



Experience & Needs
from industry

Feedback from L'Oreal R&I

Workshop on Use of QSAR
Toolbox
November 24th, 2011



L'ORÉAL



- *Introduction & General feedback on the OECD Toolbox*
- *Case-studies highlighting several issues*
 - Coverage of databases & Profilers
 - Quality /Quantity (presence/absence) of information
 - Documentation of profilers & inventories
 - Multiple databases: human vs environmental safety
 - Multiple profilers : Info available to the end-user
 - Dealing with skin and liver metabolites
 - Risk of abusing of the tool
- *Concluding remarks*
- *Acknowledgements*

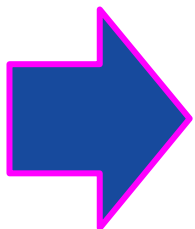


- L’Oreal has been following the development of the OECD Toolbox since 2007 as part of its global efforts to develop/implement alternative methods to animal testing
 - *Input via both the sharing of expertise and regular feedback provided when new versions were released*
 - *Interest expressed in-house by different teams which are either using the Toolbox or interested in following its development as potential future end-users :*
 - Developers of predictive computational approaches
 - Chemists
 - Eco(toxicologists)
 - Teams in charge of the REACh dossiers



General feedback

- OECD Toolbox Project: provides a public software co-developed by major stakeholders involved in the development of computational approaches to fill data gaps for regulatory use
 - ECHA
 - OECD Member States
 - Academics, institutions, experts
 - Industry
 - Etc.
- High level of complexity inherent to the process of read-across
 - Consideration of Physchem properties, chemical reactivity, metabolism, toxicology, etc.



*Multiple expertise required to use the tool adequately.
Adequate training is essential.*



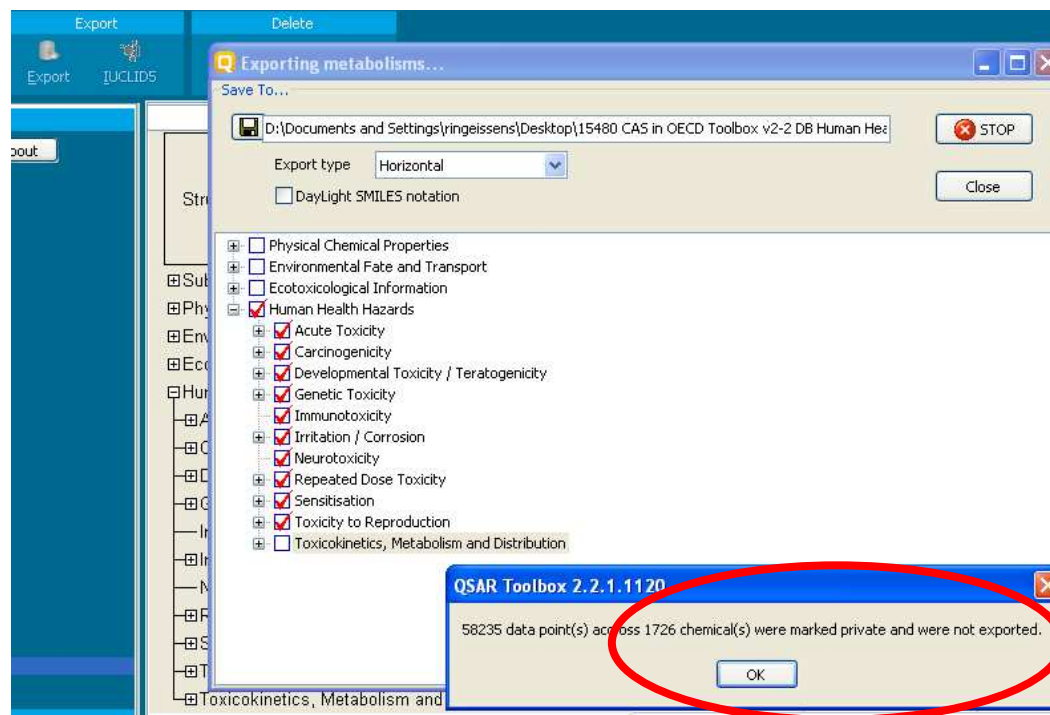
Coverage of Databases

- Some of the questions raised by chemists & safety assessors at L'Oreal regarding the ~ 50.000 chemicals present in the Toolbox & their associated safety data:
 - *What % of toxicological data being in the public domain is in the Toolbox?*
 - What public databases are not included in the current version of the Toolbox ? *Because of non authorization from institutions in charge of certain DBs, or because some data cannot at present be easily linked to adverse effects (eg HTS data)*
 - *Are there on-going DBs retrieval?*
 - *What is covered in terms of industrial use? agrochemicals (pesticides, etc), cosmetics, food ingredients, drugs, detergents, etc*
 - What do we know is missing? *Eg some chemicals used for a specific industrial application?*
 - *What % of the CAS registry is present in th toolbox?*
 - *How many chemicals have at least 1 data for human health endpoints? For Environmental endpoints?*

*Need for more
infos/statistics on
inventories/DBs included in
the Toolbox*

Coverage of Databases

- After selection of chemicals from DBs targeting human health endpoints, export of data was launched:



Eg DB REPDOSE Fraunhofer (615 chemicals): *“All copyright from the RepDose DB are owned in full by the Fraunhofer-Gesellschaft. Permission is granted to download or print material published from the RepDose DB for personal use only. This includes use of data for categorisation of chemicals via the read across or category approach. Its use for any other purpose, and in particular its commercial use or distribution, are strictly forbidden in the absence of prior written approval.”*

- Which data/quantity of data is downloadable?

Coverage of profilers

- Interest in adding to the Toolbox other profilers
 - Eg groups of flavourings defined by EFSA (interest also in adding to the Toolbox safety data on chemicals that have been assessed as food additives and food flavourings by EFSA)
 - *Boundaries of these categories (more than 30) have to be defined*



The screenshot shows the EFSA Journal website interface. At the top, there is a navigation menu with the following items: About EFSA, News & events, Topics A-Z, Publications (highlighted in orange), Panels & units, Cooperation, Applications helpdesk, and Calls & consultations. Below the menu, the breadcrumb trail reads: Home > Publications > EFSA Journal > Flavouring Group Evaluation 7, Revision 3 ... A 'Print' icon is visible on the right. On the left side, there is a sidebar menu with the following items: EFSA Journal (selected), Just Published, Latest Issue, All Issues, About the Journal, Supporting publications, and Corporate publications. The main content area features the 'EFSA JOURNAL' logo and a search bar with the text 'Search EFSA Journal'. Below the search bar is a 'Search' button and a link to 'Advanced Search'. The main article title is: 'Flavouring Group Evaluation 7, Revision 3 (FGE.07Rev3): Saturated and unsaturated aliphatic secondary alcohols, ketones and esters of secondary alcohols and saturated linear or branched-chain carboxylic acids from chemical group 5'.

Presence/Absence of information

- Read-across exercise on CAS 107-98-2
 - PGME: Propylene Glycol Monomethyl Ether
- Mutagenicity/Skin sensitization data from public sources are mentioned for the 3 analogs of PGME cited in Vink *et al.* (data captured from ECETOC and OECD reports)

Regulatory Toxicology and Pharmacology 58 (2010) 64–71

Contents lists available at ScienceDirect



Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

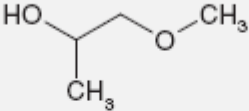
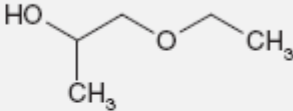
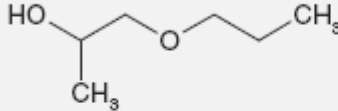
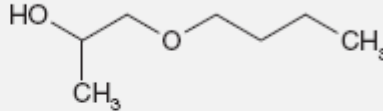


Use of read-across and tiered exposure assessment in risk assessment under REACH
– A case study on a phase-in substance

S.R. Vink^{a,*}, J. Mikkers^b, T. Bouwman^b, H. Marquart^b, E.D. Kroese^a

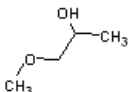
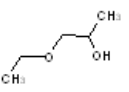
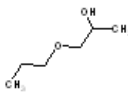
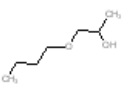
^aTNO Quality of Life, Department of Research & Development, Zeist, The Netherlands
^bTNO Quality of Life, Department of Food & Chemical Risk Analysis, Zeist, The Netherlands

Table 1
Molecular structure and physico-chemical properties of target substance PGME and its read-across candidates PGEE, PGPE and PnB.

Substance				
	1-methoxypropan-2-ol (Propylene Glycol Monomethyl Ether (PGME)) << Target chemical >>	2-propanol, 1-ethoxy- (Propylene Glycol Ethyl Ether (PGEE)) << Source chemical 1 >>	1-propoxy-2-propanol (Propylene Glycol Propyl Ether (PGPE)) << Source chemical 2 >>	1-butoxypropan-2-ol (Propylene Glycol n-Butyl ether (PnB)) << Source chemical 3 >>
CAS number	107-98-2	1569-02-4	1569-01-3	5131-66-8
Similarity		72.6% (Chem ID) 81.2% (DSSTox)	64.7% (Chem ID) 72.2% (DSSTox)	55.8% (Chem ID) 54.1% (DSSTox)

Presence/Absence of information

- The Muta/Skin sensitization data on analogs of PGME are missing in the Toolbox

	1 (Target)	2	3	4
Structure				
CAS Number	107-98-2	1569-02-4	1569-01-3	5131-66-8
Chemical Name	2-propylene glycol ... 1-methoxy-2-propanol 2-propanol, 1-meth...	1-ethoxy-2-propanol 2-propylene glycol (... 1-ethoxypropan-2-ol 2-propanol, 1-ethoxy-	propylene glycol n-... 1-propoxypropan-2-ol 2-propanol, 1-propoxy- 1-propoxy-2-propanol	1-butoxy-2-propanol propylene glycol m-... 2-propanol, 1-butoxy- 1-butoxypropan-2-ol
Structural Formula	C(C)(O)COC	C(C)(O)COCC	C(C)(O)COCCC	C(C)(O)COCCCC
Physical Chemical Properties	(4/10) M: 119 °C, -95 °C, ...	M: 131 °C	M: 150 °C, -80 °C, ...	M: 172 °C
Environmental Fate and Transport	(2/5) M: 9.20E-7 atm-m3...			M: 3.76E-11 cm3/...
Ecotoxicological Information	(3/5) M: 5 parts per milli...	M: 5 parts per million		M: 5 parts per million
Human Health Hazards				
Acute Toxicity				
Carcinogenicity				
Developmental Toxicity / Teratogenicity				
Genetic Toxicity				
Immunotoxicity				
Irritation / Corrosion	(3/3) M: 0.09		M: 1.25	M: 1.12
Neurotoxicity				
Repeated Dose Toxicity	(4/108) M: 309 mg/kg/day, ...	M: 97.7 mg/kg/day	M: 111 mg/kg/day	M: 400 mg/kg/day
Sensitisation	(1/1) M: Negative			

Information on physico-chemical and toxicological properties of the selected source substances was obtained from publicly available review documents, i.e. for PGEE and PGPE, a dataset from ECE-TOC (ECETOC, 2005), and for PGME and PnB the SIDS Initial Assessment Report on Propylene Glycol Ethers was used (OECD 2003).

Vink et al.

Read-across inconclusive due to lack of data

Quality of information (1)

- Read-across applied to PGME for the AMES test
 - Categorization: No DNA binding >> OECD HPV « Propylene glycol ethers »
 - 3/28 analogs have a data (AMES test)

QSAR Toolbox 2.2.1.1120 [Document_1]

Input Profiling Endpoint Category Definition Data Gap Filling Report

The OECD QSAR Toolbox for Grouping Chemicals into Categories
Developed by LMC, Bulgaria

Structure	1 (Target)	3	23	24
<chem>CC(O)CO</chem>	<chem>CC(O)CO</chem>	<chem>CC(O)CO</chem>	<chem>CC(O)CO</chem>	<chem>CC(O)CO</chem>
Salmonella typhimurium (3/10)	M. Negative, Negati.	M. Positive	M. Negative	

Read across prediction of "Gene Mutation", taking the highest mode from the nearest 5 neighbours, based on 10 data points from 3 neighbour chemicals. Observed target value: N/A, Predicted target value: 'Negative'

Positive
Equivocal
Negative

log Kow

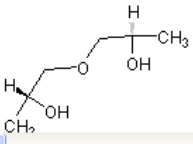
Descriptor X: log Kow

28 Propylene glycol ethers (OECD HPV Chemical Categories) | Data gap filling

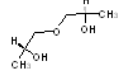
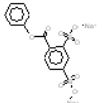
■ CAS # *0-13-1

Quality of information (2)

- Info Gathered from the Data Gap filling window on « CAS # *0-13-1 »

CAS/2D	Names	CAS/Name	2D/Name	CAS/2D	2D equality
<chem>C{P-}[C][O]COCC{P-}[C]O</chem> CAS: 131 				1: N/A 1: Genotoxicity OASIS	Base Structure

- Info. Gathered in the INPUT window > « CAS # *0-13-1 »

Selected	CAS/2D	Names	CAS/Name	2D/Name	CAS/2D
1. Yes	<chem>C{P-}[C][O]COCC{P-}[C]O</chem> CAS: 131 				1: N/A 1: Genotoxicity OASIS
2. Yes	<chem>C[=O][c1c[S(=O)](=O)O{-}.[Na]{+}]cc[S(=O)](=O)O{-}</chem> CAS: 131 	1: benzoyloxybenzene disulpha	1: N/A 1: Skin Sensitisation	1: N/A 1: Skin Sensitisation	1: N/A 1: Skin Sensitisation

« QA (CAS/2D) »

- High Quality
- High Quality, Conflict ?
- Low Quality
- Low Quality, Conflict
- Moderate Quality
- Moderate Quality, Conflict
- N/A ?
- (Vides) ?

- Understanding of what is not OK for such CAS numbers is not straight forward

Quality of information (3)

- Searching analogs of a phthalate derivative (CAS 131-17-9) by structure similarity (Tanimoto @ 95%): retrieving « CAS 110-69-0 » with the same structure assigned and a wrong chemical name/CAS given (cf oxime)

		1 (Target)	2
Structure			
Substance Identity			
CAS Number		131-17-9	110-69-0
		phthalic acid diallyl... diallyl phthalate	butanal oxime

CAS/2D	Names	CAS/Name	2D/Name	CAS/2D	2D equality
C(=O)[c1c(C(=O)OCC=O)c(C(=O)OCC=O)c1]C(=O)OCC=O CAS: 110690	1: butanal oxime	1: High Quality 1: DSSTox 2: Micronucleus ISSM 3: RepDose Fraunhofer 4: Repeated dose toxi	1: Low Quality 1: Repeated dose toxi	1: Low Quality, Conflict 1: Repeated dose toxi	Base Structure

- This CAS is in red meaning a concern with the quality of the information provided: is there a possibility to exclude such chemicals from the read-across?

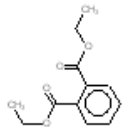


- This structure is not referenced in the Toolbox
 - Its CAS is 78418-01-6
 - Wrong information for « chemical name »

1 (Target)	
Structure	
Substance Identity	
– CAS Number	N/A
– Chemical Name	53761 9838158
– Structural Formula	C(=O)(O)c1c(O)ccc(C(=O)CCC...

Quality of information (5)

- GPMT assay not clearly indicated for CAS 84-66-2 (diethylphthalate)

1 (Target)												
Structure												
												
Data points												
Endpoint	Value	Original value	Organ	Type of method	Title	Test method / Data source	Institution and country	Year	Reference source	QA (CAS-2D)	Database name	
Skin sensitisation	Negative (Skin sensitisation II (ECETOC))	Negative (Skin sensitisation II (ECETOC))	Skin	in vivo	References Greif N, 1967. Cutaneous safety of fragrance material as measured by the maximization test. Amer. Perfum. Cosmet. 82, 54. Klecak G Geleick H and Frey JR, 1977. Screening of fragrance materials for allergenicity in the guinea-pig. 1. Comparis			01.08.1999	TR77 Skin and Respiratory Sensitisers - Reference Chemicals Data Bank.pdf	High Quality	Skin Sensitisation ECETOC	
Skin sensitisation	Negative (Skin	Negative (Skin	Skin	in vivo		LLNA	LMC,BUL	2002	Unilever	High Quality	Skin	

GPMT?

Documentation of profilers

ER Binding Profiler

Estrogen Receptor Binding (General Mechanistic) - Profiling Scheme Browser

Advanced

Estrogen Receptor Binding Category definitions

- Moderate binder, NH2 group
- Moderate binder, OH group
- Non binder, impaired OH or NH2 group
- Non binder, MW > 500
- Non binder, non cyclic structure
- Non binder, without OH or NH2 group
- Strong binder, NH2 group
- Strong binder, OH group
- Very strong binder, OH group
- Weak binder, NH2 group
- Weak binder, OH group

Profile Description

Non-ER binder due to high molecular weight.

Estrogen receptor (ER) binding is a molecular initiating event much like protein binding (1) that may lead to a series of adverse outcomes, which are typically linked to reproductive and development hazards. It is an endpoint where several comprehensive databases exist, which has led to the development of several approaches for using (Q)SARs to predict ER-binding and possible subsequent endocrine disruption (2). Popular among these are the "four phase" assessment that includes Comparative Molecular Field Analysis (CoMFA) (3) and the Common Reactivity Pattern Approach (COREPA) (4).

Since the RE-binding is a receptor mediated event, particular organic functional groups, size and shape are critical to binding potency. A schematic representation of an ER binding pocket with its three sites of interaction (A, B, C) is shown in Figure 1.

Chemicals that are too large cannot bind to the receptor regardless of structure or shape. While chemicals with a Molecular Weight of greater than 1000 are reported to be too large to bind to the receptor (2, 3) a review of the ER-binding database (ERBA OASIS) within the Toolbox reveals that no chemical with a molecular weight greater than 500 has been shown to bind to the ER receptor (Figure 2).

Need Definition of « Non binder/weak/Mod/strong/very strong »

A lot of information not easy to understand for an end-user not familiar with:

- the FDA NCCT « four phase » approach
- the ERBA OASIS database (transparent for end-users knowing OASIS software – Need for a Ref or weblink)

General intro on ER Binding: Should be moved to the « About » section. Add a sentence explaining that the ER profiler of the Toolbox is based solely on structural motifs & MW ranges?

To add:

- Reference/weblinking for the OASIS DB
- Species (Rat? Trout? Human?)
- Receptor subtype (Er α ? Er β ?)

Documentation of Inventories

- Cannot find any information on the COSING inventory

The screenshot displays a chemical profiling software interface. At the top, there are two chemical structures. Below them, a table lists various inventory affiliations and their status:

Structure	
Inventory Affiliation	COSING ECHA PR
OECD HPV Chemical Categories	(N/A)
Substance Type	Unknown struct
US-EPA New Chemical Categ...	(N/A)
DNA binding by OASIS	(N/A)
DNA binding by OECD	(N/A)
Estrogen Receptor Binding	(N/A)
Protein binding by OASIS	(N/A)
Protein binding by OECD	(N/A)

The 'Profiling results' window is open, showing a tree view of 'Inventory Affiliation' with 'COSING' and 'ECHA PR' listed. An 'Information' dialog box is displayed, stating 'Structure is present in: COSING' with a red circle around the text. The dialog box has an 'OK' button and a 'Close' button.

- Add a « Help » functionality with searches by keywords (to complement the info available on the OECD Toolbox website)?
 - Cf need to retrieve information as quickly as possible



Multiple databases

- Very important to have multiple DB but would be easier to use if DB targeting human-health safety were separated from DB targeting environmental safety

The screenshot shows a window titled "Databases" with a list of 25 databases, each with a checked checkbox. The databases listed are:

- Aquatic ECETOC
- Aquatic Japan MoE
- Aquatic OASIS
- Aquatic US-EPA ECOTOX
- Bioaccumulation Canada
- Bioaccumulation fish CEFIC LRI
- Biodegradation in soil OASIS
- Biodegradation OASIS
- Biota-Sediment Accumulation Factor
- Carcinogenic Potency Database CPDB
- Carcinogenicity & Mutagenicity ISSCAN
- ERBA OASIS
- Experimental pKa
- Eye Irritation ECETOC
- Genotoxicity OASIS
- GSH Experimental RC50
- kMdatabase
- Micronucleus ISSMIC
- Micronucleus OASIS
- Phys-chem EPISUITE
- RepDose Fraunhofer ITEM
- Repeated dose toxicity NEDO
- Rodent Inhalation Toxicity Database
- Skin Irritation
- Skin Sensitisation
- Skin Sensitisation ECETOC
- Terrestrial US-EPA ECOTOX
- Toxicity Japan MHLW
- ToxRefDB

Two red boxes highlight the following entries:

- A red box around "Carcinogenicity & Mutagenicity ISSCAN" with the text: "Name of DB: Not intuitive to an end-user not familiar with OASIS tools"
- A red box around "Skin Sensitisation" with the text: "Names of Skin sensitization DB: Confusing"



Multiple profilers (1)

- Complex for end-users not familiar with « OASIS » tools or with the latest evolutions of the Cramer classification

Profiling methods

Select All Unselect All Invert About

- Predefined**
 - Database Affiliation
 - Inventory Affiliation
 - OECD HPV Chemical Categories
 - Substance Type
 - US-EPA New Chemical Categories
- General Mechanistic**
 - DNA binding by OASIS
 - DNA binding by OECD
 - Estrogen Receptor Binding
 - Protein binding by OASIS
 - Protein binding by OECD
 - Protein Binding Potency
 - Superfragments
 - Toxic hazard classification by Cramer (original)
 - Toxic hazard classification by Cramer (with extension)
- Endpoint Specific**
 - Acute aquatic toxicity classification by Verhaar
 - Acute aquatic toxicity MOA by OASIS
 - Aquatic toxicity classification by ECOSAR
 - Bioaccumulation – metabolism alerts
 - Bioaccumulation – metabolism half-lives
 - Biodegradation fragments (BioWIN MITI)
 - Eye irritation/corrosion Exclusion rules by BFR
 - Eye irritation/corrosion Inclusion rules by BFR
 - Micronucleus alerts by Benigni/Bossa
 - Mutagenicity/Carcinogenicity alerts by Benigni/Bossa

41 Cat
115 Cat

67 Cat
122 Cat

39 Nodes
44 Nodes

No additional explanation available to the end-user to know whether to use Cramer with extensions or not

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S Ringeissen – Workshop ECHA – Helsinki - Nov 24, 2011

18



Importance of using the skin metabolism simulator

- Ethylene diamine (CAS 107-15-3)

- Use of the skin metabolism simulator

- 6 metabolites proposed for the target -> the dialdehyde glyoxal is among predicted metabolites (cf Schiff base formation leading to skin sensitization)

	1
Structure	
<ul style="list-style-type: none"> — Protein binding by OASIS 	<ul style="list-style-type: none"> 2 x No binding 4 x Schiff base formation with aldehydes 1 x 1-2-Dicarbonyls 4 x MA: Direct Acting Schiff Base For...
<ul style="list-style-type: none"> — Protein binding by OECD 	<ul style="list-style-type: none"> 4 x Mechanistic Domain: Schiff Base ... 3 x Mono-carbonyls 2 x No binding

- Primary amines undergoing oxidative deamination to aldehydes > Need infos on parent chemicals producing - via biotic metabolism - aldehydes (causing SS via Schiff Base formation) (currently missing in the simulator documentation)

Inherent complexity when dealing with metabolites (1)

- Dipropyl- (CAS 131-16-8) versus Diallylphthalate (CAS 131-17-9)

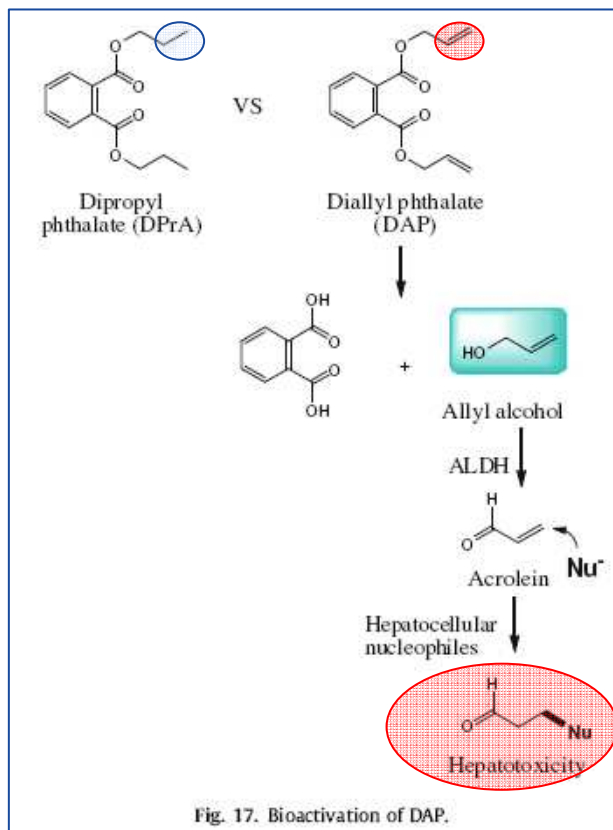
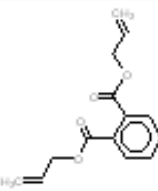
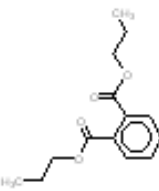


Fig. 17. Bioactivation of DAP.

S. Wu et al./Regulatory Toxicology and Pharmacology 56 (2010) 67–81

- Use of the liver metabolism simulator (cf no metabolites retrieved when using « observed liver metabolism »)
 - 12 metabolites proposed for diallylphthalate-> it becomes rapidly difficult to handle the multiple compounds (parent & metabolites) in the software

Inherent complexity when dealing with metabolites (2)

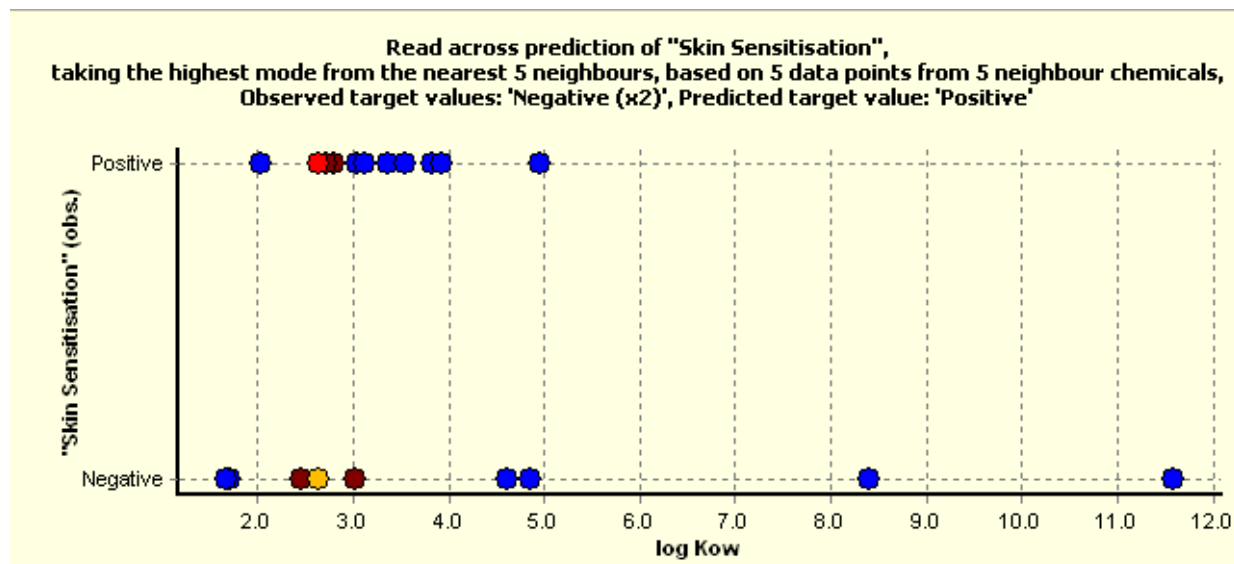
	1 (Target)	2
Structure		
Liver metabolism simulator	12 metabolites	4 metabolites
Database Affiliation	12 x (N/A)	4 x (N/A)
Inventory Affiliation	12 x (N/A)	4 x (N/A)
OECD HPV Chemical Categories	12 x (N/A)	4 x (N/A)
Substance Type	12 x Discrete chemical	4 x Discrete chemical
US-EPA New Chemical Categories	2 x (N/A) 1 x Acrylates/Methacrylates (Acute to... 2 x Aldehydes (Acute toxicity) 2 x Anionic Surfactants 2 x Epoxides 5 x Esters (Acute toxicity)	1 x Aldehydes (Acute toxicity) 1 x Anionic Surfactants 1 x Esters (Acute toxicity) 1 x Neutral Organics
DNA binding by OASIS	1 x Alpha, beta unsaturated aldehydes 2 x Epoxides, Aziridines 3 x No binding	1 x Aldehydes 3 x No binding
DNA binding by OECD	1 x Alpha, beta-unsaturated aldehydes 2 x Epoxides 2 x MA: Direct Acting Epoxides and r... 1 x MA: Direct Acting Schiff Base For... 1 x MA: Polarised Alkenes_Michael a... 1 x Mechanistic Domain: Michael addi... 1 x Mechanistic Domain: Schiff base 2 x Mechanistic Domain: SN2 1 x Mono aldehydes	1 x MA: Direct Acting Schiff Base F... 1 x Mechanistic Domain: Schiff base 1 x Mono aldehydes 3 x No Binding

Risk of abusing of the tool (1)

■ CAS 84-66-2 (diethylphthalate) : Read-across for Skin Sensitization

– Profiling:

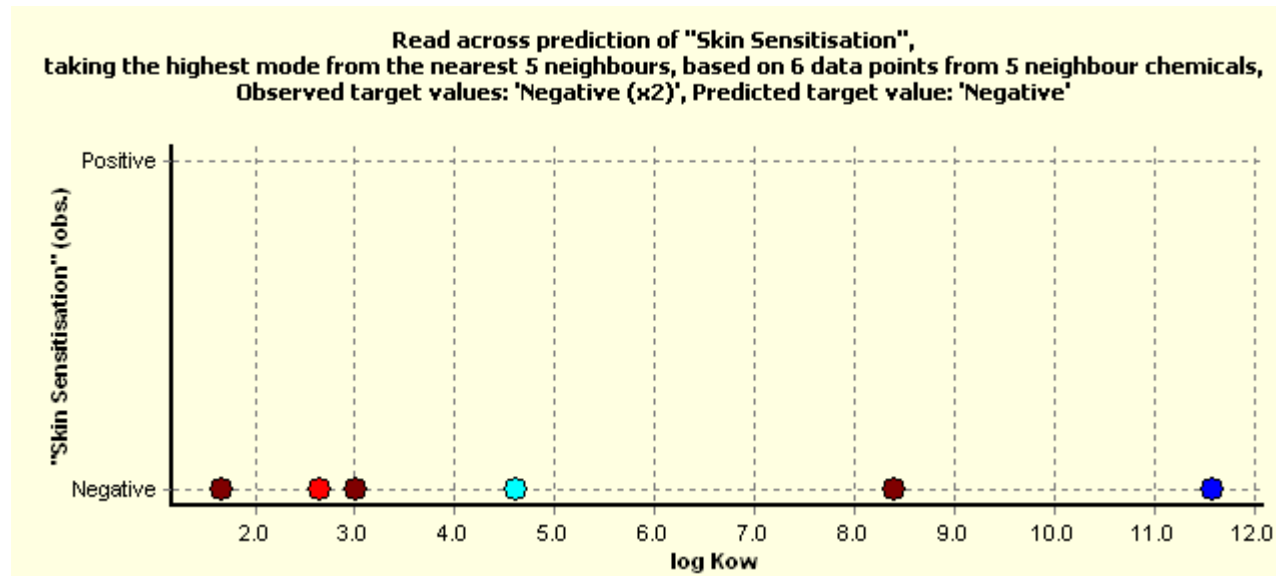
- Rq: No category « Phthalates » available
- No Protein-binding category
- ECOSAR, US EPA : category « Esters »
- Organic Functional groups (nested): « Arene, carboxylic acid ester »
 - 51/1353 analogs have a skin sensitization data
 - Sub-categorization with « chemical elements » >> 23/306 analogs have a SS data
 - » *Dimethyl & Dipropylphthalate are missing since no SS data available*
 - » *Dibutylphthalate is Neg in LLNA*



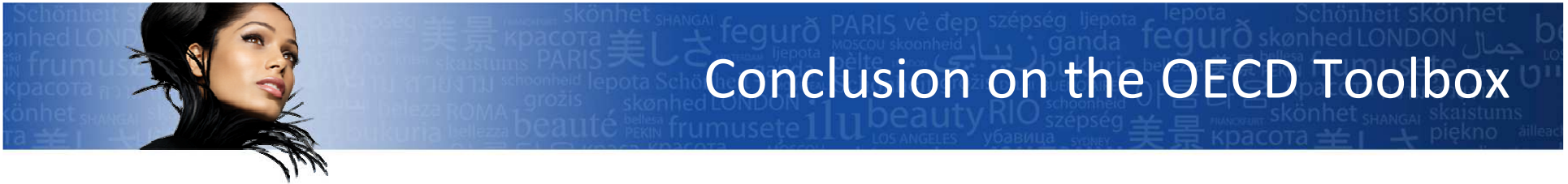


Risk of abusing of the tool (2)

- CAS 84-66-2 (diethylphthalate) : Read-across for Skin Sensitization
 - Further sub-categorization done with a focus on analogs of the category « No protein binding »
 - *Only 1/5 neighbours is a phthalate (Dibutylphthalate in blue below), other neighbours are di- or tri-carboxylates (meta or para substitutions, not ortho as it is for phthalates)*



- In the end: Read-across is conclusive? Inconclusive?



- A powerful tool made publicly available

- More development/refinement would make it more user-friendly and increase confidence in the data obtained
 - *include a reliability index to read-across outcomes?*

- Training is key to ensure as much as possible a proper use

- Appropriate use requires multiple expertise



- A special thanks to my colleagues for their feedback on the Toolbox, in particular:
 - J Clouzeau, S Morand (Safety assessors)
 - L Colombe (Ecotoxicologist)

Thanks for your attention!