Session 1: Chemical Risk Assessment in Japan;
Current Status, Challenges and Opportunities

Risk Assessment Methodology for Chemicals and Contaminants in Foods

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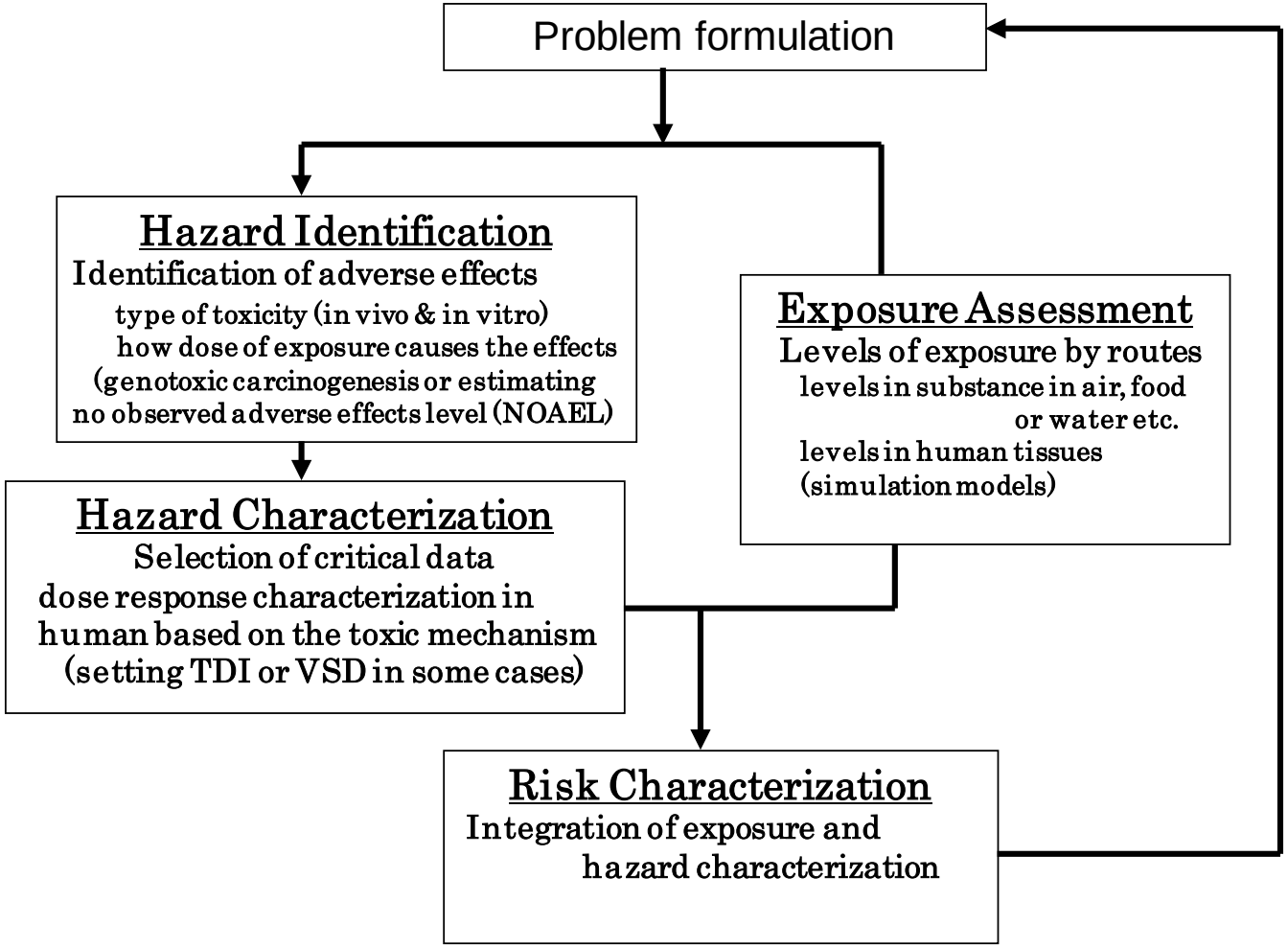
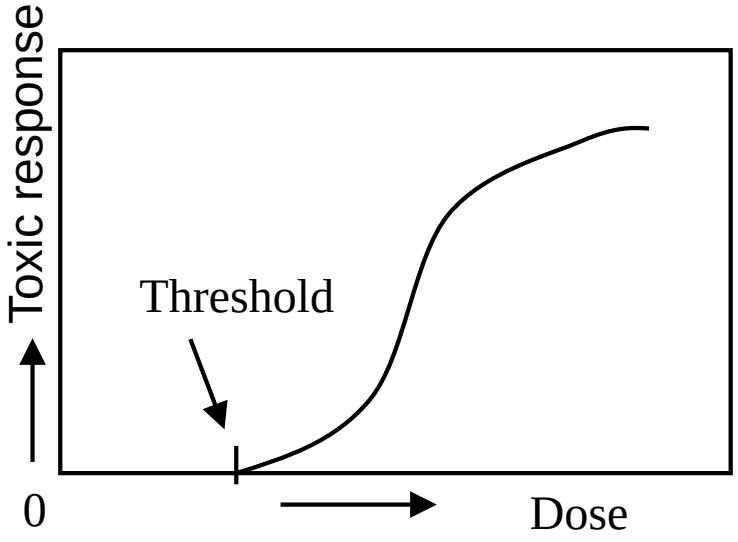
Today's topics

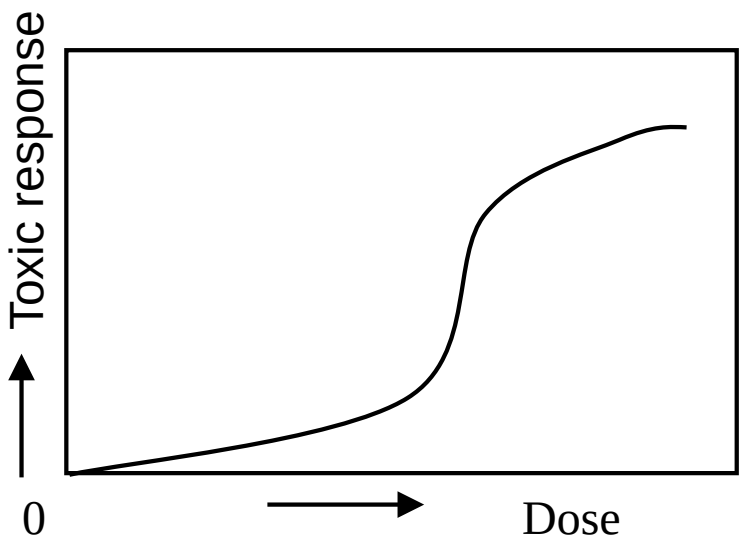
- CSAF & BMD methods
- TTC (Threshold of Toxicological Concern)
- QSAR/Category approach
- Needs of integrated risk assessment and more experts …

Traditional Risk Assessment paradigm



Hazard characterization
Dose Response Assessment





Toxic effects with threshold

↓

TDI/ADI approach
(UF approach)

Non-threshold toxicity
(DNA-direct acting chemicals)

↓

Mathematical modeling
or MOE approach

Derivation of ADI: Acceptable Daily Intake or TDI: Tolerable Daily Intake

ADI = NOAEL/SF (Safety factor)

TDI = NOAEL/UF (Uncertainty factor)

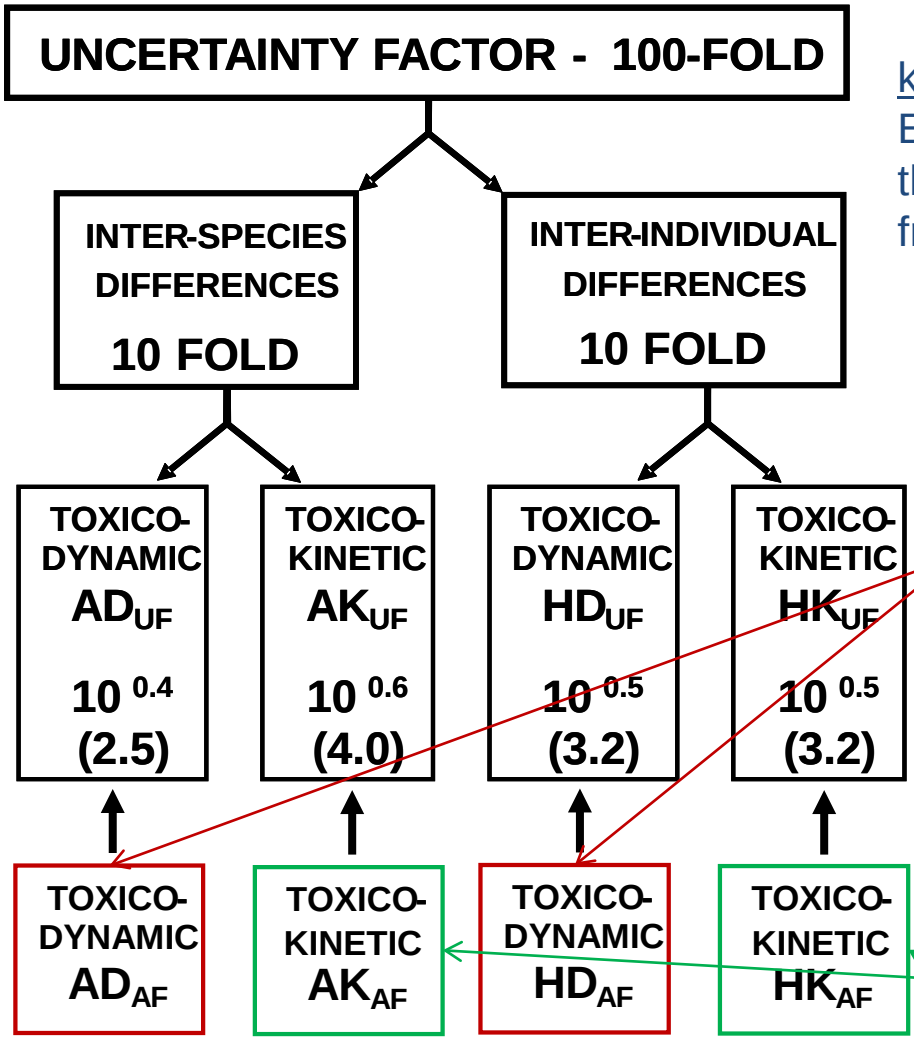
[Construction of the UF or SF]

1. **Inter species difference：10.（or allometric adjusting）**
2. **Intra species difference：10** → *to adjust scientifically*
3. **Short-term study：max. 10**
4. **LOAEL (NOAEL is not determined)：max. 10**
5. **Severity of toxicity：max. 10**

（carcinogenicity、teratogenicity、neurotoxicity etc.）

Sub-division of UF, and replacement with CSAF

（CSAF: Chemical specific adjusting factor）



The UF could be divided by toxico-kinetic and toxico-dynamic factor. Each factor could be replaced with the specific factor, which is derived from scientific evidence.

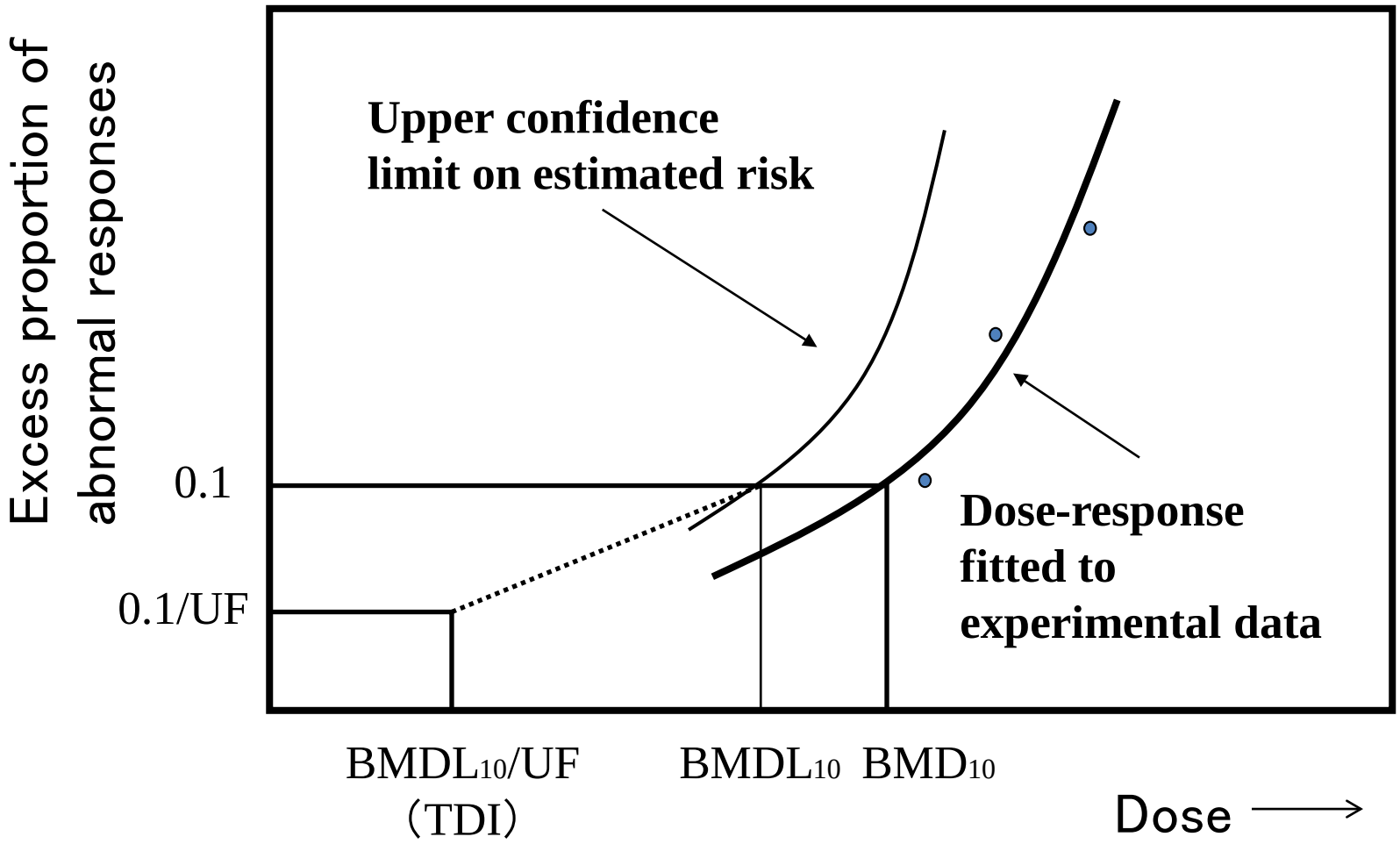
CSAFs could be estimated from in vitro or in vivo studies, when toxico-dynamic components such as target cell sensitivity has been delineated.

CSAFs could be estimated from comparison analysis such as blood concentration or AUC of the active moiety in the general circulation.

A – animal to human; H – human variability;
D – toxicodynamics; K – toxicokinetics

AF - the adjustment factor calculated from chemical-specific data

Graphical illustration of benchmark dose (BMD)



- The benchmark dose is the effective dose (or its lower confidence limit) that produces a certain increase in incidence above control levels.
- The advantages of the benchmark dose are that it takes into account the slope of the dose-response curve, the size of the study groups and the variability in the data.

Risk Characterization for each chemical

- **Direct comparison between TDI (ADI or VSD) and Daily Intake**

  Whether is “TDI” > “Total daily Intake” (or Estimated Intake), or not?

- **For derivation of guidance values (GV)**
  (health based standards for foods, drinking water or air), the below equation is usually accepted.

$$GV = \frac{TDI \times (\text{average body weight}) \times (\text{allocation factor*})}{\text{total daily intake of vehicle}}$$

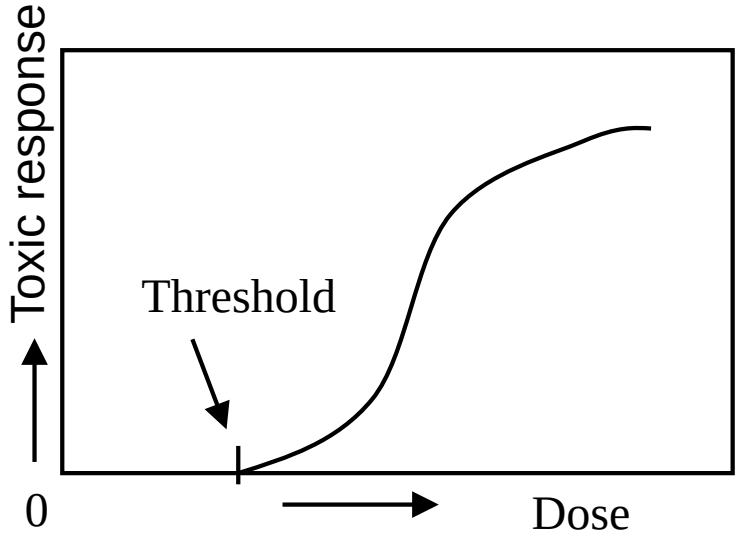
(*: the ratio of contribution via the targeted vehicle among all exposure scenarios)

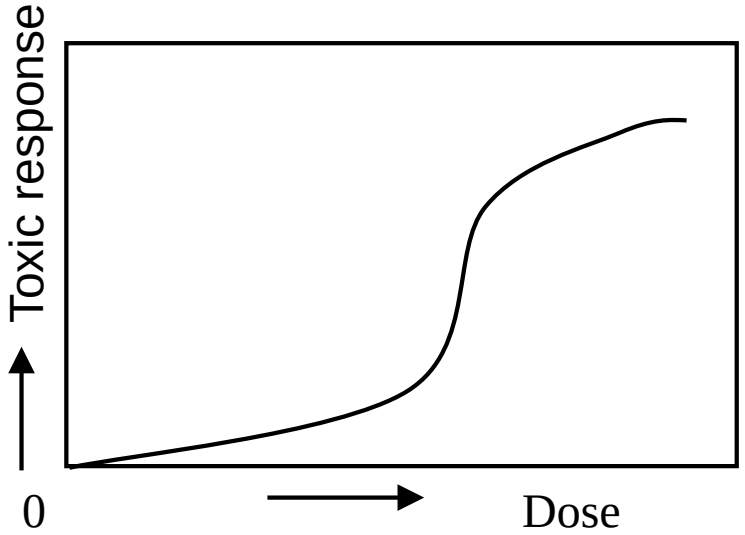
- **Margin of Exposure or Margin of Safety**

  MOE or MOS = NOAEL / Human Exposure level

  (The value of MOE may be used for chemical management prioritization or political decision etc.)

Hazard characteriztion
Dose Response Assessment





Toxic effects with threshold

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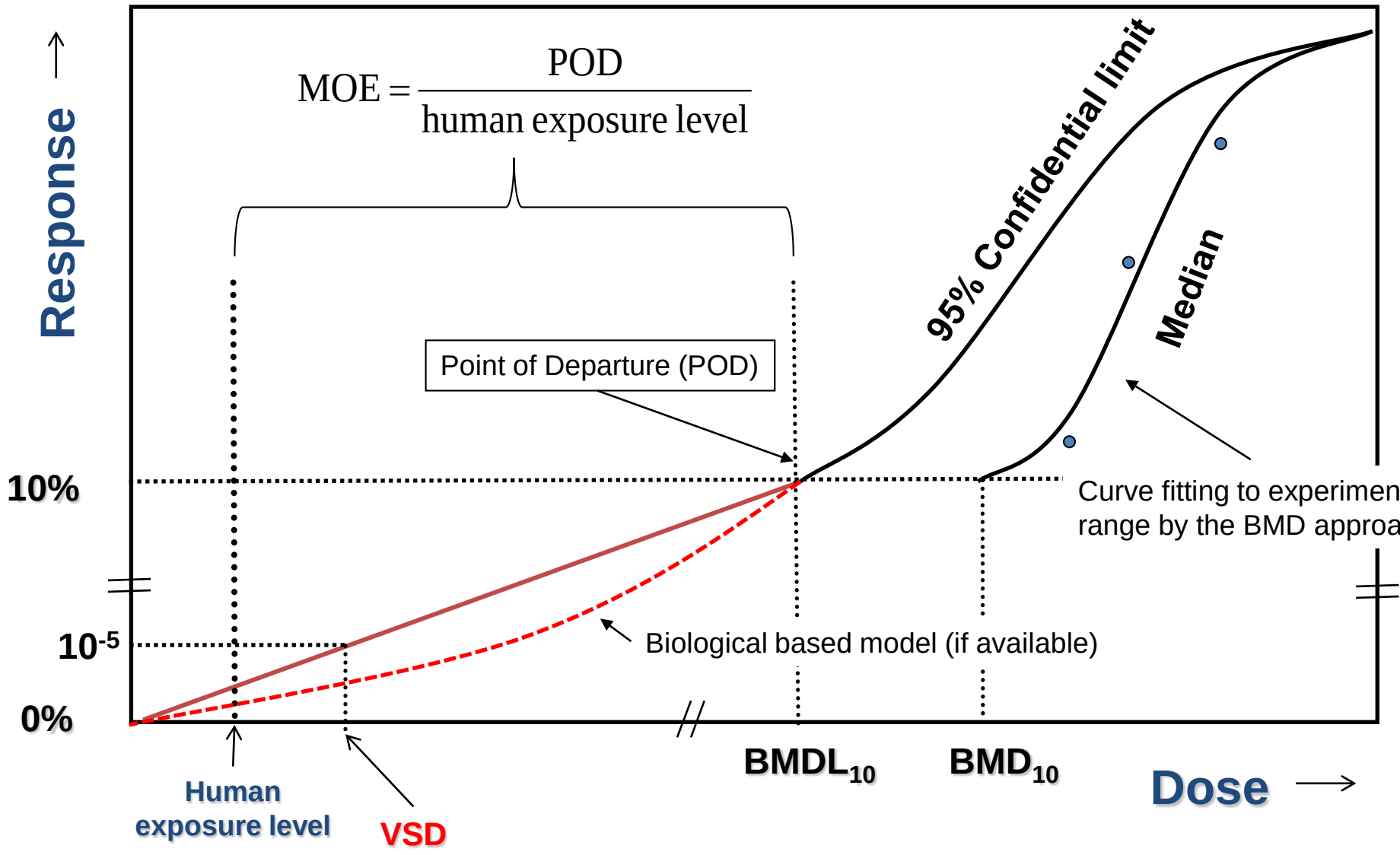
Genotoxic carcinogen risk assessment by using BMD method



BMD method is also used for derivation of the POD in the genotoxicity risk assessment

Problem of the risk assessment of plastics for food container

(In case of very low level exposure and limited toxicity information)

- **What is targets chemicals?**

  Plastics as high molecular weight polymer could not be absorbed into the body. → no health concern.

  But, foods might be contaminated with eluted chemical from plastics

  → Plastics might contain additives, by-products, catalysts, monomer, impurities, degradation products, etc.

- **How to assess safety for many kinds of chemicals in plastics?**

  It is not realistic to assess fully the potential risks of all chemicals.

  Toxicological information for most of the chemicals are limited.

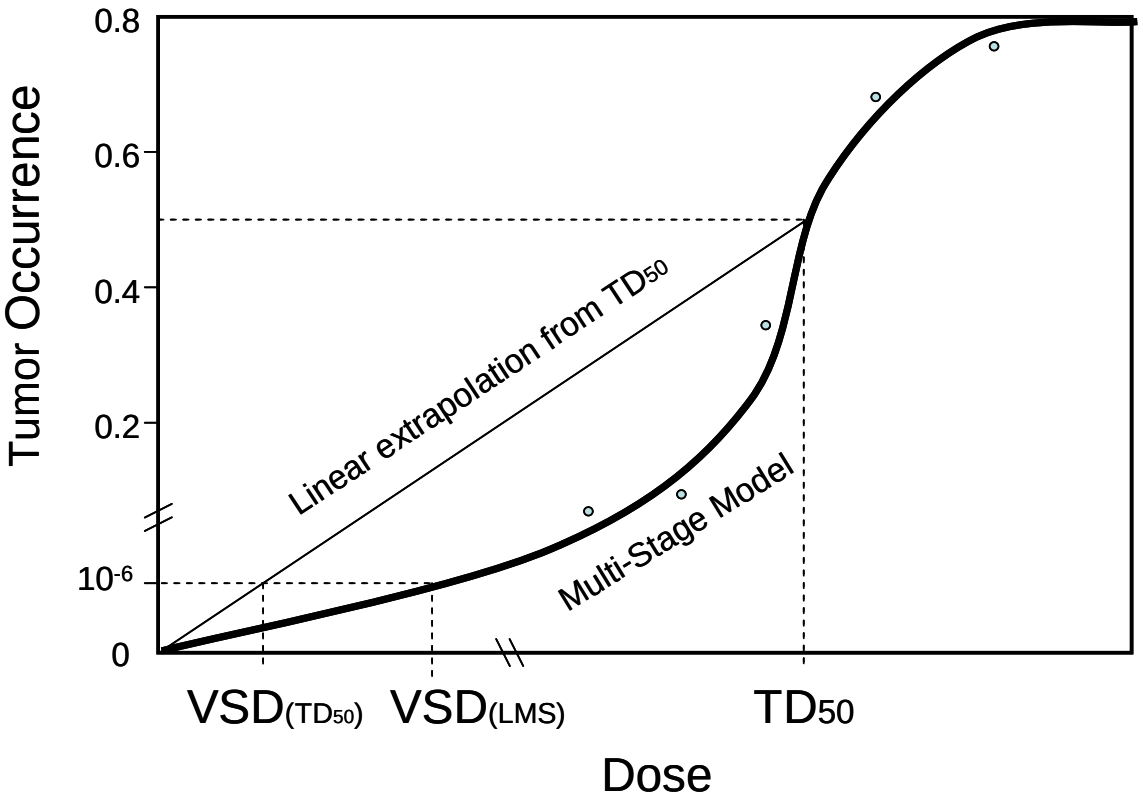
  →The toxicity testing schemas depending on migration levels are required. (Threshold of exposure level is necessary)

Summary table of minimum required toxicity tests

levels of migrant (intake estimate at 3 kg of total diet in case of FDA)	U.S. FDA	EFSA
≦0.5 ppb (≦1.5 ug/day)	No safety studies are recommended ; evaluation of structural similarity to known toxicants	• 3 genotoxicity studies in vitro: i) A test for induction of gene mutations in bacteria ii) A test for induction of gene mutations in mammalian cells in vitro (preferably the mouse lymphoma (ML) to assay) iii) A test for induction of chromosomal aberrations in mammalian cells in vitro
0.5 ～ 50 ppb (1.5 ～ 150 ug/day) First threshold of regulation (TOR) by FDA	2 genotoxicity studies in vitro: i) a test for gene mutations in bacteria and ii) an in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk± assay	
50 ppb ～ 1 ppm (150 ～ 3000 ug/day)	・Above 2 tests＋an in vivo test for chromosomal damage using rodent hematopoietic cells ・2 subchronic oral toxicity tests (a rodent and a non-rodent species).	• Above 3 mutagenicity tests • A 90-day oral toxicity study • Data to demonstrate the absence of potential for accumulation in man
＞1 ppm ～5 ppm	food additive petition should be submitted	
＞5 ppm		• Above tests • Studies on absorption, distribution, metabolism and excretion • Studies on reproduction in one species, and developmental toxicity, normally in two species • Studies on long-term toxicity/carcinogenicity, normally in two species

Derivation of Threshold of Toxicological Concern: TTC

The first TTC of the TOR (Threshold of Regulation) in the U.S.FDA was developed by using the calculate VSD (Virtual Safety Dose) from TD_{50} in the Carcinogenic Potency Database (CPDB)



The value of the VSD linearly extrapolated from TD50 is more conservative than the value of the VSD calculated with the LMS (linearized multistage) model.

Summary table of minimum required toxicity tests based non-carcinogenic endpoints

levels of migrant (intake estimate at 3 kg of total diet in case of FDA)	U.S. FDA	EFSA	Proposal	Estimated Exposure
≦0.5 ppb (≦1.5 ug/day)	No s… reco… stru… toxi…	…udies in …on of gene …eria	No safety studies are recommended ; evaluation of structural similarity to known toxicants	≦1.5 ug/day (0.5 ppb)
0.5 ～ 50 ppb (1.5 ～ 150 ug/day)	2 genotoxicity studies in vitro: ⅰ) a test for gene mutations in bacteria and ⅱ) an in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk± assay	ii) A test for induction of gene mutations in mammalian cells in vitro (preferably the mouse lymphoma (ML) tk assay) iii) A test for induction of chromosomal aberrations in mammalian cells in vitro	2 of 3 tets i) Ames test ii) CA test in mammalian cells *in vitro* ⅲ) ML assay	>1.5 ～ 100 ug/day (50 ppb)
50 ppb ～ 1 ppm (150 ～ 3000 ug/day)	・Above 2 tests+an in vivo test for chromosomal damage using rodent hematopoietic cells ・2 subchronic oral toxicity tests (a rodent and a non-rodent species).	・Above 3 mutagenicity tests ・A 90-day oral toxicity study ・Data to demonstrate the absence of potential for accumulation in man	Above 3 tests A 90 day oral toxicity study (except of organophosphate)	>100 ～ 2000 ug/day (1 ppm)
>1 ppm ～5 ppm >5 ppm	food additive petition should b…	one species, and developmental toxicity, normally in two species ・Studies on long-term toxicity/carcinogenicity, normally in two species	Adequate toxicity information for the compound specific risk assessment (usually all toxicity tests for food additive petition)	>2000 ug/day (1 ppm)

Threshold of concern for non-carcinogenic toxicity

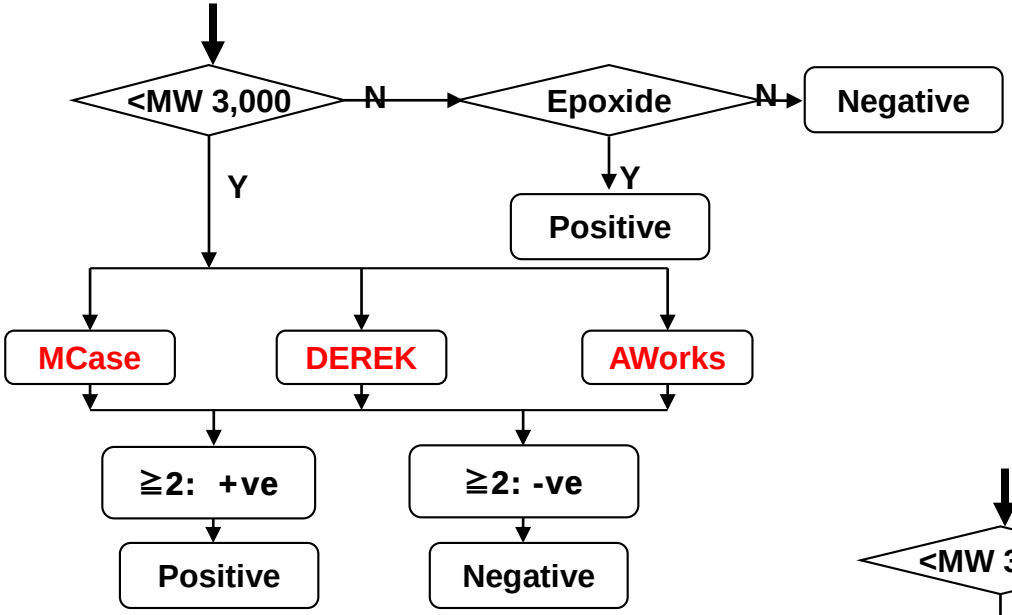
Threshold of concern for specific toxicities (ex. reproductive and developmental toxicity)

Discussion for application of the TTC concept

- The proposed thresholds for toxicity testing schema based on the TTC concept is considered to be similar to other authorities which were traditionally established.
- Development of genotoxicity QSAR system for helping TOR decision would be necessary
- In addition, more precise research on the advancing structural categorization, especially for repeated-dose or developmental toxicities categorization, for developing specific TTCs would be required in future.

Combination (Q)SAR approach

with three mutagenicity (Q)SAR models



Combination 1 of in silico outcomes

Ames test	In silico >++	In silico >--	Total
+	19	7	26
-	23	147	170
	42	154	196

Sensitivity 73.1 %

Specificity 86.5 %

Concordance 84.7 %

Applicability: 95.1% (196/206)

Combination 2 of *in silico* outcomes

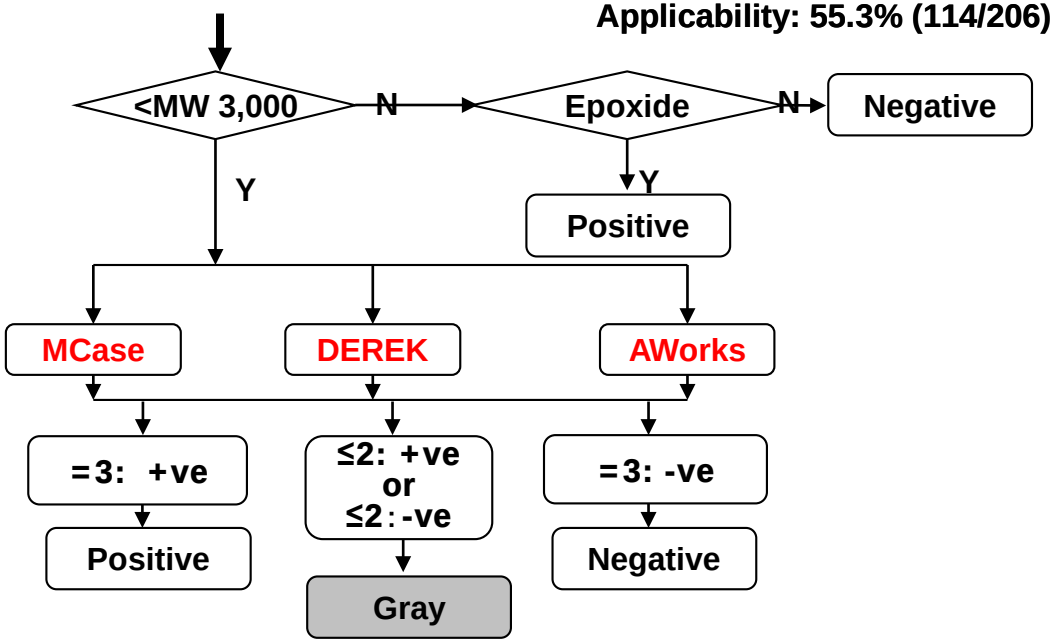
Ames test	*In silico* +++	*In silico* - - -	Total
+	13	2	15
-	5	94	99
	18	96	114

Sensitivity 86.7 %

Specificity 94.9 %

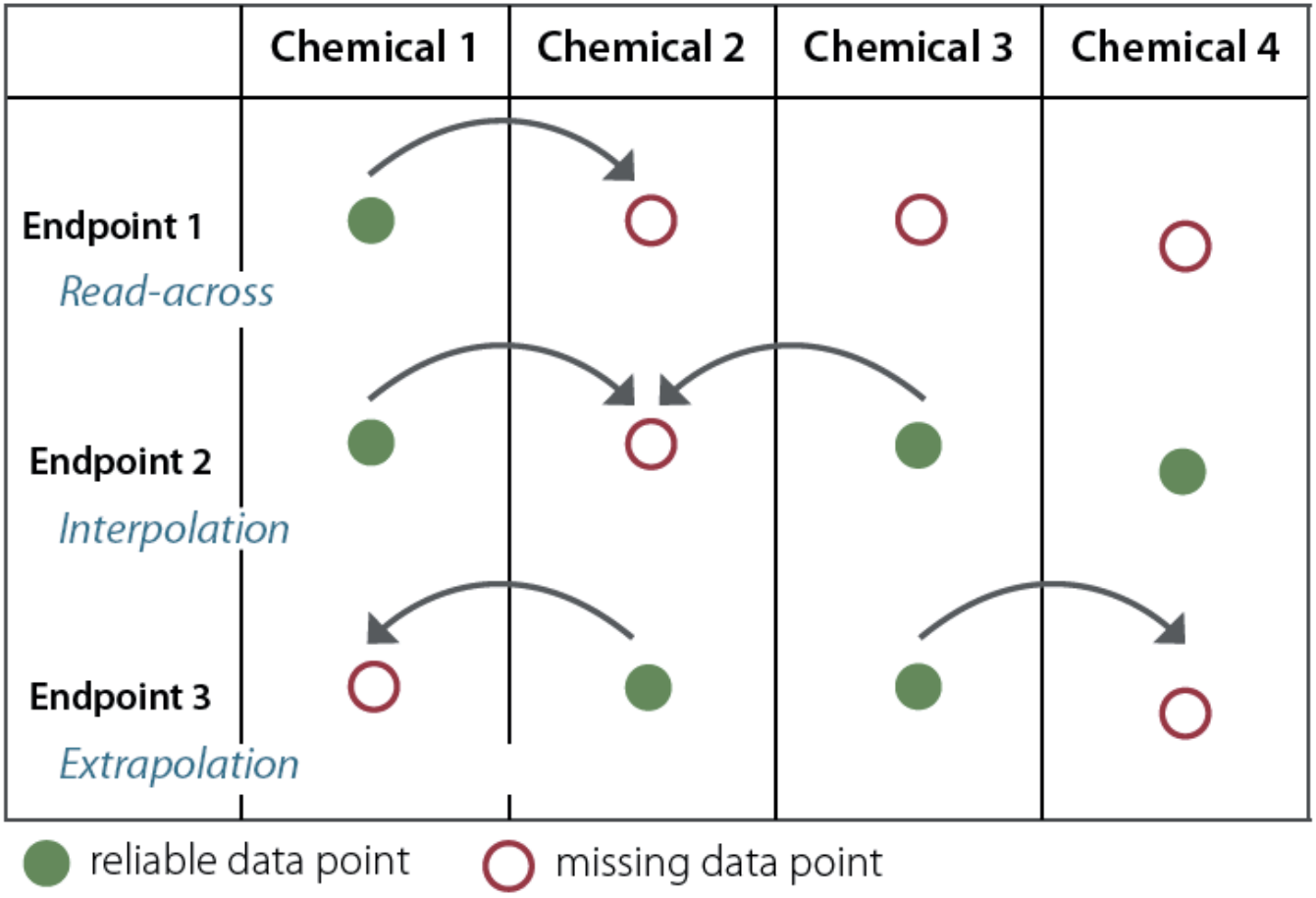
Concordance 93.9 %

Applicability: 55.3% (114/206)



Acknowledgements: This work was supported by Health and Labour Sciences Research Grants of MHLW, Japan

QSAR/Category approach

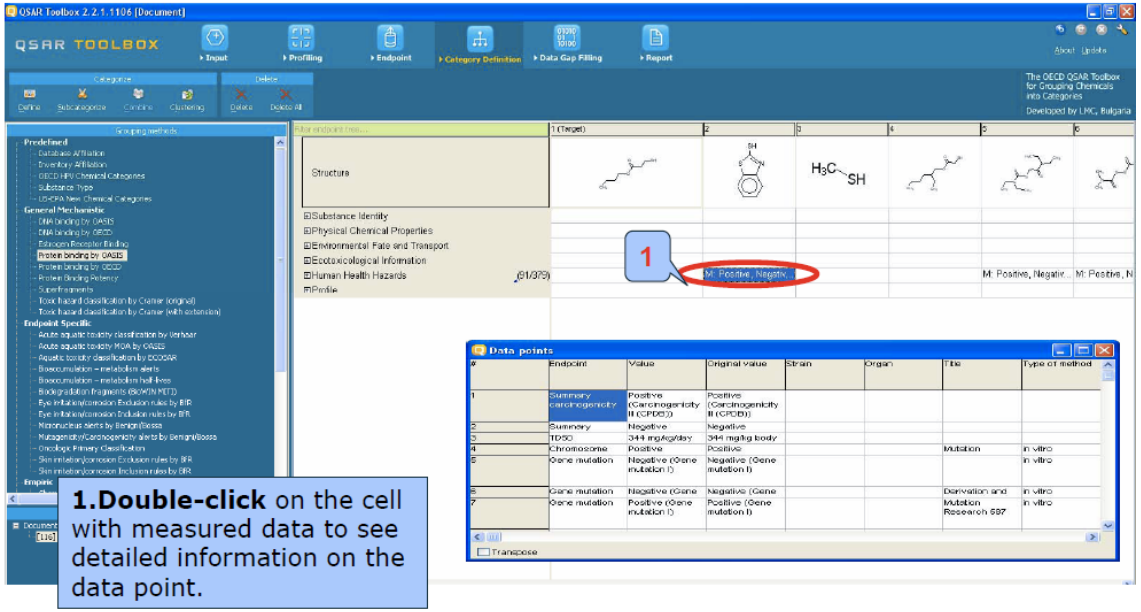


A chemical category can be represented graphically as a two-dimensional matrix in which category members occupy different columns, and the category endpoints occupy different rows.

Data gaps may be filled by read-across from a tested to an untested chemical or by trend analysis.

The OECD QSAR Toolbox for Grouping Chemicals into Categories

Category definition
Side-bar of experimental data



1.Double-click on the cell with measured data to see detailed information on the data point.

In cooperation:

OECD
BETTER POLICIES FOR BETTER LIVES

ECHA
EUROPEAN CHEMICALS AGENCY

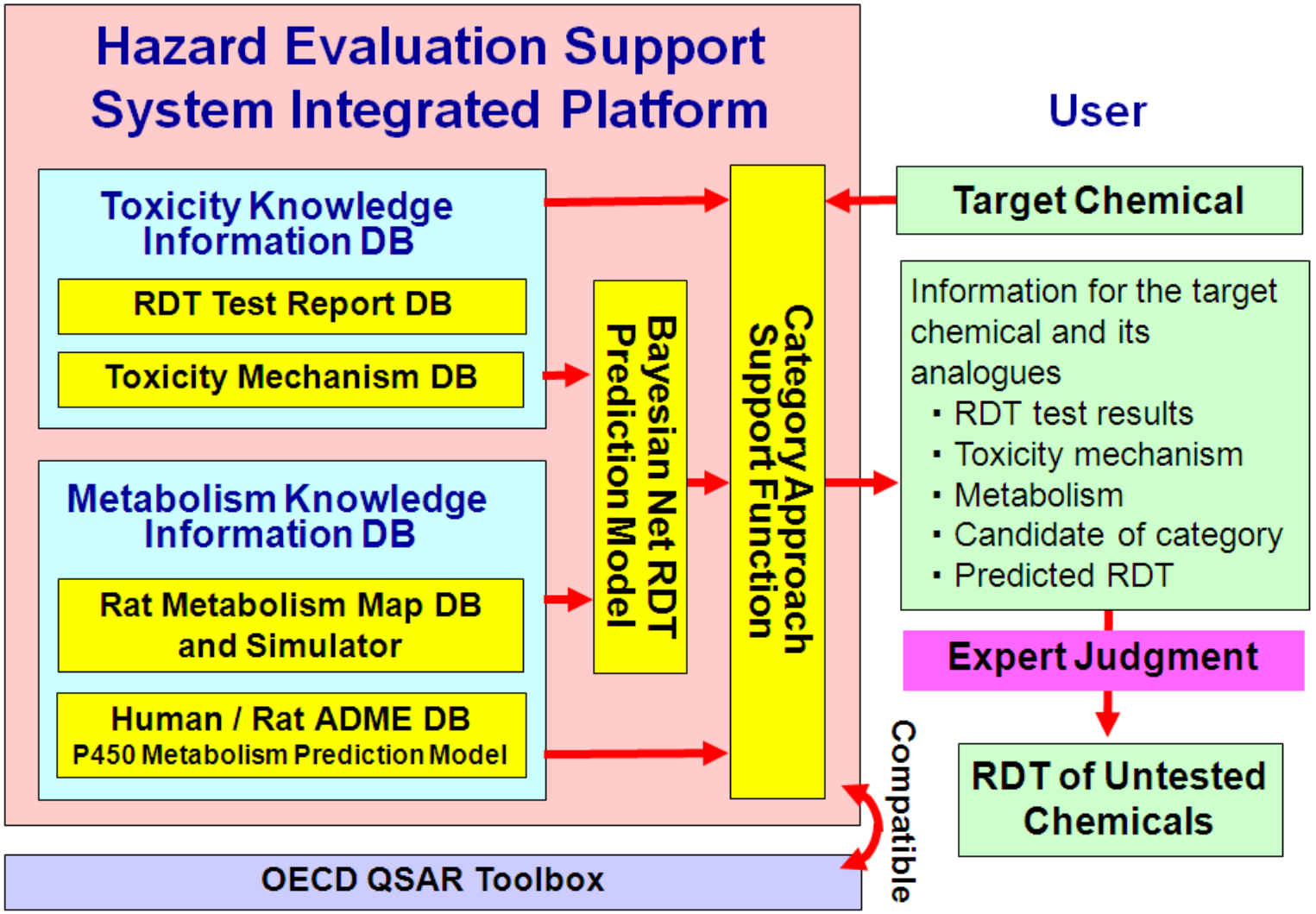
http://www.qsartoolbox.org

Donation (Version 2.1) of database, profiler or QSAR from:

U.S. EPA
Istituto Superiore de Sanita, Italy
European Commission
Environment Canada
Danish EPA
RIVM, the Netherlands
MOE, Japan
MHLW, Japan
METI, Japan
NEDO,Japan
Fraunhofer Institute, Germany
LMC, Bulgaria
BfR, Germany
Istituto Superiore de Sanita, Italy;
Office of Public Health, Switzerland
University of Vienna, Austria
University of Tennessee, Knoxville,
ECETOC
CEFIC
RIFM
International QSAR Foundation
Multicase Inc.; ChemAxon;
Exxon Mobil; Unilever;
P&G; L'Oréal; Dow Chemical;

HESS System

http://www.safe.nite.go.jp/english/kasinn/qsar/hess-e.html



Hayashi, M. and Sakuratani, Y. 2011. Development of an Evaluation Support System for Estimating Repeated Dose Toxicity of Chemicals Based on Chemical Structure. In: New Horizons in toxicity Prediction. Wilson, A. G. E. ed., Royal Society of Chemistry: Chap. 3.

Development of Hazard Evaluation Support System (HESS) and the attached database (HESS DB)②

Data (Collected by NIHS, NITE, Tohoku Univ., Bourgas Univ.)

- Toxicity test reports (545 reports for 515 chemicals, GLP standards)
  - 28d repeated dose toxicity (RDT) studies under Japan's Chemical Substances Control Law (CSCL)
  - Combined RDT and reproductive/developmental toxicity studies under CSCL
  - 13w NTP studies etc.
- Toxicity profiles (530)
  - judged by the committee of Japan's CSCL or created by the experts in toxicology
- Related references (ADME, toxicity mechanism)

HESS (Developed by LMC, Bourgas Univ.)

- Chemical
- Repeated dose toxicity DB
  - LOEL and NOEL for each endpoint
- Category library
- Metabolism map & simulator
- Compatible to OECD QSAR Toolbox

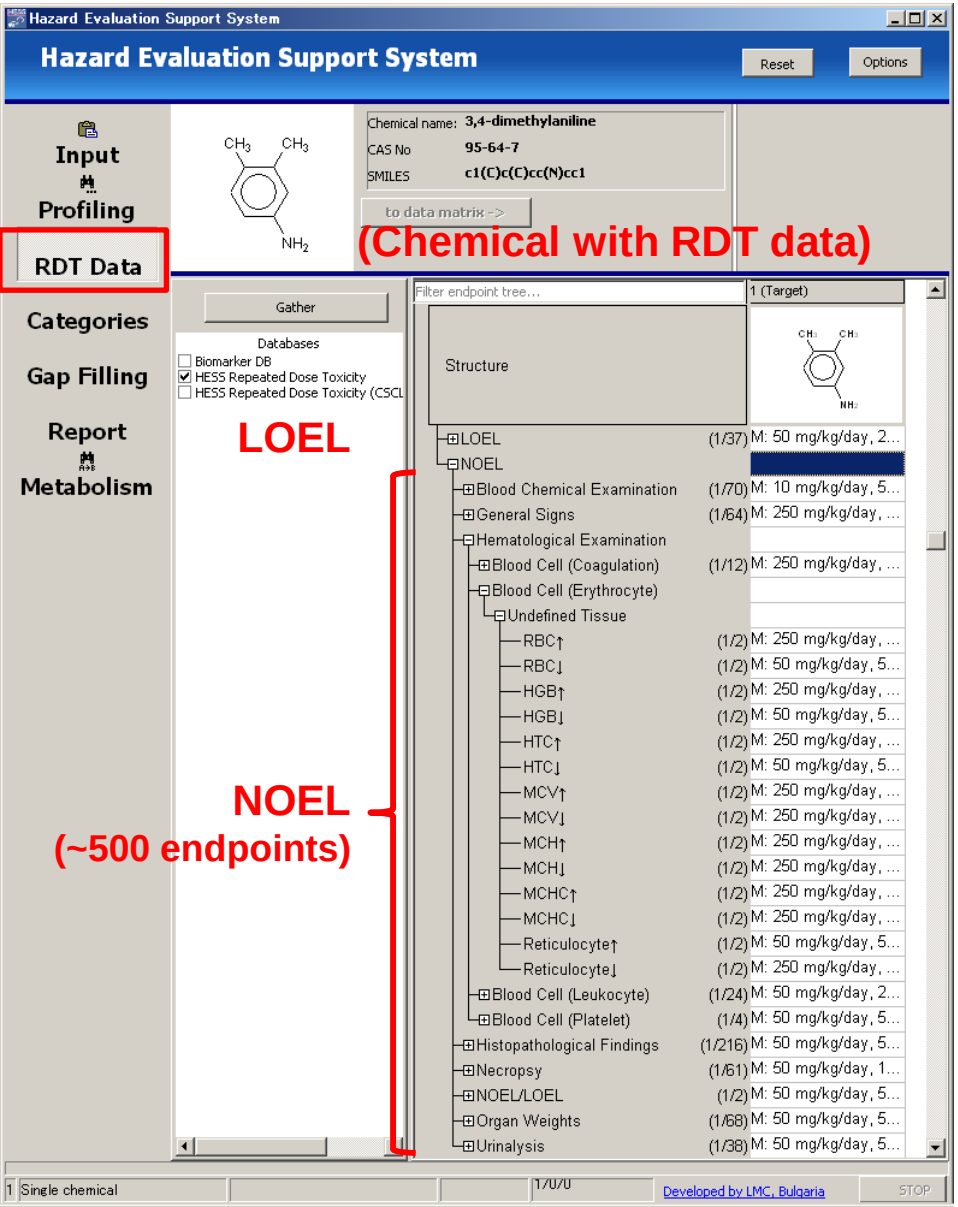
Link

HESS DB (Developed by Fujitsu Limited)

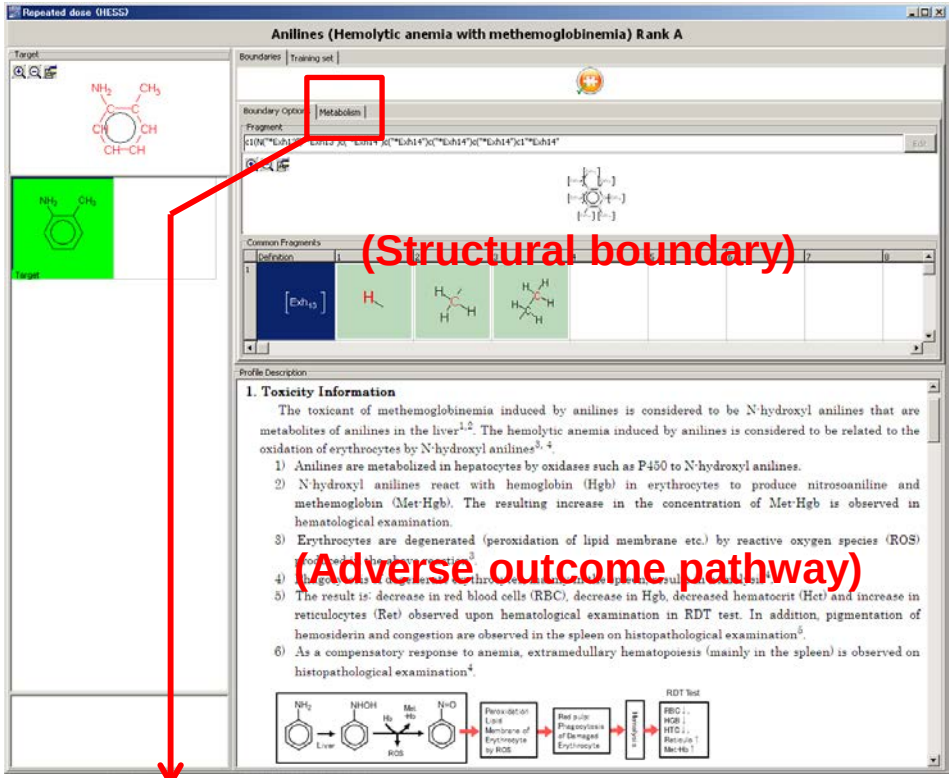
- Chemical
- Toxicity test report DB
  - Toxicological profiles
  - Measured data
- ADME DB
- Toxicity mechanism DB

HESS (Data structure)

Repeated dose toxicity data



Category information



Boundaries | Training set

(Training set)

CAS	Name	SMILES
108-44-1	3-methylaniline; m-toluidine	c1(N)cc(C)ccc1
87-59-2	2,3-dimethylaniline	c1(N)c(C)c(C)ccc1
95-68-1	2,4-dimethylaniline	c1(N)c(C)cc(C)cc1
87-62-7	2,6-dimethylaniline	c1(C)c(N)c(C)ccc1
95-64-7	3,4-dimethylaniline	c1(C)c(C)cc(N)cc1
108-69-0	3,5-xylidine; 3,5-dimethylaniline	c1(N)cc(C)cc(C)c1
99-88-7	aniline 4- (1-methylethyl-); 4-isoprop...	c1(C(C)C)ccc(N)cc1
95-51-2	2-chloroaniline; o-chloroaniline	c1(Cl)c(N)cccc1
108-42-9	3-chloroaniline; m-chloroaniline	c1(Cl)cc(N)ccc1
20265-96-7	p-chloroaniline hydrochloride	c1(Cl)ccc(N)cc1_Cl
536-90-3	3-benzoxyaniline; 3-methoxybenzen...	c1(OC)cc(N)ccc1
156-43-4	4-ethoxybenzenamine	c1(OCC)ccc(N)cc1
100-61-8	n-methylaniline	c1(NC)ccccc1
103-69-5	n-ethylaniline	c1(NCC)ccccc1
121-69-7	n,n-dimethylaniline	c1(N(C)C)ccccc1
99-09-2	3-nitroaniline; 3-nitrobenzenamine; ...	c1(N)cc(N(=O)=O)ccc1
97-52-9	4-nitro-o-anisidine; benzenamine, 2-...	c1(N)c(OC)cc(N(=O)=O)cc1
89-63-4	aniline, 4-chloro-2-nitro-; 4-chloro-2-...	c1(N)c(N(=O)=O)cc(Cl)cc1

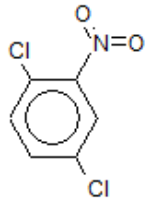
Currently 33 categories included

Hazard Evaluation Support System

Hazard Evaluation Support System

Reset | Options

Input
Profiling
RDT Data
Categories
Gap Filling
Metabolism
Report



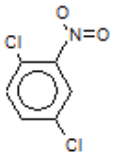
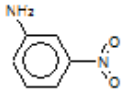
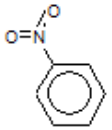
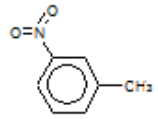
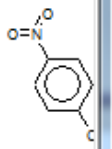
Chemical name:
CAS No 89-61-2
SMILES c1(Cl)c(N(=O)=O)cc(Cl)cc1

to data matrix ->

Target

Analogs

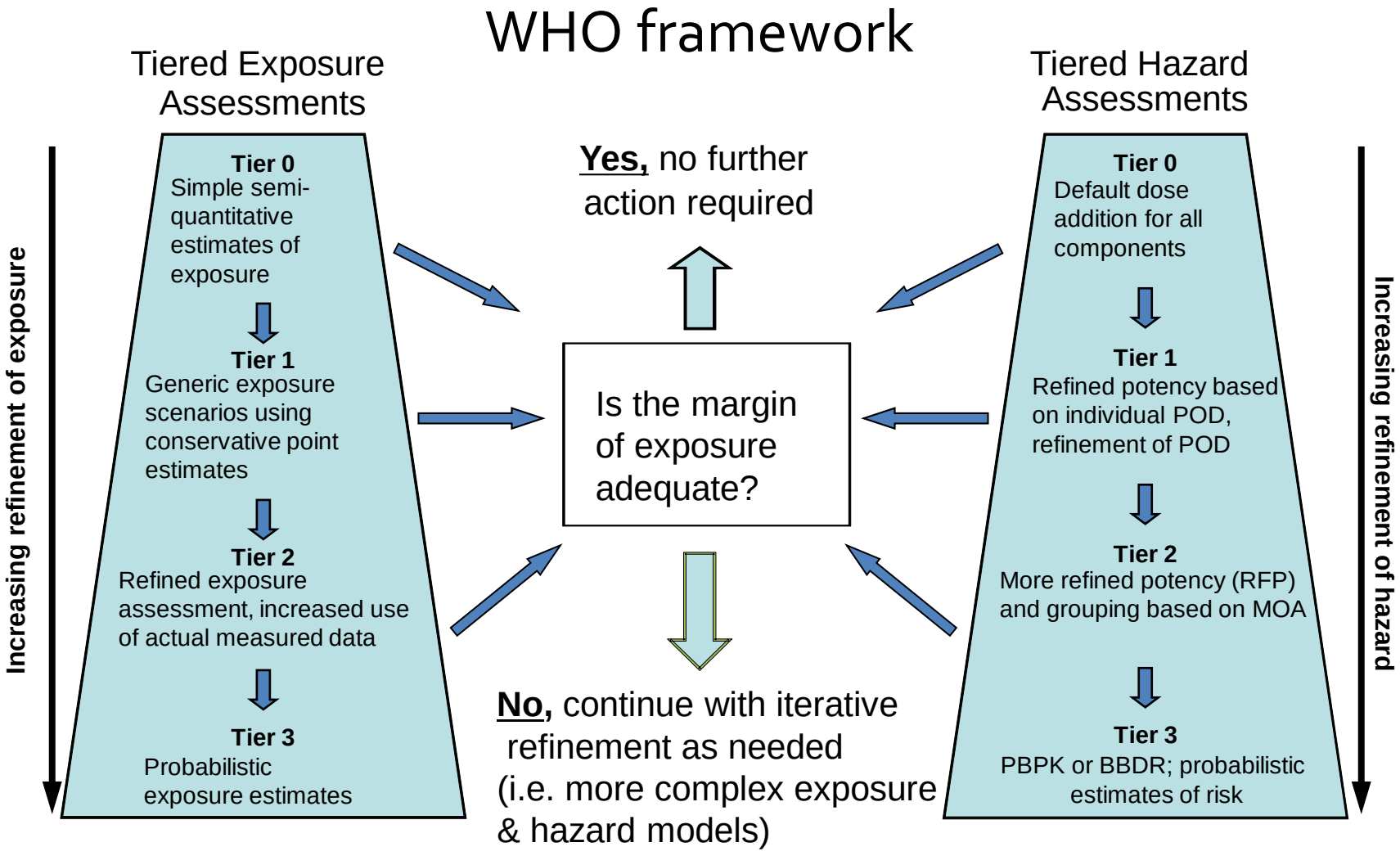
Filter endpoint tree...

Structure	1 (Target)	2	3	4	5
					
Substance Identity					
Repeated Dose Toxicity					
LOEL Min		M: 15 mg/kg/day	M: 5 mg/kg/day	M: 625 mg/L	M: 625 mg/L
Blood Chemical Examination (3/4)			M: 20 mg/kg/day		
Hematological Examination					
Blood Cell (Erythrocyte)					
Undefined Tissue					
RBC↓ (9/16)		M: 15 mg/kg/day, 1...	M: 20 mg/kg/day, 2...	M: 1E4 mg/L, 1E4 ...	M: 5E3 mg/L
HGB↓ (10/17)		M: 15 mg/kg/day, 1...	M: 20 mg/kg/day, 2...	M: 1E4 mg/L, 1E4 ...	M: 5E3 mg/L
HTC↓ (10/17)		M: 15 mg/kg/day, 1...	M: 20 mg/kg/day, 2...	M: 1E4 mg/L, 1E4 ...	
Reticulocyte↑ (10/14)		M: 50 mg/kg/day, 5...	M: 60 mg/kg/day	M: 5E3 mg/L, 1E4 ...	
Methemoglobin↑ (5/9)			M: 20 mg/kg/day	M: 2.5E3 mg/L, 1E...	M: 1E4 mg/L,
Histopathological Findings (9/39)		M: 15 mg/kg/day, 1...	M: 5 mg/kg/day, 5 ...	M: 625 mg/L, 2.5E...	M: 625 mg/L,
Organ Weights (5/16)		M: 15 mg/kg/day, 5...	M: 20 mg/kg/day, 2...		
NOEL (12/240) Min		M: 15 mg/kg/day	M: 0 mg/kg/day	M: <625 mg/L	M: <625 mg/L
Profile					

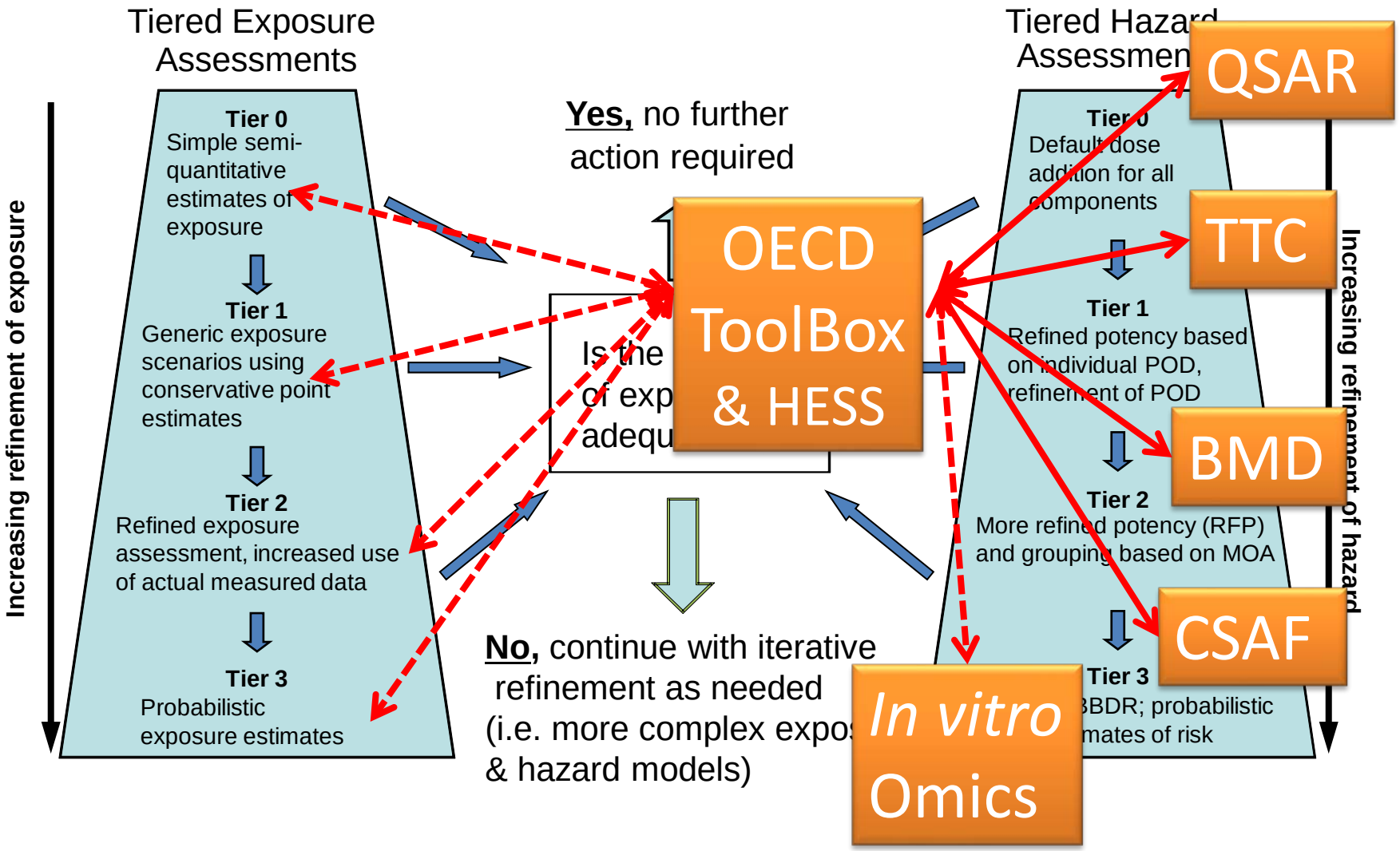
LOEL for RGB↓

12 Nitrobenzenes (Hemolytic anemia with me...

How to assess the risk of the mixture exposure ?



WHO framework



Certified toxicologists as experts for hazard assessment

DABT: Diplomat of American Board of Toxicology (USA)
DJSOT: Diplomat of Japanese Society of Toxicology (Japan)
ERT: European Registered Toxicologist (EU)
ATS: Fellow of the American Toxicological Society (USA)
DKBT: Diploma, Korean Board of Toxicology (Korea)
Expert in Toxicology, DGPT: sponsored by the German Society of Experimental and Clinical Pharmacology and Toxicology (Germany)
UK Register of Toxicologists: sponsored by the Society of Biology and the British Toxicology Society (United Kingdom)
DCST: Diplomat of the Chinese Society of Toxicology (China)