

**Future Challenges in Developing Assessment Methodologies  
for Human Health Effects**  
November 14, 2018  
Tokyo, Japan

# **Category-based Read-across Approach, Considering Human Relevance**

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**The views expressed are those of the speaker and not an official position of NIHS.**

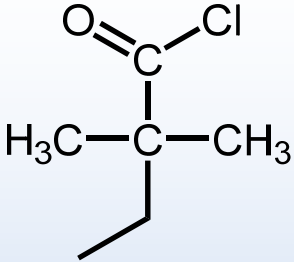
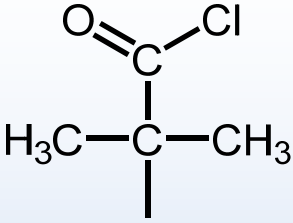
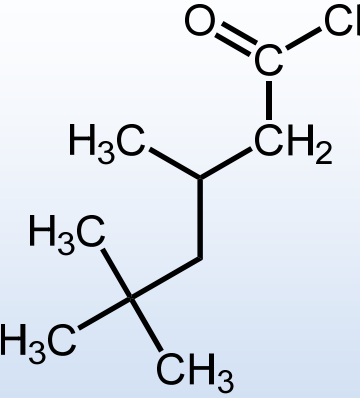
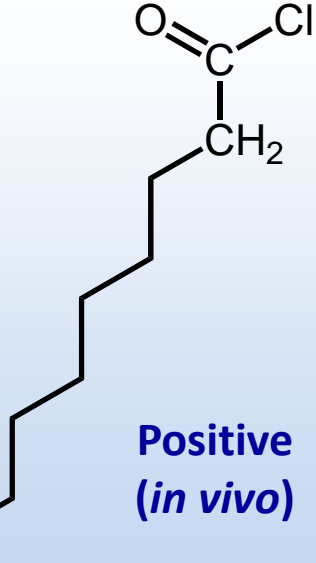
# Topics of This Talk

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- Databases and computational tools to support read-across approach
- Our recent read-across experiences in OECD IATA Case Studies Project

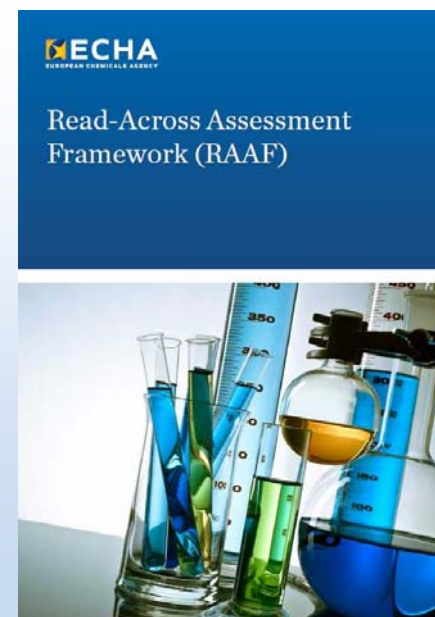
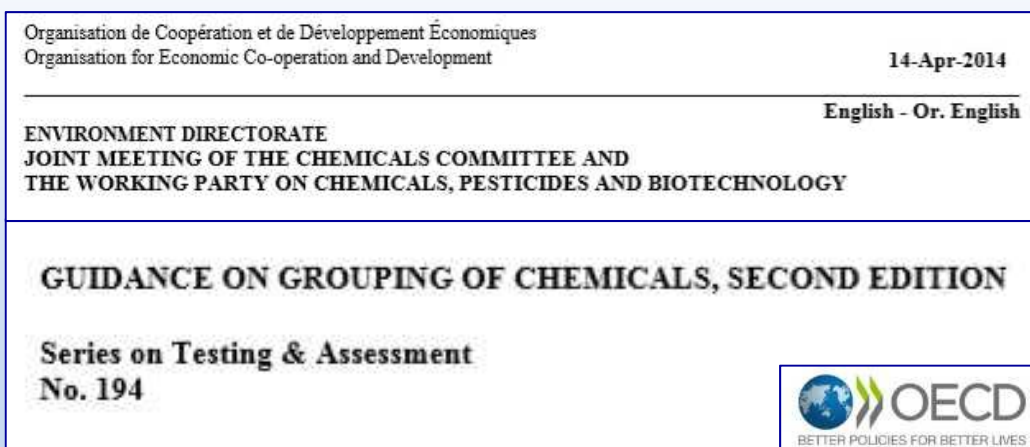
# Read-across

Read-across is regarded as a technique for predicting endpoint information for one substance (**target substance**), by using data from the same endpoint from (an)other substance(s) (**source substance(s)**).

	Target substance		Source substances	
Structure				
Endpoint (Skin Sensitization)	<b>Positive (read-across)</b>	<b>Positive (in vivo)</b>	<b>Positive (in vivo)</b>	<b>Positive (in vivo)</b>

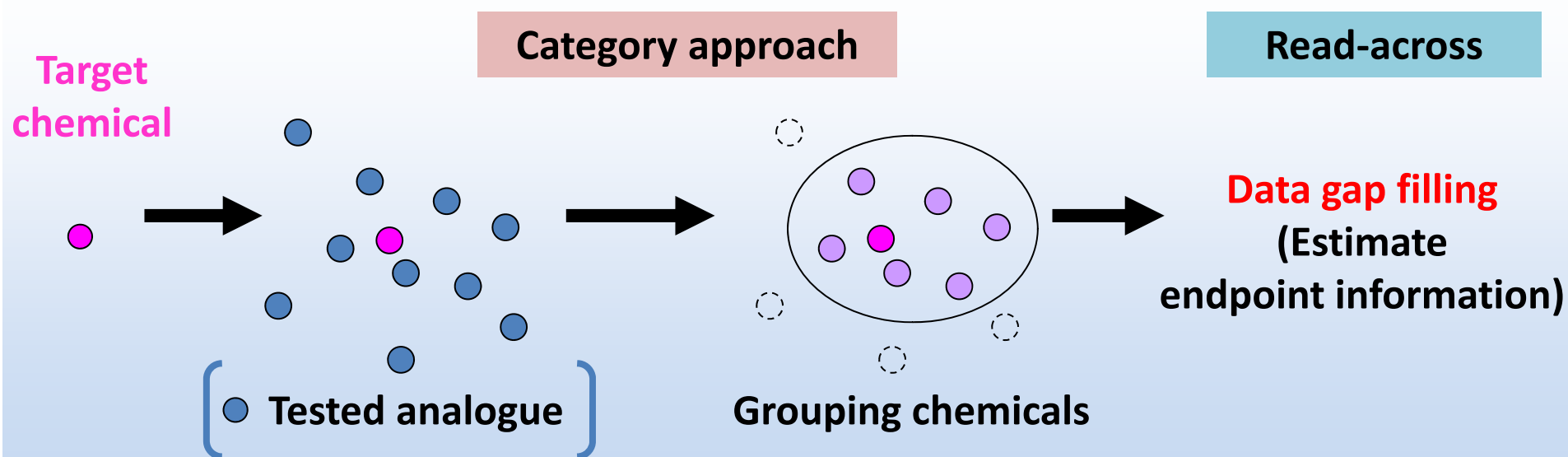
# Benefits

- Read-across saves resources and animals
- Not a new concept, but gains importance
  - -as the number of assessed chemicals increase
  - -animal welfare gains importance
- OECD Guidance, strongly emphasized in REACH Guidance



# Terminology

- Category approach and analogue approach
  - Techniques for grouping chemicals
- Read-across
  - Technique of filling data gap in either approach





Contents lists available at ScienceDirect

# Computational Toxicology

journal homepage: [www.elsevier.com/locate/comtox](http://www.elsevier.com/locate/comtox)



## Navigating through the minefield of read-across tools: A review of in silico tools for grouping



Grace Patlewicz<sup>a,\*</sup>, George Helman<sup>a,b</sup>, Prachi Pradeep<sup>a,b</sup>, Imran Shah<sup>a</sup>

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<sup>b</sup> Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN, USA

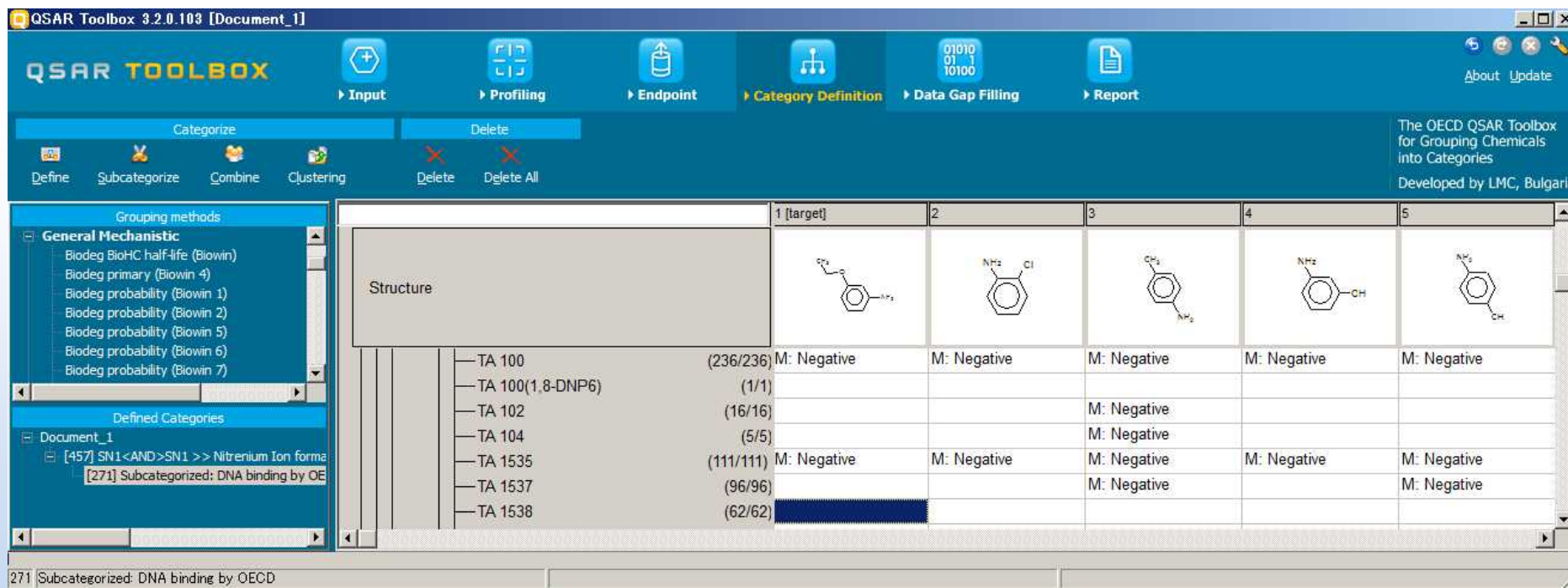
Publicly available tools

	Workflow steps Data Gap Analysis	Analogue Identification	Analogue Evaluation	Data Gap Analysis	Data Gap Filling	Uncertainty Assessment
AIM						
AMBIT		Find Analogues				
CBRA	Collect Structures	Endpoint Data Used		Assessment Details		
CIIP		Compute CBRA			Compute CBRA, cont.	
CIIP	CIIProfiler			CIIPredictor		
QSAR Toolbox						
Toxmatch	Profiling Endpoint		Category Definition		Data Gap Filling	
ToxRead	Explore Descriptor Space	Calculate Similarity	Similarity Assessment		Explore Similarity Assessment Results	
		Select Endpoint			Read-Across Chart	

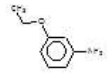
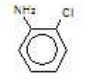

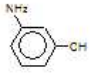
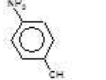
Strength and limitations

# OECD QSAR Toolbox

- Free software to support data gap filling by category approach (<http://www.qsartoolbox.org/>)
- Functionality of finding analogues and the test data for regulatory endpoints are equipped.



The screenshot displays the OECD QSAR Toolbox software interface. The main window shows a table with columns for chemical categories and their associated test data. The table is organized into columns labeled 1 [target], 2, 3, 4, and 5. The first column contains chemical structures and their corresponding test data. The other columns show the results of the data gap filling process, with 'M: Negative' indicating that the test data is not available for those categories.

Structure	1 [target]	2	3	4	5
Structure					
—TA 100 (236/236)	M: Negative	M: Negative	M: Negative	M: Negative	M: Negative
—TA 100(1,8-DNP6) (1/1)					
—TA 102 (16/16)			M: Negative		
—TA 104 (5/5)			M: Negative		
—TA 1535 (111/111)	M: Negative	M: Negative	M: Negative	M: Negative	M: Negative
—TA 1537 (96/96)			M: Negative		M: Negative
—TA 1538 (62/62)					

271 | Subcategorized: DNA binding by OECD

# QSAR Toolbox : Databases and Profilers for Human Health Hazard

## Databases

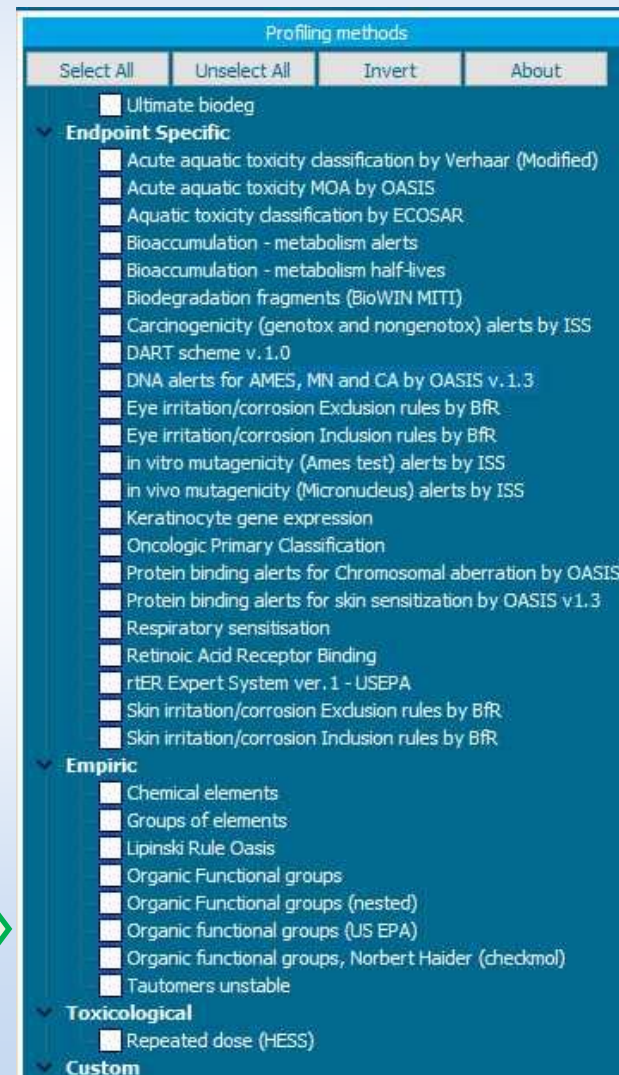


**Bacterial mutagenicity,  
Carcinogenic potency,  
Cell transformation,  
Developmental and  
reproductive toxicity,  
Genotoxicity  
etc.**

**Repeated dose toxicity**



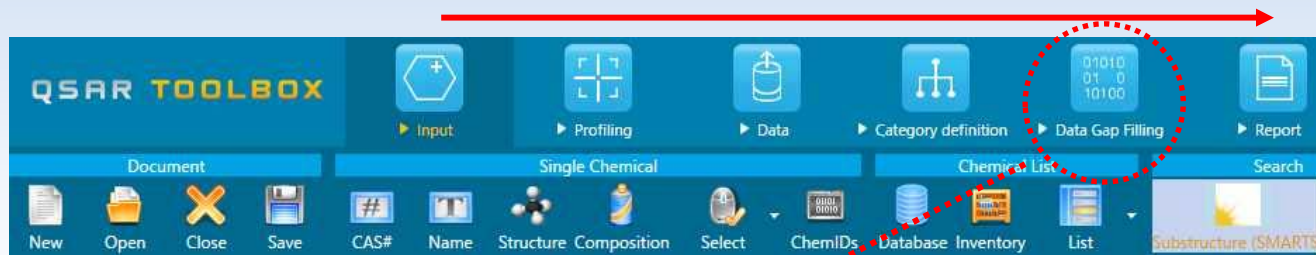
## Profilers



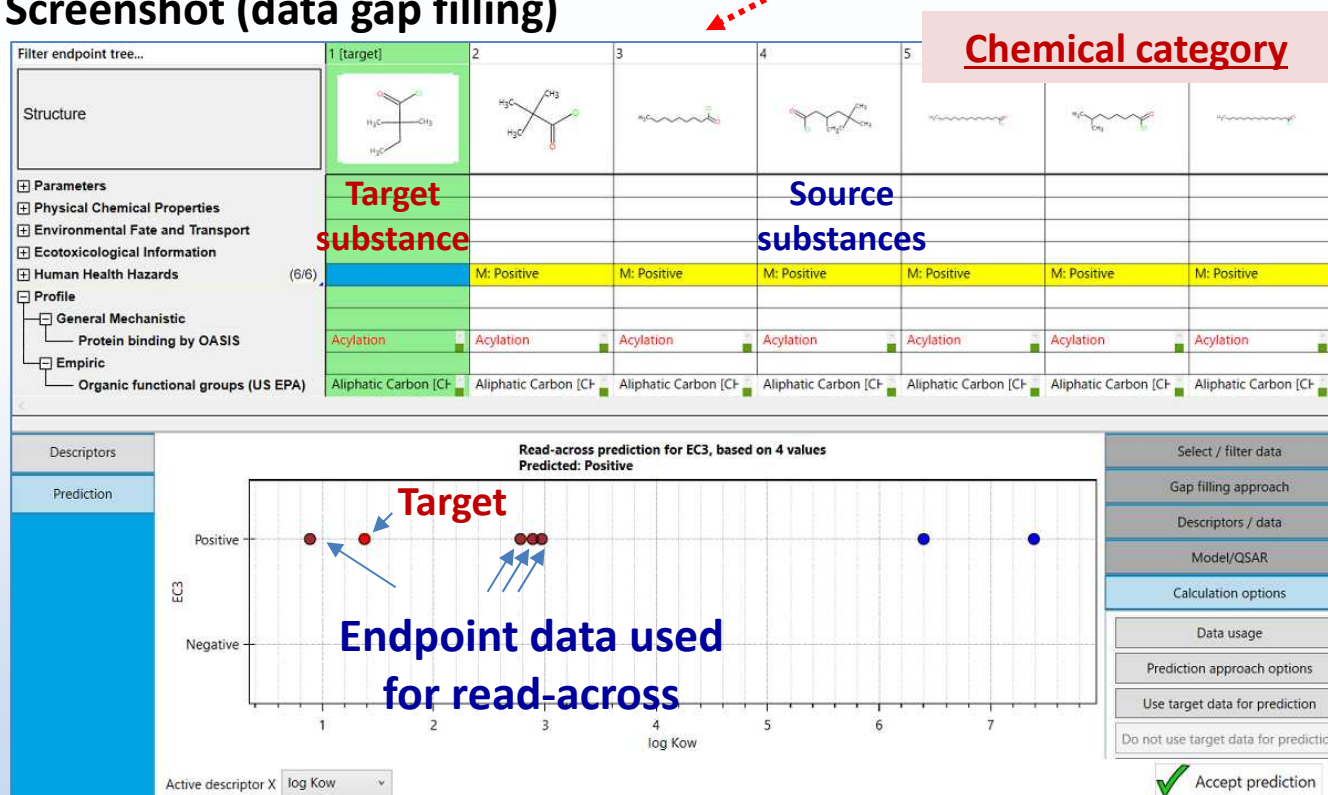


# Chemical Category and Read-across

## (A) Workflow



## (B) Screenshot (data gap filling)



# Importing a Custom Database

QSAR Toolbox 4.1 [Document 1]

QSAR TOOLBOX

Input Profiling Data Category definition Data Gap Fill

Data Import Export

Gather Import IUCLID6 IUCLID6

Documents

Databases

Options

Select All Unselect All Invert

- ECOTOX
- Human Health Hazards
  - Acute Oral toxicity
  - Bacterial mutagenicity ISSSTY
  - Biocides and plant protection ISSBIOC
  - Carcinogenic Potency Database (CPDB)
  - Carcinogenicity&mutagenicity ISSCAN
  - Cell Transformation Assay ISSCTA
  - Dendritic cells COLIPA
  - Developmental & Reproductive Toxicity
  - Developmental toxicity ILSI
  - ECHA CHEM
  - ECOTOX
  - ECVAM Genotoxicity & Carcinogenicity
  - Eye Irritation ECETOC
  - Genotoxicity OASIS
  - Human Half-Life
  - Keratinocyte gene expression Givaudan
  - Keratinocyte gene expression LuSens
  - Micronucleus ISSMIC
  - Micronucleus OASIS
  - MUNRO non-cancer EFSA
  - RDT Data FSCJ\_2018forQSARToolbox\_1**
  - BEACH Skin sensitization database (no)
  - Rece

import

Filter endpoint tree...

Structure

Photoinduced

Repeated Dose

LOEL

Rat

Blood cell (Coagulation) (14/40)

Blood cell (Erythrocyte) (42/98)

HCT↓ (42/98) M: 353 mg/kg bdwt

HGB↑ (1/1)

Importing to RDT Data FSCJ\_2018forQSARToolbox\_1

Import mode

Vertical  Horizontal  I have a header row

Preview of file

Strain	Test category	Test group	Test organisms species	Year reported	Max dose	Min dose	Tissue	Recovery period	Purity	Comment	Reliability
Wistar	RDT	main	Rat	2004	1.2E3 mg/kg/day	120 mg/kg/day					
Wistar	RDT	main	Rat	2004	1.2E3 mg/kg/day	120 mg/kg/day					
Wistar	RDT	main	Rat	2004	1.2E3 mg/kg/day	120 mg/kg/day					
Wistar	RDT	main	Rat	2004	1.2E3 mg/kg/day	120 mg/kg/day					
Wistar	RDT	main	Rat	2000	688 mg/kg/day	27 mg/kg/day					
Wistar	RDT	main	Rat	2000	688 mg/kg/day	27 mg/kg/day					
Wistar	RDT	main	Rat	2000	816 mg/kg/day	33 mg/kg/day					
Wistar	RDT	main	Rat	2000	816 mg/kg/day	33 mg/kg/day					
Wistar	RDT	main	Rat	2000	816 mg/kg/day	33 mg/kg/day					
Wistar	RDT	main	Rat	2000	816 mg/kg/day	33 mg/kg/day	Hepatocyte				
Wistar	RDT	main	Rat	2000	816 mg/kg/day	33 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day	Hepatocyte				
F344	RDT	main	Rat	2004	2.96E3 mg/kg/day	176 mg/kg/day					

Food Safety Commission Japan Tox database (repeated-dose toxicity)

Back Next Import

# HESS: A tool to find analogues and the repeated-dose toxicity test data for read-across

**Input**

Chemical name: allyl valerate; pentanoic acid, 2-propenyl ester  
 CAS No: 6321-45-5  
 SMILES: C(=O)(CCCC)OCC=C

**Findings**

- Substance Identity
- Repeated Dose Toxicity
  - LOEL (3/52)
  - NOEL
    - Blood Chemical Examination (1/26)
    - General Signs (3/195)
    - Hematological Examination (1/57)
    - Histopathological Findings (3/1089)
    - Necropsy (3/261)
    - NOEL/LOEL (3/6)
    - Organ Weights (1/56)
- Profile
  - Study No. (Link to SSRDT)
  - Chemical No. (Link to HESS DB)
  - RDT Report No.
  - Rat Liver Metabolism Database
  - Repeated dose (HESS)

**RDT test data (NOEL/LOEL)**

1 (Target)	2	3	4
M: 12 mg/kg/day, ...	M: 25 mg/kg/day, ...	M: 400 mg/kg/day, ...	M: 31 mg/kg/day, ...
M: 12 mg/kg/day, ...	M: 12 mg/kg/day, ...	M: 25 mg/kg/day, ...	M: 31 mg/kg/day, ...
M: 100 mg/kg/day, ...	M: 400 mg/kg/day, ...	M: 250 mg/kg/day, ...	M: 250 mg/kg/day, ...
M: <6 mg/kg/day, ...	M: 25 mg/kg/day, ...	M: 31 mg/kg/day, ...	M: 31 mg/kg/day, ...
M: 25 mg/kg/day, ...			
318	356	349	
304	334	329	
304	336	329	
N/A	Root of map No. 255	Root of map No. 454	Root of map No. 449
Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)	Allyl esters (Hepatotoxicity)

# Link to Metabolism/Mechanistic Information and Toxicity Test Results of Analogues

**Metabolism map, ADME**

Form mechanism-based category

Confirm the test data of the nearest analogue for read-across

**Possible mechanism**

the liver (Ghilarducci and Tjeerdema, 1995). It readily forms a conjugate with glutathione (GSH), acrolein-GSH adduct (Olano *et al.*, 1985; Adams and Klaidman, 1993), which is followed by GSH depletion (Olano *et al.*, 1985; Silva and O'Brien, 1989), oxygen radical formation (Silva and O'Brien, 1989; Adams and Klaidman, 1993), and lipid peroxidation (Silva and O'Brien, 1989; Watanabe *et al.*, 1992). Acrolein is also capable of reacting with sulfhydryl groups of macromolecules nonenzymatically via a Michael addition. Reaction with critical intracellular sulfhydryl groups is proposed as one component of the cytotoxicity of acrolein (Cooper *et al.*, 1992; Kehrer and Biswal, 2000). Oxidative stress subsequent to the loss of GSH may be associated with mitochondrial dysfunction (Watanabe *et al.*, 1992; Arumugam *et al.*, 1999). Direct action of acrolein on mitochondria is possible (Sun *et al.*, 2006), but seems unlikely at low doses due to the presence of abundant cellular GSH. The mechanism of bile duct hyperplasia induced by allyl acetate and allyl alcohol is less well understood.

Figure 1. Hepatotoxic pathway induced by allyl acetate.

**Toxicity test report database**

DOSE	mg/kg	6			12			25									
		mean	SD	s...	F1	F3	mean	SD	s...	F1	F3	mean	SD	s...	F1	F3	
BUN	mg/dL	16.2	0.6		14.3	0.4	*	15.5	0.3		15.7	0.5					
CRN	mg/dL	0.77	0.02		0.70	0.00	**	0.74	0.02		0.71	0.01	*				
T-CHO																	
TG																	
PL																	
T-BIL																	
CLUC																	
TP	g/dL	6.5	0.1		6.4	0.1		6.5	0.1		6.5	0.0					
BA	µmol/L	18.0	1.0		21.8	2.0		19.8	2.7		21.2	2.2	Δ				
ALB	g/dL	4.8	0.1		4.6	0.0		4.7	0.0		4.7	0.0					
A/G																	
Protein % ALB																	
Protein % α1-glo																	
Protein % α2-glo																	
Protein % α3-glo																	
Protein % β-glo																	
Protein % γ-glo																	
AST(GOT)																	
ALT(GPT)	IU/L	88	3		83	3		95	4		99	5					
ALP	IU/L	626	16		588	9		597	7		583	11	*				
LDH																	
CPK-MB	U/L	70	1		70	1		70	1		70	1					

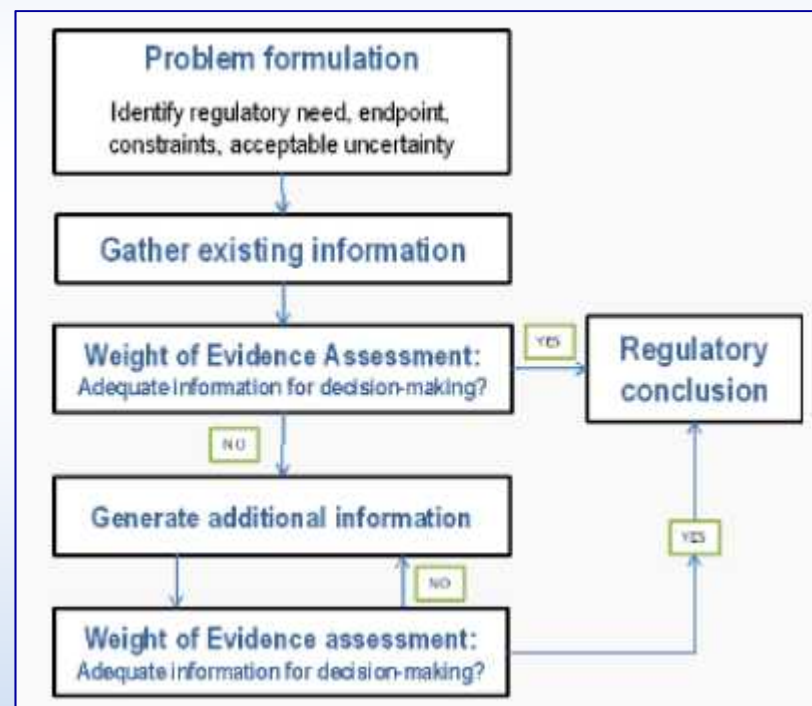
**(Dose-response data)**

# OECD IATA Case Studies Project (2015-)

- IATA (Integrated Approaches to Testing and Assessment)
- Combinations of in silico, in chemico, in vitro approaches.

Read-across is a part of IATA.

- Provide a forum to increase experience with the use of IATA for regulatory purpose
- Develop guidance
- Project team: Australia, Canada, Denmark, Japan, Netherlands, Sweden, United States, EU (EC), EU (JRC), EU (ECHA), BIAC and ICAPO



# Development of Case Studies by Member Countries/Bodies

## FY2015

- In Vitro Mutagenicity of 3,3' Dimethoxybenzidine (DMOB) based Direct Dyes [Canada & US]
- Repeated Dose Toxicity of Substituted Diphenylamines (SDPA) [Canada]
- Hepatotoxicity of Allyl Ester Category [Japan]
- Bioaccumulation Potential of Biodegradation Products of 4,4'-Bis (chloromethyl)-1,1'-biphenyl [Japan]

ENVIRONMENT, HEALTH  
& SAFETY NEWS



Just released!

October, 2016

### First Four Case Studies from the Integrated Approaches to Testing and Assessment (IATA) Case Studies Project

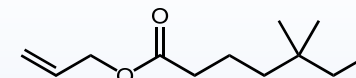
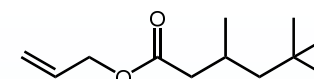
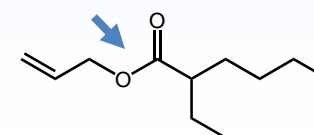
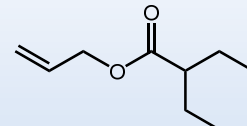
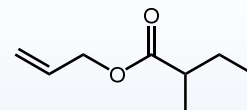
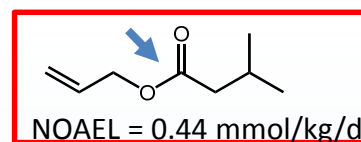
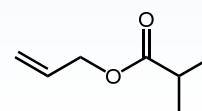
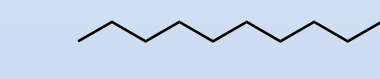
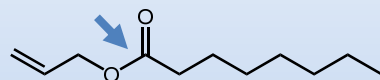
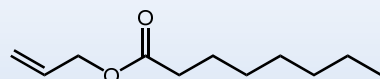
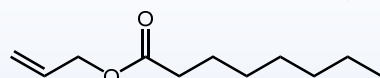
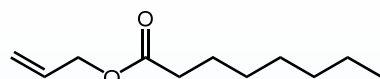
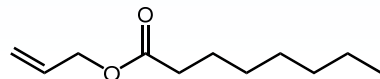
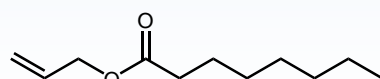
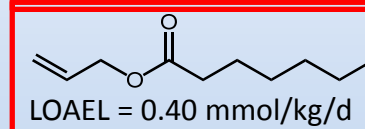
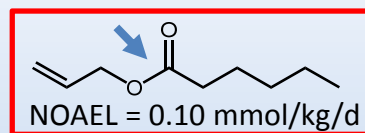
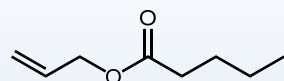
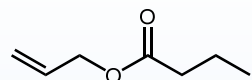
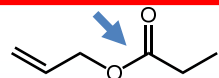
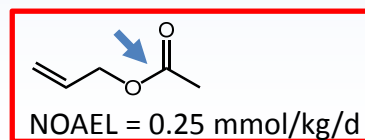
The first four case studies from the IATA Case Studies Project were published along with a reporting template and considerations document highlighting the learnings from the first review cycle.

The IATA Case Studies Project was launched in 2015 to increase experience with the use of IATA by developing case studies, which constitute examples of predictions that are fit for regulatory use.

**From 2015 to 2018 cycles, 15 case studies were developed by the member countries/bodies and reviewed by the project team.**

# Case Study 1 Developed by JP

**Purpose:** to assess repeated-dose toxicity of allyl ester category using toxicity data of the tested analogues. NO(A)EL values are derived for the hazard classification under the Chemical Substances of Control Law (CSCL).

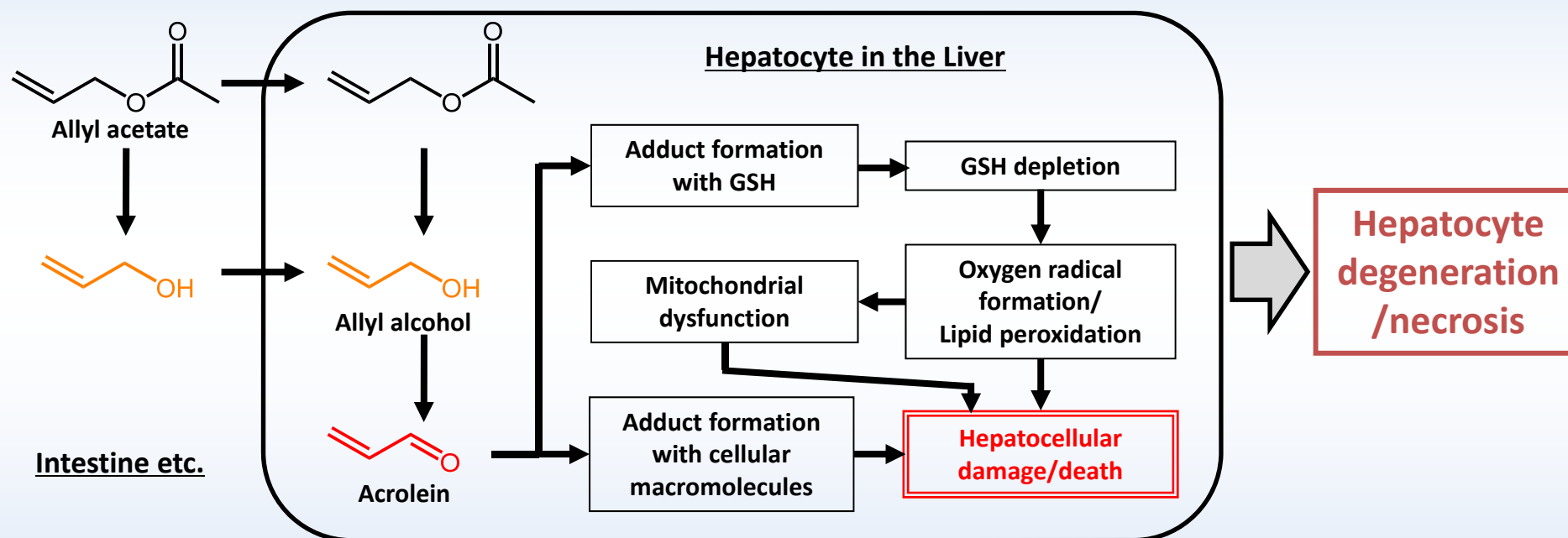


Source chemical  
Empirical metabolism  
data available

Primary toxicity of the category:  
Presumed NOAEL:

# Similarity Hypothesis (Case Study 1)

## Possible Mode of Action/Adverse Outcome Pathway



The mechanism of **bile duct hyperplasia** is not understood, although allyl alcohol formation is apparently linked to the toxic response.

**Similarity hypothesis:** Allyl esters that can be predictably metabolized to allyl alcohol are likely to produce hepatotoxicity.



# Category Justification (Case Study 1)

## ■ Toxicity

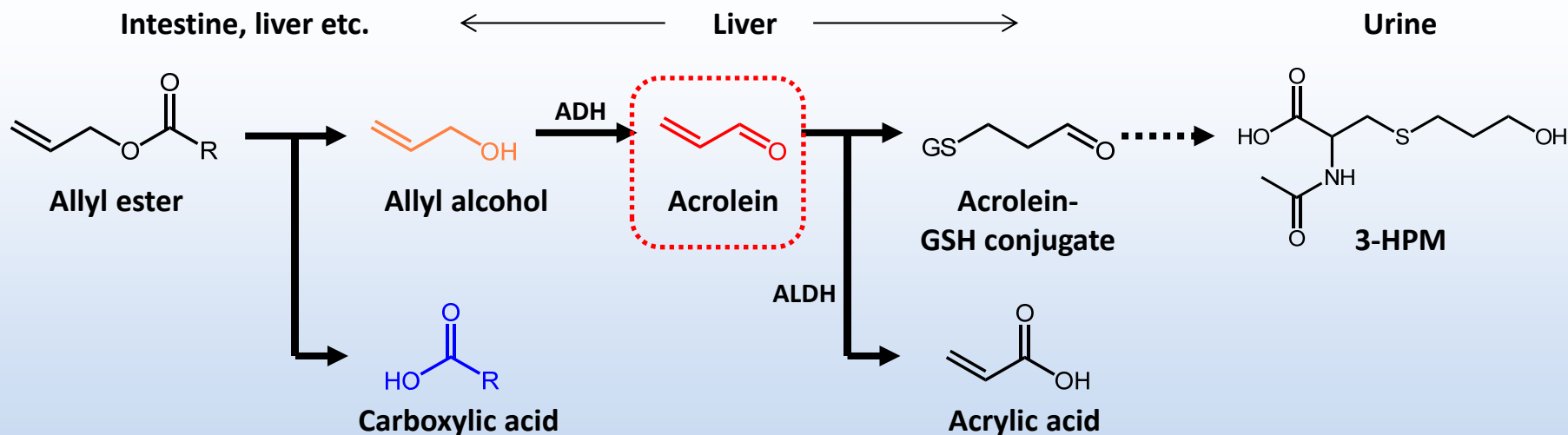
- ✓ Primary toxicity: hepatotoxicity

## ■ ADME

- ✓ In vitro: hydrolysis in intestine
- ✓ In vivo: 3-HPM in urine
- ✓ Hydrolytic rate (linear vs branched)

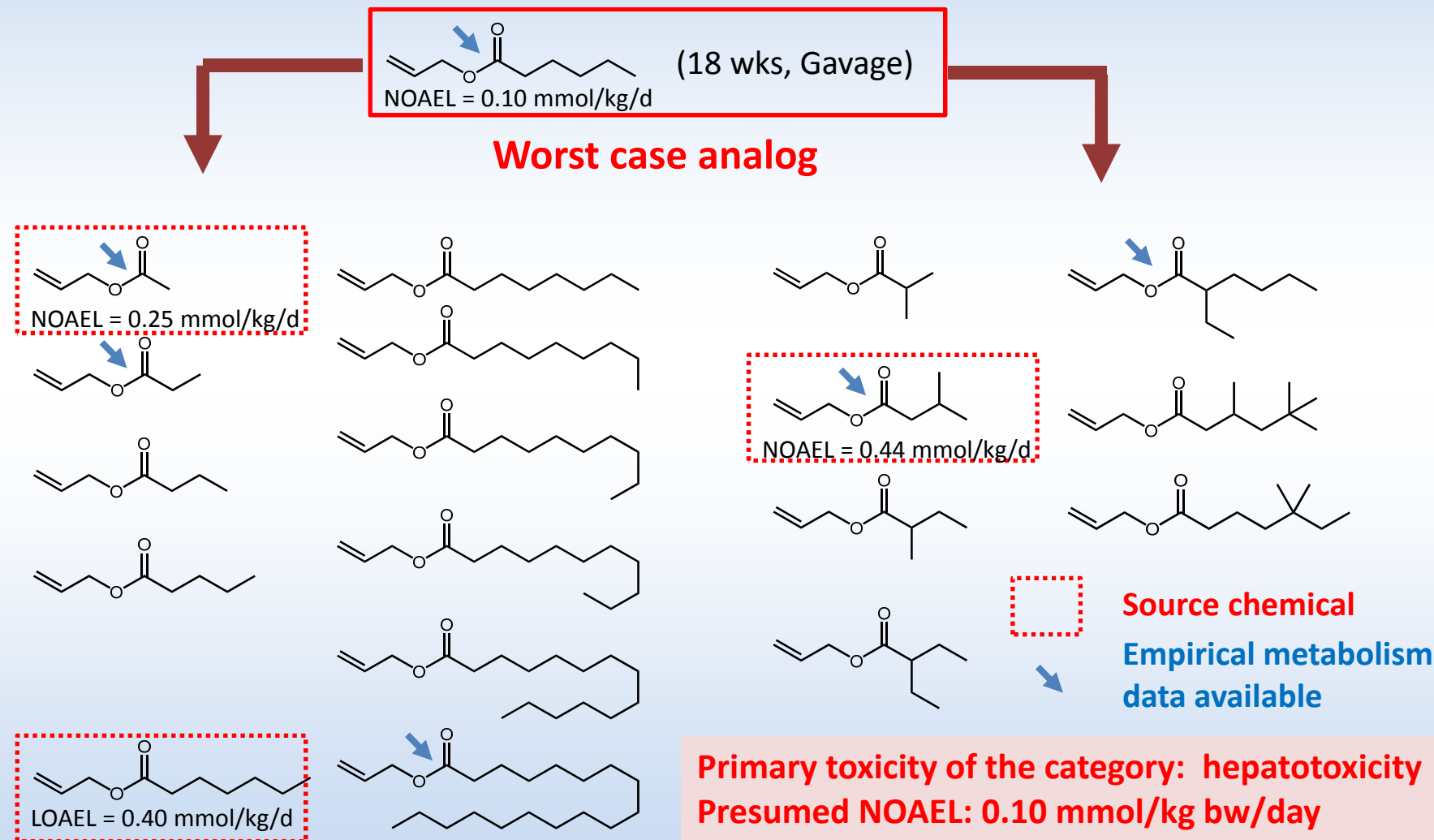
◆ Why and how is structural similarity associated with similar biological properties?

◆ Why read-across for the property under consideration is acceptable despite of the structural dissimilarities?



(Data is gathered and is used to connect category members.)

# Read-across (Case Study 1)



# Reviewers' Comments (Case Study 1)

---

## Uncertainty analysis

- Is the hepatotoxic effect the **critical effect** of these substances?
- How does the steric hindrance influence the toxicity?
- Not convinced that the **other part of the metabolites**, the carboxylic acids, do not have **other toxic properties**.
- More clarify category boundary (for branched subcategory)
- Ideally, **the hypothesis should allow a quantitative estimation** of the toxicity.
- Is this is also **relevant for humans**? A more in depth discussion of this would be valuable.

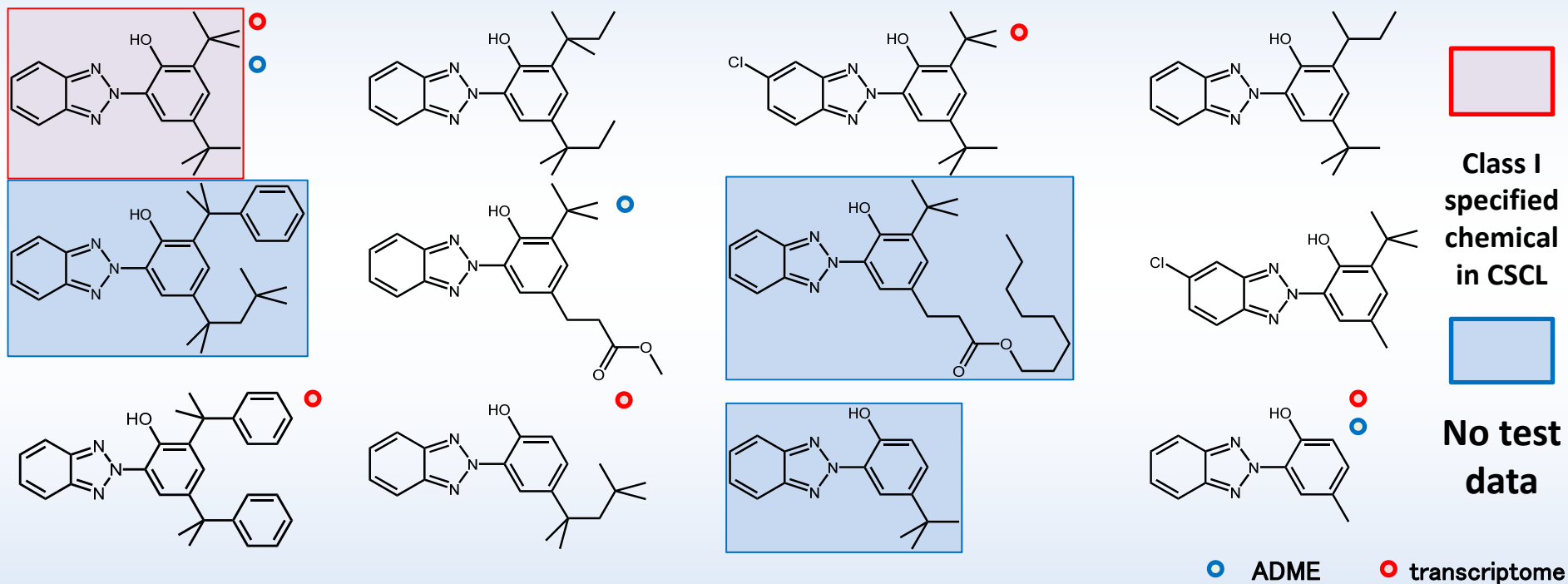
# Discussion on Human Relevance

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- Lack of toxicological information on allyl esters in humans
- Plausible to assume that **hydrolytic enzymes in the intestine** and other tissues, as well as **ADHs in the liver**, participate in metabolic activation **in humans**
- Acrolein was detected in the blood, bile and urine of man who had **accidental oral ingestion of allyl alcohol** (Toennes et al., 2002).
- Allyl alcohol and acrolein showed **cytotoxic effects in human hepatocytes** (Dvorák et al., 2003, Mohammad et al., 2012).

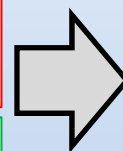
# Case Study (2) Developed by JP

## Screening assessment of phenolic benzotriazole category for repeated-dose toxicity

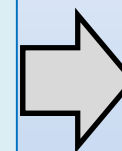


**Primary toxicity: hepatotoxicity**  
**NOEL: 0.1 – 100 mg/kg bw/day**

**Structural differences affect toxicity levels**



**Use of ADME and liver transcriptomic data for subcategorization**



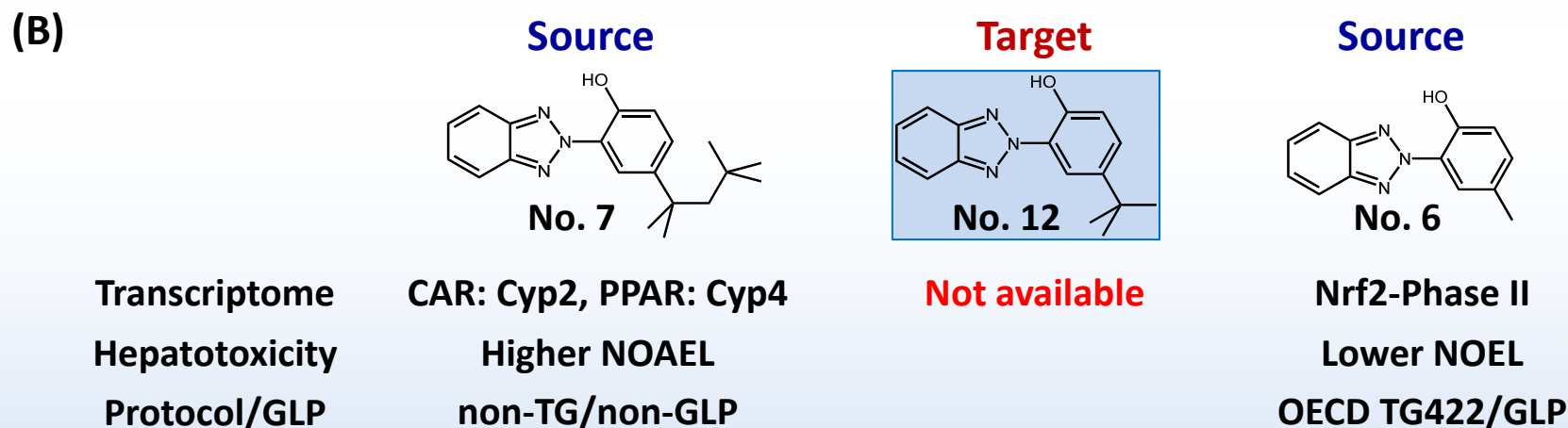
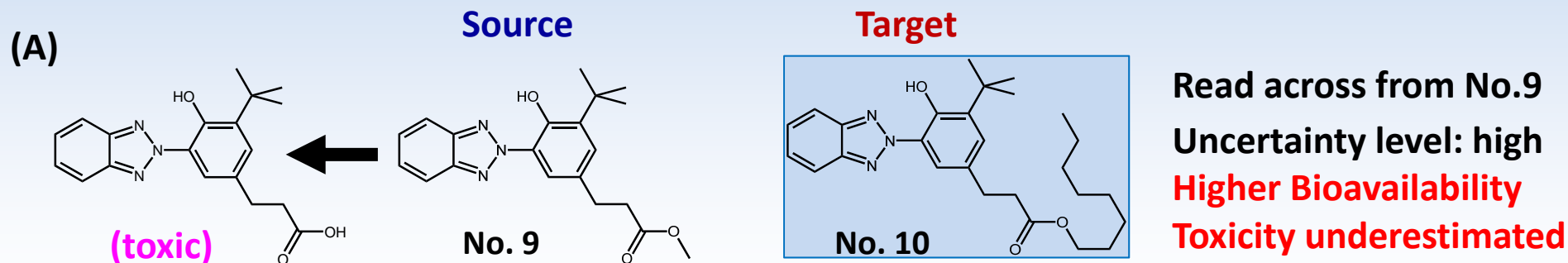
**Read-across**

## Reviewers' Comments (Case Study 2)

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- A proper hypothesis should describe the basis on which it is considered that **the members of the (sub)categories will share the same properties as far as the repeated-dose toxicity is concerned.**
- Better explanation is needed on **why only hepatotoxicity was considered and not nephrotoxicity.** Are we sure that for the target substances the effects on kidney will be induced at higher doses than the hepatotoxic effects?
- The reason for conducting **the transcriptome tests for this category should be stated relating to the hypothesis.** A short discussion of the selected genes and how they relate to **hepatotoxicity** is needed.

# Uncertainties in Selecting an Analogue and Performing Read-across (Case Study 2)



- **Uncertainty level is high: Transcriptome and kinetics data not uniformly available**
- "high uncertainty" may be an obstacle to derive prediction.
- No.6 recommended as a source: better quality study and more conservative for screening, and mention the branching difference as an uncertainty for the effect

# Areas Identified for Guidance Development

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- Definition of analogues/category boundaries
  - Lacked a discussion on the structural differences whereas their structural similarities were well documented
- Uncertainty analysis and reporting
  - Each case study contains different uncertainties because of limited data or resource
  - Uncertainty analysis helps reviewers to consider the acceptable degree of uncertainty to the specified purposes.
  - There are several studies on uncertainty of read-across, but no international guidance. (Blackburn, 2014; Schultz, 2015)



# Lessons Learned from Our Case Studies

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- Read-across is conceptually simple but practically difficult.
- It takes a lot of time to gather information for preparing read-across cases.
- Crucial factors for regulatory acceptance
  - Transparency and reproducibility
  - Increase in confidence: similarity hypothesis based on mechanism, and quality and quantity of test data used for read-across
  - Decrease in uncertainty: supporting data of TK, in vitro testing, omics or related information for bridging chemicals

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**Thank you very much  
for your attentions!**