Promotion and Issues of the NAMs Approach in Food Risk Assessment in Japan

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What is NAM (New Approach Methods)?

In chemical safety assessment methods, especially in laboratory animal alternative methods, NAM stands for new approach methods and refers to the strategy of evaluating the hazard or risk assessment of a chemical with an emerging technology, methodology, approach, or combination of alternative methods when it is not possible to replace them with only one alternative method compared to animal testing.

- *In vitro* methods: *in vitro* testing with cells and tissues
- *In chemico* methods: methods for measuring chemical reactions, molecular interactions, and physicochemical properties
- *In silico* methods: methods for assessing and testing with mathematical models and computer simulations

What kind of Technology are used as a NAM Approach?

- *In vitro* methods: Cellular toxicity assay, Cell transformation assay, Cell proliferation assay, Cytokine release assay, Nuclear receptor (anti-)activation assay, MPS (Micro-physiological system), Organoid, etc.
- *In chemico* methods: Protein-binding assay, Enzyme activity assay, Direct peptide reaction assay, measuring physicochemical property, etc.
- *In silico* methods: TTC (Threshold of Toxicological Concern) approach, QSAR (Quantitative Structure Activity Relationship), PBPK (Physiologically based pharmacokinetic) model, IVIVE (*in vitro* to *in vivo* extrapolation), Omics (Toxicogenomics, Proteomics, metabolomics, etc.) data mining, AI-based prediction, etc.

Outline of the Presentation

- Current status of risk assessment by the Food Safety Commission
- Initiatives in technical research on food health impact assessment
- Issues in applying of NAM to risk assessment in Japan
- Necessity of cooperation within Japan and with overseas

NAM-related Approaches Implemented in "FSCJ Guidelines" for the Risk Assessment

- **Food additives**
  - Allergenicity: *in vitro* OECD guideline studies for Integrated Approaches to Testing and Assessment (IATA) of allergenicity
  - Processing aids : TTC-based tiered assessment
  - Enzymes : *in chemico* studies for physicochemical stability tests and IgE-binding activity; *in silico* method for homology search of amino acid sequence for allergenicity assessment
- **Flavoring substances** : QSAR prediction for genotoxicity and TTC approach on general toxicity for the tiered assessment
- **Food contact materials** : TTC-based tiered assessment

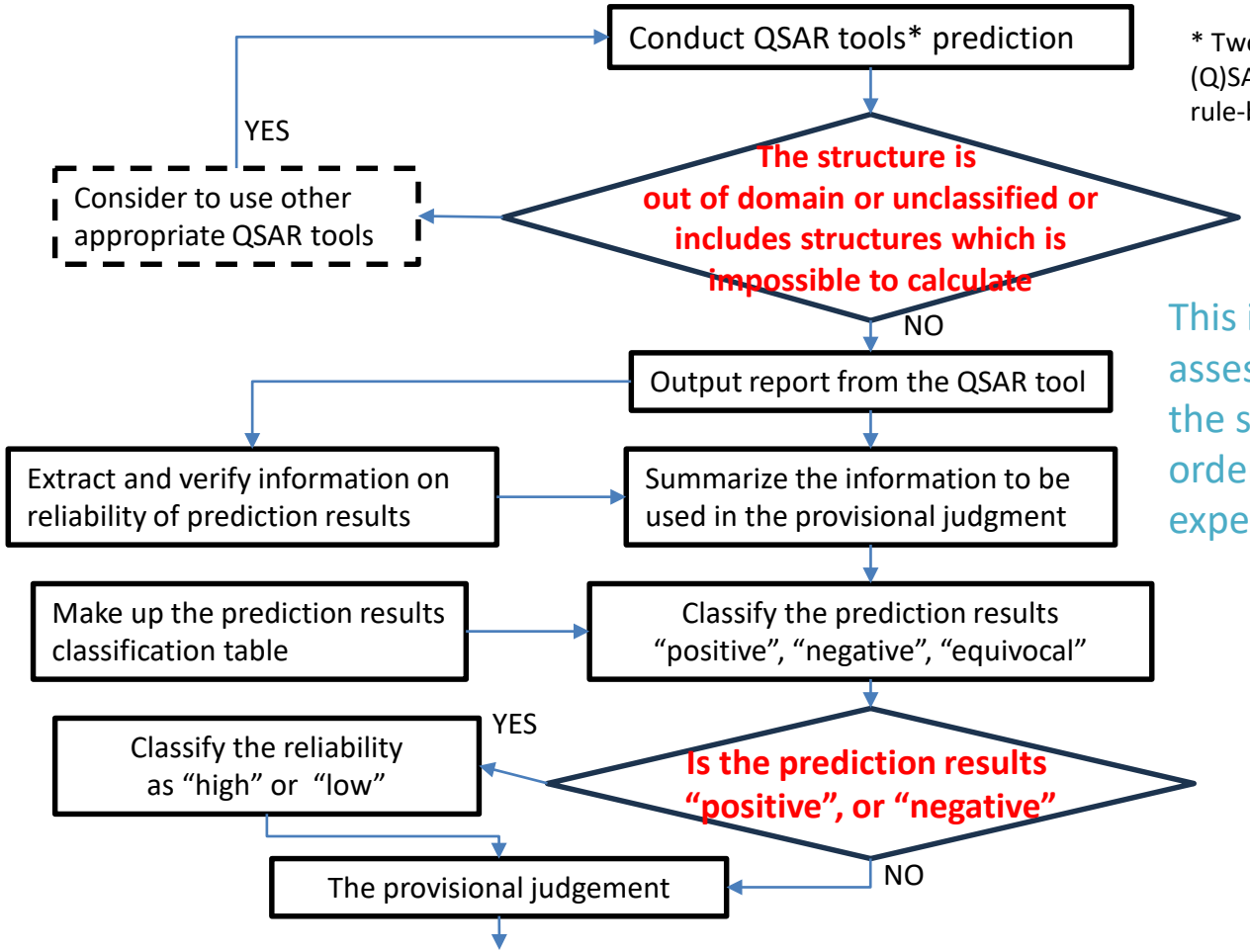
EXAMPLE:
TTC for Assessment of Processing Aids

Estimated intake class		Required tests
Class a	90 µg/human/day or less	• Genotoxicity
Class b	more than 90 µg/human/day, 2,000 µg/human/day or less	• Genotoxicity • Sub-chronic toxicity*
Class c	more than 2,000 µg/human/day	• ADME • Genotoxicity • Repeated dose toxicity • Carcinogenicity • Reproductive toxicity • Developmental toxicity • Allergenicity

*in principle, repeated dose toxicity tests for 90 days

Guidance for QSAR Application to Genotoxicty Evaluation

Developed by assessment technology WG in 2021



This is a part of assessment flow for the secretariate in order to assist the expert judgment

Initiatives of NAM Application in Research and Survey Projects Funded by FSCJ

The FSCJ has implemented “Research Grant Program for Risk Assessment on Food Safety” and “Survey Program for Collecting Data on Food Safety,” in order to support research and survey for accumulating the scientific findings and knowledge, and developing and improving risk assessment methodologies.

Some of research projects had directly contributed to establish or amend the current guidelines or guidances

ex. Study on migration test in risk assessment for synthetic resin for apparatus, containers and packaging*. (FY2017-2019) ⇒ “Guidelines for the Risk Assessment of Food Apparatus, Containers, and Packaging*” (May 2019) and its revision (October 2020)

But only use of TTC approach and genotoxicity QSAR prediction

* apparatus, containers and packaging = food contact materials

Recent NAM-related Research Projects

The below research project results are still in research phase

- (FY2016-2017) Construction of the database of *in vivo* toxicity tests and its application to the *in silico* prediction and evaluation of *in vivo* toxicity
- (FY2018-2019) Development of new evaluation support technology: Examination of database utilization method for toxicity prediction
- (FY2020-2021) Research for refinement of prediction approach of hepatotoxicity by introducing *in silico* methods
- (FY2019-2022) Study on risk assessment methods of metabolites from pesticide residues
- (FY2023-2024) Research for the application and reliability of the read-across assessment of food-related chemicals

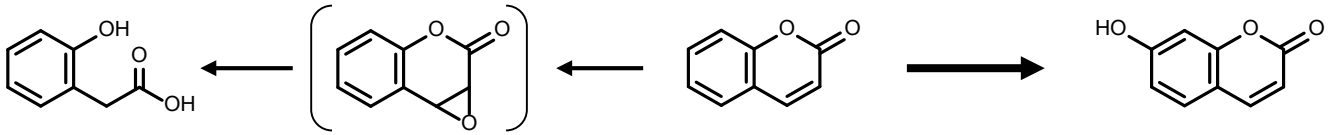
Prediction of Hepatotoxicity by Using *in silico* Methods

- **Case study of integrated risk assessment of coumarin in Food**

Existing Data on coumarin
- Clinical and animal studies
- ADME

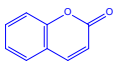
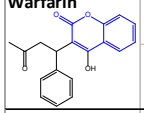
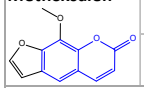
Data of *in silico* models
- Human PBPK model
- FDA DILI score model

Combined and assessed

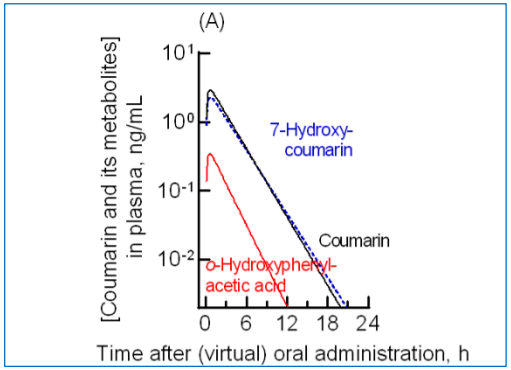


o-HPA (active metabolite) — Coumarin — 7-Hydroxycoumarin

DILI Prediction model by NCTR/FDA

Chemicals	Daily Dose (mg/day)	logP	RM formation	DILI score	DILI risk (estimated)	DILI (observed)
Coumarin 	2.5	1.39	Yes (1)	3.71	Moderate	(+)
	25			5.11	Moderate	
Warfarin 	2	2.44	No (0)	0.98	Low	-
	10			1.95	Low	
Methoxsalen 	3	1.93	Yes (1)	3.92	Moderate	+
	40			5.51	Moderate	

TK Prediction in human

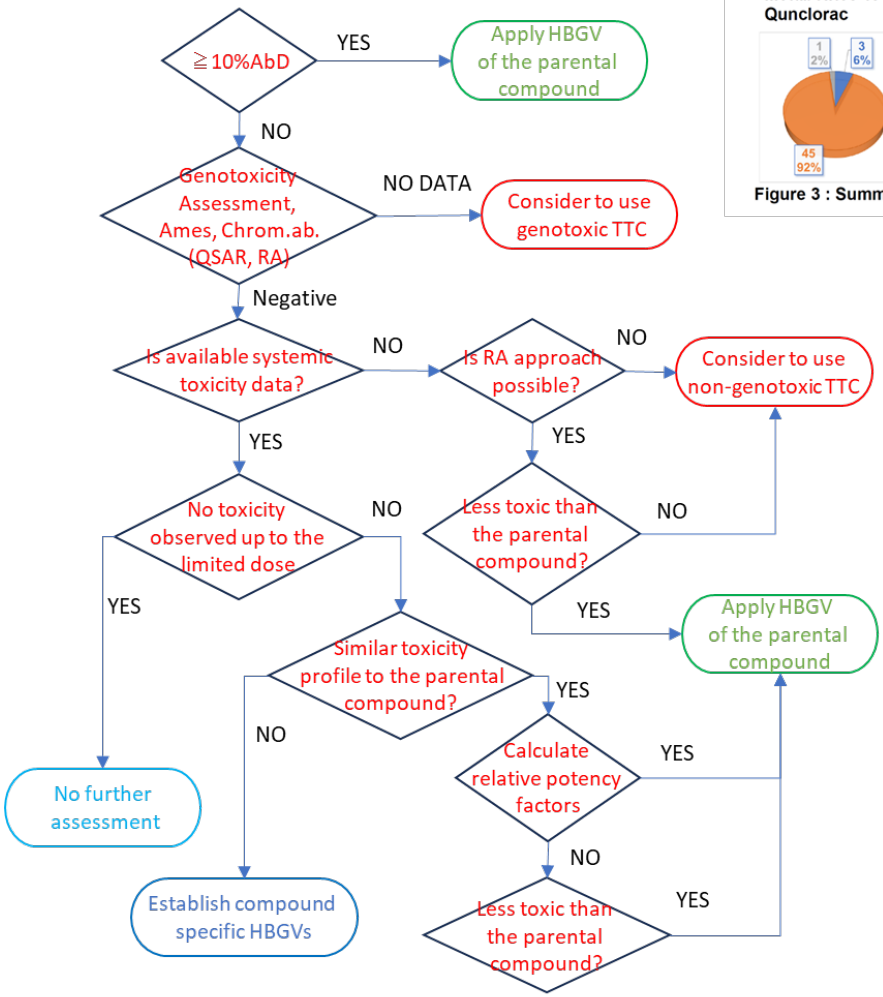


Qualitative assessment + **Quantitative assessment**

<data provided by Dr. Takashi Yamada>

Evaluation of *in silico* Mutagenicity Prediction and TTC approach for Pesticides and Their Metabolites

Analysis total 416 metabolites of 15 pesticides



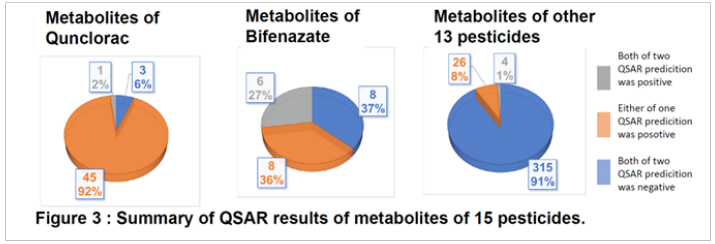


Figure 3 : Summary of QSAR results of metabolites of 15 pesticides.

↑ Only a few metabolites were predicted as positive in 13 pesticides. But the large number of metabolites of quinclolac and bifenazate were predicted as positive. All of quinclolac and many of bifenazate metabolites have same structural alert with parent compounds. This may lead to false positive prediction.

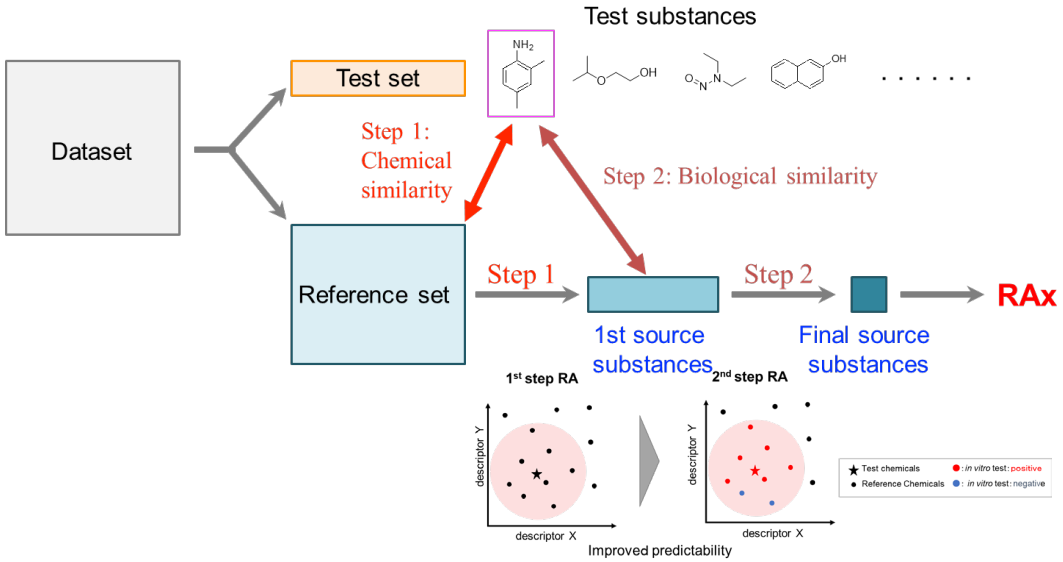
<= Propose the assessment scheme of major residues by using QSAR and Read across approach combined with TTC threshold.

Determine the presence or absence of a genotoxicity alert for the parent pesticides substance and assess the system toxicity compared with the parent substance by using read across (RA) approach.

<data provided by Dr. Atsushi Ono>

In silico Approach for Hepatoxicity Read-across Prediction by Using *in vitro* Data

Development of an objective read-across method for evaluation of systemic toxicity and carcinogenicity



Association between tumors and in vitro assay results

Assay	Liver	Thyroid	Testis	Uterus	Ovary	Breast	Nasal cavity	Stomach	Bladder/ urethra	Cytotoxic
LDH	1.0000	0.8421	1.0000	1.0000	0.5006	1.0000	0.1748	0.0904	0.2428	0.0146
CellTiter	1.0000	0.1570	1.0000	1.0000	0.5087	0.5087	0.6491	0.0818	0.1303	0.0292
GSH	1.0000	1.0000	0.5570	1.0000	1.0000	0.5246	0.0692	0.0296	0.7078	0.1667
AHR	1.0000	0.7716	0.0015	0.5955	1.0000	1.0000	1.0000	0.5846	0.0492	0.2261
PXR	0.6832	0.0073	1.0000	1.0000	0.5130	1.0000	1.0000	1.0000	0.6995	1.0000
PPARα	1.0000	1.0000	0.0395	0.5957	1.0000	1.0000	0.0960	1.0000	1.0000	1.0000
RXRα	0.1122	0.6815	0.5926	1.0000	1.0000	1.0000	1.0000	1.0000	0.3756	1.0000

<data provided by Dr. Kouichi Yoshinari>

Issues in Applying of NAM to Risk Assessment in Japan

- Only TTC and Ames QSAR are administratively accepted. Application of read-across (RA) is also recommended, but expert involvement is required.
- Some studies have shown the usefulness of other techniques (PBPK, genomics, and *in vitro* data). However, application examples are limited and cannot be generalized yet at present.
- There are many similarity metrics underlying read-across, and there is no standard RA approach.
- Resources or researcher are very limited (especially in Japan).

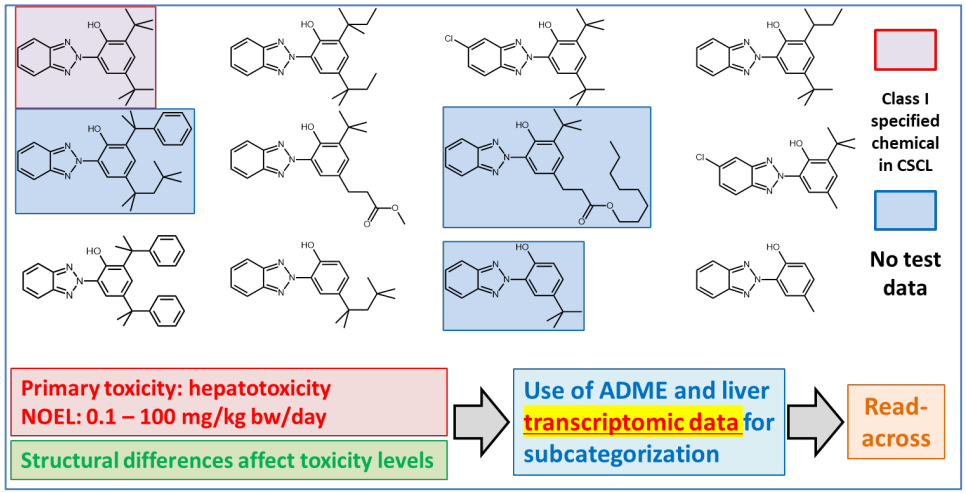
Necessity of Cooperation Within Japan and With Overseas

Other agencies or ministries than FSCJ in Japan have been conducting research projects for safety evaluation of chemicals or medicine by using NAM based approach. The below are some examples.

- IATA case project (MHLW-NIHS)
- AI-SHIPS project (METI)
- AMED-MPS project (AMED)
- Tox-GAN projects (US FDA) (learning data of AI used “OPEN TG-Gates” published by MHLW-NIHS)

Toxicogenomic data to support read-across assessment of hepatotoxicity

OECD IATA case studies project (2nd review cycle, 2016)



Toxicogenomic data from NIHS

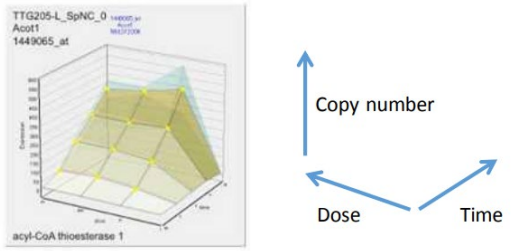
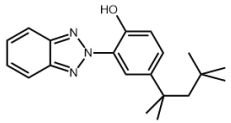
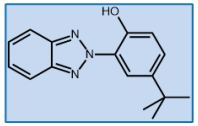
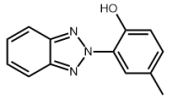


Figure 2. Three-dimensional surface expression of Percellome data

Read-across

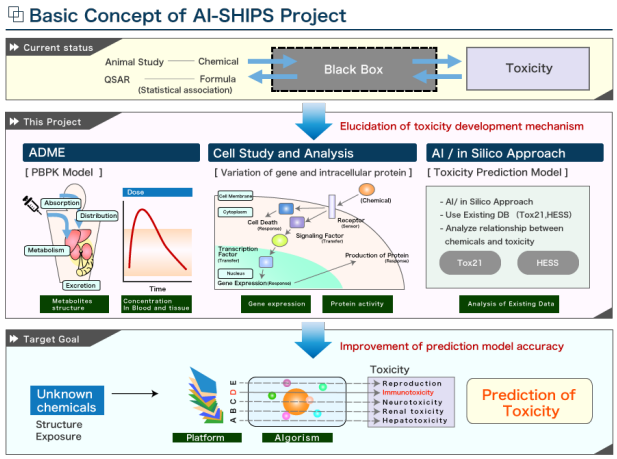
	 Member 7	 Member 12	 Member 6
Repeated-dose toxicity	NOAEL=5658 mg/kg bw/day No effects 30-day, Feeding (1968), Non-GLP	Read-across ←	NOEL=<30 mg/kg bw/day Liver; weight increase, hypertrophy (30 mg/kg bw/day in male, 42-day, Gavage (2007), GLP
Transcriptome	CAR: Cyp2, PPAR: Cyp4	Not available	Nrf2-Phase II

Repeated-Dose Toxicity of Phenolic Benzotriazoles (Japan)

Development for Application of NAM to Safety Evaluation by METI* and AMED**

* The Ministry of Economy, Trade and Industry
** The Japanese Agency for Medical Research and Development

- AI-based Substances Hazard Integrated Prediction System (started in 2017 to 2022)

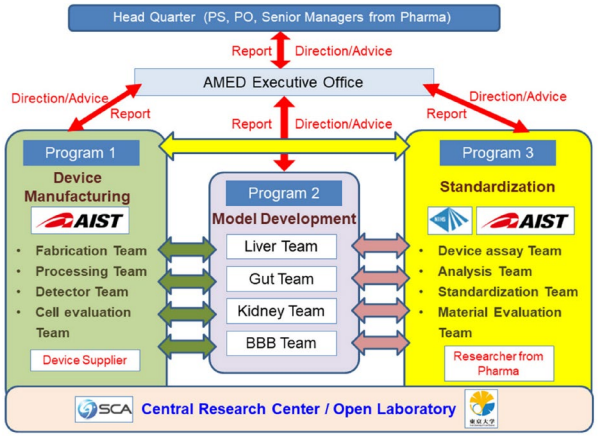


The system was originally developed for chemical registration evaluation under the METI* project. About 46 *in vitro* assays have been performed on about 326 industrial chemicals. Model building has been completed and is being prepared for public use.

(AI-SHIPS Web page : http://www-dsc.naist.jp/ai-ships/en/project/)

- AMED**-MPS project (launched in 2017)

The Project focused on developing key evaluation technology of MPS. Research project for practical application of regenerative medicine. Research on development of new drug.

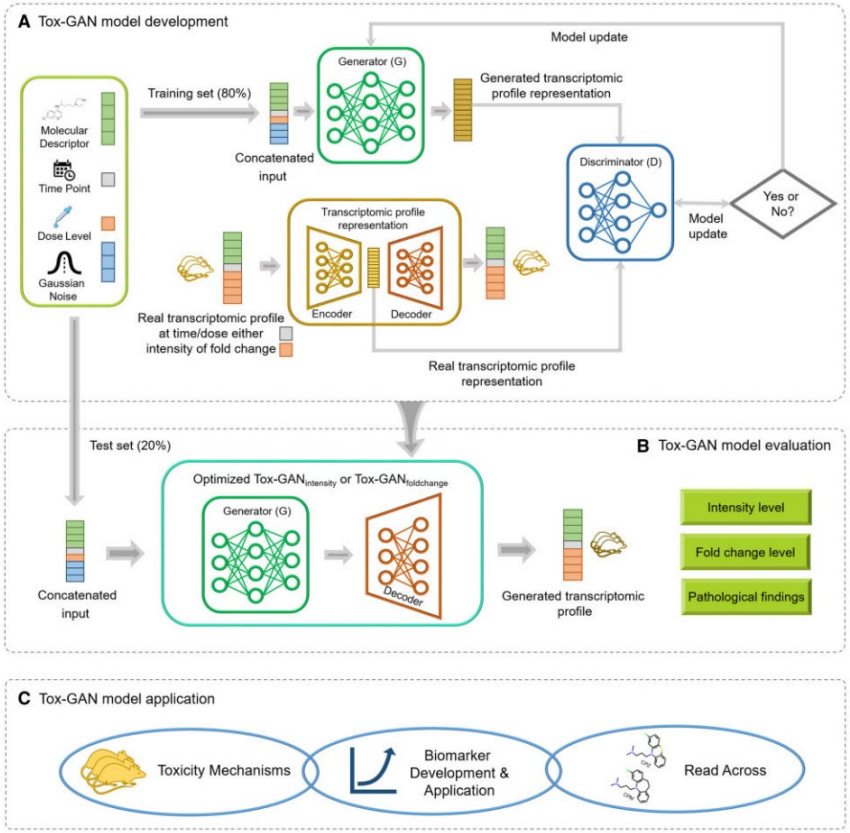


(Seiichi Ishida, Research and Development of Microphysiological Systems in Japan Supported by the AMED-MPS Project. Front Toxicol. 2021; *3*: 657765.)

AI Approach Alternative to Animal Studies (NCTR/FDA)

AnimalGAN program in AI4TOX (*AI Program for Toxicology at NCTR, US FDA*)

Tox-GAN : A Case Study with Toxicogenomics



Training
ToxGen data
(Open TG-GATES)
Provided by
NIHS/MHLW

Xi Chen et al., TOXICOLOGICAL SCIENCES, 186(2), 2022, 242–259

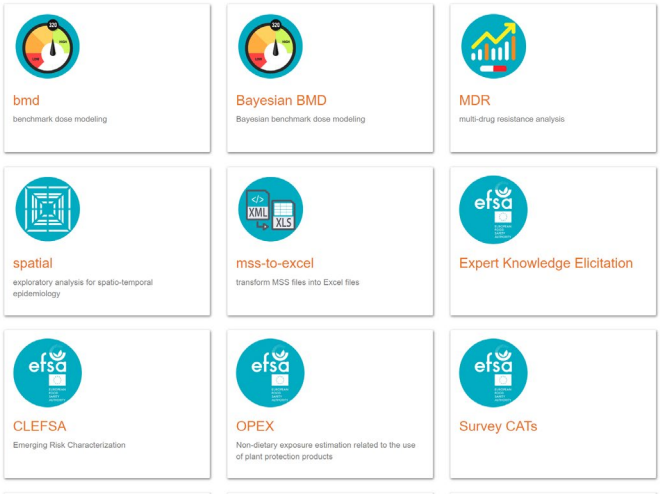
Necessity of Cooperation Within Japan and With Overseas

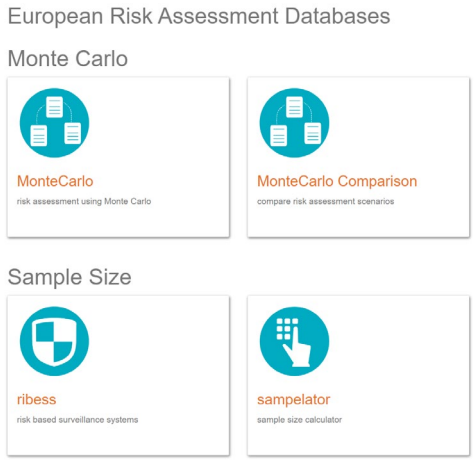
- In areas other than food area, projects and research related to the NAM have been conducted, but budgets and targeted substances differ. However, even in those projects, the technology has not yet been generalized for regulatory use, still challenging for improving accuracy and standardization.
- In the food sector (especially in Japan) , the main objective of risk assessment is focused on setting reference values (ADI, TDI, etc.) for risk management, and the hurdles to use the NAM approach are higher than in other areas because quantitative evaluation by NAM is required.
- Additionally, limitation for human and budgetary resources in the Food risk assessment and management area is an important problem.

Examples of *in silico* Tools Sites in US and EU

On the ICE site (right), in silico tools developed by U.S. assessment agencies, chemical properties prediction, in vitro data retrieval, PBPK and IVIVE are compiled in one place.
On the European Risk Assessment Database site (lower), tools for statistical analysis of dose response assessment and exposure assessment, and basic data on national exposure are compiled. Together, quantitative risk assessment predictions are made possible in one place in the EU and US.

https://ice.ntp.niehs.nih.gov/





https://r4eu.efsa.europa.eu/

Global Collaboration Needed?

- Even if NAM technology from other sectors can be introduced into the food sector, various *in silico* tools, especially high-quality quantitative prediction tools, need to be developed.
- More mechanistic data and AOPs will need to be accumulated to more accurately predict complex biological responses. This may require sharing the research burden internationally.
- In the future, it is necessary to develop robust AI-based prediction systems based on the above accumulated data and developed tools.

Thank you for your attention.

Acknowledgements:

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