Issues in and Ideas for facilitating application of *in silico* method

July 2016

Investigative Commission on *In Silico* Methods for Chemical Assessment

Table of contents

1. About the Investigative Commission on *In silico* Methods for Chemical Assessment

1.1 Purpose of the Commission

In attaining the 2020[1] goal of the World Summit on Sustainable Development on ensuring chemical safety around the world, a major challenge is to assess the safety of the vast number of chemicals lacking experimental data. To address this issue, regulatory authorities of various countries have vigorously promoted the development and application of *in silico* methods. For instance, in Europe, the application range of *in silico* methods is planned to be significantly expanded within a few years, along with the shift of the main target of assessment to low production volume chemicals under the Regulation concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). Moreover, for the sake of animal welfare, the trend towards the reduction or elimination of animal testing has rapidly grown over the last few years, which has led to the full ban on the marketing of cosmetic products and materials tested on animals in 2013.

Along with such regulatory trends, it has become more and more important to improve the technical levels of the existing *in silico* methods and expand their application range. The United States and European countries, as well as international organizations such as OECD, are currently proceeding with large projects on technical development of *in silico* methods.

Japan has also implemented several projects on developing *in silico* methods using the data obtained under the Japanese Chemical Substance Control Law (CSCL), and their outcomes are partially applied to the operation of the CSCL, but only to a limited extent. Currently, our country is far behind the international movement mentioned above and is urgently required to take effective measures for improving the utilization of *in silico* methods in domestic regulation of chemical substance management.

Under these circumstances, "Investigative Commission on *in Silico* Methods for Chemical Assessment" has been organized by the National Institute of Technology and Evaluation (hereinafter referred to as NITE). Experts from relevant industries and academic research institutes, as well as government officials, gathered to review the domestic and foreign research trends on *in silico* methods and their status of application, identify the technical and operational obstacles to effective utilization of *in silico* methods in regulation of chemical substances management in Japan and had an open-minded discussion to develop possible measures to address these obstacles and facilitate the application of *in silico* methods. This document provides a summary of the discussion.

1.2 Subjects for discussion

The Commission reviewed and discussed the following subjects:

- Current status of utilization of *in silico* methods in regulation of chemical substance management in Japan and other countries
- Trend of research and development of *in silico* techniques in relevant areas
- Measures for facilitating utilization of *in silico* methods in regulation of chemical substance management in Japan

[1] World Summit on Sustainable Development, "chemicals are produced and used in ways that minimize significant adverse effects on human health and the environment by 2020"

- Specific applications of *in silico* methods in regulation of chemical substance management in Japan
- Research and development subjects required in future on *in silico* methods
- Framework for utilizing *in silico* methods in regulation of chemical substance management in Japan
- Others

* The Commission uses the term "*in silico* methods" to describe a wide array of assessment approaches, including not only computational simulation techniques but also computational toxicology and computational chemistry that utilize the existing *in vitro* and *in vivo* data or implicit knowledge, such as:

- category approach
- TTC (Threshold of Toxicological Concern)
- ADME (Adsorption, Distribution, Metabolism, Elimination) prediction
- mechanism-based toxicity prediction in humans
- integration of data/information, systematization of knowledge including implicit knowledge
- Quantitative Structure-Activity Relationship (QSAR)

1.3 Participants of the Meetings (1st – 6th)

[Commission members]

Makoto Hayashi (Chairman)	President Emeritus, Public Interest Incorporated Foundation Biosafety Research Center
Fusae Harada	Director of Human & Environmental Safety Evaluation Center, Research & Development Headquarters, Lion Corporation
Nozomu Hatakeyama	Associate Professor, New Industry Creation Hatchery Center, Tohoku University
Toshihide Hida	Director, Chemical Safety Office, Chemical Management Policy Division, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry (6th meeting)
Akihiko Hirose	Director, Division of Risk Assessment, National Institute of Health Sciences
Masamitsu Honma	Director, Division of Genetics and Mutagenesis, National Institute of Health Sciences
Toshio Kasamatsu	Senior Principal Research Scientist, R&D Core Technology – Safety Science, Kao Corporation
Akira Miyamoto	Professor, New Industry Creation Hatchery Center, Tohoku University
Fumiaki Shono	Executive Director, Japan Chemical Industry Association
Hideaki Tanaka	Director, Chemical Safety Office, Chemical Management Policy Division, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry (METI) (1st – 5th meetings)
Itaru Yasui	Honorary Advisor, National Institute of Technology and Evaluation

[Observers]

Tatsuya Mizukoshi	General Manager, Chemicals Management Department, Japan Chemical Industry Association
Kazuhiro Kaneko	General Manager, Chemicals Management Department, Japan Chemical Industry Association
Masanori Imamura	Deputy Director, Chemical Safety Office, Chemical Management Policy Division, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry
Akira Ohkubo	Deputy Director, Chemical Safety Office, Chemical Management Policy Division, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry
Naohito Otaki	Assistant Director, Chemical Risk Assessment Office, Chemical Management Policy Division, Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry
Takehiko Fukushima	Director, Chemicals Evaluation Office, Policy Planning Division, Environmental Health Department, Environmental Policy Bureau, Ministry of the Environment
Ryosuke Takahashi	Deputy Director, Chemicals Evaluation Office, Policy Planning Division, Environmental Health Department, Environmental Policy Bureau, Ministry of the Environment
Kei Sasahara	Deputy Director, Chemicals Evaluation Office, Policy Planning Division, Environmental Health Department, Environmental Policy Bureau, Ministry of the Environment
Yosuke Takasaki	Director for Risk Assessment Coordination, Food Safety Commission Secretariat, Cabinet Office

[Secretariat]

Yasuo Kii	Director-General, Chemical Management Center, National Institute of Technology and Evaluation
Takashi Fukushima	Deputy Director-General, Chemical Management Center, National Institute of Technology and Evaluation
Mariko Murata	Director, Chemical Management Center, National Institute of Technology and Evaluation
Chie Hamaguchi	Director, Safety Assessment Division, Chemical Management Center, National Institute of Technology and Evaluation
Ruriko Nakamura	Senior Chief, Data Analysis Division, Chemical Management Center, National Institute of Technology and Evaluation
Yuki Sakuratani	Chief, Safety Assessment Division, Chemical Management Center, National Institute of Technology and Evaluation
Takashi Yamada	Chief, Safety Assessment Division, Chemical Management Center, National Institute of Technology and Evaluation
Yutaka Ikenaga	Chief, Safety Assessment Division, Chemical Management Center, National Institute of Technology and Evaluation
Tomoko Aoyagi	Chief, Safety Assessment Division, Chemical Management Center, National Institute of Technology and Evaluation
Yuri Zaitsu	Staff, Safety Assessment Division, Chemical Management Center, National Institute of Technology and Evaluation

Takaaki Yamaguchi	Technical Staff, Chemical Management Center, National Institute of Technology and Evaluation
Kenichiro Suzuki	Research Staff, Genotoxicity Laboratory, Public Interest Incorporated Foundation Biosafety Research Center

1.4 Schedule and progress of the meetings

The Investigative Commission held six meetings in total over two years from FY2014 to FY2015. In the 1st meeting, subjects to be discussed and plans for the meetings were confirmed.

From the 2nd to 5th meetings, topics related to the subjects of discussion listed above in 1.2 were presented by each Commission member and some observers and were discussed by the participants.

Then, additional hearings were conducted on each Commission member by NITE, which were also included in the summary discussion at the 6th meeting.

[Investigative Commission meeting schedule]

1st July 15, 2014 (Tue)
2nd November 7, 2014 (Fri)
3rd February 25, 2015 (Wed)
4th June 8, 2015 (Mon)
5th July 8, 2015 (Wed)
6th February 16, 2016 (Tue)

2. Outline of investigation by the Commission

2.1 Current status of application of *in silico* methods in regulation of chemical substance management in Japan and other countries

2.1.1 Current status of application to review of new chemical substances under the CSCL

In the hazard assessment of new chemical substances, on the condition that rationality of the assessment is ensured, *in silico* methods have been actively utilized, particularly in bioaccumulation assessment.

1) Biodegradability and bioaccumulation assessments based on read-across approaches are conducted on a case-by-case basis, and criteria for using read-across in bioaccumulation assessment has been consulted to be stipulated (Fig. 1).

Guidance for Bioaccumulation Assessment by using QSAR

1. Bioaccumulation Assessment by using QSAR and Read-Across

If chemical A meets following criteria, chemical A can be assessed to be not highly bioaccumulative:

(1) Chemical A is similar in structure to Chemical B (specifically as follows):
- i. Chemical A has the same basic skeleton as Chemical B and chemical A's structure is partially changed from compound B, or
- ii. Chemical A is an isomer of Chemical B.

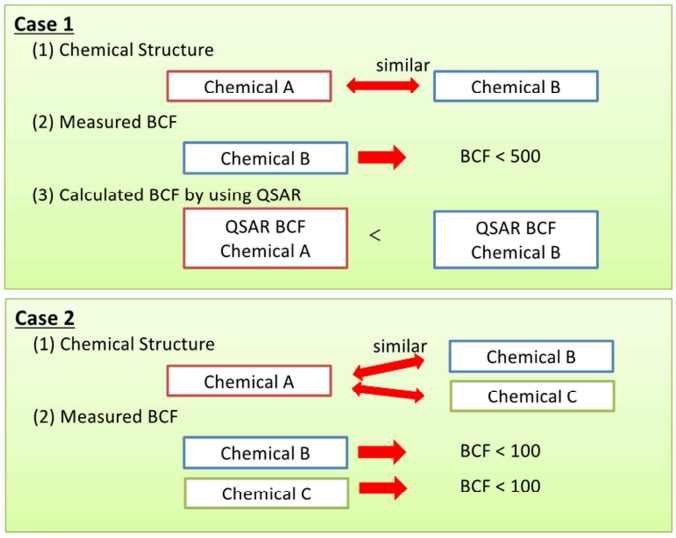
(2) Measured BCF (bioconcentration factor) of chemical B < 500.

(3) Bioaccumulation of chemical A is estimated in a rational way to be almost the same as or lower than chemical B based on their chemical structure. (specifically as follows)
- i. Calculated BCF by using QSAR of chemical A is almost the same as or lower than measured and calculated BCF of chemical B.
- ii. Two or more similar chemical B have measured BCF <100.

※ Recommended QSAR model is either BCFBAF (EPI SUITE) or BCF base-line model (OASIS Catalogic).
※ Japan added the published measured BCF data on the website in Sep. this year in order to facilitate the above approach. Following NITE website is very useful to search measured BCF data because it includes how to use it by OECD QSAR toolbox. (both in Japanese…)
http://www.nite.go.jp/chem/qsar/bunchiku_qsar.html

29

Example (1)



Chemical A is not highly bioaccumulative in both cases.

30

Fig. 1 Read-across-based bioaccumulation assessment of new chemical substances under the CSCL [2]

[2] Recent Progress of Chemical Substances Control Law (CSCL) (FY2014) (Chemical Safety Office Chemical Management Policy Division Ministry of Economy, Trade and Industry (METI), December 2015) http://www.meti.go.jp/policy/chemical_management/english/cscl/files/about/02Progres.pdf

2) For accumulating knowledge towards further utilization of *in silico* methods, QSARs concerning biodegradability, bioaccumulation, eco-toxicity and genotoxicity are calculated for new chemical substances, which are the target substances to be reviewed under the CSCL, and submitted for reference to the Review Committee of the Chemical Substances Council.

3) For the purpose of facilitating data utilization to chemical assessment based on *in silico* methods and reducing redundancy of testing on identical substances, test results used for hazard assessments of the published new chemical substances and their assessment results are sequentially disclosed.

2.1.2 Current status of utilization to risk assessment of Existing Chemical Substances under the CSCL

Data used in the screening assessment or risk assessment (Primary) Assessment I must meet the following reliability criteria (Table 1).

1) Physico-chemical properties, biodegradability, bioconcentration data (METI)[3] must be either: (i) test data[4] conducted by internationally accepted testing methods or those specified in the CSCL (including estimation methods), or (ii) test data[5] that have been reviewed or are regarded as being reviewed by experts. When test data meeting criteria (i) or (ii) is not available, data gap filling based on QSAR models (EPI Suite) or read-across is accepted for the endpoints provided in Table 1.

[3] Regarding the reliability assessment etc. of data concerning physico-chemical property, biodegradability and bioconcentration under the Japanese Chemical Substances Control Act (September 15, 2011) http://www.meti.go.jp/policy/chemical_management/kasinhou/files/information/ra/reliability_criteria02.pdf

[4] Obtained in compliance with testing guidelines of the Japanese Chemical Substances Control Act or OECD test guidelines and in compliance with GLP

[5] Obtained in compliance with testing guidelines of the Japanese Chemical Substances Control Act or OECD test guidelines but not in compliance with GLP or its compliance status is unknown

Table 1 Endpoints for which the use of QSAR or read-across is approved in the screening assessment or risk assessment (Primary) Assessment I

Endpoint	Estimation method (QSAR)	Acceptance of read-across
Melting point	MPBPVP (EPI Suite)	–
Boiling point	MPBPVP (EPI Suite)	–
Vapor pressure	MPBPVP (EPI Suite)	–
Solubility in water	WSKOW (EPI Suite)	–
Soil adsorption coefficient normalized to organic carbon (Koc)	KOCWIN (EPI Suite)	–
Partition coefficient between 1-octanol and water (logPow)	KOWWIN (EPI Suite)	–
Henry constant	HENRYWIN (EPI Suite)	–
Biodegradability	BIOWIN3 (EPI Suite)	○
Bioconcentration factor (BCF)	BCFBAF (EPI Suite)	○ (only for not highly bioconcentrative substances.)

2) Hazard data concerning eco- toxicity (Ministry of the Environment)[6]: In principle, data should be obtained through testing. However, when this is difficult, the validity of the data can be judged by experts. In one case, application of QSAR prediction to fish acute toxicity assessment was discussed for a specific substance assigned "medium" priority by the screening assessment, but it was not put into practice as the structure of the substance to be assessed was outside the applicability domain of QSAR models. In FY 2015, the Ministry of the Environment has organized an expert committee to study the application status of *in silico* methods in foreign countries as well as to seek their practical application under the CSCL.

3) Hazard data concerning effects on human health (MHLW)[7]: Data should be obtained through testing. However, to promote efficiency and acceleration of the assessments, as well as to meet the social demands for reduction of animal testing, it is necessary to explore the extent to which the existing *in silico* methods can be applied.

For biodegradability, bioaccumulation and physico-chemical properties, estimation and read-across based on *in silico* methods have been utilized. Meanwhile, for effects on the ecosystem and human health, "Basic Concept of the Risk Assessment of Priority Assessment Chemical Substances (PACSs) under the Japanese Chemical Substances Control Low" states that it is an urgent task to explore the aspects of risk assessment in which these approaches can be utilized.

---

[6] Reliability assessment etc. of hazard data concerning ecological toxicity under the Japanese Chemical Substances Control Act (September 15, 2011)
http://www.meti.go.jp/policy/chemical_management/kasinhou/files/information/ra/reliability_criteria04.pdf

7 Reliability assessment etc. of hazard data concerning effects on human health under the Japanese Chemical Substances Control Act (September 15, 2011)
http://www.meti.go.jp/policy/chemical_management/kasinhou/files/information/ra/reliability_criteria03.pdf

2.1.3 Current status of utilization under the U.S. Toxic Substances Control Act

The Toxic Substances Control Act (TSCA) requires those who plan to manufacture or import any new chemical substances not listed on the Inventory for commercial purposes to seek approval by submitting pre-manufacture notification at least 90 days before initiating the activity. For performing risk assessment for these new chemical substances, the U.S. Environmental Protection Agency (EPA) has developed and released Estimation Programs Interface Suite (EPI Suite), a free QSAR software for estimating eco-toxicity and environmental fate (Fig. 2). Under the TSCA, substances to be added to the priority testing list are identified by the following two QSAR-based approaches.

1) Biodegradability and bioconcentration are estimated for individual substances to identify the candidate substances to be added to the testing list.

2) Substances are individually classified into chemical categories, and their eco-toxicity potentials are estimated to identify the candidate substances to be added to the testing list.

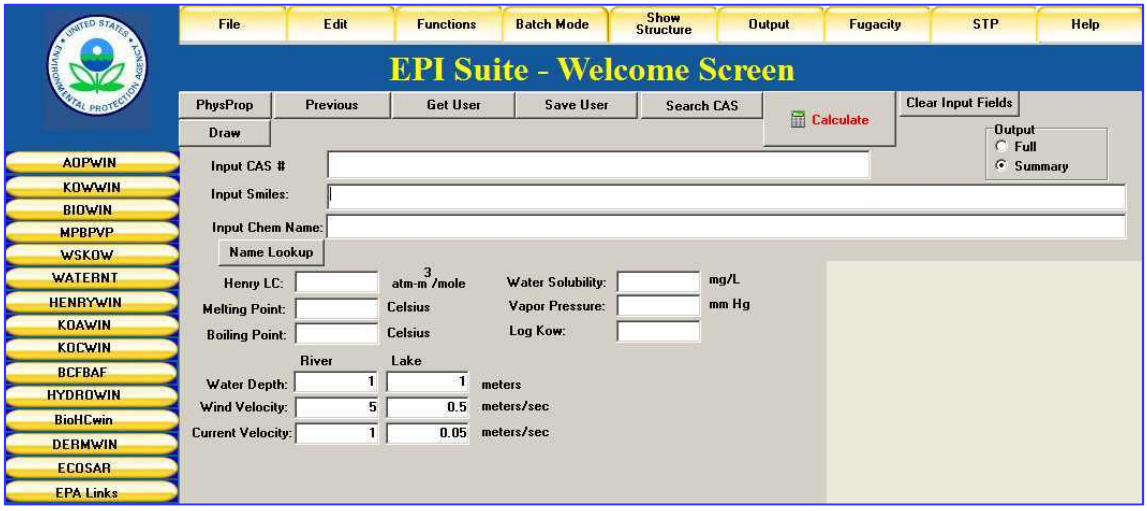


Fig. 2 Estimation Programs Interface Suite (EPI Suite[8]) developed by the U.S. EPA

2.1.4 Current status of application under EU REACH

In principle, REACH only permits animal testing as a last resort and allows companies to use various alternative testing methods to fulfill hazard information requirements for the registration. The following four methods are accepted as alternatives to animal testing:

1) Use of relevant information from analogous substances (grouping, read-across)

2) Weight-of-evidence approaches based on various information sources

3) *In vitro* tests using cells, parts or organs

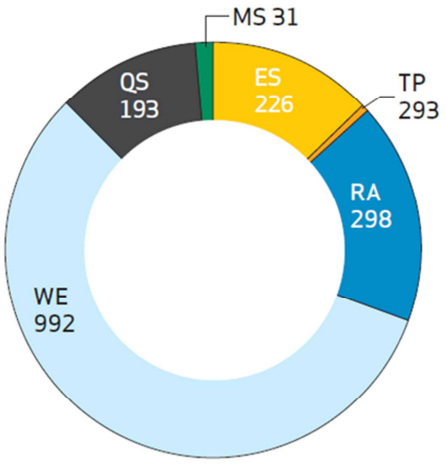
4) Computational modeling (QSAR)

In silico methods (e.g. read-across, weight-of-evidence) were used in the bioaccumulation and repeated dose toxicity assessments for about 85% (read-across: 17.1%, weight-of-evidence: 57%,

[8] EPI Suite™-Estimation Program Interface (U.S. EPA)
https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

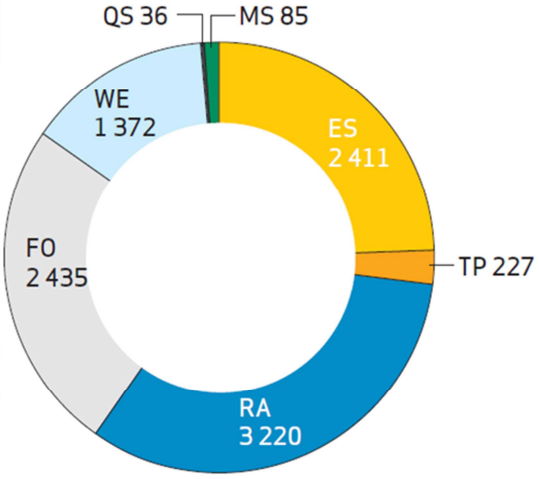
QSAR: 11.1%) and about 47% (read-across: 32.9%, weight-of-evidence: 14%, QSAR: 0.4%) of the registered chemicals, respectively (Fig. 3). However, it should be noted that these numbers and percentages represent the *in silico* data submitted for REACH registration and not those actually approved as valid data.

Bioaccumulation – fish (ENV)		
	No. ESR	% ESR
ES	226	13
TP	9	0.5
RA	298	17.1
FO	0	0
WE	992	57
QS	193	11.1
MS	23	1.3
Total	1 741	100



ESR – Endpoint Study Record
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

RDT – all routes, all study durations (HH)		
	No. ESR	% ESR
ES	2 411	24.6
TP	227	2.3
RA	3 220	32.9
FO	2 435	24.9
WE	1 372	14.0
QS	36	0.4
MS	85	0.9
Total	9 786	100



ESR – Endpoint Study Record
ES – Experimental studies
TP – Testing proposal
RA – Read-across
FO – IUCLID flags to omit the study
WE – Weight of Evidence approach
QS – (Q)SAR studies
MS – Miscellaneous

Fig. 3 Bioaccumulation (upper figure) and repeated dose toxicity (RDT, lower figure) data registered under REACH (100 - 1,000 ton/year)[9]

[9] The Use of Alternatives to Testing on Animals for the REACH Regulation (European Chemicals Agency (ECHA), June 2, 2014)
http://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf

2.1.5 Application to risk assessment and management of genotoxic impurities in pharmaceuticals

In June 2014, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) developed the ICH guideline M7[10] on "Assessment and Control of DNA Reactive (mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk". The guideline accepts the use of QSAR in initial screening for the genotoxicity assessment of reaction impurities with exposure at low dose levels, including reagents, reaction intermediates and by-products in the process of synthesis or degradation products of pharmaceuticals (Fig. 4). Since impurities in pharmaceuticals generally exist in extremely low amounts and their separation and purification are difficult or require large costs, it seems reasonable to use QSAR for their safety assessments. The guideline specifies that two complimentary QSAR methodologies (knowledge-based and statistics-based) should be used to predict mutagenicity, which is the first case of official approval of QSAR-based toxicity assessment.

Fig. 4 ICH-M7 guideline and key points (Safety)[11]

2.1.6 Efforts made by the Food Safety Commission

In April 2015, the Food Safety Commission Secretariat of the Cabinet Office established the Assessment Methodology Development Office for strengthening their functions in developing and planning new assessment methods contributing to rapid and reliable risk assessment

[10] About the Guideline on Assessment and Control of DNA-Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risks (MHLW, November 2015)
https://www.pmda.go.jp/files/000208287.pdf

[11] Materials from the 2nd meeting of Investigative Commission on *In Silico* Methods for Chemical Assessment (undisclosed)

(introducing and utilizing computer-based methods etc.) as well as food assessment methods adopting new techniques (e.g. regenerative medicine techniques).

Moreover, in April 2016, the Food Safety Commission organized the Working group for developing assessment methodology, which is currently discussing the applicability of *in silico* methods (prediction of genotoxicity or repeated dose toxicity by (Q) SAR, read-across and TTC) to the Assessment of the Effect of Food on Health.

In addition to the establishment of such organizational frameworks, in FY 2015, the Food Safety Commission implemented the Comprehensive Study for Ensuring Food Safety to learn the relevant efforts made in various countries and identify the issues associated with the introduction of *in silico* methods into the food safety area. In FY 2016 and thereafter, the Commission will aim at establishing *in silico* methods for safety assessment by building a database of toxicity data provided in the Food Safety Commission Risk Assessment Reports under the Research Program for Risk Assessment Study on Food Safety.

Potential applications of *in silico* methods include priority setting and screening, particularly in cases where: 1) a vast number of substances need to be assessed, such as raw materials for utensils, containers and packaging, 2) substances have extremely low exposure levels, such as flavoring agents and impurities, and 3) they can be used to complement the lack of data.

As for the recent trends in food safety in other countries, the U.S. and Europe are taking the lead in implementing projects on *in silico* methods, promoting the use of toxicogenomics data and AOP approaches for performing simulation. The European Food Safety Authority (EFSA) has developed a Monte Carlo simulation tool for assessing exposure. Under the current situation where Japan is far behind the U.S. and Europe in the area of *in silico* methods for assessment of food safety, the Food Safety Commission recognizes the importance of developing close relationships with the relevant U.S. and European authorities including EFSA.

2.2 Trends in research and development of *in silico* techniques in relevant areas

2.2.1 NEDO / Ministry of Economy, Trade and Industry (METI)

Since 2000, NEDO's research and development in the area of comprehensive assessment and management of chemicals has focused on the establishment of approaches for comprehensively assessing and managing risks throughout the life cycle of chemicals as well as the development of processes, approaches and intellectual infrastructures contributing to risk reduction. Projects particularly related to *in silico* techniques were the development of biodegradability/bioaccumulation prediction methods (FY 2000-2006) and the development of a prediction support system for 28-days repeated dose toxicity (FY 2007-2012) (Fig. 5). A biodegradability/bioaccumulation prediction model (CERI Biodegradation Prediction System), developed by the Chemicals Evaluation and Research Institute, had been available online for free since 2005 but is no longer used. The Hazard Evaluation Support System Integrated Platform (HESS), a repeated dose toxicity prediction system developed at the initiative of NITE, was the world's first (at the time of its release in 2012) prediction support system with a function enabling efficient reference to information concerning toxicity tests, metabolism and mechanism of action related to repeated dose toxicity of chemicals. NITE is currently in charge of its data update, system release and operation and dissemination of application examples. The development of HESS was an industry-government-academia collaboration, which involved researchers from various areas including chemistry, biology, medicine, statistics and IT as well as support from other ministries. However, large public research and development projects related to *in silico* techniques have not been implemented after FY 2013. Usually, a newly developed technique or tool cannot be put into practical use immediately after the project is completed, so continuous

follow-up must be provided to make effective use of the research and development outcome; this applies not only to this particular project but also to other projects.

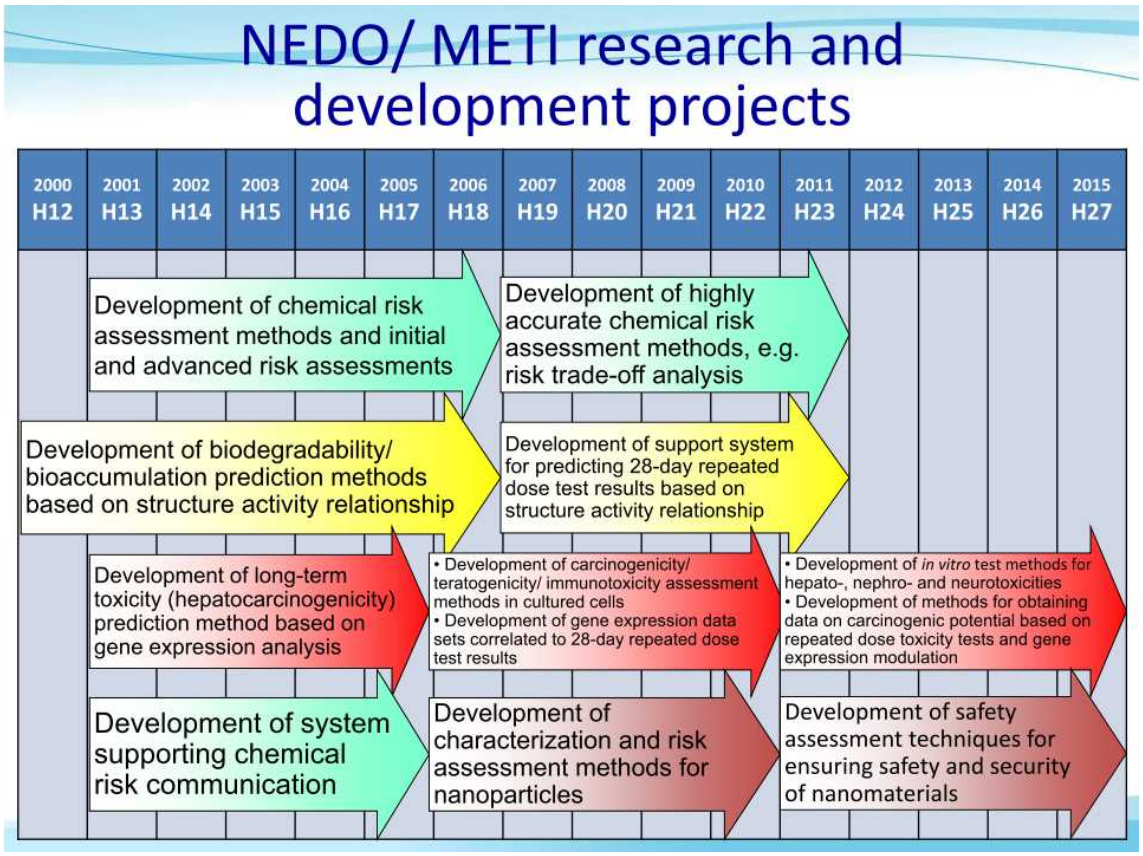


Fig. 5 NEDO / METI research and development projects in the area of comprehensive assessment and management of chemicals[12]

2.2.2 U.S. ToxCast program

The goal of the ToxCast program initiated by the U.S. EPA is to develop an *in silico* toxicity prediction model based on diverse bioactivity profiles obtained by testing a large library of chemicals consisting of up to 10,000 substances using *in vitro* high-throughput assays (Fig. 6). Since the program's launch in 2007, 300 and 1,800 substances have been comprehensively analyzed in Phases 1 and 2, respectively, using 700 or more high-throughput assays. The analysis is ongoing using a decreased number of test systems and an increased number of substances. The total research and development budget calculated in yen is estimated to be 200 billion yen or more. So far, successful results have been achieved in prioritization of endocrine disrupters for further screening and assessment.

[12] Materials from the 1st meeting of Investigative Commission on *In Silico* Methods for Chemical Assessment (undisclosed)

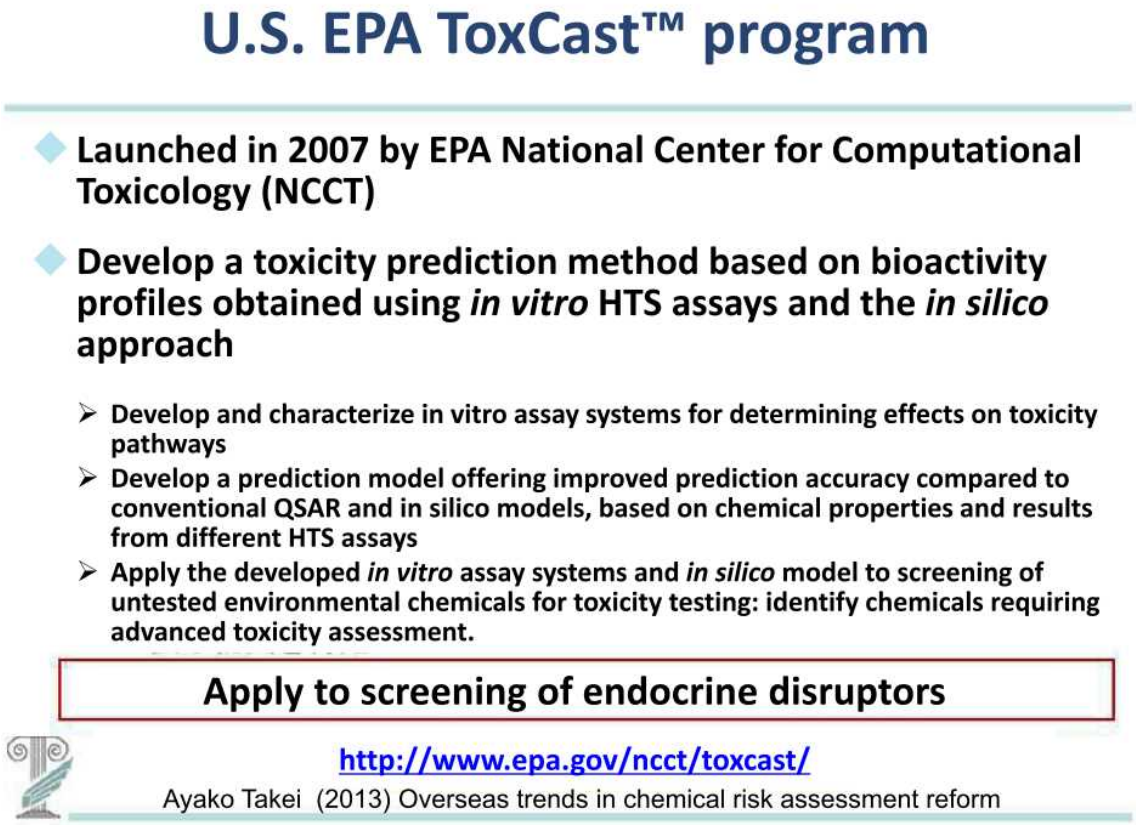


Fig. 6 Summary of the ToxCast program[13]

2.2.3 SEURAT-1 in Europe

SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing-1) is a large collaboration project among industry, government and academia, which was launched for developing alternative methods for repeated dose toxicity tests to prepare for the enforcement of a full ban of animal testing under the EU Cosmetics Directive (Fig. 7). The project consisted of 6 research programs, mainly focusing on the development of *in vitro* test systems but also addressing the development of *in silico* models for predicting long-term toxicity of cosmetic ingredients[14]. This research program has committed to the establishment of databases related to repeated dose toxicity and genotoxicity and the development of *in silico* methods such as category approach, read-across and Threshold of Toxicological Concern (TTC) approaches.

[13] Overseas trends in chemical risk assessment reform (FoRAM Study Group, February 21, 2013) https://staff.aist.go.jp/kyoko.ono/FoRAM/FoRaAM_022113_Takei.pdf

[14] COSMOS - Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety: http://www.cosmostox.eu/home/welcome/

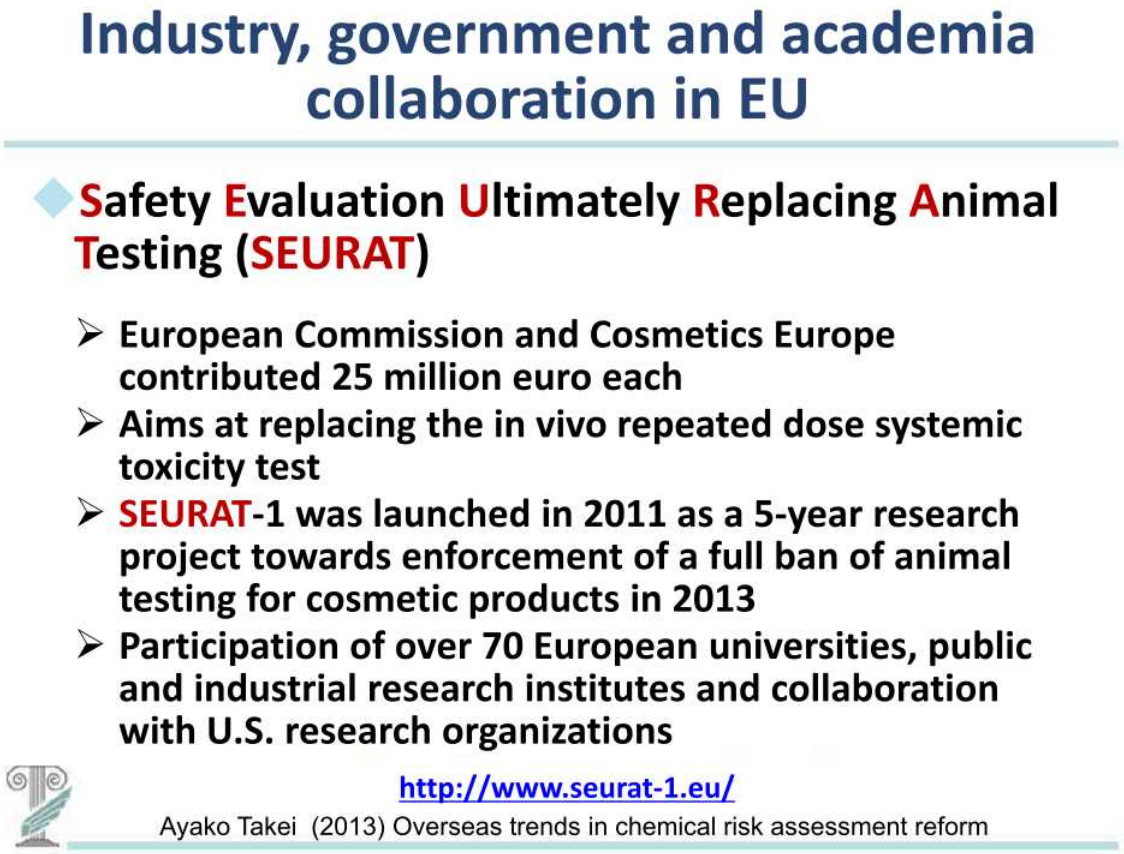


Fig. 7 Summary of SEURAT-1[13]

2.2.4 Effective utilization of existing data – Modification of Ames QSAR –

In response to the movement towards the establishment of the ICH guideline M7 on "Assessment and Control of DNA Reactive (mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" (see 2.1.5), the National Institute of Health Sciences (NIHS) has launched an international collaborative project for improving Ames QSAR models using data for approximately 13,000 substances, which were obtained from Ames assays conducted according to the Japanese Industrial Safety and Health Act and can be used for developing QSAR. NIHS will disclose the assay data in multiple phases over three years and call on QSAR builders worldwide to improve their QSAR models. The utilization of the world's largest high-quality database containing a larger number of Ames assay data compared to those previously available to the public is attracting attention from relevant parties.

2.2.5 Current status and examples of development and utilization of *in silico* methods by companies

・Lion Corporation utilizes AIST-Standardized Hydrology-based Assessment tool for chemical Exposure Load Ver.2.5, a mathematical model for estimating chemical concentration in river water. The model has been verified by a match between the predicted environmental concentration (PEC) of linear alkylbenzenesulfonate (LAS) etc. based on monitoring data and the PEC calculated by the mathematical model (monitoring data: 29 μg/L, mathematical model: 0.02 – 24 μg/L). Collection of monitoring data with temporal and spatial details is both technically and economically difficult, so the use of mathematical models in exposure assessments has great advantages in terms of wide application range and low cost. The company also uses a QSAR

model (BIOWIN5) for predicting the biodegradability of their newly developed chemicals. Before starting its application in the development stage of new chemical substances, the company set a limitation on the range of chemicals to which BIOWIN5 could be applied to improve its accuracy, based on the data they had for 72 substances (mainly surfactants).

• Kao Corporation promotes the development of efficient and reliable assessment by *in silico*/ *in vitro* methods to respond to the global movement towards animal protection as well as to overcome the species differences between animals and human. With the aim of developing *in silico* methods applicable to systemic toxicity assessment, the company is working on the improvement of assessment methods by collecting and organizing existing information on chemical structure and hepatotoxicity of pharmaceuticals etc. from various sources, such as toxicology books, and combining them with the data from HESS, the computer software developed by NITE and its collaborators for supporting repeated dose toxicity assessment. The company is also developing toxicity assessment systems based on *in vitro* assays and the combination of *in silico*/ *in vitro* methods. The company recognizes the importance of understanding the limits of these assessment systems before seeking their practical application to assessments.

2.2.6 HESI RISK21 project

The RISK21 project of the Health and Environmental Sciences Institute (HESI), an international non-profit organization, developed an assessment scheme consisting of the following steps (Fig. 8): (i) formulating problems in accordance with risk management policies; (ii) identifying exposure scenarios and conducting exposure assessment; (iii) conducting hazard assessment; (iv) visualizing risks by plotting the data on exposure-hazard matrices and drawing conclusions by considering the uncertainties in the assessment results, or identifying the missing data required for the assessment and, if necessary, collecting additional data to conduct detailed assessment. The key is to conduct exposure assessment first; if the exposure level is sufficiently low, the risk is assumed to be low (plotted within the green area of the matrix in the figure), even if the hazard level is high (or if the hazard level is associated with large uncertainty), so low-cost methods such as TTC and QSAR can be used for the assessment (the red triangle in the figure shows that the cost becomes smaller toward the peak at the bottom and larger toward the base at the top), demonstrating the flexibility of this risk-based assessment scheme.

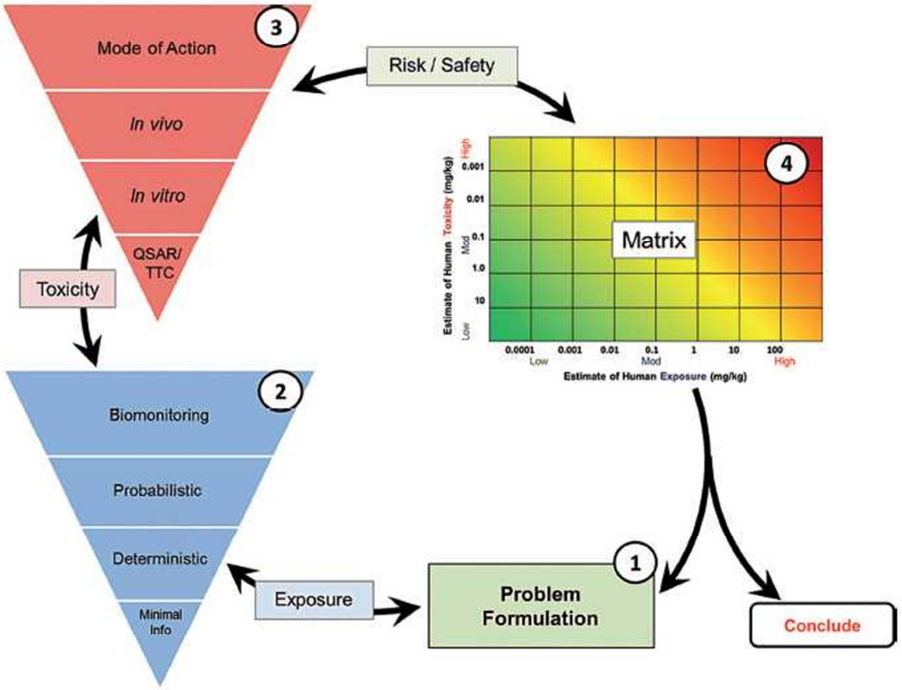


Figure 1. The RISK21 roadmap. This diagram is a schematic representation of a multifunctional tool that provides a transparent process for obtaining rational risk-related decision points. The inverted triangles for exposure and toxicity represent the proportional investment of resources needed for each tier. The following steps describe the use of the roadmap and are described in additional detail in Embry et al. (2014): 1) *Problem formulation*: Define problem. This initial step is reevaluated throughout the iterative process; 2) *Exposure estimate*: Obtain tiered estimate of exposure BEFORE assessing toxicity. Use existing knowledge. Express as range; 3) *Toxicity estimate*: Obtain tiered estimate of toxicity. Use existing knowledge. Develop data only as needed. Express as range; 4) *Matrix*: Intersect exposure and toxicity estimates on the matrix.

Fig. 8 HESI RISK21 roadmap[15]

2.2.7 Development of AOP and IATA

AOP (Adverse Outcome Pathway) illustrates the sequence of events from chemical exposure of organisms or populations to the final expression of adverse effects at the organism or population level, and includes events at molecular, cellular, organ, organism and population levels (Fig. 9). For complex toxicological endpoints that are difficult to predict by structure-based QSAR, OECD has proposed an AOP-based concept for making assessments. IATA (Integrated Approaches to Testing and Assessment) is a chemical safety assessment approach that combines *in silico* methods and *in vitro* and *in vivo* assays (which may include those without any test guidelines). In recent years, OECD has placed great emphasis on the development of AOP and IATA, which enable maximum utilization of existing knowledge including the structure-activity relationship (SAR) as well as flexible incorporation of data from novel assay systems (Fig. 10) and are thus expected to serve as useful tools for chemical safety assessment. AOPs are developed for individual endpoints. Although IATA is an approach that utilizes various types of assay and prediction results that are available for performing chemical safety assessment, rational, AOP-based methods are likely to become the main assessment methods in the future. Therefore, instead of inappropriately applying the existing methods, we need to develop rational assay methods suited for AOP.

---

[15]"A 21st century roadmap for human health risk assessment", *Critical Reviews in Toxicology*, 2014; **44**(S3):1–5 (open access)
http://www.tandfonline.com/doi/full/10.3109/10408444.2014.931923

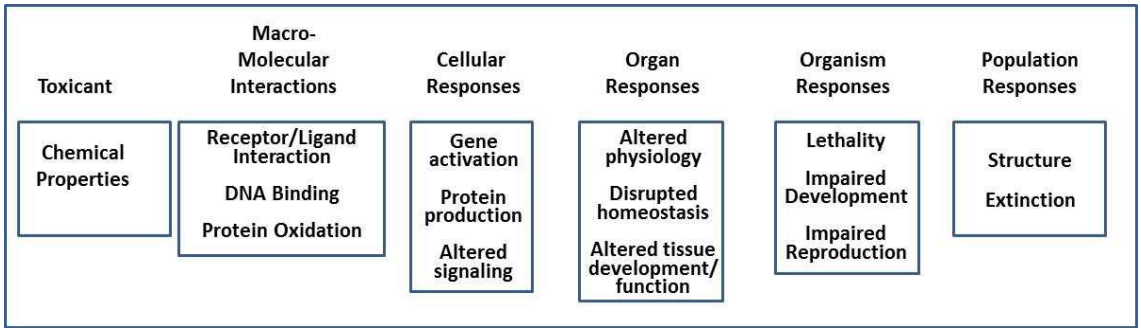


Fig. 9 AOP proposed by OECD (translated by the secretariat)[16]

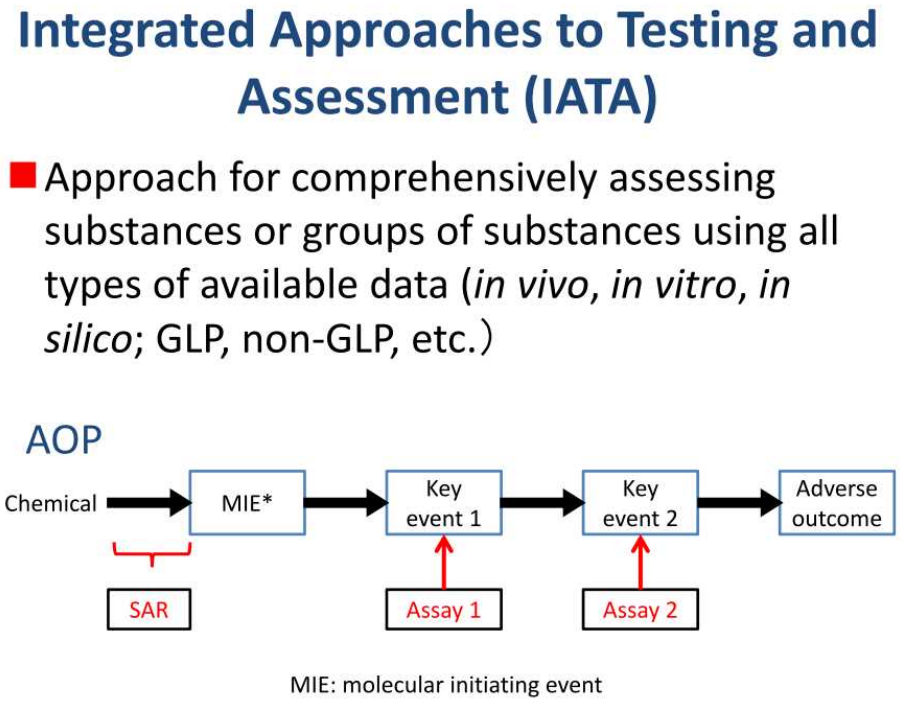


Fig. 10 AOP and SAR/ *in vitro* data utilized in IATA[17]

2.2.8 TTC approach

As an *in silico* method for hazard assessment, the Threshold of Toxicological Concern (TTC) approach has been utilized in other countries for assessing trace substances migrating from food packaging materials and flavoring agents, and its application is also considered in Japan. The establishment of thresholds is based on statistical analyses of databases (Fig. 11), and the knowledge on the structure-activity (toxicity) relationship is used for elaborating compound classification and selecting structures to be excluded from TTC application.

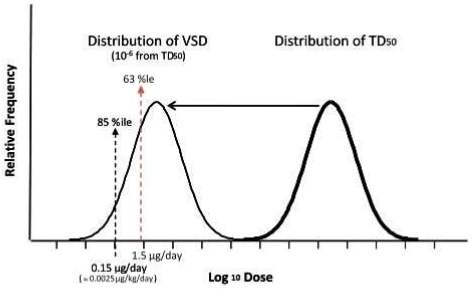
---

[16] Guidance document and template for developing and assessing adverse outcome pathways, ENV/JM/MONO(2013)6
http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en

[17] USERS' HANDBOOK SUPPLEMENT TO THE GUIDANCE DOCUMENT FOR DEVELOPING AND ASSESSING AOPs (OECD 2014)
http://aopkb.org/common/AOP_Handbook.pdf

Threshold of Toxicological Concern（TTC) is a human exposure threshold value for chemicals below in which no apparent adverse effect can be observed.

Assuming that carcinogenicity is usually the most sensitive toxicity endpoint, TTC is determined by examining the distribution of VSDs (10^{-6} risk) obtained by linear extrapolation from TD50 values of 477 chemicals in the carcinogenicity database.



Proportions of potential carcinogens and of those representing less than 10^{-6} risk at various threshold values

Threshold value (μg/day)	Percentage of chemicals presumed carcinogenic							
	100%	50%	20%	10%	100%	50%	20%	10%
	10^{-6} Target risk				10^{-5} Target risk			
0.15	86	93	97	99	96	98	99	>99
0.3	80	90	96	98	94	97	99	99
0.6	74	87	95	97	91	96	98	99
1.5	63	82	93	96	86	96	97	99
3	55	77	91	95	80	90	96	98
6	46	73	89	95	74	87	95	97

1.5 μg/person/day ≈ 0.025 μg/kg·bw/day ≈ 0.5 ppb

Fig. 11 Threshold of Toxicological Concern (TTC)[18]

3. Issues in and ideas for facilitating application of *in silico* methods in regulation of chemical substance management in Japan

3.1 Issues in promoting application of *in silico* methods

3.1.1 Validation of QSAR models

- For new chemical substances, which are the target substances to be reviewed by the Chemical Substances Council under the CSCL, actual measured values submitted by the applicants and QSAR prediction results have been compiled for biodegradability, bioaccumulation, eco-toxicity and Ames assay, but model validation by comparing the measured values and the predicted values has not been sufficiently conducted. There must be cases where the prediction results greatly differ from the actual test results, and there must be a cause for such difference. In order to utilize the prediction results obtained by *in silico* methods, discussion on validation of QSAR models will be needed. Repeating such model validation to define the reliability, limitations and applicability domain of the models is crucial for promoting their application.

3.1.2 Handling of company-owned data

- Companies cannot disclose their data without permission from the intellectual property departments, which needs to be overcome. Once there was a case where an industry organization (Japan Chemical Industry Association) asked its member companies to provide information on a 28-days repeated dose toxicity test; however, the companies refused to

[18] Materials from the 4th meeting of Investigative Commission on *In Silico* Methods for Chemical Assessment (undisclosed)

submit their data, whether "positive" or "negative", due to the opposition from their intellectual property departments. Companies are reluctant to submit their data, not only for the sake of securing the intellectual property but to hedge against the risk of disclosing any data that might affect their image.

3.1.3 Collaboration between companies and academic research institutes

- One disadvantage for Japan is the lack of collaboration between companies having the experimental data obtained from animal testing etc. and universities or academic research institutes willing to use that data. Particularly, in the area of *in silico* methods for assessment, if researchers in academic research institutes equipped with skills and ideas could access the data measured by companies, they may come up with new ideas that could potentially lead to the improvement of prediction theories or their accuracy. In Europe, companies often approach academia with collaboration offers. In Japan, on the other hand, exchange of opinions between academic research institutes and companies has not been very successful, preventing the introduction of innovative ideas of the academic research institutes into companies.

3.1.4 Cost-related issues

- From the aspect of cost, it is less expensive for companies to conduct the actual tests, for example, the required *in vitro* assays, than to conduct assessments by *in silico* methods. For small and medium-sized companies assessing only one or two substances per year, it is cheaper to conduct actual tests such as Ames assay, although this does not apply to large companies that need to assess thousands of substances per year. Discussion on the validity of the assessment results is indeed important, but under the current circumstances where cost may be imposing a substantial obstacle to the application of *in silico* methods, measures for improving cost performance also needs to be considered, such as collectively conducting prediction assessments in one place.

3.1.5 Significance of data and database

- Validation using experimental data is necessary for improving the existing systems; unfortunately, however, there are not sufficient budgets for this. We must continue our efforts to devise project plans to cultivate domestically developed systems. It is inevitable that prediction accuracy is initially low; we must continue to accumulate more and more data.
- Japan has the advantage of having an abundance of high quality uniform test data. QSAR experts in other countries, who place great emphasis on the enrichment of databases, have great interest in the Japanese data, which is difficult to search in English. Meanwhile, even if a database contains a large amount of data, contamination of low-quality, unreliable data can prevent the user from reaching the right conclusions. Thus, it is always important to accumulate high-quality data. The Food Safety Commission releases reports on the safety assessments conducted on pesticides etc. They recognize the importance of compiling the data provided in the assessment reports into a database and using it to promote the application of *in silico* methods, and are planning to take the necessary measures sequentially.

3.1.6 Importance of giving serious thoughts about *in silico* methods in Japan

- Europe is serious about the elimination of animal testing because they believe that is right. Japan, on the other hand, does not seem so serious. The above belief makes European countries think that they should ultimately depend on *in silico* methods, but not Japan. This makes a big difference.

- We have not yet made enough consideration on how we can use the *in silico* methods introduced in this Investigative Commission, but maybe we will when we have our back against the wall. In the current situation, there is a national review system and we just need to conform to it, but how would we think if the system were abolished? Would this not raise strong motivation?
- Since the assessment system has been shifted from a hazard-based system to a risk-based system, we must predict the outcome of human exposure via the environment, considering the actual cases provided under the CSCL. The 28-days repeated dose toxicity tests performed today may be prohibited in the future. Since toxicity tests are generally conducted using doses as high as possible, toxicity data at lower doses often does not exist, so NOELs/NOAELs required for risk assessment can only be "extrapolated". However, when extrapolating the NOELs/NOAELs from data obtained at higher doses, we must consider the validity of such extrapolation itself. Then, what should *in silico* methods aim for? If we consider applying *in silico* methods to human health effects, the 28-days repeated dose toxicity data, which is currently used for screening, seem insufficient for representing the wide range of issues concerning human health effects and may mislead the risk assessment in some cases.

3.2 Ideas for promoting application of *in silico* methods

3.2.1 Combination of knowledge and techniques

- As an overall procedure, combining the knowledge from experts in the biology area with those in the chemistry and IT areas may produce interesting results unique to Japan. The recent improvement in the computing power of computers has brought great changes to chemistry and life sciences research. For example, by using the ultra-accelerated quantum chemical molecular dynamics method, complex interactions between biopolymers (e.g. interactions between DNA and DNA-binding proteins) can be calculated on computers (Fig. 12), which enables us to explain the mechanism of such biological phenomena at the molecular level. It should be interesting to apply such methods to chemical safety assessment. Moreover, although it has been difficult to make negative prediction for genotoxicity, if we could calculate the covalent binding potential of chemicals to DNA, we would be able to determine that the chemicals not forming adducts with DNA are not genotoxic. Statistics-based QSAR has a limitation on its applicability domain, so we need to set mechanism-based, molecular-level endpoints when developing *in silico* models.
- In the long term, it is important to develop an assessment system capable of predicting mutagenicity by a scheme based on the mechanism of mutagenesis involving the interaction between the damaged sites in mutated DNA and the repair proteins. We need to select appropriate endpoints for AOP and select appropriate assay methods for such endpoints before predicting their targets; otherwise, it may not be accepted in the future.

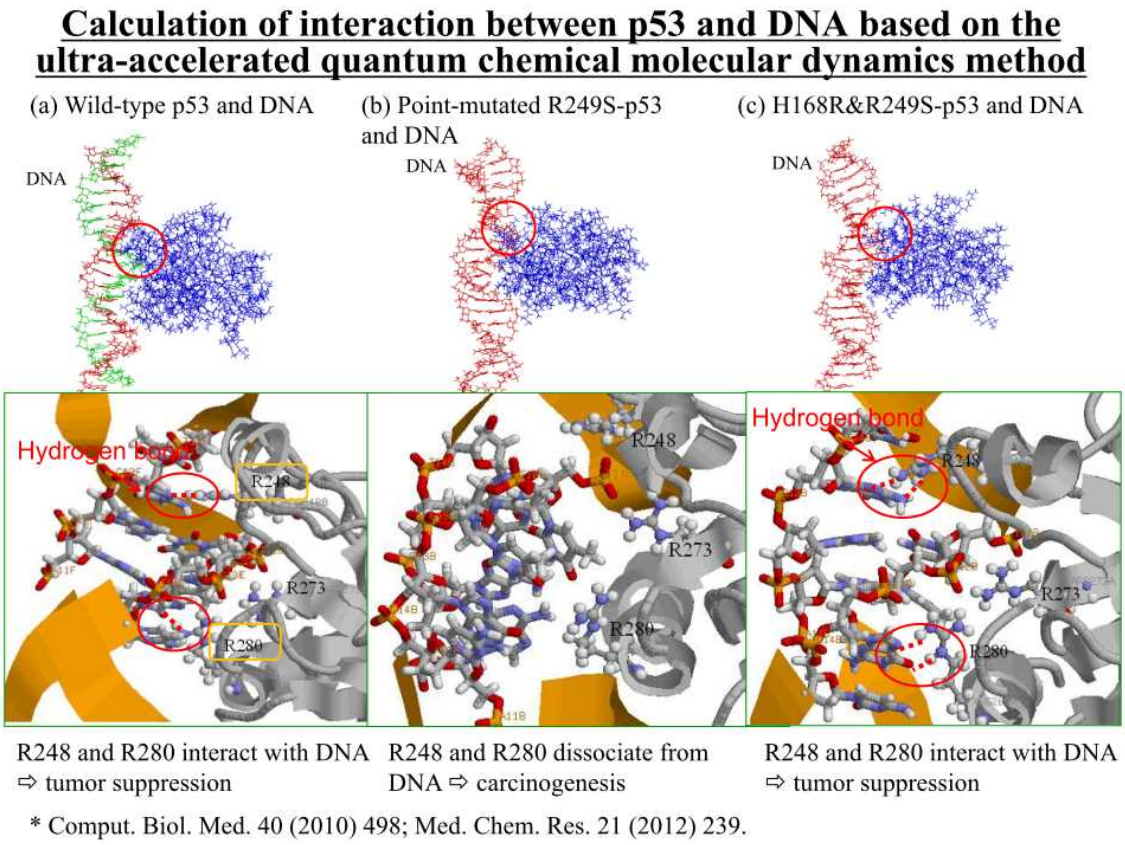


Fig. 12 Simulation of biopolymer interaction based on quantum molecular dynamics[19]

- We hope to make contribution by internationally showcasing the academic and technical potential of Japan. Combining the existing methods with new methods such as molecular dynamics is expected to increase our international standing.
- The Investigative Commission has prompted one of its members, who specializes in quantum molecular dynamics, to start collaborative research with another member specializing in genotoxicity. They will analyze the interaction between the damaged sites in chemically mutated DNA and the repair enzymes. One of the purposes of this Commission was to promote interaction between people from different areas. In order to achieve successful results from new research and development, it is important to mobilize resources from different areas and induce synergy.
- For involving people from other areas, it is important that we explain matters in ways they can easily understand. Once, a member specialized in genotoxicity provided us with an easy-to-understand lecture on a matter that was taken for granted in their area but unfamiliar to others. Such lecture is a good opportunity for people in other areas to recognize how they can contribute to the matter with their knowledge and techniques, which should motivate them to make suggestions on which part should be modified to improve safety.

[19] Materials from the 3rd meeting of Investigative Commission on *In Silico* Methods for Chemical Assessment (undisclosed)

3.2.2 Application of *in silico* methods to safety assessment in Japan

- Regarding low-volume new chemical substances for which safety has not been previously assessed, assessment by QSAR may only give low accuracy results but would provide some check. In that sense, maybe we should use QSAR without being too stringent.
- It is assumed that new chemical substances with large production volumes will be rare. On the other hand, the number of registration of low-volume new chemical substances shows an increasing trend, which gives rise to a demand for efficiently assessing such low-production substances. Considering the large cost for conducting risk assessment by obtaining actual data for each of the diverse, small-lot substances, and also from the standpoint of avoiding animal testing, *in silico* methods must be utilized.
- The abolishment of the national review system might accelerate the utilization of *in silico* methods. This may be an extreme argument, but by viewing it from the opposite angle, we may come up with an entirely new idea when we have our back against the wall.
- We hear some opinions from other commissions that QSAR should not be used unless it produces 100% accurate results, but even if QSAR is not that accurate, it can still be used in some applications. We need to distinguish between the different nuances of "using" *in silico* methods.
- It may be effective to modify the CSCL, i.e. the substances are first assessed by QSAR before the risk assessment (Primary) and, if a substance is judged as hazardous by QSAR and added to the priority list but then proved to have no hazard by experimental data etc., it is returned to the General Chemical Substances category. This should make QSAR easier to use. The problem of the current system is that, due to its hazard-based standpoint, once a substance is placed under Priority Assessment Chemical Substances, it cannot return to General Chemical Substances until its detail assessment is conducted.
- Considering the errors within tests and the differences between human and experimental animals, there is not much significance in discussing the accuracy of *in silico* methods in predicting human health effects, merely by comparing the results from animal tests with those from *in silico* methods.

3.2.3 Research and development seeds

- How about developing a 3D model, like the so-called virtual idol "Hatsune Miku (a vocaloid software using a voice synthesizer)", and incorporating factors such as in vivo dynamics and total exposure volume to prepare a visual simulation of how a substance is ingested, carried by the bloodstream, and accumulated in organs to exert its toxicity?
- How about using artificial intelligence, like IBM Watson? We must do something to move beyond the status quo.
- Experts are capable of predicting the presence of toxicity in one glance at the molecular structure, which is close to human's pattern recognition. If this recognition ability can be input into computers like artificial intelligence, it may be applicable to the development of pharmaceuticals.
- Another option is to develop an artificial intelligence or an android (simulation). Japan is strong in such areas. If we are going to perform a simulation, the target of toxicity assessment should be human instead of rats. Based on such options, we need to establish an ultimate goal and make necessary adjustments by considering what kinds of techniques and systems are needed to achieve this goal.
- It is suggested that we perform material flow analysis. If the application of substances is at least partly determined, we could assess their toxicity by their application. Perhaps, material flow has never been identified for any chemical. Metals should be relatively easy to monitor, but chemicals are difficult because they change their forms. It is worth a try to monitor the

material flow of one or more chemicals. The obtained results would be compiled into databases and handled as big data.

3.2.4 Measures for appealing to relevant parties and fostering their awareness

- We will disseminate what we have discussed in this Commission, domestically by explaining to the members of the Chemical Substances Council that conduct reviews and assessments under the CSCL, and internationally by giving presentations at international conferences and workshops planned in the future.
- Conservation and safety of the environment can only be achieved through international cooperation and not through competition, so Japan should propose collaboration to other countries. It would be great if Japan could demonstrate its will to take leadership in this area to the global society, for example, by holding an international conference under the theme of "science for safe and secure society".
- Becoming board members of international conferences should facilitate information sharing, such as the direction of international argument, with the domestic industries. For that purpose, we need to train Japanese representatives who can defend their standpoints at international meetings, which requires daily efforts in compiling of data, studying of methodologies and discussion. We should be able to contribute to developing international order by leading the board to assign an important role to the representative of each country.

3.3 Points of concern in research and development

3.3.1 Concerns for domestic research and development budgets

- Nowadays (2016), it is difficult to acquire a budget without using the term "innovation". Even if the research aims at developing a new assessment method, those linked to existing assessments are regarded as "continuation of existing techniques" and can rarely acquire budget. Under the current conditions, basically, only novel "exciting" ideas can acquire budget.
- If METI will hold a discussion on technical development of *in silico* methods, they should incorporate (i) an additional resolution to the CSCL, (ii) IoT and (iii) product development (chemical designing) into their discussion.
- We are proud of the fact that HESS has been developed as a cross-ministry project. There was a discussion on how HESS should be used to move on to the next step. We will need to get a budget to improve it for use, but it could be difficult if it is regarded as maintenance of HESS.

3.3.2 Points to consider when planning new research and development projects

- In Japan, we have just completed conducting some projects related to research and development of novel assessment methods including *in silico* methods and established a technical platform. Meanwhile, other countries are proceeding into a new phase, developing assessment methods that integrate various techniques, including the newly developed techniques.
- If we should take on any large projects in the future, we must have a clear view on how chemicals should be managed in the future and how *in silico* and *in vitro* data should be dealt with. Without such views, we could end up spoiling the success of the project, being unable to make use of the outcome.
- In the long run, it is better that we set a goal (point of compromise) for how we should use *in silico* methods.

- The European industry-academia collaboration project SEURAT-1, which was referred to in 2.2.3, began to place more and more emphasis on academic methods along with its progress; this raised strong concerns among the companies about how long they must wait until it could be put into use. As a consequence, Cosmetic Europe, which initially sponsored half of the project, virtually withdrew from the project and launched a new original project, Long Range Science Strategy (LRSS), to develop a more practical assessment method.
- In industry-government-academia collaboration, the universities and governments working together must always place their emphasis on the usefulness of *in silico* methods from the standpoint of companies. *in silico* methods are somewhat related to areas such as sensors, IoT and analysis systems and are also in line with the future direction of development of the society. When placed in the social context, such efforts should gain momentum to bear fruit that can enhance the value of Japan.

4. Conclusion

The Investigative Commission has been launched to create an opportunity where a small number of people from governmental agencies, national research institutes, universities and companies can have an open-minded discussion on the issues concerning *in silico* methods in chemical safety assessment and the measures for their application in regulation of chemical substance management in Japan. Discussion at the meetings covered a wide range of topics, including not only the CSCL but also pharmaceutical- and food-related topics. Attendance gradually increased at each meeting, and valuable comments were provided by not only the Commission members but also the observers and secretariat members, contributing to the active exchange of diverse views.

Rather than the technical details of *in silico* methods for chemical assessment or specific approaches for application of *in silico* methods under the CSCL, the Commission discussed more general issues, such as the identification of problems associated with *in silico* methods or measures for exploring and promoting their utilization. The suggested ideas are merely rough sketches, and, as the next step, their essences need to be extracted and subjected to more elaborate discussion. We expect that the discussions made in this Investigative Commission will be shared among many people interested in *in silico* methods and will serve as a trigger for further discussion towards the practical application of *in silico* methods.

April, 2016