Use of Alternative Methods to Animal Testing in Chemical Assessment under CSCL

March 7, 2019

Chemical Management Center

National Institute of Technology and Evaluation

Japan

1) Introduction of HESS Database

HESS Project

(Aug. 2007- Feb. 2011)

Sponsors :

New Energy and Industrial Technology Development Organization (NEDO)
Ministry of Economy, Trade and Industry (METI)

Aim :

Development of Hazard Evaluation Support System Integrated Platform (HESS) for predicting repeated dose toxicity of chemicals based on category approach

Project Leader:

Dr. Makoto Hayashi, Biosafety Research Center (BSRC)

Contractors:

National Institute of Health Sciences (NIHS)
National Institute of Technology and Evaluation (NITE)
Fujitsu Limited
Bourges" Prof. Assen Zlatarov " University
Tohoku University
Kwansei Gakuin University

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.

Overview of HESS and HESS DB

User

Hazard Evaluation Support System（HESS）

RDT Test DB (summary)

Rat Metabolism Map DB and Simulator

HESS DB

RDT Test DB (detail)

Toxicity Mechanism DB

Human / Rat ADME DB

Category Approach Support Function (Category Library)

Input

Target chemical
- CAS No.,
- chemical name
- structure

Output

Candidate of category and support data

Expert judgement

Prediction

Not used for CSCL examination

OECD QSAR Toolbox（HESS data & Categories）

compatibility

Category Library

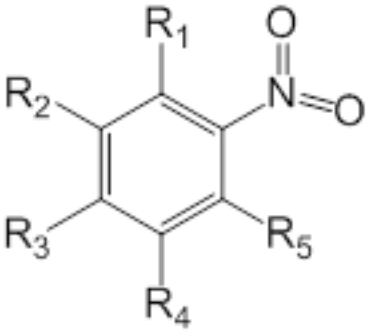
145 categories for RDT are defined based on existing knowledge including mechanism information such as MoA/AOPs.

Category (effect)	Number of category members	LOEL for target effect (mg/kg/day)	Reliability ranking
Azobenzenes (Hemolytic anemia)	2	0.6±5.7	B
Imidazole-2-thione derivatives (Thyrotoxicity)	2	5.5±5.8	B
Diphenyl disulfides (Hemolytic anemia)	1	30	B
Hydrazines (Hemolytic anemia)	2	20±127	B
Acrylamides (Neurotoxicity)	2	21±111	B
Oximes (Hemolytic anemia)	3	23±7	B
Aliphatic nitriles (Hepato toxicity)	4	33±46	B
Nitrobenzenes (Hemolytic anemia)	12	54±82	A
Hydroquinones (Hepatotoxicity)	2	55±64	B
p-Aminophenols (Renal toxicity)	2	63±476	B
Phenyl Phosphates (Lipidosis of adrenocortical cells)	4	70±34	C
Anilines (Hemolytic anemia)	18	72±40	A
4,4'-Methylenedianilines/Benzidines (Hepatobiliary toxicity)	5	75±156	B

Sakuratani, Y. Zhang, H. Q. Nishikawa, S. Yamazaki, K. Yamada, T. Yamada, J. Gerova, K. Chankov, G, Mekenyan, O. and Hayashi, M. 2013. Hazard Evaluation Support System (HESS) for Predicting Repeated Dose Toxicity Using Toxicological Categories. SAR QSAR Environ. Res. **24**: 617-629.

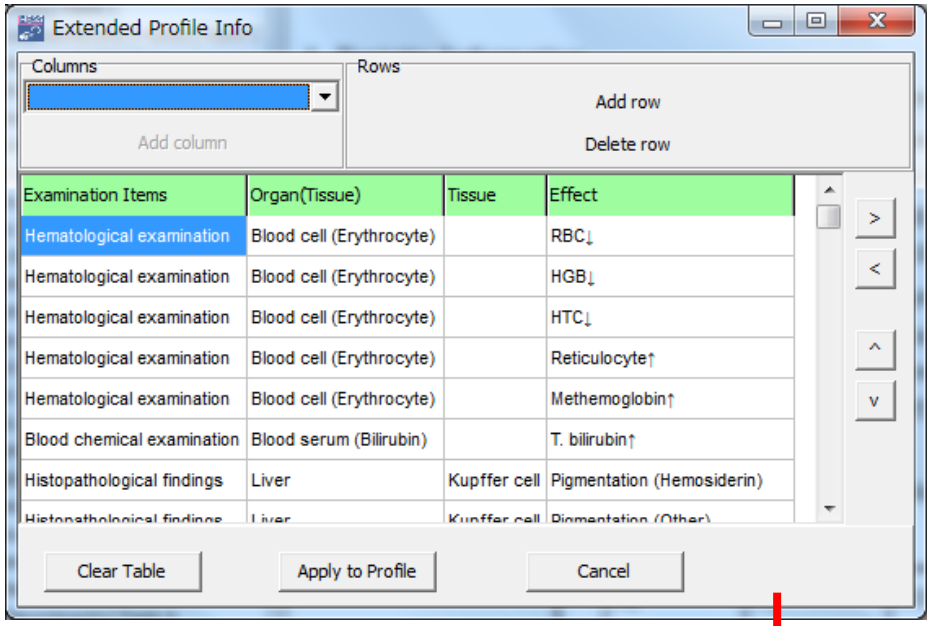
Example of a Category Based on AOP

Structural Boundary



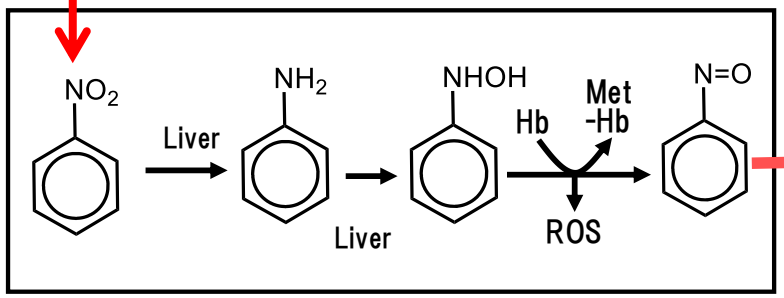
$R_1 \sim R_5$ = H, alkyl, halo, alkoxy, NH_2 or NO_2.

RDT findings related to anemia



AOP

Hemolytic anemia



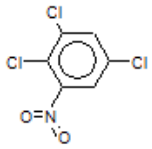
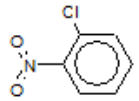
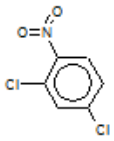
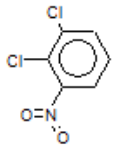
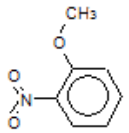
Peroxidation Lipid Membrane of Erythrocyte by ROS

Hemolysis

RBC↓, HGB↓, HTC↓
Reticulo↑, Met-Hb↑
T-bil↑
Spleen
-hemosiderin
-Pigmentation
etc.

Sakuratani, Y. Zhang, H. Q. Nishikawa, S. Yamazaki, K. Yamada, T. Yamada, J. and Hayashi, M. 2013. Categorization of nitrobenzenes for repeated dose toxicity based on adverse outcome pathways. SAR QSAR Environ. Res. **24**: 35-46.

Category Approach Support Function

Filter endpoint tree...	1 (Target)	2	3	4	5
Structure					
⊞Substance Identity					
⊟Repeated Dose Toxicity	Target	Analogues			
⊟LOEL					
⊞Blood Chemical Examin... (4/6)		M: 2E3 mg/L, 2E3 ...	M: 200 mg/kg/day		
⊟Hematological Examination					
⊟Blood Cell (Erythrocyte)					
⊟Undefined Tissue					
RBC↓ (4/8)			M: 8 mg/kg/day, 3...		M: 154 mg/kg/day, ...
HGB↓ (7/13)		M: 63 mg/L, 250 mg/L	M: 40 mg/kg/day, 1...	M: 100 mg/kg/day	M: 154 mg/kg/day, ...
Reticulocyte↑ (6/11)		M: 1E3 mg/L, 1E3 ...	M: 40 mg/kg/day, 2...	M: 100 mg/kg/day	M: 15.4 mg/kg/day,...
HCT↓ (7/13)		M: 1E3 mg/L, 1E3 ...	M: 40 mg/kg/day, 2...	M: 100 mg/kg/day	M: 154 mg/kg/day, ...
⊟Histopathological Findings					
⊟Liver					
⊟Kupffer Cell					
Pigmentation (He... (3/5)		M: 1E3 mg/L, 1E3 ...			M: 1.38E3 mg/kg/d...
⊞Spleen (7/23)		M: 250 mg/L, 250 ...	M: 8 mg/kg/day, 2...	M: 25 mg/kg/day, 1...	M: 462 mg/kg/day, ...
⊞Organ Weights (5/16)		M: 1E3 mg/L, 1E3 ...	M: 2E3 mg/L, 2E3 ...		M: 462 mg/kg/day, ...
⊞NOEL (7/190)		M: 63 mg/L, 63 mg/...	M: 8 mg/kg/day, 8 ...	M: 25 mg/kg/day, 2...	M: 46.2 mg/kg/day,...
⊟Profile					
Study No. (Link to SSRDT)		894	228 892	250	427 845
Chemical No. (Link to HESS DB)		729	222	244	404
Repeated dose (HESS)	Nitrobenzenes (He... Nitrobenzenes (Hep...	Nitrobenzenes (He... Nitrobenzenes (Hep...	Nitrobenzenes (He... Nitrobenzenes (Hep...	Nitrobenzenes (He... Nitrobenzenes (Hep...	Nitrobenzenes (He... Nitrobenzenes (Hep...
⊟Metabolism					
⊞Observed Rat Liver metabolism		3 metabolites	1 metabolites		9 metabolites

RDT Summary data

Link to HESS DB

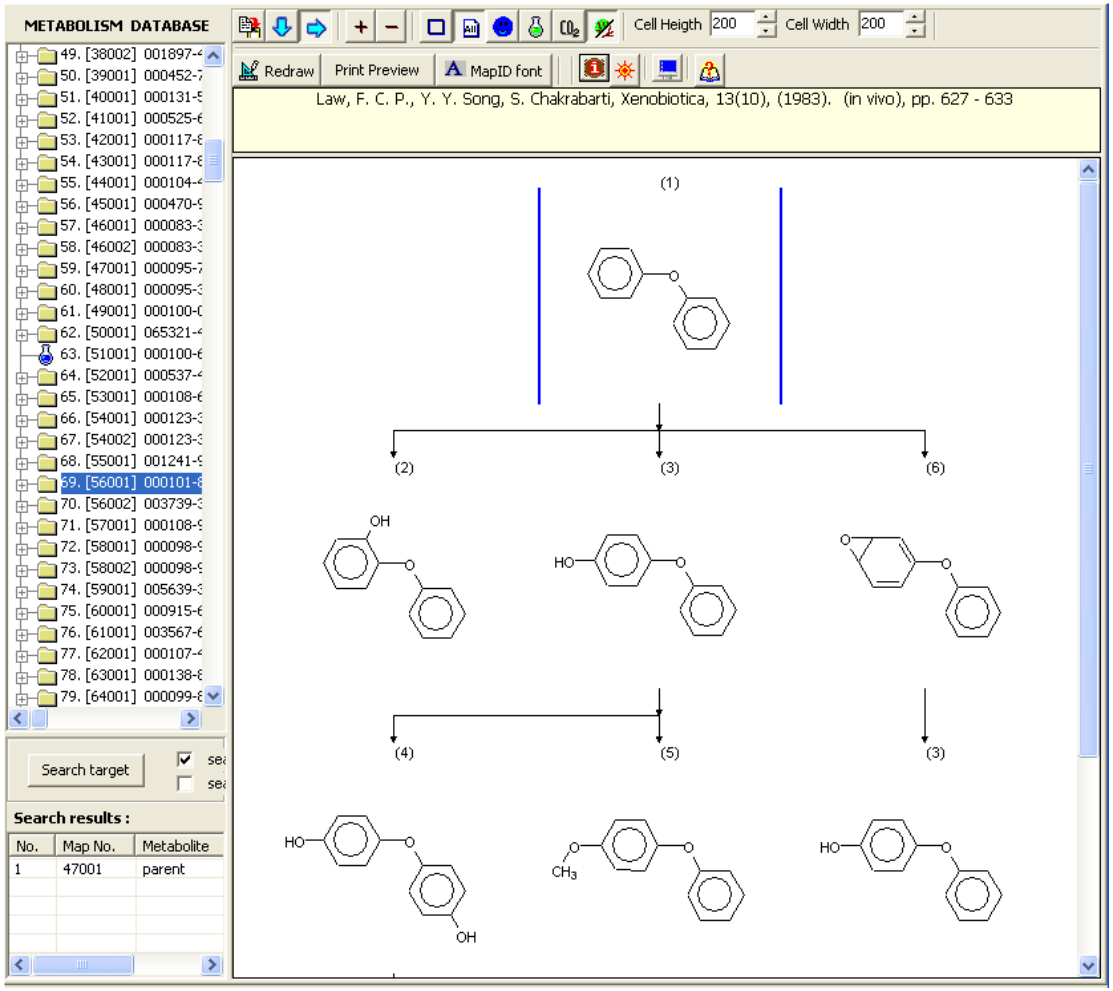
Link to Category description

Link to Rat Metabolism Map DB

RDT Test Data in HESS and HESSDB

DB Name	# of chemicals	Data Source	HESS	HESS-DB
HESS Repeated Dose Toxicity	700	• MHLW/NIHS safety examination of existing chemicals under CSCL in Japan: 362 studies • NITE toxicity test (OECD TG407): 27 studies • METI toxicity test (OECD TG422): 104 studies • NTP short term studies: 59 studies • NTP long term studies (dose selection studies): 141 studies *etc.*	○	○
HESS Repeated Dose Toxicity (CSCL New Chemicals)	49	RDT test data used for the examination of new chemical substances under CSCL in Japan.	○	
HESS RDT DB (HPV Chemicals)	130	OECD SIDS	○	
HESS RDT DB (Inhalation)	29	OECD SIDS	○	
Drug Repeated Dose Toxicity	50	Papers published for drug registration in Japan		○
TGP Repeated Dose Toxicity	124	TGP (Toxicogenomics Project by NIBIOHN, Japan)	○	
COSMOS DB	852	COSMOS DB	○	
ToxRefDB	493	ToxRef DB	○	
Tox-Omics RDT DB	31	Tox-Omics project (CERI, Japan)	○	○

Rat Metabolism Map DB and Simulator

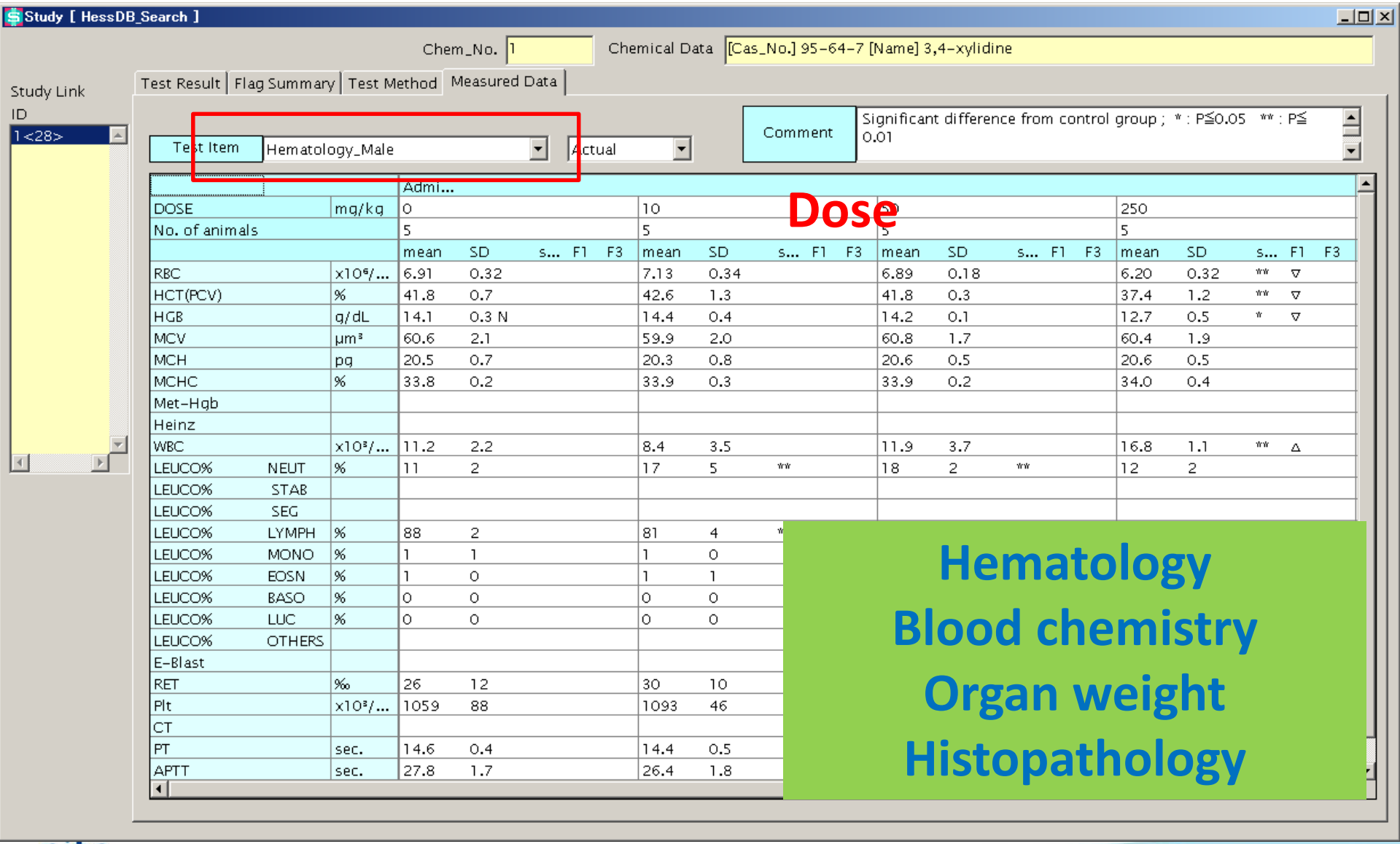


DB of the metabolism maps mainly in the rat liver (1199 maps）。

Metabolism simulator for the rat liver* (in vitro / in vivo)

*Mekenyan, O.G. Dimitrov, S. Dimitrova, N. Dimitrova, G. Pavlov, T. Chankov, G, Kotov, S. Vasilev, K. and Vasilev, R. 2006. Metabolic activation of chemicals: in-silico simulation, SAR QSAR Environ. Res. 17:107-120.

RDT Test DB (detailed)



			Admi...															
DOSE		mg/kg	0				10				50				250			
No. of animals			5				5				5				5			
			mean	SD	s...	F1 F3	mean	SD	s...	F1 F3	mean	SD	s...	F1 F3	mean	SD	s...	F1 F3
RBC		x10⁶/...	6.91	0.32			7.13	0.34			6.89	0.18			6.20	0.32	**	▽
HCT(PCV)		%	41.8	0.7			42.6	1.3			41.8	0.3			37.4	1.2	**	▽
HGB		g/dL	14.1	0.3 N			14.4	0.4			14.2	0.1			12.7	0.5	*	▽
MCV		µm³	60.6	2.1			59.9	2.0			60.8	1.7			60.4	1.9		
MCH		pg	20.5	0.7			20.3	0.8			20.6	0.5			20.6	0.5		
MCHC		%	33.8	0.2			33.9	0.3			33.9	0.2			34.0	0.4		
Met-Hgb																		
Heinz																		
WBC		x10³/...	11.2	2.2			8.4	3.5			11.9	3.7			16.8	1.1	**	△
LEUCO%	NEUT	%	11	2			17	5	**		18	2	**		12	2		
LEUCO%	STAB																	
LEUCO%	SEG																	
LEUCO%	LYMPH	%	88	2			81	4	*									
LEUCO%	MONO	%	1	1			1	0										
LEUCO%	EOSN	%	1	0			1	1										
LEUCO%	BASO	%	0	0			0	0										
LEUCO%	LUC	%	0	0			0	0										
LEUCO%	OTHERS																	
E-Blast																		
RET		‰	26	12			30	10										
Plt		x10³/...	1059	88			1093	46										
CT																		
PT		sec.	14.6	0.4			14.4	0.5										
APTT		sec.	27.8	1.7			26.4	1.8										

Toxicity Mechanism DB

Mechanism information for the critical toxicity such as necrosis of hepatocytes observed in RDT was built in.

<Mechanism Information>
- Toxicity
- Possible Chemistry Reaction /Metabolism
- Possible Toxicant
- Possible Interaction with Target Molecule
- Possible Effects
- Target Organ/Tissue/Cell etc.

<Mechanism Summary>
- Possible Mechanism Summary

<Experimental Information>
- Species
- Experimental Design
- *in vitro* / *in vivo* / *ex vivo*
- Dose / Concentration Employed
- Effective Dose / Concentration

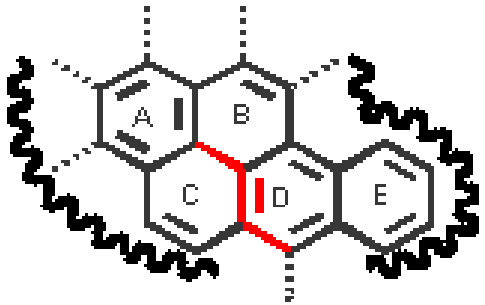
<Other information>
- Other Compounds studied
- Additional Information
- Authors' Suggestion

Human / Rat ADME DB

Information for chemical substances related to human/rat metabolism that are useful for comparing species differences in toxicity between human and rat.

Absorption	Absorption rate, Cmax, Tmax Imvolvement of transporter
Distribution	Apparent volume of distribution Time-dependent changes by repeated doses Brain → Blood-brain barrier, Adipocyte → strage Liver → Metabolism, Kidney → Urinary excretion Kidney → Binding to protein Organs with higher concentration of chemicals than blood Imvolvement of transporter
Metabolism	Related enzyme and molecular information Contribution ratio, Cellular fraction, Metabolite Spaces differences, Strain differences
Excretion	Excretion rate, Imvolvement of transporter Spaces differences, Strain differences
Result of interaction, inhibition of enzyme , enhanced of enzyme test	
Relationship of toxicity	

P450 Metabolism Prediction Model based on Ligand Structure*

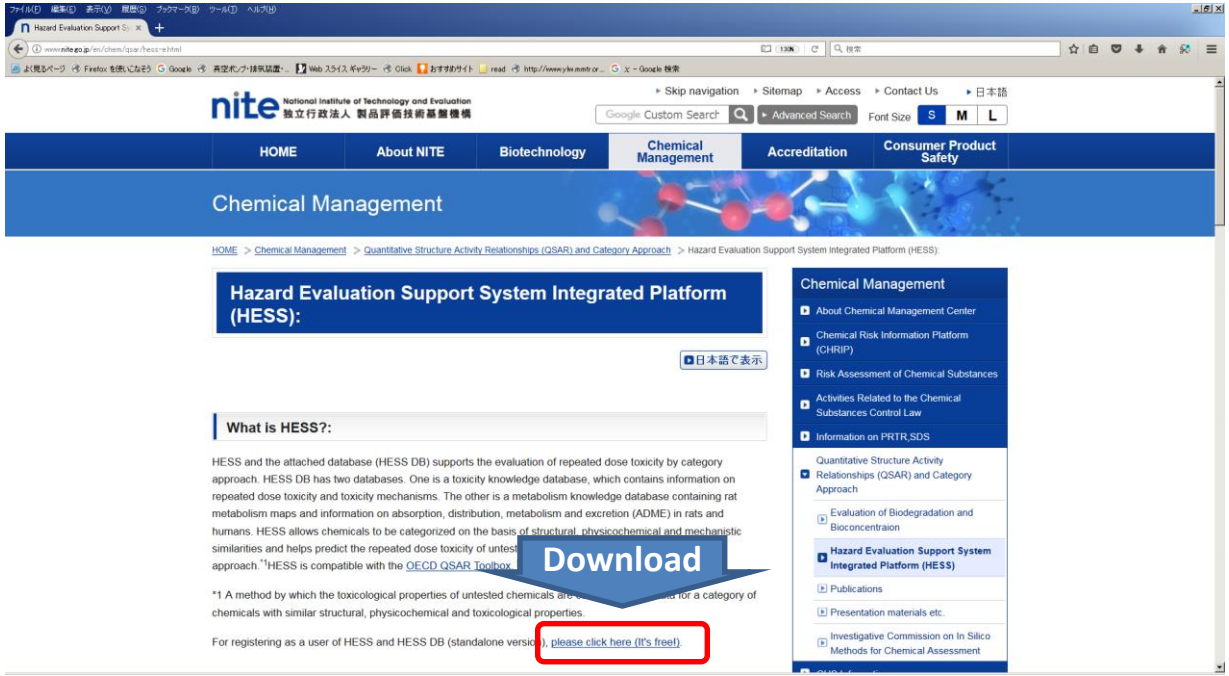


Human CYP2E1 model

*Yamazoe, Y. Ito, K. Yoshinari. K. 2011. Construction of a CYP2E1-template system for prediction of the metabolism on both site and preference order. Drug Metabol. Rev. 43: 409-439.

Download Site

http://www.nite.go.jp/en/chem/qsar/hess-e.html



If you have any questions on HESS, please don't hesitate to contact us (hess@nite.go.jp).

OECD QSAR Toolbox（containing HESS data and categories）

http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm

2) Read-across Rules for Bioaccumulation under CSCL

Alternative Testing Methods Allowed

Biodegradation Test (OECD301C; BOD≥60%) →

- **Read-across**

Bioconcentration Test (OECD305; BCF<5,000) →

- **Log P < 3.5**
- **Log D < 2.5 (for ionic substances)**
- **MW ≥ 800 (MW ≥ 1,000, for substances with two or more halogens)**
- **Read-across (based on specific rules)**

Read-across Rules for Bioaccumulation

- **The evaluation of read-across results under CSCL had previously been conducted case-by-case.**
- **Read-across rules for bioaccumulation were defined in 2013 to clarify the criteria for the acceptable read-across results**

Rule 1: Based on structural similarity including QSAR predictions

Rule 2: Based on hydrophilicity data by HPLC

http://www.meti.go.jp/policy/chemical_management/english/files/laws/bioaccumulation_analog_approach.pdf

Rule 1: Description

If Chemical A meets the following criteria, Chemical A may be judged to be **"not highly bioaccumulative"**:

- Chemical A and B are similar in structure to each other:
  - Chemical A has the same basic skeleton as Chemical B, or
  - Chemical A is an isomer of Chemical B.
- Measured BCF of Chemical B is less than 500.
- The bioaccumulation of Chemical A is estimated to be almost equal to or lower than Chemical B based on their chemical structure.
  i. The estimated BCF by QSAR* for Chemical A is almost equal to or lower than both the measured and estimated BCF for Chemical B.
  ii. There exist two or more structurally similar substances whose measured BCF are less than 100.

* BCFBAF (EPI SUITE) or BCF base-line model (OASIS CATALOGIC).

Rule 1: Summary

Case i)

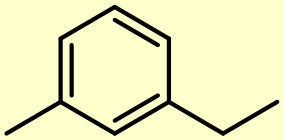
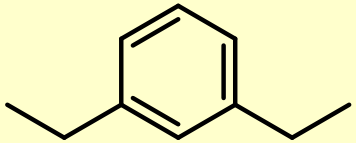
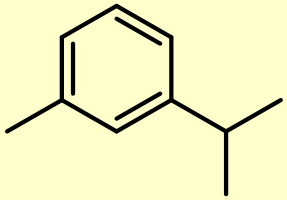
	New Chemical A (Target)	Chemical B (Source)
Structure	Similar (Same basic skeleton or isomer)	
Measured BCF	?	<500
Estimated BCF (QSAR)	$BCF_A < BCF_B$	

Case ii)

	New Chemical A (Target)	Chemical B_1 (Source)	Chemical B_2 (Source)
Structure	Similar (Same basic skeleton or isomer)		
Measured BCF	?	<100	<100

Rule 1: Example

Case i)

	New Chemical A (Target)	Chemical B_1 (Source)	Chemical B_2 (Source)
Structure			
Measured BCF	?	481	485
Predicted BCF (BCFBAF)	196	481	433

Rule 2: Background

-Evaluation of Biodegradation Products Under CSCL-

The chemical structure of stable biodegradation products (≥ 1 w%) in biodegradation tests need to be identified as possible.

Following tests such as a bioconcentration test need to be conducted for all the stable biodegradation products.

Extra Costs for Industry

Rule 2: Description

If the substance of concern (Chemical A) has a similar structure to Chemical B* whose bioaccumulation is known and further, if it is observed that Chemical A is more hydrophilic (polar) than Chemical B by reverse-phase HPLC, only when Chemical B is not highly bioaccumulative**and has a certain level (or higher level) of hydrophilicity, Chemical A may be assessed to be **"not highly bioaccumulative"**.

* Chemical A and B are similar in structure to each other:
  - Chemical A has the same basic skeleton as Chemical B, or
  - Chemical A is an isomer of Chemical B.

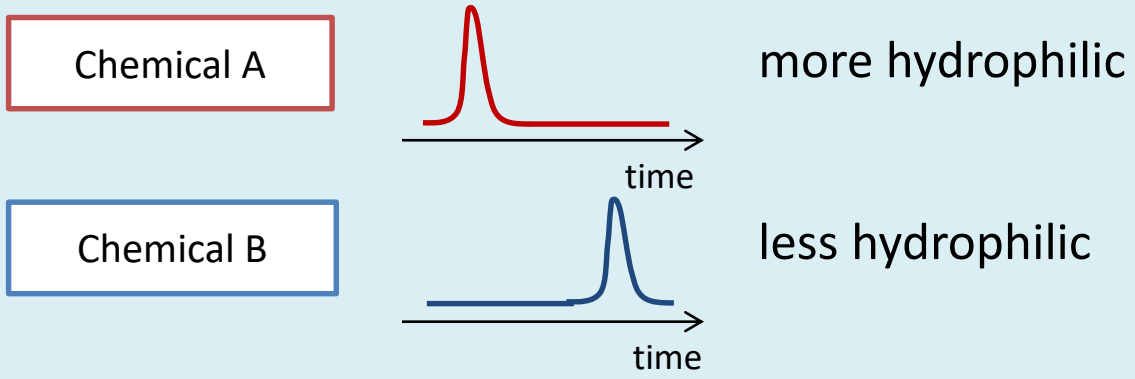
**Measured BCF of Chemical B is less than 500.

This analogous method does not apply to surfactants, organic metallic compounds, low purity compound and inorganic compound.

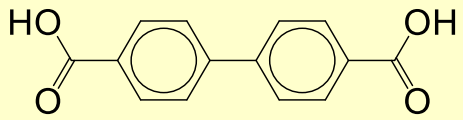
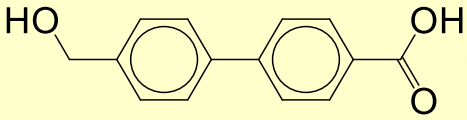
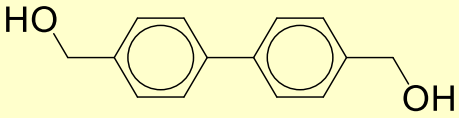
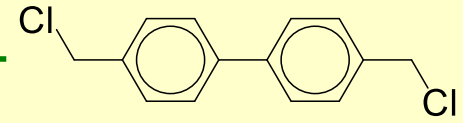
Rule 2: Summary

	New Chemical A (Target)	Chemical B (Source)
Structure	Similar (Same basic skeleton or isomer)	
Measured BCF	?	<500
Hydrophilicity (HPLC)	$Hydrophilicity_A$ > $Hydrophilicity_B$	

Comparison of Hydrophilicity by reversed-phase HPLC

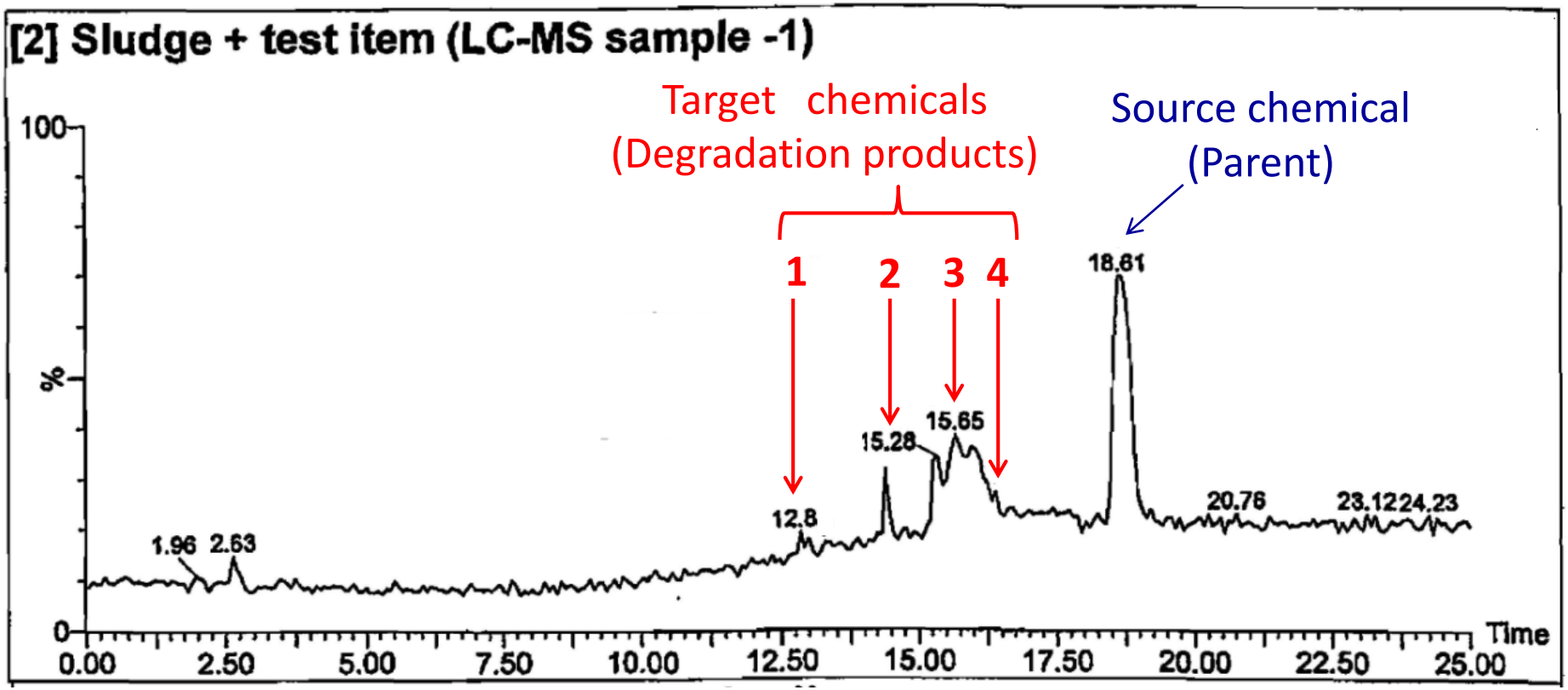


Rule 2: Example

	New Chemical A (Target)	Chemical B (Source)
Structure	1  2  3  4 **Unidentified Structure**	*Degradation Prodcuts* 
Measured BCF	?	<48
Hydrophilicity (HPLC)	$Hydrophilicity_{A1-4} > Hydrophilicity_{B}$	

* OECD Series on Testing and Assessment No.254: Case Study on the Use of an Integrated Approach for Testing and Assessment of the Bioaccumulation Potential of Degradation Products of 4,4'-Bis(Chloromethyl)-1,1'-Biphenyl ENV/JM/MONO(2016)52.

Comparison of Hydrophilicity by HPLC



Considerations from our Review Experience of the Read-across Results of New Chemicals Submitted to CSCL

- **Read-across rules for the evaluation of bioconcentration facilitate the use of read-across in the notification of new chemicals.**
- **Reduction of uncertainties by additional support evidence can extend the applicability of read-across (e.g. using IATA approach).**
- **Skill levels on read-across (e.g. selection of appropriate source chemicals) vary by companies. (NITE supports industry to master read-across.)**