Venture 1st 호서대학교 AI4U 호서대학교

HOSEO UNIVERSITY

통합시험평가접근법 (IATA) 연구동향

호서대학교 오승민 교수

HOSEO UNIVERSITY

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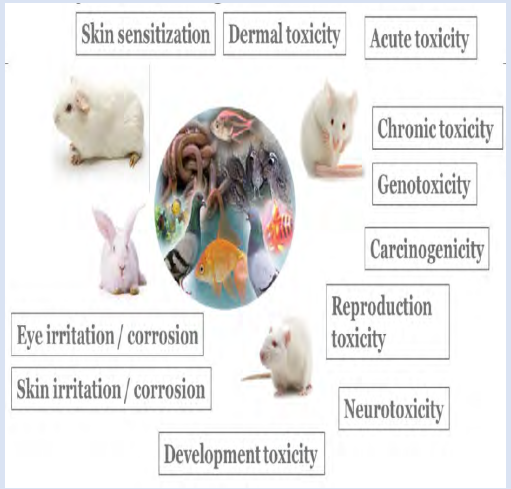
Contents

Traditional animal tests → **3R Alternative Methods**



- Refinement
- Reduction
- Replacement:
  - → in vitro assay
  - → In Silico
  - → QSAR

PROS and CONS — UNCERTAINTY — Health Effects

Integrated Approaches → Regulatory decision

Combination of methods / integrating results from one or many methodological approaches

01 | Regulators want to ……

~~UNCERTAINTY~~

- Have more data
- Make better use of existing data
- Access new, more predictive information
- Accelerate the pace of Chemical risk assessment

Information requirements

IATA

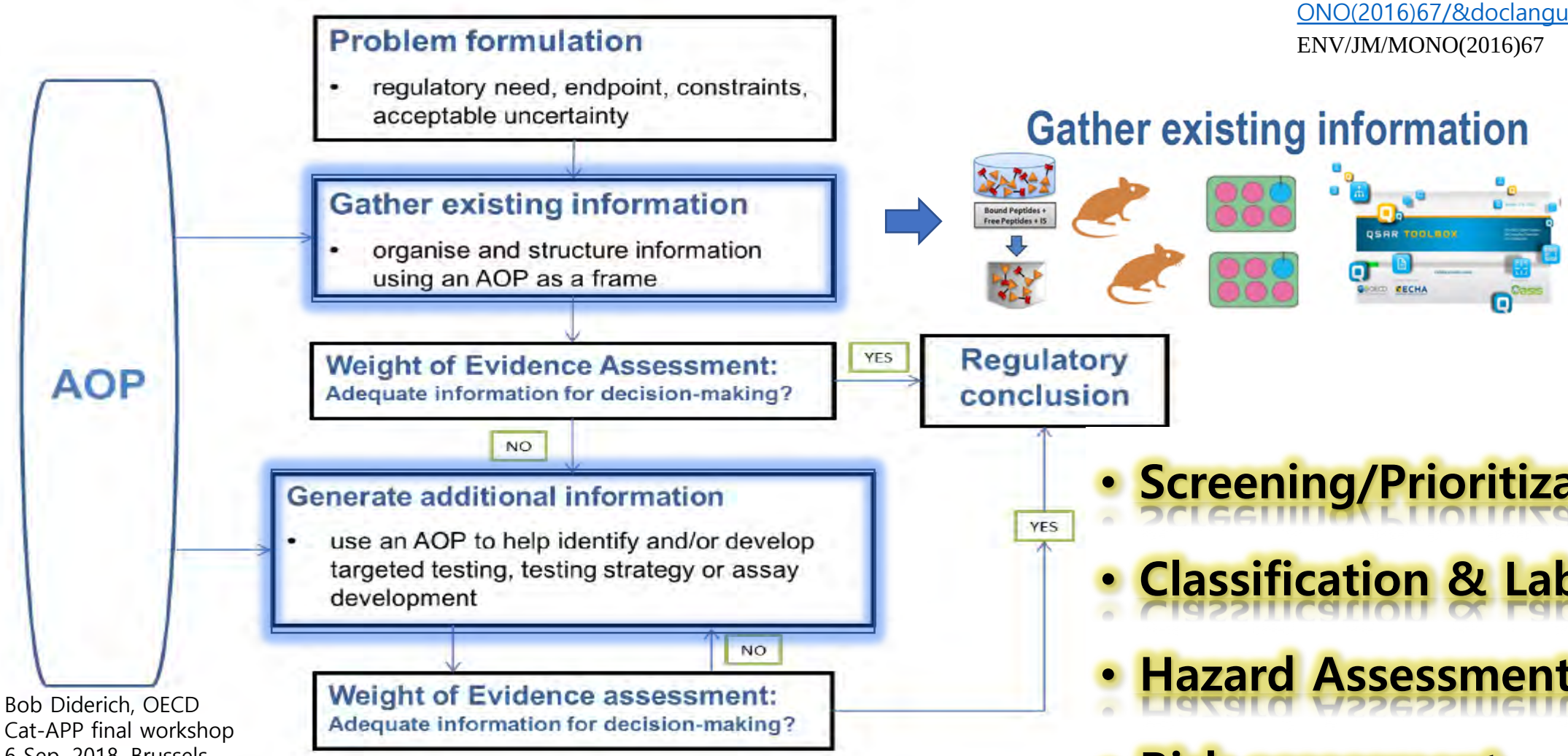
Integrated Approaches Testing Strategies

Application in regulatory decision-making

01 | IATA FRAMEWORK

http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2016)67/&doclanguage=en

ENV/JM/MONO(2016)67



Bob Diderich, OECD
Cat-APP final workshop
6 Sep, 2018, Brussels

- **Screening/Prioritization**
- **Classification & Labeling**
- **Hazard Assessment**
- **Risk assessment**

Flexible

Judgement-Based

Prescriptive

Rules-Based

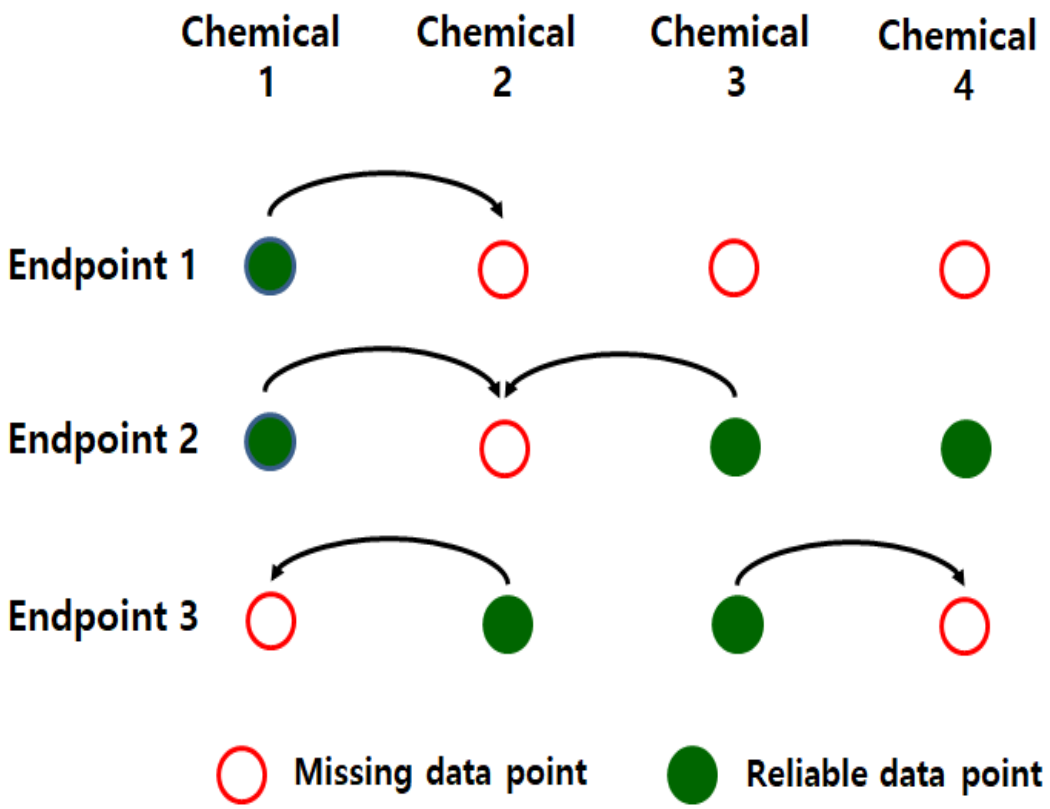
Non-formalized approaches

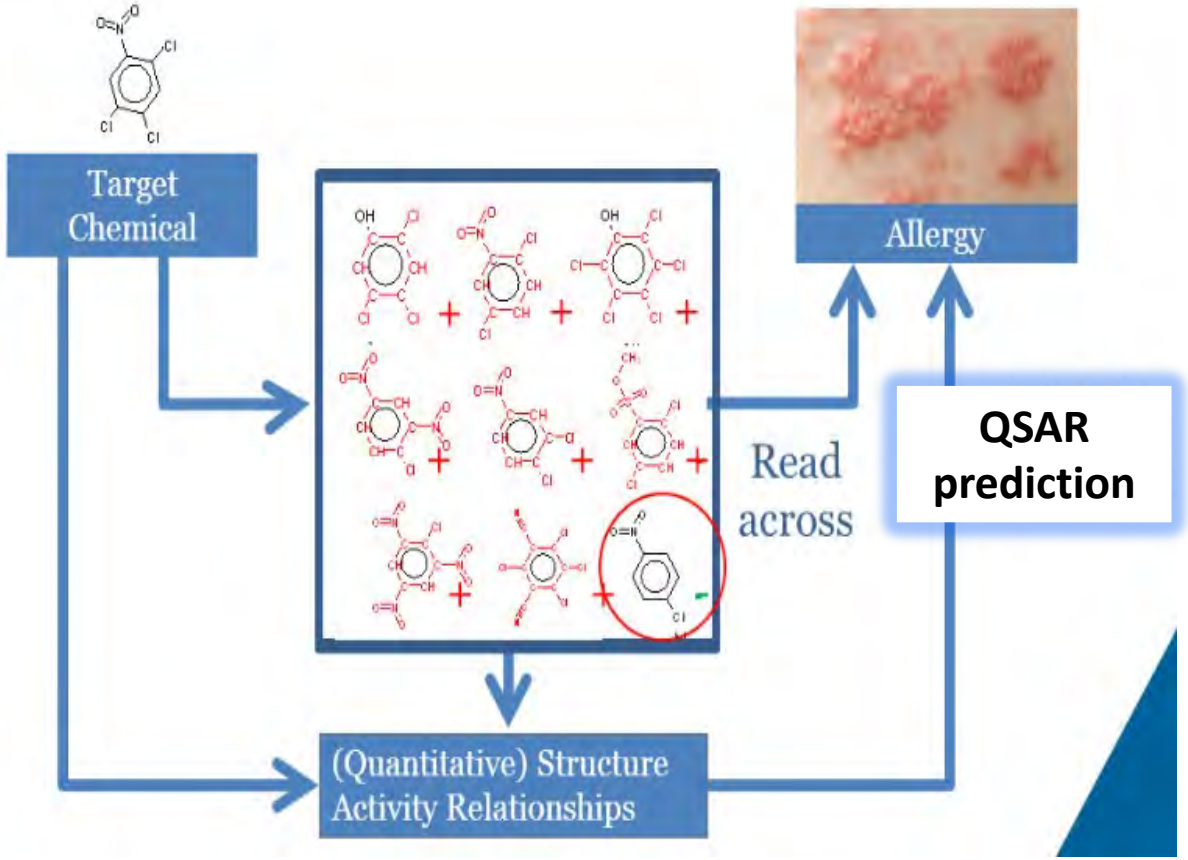
- **Grouping**
- **Read-across**

Structured approaches

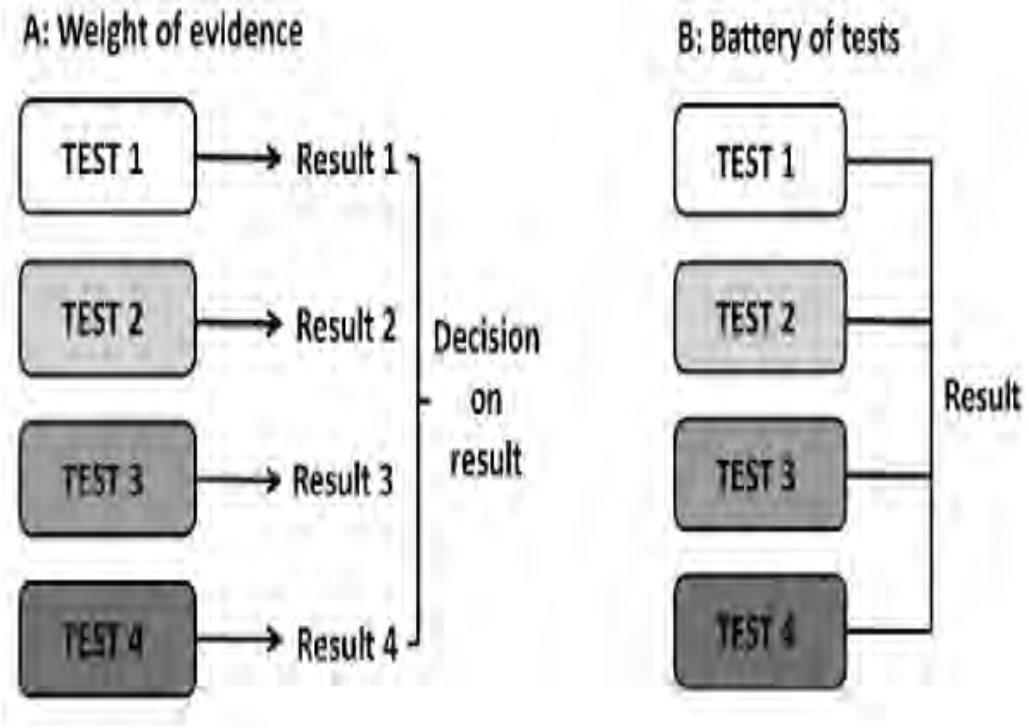
- **Integrated Testing Strategy (ITS)**
- **Weight of Evidence**

Uncertainty → **Computational analysis**

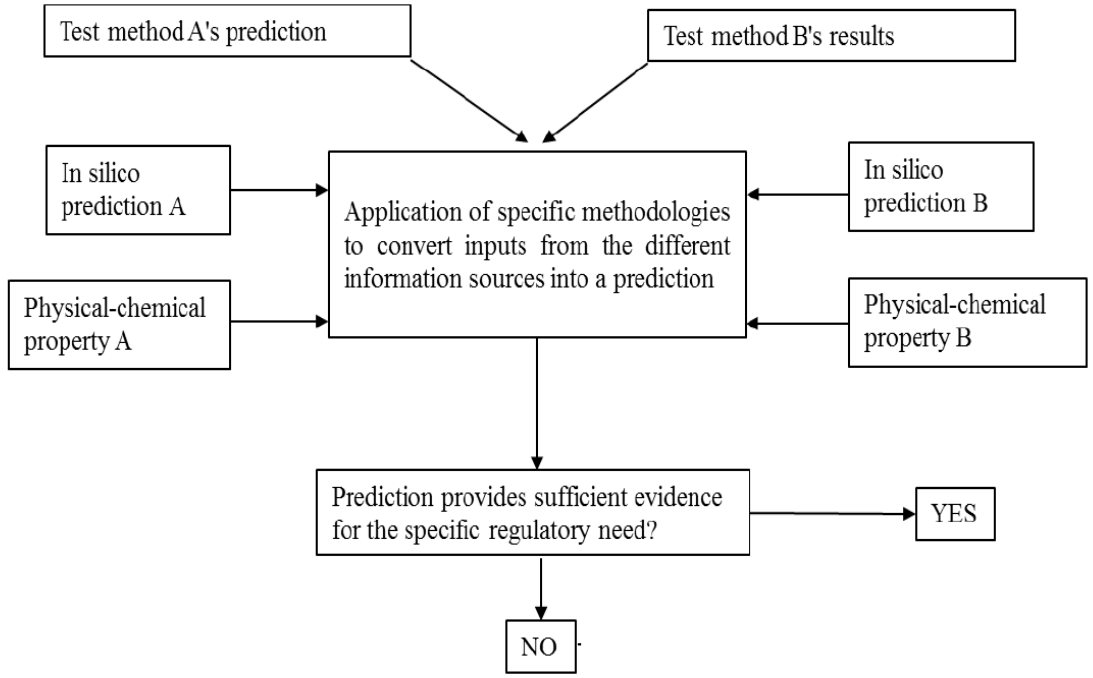


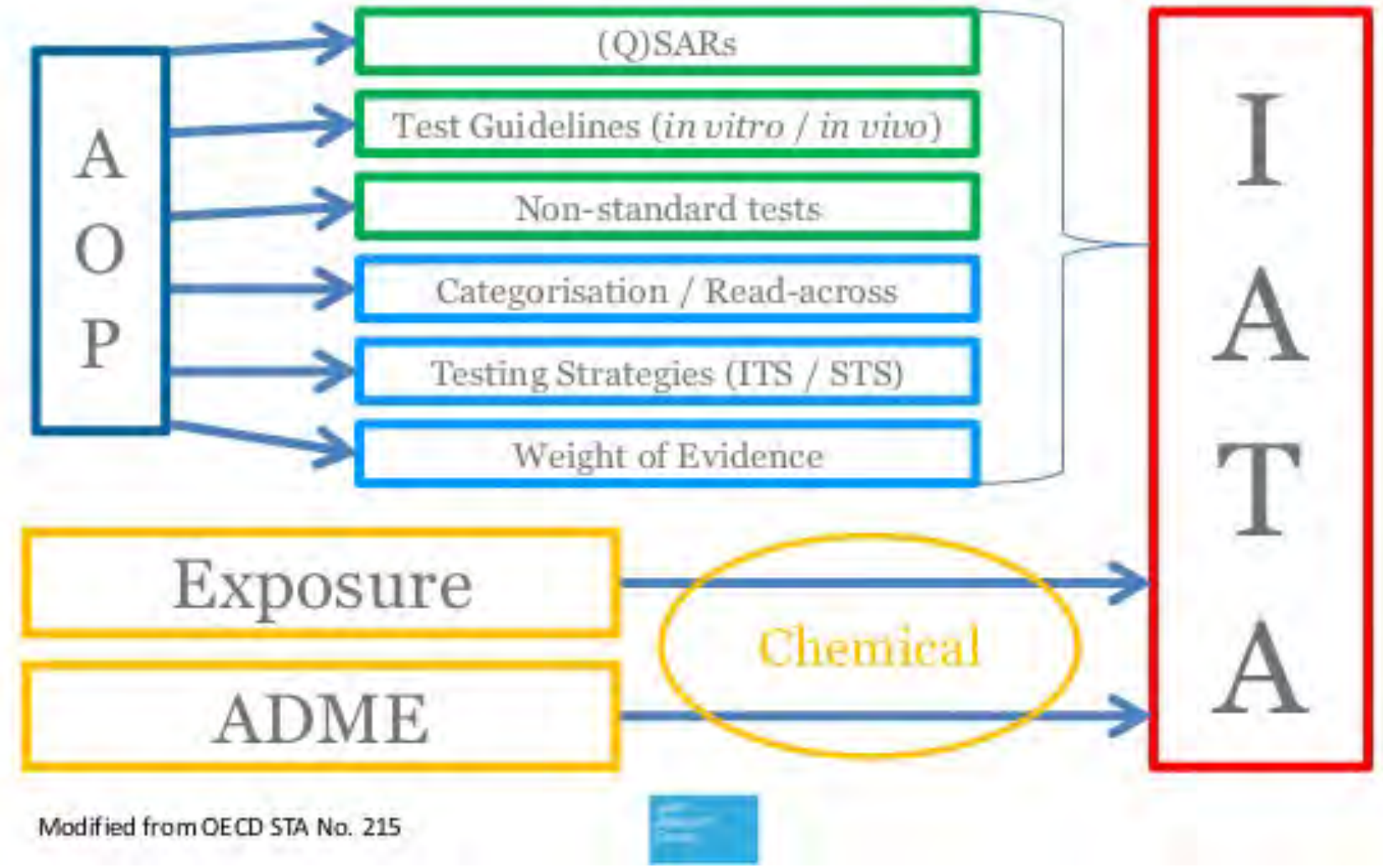


Adapted from Worth (2008).



Integrated Testing Strategy (ITS)





- Screening/Prioritization
- Classification & Labeling
- Hazard Assessment
- Risk assessment

02_ IATA CASE STUDIE LISTS

02 | IATA CASE STUDIES – PUBLISHED

Review Year	No	Title	Type assessment	Endpoint	Status
2020	1	Case Study on the use of Integrated Approaches for Testing and Assessment for the Systemic Toxicity of **Phenoxyethanol** when included at 1% in a body lotion	Safety Assessment workflow	Repeated Toxicity	Under Review
2019	1	Case Study on the use of an Integrated Approach to Testing and Assessment (IATA) and New Approach Methods to inform a Theoretical Read-Across for Dermal Exposure to **Propylparaben** from Cosmetics	Safety Assessment workflow	Reproductive toxicity	Published
	2	Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to **Caffeine**	Safety Assessment workflow	Repeated dose toxicity	Published
	3	Case Study on the Use of Integrated Approaches for Testing and Assessment for 90-Day Rat Oral Repeated-Dose Toxicity of **Chlorobenzene-Related Chemicals**	Grouping (Read-across)	Repeated dose toxicity	Published
	4	Case Study on the Use of Integrated Approaches for Testing and Assessment to Inform Read-across of **p-Alkylphenols**: Repeated-Dose Toxicity	Grouping (Read-across)	Repeated dose toxicity	Published
	5	Prediction of a 90 day repeated dose toxicity study (OECD 408) for **2-Ethylbutyric acid** using a read-across approach from other branched carboxylic acids	Grouping (Read-across)	Repeated dose toxicity	Published

(source) OECD Integrated Approaches to Testing and Assessment (IATA)

02 | IATA CASE STUDIES – PUBLISHED

Review Year	No	Title	Type assessment	Endpoint	Status
2019	6	Read-across based filling of developmental and reproductive toxicity data gap for **methyl hexanoic acid**	Grouping (Read-across)	Developmental toxicity	Published
	7	Identification and characterisation of parkinsonian hazard liability of **deguelin** by an AOP-based testing and read across approach	Grouping (Read-across)	Neurotoxicity	Published
	8	Mitochondrial Complex-III-mediated neurotoxicity of - Read-Across to other **strobilurins**	Grouping (Read-across)	Neurotoxicity	Published
2018	1	Case Study on the use of Integrated Approaches for Testing and Assessment for Testicular Toxicity **of Ethylene Glycol Methyl Ether (EGME)-Related Chemicals**	Grouping (Read-across)	Reproductive toxicity	Published
	2	Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying **Estrogen Receptor Active Chemicals**	Screening and prioritisation	Endocrine disruption	Published

(source) OECD Integrated Approaches to Testing and Assessment (IATA)

02 | IATA CASE STUDIES – PUBLISHED

Review Year	No	Title	Type assessment	Endpoint	Status
2017	1	**Estrogenicity** of Substituted Phenols No. 290.	Prioritization and hazard characterization	Endocrine disruption	Published
	2	Prioritization of chemicals using the Integrated Approaches for Testing and Assessment (IATA)-based **Ecological Risk Classification No. 291**	Prioritization of chemicals	Ecotoxicity	Published
	3	Case study on grouping and read-across **for nanomaterials genotoxicity of nano-TiO2 No. 292**	Grouping (Read-across)	Genotoxicity	Published
	4	A Case Study on the Use of Integrated Approaches for Testing and Assessment for **Sub-Chronic Repeated-Dose Toxicity of Simple Aryl Alcohol Alkyl Carboxylic Esters: Read-Across**	Grouping (Read-across)	Repeated dose toxicity	Published

(source) OECD Integrated Approaches to Testing and Assessment (IATA) http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm#Project

02 | IATA CASE STUDIES – PUBLISHED

Review Year	No	Title	Type assessment	Endpoint	Status
2016	1	Repeated-Dose Toxicity of **Phenolic Benzotriazoles**	Grouping (Read-across)	repeated dose toxicity	published
	2	**Pesticide** Cumulative Risk Assessment & Assessment of Life stage Susceptibility	Cumulative risk assessment	Neurotoxicity	published
	3	90-Day Rat Oral Repeated-Dose Toxicity for **Selected n-Alkanols: Read-Across**	Grouping (Read-across)	repeated dose toxicity	published
	4	90-Day Rat Oral Repeated-Dose Toxicity for **Selected 2-Alkyl-1-alkanols: Read-Across**	Safety assessment workflow	repeated dose toxicity	published
	5	Chemical Safety Assessment Workflow Based on Exposure Considerations and Non-animal Methods	Grouping (Read-across)	repeated dose toxicity	published
2015	1	In Vitro Mutagenicity **of 3,3'Dimethoxy- benzidine (DMOB**) Based Direct Dyes	Grouping (Read-across)	Mutagenicity	published
	2	Repeat Dose Toxicity of **Substituted Diphenylamines (SDPA)**	Grouping (Read-across)	repeated dose toxicity	published
	3	Hepatotoxicity of **Allyl Ester Category**	Grouping (Read-across)	repeated dose toxicity	published
	4	Bioaccumulation Potential of Biodegradation Products of **4,4'-Bis(chloromethyl)-1,1'-biphenyl**	Grouping (Read-across)	bioaccumulation	published

(source) OECD Integrated Approaches to Testing and Assessment (IATA)

02 | IATA CASE STUDIES – PUBLISHED

Year	2020	2019	2018	2017	2016	2015
Publication	1 Under review	8	2	4	5	4
Repeated toxicity	1	4		1	4	2
Genotoxicity & Mutagenicity				1		1
Reproductive toxicity						
Development toxicity		1				
Neurotoxicity		2			1	
Endocrine disruption			1	1		
Ecotoxicity				1		
Bioaccumulation						1

Type assessment	
Safety Assessment workflow	4
Grouping (read-across)	16
Prioritization and hazard characterizaiton	1
Prioritization of chemicals	1
Screening and Prioritisation	1
Cumulative risk assessment	1

(source) OECD Integrated Approaches to Testing and Assessment (IATA)

03_ Application of IATA: CASE STUDIES

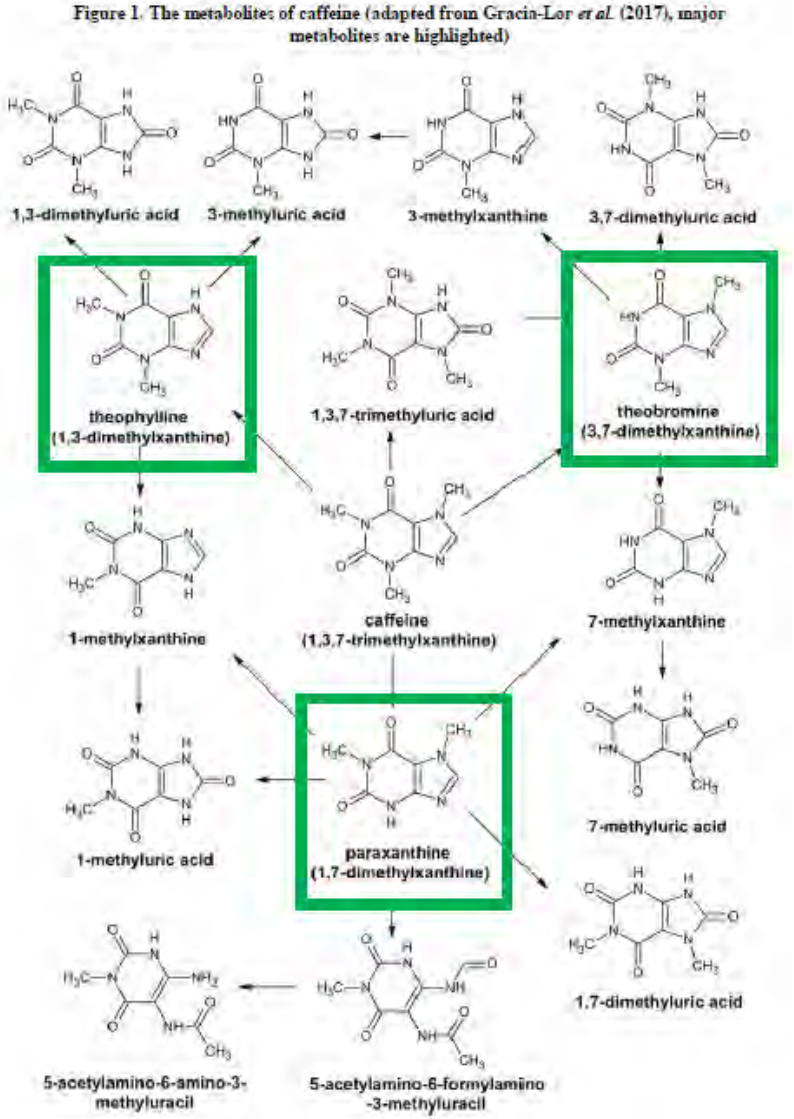
03-1 | Safety Assessment Workflow: 반복독성

2	Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to **Caffeine**	Safety Assessment workflow	Repeated dose toxicity	Published

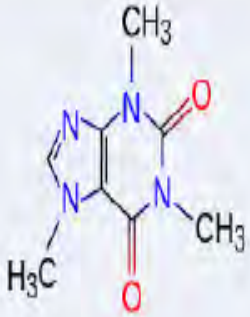
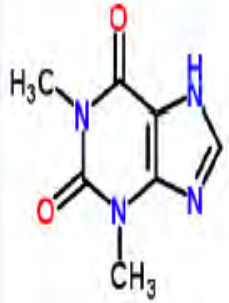
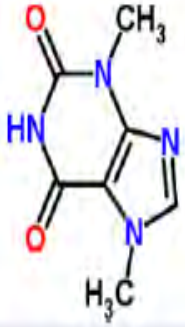
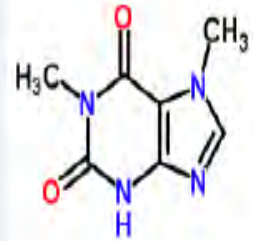
- **목적**: to assess the potential risk from consumer (cosmetics) exposure to caffeine
- **방법**: a read-across approach
  which utilised *in vivo* data from close structural analogues, paraxanthine, theobromine and theophylline, while assuming that no *in vivo* repeated dose toxicity data were available for caffeine.
- **노출평가**: exposure from dermal (cosmetics) and oral (food/drink) exposure.
- **카페인의 내부농도 측정**
  the read-across approach, *in silico*/*in vitro* biokinetic and toxicodynamic data were generated and a physiologically-based biokinetic (PBBK) model
- **대사체 분석** Based on the toxicity data for the metabolites of caffeine, read-across
- **독성 데이터 수집:** Toxcast 등 다양한 독성 데이터 수집
- **최종적 결과물 도출 (통합적 결과 도출)**
  a no-observed-adverse-effect- level (**NOAEL**) modelled plasma concentration was derived which was compared with the modelled plasma concentrations resulting from human dermal and oral exposure to achieve a Margin of Internal Exposure (**MoIE, 내부 노출한계**) value for the safety assessment.

03-1 | Safety Assessment Workflow: 반복독성

대사체분석



대사체 구조적 특성 분석

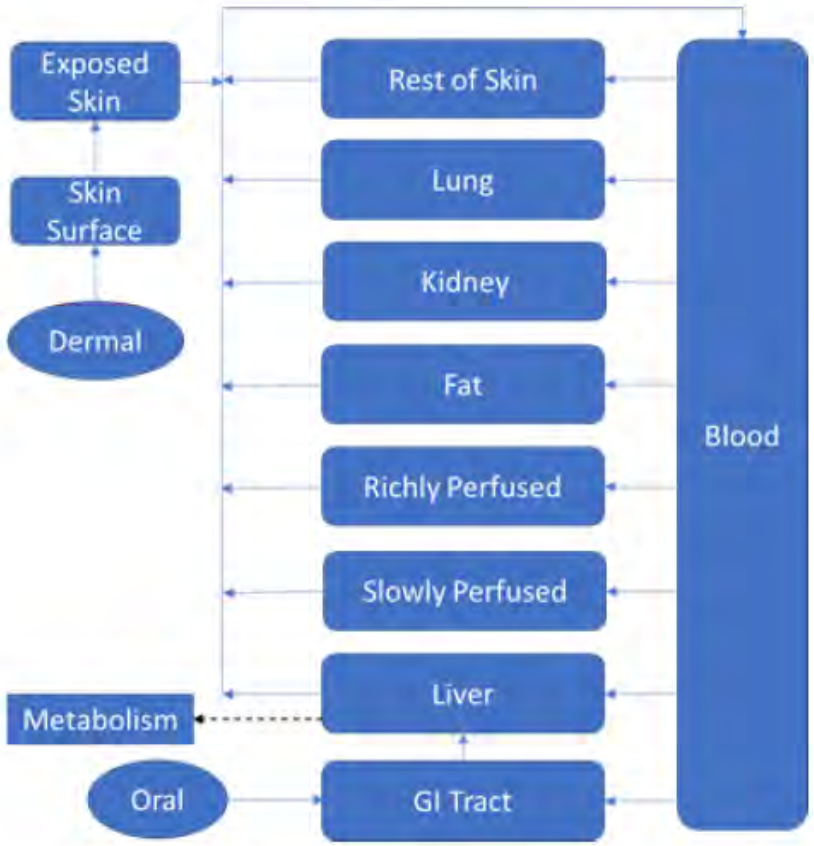
	Target Substance	Analogue 1	Analogue 2	Analogue 3
Name	Caffeine	Theophylline	Theobromine	Paraxanthine
CAS No:	58-08-2	58-55-9	83-67-0	611-59-6
2D Structure				
Molecular Formula	$C_8H_{10}N_4O_2$	$C_7H_8N_4O_2$	$C_7H_8N_4O_2$	$C_7H_8N_4O_2$
Chemical Class	Purine	Purine	Purine	Purine
Chemical Sub-Class	Methyl xanthine	Methyl xanthine	Methyl xanthine	Methyl xanthine
Chemical Sub-Class	1,3,7-trimethyl xanthine	1,3-dimethyl xanthine	3,7-dimethyl xanthine	1,7-dimethyl xanthine

독성 결과값 수집 (ToxCast data)

Name	CAS	Similarity Cutoff (Isentris)	Result Count (All)	Result Count (Hit)	Positive hits details
Caffeine	58-08-2	100	663	21	2 background measurement, 5 nuclear receptor, 6 DNA binding, 2 cell cycle, 3 gpcr, 2 esterase, 1 signalling
Theophylline	58-55-9	>80	770	13	2 background measurement, 2 nuclear receptor, 4 DNA binding, 1 cell cycle, 3 gpcr, 1 esterase
Theobromine	83-67-0	>90	716	12	1 nuclear receptor, 1 DNA binding, 2 gpcr, 2 oxidoreductase, 2 cyp, 1 protease, 1 ion channel, 1 transporter, 1 misc
Etofylline	519-37-9	>90	109	2	1 background measurement, 1 DNA binding
Theofibrate	54504-70-0	>80	64	2	1 nuclear receptor, 1 cell cycle
Dimenhydrinate	523-87-5	>70	113	5	2 background measurement, 2 DNA binding, 1 nuclear receptor
Valacyclovir hydrochloride	124832-27-5	>70	109	1	Background measurement
8-Bromotheophylline	10381-75-6	>80	109	1	Nuclear receptor
Enprofylline	41078-02-8	>80	64	1	Cell cycle
Isbufylline	90162-60-0	>90	109	1	Background measurement

체내동태 분석: physiologically-based biokinetic (PBBK) model

Figure 3. PBBK model schematic for caffeine showing the representation of the main organs considered with various sub-compartments in the skin and GI tract for oral and dermal exposure



in Berkeley Madonna software (version 8.3.18; University of California, Berkeley, CA; www.berkeleymadonna.com).

불확실성 확인

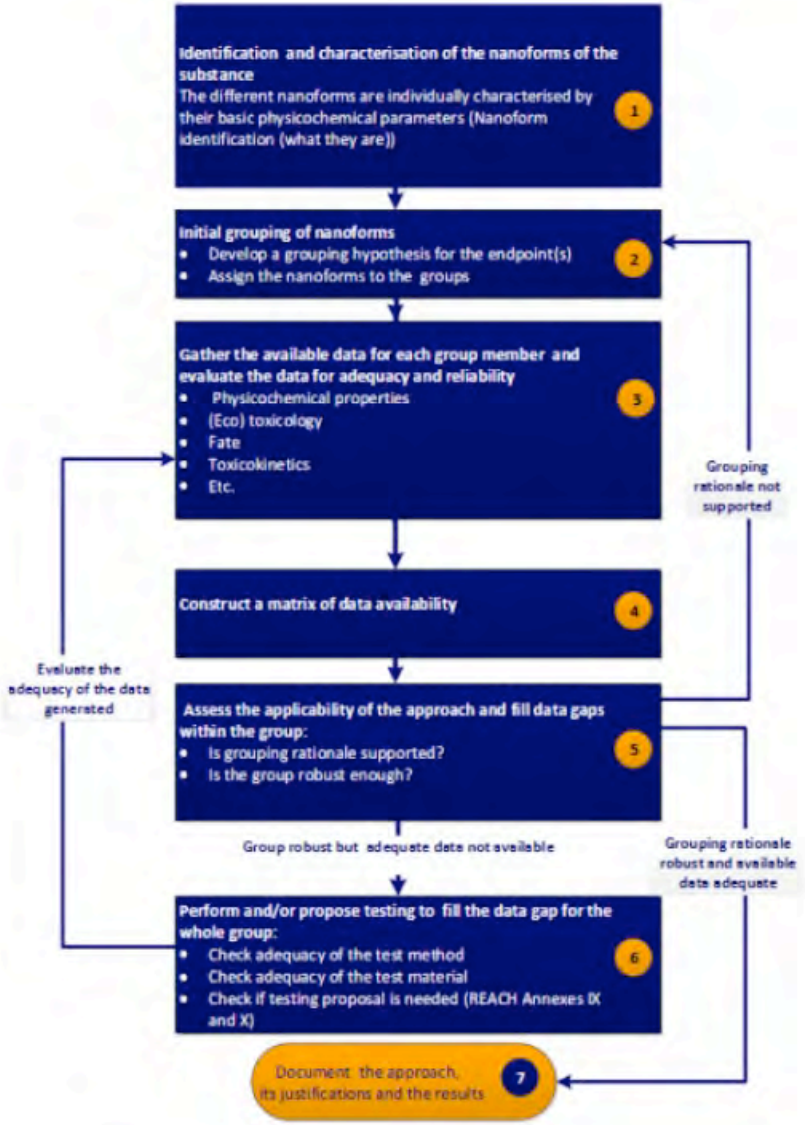
Factor	Uncertainty (low, medium, high)	Comment
Hypothesis used for the read across	Low	Three analogues (theophylline, theobromine, paraxanthine) have been found on the basis of comparable structural, physico-chemical and molecular properties. In addition, based on available data they are considered to have a common mode of action (MOA) with different potencies
Structural similarity	Low	High level of structural similarity (all are methyl xanthines). Tanimoto score >0,9 based on CE-ToxGPS software.
Similarity of physico- chemical properties	Low	The 4 chemicals (caffeine, theophylline, theobromine, paraxanthine) have similar physico-chemical properties (similar MW and logP range, similar negligible volatility and similar solubility classes), all are predicted to be bioavailable and to have a good skin penetration
Toxicokinetic similarity	Low	Following oral intake, caffeine, theophylline, theobromine and paraxanthine are readily and completely absorbed. The biotransformation is catalysed by the cytochrome P-450 monooxy-genase system and leads to the formation of demethylated compounds or, through oxidation, to the urate and/or hydration to the diaminouracil.
Mode of action (MoA)	Low	The common pivotal MoA between caffeine and the source chemicals was considered to be antagonism of the A1-adenosine receptor based on pharmacological investigations. The differences in the relative potency were taken into account.
Similarity of other supportive data (ToxCast data)	Low	Theophylline and theobromine have been identified as the closest structural analogues among all the chemicals in ToxCast. The categories of positive hits are similar to those of caffeine.
Number of analogues used for the read across	Low	The number of analogues used for the read- across was low, but considered sufficient. Valid *in vivo* data are available for theophylline that was considered the most similar analogue based on ToxCast and Tanimoto coefficient
Quality of the endpoint data used for the read across	Low	Several supporting *in vivo* studies with Klimisch score 1 & 2 available for all three analogues. The rabbit prenatal developmental toxicity study with theophylline was used since it was an intravenous study (100% bioavailability) with data on internal plasma levels
Similarity of the endpoint data (among source chemicals)	Medium	Differences were most probably due to different pharmacological potencies
Concordance and weight of evidence of all data used for justifying the hypothesis	Low	Combination of similarity in structures, ADME, biological and kinetic properties (see Annex 2: QSAR Toolbox v 4.3)
Overall level of confidence in the read- across approach	Low	Available *in vivo* data on caffeine support the read - across approach. Moreover, internal plasma levels derived from a reliable (Klimisch score 2) *in vivo* study with theophylline, the most potent analogue, were used as point of departure (NOAEL). The final Margin of Internal Exposure (MoIE) calculation was considered sufficiently conservative since it was based on estimated internal exposure with a PBBK model, rather than on external doses with inherent uncertainty related to route-to-route extrapolation and species/strain differences

3	Case study on grouping and read-across **for nanomaterials genotoxicity of nano-TiO2 No. 292**	Grouping (Read-across)	Genotoxicity	Published

- **목적**: to determine the genotoxic hazard potential of **two nano-TiO2** target substances
- **방법**: by **reading across *in vitro* comet assay results**.
- The case study is also evaluating **the applicability of the workflow for grouping and read-across** proposed in the REACH guidance update for nanomaterials (NMs) (ECHA 2017a) and identifying sources of uncertainty associated with the read-across, exploring the extent to which ECHA's Read-Across Assessment Framework (RAAF) (ECHA 2017b) captures the different sources of uncertainty for nanoforms.
- **물질선별**: The dataset of the analogues includes **six NMs with different properties**: different primary and crystallite sizes (5-100nm), different crystalline types (anatase and rutile) and surface characteristics (some are coated and some uncoated).
- **DATA 수집**:

a) physicochemical characterisation, fundamental behaviour and reactivity of the identified NMs
b) toxicological data of relevant REACH endpoints/assays.

- **최종적 결과물 도출:** the practical application of the ECHA guidance for grouping and read-across of NMs.
  The outcome of the *in vitro* comet assay was predicted for two target TiO2 NMs, negative for the coated one, and positive for the uncoated one. These results were verified by experimental literature data.
  The conclusions obtained for specific substances should not be regarded as recommendations for regulatory action.



물리화학적 특성 분석

Property	NM100	NM101	NM102	NM103	NM104	NM105
Crystal type	Anatase	Anatase	Anatase	Rutile	Rutile	83% anatase 17% rutile
Other info	Dry-milled	Semiconductor catalyst used in photocatalytic process	photocatalytic	hydrophobic	hydrophilic	-
Total non-TiO_2 content including coating and impurities (% w/w)	1.5	9	5	11	11	0.11
Surface chemistry (as declared by manufacturer)	uncoated	uncoated	uncoated	Al_2O_3 and SiO_2	Al_2O_3 and SiO_2	uncoated
Surface coating (% w/w)	0	0	0	8	8	0
Organic matter (%)	0	8	0	2	2	0
Primary particle diameter (TEM) (nm)	93 ± 23	5 ± 1	22 ± 10	24 ± 2	24 ± 2	20 ± 3
Crystallite size (XRD) (nm)[a]	117 ± 40	7 ± 2	24 ± 5	24 ± 4	25 ± 4	22 ± 5
Particle Size Distribution average (nm)[b]	210 ± 10	278	440 ± 37	135 ± 25	145 ± 35	177 ± 39
Shape	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal
Aspect ratio	1.53	1.53	1.53	1.7	1.53	1.36
Specific surface area (m^2/g)	9	242 ± 73	77 ± 10	54 ± 4	54 ± 2	47 ± 0.5

	Name	NM-100	NM-101	NM-102	NM-103	NM-104	NM-105
In vitro	Micronucleus assay	-	-	3/10	3/8	3/8	4/18
	Comet	2/2	2/6	5/8	0/6	0/6	10/14
	Chromosomal aberration	-	-	-	-	-	0/1
In vivo	Micronucleus assay	-	0/3	0/6	0/5	0/5	2/9
	Comet	-	1/5	2/13	1/12	2/12	4/15
	Chromosomal aberration	-	-	0/2	-	-	-

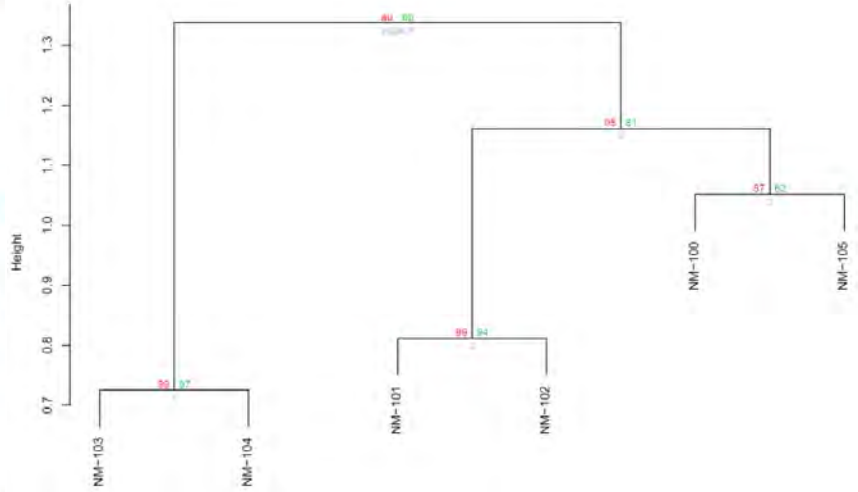
- **독성정보수집**

- Data available from public OECD dossiers (OECD 2015)
- REACH registration dossiers as of March 2016
- report on nano-TiO_2 proposal for classification from ANSES (ANSES 2016),
- JRC Repository (Rasmussen et al. 2014).
- Nanogenotox FP7 project (Nanogenotox 2012).

- 그룹핑

Table 4. Assignment of the analogues to the two groups identified regarding DNA damage potential measured with the *in vitro* comet assay, depending on the NM characteristics.

	Naked particle	Particle with high non-TiO_2 content (coating and impurities)
NMs	NM-100, NM-102, NM-105	NM-103, NM-101, NM-104
DNA damage	+	-



If nano-TiO_2 is uncoated, it has the potential to damage DNA. If a non-reactive coating and large amounts of impurities are present on the surface of the NM, this can mask the effect and no DNA damage is caused.

불확실성 확인

	RAAF Assessment Element (Scenario 6)	Uncertainties in the TiO_2 case study	Nanospecific issues
C.1	Substance characterisation	• Measured physicochemical characteristics of the NMs vary: measurement uncertainty. Is there an influence on other properties of the nanomaterials? • Impurity information not always available or inconsistent	• Physicochemical characterisation of NMs: high variability of measurements (influence of different experimental conditions)
C.2	Structural similarity and category hypothesis	• NM-101 is not declared as coated, but has 9% organic impurities corresponding to a coating. Thus it was considered coated. • Different composition of the coatings/impurities (e.g. some containing Al_2O_3) • Possible influence of crystal type if particle not coated. • Uncertainty of reading across a spherical to a rod-shaped particle.	• For NMs, the similarity cannot be based on chemical (e.g. molecular) structure as for conventional chemicals, but should consider physical form and key physicochemical properties
C.3	Link of structural similarities and structural differences with the proposed property	• Little is known about the mechanisms of toxic action, making it challenging to link similarity to the property (genotoxicity) considered	
C.4	Consistency of effects in the data matrix	• Uncertainty in applying existing testing protocols to nanomaterials and thus uncertainty in assessment of quality, reliability and relevance to human health endpoints of measured toxicity data	• Artefacts affecting the results of toxicity assessment of NMs are discussed in the literature
C.5	Reliability and adequacy of the source study(ies)		
C.6	Bias that influences the prediction	• Selection of analogues based only on data availability	
6.1	Compounds the test organism is exposed to	• The mechanism of genotoxicity of TiO_2 is not well defined. It is also possible that several effects take place at the same time.	• For conventional chemicals, either the parent molecule or (bio)transformation products are the indirect/direct toxicants; for NMs the considerations extend to coating, released metals etc
6.2	Common underlying mechanism, qualitative aspects		
6.3	Common underlying mechanism, quantitative aspects		
6.5	Occurrence of other effects than covered by the hypothesis and justification		
6.4	Exposure to other compounds than to those linked to the prediction	• For example the presence of reactive transition metals may also contribute to oxidative DNA damage induction.	

Summary of uncertainties in the case studies

The major uncertainties encountered in the nanomaterial case studies were related to:

- Complexity of nanostructures: similarity, category boundaries and members
- Identification of nanomaterials
- Quality and inconsistency/reproducibility of study data; missing protocols or uncertainty in the applicability of protocols to nanomaterials, both for material characterisation and toxicity assays
- Physicochemical properties driving the toxicity
- Limited datasets
- Read-across of negative effects
- Possible combination of physicochemical properties affecting toxicity

The following nanospecific issues have been identified related to a read-across for nanomaterials:

- Similarity based on chemical structure to be replaced with appropriate and relevant other parameter(s), i.e. consideration of physical form and key physicochemical properties
- Definition of the "identical or different" compounds in the RAAF should be adapted to nanoforms and consider factors such as coating, size, and length
- Definition of the compound the organism is exposed to and leading to an adverse effect: In the case of nanomaterials it is not only a distinction between parent compounds and (bio)transformation products such as metabolites, but for example needs to consider either the nanomaterial as such, or impurities/coating, released metals.
- Are there specific nanotoxicity mechanisms or kinetics of exposure which have to be taken into consideration? High variability of the measurements and possible nanospecific artefacts in assays are issues specific to NMs to be considered in the assessment.

03-3 | Screening and prioritisation: 내분비장애독성

2	Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying **Estrogen Receptor Active Chemicals**	Screening and prioritisation	Endocrine disruption	Published

- **목적**: **screening and priority setting of environmental chemicals based on their ER agonist activity** and for further evaluation of endocrine-related activity in higher tier *in vivo* tests (e.g., female pubertal assay, two generation reproductive toxicity study).
- **방법**: uses a combination of ***in vitro* HTS assays** and a computational model for **ER activity**, which could serve as an alternative to low and medium throughput *in vitro* and *in vivo* tests.
- **물질선별**: The target chemicals are environmental and commercial chemicals.
- **ENDPOINT/DATA 수집**: as measured in a subset of *in vitro* HTS assays (16) run in US EPA's ToxCast program.
- **최종적 결과물 도출:** this case study presents an IATA which is an ITS for the identification of potential endocrine disruption through ER agonist activity by a substance.
- The results of the analysis of this IATA gives scientific support for the use of these HTS assays as alternatives for existing OECD TGs, for potential use in regulatory decisions related to estrogenic activity.

03-3 | Screening and prioritisation: 내분비장애독성

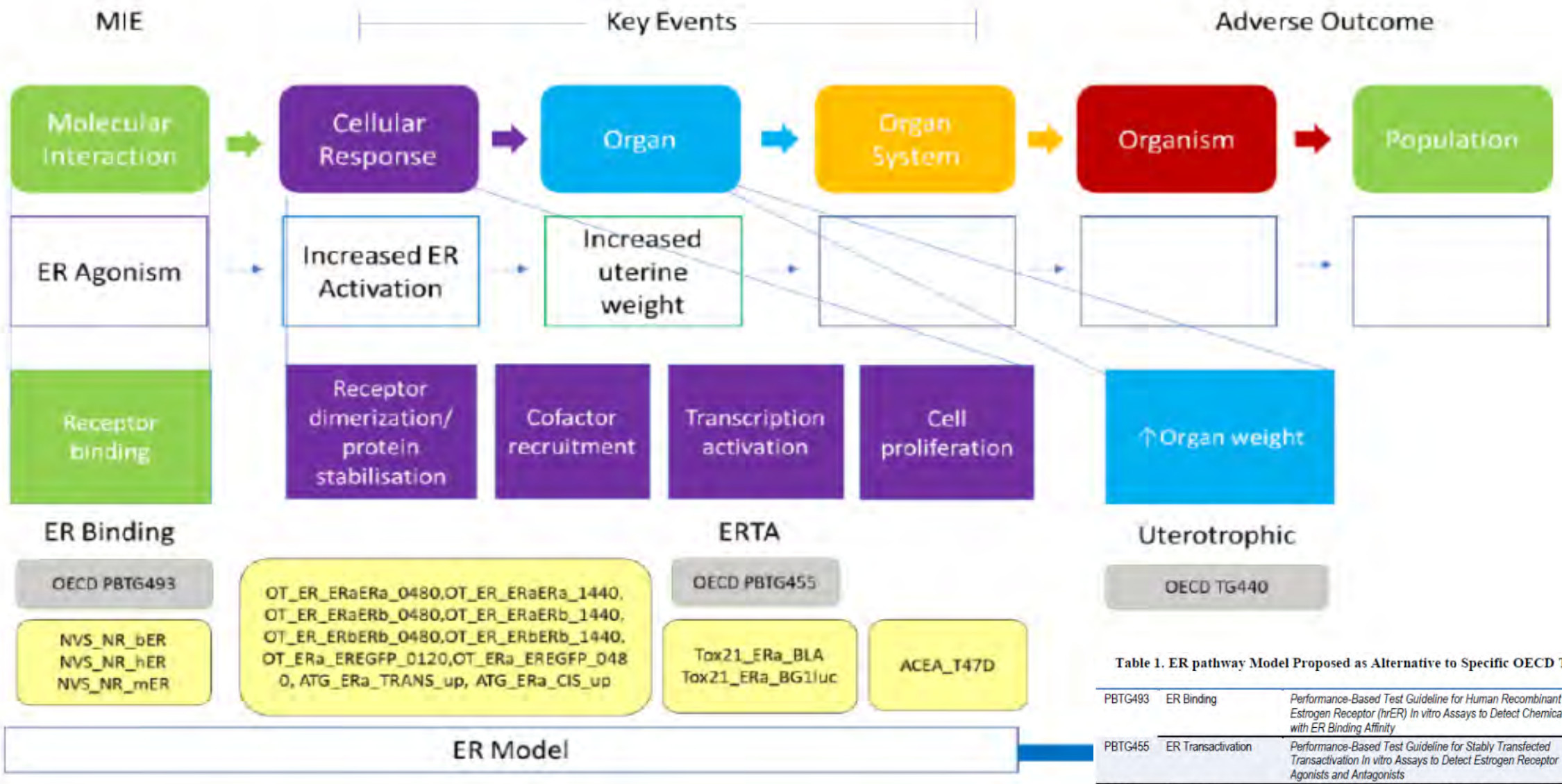
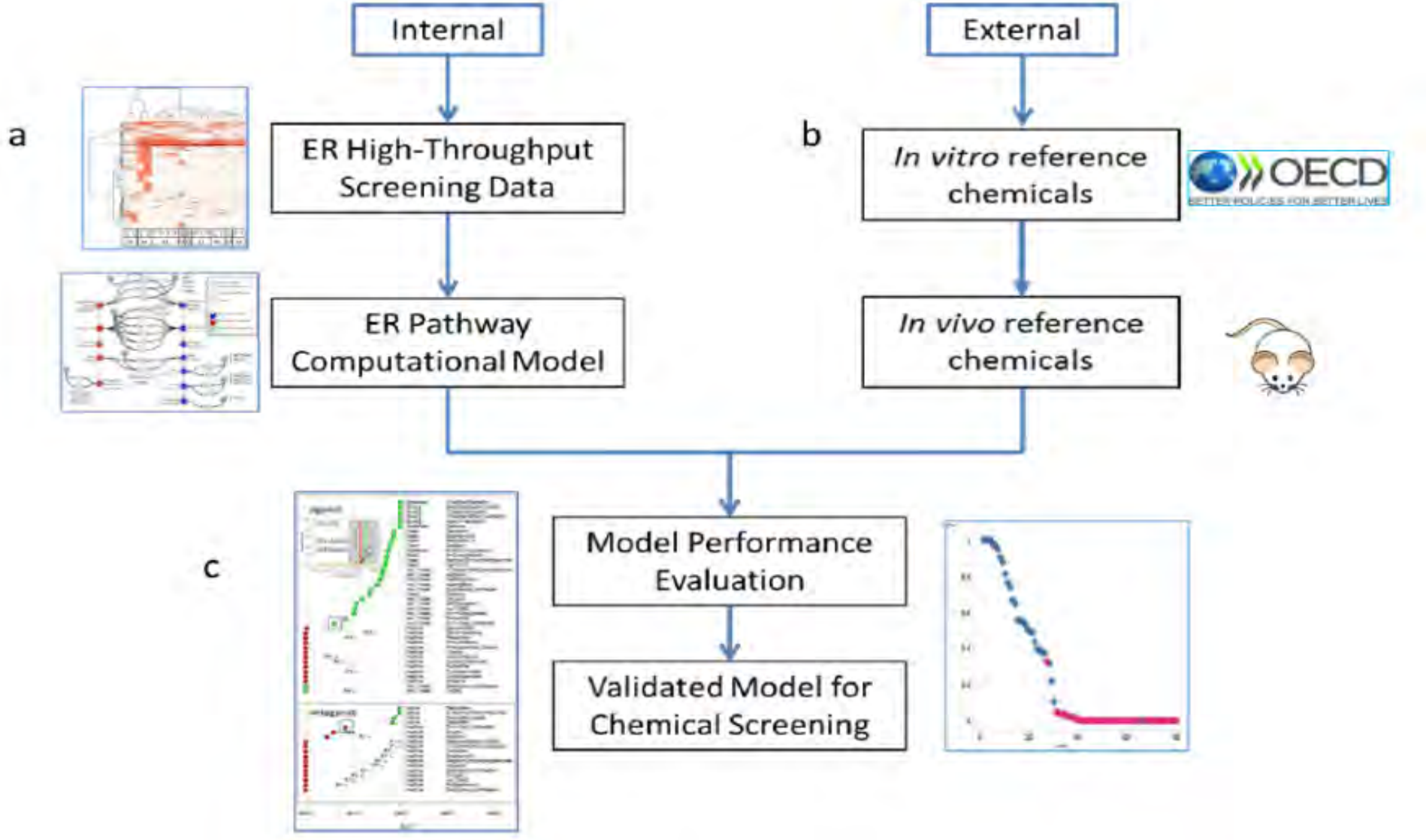


Table 1. ER pathway Model Proposed as Alternative to Specific OECD Test Guidelines

PBTG493	ER Binding	*Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In vitro Assays to Detect Chemicals with ER Binding Affinity*	OECD, 2015b
PBTG455	ER Transactivation	*Performance-Based Test Guideline for Stably Transfected Transactivation In vitro Assays to Detect Estrogen Receptor Agonists and Antagonists*	OECD, 2015a
TG440	Uterotrophic assay	*Uterotrophic Bioassay in Rodents: A Short-Term Screening Test for Oestrogenic Properties*	OECD, 2007


Internal
External
a
b
ER High-Throughput Screening Data
ER Pathway Computational Model
In vitro reference chemicals
In vivo reference chemicals
OECD
c
Model Performance Evaluation
Validated Model for Chemical Screening

03-3 | Screening and prioritisation: 내분비장애독성

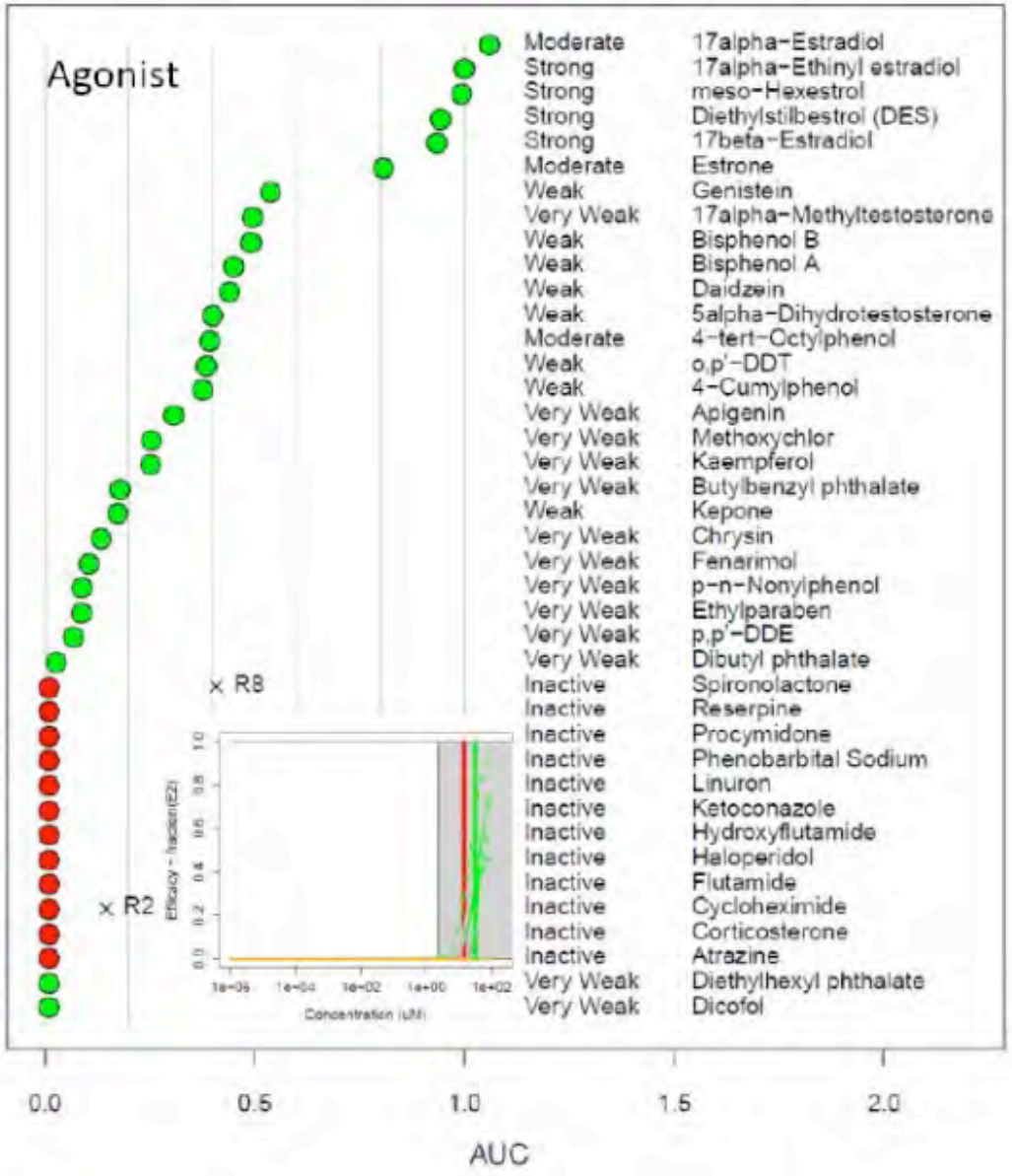


Figure 4B. Activity of *in vivo* reference chemicals (Browne et al., 2015).

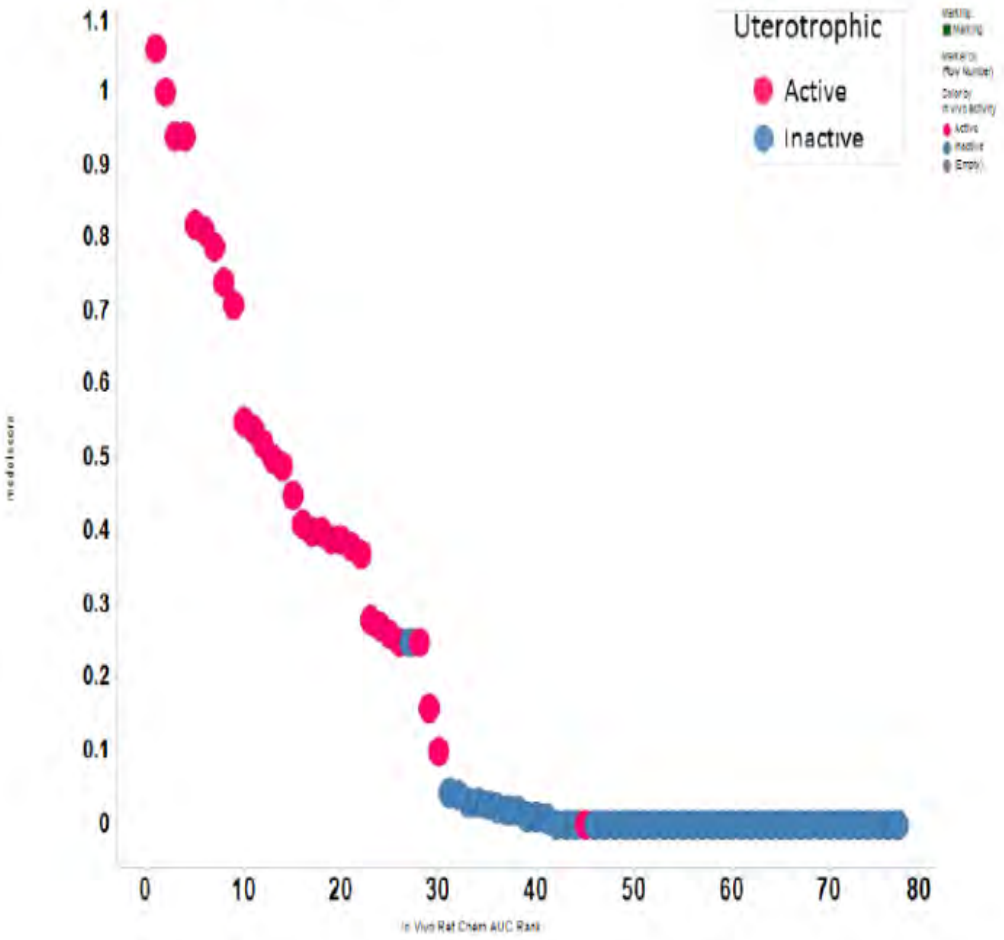


Table 5. Uncertainty of Defined Approach

Factor	Uncertainty (low, medium, high)	Comment
Hypothesis used for the Defined Approach case study	Low	The hypothesis for performing this IATA was to use a pathway-based, computational model (based on *in vitro* HTS assays) to be able to screen and prioritise chemicals for further assessment, based on whether or not they demonstrated ER agonist activity. This methodology allows for a more rapid analysis of a larger number of chemicals than was possible with traditional, low-throughput *in vitro* or *in vivo* assays.
Assumptions related to key events of ER pathway	Low	Pathway frameworks are built upon assumptions of a relationship between the MIE, subsequent KEs and the proposed apical effect or adverse outcome. The use of these pathways may not consider that there are unknown pathways that may impact the decisions made about a specific substance. While it is important to consider the assumptions related to specific pathways during any analysis, pathways are useful as a framework for organizing the data to aid in highlighting where existing assays can be used to inform decisions, and where gaps may exist that require the development of new assays.
Reference chemicals for performance evaluation of the ER model	Low	Number of reference chemicals used for the performance evaluation of the ER model is sufficient. Well documented curation of reference chemicals based on their ER activity is included in the references cited in this case study. Study quality was evaluated but should be noted that this evaluation is not necessarily equivalent to a thorough assessment of overall study quality.
In vitro HTS assays and computational model	Low-medium	All testing methods have some element of uncertainty. In the analysis of the HTS assays, statistical methods are being developed to establish uncertainty bounds around potency and efficacy values. These statistical methods involve resampling the data and refitting the concentration response curves thousands of times to quantitatively estimate the uncertainty. Concentration-response parameters such as potency and efficacy are extracted from HTS data using nonlinear regression, and

Summary of Screening and prioritisation

CASRN	Chemical name	Active [a]	Inactive [a]	Bioactivity	ER pathway model score
57-91-0	17α-Estradiol	2	0	active	1.06
57-63-6	Ethinyl Estradiol	59	0	active	1
56-53-1	Diethylstilbestrol (DES)	8	1	active	0.94
50-28-2	Estradiol	25	0	active	0.94
474-86-2	Equilin	2	0	active	0.82
53-16-7	Estrone	9	0	active	0.81
50-27-1	Estriol	4	0	active	0.79
72-33-3	Mestranol	3	0	active	0.74
17924-92-4	Zearalenone	4	0	active	0.71
1478-61-1	Bisphenol AF	4	0	active	0.55
446-72-0	Genistein	27	1	active	0.54
68-22-4	Norethindrone	2	0	active	0.52
58-18-4	Methyltestosterone	3	0	active	0.50
77-40-7	Bisphenol B	2	0	active	0.49
80-05-7	Bisphenol A	37	6	active	0.45
104-43-8	4-Dodecylphenol	3	0	active	0.41
521-18-6	Dihydrotestosterone	3	0	active	0.4
131-55-5	Benzophenone-2	6	0	active	0.40
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	3	1	active	0.39
789-02-6	o,p'-DDT	15	1	active	0.39
599-64-4	p-Cumylphenol	2	0	active	0.38
5153-25-3	Benzoic acid, 4-hydroxy-, 2-ethylhexyl ester	2	0	active	0.37
80-46-6	4-(1,1-Dimethylpropyl)phenol	4	0	active	0.28
131-56-6	2,4-Dihydroxybenzophenone	3	0	active	0.27
80-09-1	Bisphenol S	2	0	active	0.26
72-43-5	Methoxychlor	18	1	active	0.25
94-26-8	Butylparaben	8	2	active	0.25
98-54-4	p-tert-Butylphenol	2	0	active	0.16
104-40-5	Nonylphenol	5	4	active	0.10
556-67-2	Octamethylcyclotetrasiloxane	3	0	active	0
520-18-3	Kaempferol	0	3	inactive	0.25
84-74-2	Dibutyl phthalate	0	2	Inactive	0.03
84-61-7	Dicyclohexyl phthalate	0	2	inactive	0.02
84-75-3	Dihexyl phthalate	0	2	inactive	0.01
51630-58-1	Fenvalerate	0	2	inactive	0.01
103-23-1	Bis(2-ethylhexyl)hexanedioate	0	2	inactive	0
117-81-7	Bis(2-ethylhexyl)phthalate	0	2	inactive	0
1461-22-9	Tributylchlorostannane	0	2	inactive	0
1912-24-9	Atrazine	0	2	inactive	0
61-82-5	Amitrole	0	2	inactive	0
84-66-2	Diethyl phthalate	0	2	inactive	0
87-86-5	Pentachlorophenol	0	2	inactive	0
99-96-7	4-Hydroxybenzoic acid	0	2	inactive	0

CONCLUSION

- Pragmatic, Science-based approaches for **chemical hazard characterization**
- Follow an Iterative, Integrative approach to **answer a defined question in a specific regulatory context**
- **Combination of methods / integrating results** from one or many methodological approaches
  - Take into account the acceptable level of **uncertainty** associated with the decision context
- **Hazard assessment, Screening & prioritization, Risk assessment**


Thank
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