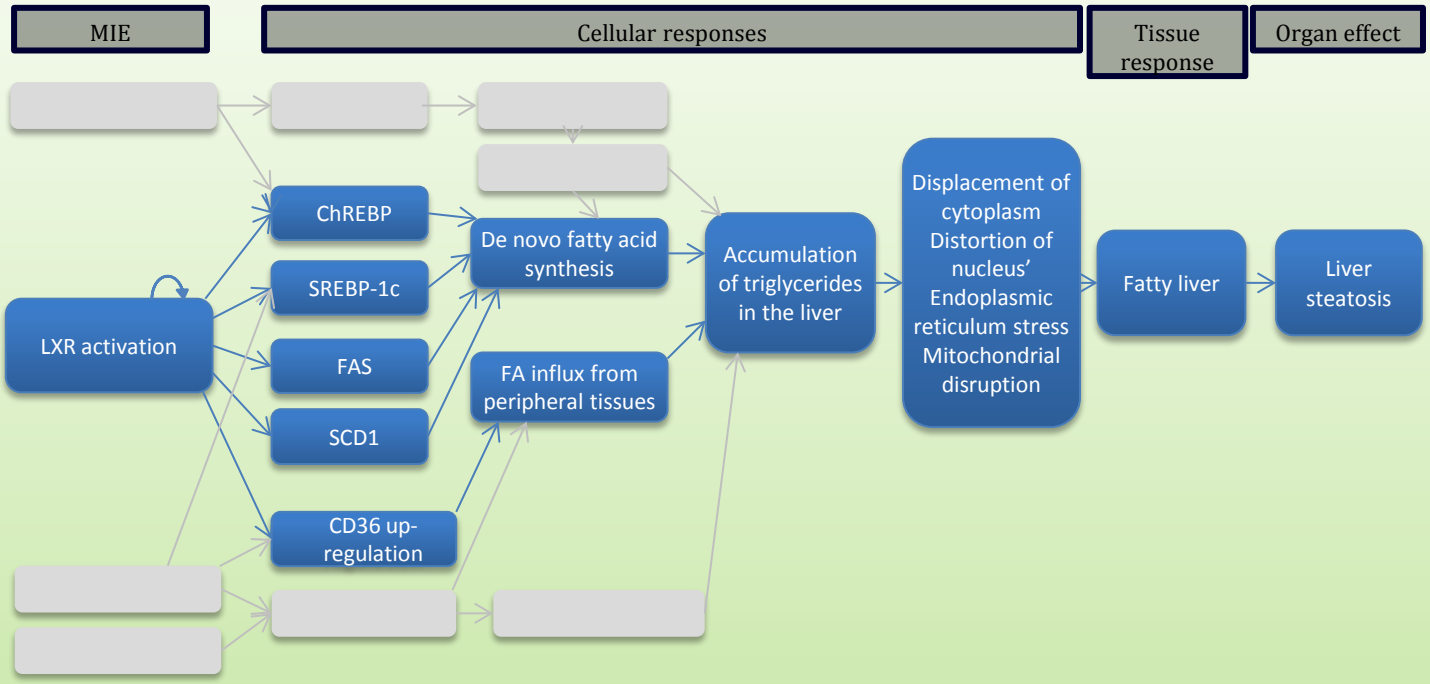
Landesmann, B., Goumenou, M., Munn, S., and Whelan, M. (2012). *Description of Protoype Modes-of-action related to repeated dose toxicity.* JRC Scientific and Policy Report 75689 DOI: 10.2788/71112.

Fibrosis



- MIE: well documented, sufficient for read-across (applicability may be narrow)
- Apoptosis is an intermediate event, but not all causes of apoptosis lead to fibrosis: ***rate*** is critical
- Further characterization of IEs is necessary for ID of KEs
- Some temporal understanding of IEs; dose-response data needed
- Good to compare other protein alkylating chemicals that do not cause fibrosis
- Is a solid framework for further ID of IEs and KEs

Steatosis



- Potentially several competing pathways, including multiple NR-dependent pathways
- Microvesicular steatosis is very serious, macrovesicular steatosis is associated with many physiological syndromes and are caused by separate mechanisms
- Steatosis pathway should have separate branches for micro- and macro-vesicular
- This is a good start but narrow and needs development

Problem Areas Identified in Prototype Development

- Systematic reviews and data collection
  - How to capture relevant information
  - How to address gaps
- Incorporation of feed-back loops and other forms of homeostasis
- Identification of key events from the set of intermediate events
- Incorporation of quantitative (dose-response) relationships

Summary: General Principles

1. Use of an AOP is critical aspect of design
   - e.g. for QSAR modeling focus is on MIE and upstream events
2. Pathways are not linear: the usefulness of forcing an AOP to be a single MIE leading to a single AO remains to be seen
   - may prove useful initially
   - branch points will be identified to link pathways
   - helpful to group pathways with common anchors
3. Different uses require different levels of detail
   - e.g. quantitative information about key event relationships is necessary for use in risk assessment
4. Biomarkers can both inform pathways and be supported by pathways
5. It is likely that assays needed to build the pathways are different from those used to query the identified key events for chemical evaluation
6. In general, initial key events are chemical-specific, whereas later intermediate events are disease-specific

Critical Needs for Use in Safety Assessment

- Need to describe the causal relationships between key events
- Quantitative information is necessary for use in risk assessment
  - to understand degree of perturbation required to provoke the next step
  - to differentiate between conditions within homeostatic bounds from adversity
  - to determine likelihood of thresholds
- Once a pathway is quantified, it may be possible to use upstream event as point of departure
- Need to describe context surrounding AOP
  - biological specificity of pathway: species, sex, developmental stage
  - chemical specificity: ADME
  - link to consumer/patient/environmental exposure

Summary

- Best to start with what we know, build pathways using case studies
  - Begin linear or near linear
  - Prototypes are essential for building real experience
  - Consulting a broad spectrum of expertise is critically important
  - Collect related AOPs and connect though shared IMs
  - → network of interrelated pathways

A strength of the AOP approach is that you can take information from many different sources, and ultimately bridge the needs of the different communities

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Thank you!

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