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Federal Department of Home Affairs FDHA
Federal Food Safety and Veterinary Office FSVO
Risk Assessment Division

Toxicological assessment of NIAS from FCM in food: which way to go?

Beat Brüschweiler

3rd EFSA FIP Network FCM Meeting
Parma, 24.-26.5.2016

Overview

- Background
- Guidance and critical aspects in the NIAS evaluation:
- Accordance with “internationally recognized scientific principles on risk assessment” (EC/10/2011, Article 19)
- 5 real cases of NIAS evaluations:
  - by-product from photoinitiator for packaging inks
  - impurity in printing inks
  - degradation product of an additive
  - epoxy coating reaction product
  - polyamide reaction product
- Conclusions and recommendations

Starting substances (e.g. monomers, prepolymers, additives, solvents etc.)

IAS

Oligomers

NIAS

Impurities

Reaction intermediates

Contaminants

By-products

Degradation products

Guidance documents

- **EFSA Opinion on recent developments in the RA of chemicals in food and their potential impact on the safety assessment of substances used in FCM (2016)**
  Update of the SCF guidance (2001), EFSA Note for Guidance FCM (2008)

  Points to be taken into account:
  - sensitive population (children)
  - food consumption
  - migration of FCM substances into food
  - TTC approach

- **PlasticsEurope Risk assessment** of non-listed substances (NLS) and NIAS under Article 19 (EC/10/2011) (2013)

- **ILSI Guidance** on best practices on the risk assessment onf NIAS in food contact materials and articles (2015)

Categories of NIAS

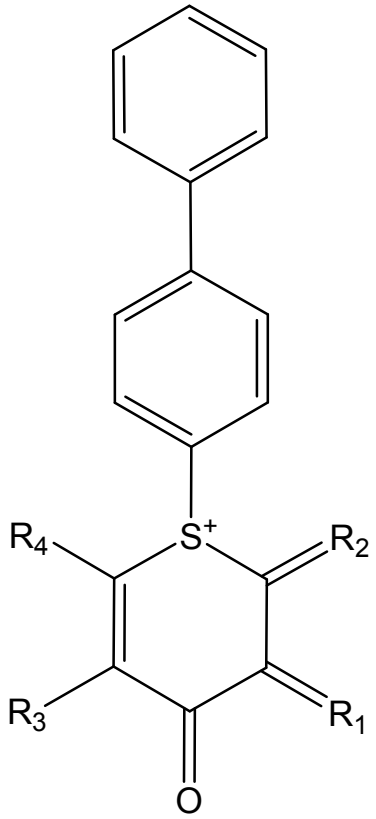
a) Identified NIAS, known chemical structure, experimental toxicity data available
   Case 1: toxicological evaluation already available

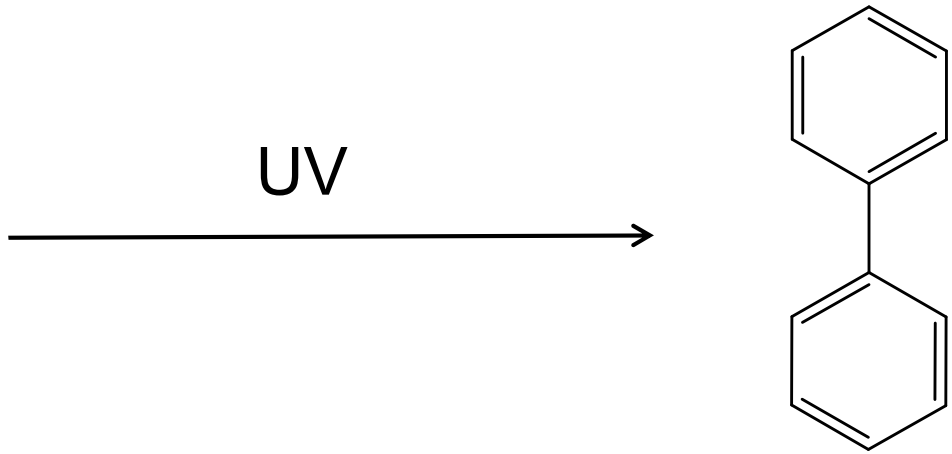
b) Identified NIAS, known chemical structure, no or insufficient experimental toxicity data
   Case 2: „pure TTC“
   Case 3: „structural alert for genotoxicity“
   Case 4: „read-across“ supplemented with molecular modeling
   Case 5: TTC supplemented with SAR and molecular modeling

c) Detected NIAS, chemical structure not identified
d) NIAS, not detected yet

Case 1: Sulphonium salt photoinitiator

(according to Green, 2010)





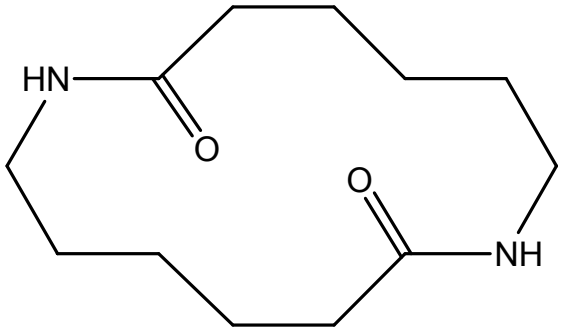
Biphenyl as by-product

Biphenyl was/is also used as pesticide, food additive and flavouring.

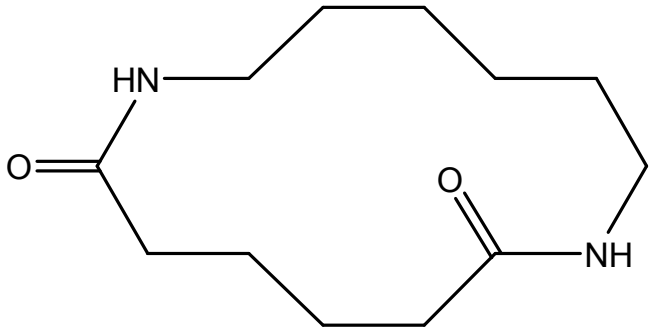
PTDI = 38 µg/kg bw/day (JECFA, 2006; EFSA, 2010)
10% allocation of exposure via food packaging
Intervention value = 0.23 mg/kg

Case 2: Cyclic polyamides

(described in Heimrich et al. 2012, 2015)



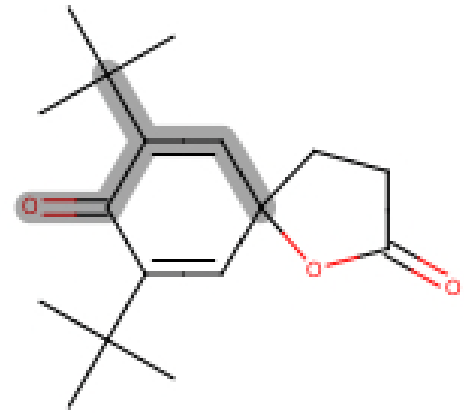
1,8-Diazacyclotetradecan-2,9-dione (cyclic dimer of PA6)



1,8-Diazacyclotetradecan-2,7-dione (cyclic dimer of PA66)

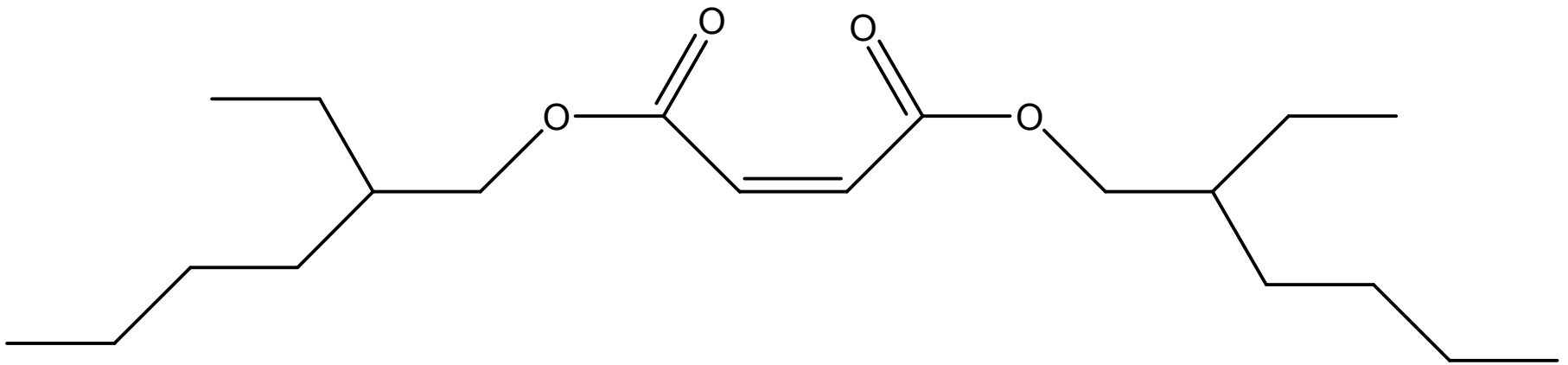
- Migration of 430 µg/kg (cyclic PA6 dimer) from artificial casings into sausages and up to 1500 µg/kg (cyclic PA66 dimer) from kitchen utensils (LUA Sachsen, Germany)
- Both substances were provisionally evaluated by BfR in 2012:
  - No structural alerts for genotoxicity; Cramer class III, 90 µg/person/day
  - Exposure estimate for cyclic PA6 dimer < 90 µg/person/day and for cyclic PA66 dimer > 90 µg/person/day
  - Need to take action for cyclic PA66 dimer (BfR, 2012)
  - Toxicity tests according to EFSA note for guidance FCM are required for a definite risk assessment
  - Data on hydrolysis of cyclic polyamides is needed (BfR research project; Prof. Simat, Dresden D)

Case 3: 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione



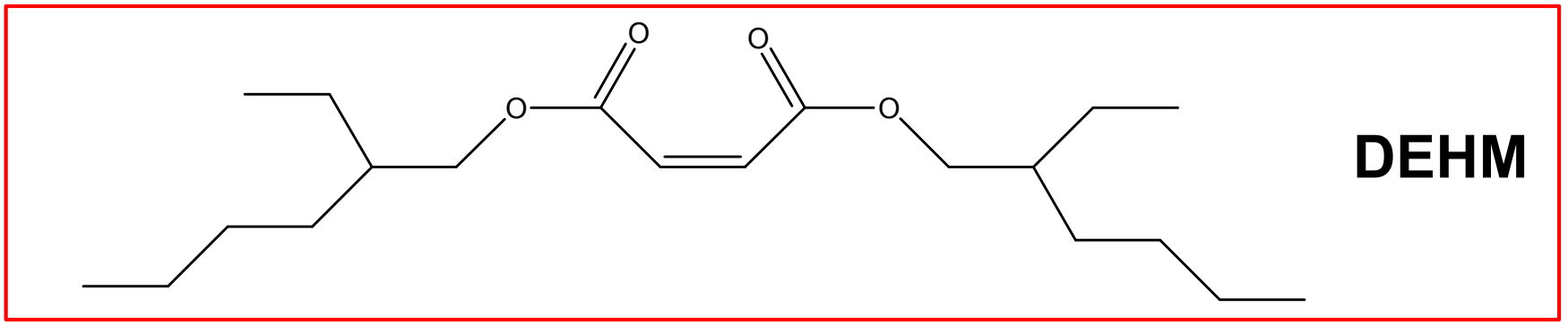
- Degradation product of primary phenolic antioxidants
- e.g. from sealing gaskets (polyethylene) for lids of mineral water bottles
- Toxtree (v2.6.0):
  - **structural alert** for genotoxic carcinogenicity
  - **structural alert** for *S. thyphimurium* mutagenicity
  - at least **one positive structural alert** for the micronucleus assay
    - alpha,beta-unsaturated aldehyde
  - DNA binding alert (Alert for Michael acceptor identified)
- Derek (Version 4.0.5):
  - Chromosome damage *in vitro* in mammal is **EQUIVOCAL**
    - Alert: alpha,beta-unsaturated ketone
  - Mutagenicity *in vitro* in bacterium is **INACTIVE**
    - No misclassified or unclassified features
- Sarah: Mutagenicity **NEGATIVE** (36% confidence)
- Definite answers by *in vitro* genotoxicity test(s)

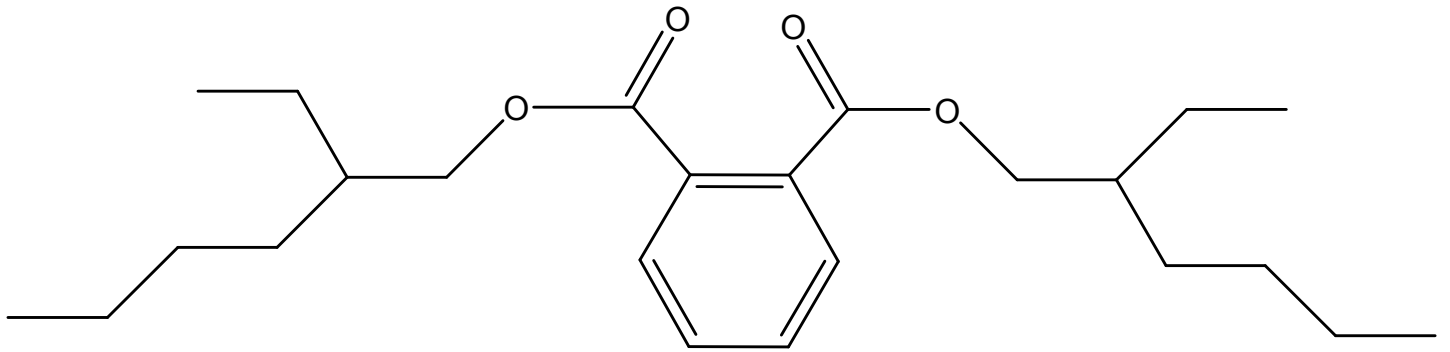
Case 4: Di(2-ethylhexyl)maleate (DEHM)



- Impurity of di(2-ethylhexyl)sulfosuccinate, an anionic emulsifier and surfactant, which is used in printing inks and adhesives for cardboard boxes
- Concentrations of up to 1500 µg/kg food (rice) (spring 2009) (Kantonales Labor Zurich; Fiselier et al., 2010)

Case 4: Di(2-ethylhexyl)maleate (DEHM)

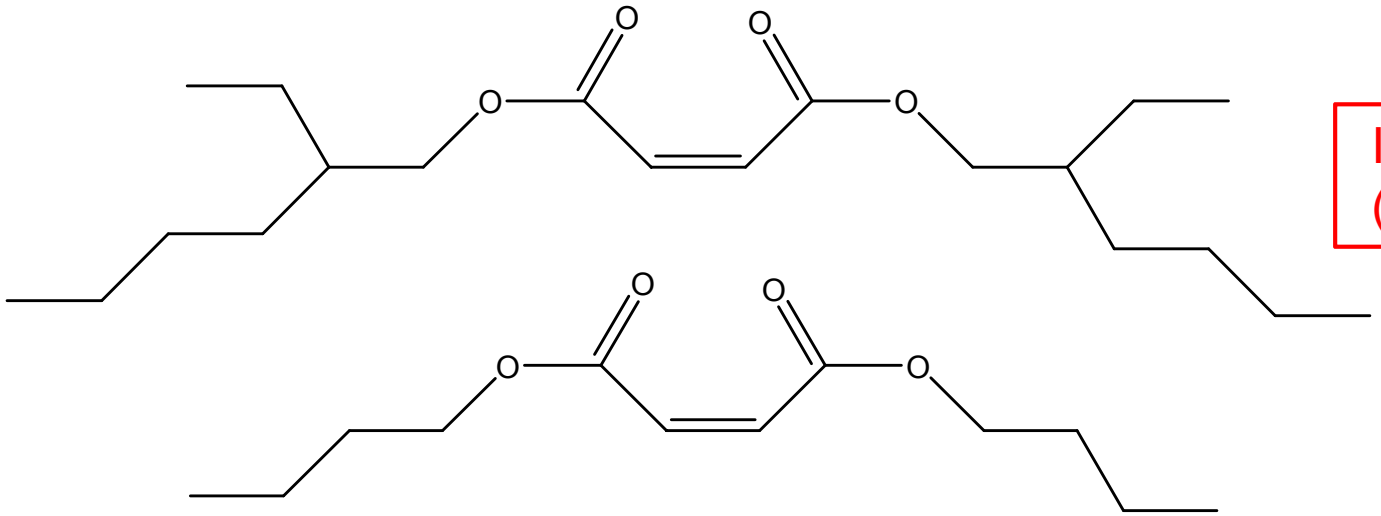




DNEL oral = 34 µg/kg bw/day (ECHA, 2013)
SML = 1.5 mg/kg (with restrictions)

DEHM and DEHP are classified into Cramer Class I (TTC = 30 µg/kg bw/day), but also dibutylphthalate (TDI = 10 µg/kg bw/day)

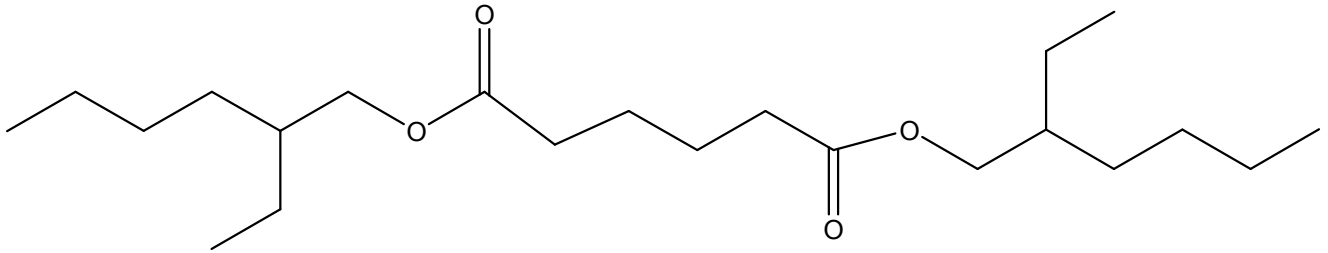
Case 4: Di(2-ethylhexyl)maleate (DEHM)



DEHM

Intervention value = 3 mg/kg
(Swiss Authority, July 2009)

Dibutylmaleate (DBM)



Di(2-ethylhexyl)adipate

- Read-across from DBM to DEHM
- Data for DEHM and DBM available on the ECHA homepage (DEHM: 1‘000-10‘000 t per year, full registration type)

Case 4: Read-across to determine an intervention value for DEHM

Endpoint	DEHM	DBM
	2009	2009
***In vitro* genotoxicity:**		
- Ames test	O	negative
- mouse lymphoma assay	O	O
- *in vitro* chromosome aberration assay	O	O
***In vivo* genotoxicity:**		
- mouse micronucleus	O	negative
Repeated dose toxicity:		
- 28 day study	O	OECD 422 (NOAEL = 95 mg/kg bw/day)
- 90 day study	O	O
Developmental / reproductive toxicity study	O	OECD 422 (NOAEL = 30 mg/kg bw/day)
Hydrolysis stability	- in the liver: rat liver S9 fraction * - intestinal fluid simulant, including. porcine pancreas lipase *	

Case 4: Read-across to determine an intervention value for DEHM

Endpoint	DEHM		DBM	
	2009	2014	2009	2014
***In vitro* genotoxicity:**				
- Ames test	O	negative	negative	see 2009
- mouse lymphoma assay	O	negative	O	negative
- *in vitro* chromosome aberration assay	O	negative	O	positive
***In vivo* genotoxicity:**				
- mouse micronucleus	O	O	negative	see 2009
Repeated dose toxicity:				
- 28 day study	O	OECD 422 (NOAEL = 300 mg/kg bw/day)	OECD 422 (NOAEL = 95 mg/kg bw/day)	see 2009
- 90 day study	O	O	O	OECD 408 (LOAEL = 30 mg/kg bw/day)
Developmental / reproductive toxicity study	O	OECD 422 (at least 1000 mg/kg bw/day)	OECD 422 (NOAEL = 30 mg/kg bw/day)	see 2009
Hydrolysis stability	- in the liver: rat liver S9 fraction * - intestinal fluid simulant, including. porcine pancreas lipase *	see 2009		

Case 4: Di(2-ethylhexyl)maleate (DEHM)

Binding affinity to different target proteins (VirtualToxLab, Prof. Vedani, Basel, 2009)

Target protein	**Calculated binding affinity**		
	(RR)-Isomer	*(RS)-Isomer*	*(SS)-Isomer*
Androgen receptor	not binding	not binding	not binding
Arylhydrocarbon receptor	not binding	not binding	not binding
CYP 2A13	not binding	280 µM **	not binding
CYP 3A4	40 µM **	25 µM **	11 µM **
Estrogen receptor α	not binding	1.8 mM **	220 µM **
Estrogen receptor β	not binding	68 µM ***	7.3 µM **
Glucocorticoid receptor	58 nM ***	42 nM **	300 nM ***
Liver X receptor	not binding	not binding	not binding
Mineralocorticoid receptor	not binding	not binding	not binding
PPAR γ (Peroxisome proliferator-activated receptor γ)	not binding	not binding	not binding
Thyroid receptor α	not binding	not binding	not binding
Thyroid receptor β	not binding	not binding	not binding
Toxic potential	**0.214 = low**	**0.245 = low**	**0.363 = low to medium**

Standard deviation of the prediction: *** (low), ** (medium), * (high), ~ (very high)

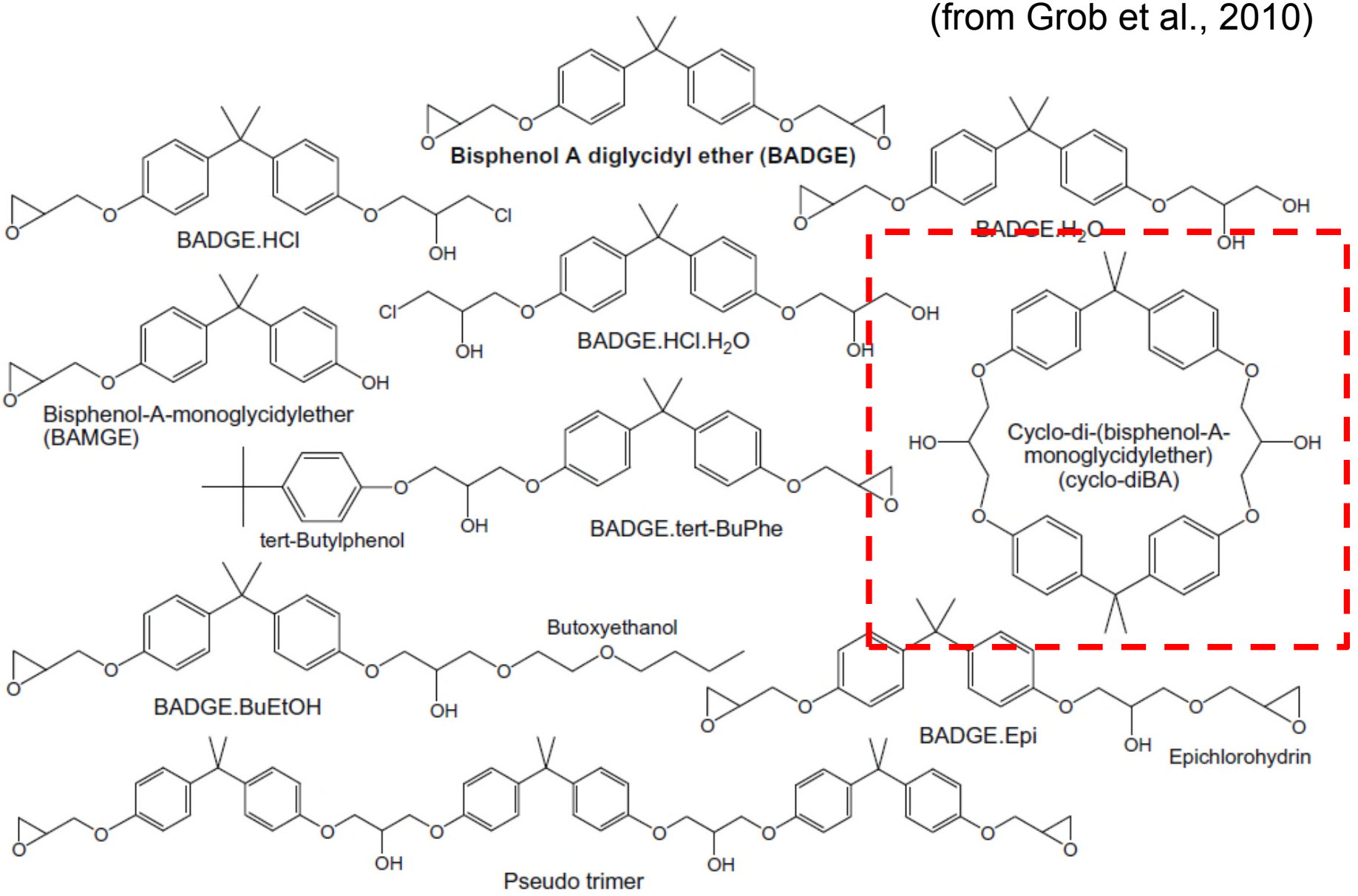
Case 4: Di(2-ethylhexyl)maleate (DEHM)

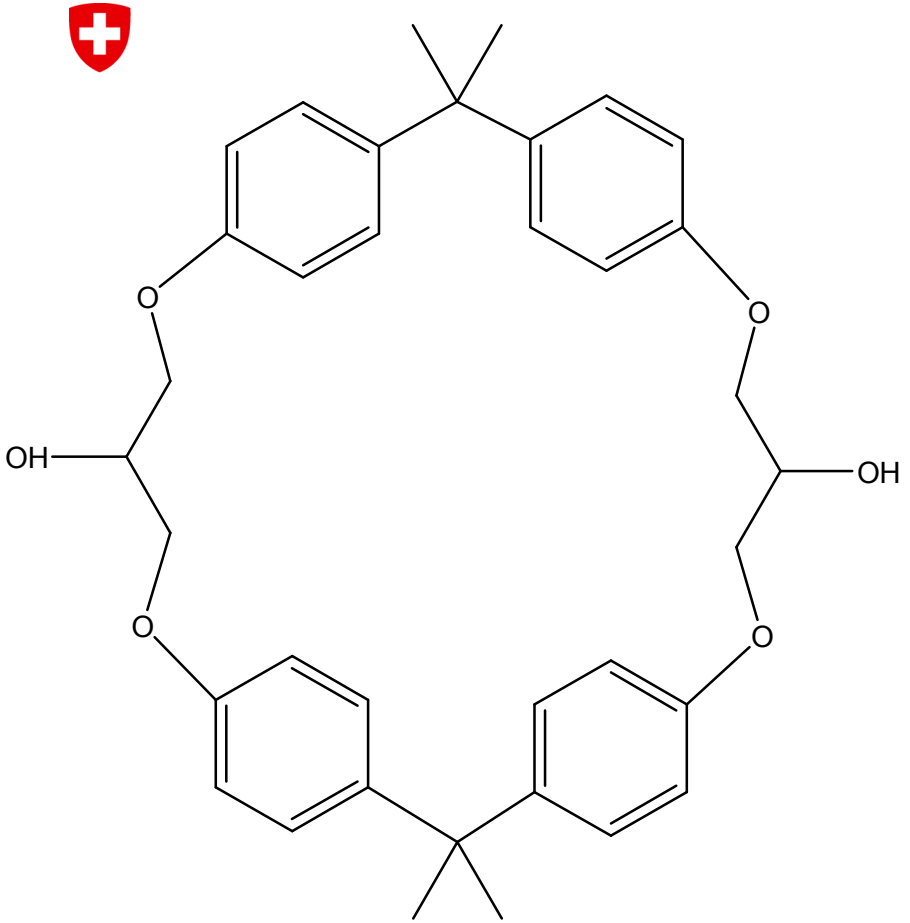
In a later stage of the evaluation process, an industry-sponsored hydrolysis study became available.
The study demonstrated that DEHM is completely degraded in intestinal fluid simulant after 3 h incubation.
The probable degradation products, maleic acid and diethylhexanol, should not be of toxicological concern.

(BfR, 4th BeKo-Meeting, 2009)

Case 5: Cyclo-di-BADGE, a reaction products formed during epoxy resin production

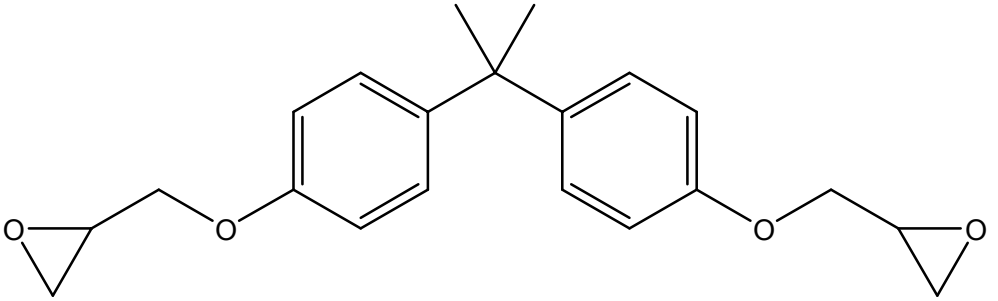
(from Grob et al., 2010)



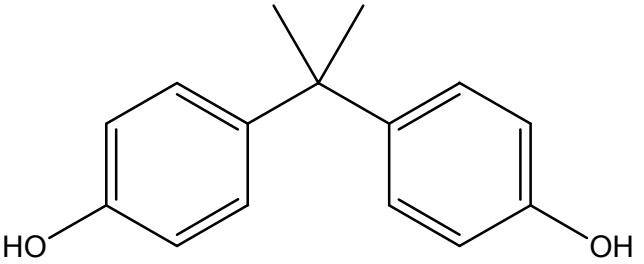


Cyclo-di-BADGE
(also referred to as cyclo-diBA)

Bisphenol-A-diglycidyl ether (BADGE)



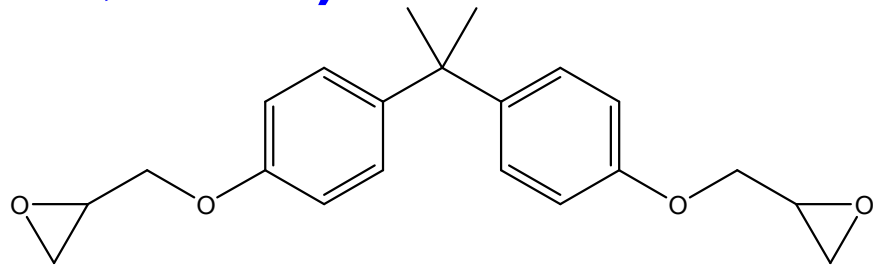
TDI = 150 µg/kg bw/d (EFSA, 2004)



Bisphenol A (BPA)

t-TDI = 4 µg/kg bw/d (EFSA, 2015)
Previous TDI = 50 µg/kg bw/d (EFSA, 2006)

BADGE evaluation (EFSA, 2004)



- "In summary, the Panel concluded that BADGE and its chlorohydrins (BADGE.2HCl, BADGE.HCl and BADGE.H_2O.HCl) do not raise concern for carcinogenicity and genotoxicity *in vivo,* respectively."
- "The Panel is aware that other BADGE reaction products other than chlorohydrins, with undefined toxicological properties and chemical identity, may be found at low levels in the migrate from epoxy coatings.
  For the assessment of these, and in general of minute amounts of unknown migrants from food contact materials, a general approach is currently under consideration by the Panel."

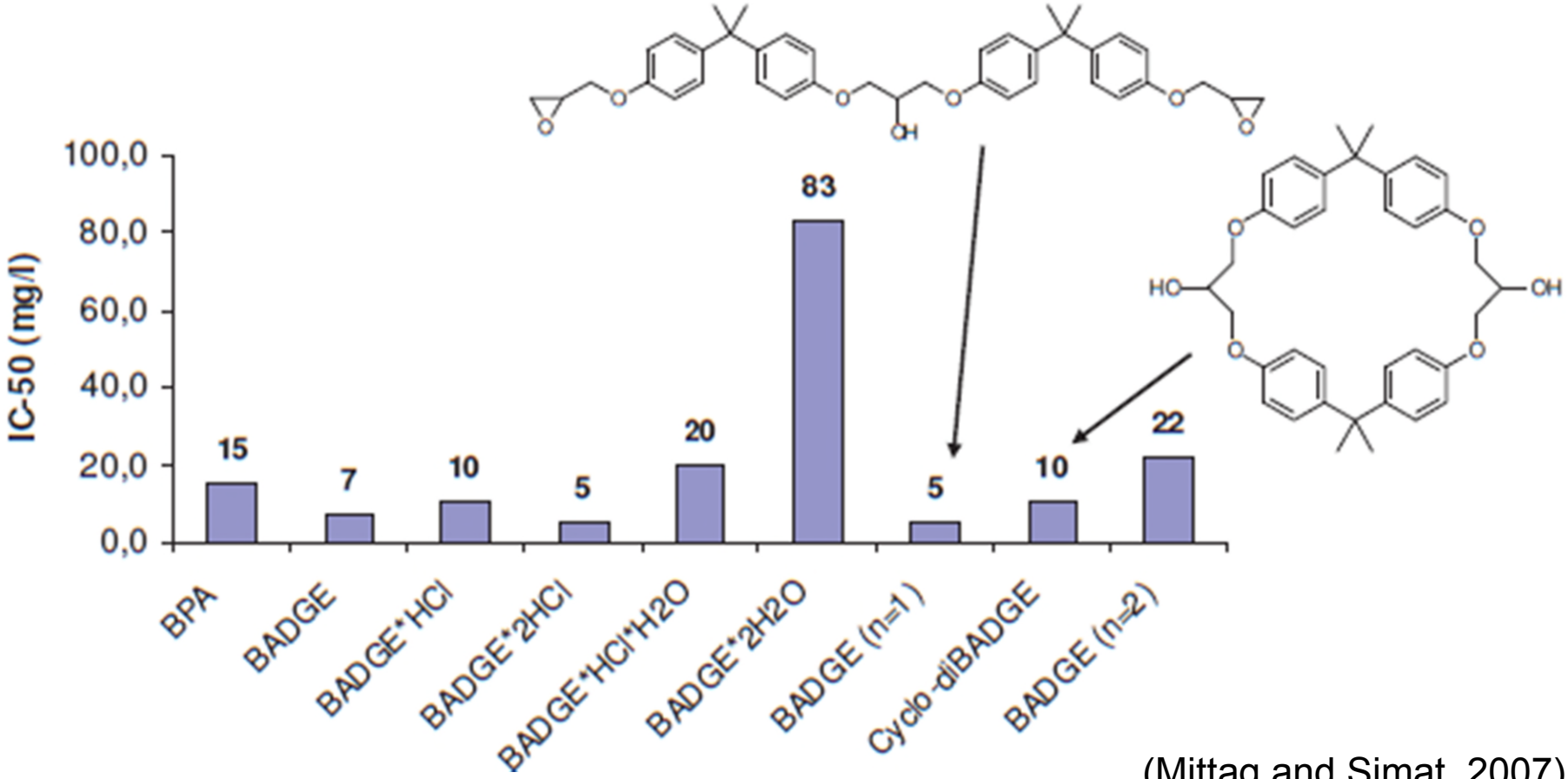
SML (EU): BADGE, BADGE.H_2O and BADGE.$2H_2O$ = 9 mg/kg;
BADGE chlorohydrins = 1 mg/kg; Regulation EC/1895/2005

SML (CH): BAGDE and its derivatives (BADGE.H_2O, BADGE.HCl, BADGE.2HCl, BADGE.H_2O.HCl) = 1 mg/kg

BPA: SML (EU, CH) = 0.6 mg/kg

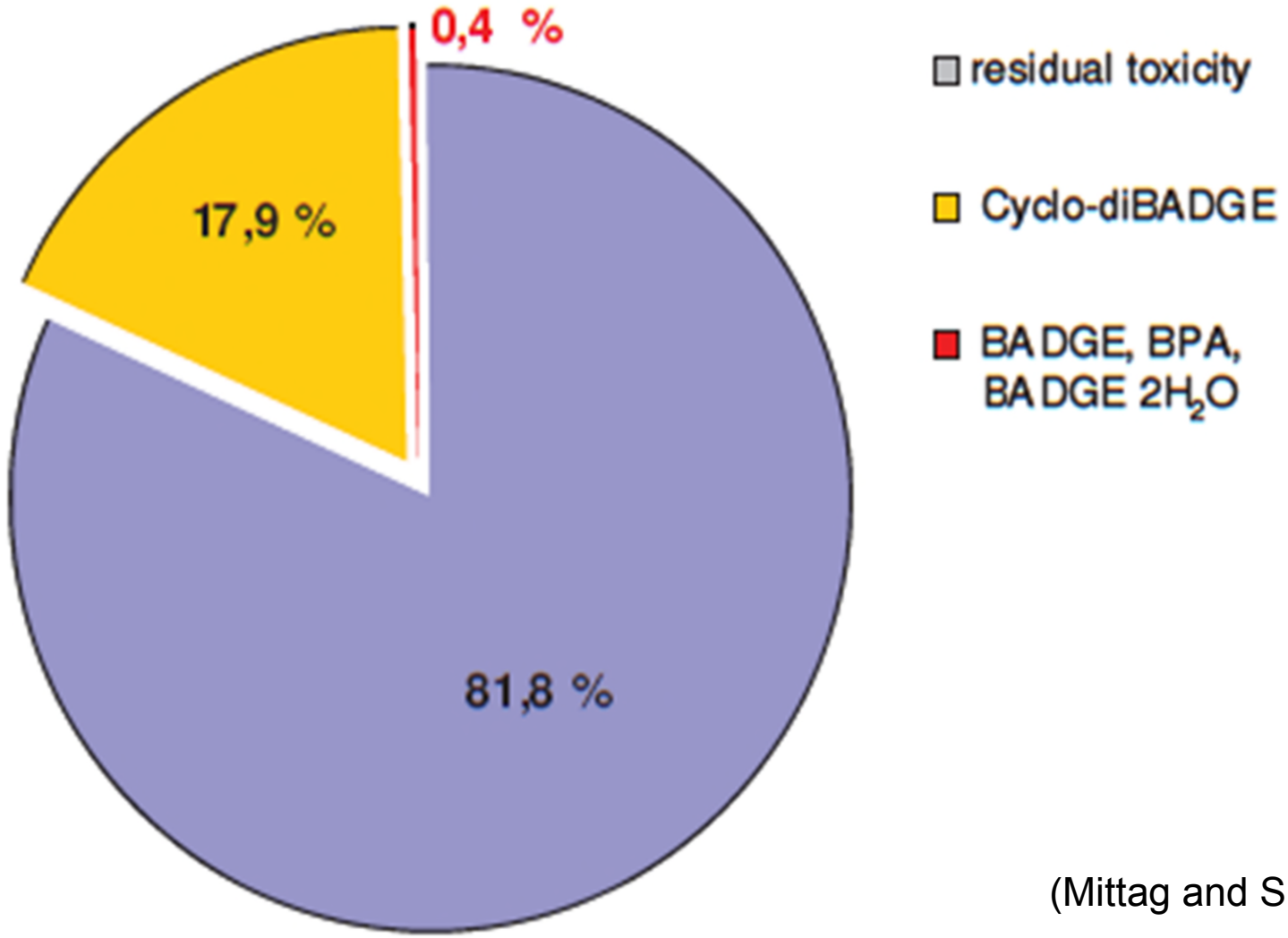
No experimental toxicity data for Cyclo-di-BADGE

Cytotoxicity: Neutral red assay in Hep-G2



(Mittag and Simat, 2007)

Contribution of Cyclo-di-BADGE to the total cytotoxicity of the epoxy coating migrate



(Mittag and Simat, 2007)

SAR and Cramer structural class for Cyclo-di-BADGE

Derek (Lhasa, version 12.0.0)

- No structural indications for genotoxic or carcinogenic properties.
- Weak indications for alpha-2-microglobulin nephropathy in mammals, including rats and rodents. Not relevant for humans.

ToxTree (Ideaconsult, version 1.51)

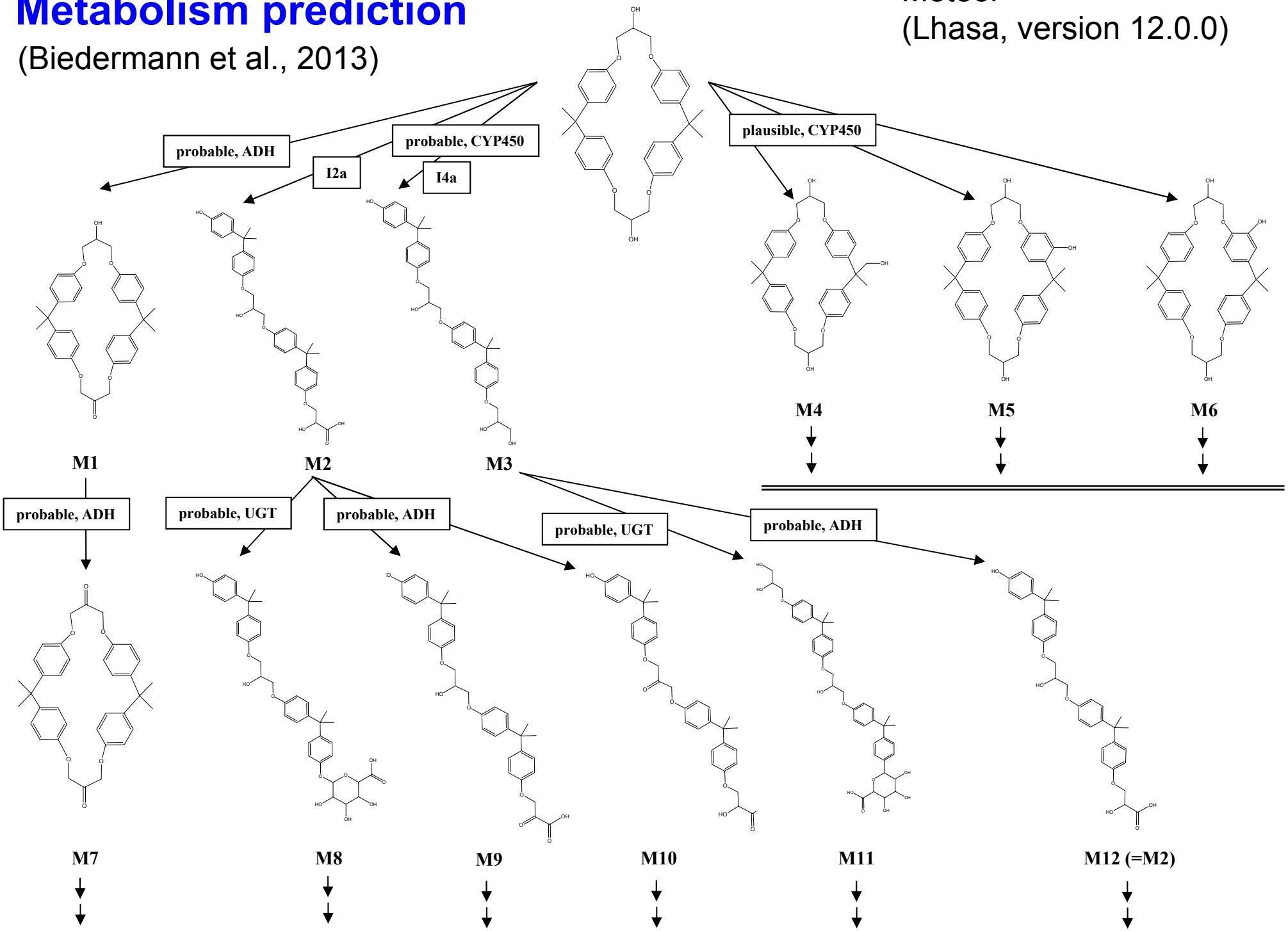
- Cramer structural class III
- No indication of carcinogenic activity (Benigni/Bossa rulebase)

(Biedermann et al., 2013)

Metabolism prediction

(Biedermann et al., 2013)

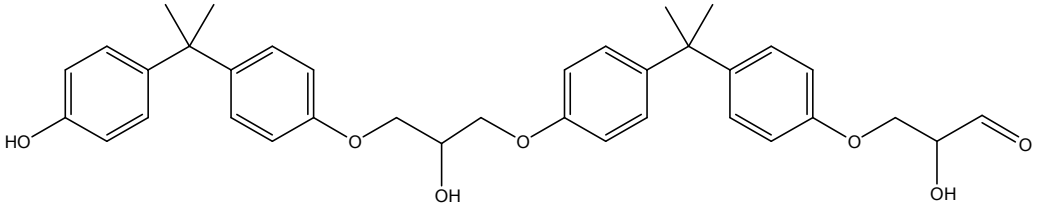
Meteor
(Lhasa, version 12.0.0)



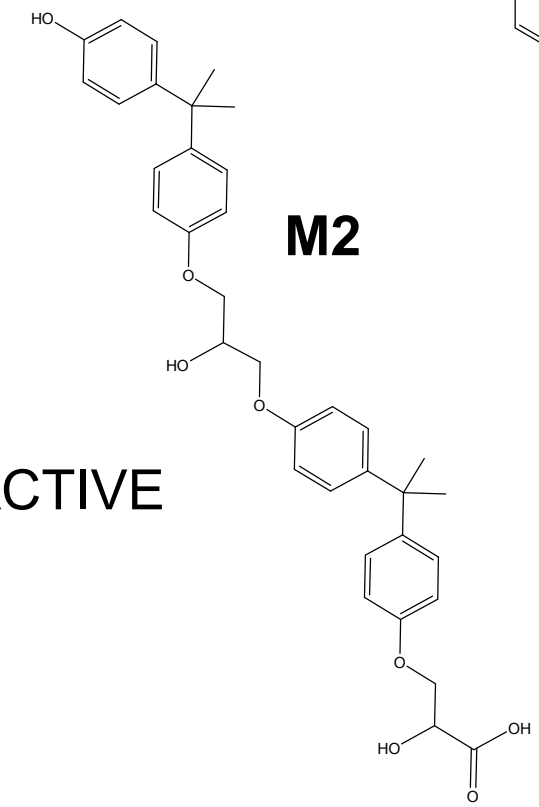
Genotoxicity and carcinogenicity *(1/2)*

- Cyclo-di-BADGE: no structural indication for a genotoxic or carcinogenic potential (Derek v 4.0.5 and Benigni/Bossa rulebase)
- Cyclo-di-BADGE metabolites:

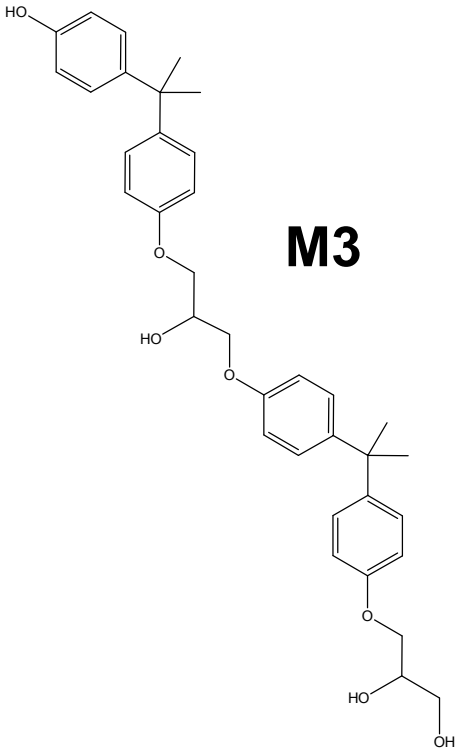
Intermediate I2a/I4a



M2



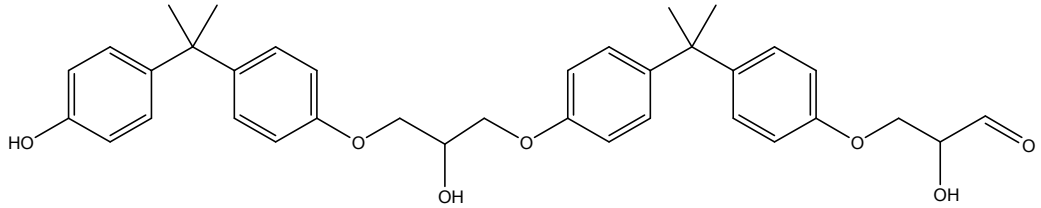
M3



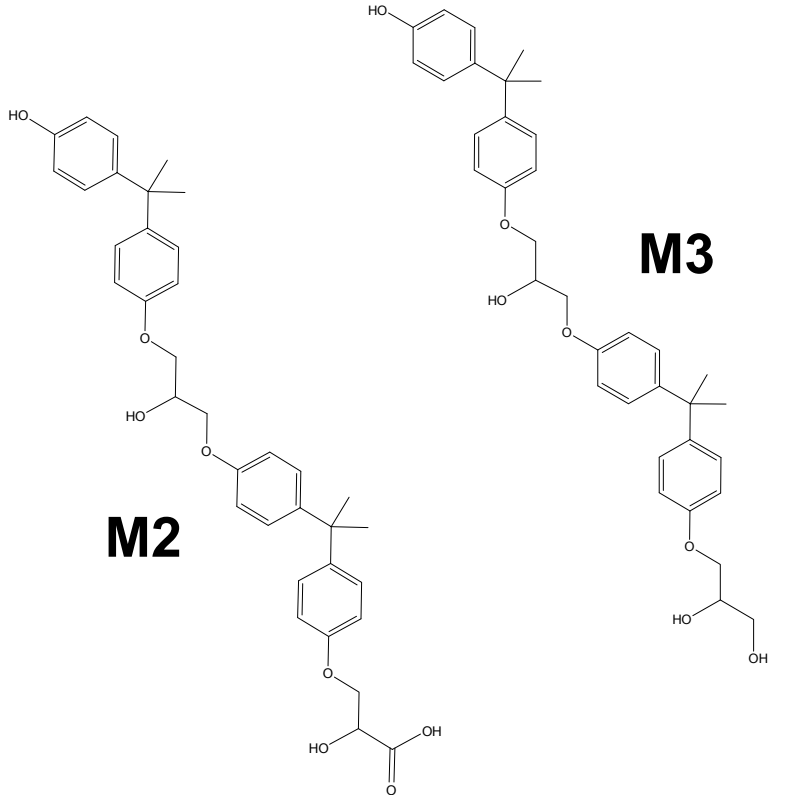
- Mutagenicity *in vitro* in bacterium is INACTIVE (Derek v 4.0.5)
- Indication of genotoxic carcinogenicity (Benigni/Bossa rulebase)

(Biedermann et al., 2013)

Genotoxicity and carcinogenicity *(2/2)*



Intermediate I2a/I4a



M2

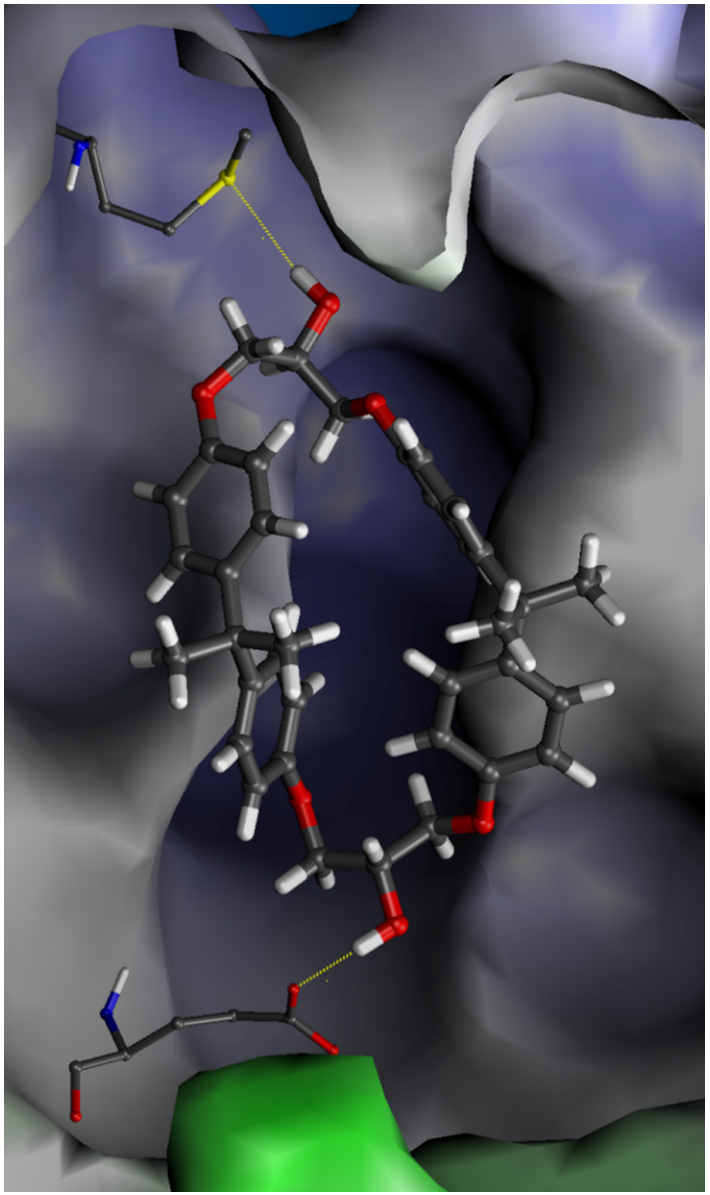
M3

Because the Cyclo-di-BADGE metabolites exhibit structural similarities with metabolites of BADGE derivatives, BADGE itself is not genotoxic *in vivo* and no carcinogenic potential could be determined in the gastrointestinal tract or in other tissues in a chronic toxicity/carcinogenicity study with rats after oral administration (EFSA 2004), it can be assumed that these Cyclo-di-BADGE metabolites are likewise not genotoxic and not carcinogenic *in vivo.*

(Biedermann et al., 2013)

Prediction of toxic potential by VirtualToxLab

Binding to Estradiol Receptor β



Compound	Isomers	Toxic Potential	Target
Parent compound			
Cyclo-di-BADGE	2	cis = 0.477 trans = 0.377	ERβ PR
Cyclic metabolites			
M1	1	0.380	PR
M4	4	0.339–0.621	ERβ
M5	4	0.371–0.625	GR
M6	4	0.267–0.295	GR
M7	1	0.369	CYP3A4
Acyclic metabolites			
M2	4	0.359–0.587	PR
M3	4	0.420–0.641	GR
Reference compound			
Bisphenol A [1]		0.470	ERβ [1]

[1] Calculated binding affinity = 120 nM (Exp. = 93 nM)

(Biedermann et al., 2013)

Oral bioavailability of Cyclo-di-BADGE

- Lipinski's Rule of Five
  - MW of >500 D (569 D)
  - LogKow) of >5 (7.56)

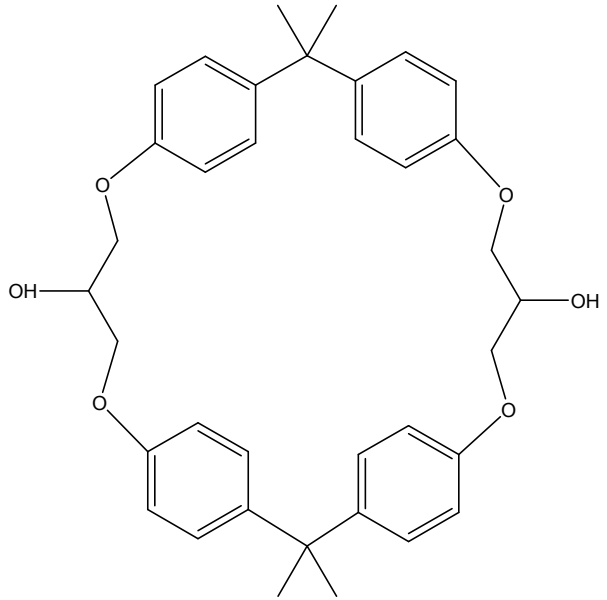
  ⇒ highly likely to have poor oral bioavailability

- Intestinal absorption prediction (Univ. Kent, UK) Regression models:

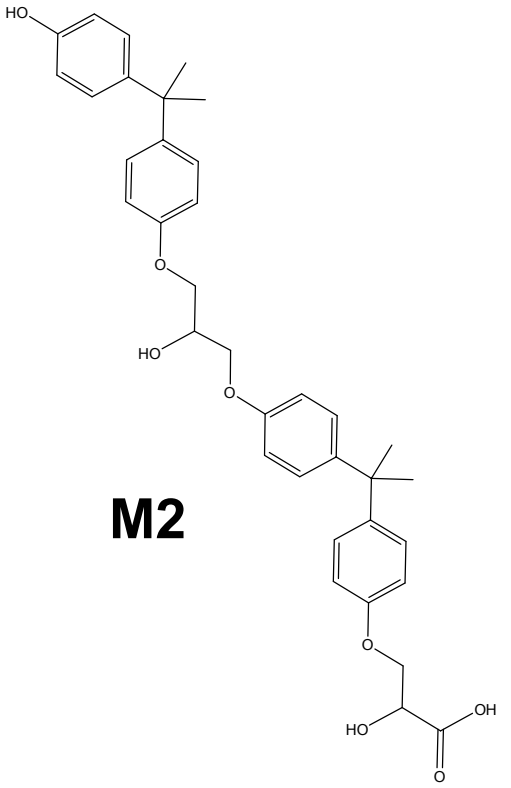
  ⇒ will be highly absorbed (>50%)

Potential for accumulation in man

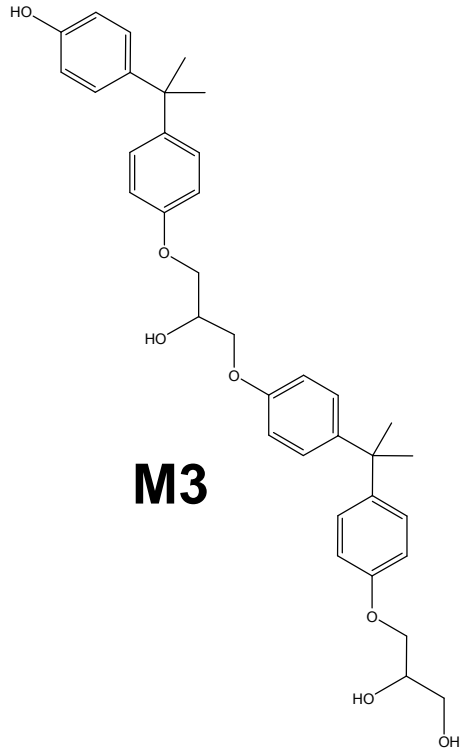
	$\underline{LogK_{ow}}$ (EpiSuite, US EPA)
Cyclo-di-BADGE	7.56
Metabolite M2	6.51
Metabolite M3	6.37



Cyclo-di-BADGE

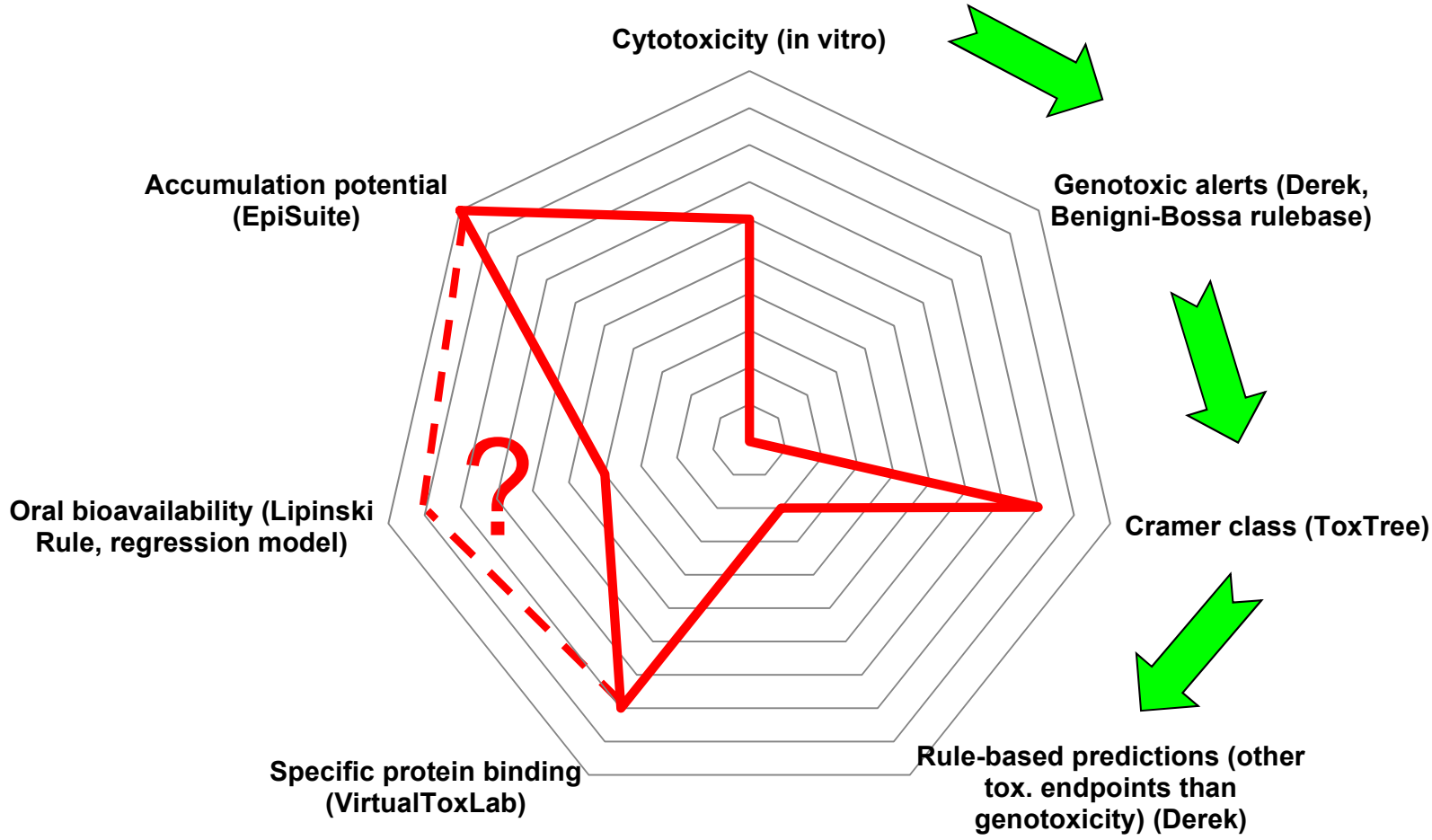


M2



M3

Toxicity profile of Cyclo-di-BADGE



Tests to reduce the existing uncertainties in the hazard assessment of Cyclo-di-BADGE

- ADME study:
  - Oral bioavailability
  - Metabolism *in vivo*
  - Accumulation

- *In vitro* experiments on Cyclo-di-BADGE for estrogenic and anti-estrogenic activities in a cell-based test system (e.g. CALUX®)
  - Receptor binding (ER, PR, GR, MR)

- ~~90-day oral toxicity study~~
  - ~~Subchronic toxicity ⇒ NOAEL ⇒ TDI ⇒~~ Intervention value

6 kg test material needed !

TTC approach as described in the EFSA opinion (2012) „should not be used for:

- Substances that are known or predicted to bioaccumulate
- Mixtures of substances containing «unknown chemical structures»

Questions:

- How to handle (potentially) endocrine disruptors?
  - how to identify them?
  - how to proceed if identified?
  EC criteria for identification of endocrine disruptors are expected before summer 2016

- TTC-level for substances with structural alerts for genotoxicity is extremely low (2.5 ng/kg bw/day).
- Its derivation is not in line with the EFSA opinion on genotoxic and carcinogenic substances (EFSA, 2005).
  MOE to $BMDL_{10}$ < 10‘000 is considered of low health concern vs. extrapolation from TD_{50} to a 1 in a million risk.

Conclusions and recommendations *(1/2)*

For the toxicological evaluation **available experimental data and existing evaluations** should be used at first place (case 1 biphenyl).

- In-depth literature and data search search engines
- REACH data become more important (check ECHA homepage) for substance evaluation and read-across (case 4 DEHM); Availability of study reports and raw data; Copyright question to use REACH data for FCM evaluations

TTC approach is an **extremely useful tool** in case that no or insufficient toxicity data are available for a (provisional) assessment.

- More guidance how to proceed in the prediction of genotoxic alerts in case of discrepancies between different tools (case 3 oxaspiro compound)
- Comparison and validation of the silico tools is requested
- TTC approach may not always be the appropriate method of choice (see phthalates and structurally related compounds (case 4 DEHM)

  $\Rightarrow$ Read-across approach

Conclusions and recommendations *(2/2)*

Toxicological profiling

TTC supplemented with (Q)SAR and molecular modeling:

- Metabolism prediction etc.
- Potential for endocrine disrupting properties by molecular modeling of critical target protein binding (case 4 DEHM, case 5 Cyclo-di-BADGE)
- Need for validated (Q)SAR tools to predict **oral bioavailability** and **potential to accumulate in human** (case 5 cyclo-di-BADGE)
- Identify critical steps and uncertainties
- Perform a plausibility check

Develop appropriate **testing strategies**, identify appropriate **surrogate compounds** for critical substance categories

Interesting compound group are **cyclic dimers and trimers.**

More exchange of knowledge and information on NIAS is needed:

- More transparency on NIAS for FCM linked to specific applications (lists of identified NIAS)
- Existing evaluations of NIAS should be published

Thank you for your attention !

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