GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

20 YEARS

BfR
German Federal Institute for Risk Assessment

AOPs as a concept - (regulatory) implications and consequences

Dr. Tewes Tralau

Regulatory *Status Quo*: Where do we stand?

Pesticides & Biocides:
Harmonised guidelines (OECD, EU), mostly ***in vivo***,
mechanistic data only as “add-on”.

Food & Feed:
Most testing is done ***in vivo***,
non-animal methods comprise PBTK modelling and TTC.

Consumer products / Contaminants:
Heterogeneous (**REACH, product-specific regulations, ALARA**),
cave: *in vivo* ban (cosmetics), nanotox, EDCs, mixtures ...

REACH & CLP:
Harmonised guidelines, mostly ***in vivo***,
mechanistic studies currently treated as “add-on”. Cautious attempts to make potential use of mechanistic data for "read across“ and grouping.

Apart from sensitisation, some selected read across projects and occasional justifications in the context of the assessment of ED-properties and non-genotoxic carcinogenicity for plant protection products there is currently little regulatory use of mechanistic data or AOPs!

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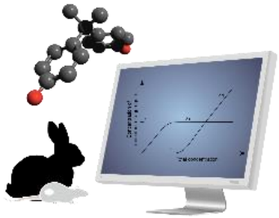
Yet, beyond the regulatory domain mechanistic concepts have been around for more than 20 years. Uses comprise
- **functional pre-screens,**
- **evaluation of (toxicological) mechanisms,**
- **hazard characterisation and prioritisation,**
- **biomarker identification and others.**

Primary objective is public health protection!



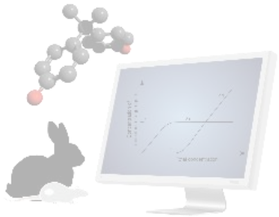
This means data/tests must allow

- clear conclusions regarding the potential ***adversity in an intact organism***,
- clear conclusions regarding ***dose-response relationships***,
- provide ***legal certainty***,
- refer to set and accepted testing guidelines,
- and, ideally, should have earned some trust (*e.g.*, by standing the test of time).

⇒ *Also, mind that regulatory toxicology not only has been around for some odd 100 years but that its primary objective together with its close interconnection with legal acts and proceedings inherently sets a slower and more conservative pace with regard to adaptation.*

Regulatory data requirements

Primary objective is public health protection!



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Current system - PROs:

a) Established methods,
b) mostly harmonised legal framework,
c) long experience,
d) trusted results,
e) predictability of legal procedures.

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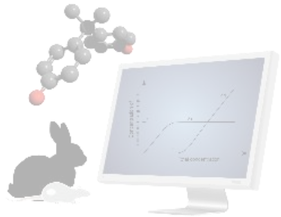

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Current system - CONs:

a) Capacity issues:
> 80,000 chemicals, increasing by ~ 2,000/a

b) Not all questions or endpoints addressed/addressable (*e.g.*, residues, mixtures, low dose effects, neurotoxicity, ED- or epigenetics…).

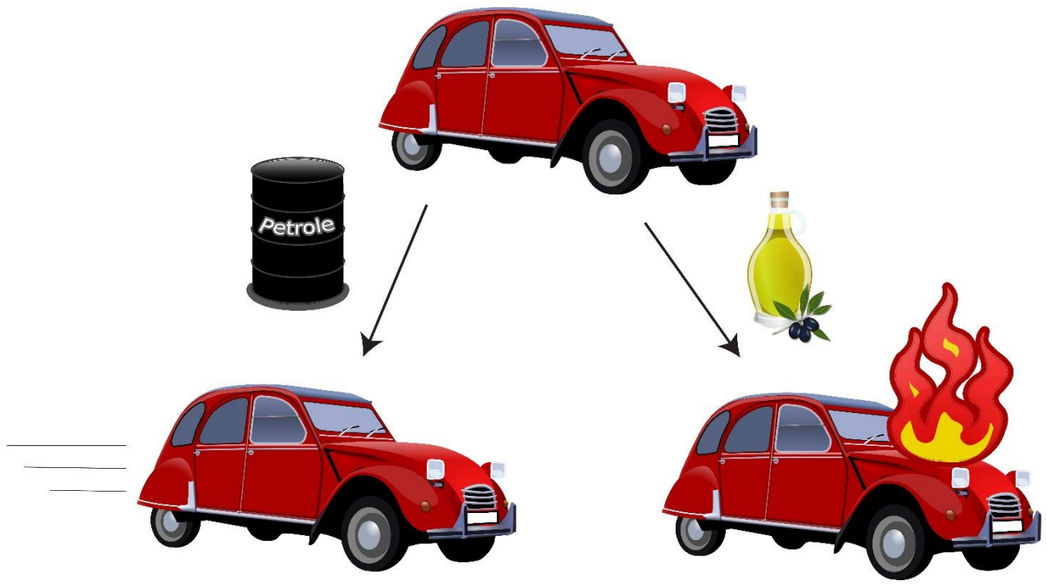
c) Worries about species specific effects (metabolism/microbiome).

d) Societal pressure/ethics.

Toxicological assessment from a (very much) simplified perspective

- Is it dangerous? (Is there a hazard?)

- Under which conditions? (Quantification)



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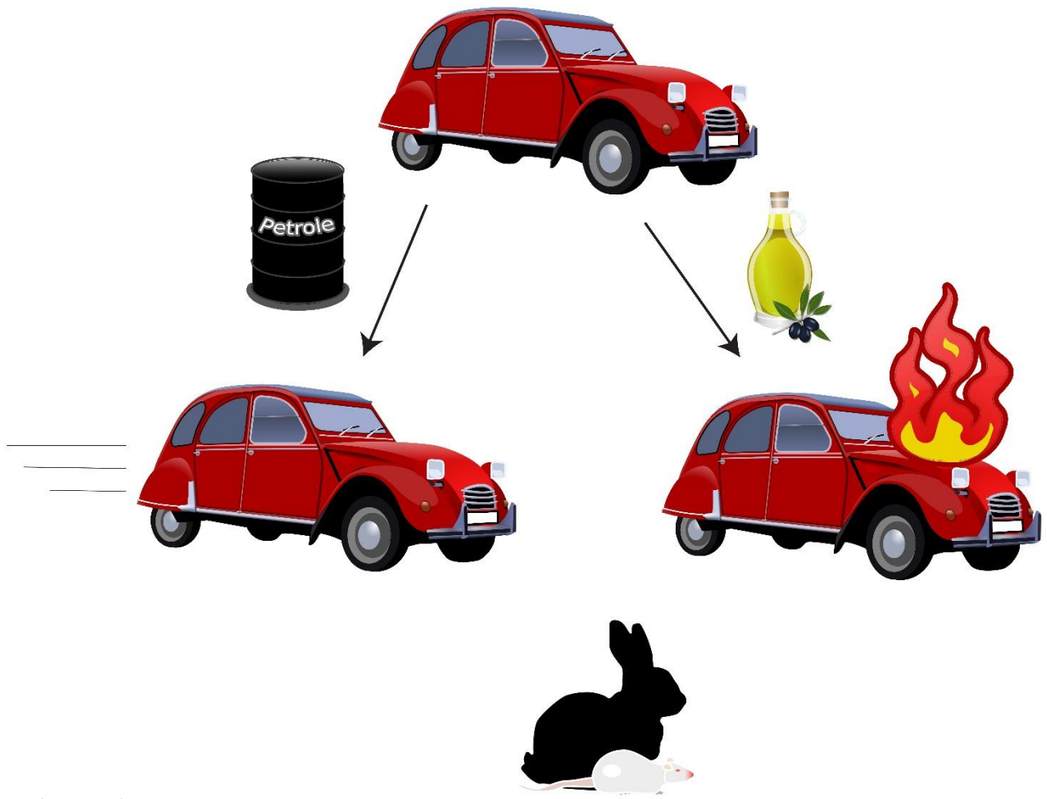

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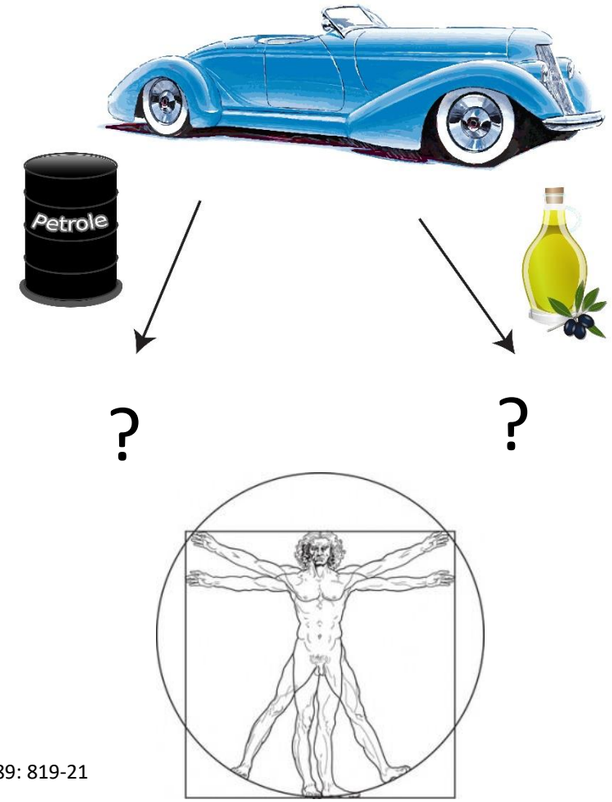

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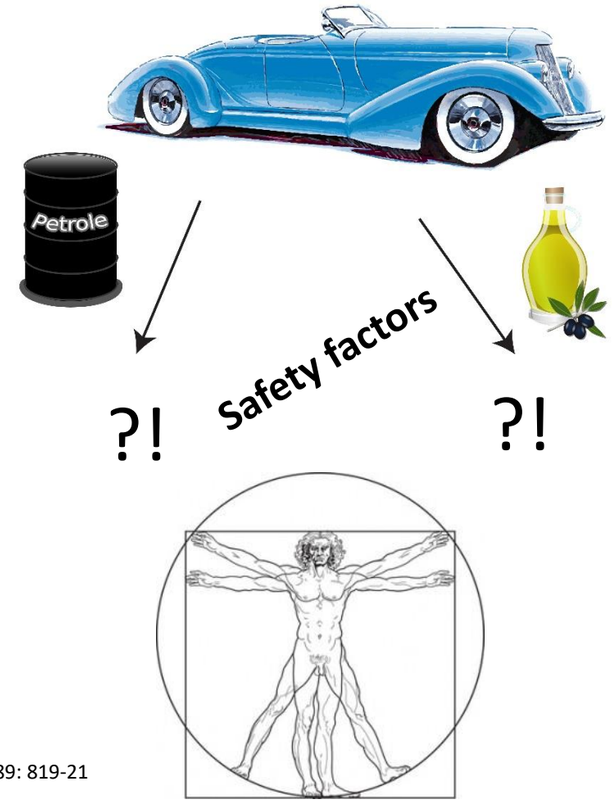

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Adversity - aetiology of toxicological connectivity

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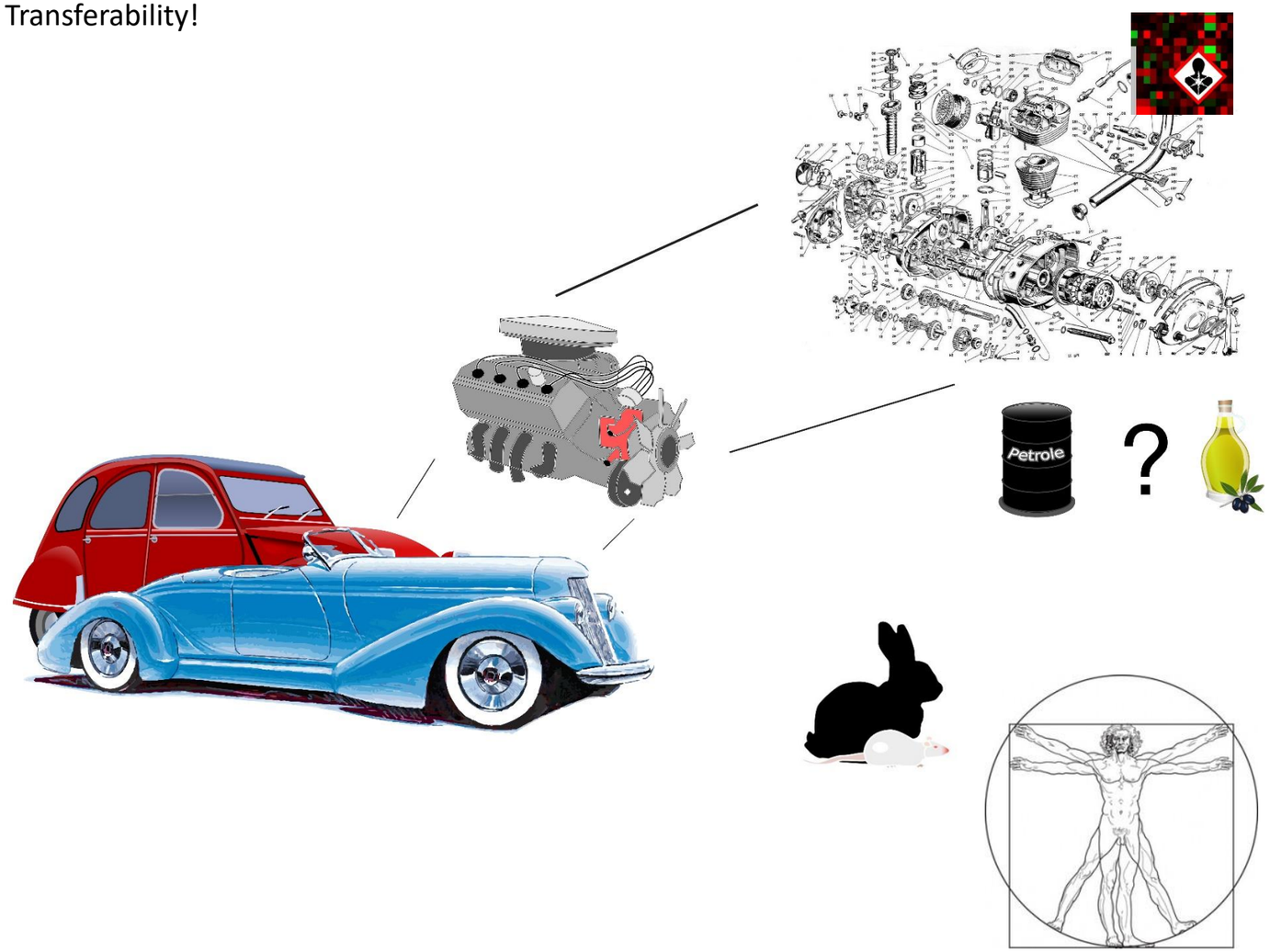

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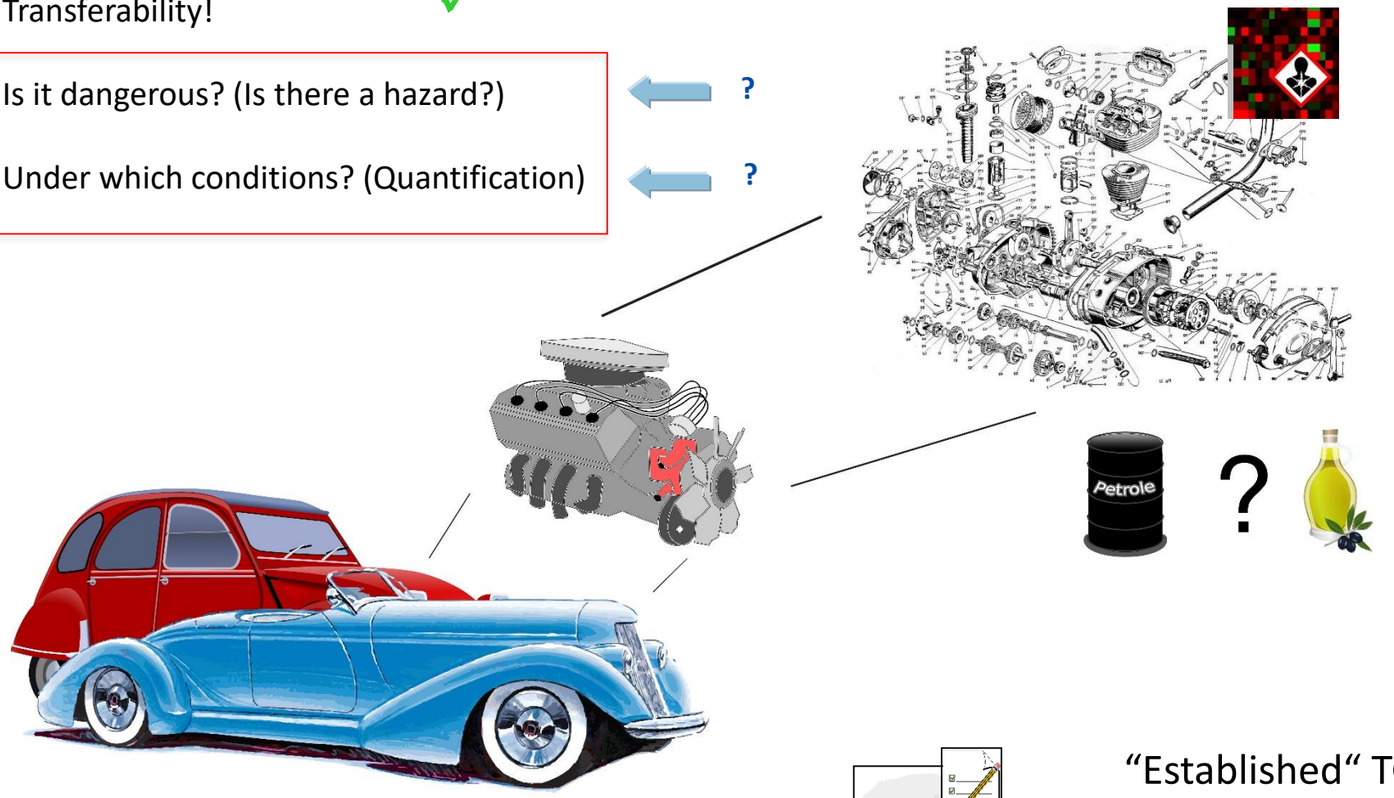
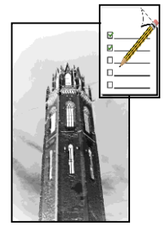




NAMs/AOPs

vs.

"Established" TG

OECD
BETTER POLICIES FOR BETTER LIVES

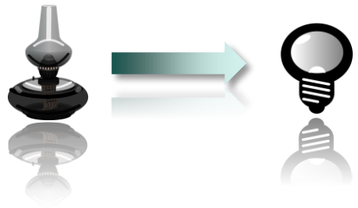
Adapted from Tralau & Luch, Arch. Toxicol. 2015, 89: 819-21

Mechanistic data allow to:

- Characterise molecular targets and the respective pathways affected.
- Classify and rank substances according to their potential for cellular interference.
- Identify substances with no effect.
- Identify biomarkers and signatures.
- Build testing strategies by using a versatile modular setup.

Inherent issues:

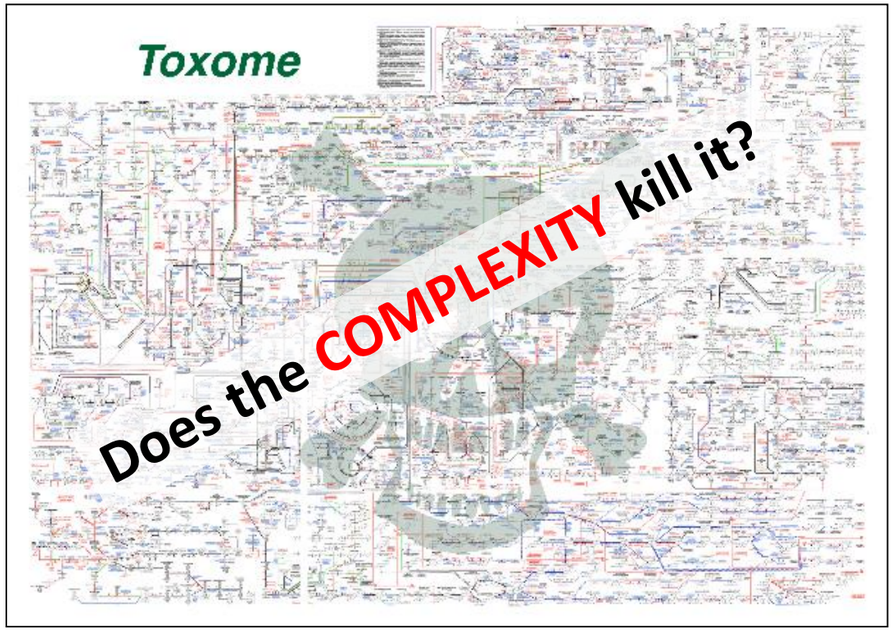
- Limited compatibility to the grown histopathological picture of adversity.
- Such data **do not easily** translate into established **quantitative** measures of risk assessment.
- Classical endpoints comprise a compact readout for a plethora of underlying pathways…



Mechanistic data - considerations & concepts

"**Relevant Pathways of Toxicological Concern**"

*Entity of pathways with the potential for adversity, the network of which is the "**toxome**".*



=> Not necessarily! In the end adversity will rely only a limited number of pathways, many of them interconnected and thus sharing molecular components. Also, similar to classical endpoints molecular readouts can likewise subsume several pathways/cellular states.

(Ideally) AOPs should help to break down the complexity

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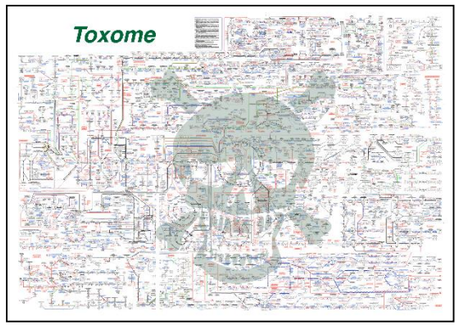

"Adverse Outcome Pathways"

Identification of endpoint-specific events & signalling cascades suitable to define adverse outcome

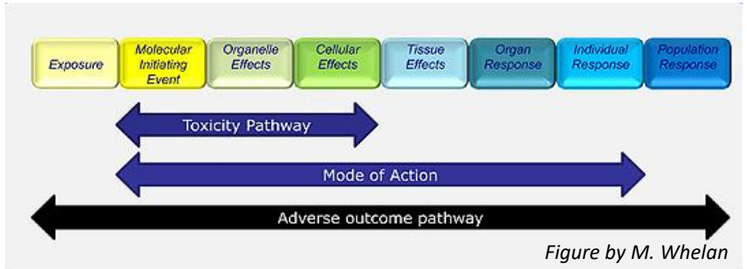

Figure by M. Whelan

And allow for integrated approaches to testing and assessment

“Adverse Outcome Pathways”

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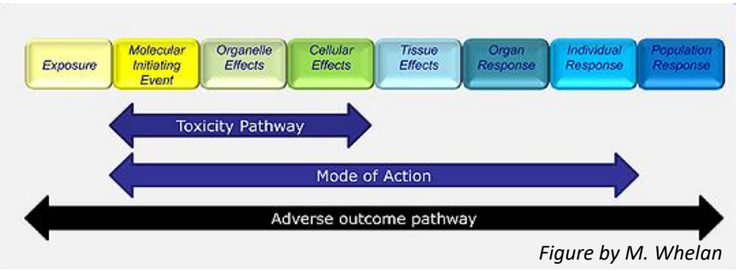
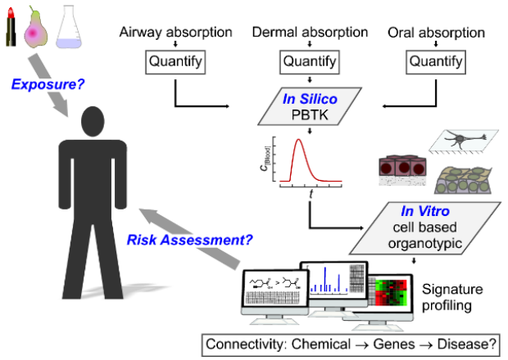


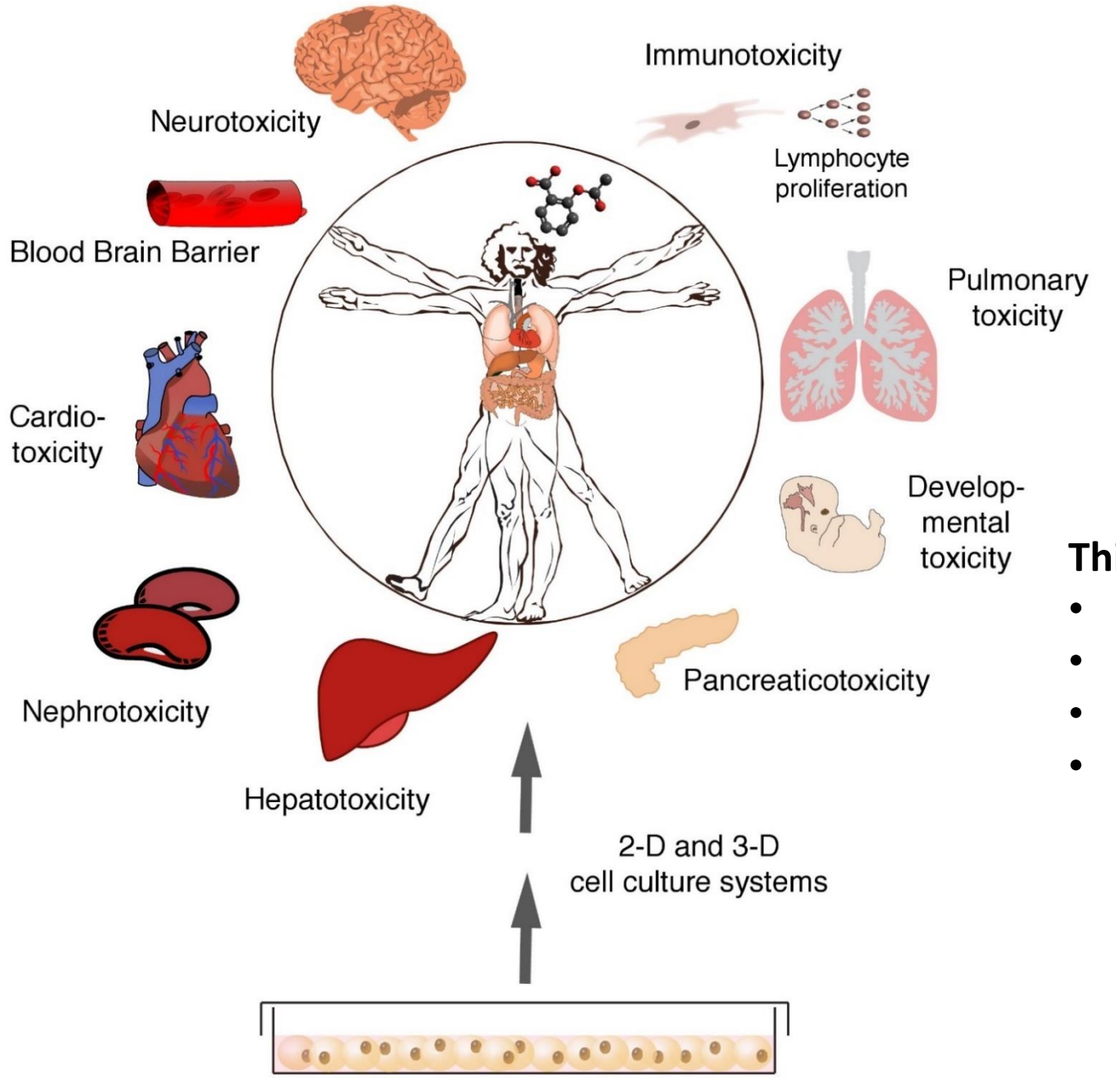
Figure by M. Whelan

“Mechanistic Integrated Testing” (Tox Test Dummy)



Tralau *et al.*, EHP 2012, 120: 1498-94

Current state of the art



Adapted from Tralau & Luch, TiPS 2012, 33: 353-64

Cellular and biochemical assays for ≥ 600 endpoints

This includes:

- Molecular & biochemical assays
- Cell lines
- Primary cells
- Designed cells (transformed)

In addition there are

- *in silico* approaches and models,
- organ-mimicking systems and
- of course *in vivo* data.

What does this mean for AOPs and their regulatory use?

By conceptual definition AOPs should be

Ankley *et al.*, Environ. Toxicol. Chem. 2010, 29: 730-41; Villeneuve *et al.*, Toxicol. Sci. 2014, 142: 312-20

- an information tool,
- that is universal and not specific for certain chemicals,
- systematically characterises the processes underlying adverse processes based on the information available (*in silico*, *in vitro* and *in vivo*),
- is integrative across the hierarchy of the biological levels of organisation,
- is modular, and
- constitutes living documents.

As handled this comprises

- a collection of a multitude of biological pathways and mechanisms,
- which are preferably kept simple,
- are more or less well linked to potentially adverse outcomes, and
- which often require further validation and integration for further use.

What does this mean for AOPs and their regulatory use?

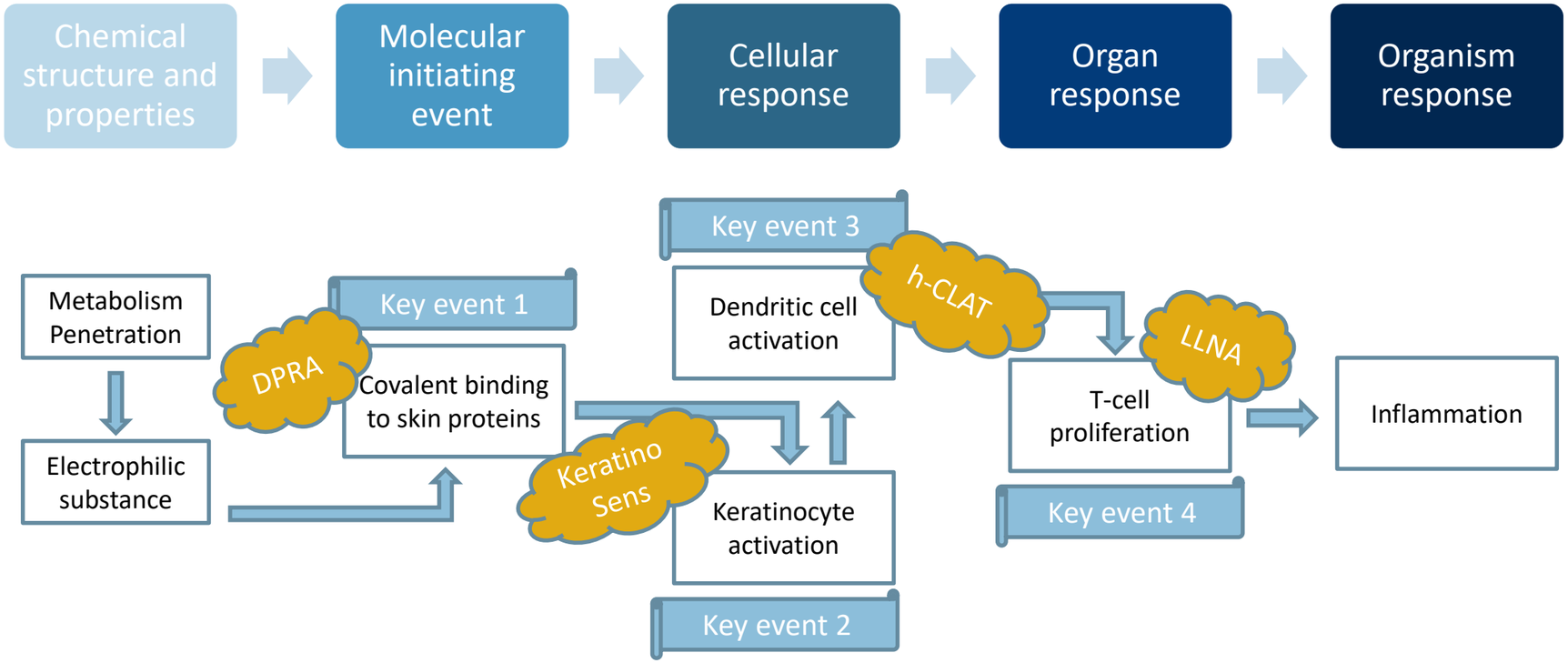
However, for an anticipated wider regulatory use

- pathways need not only to capture (key) events underlying adversity,
- but be sufficiently complex to do so with a wider scope, and
- provide interlinkage to other AOPs (related or unrelated) as to
- fit into the wider network of endpoints relevant for regulatory hazard assessment.
- Also, the respective pathways should ideally allow for quantitative assessments (qAOPs).

This means

- while AOPs can serve various stakeholders,
- one should be clear about their respective application domains, ideally right from the start.
- Anticipated regulatory use leads to other information requirements than when merely establishing causal links of biologically relevant pathways (*e.g.*, sensitisation vs. reduced stress tolerance due to GSH-depletion).
- Developing and keeping regulatory AOPs “alive” (as documents as well as in terms of assays) requires dedicated resources and organisational structures as well as a highly structured design and documentation. This is a precondition for making AOPs workable for regulation and to keep up with the pace of scientific developments.

Example functional adversity - sensitisation



Based on: OECD Series on Testing and Assessment No. 168: The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins - Part 1, Part 2

Courtesy of D. Bloch

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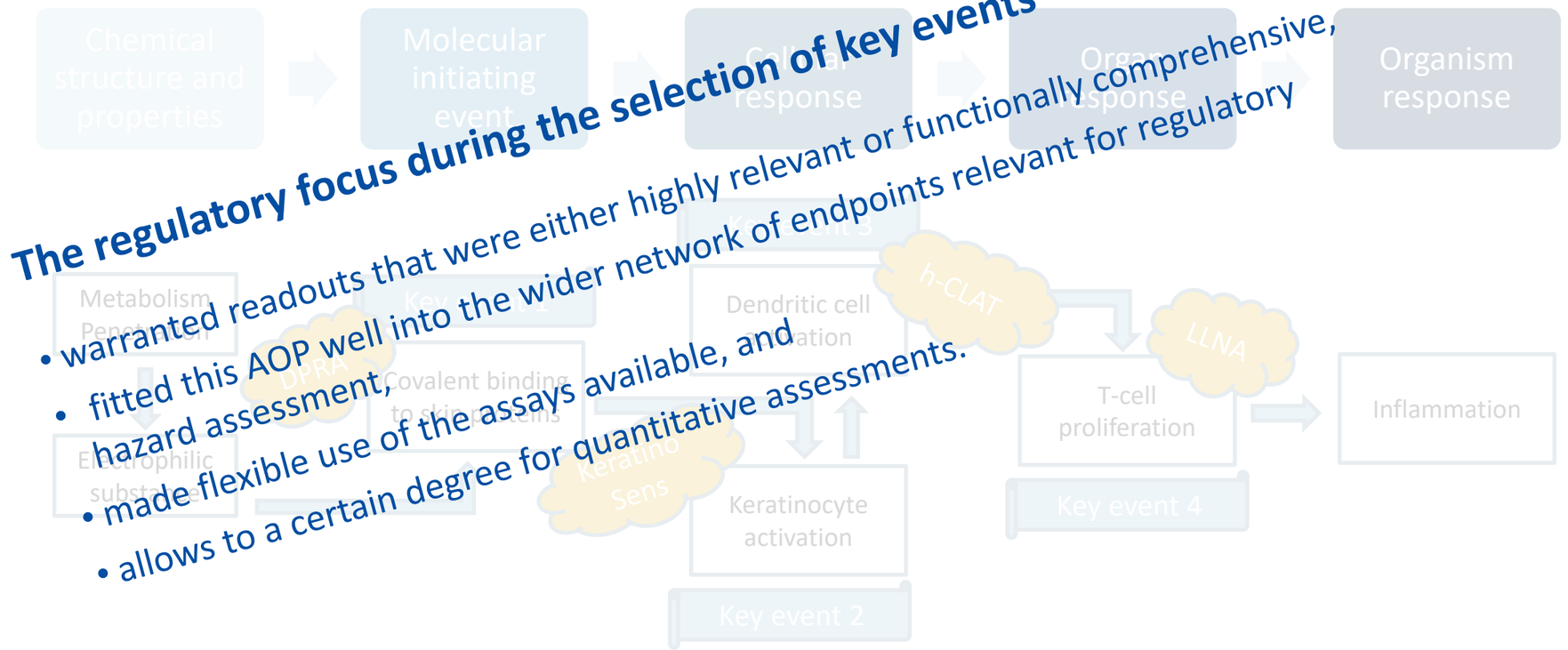

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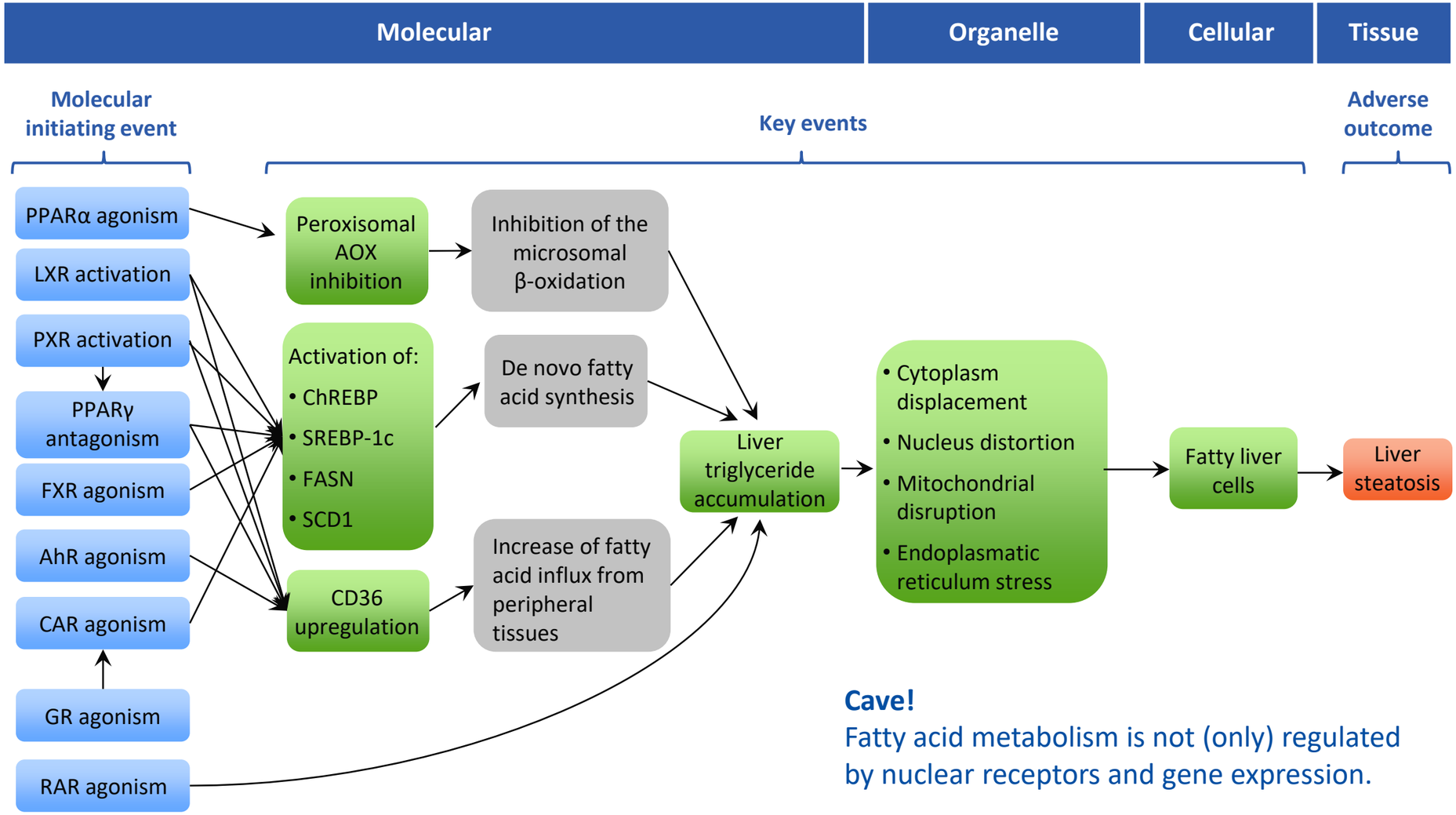
Example molecular adversity – liver steatosis

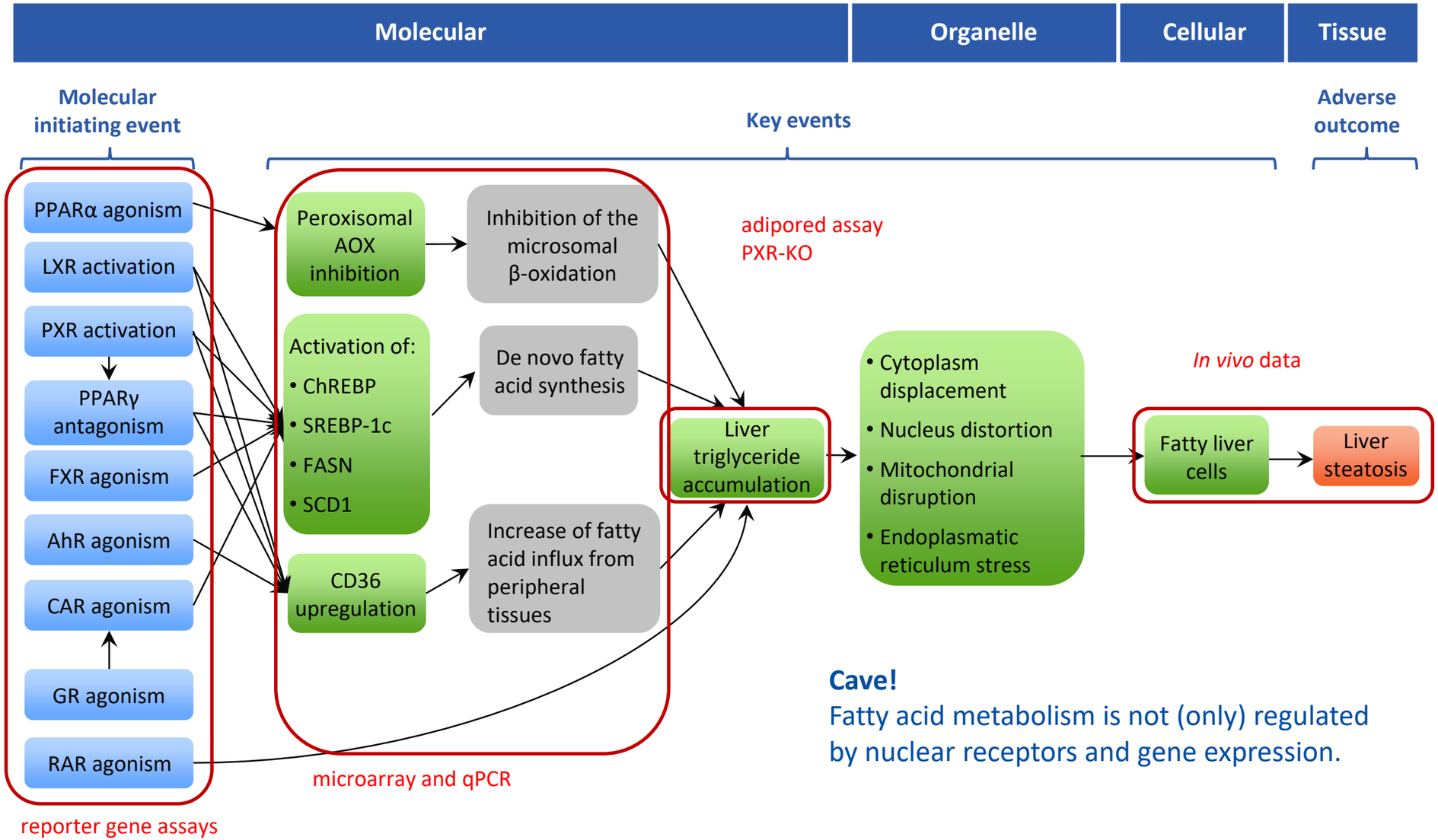


Cave!
Fatty acid metabolism is not (only) regulated by nuclear receptors and gene expression.

Courtesy of P. M. Stoelting

(modified after Vinken Chem Res Toxicol. 2015 and Mellor et al. Crit Rev Toxicol. 2016)

Example molecular adversity – liver steatosis



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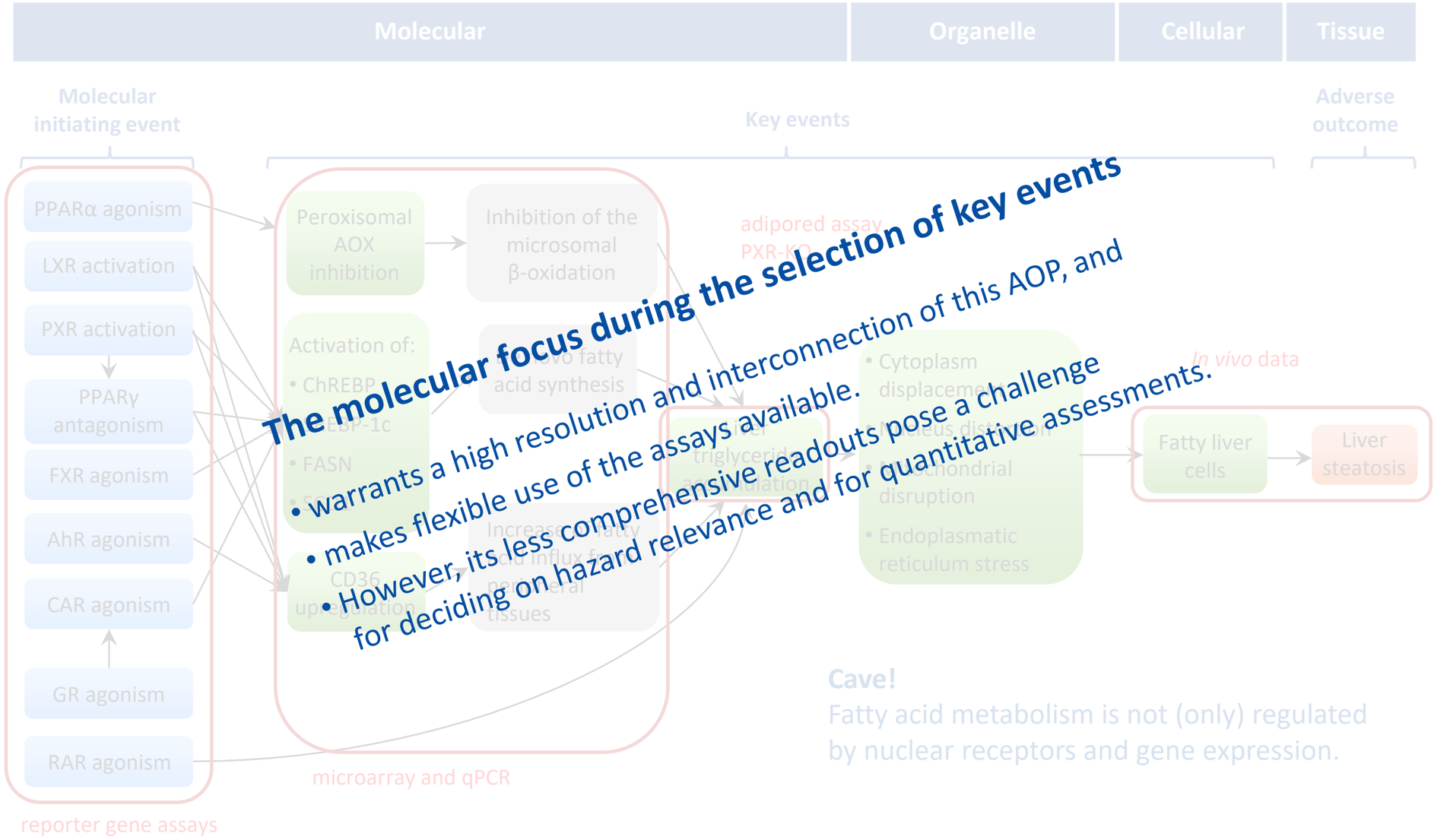

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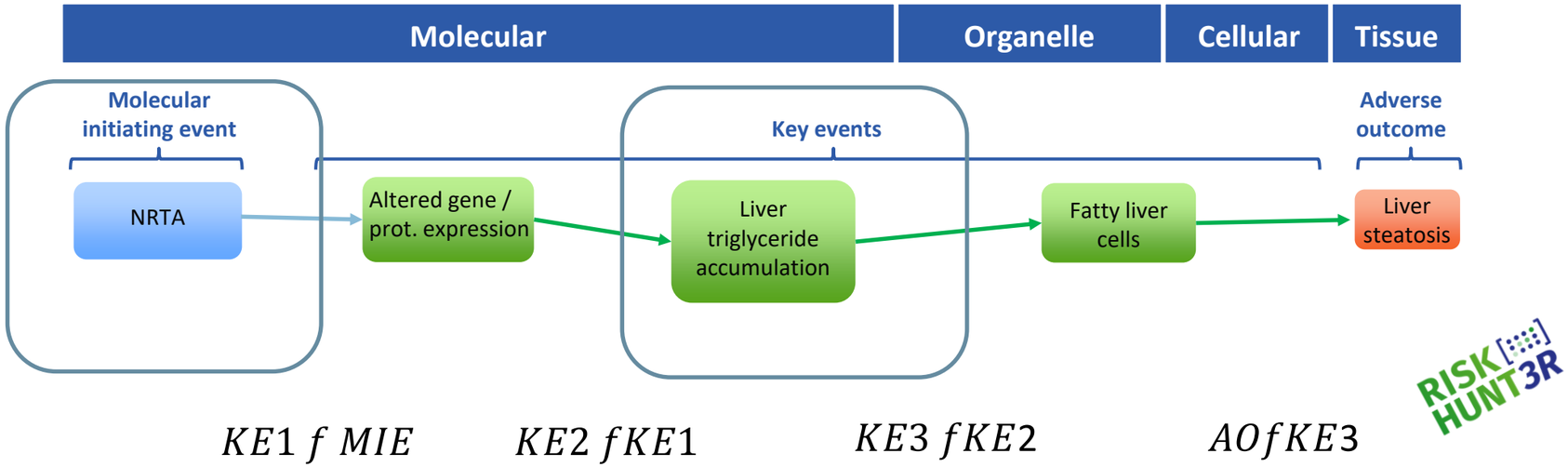
Example molecular adversity – liver steatosis

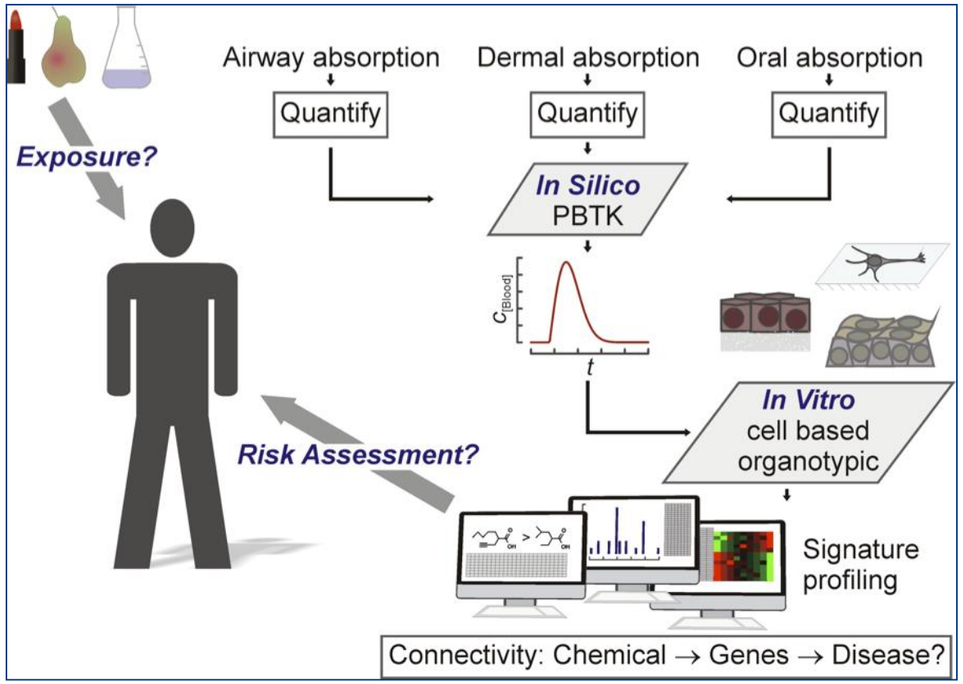
Solutions:

- Reduce complexity, by identifying and focussing on key MIEs,
- focus on late KE, analyse this in vitro and build a quantitative model for $AO\ f\ KE$, or
- do both and select the better model



Courtesy of P. M. Stoelting

So, where do we stand with regard to an AOP-based Tox-test dummy?



Tralau *et al.*, EHP 2012, 120: 1498-94

- From a **scientific** perspective current state of the art AOPs are fit to deliver data suitable for assessing many aspects of potential **organ toxicity** and **aspects of chronic toxicity**.
- However, the more complex endpoints often still need further development in terms of coverage and assays.
- Also, for moving beyond hazard characterisation we still need to **find (standardisable) concepts for designing qAOPs** and of **integrating exposure**. Other issues to be considered during AOP selection and design are **validation and how to identify most relevant/sensitive endpoints**.

Principal regulatory issues to address

(Regulatory) acceptance

- ***Validation does not necessarily warrant acceptance***… The latter depends on trust into the method. This should be addressed by ***blinded cross-validation trials*** alongside established assays. Also, there is need to reduce the **skill gap on both sides** (regulatory as well as academic).

Adversity & Plasticity

- Need to ***convert molecular measures into quantitative (or probabilistic) measures*** suitable for risk assessment.
- Need to (partly) ***rethink adversity*** (biomarker based *vs.* histopathology-based).
- How to select for the ***most sensitive endpoints/AOPs***?
- How to address ***plasticity***? Can one agree on biomarkers that ***define the 'point-of-no-return'***?
- Need for improved **in vitro/in vivo *correlations*.**

Test System Physiology

- Test systems used should be adequate, sufficiently complex and suitable for higher throughput.

Test System Validation

- ***How do we validate and do we do so against the right standards?*** How "golden" are they? Informed decisions on method performance and testing reliability require sound comparable performance parameters.

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Fields of action

- ***Need of joint and active engagement of all parties involved.*** Foster a culture of dialogue and better understanding for what is possible, what is required and what current systems can deliver.

- ***Identification of quantifiable readouts and relevant key events.***

  Readouts should ideally differentiate between background, preadverse and adverse reactions as well as adaptative processes. In doing so they should allow for some (semi)quantitative comparisons. Respective approaches could follow a classic deterministic lead or, alternatively, rely on probabilistic methods.

- ***Systematic performance review of the available AOPs and methods*** in order to facilitate scientifically based decisions on the best testing strategies.

  Predictivity and reliability are key for the acceptability of testing methods. Yet, particularly for many of the established methods these parameters have never been evaluated systematically. This often makes a quantifiable comparison impossible and introduces a “gold standard”-bias which not only flaws the judgement on test performance but at worst prevents better testing and assessments.

- ***(Pre)validation of existing and new systems in blinded trials*** vs. (existing) *in vivo* data. Reliance on performance standards and pilot studies before thinking of full blown validation trails.

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