Joint Research Centre

The European Commission's in-house science service

www.jrc.ec.europa.eu

Adverse Outcome Pathways: From Research to Regulation

September 3rd 2014
National Institutes of Health
Bethesda

Serving society
Stimulating innovation
Supporting legislation

AOP development: after the heights of the mountains – the hardship of the plains; the example of liver fibrosis

"Die Mühen der Berge haben wir hinter uns,
vor uns liegen die Mühen der Ebenen."
B. Brecht

Brigitte Landesmann

AOP development
protein alkylation leading to liver fibrosis:

- Why
- How
- Difficulties
- Open questions
- Further plans

AOP development
protein alkylation leading to liver fibrosis:

- **Why**
- How
- Difficulties
- Open questions
- Further plans

SEURAT-1

"**S**afety **E**valuation **U**ltimately **R**eplacing **A**nimal **T**esting"

The **SEURAT vision** is to fundamentally change the way we assess the safety of chemicals, by superseding traditional animal experiments with a predictive toxicology that is based on a comprehensive understanding of how chemicals can cause adverse effects in humans.

The **SEURAT strategy** is to adopt a toxicological AOP framework to describe how any substance may adversely affect human health, and to use this knowledge to develop complementary theoretical, computational and experimental (in vitro) models that predict quantitative points of departure needed for safety assessment.

proof-of-concept on three levels

- **Theoretical level:** description of selected AOPs to a sufficient extent so that they can be used as blueprints for system design
- **Systems level:** demonstration of integrated systems for associating a chemical with an AOP category and for predicting the points of departure of a pathway of toxicity
- **Application level:** use of the information derived from predictive systems to support safety assessment and decision-making processes.

Liver - Fibrosis

The liver is the potential most affected organ by toxicants, due to its role of xenobiotic biotransformation.

Hepatotoxicity in general therefore is of special interest for human health risk assessment.

Liver fibrosis in particular is a typical result of chronic or repeated-dose toxic injury and an important human health issue associated with chemical exposure.

A sufficiently detailed description of the AOP to liver fibrosis might support chemical risk assessment by indicating early (upstream) markers for downstream events and facilitate a testing strategy without the need for a sophisticated cell model.

AOP development
protein alkylation leading to liver fibrosis:

- Why
- **How**
- Difficulties
- Open questions
- Further plans

Methodology:

- Selection of the Adverse Outcome (top down approach)
- Selection of the Molecular Initiating Event
- Study of the relevant biology and physiology
- Identification of Key Events
- Description of Key Event Relationships
- Graphic representation of the AOP
- Evaluation
- Report / Insertion into the AOP Wiki

Methodology:

- Selection of the Adverse Outcome
- **Selection of the Molecular Initiating Event**
- Study of the relevant biology and physiology
- Identification of Key Events
- Description of Key Event Relationships
- Graphic representation of the AOP
- Assessment
- Report / Insertion into the AOP Wiki

Selection of the Molecular Initiating Event

AOPs are not chemical specific; chemicals are necessary to get data and for understanding the patterns of response

The two SEURAT-1 reference chemicals ("Gold Compounds") for liver fibrosis are allyl alcohol and carbon tetrachloride.
Their common MIE is **protein alkylation**

Chemical properties:
A compound or its metabolite that is capable of alkylating proteins.

Methodology:

- Selection of the Adverse Outcome
- Selection of the Molecular Initiating Event
- **Study of the relevant biology and physiology**
- **Identification of Key Events**
- Description of Key Event Relationships
- Graphic representation of the AOP
- Assessment
- Report / Insertion into the AOP Wiki

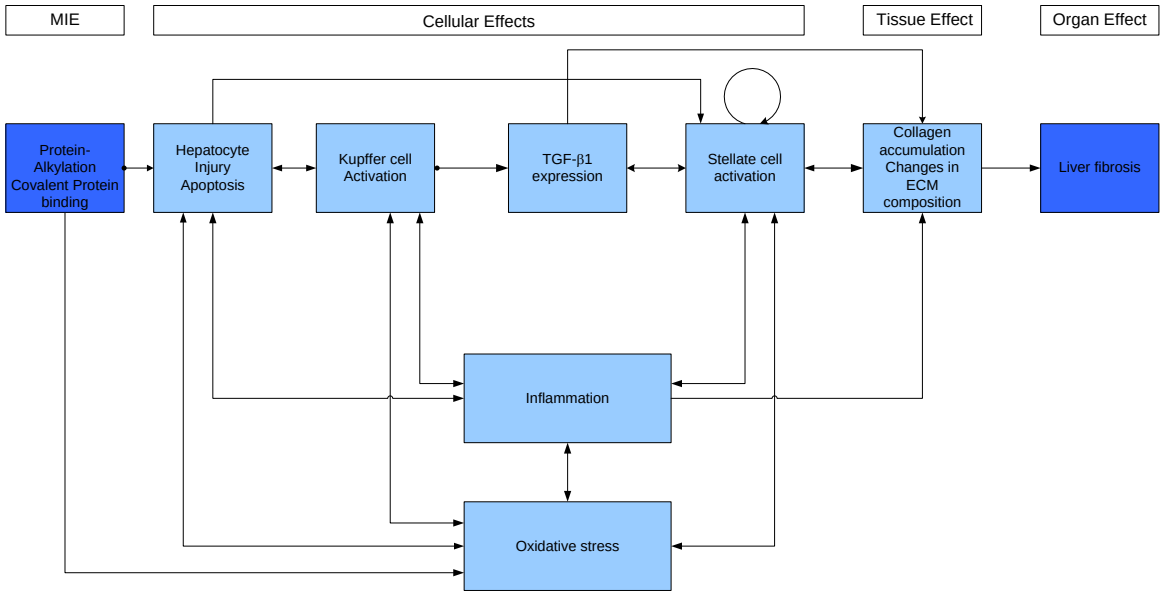
Identification of Key Events:

Literature study of relevant biology and physiology

A systematic literature search with emphasis on key studies and review papers and consecutive analysis according to different levels of biological organization.

Choice of the relevant level of detail

Key events

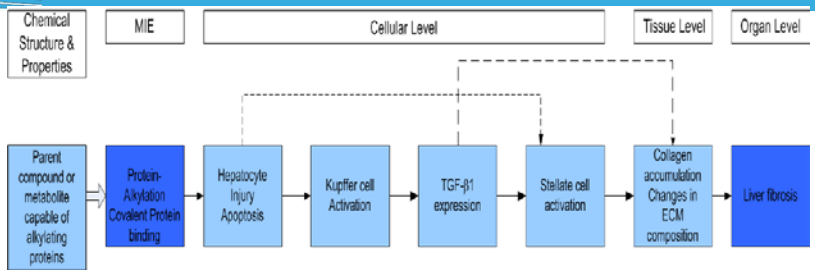


- Hepatocyte injury/death
- Kupffer cell activation
- TGF-β 1 expression
- Stellate cell activation
- Collagen accumulation
- Inflammation
- Oxidative stress

Methodology:

- Selection of the Adverse Outcome
- Selection of the Molecular Initiating Event
- Study of the relevant biology and physiology
- Identification of Key Events
- **Description of Key Event Relationships**
- Graphic representation of the AOP
- Assessment
- Report / Insertion into the AOP Wiki

Description of KERs

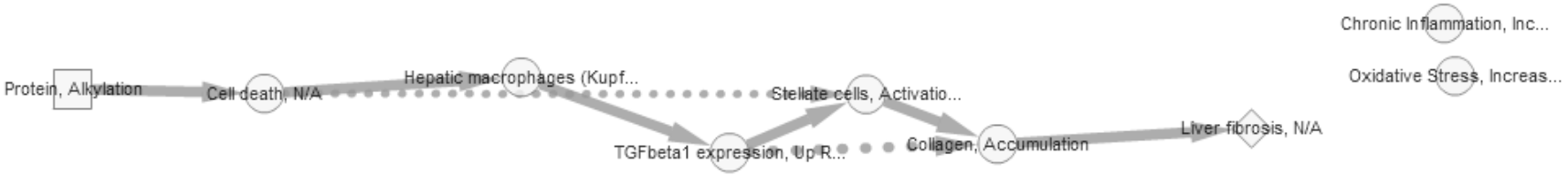


Relationships Among Key Events and the Adverse Outcome

Add record to table

Step	Event	Description	Triggers	Weight of Evidence	Quantitative Understanding	
1	Protein, Alkylation	Directly Leads to	Cell death, N/A	Strong		
2	Cell death, N/A	Directly Leads to	Hepatic macrophages (Kupffer Cells), Activation	Strong		
3	Cell death, N/A	Indirectly Leads to	Stellate cells, Activation	Moderate		
4	Hepatic macrophages (Kupffer Cells), Activation	Directly Leads to	TGFbeta1 expression, Up Regulation	Strong		
5	TGFbeta1 expression, Up Regulation	Directly Leads to	Stellate cells, Activation	Strong		
6	TGFbeta1 expression, Up Regulation	Indirectly Leads to	Collagen, Accumulation	Strong		
7	Stellate cells, Activation	Directly Leads to	Collagen, Accumulation	Strong		
8	Collagen, Accumulation	Directly Leads to	Liver fibrosis, N/A	Strong		

Network View



Research Centre

Methodology:

- Selection of the Adverse Outcome
- Selection of the Molecular Initiating Event
- Study of the relevant biology and physiology
- Identification of Key Events
- Description of Key Event Relationships
- **Graphic representation of the AOP**
- Assessment
- Report / Insertion into the AOP Wiki

Methodology:

- Selection of the Adverse Outcome
- Selection of the Molecular Initiating Event
- Study of the relevant biology and physiology
- Identification of Key Events
- Description of Key Event Relationships
- Graphic representation of the AOP
- **Assessment**
- Report / Insertion into the AOP Wiki

Assessment

strength of scientific evidence

Event	Scientific Support	Strength of Evidence
MIE Protein Alkylation	Liebler DC "Protein Damage by Reactive Electrophiles: Targets and Consequences" Chem. Res. Toxicol. 2008; 2221(1): 117-128 Kehrer JP, Biswal S. "The Molecular Effects of Acrolein" Toxicol. Sciences 57, 6-15 (2000)	well established A well-accepted toxic mechanism for causing cell injury.
KE Hepatocyte Injury and Apoptosis	Malhi H. et al. "Hepatocyte Death: A Clear and Present Danger" Physiol. Rev. 90:1165-1194. 2010 Canbay A. et al. "Apoptosis: The Nexus of Liver Injury and Fibrosis" Hepatology, Vol. 39, No. 2, 2004 Orrenius S.et al. "Cell Death Mechanisms and Their Implications in Toxicology" Toxicol. Sciences 9(1);3-19(2011) Jaeschke H. "Inflammation in Response to Hepatocellular Apoptosis" Hepatology 2002;35:964–966	very strong Emerging concepts implicate apoptosis as a keystone in the genesis of hepatic inflammation and fibrogenesis.
KE Activation of Hepatic Macrophages (Kupffer cells)	Kolios G. et al. "Role of Kupffer Cells in the Pathogenesis of Liver Disease" World J Gastroenterol 2006 December 14; 12(46): 7413-7420 Kisseleva T, Brenner D. "Mechanisms of Fibrogenesis" Minireview Experimental Biology and Medicine 2008,233:109-122	very strong Kupffer cells are the main source of TGF-β1 and a major source of ROS and inflammatory mediators. Their activation is directly related to hepatocyte injury and apoptosis.
KE TGF-β1 Expression	Liu X. et al. "Therapeutic Strategies Against TGF-β Signaling Pathway in Hepatic Fibrosis" Review Liv Int 2006:26: 8-22 Gressner et al. "Roles of TGF-β in Hepatic Fibrosis" Front Biosci. 2002 Apr 1;7:d793-807	very strong TGF-β1 is considered the most potent profibrogenic cytokine and several reviews assign this cytokine a central role in fibrogenesis, especially in stellate cell activation.

AOP WoE assessment

Subset of Evolved Bradford Hill Considerations (Meek et al. 2014)

1. Biological plausibility – KERs
   a) Biological concordance
   b) Is there a mechanistic (i.e. structural or functional) relationship between KE_{up} and KE_{down}

2. Essentiality – KEs

3. Empirical support – KERs
   Concordance of empirical observations: DR, temporality, incidence, consistency

4. Inconsistencies and uncertainties - KERs

Methodology:

- Selection of the Adverse Outcome
- Selection of the Molecular Initiating Event
- Study of the relevant biology and physiology
- Identification of Key Events
- Description of Key Event Relationships
- Graphic representation of the AOP
- Assessment
- **Report / Insertion into the AOP Wiki**

JRC SCIENTIFIC AND POLICY REPORTS

Description of Prototype Modes-of-Action Related to Repeated Dose Toxicity

Brigitte Landesmann
Marina Goumenou
Sharon Munn
Maurice Whelan

2012

Joint Research Centre

Main Page

http://aopwiki.org

Navigation
Main page
AOP List
Help
FAQ
Recent changes
Release notes
Actions
Feedback
Tools

Announcements [edit]

The latest version of the AOP Wiki was released on April 8, 2014. The templates and widgets for this release match the latest version of the OECD AOP Development Handbook: File:AOP Handbook.pdf. Screenshots accompanying the handbook are available File:AOP HBScreenshots.pdf. The wiki help pages are being updated currently, so you may find some discrepancies until that process has been completed.

We have automatically migrated existing pages to the new template, but please check over your pages to make sure the changes make sense. If you had previously entered additional section headers, it is more likely we may have rearranged the sections in an awkward manner. As always, all changes were tracked, so it is possible to see the page before and after the changes as well as the differences. If you need assistance in correcting errors you find on your page, please email us at aopwiki@googlegroups.com. If you find any bugs with the new code, you can send them to the same email or report them using the Feedback section on the sidebar.

Welcome to the Collaborative Adverse Outcome Pathway KnowledgeBase (AOP-KB) Wiki [edit]

Protein Alkylation to Liver Fibrosis

AOP Title [edit]

Protein Alkylation leading to Liver Fibrosis

Authors [edit]

Brigitte Landesmann
Systems Toxicology, Institute for Health and Consumer Protection
Joint Research Centre, European Commission
Brigitte.LANDESMANN@ec.europa.eu

Status [edit]

Under development: Do not distribute or cite.
last updated: July 2014

Abstract [edit]

Hepatotoxicity in general is of special interest for human health risk assessment. Liver fibrosis in particular is an important human health issue associated with chemical exposure and predictive assays are lacking; it is a typical result of chronic or repeated-dose toxic injury and one of the considered endpoints for regulatory purposes. It is a long-term process in which inflammation, tissue destruction, and repair occur simultaneously, together with sustained production of growth factors and fibrogenic cytokines due to a complex interplay between various hepatic cell types, various receptors and signaling pathways which lead to an imbalance between the deposition and degradation of extracellular matrix (ECM) and a change of ECM composition. Due to this complex situation an adequate cell model is not available and an in vitro evaluation of fibrogenic potential is therefore not feasible. A sufficiently detailed description of the AOP to liver fibrosis might support chemical risk assessment by indicating early (upstream) markers for downstream events and facilitate a testing strategy without the need for a sophisticated cell model.

This AOP describes the linkage between hepatic injury caused by protein alkylation and the formation of liver fibrosis. The MIE is protein alkylation, leading to structural and functional cell injury and further to cell death, the first KE. Apoptotic hepatocytes undergo genomic DNA fragmentation and formation of apoptotic bodies. Upon engulfment of apoptotic bodies Kupffer cells (KCs) are activated, the next KE along the pathway. Activated KCs are the main source of TGF-β1, the most potent profibrogenic cytokine. TGF-β1 expression therefore is considered a KE that causes the next KE, hepatic stellate cell (HSCs) activation, meaning the transdifferentiation from a quiescent vitamin A–storing cell to a proliferative and contractile myofibroblast, the central effector in hepatic fibrosis. Activated HSCs cause progressive collagen accumulation, which together with changes in ECM composition signifies the KE on tissue level. Collagen bands progress further to bridging fibrosis, finally affecting the whole organ (the adverse outcome on organ level) and eventually leading to cirrhosis. Fibrous bands may disrupt normal blood flow, leading to portal hypertension and extensive scarring, which is the setting for unregulated growth and neoplasia. The inflammatory response plays an important role in driving fibrogenesis, since persistent inflammation precedes fibrosis. Inflammatory signaling stems from injured hepatocytes, activated KCs and HSCs. Inflammatory and fibrogenic cells stimulate each other in amplifying fibrosis. Chemokines and their receptors provoke further fibrogenesis, as well as interacting with inflammatory cells to modify the immune response during injury. Oxidative stress, as well, plays a crucial role in liver fibrogenesis by inducing hepatocyte apoptosis, activation of KCs and HSCs and fuelling inflammation. ROS contributing to oxidative stress are generated by hepatocytes, KCs, HSCs and inflammatory cells.

This purely qualitative AOP description is plausible, the scientific data supporting the AOP are logic, coherent and consistent and there is temporal agreement between the individual KEs. Quantitative data on dose-response-relationships and temporal sequences between key events are still lacking; the provision of quantitative data will strengthen the weight of evidence and make the AOP applicable for chemical risk assessment purposes.

Summary of the AOP

Molecular Initiating Event

Add Molecular Initiating Event to Table

Molecular Initiating Event	Support for Essentiality	
Protein, Alkylation	Strong	

Key Events

Add Event to Table

Event	Support for Essentiality	
Hepatic macrophages (Kupffer Cells), Activation	Very Strong	
TGFbeta1 expression, Up Regulation	Very Strong	
Stellate cells, Activation	Very Strong	
Collagen, Accumulation	Very Strong	
Oxidative Stress, Increase	Strong	

AOP development
protein alkylation leading to liver fibrosis:

- Why
- How
- **Difficulties**
- Open questions
- Further plans

Difficulties

MIE

MIE = a measureable change in biological state as result of a direct interaction between a chemical and some biomolecule in an organism.

The target biomolecule may be specific, as a particular enzyme or receptor or it may be non-specific, as is the case with many reactive mechanisms.

Non-specific MIEs can only be described more generally without specifying distinct biomolecules that are involved.

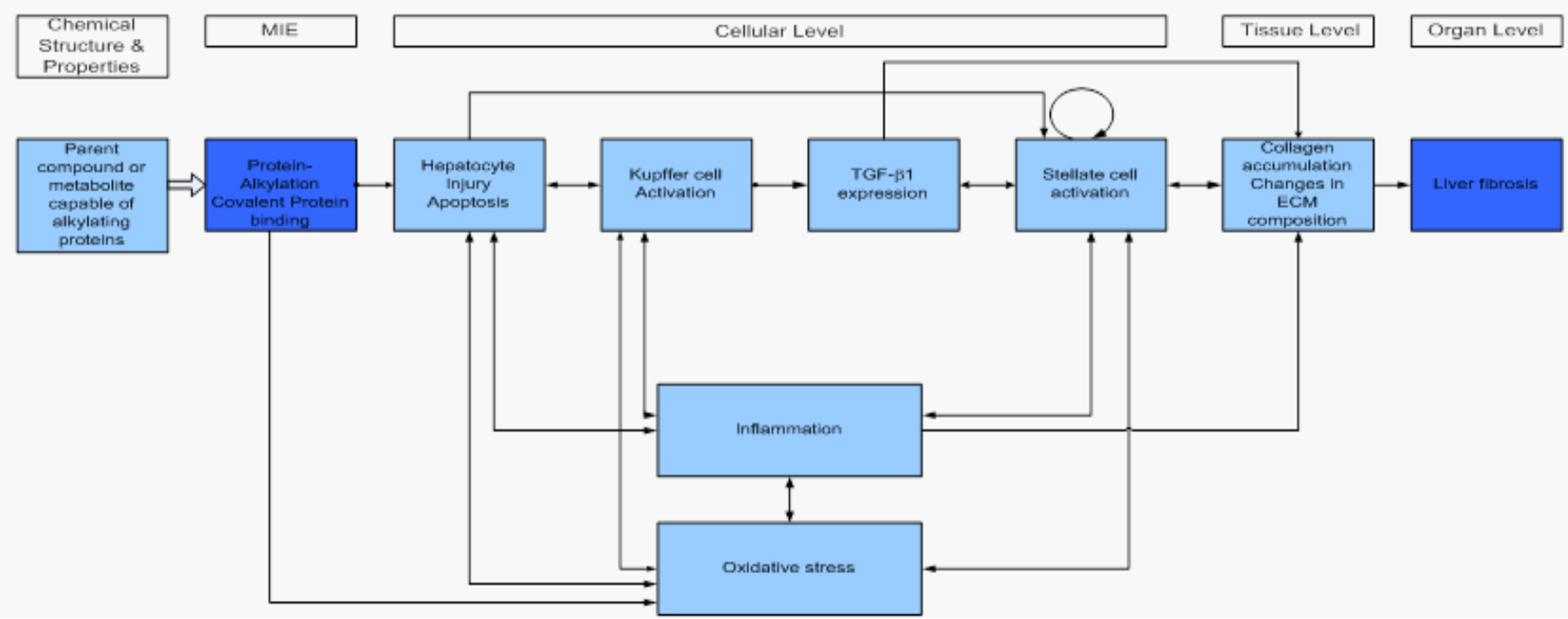
Therefore it is difficult

- to establish a relationship between a distinct biomolecule and a downstream event
- to define the chemical initiators
- to use the MIE for profiling and categorising of chemicals.

Difficulties
Linearity

AOPs are an artificially linear construct, defined as a linear, non-branching and directed sequence of KEs, connecting a single MIE to an AO.
Linear AOPs are the basis for the WoE evaluation.

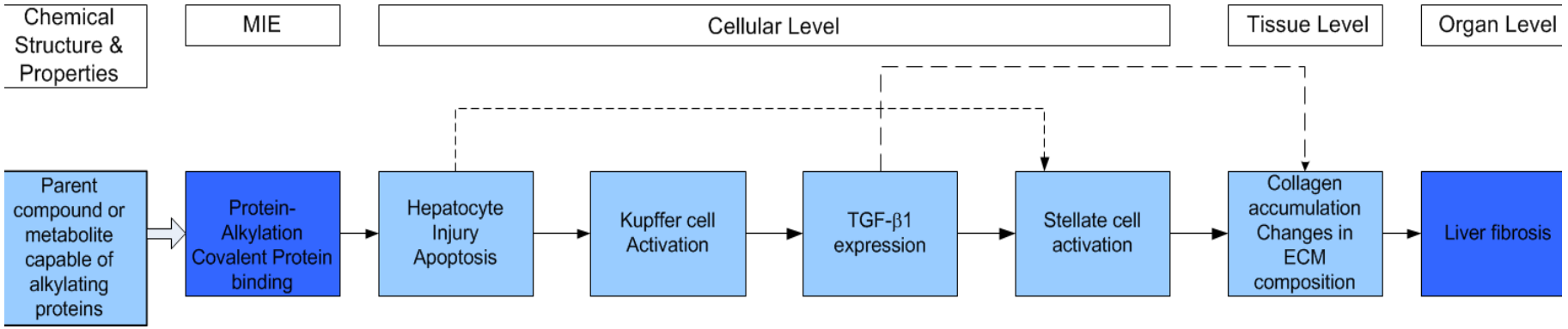
BUT: what about feed-back and feed-forward loops and inter-relations ?



Linearity:

European Commission

Possible solution:



Difficulties

Taxonomy

The description of the taxonomic relevance of each KE, most notably the conservation of the molecular target (MIE) across taxa

helps to understand the taxonomic relevance of the AOP as a whole and how broadly the data represented by a key event measurement may be extrapolated.

Data from humans, rodents,…..
how broad and deep to go?

AOP development
protein alkylation leading to liver fibrosis:

- Why
- How
- Difficulties
- **Open questions**
- Further plans

Open questions

- Is protein alkylation/binding to certain proteins required?
  - Do particular proteins and various binding sites influence the further downstream process?
- Hepatocyte injury/death - a converging KE – the one that really initiates the pathway for fibrogenesis?
  - Receptor-specific and non-specific MIEs – does it make a difference?
- Which cellular responses to stress/injury (apoptosis, necrosis, senescence, transdifferentiation/transition, recovery) ultimately progress liver fibrosis?
  - Is hepatocyte insult/injury, rather than death, sufficient to trigger and progress fibrosis?
  - Threshold values: how many dead/injured hepatocytes over which time frame are necessary to initiate the pathway (for activating a sufficient amount of Kupffer cells)?
- Valid biomarkers for the fibrogenic process TGF –β1, HSC activation, collagen deposition, or..?

AOP development
protein alkylation leading to liver fibrosis:

- Why
- How
- Difficulties
- Open questions
- **Further plans**

Future Plans

Further AOP development - applicability for risk assessment

WHAT:

chemical space: specific properties, other MIE's, common KEs

quantitative data: threshold values, dose response-relationships

temporal data: KE duration, rate

HOW:

Experiments with co-culture models (and HepaRGs)

Literature research for quantitative information

Mining of DBs with rodent data to evaluate the species concordance and relevance for humans and to identify convergent biomarkers (rodents-humans) related to fibrosis using genomics.

WHO:

Collaborators

Scott Auerbach, Steve Ferguson, Alex Merrick, Michael Devito, Ray Tice (NIEHS)

Ed LeCluyse (The Hamner)

Pau Sancho, Leo van Grunsven, Sofia Batista Leite (SEURAT-1 partners)

Acknowledgments

Dan Villeneuve
and all members of WG 2 / workshop in Somma Lombardo

Sharon Munn
Alfonso Lostia
Tomislav Horvat

Clemens Wittwehr

THANK YOU for your attention