令和2年(2020年）9月8日

全身毒性代替法の開発に向けた最新動向
-海外の動きを中心にして-

国立医薬品食品衛生研究所
小島　肇

本発表は、個人的な見解であり、必ずしも他機関や国立衛研の公式見解ではありません。また、発表に利益相反はありません。

内容

- 各国のNAMの開発状況について
- 国際機関の動向
  OECD，ICH，　ICCR
- 我が国におけるNAMの開発状況について

各国のNAMの開発状況について

The Transatlantic Divide

Top-down development
of new toxicological tools

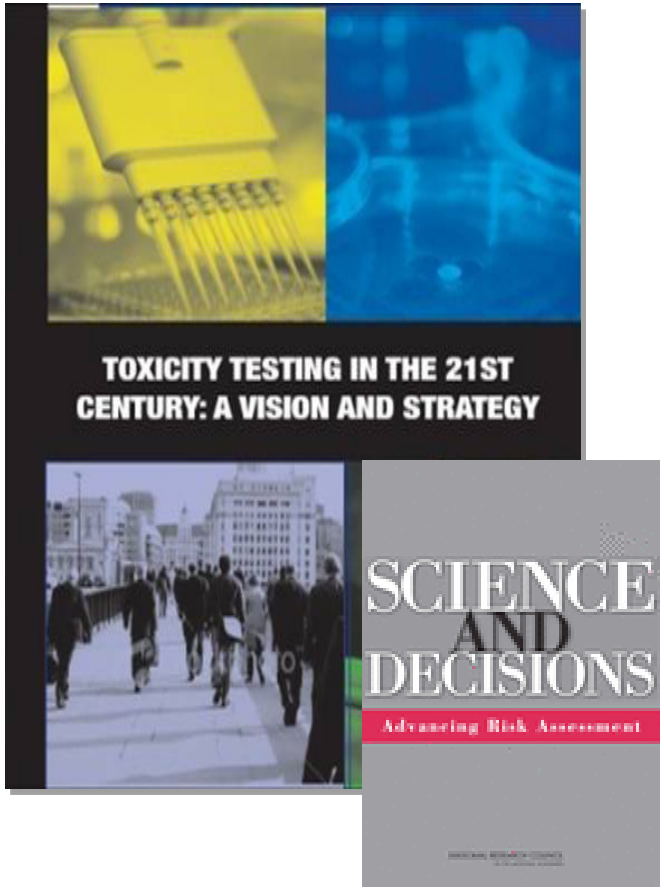
Tox-21c

Center for Alternatives to Animal Testing

3Rs

Bottom-up support to
alternative methods and
legislative pressure

The NTP Roadmap are consistent with the recent NAS Report



- **2007 NRC Report:**
  - Calls for transforming toxicology: *"from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."*
  - Envisions pathway-based toxicology, where pathway perturbations are used to predict adverse effects
- **2009 NRC report**: *"the realization of the promise [of the 2007 report] is at least a decade away"*

National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: National Academy of Sciences. Available: http://books.nap.edu/catalog.php?record_id=11970

EPA
United States
Environmental Protection
Agency

ToxCast and Tox21 High Throughput Screening of Chemical Bioactivity

- Addresses chemical screening and prioritization needs for chemicals regulated by EPA

- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures

- Committed to stakeholder involvement and transparency
  - Communities of Practice- Chemical Prioritization; Exposure
  - Release of all data upon peer review publication

High throughput testing

Compound management

Large datasets using the standard metabolic competent system

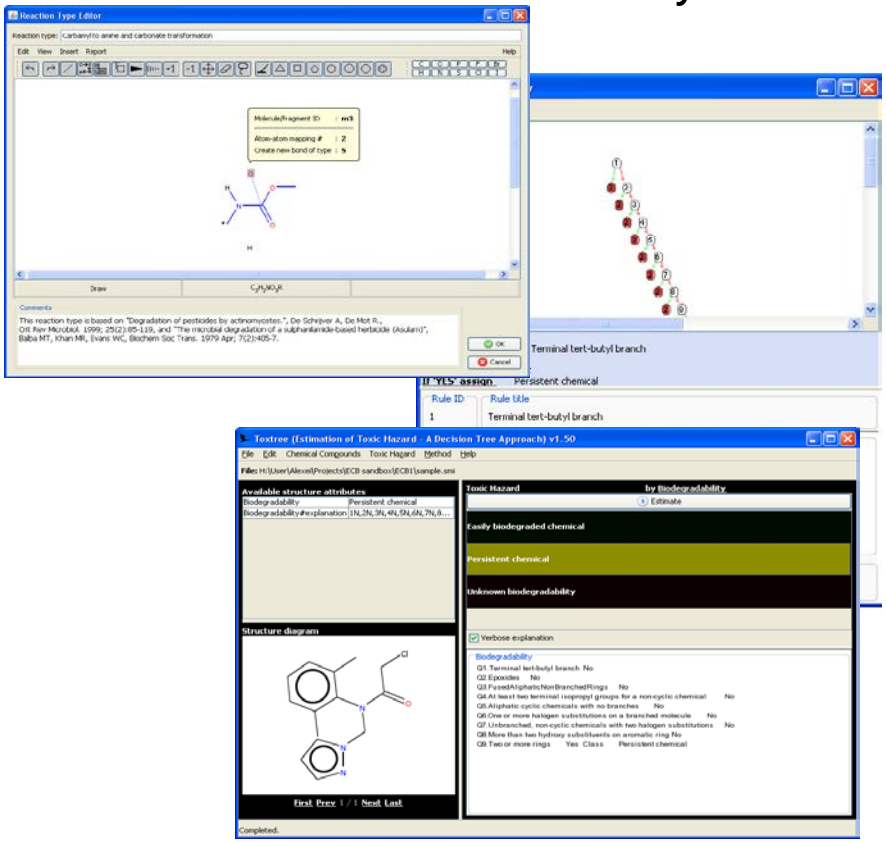
Liquid handling robot

Cell culturing

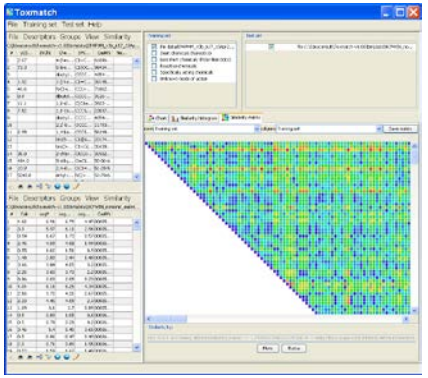
Compound Repository

Computational Toxicology: physicochemical and reactivity profiling of compounds/metabolites

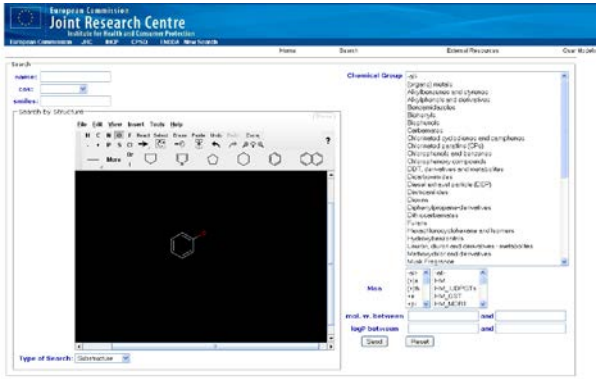
CRAFT - Chemical Reactivity And Fate Tool



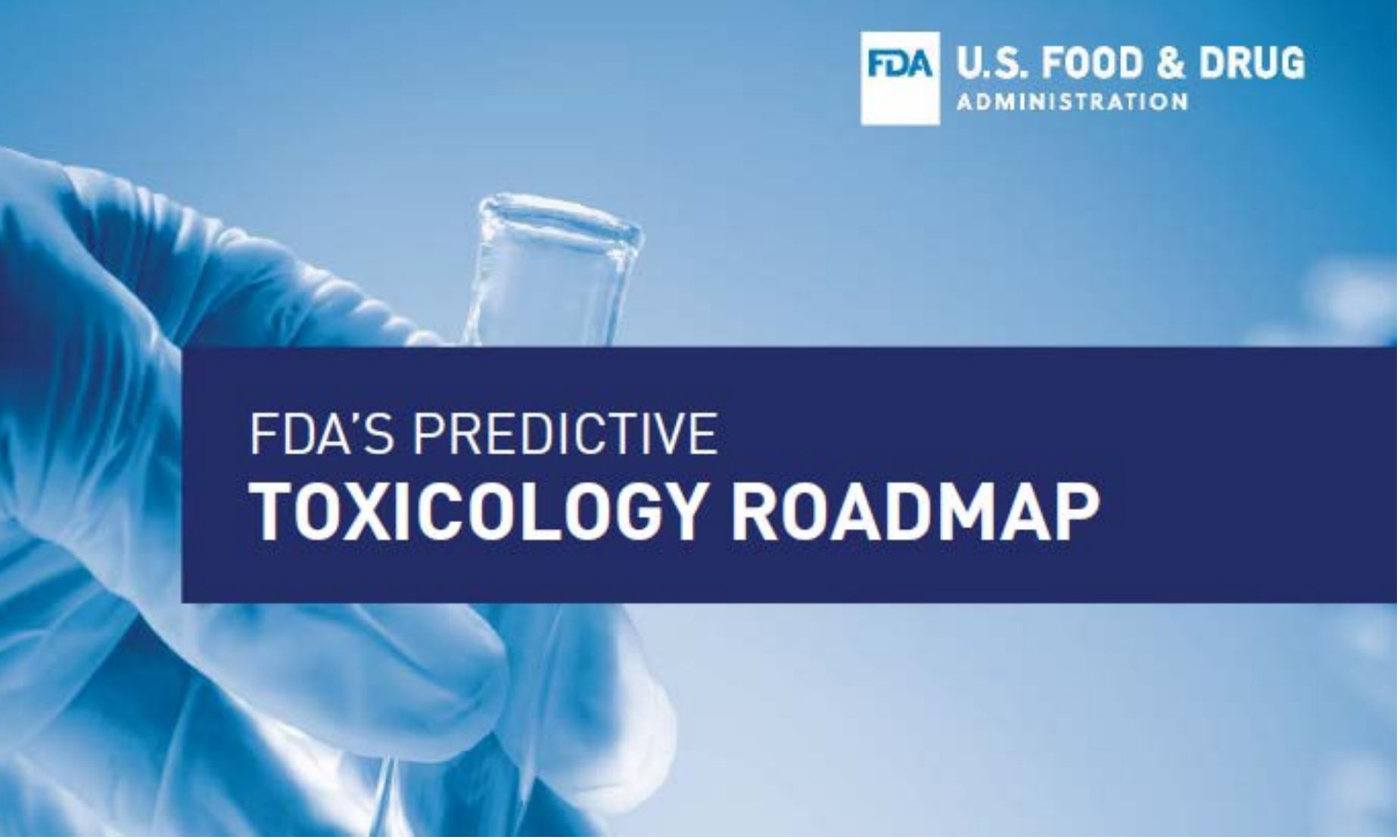
Toxtree – Hazard estimation



Toxmatch – Chemical grouping



Endocrine-Active Chemicals Database

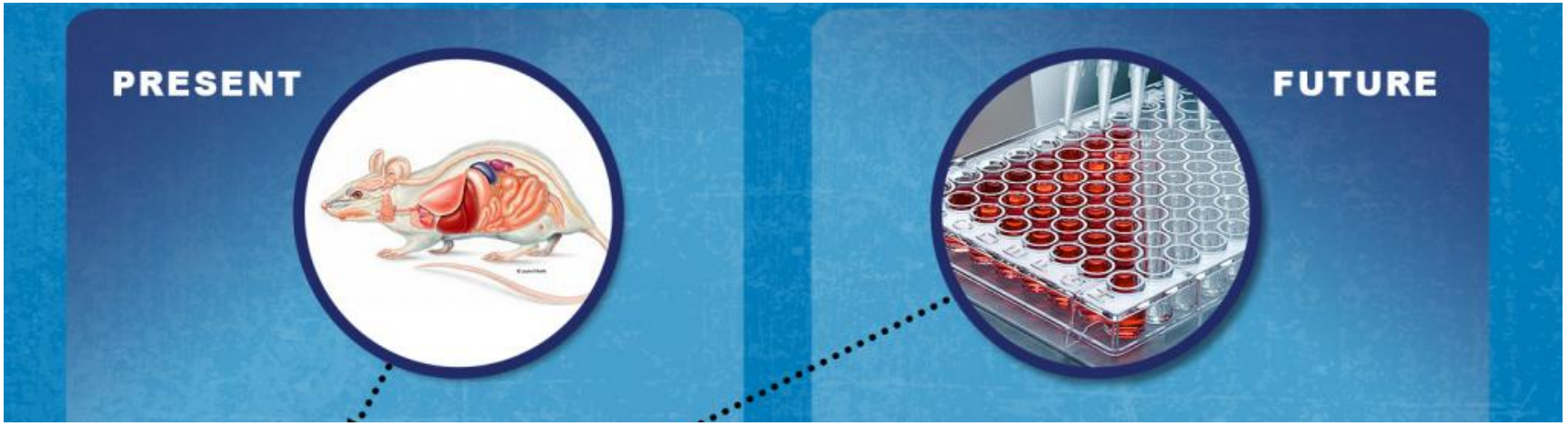


Alternative test methods for reproductive toxicity testing

FDA's Center for Drug Evaluation and Research is working through the International Conference on Harmonisation (ICH) to consider the regulatory use of alternative test methods for reproductive toxicity testing, as outlined in the Step 2 draft guidance ICH S5(R3) available at www.ich.org.

Advancing Alternative Methods at FDA

FDA's Alternative Methods Working Group



Objectives of FDA's Alternative Methods Working Group

- Discuss FDA-wide new in vitro, in vivo, and in silico methods, including research, training, and communication.
- Engage with U.S. Federal partners and global partners to promote discussion, development, and acceptance of regulatory performance criteria for such assays.
- Establish a dialogue and develop partnerships with FDA stakeholders to explore regulatory science applications for such technologies.
- Identify the performance criteria of microphysiological systems by engaging with FDA experts and FDA stakeholders through public-private partnerships.

EPA （米国環境保護庁）は2019年、動物実験削減に関する具体的数値目標を掲げた

Environmental Topics | Laws & Regulations | About EPA | Search EPA.gov

Efforts to Reduce Animal Testing at EPA

On September 10, 2019, EPA Administrator Andrew Wheeler signed a directive that prioritizes efforts to reduce animal testing. The memorandum calls for the agency to:

- reduce its requests for, and funding of, mammal studies by 30 percent by 2025, and
- eliminate all mammal study requests and funding by 2035.

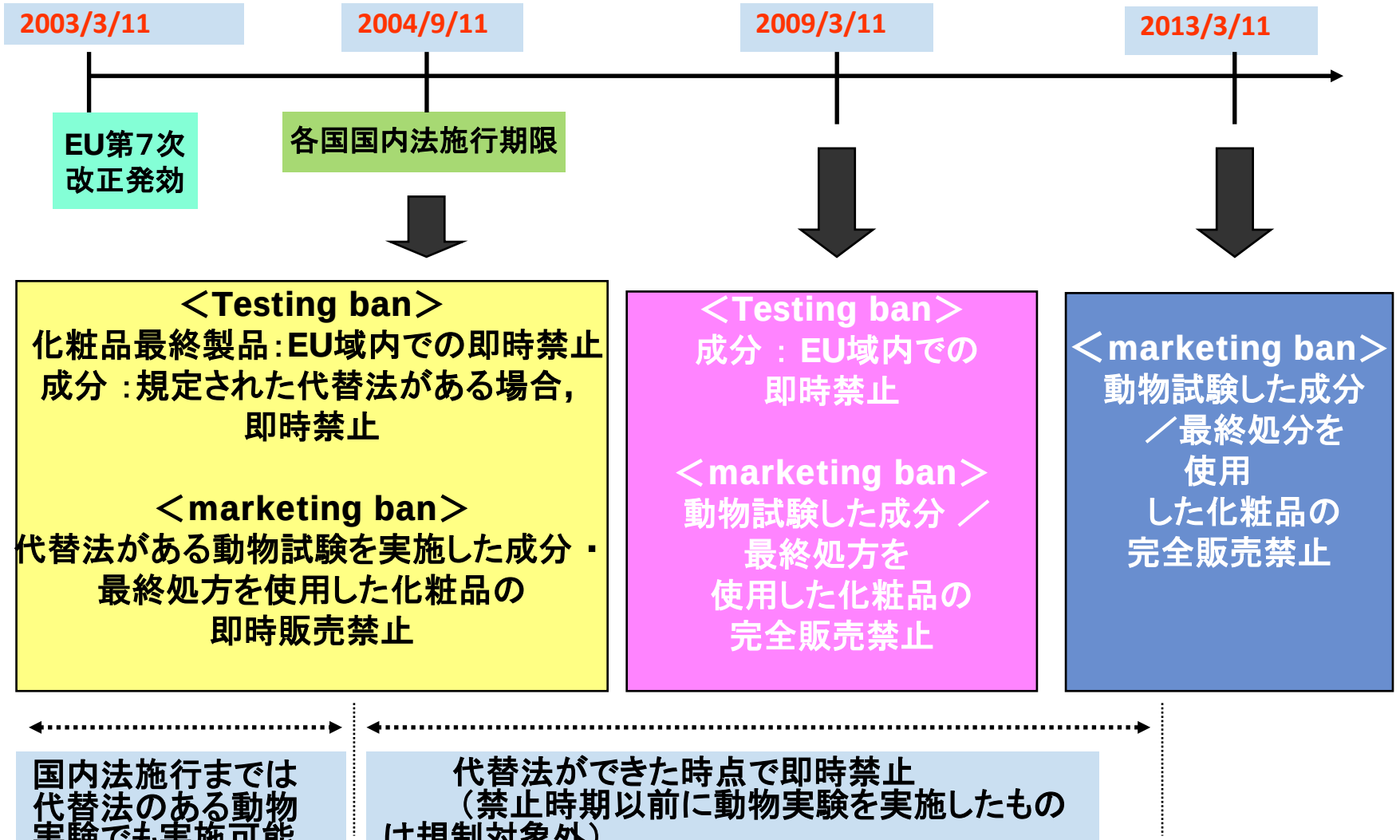
Any mammal studies requested or funded by EPA after 2035 will require administrator approval on a case-by-case basis.

It also directs the Office of Chemical Safety and Pollution Prevention and the Office of Research and Development to prioritize ongoing efforts, and to direct existing resources, toward additional activities that will demonstrate measurable impacts in the reduction of animal testing while ensuring protection of human health and the environment.

Administrator Wheeler also announced $4.25 million in funding to five universities to research the development and use of alternative test methods and strategies that reduce, refine, and/or replace vertebrate animal testing.

・2025年までに哺乳類の動物実験の要求と資金提供を30％削減
・2035年までにすべての哺乳類の動物実験の要求と資金提供の廃止

ＥＵ化粧品における動物実験規制



Fragrance journal, 2005-2

Major Achievements of the SEURAT-1 Research Initiative

Development of a research strategy based on generating and applying knowledge of mode-of-action

Development of highly innovative tools and methodology that can ultimately support regulatory safety assessment

Sustainable collection of data and standard operating procedures in a Data Warehouse

Development of a conceptual framework to combine evidence derived from predictive tools to support a safety assessment decision in a biologically rational manner

Application of the tools and concept developed in case studies addressing three scenarios:

- A Threshold of Toxicological Concern (TCC) case study, expanding the applicability domain of the established TTC approach towards cosmetic ingredients applied dermally

- A read-across case study demonstrating use of *in vitro* and *in silico* tools in regulatory toxicology

- An *ab initio* case study, illustrating proof of concept of how risk assessment for a cosmetic ingredient might be carried out without animal testing

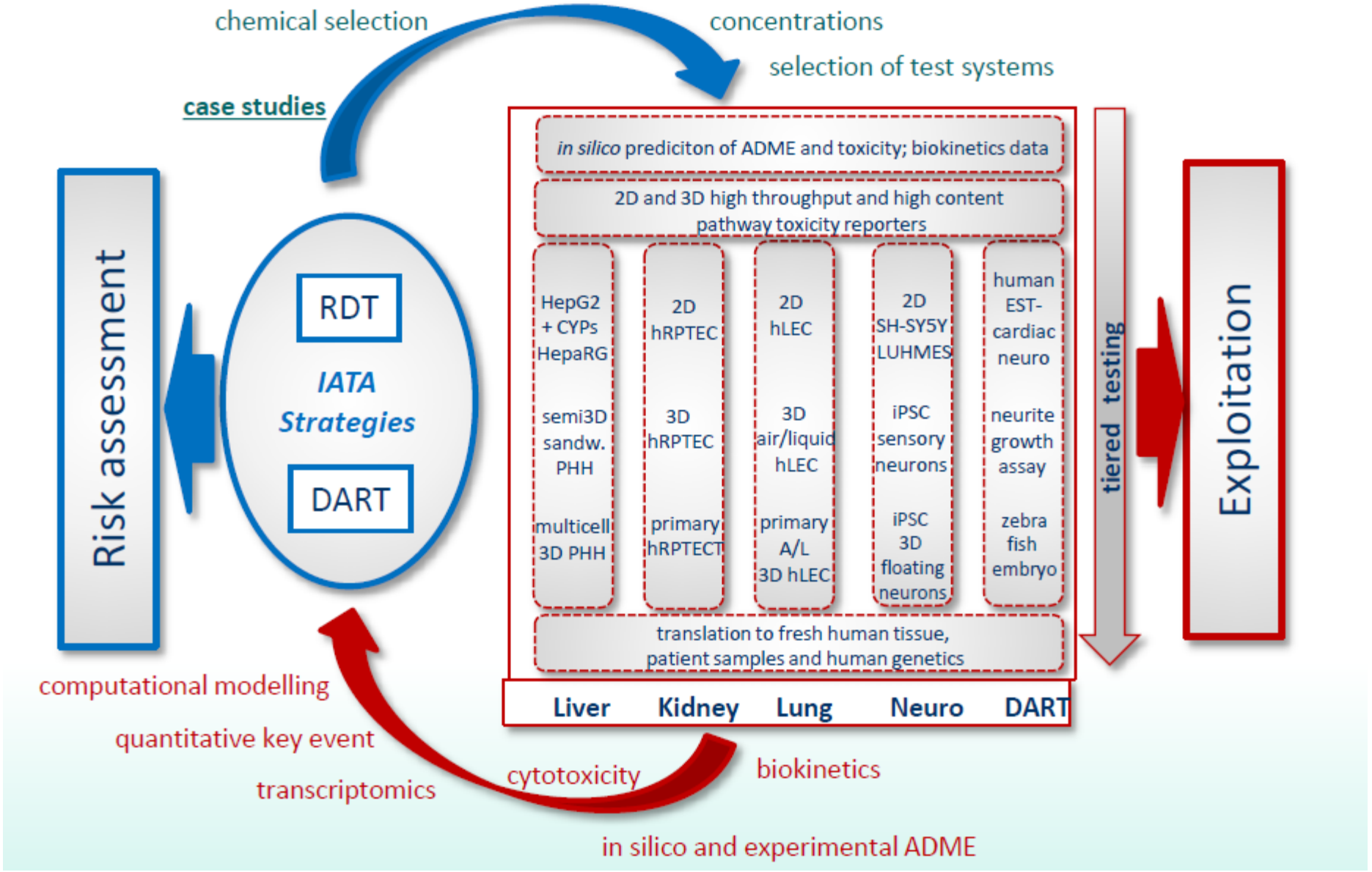
EUTOXRISK

A European Union Horizon 2020 Research and Innovation Project • February 2017, No. 1 • http://www.eu-toxrisk.eu

The Project Turns One

I am proud to introduce to you the first issue of the *EU-ToxRisk Newsletter*. After a highly dynamic first year the EU-ToxRisk project just entered already its second year. As coordinator of the project it has been an exciting year where the various aspects of the project have started. This included aligning technologies as well as testing novel advanced model systems. These different technologies and model systems will ultimately cluster together in integrated testing strategies. In particular, we have been working in the last six months to initiate the different case studies that will ultimately drive the selection of technologies and models that are fit-for-purpose for regulatory decision making. I am particularly proud that we have received highly positive responses from various regulatory authorities that will provide advice to the case studies during the project. The experimental work within these case studies have now started intensively. Also, our collaboration with our US Tox21 partners is taking shape. With this regular *EU-ToxRisk Newsletter* we want to keep you up to date on the development of the project and the successes we achieved. In this first issue, I am happy to introduce you to the overall project concepts, strategies. and models that make and (will) shape EU-ToxRisk.

Bob van de Water, Leiden Academic Centre for Drug Research (LACDR), Leiden University (The Netherlands), and coordinator of the EU-ToxRisk project


chemical selection
concentrations
selection of test systems
case studies
in silico prediciton of ADME and toxicity; biokinetics data
2D and 3D high throughput and high content pathway toxicity reporters
HepG2 + CYPs HepaRG
semi3D sandw. PHH
multicell 3D PHH
2D hRPTEC
3D hRPTEC
primary hRPTECT
2D hLEC
3D air/liquid hLEC
primary A/L 3D hLEC
2D SH-SY5Y LUHMES
iPSC sensory neurons
iPSC 3D floating neurons
human EST-cardiac neuro
neurite growth assay
zebra fish embryo
translation to fresh human tissue, patient samples and human genetics
Liver
Kidney
Lung
Neuro
DART
tiered testing
Exploitation
Risk assessment
RDT
IATA Strategies
DART
computational modelling
quantitative key event
transcriptomics
cytotoxicity
biokinetics
in silico and experimental ADME

The EU-ToxRisk Harvest Season Has Begun

From its start, the EU-ToxRisk project has been fostering a robust toxicity testing strategy, integrating state-of-the-art *in vitro* and *in silico* technologies for mechanistic, animal-free safety assessment, applicable across industry sectors and acceptable for regulatory purposes.

Further uptake was galvanized via open discussions with regulators, industry stakeholders, and international toxicology programs. These knowledge exchanges fed into various tangible project outcomes.

To date, the consortium produced over 85 publications based on almost 800 deposited datasets from over 150 different new approach methodologies (NAMs). These methods have been extensively characterized and described, adhering to high-level quality parameters and have given rise to a publicly accessible methods database (see the previous newsletter edition for more details).

For this newsletter, we are thrilled to introduce two key outcomes of the project:

1. The EU-ToxRisk NAM-enhanced Read-Across (RAx) Advisory Document

2. The EU-ToxRisk Integrative Testing Platform

The EU-ToxRisk NAM-enhanced RAx Advisory Document was built on the foundations of the first round of project RAx case studies (CSs). The Advisory Document is the consolidated result of feedback, critical observations, and endorsements on taken approaches as drawn from the EU-ToxRisk Regulatory Advisory Board, from the OECD, and from multiple regulatory toxicologists involved in the discussions.

The Advisory Document targets the broader toxicology community and contains practical instructions on its applications in different regulatory contexts. Its application will improve submission quality of read-across cases by registrants and thereby increase successful acceptance rates of non-animal approaches.

Parallel to improving read-across procedures, the EU-ToxRisk project also worked on establishing IATAs for both repeated-dose toxicity (RDT) as well as developmental and reproductive toxicology (DART) testing. The collected experience and expertise form the basis of the EU-ToxRisk Integrative Testing Platform.

The EU-ToxRisk Integrative Testing Platform will offer on-demand, fit-for-purpose packages to interested stakeholders. The platform will integrate results from different sources and use the integrated results in both safety assessment and investigative toxicology. It is based on NAM-based risk assessment, as developed in the EU-ToxRisk project, and will commercially be offered to end-users as testing services and expert consultancy to the larger risk assessment community.

Involved EU-ToxRisk experts will explain these exciting tools in this issue.

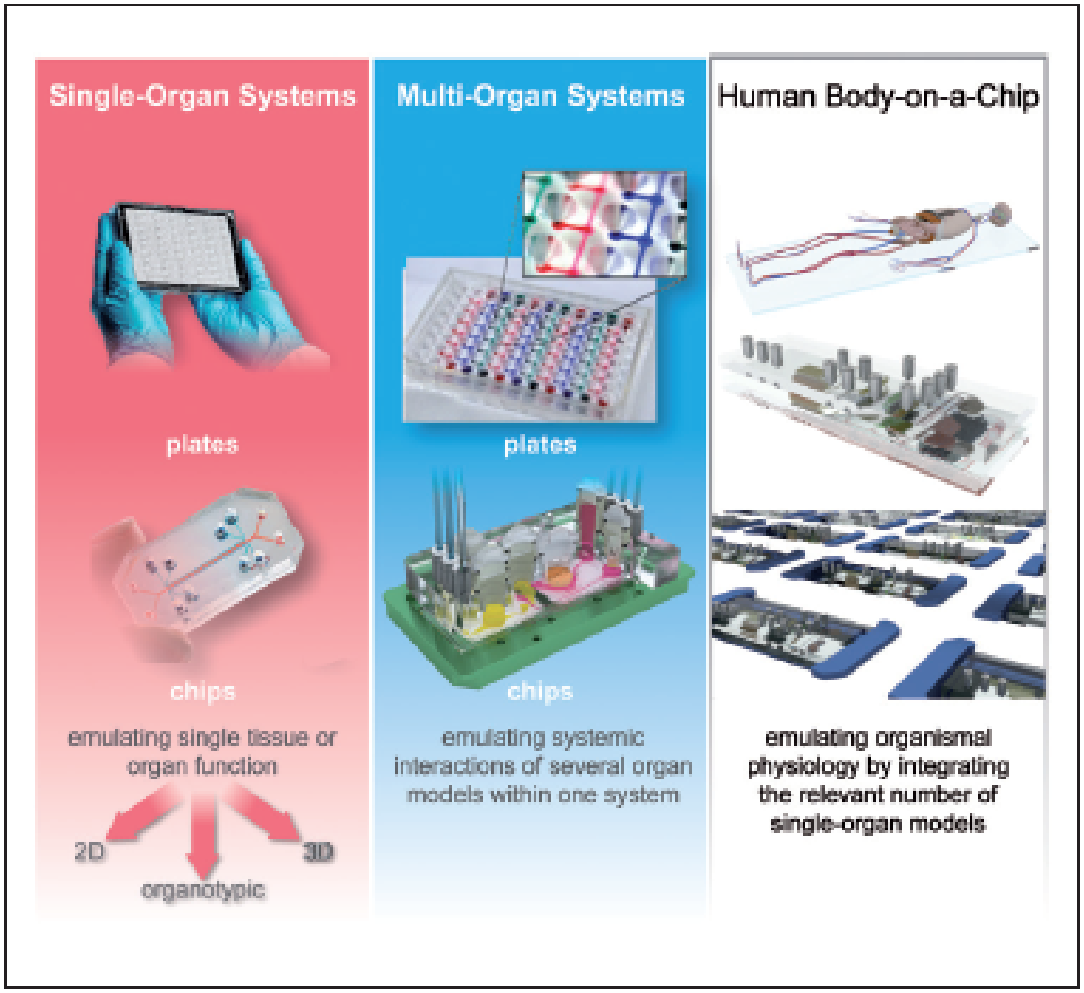


Fig. 3: Types of MPS used for emulation of human biology *in vitro*

Mark U., et al, ALTEX、33(3)272-321（2016）

Conference plan

1st Microphysiological Systems World Summit, MPS-WS-1, New Orleans, LA, USA, 12/13-12/16/2021

<table>
<tr><th></th><th colspan="5">MONDAY</th><th colspan="2">TUESDAY</th><th colspan="2">WEDNSDAY</th><th colspan="2">THURSDAY</th></tr>
<tr><td>8 am</td><td colspan="2"></td><td colspan="3"></td><td colspan="2">Key note: Michael Shuler</td><td colspan="2">Key note: Denise Hinton (tbc)</td><td colspan="2">Key note: tbc</td></tr>
<tr><td>9am-11am</td><td colspan="2" rowspan="2">Hands-on training: Cell models for MPS
Hands-on training: technologies/bio engineering</td><td colspan="3" rowspan="2">Hands-on training: technologies/bio engineering</td><td>S3: Organ Models: use case studies (e.g. Nociception, addiction, regenerative medicine)</td><td>S4: Reproducibility and robustness; Endpoint readouts & analytical tools standardization and harmonization (best practices)</td><td>S7: Models: Predictive toxicology, determine adversity, link to AOP</td><td>S8: Advances in MPS by introducing Immune system and vascularization</td><td>S11: Models: Precision medicine and clinical trial on chip planning</td><td>S12: MPS for Rare diseases</td></tr>
<tr><td rowspan="3">11:30 – 2 pm</td><td colspan="2" rowspan="2">Debate: Concordance MPS vs. in vivo animal and human data</td><td>Workshop: Human cell sourcing and ethical considerations</td><td>Computational modeling and MPS: AI, machine learning , virtual organs/patient</td><td colspan="2">Poster sessions</td></tr>
<tr><td colspan="5" rowspan="2">Opening ceremony,
Keynote: Don Ingber</td><td colspan="2" rowspan="2">Poster session</td><td colspan="2" rowspan="2">Poster session</td><td>Round table: Data; collection, storage, management and dissemination</td><td>Round table: Partnerships and Collaborative Efforts towards the society</td></tr>
<tr><td>MPS/MPPS: live</td><td>MPS/MPPS: brain</td><td>MPS/MPPS: lung</td><td>MPS/MPPS: skin</td><td colspan="2">Closing ceremony</td></tr>
<tr><td>2:30-4:30 pm</td><td>MPS/MPPS: kidney</td><td>MPS/MPPS: heart</td><td>MPS/MPPS: gut</td><td>Multi-organ-on chip</td><td>S5: Reproducibility and robustness; Analytical qualification and validation standardization and harmonization (best practices) , GCCP</td><td>S6: PBPK, IVIVE, metabolism for MPS</td><td>S9: Regulatory acceptance: where we are</td><td>S10: Models: Drug Efficacy</td><td colspan="2" rowspan="2">Satellite meetings</td></tr>
<tr><td>5 pm</td><td colspan="5">Welcome reception</td><td colspan="2">Poster session and get together (music social event)</td><td colspan="2">Posters
Social event/dinner</td></tr>
</table>

Japanese Center for the Validation of
Alternative Methods (JaCVAM)
Center for Biological Safety and Research (CBSR)
National Institute of Health Sciences (NIHS)
3-25-26, Tonomachi, Kawasaki-ku, Kawasaki 210-9501 JAPAN
URL: http//jacvam.jp/

15 May, 2020

Prof. Bob van de Water,
Division of Drug Discovery and Safety /
Leiden Cell Observatory High Content Imaging Screening Facility

We are aware of your initiative of coordinating the RISK-HUNT3*R* proposal that will be submitted for funding under the H2020 topic "Advancing the safety assessment of chemicals without the use of animal testing" (SC1-BHC-11-2020). This proposal is in line with the overall long term goals of JaCVAM for the development and application of new approach methodologies for next generation risk assessment of chemical entities.

stay closely associated with the project when funded. At the earliest convenience after start of the project we would greatly look into possibilities for close collaboration. In this context we have already been discussing about the planning of a workshop to align our joint research capabilities and objectives at an international level that would enhance the animal-free testing approaches and translates our findings closer to an in vivo human setting. Topics for such a workshop and further collaboration could particularly including in vitro-in vivo extrapolation (IVIVE), transcriptomics and cell and tissue culture as a disease model.

We look forward to work with the RISK-HUNT3*R* project partners, as human-relevant next-generation risk assessment, which your consortium aims to pursue through innovative scientific and regulatory strategies, is a highly relevant topic that requires strong initiatives.

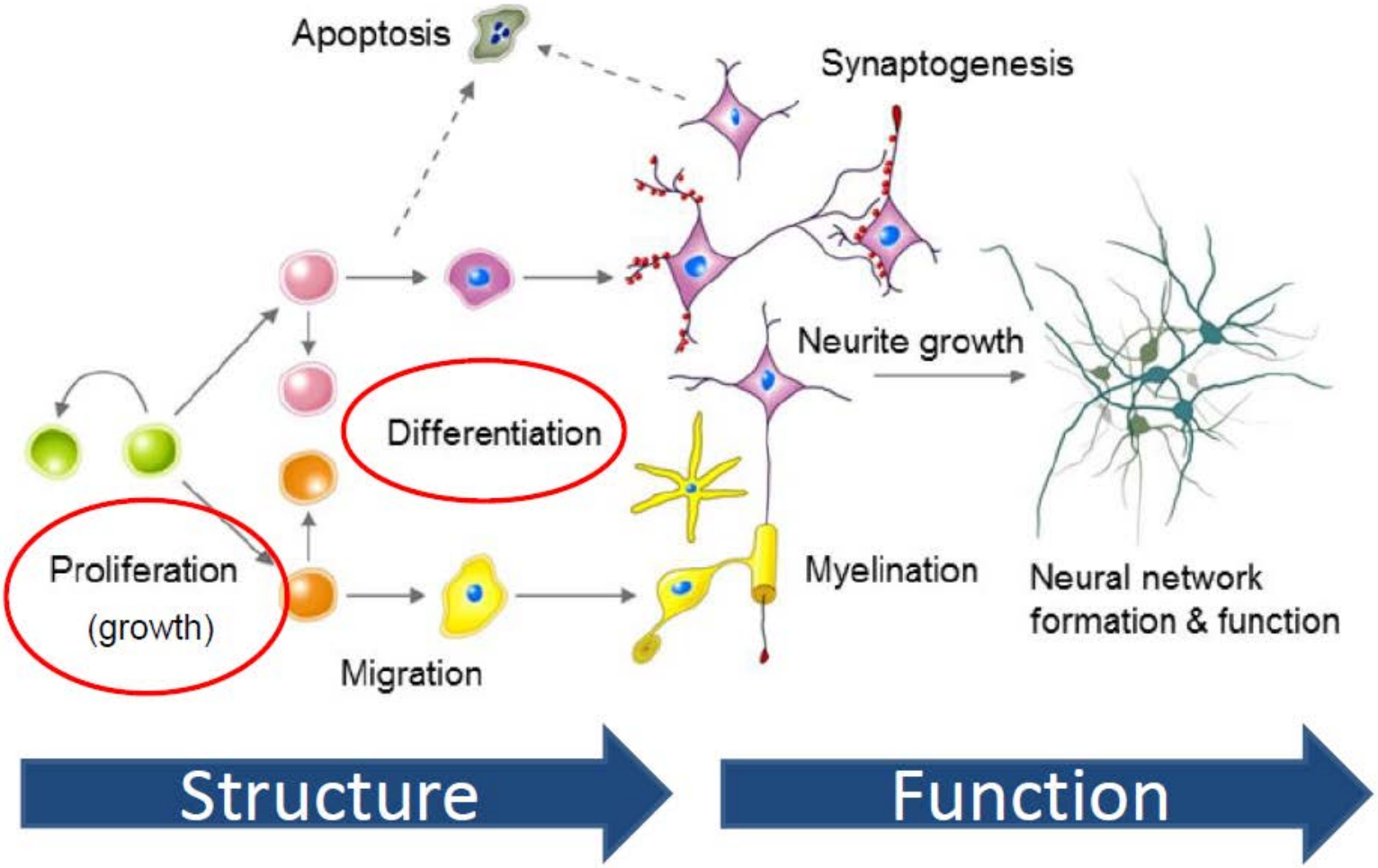
Sincerely yours,

Yoko Hirabayashi

Yoko Hirabayashi, MD
Head of JaCVAM
Director of CBSR
NIHS

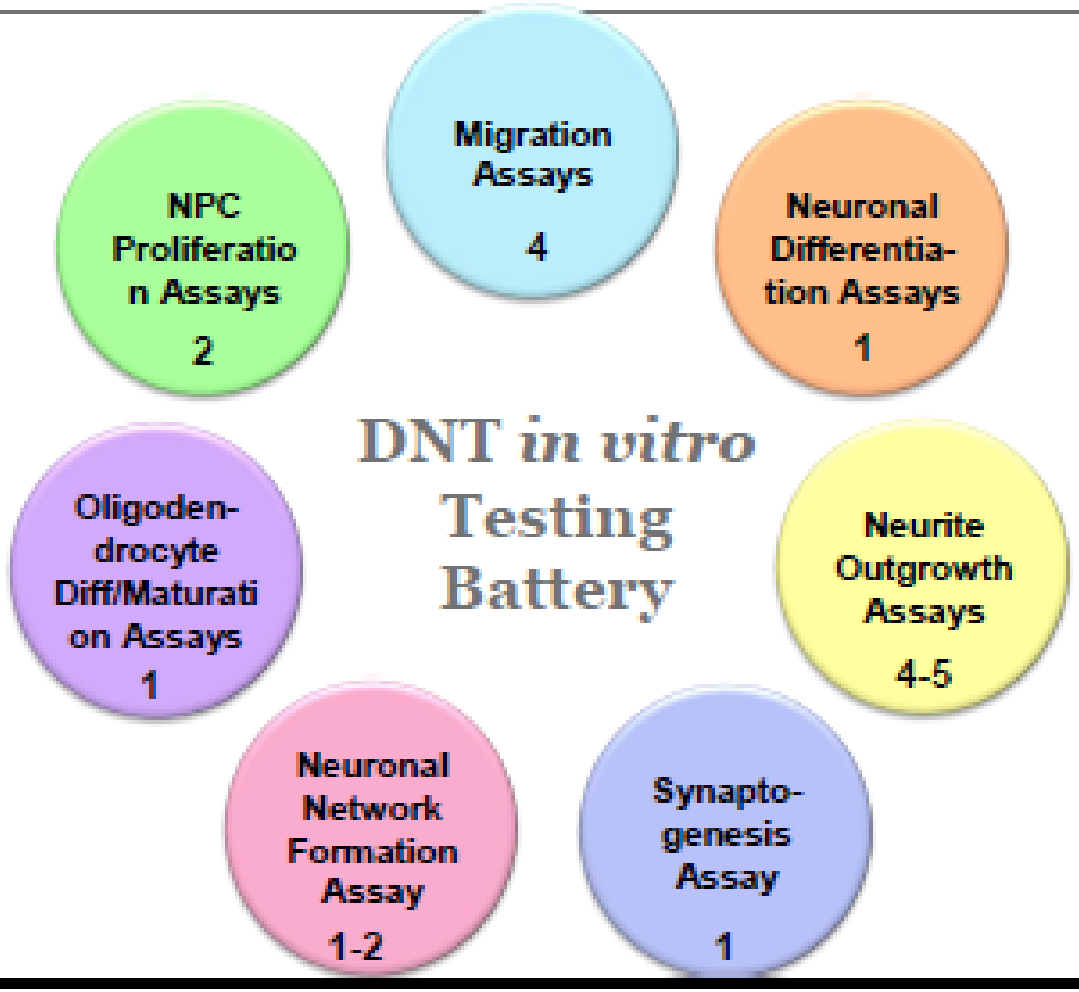
OECDの動向

Structural and functional aspects of DNT using iPSC technology



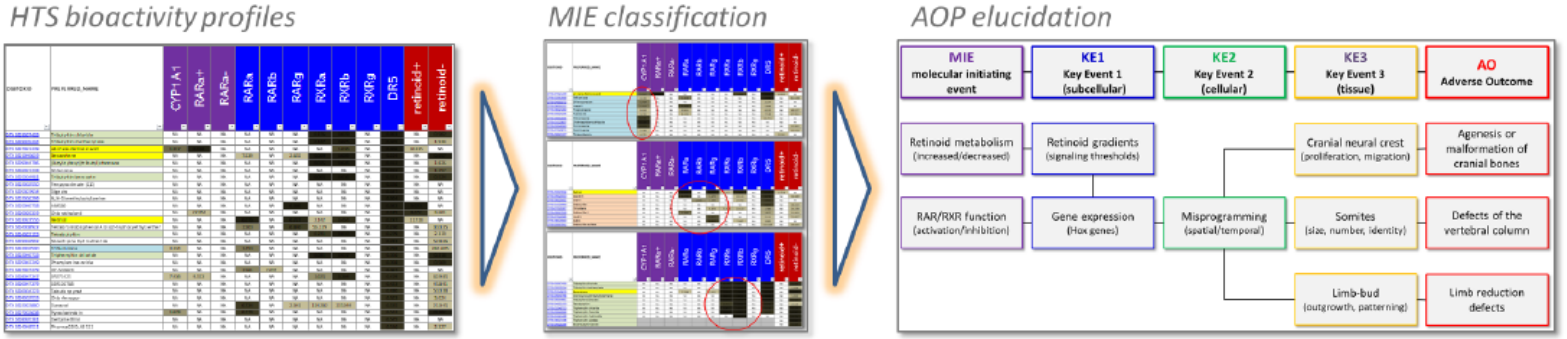
ALTEX. 2017;34(1):49-74.

The current DNT testing strategy



n = number of assays

In vitro profiling for bioactivity on the retinoid system

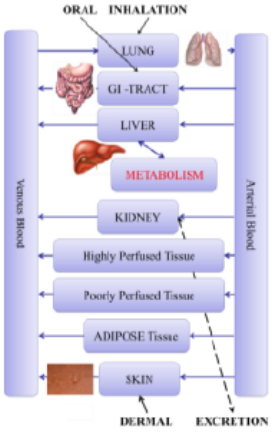


- ToxCast/Tox21 portfolio https://comptox.epa.gov/dashboard/assay_endpoints/
- 1984 active hit calls on 12 assays; 261 where AC50 is < 2 µM in one or more assays
- picks up potential MIEs for:
  - triazole effects on ATRA homeostasis
  - organochlorine effects on RARg and RARE-transactivation
  - preferential activation of RXR and RARE by organotin biocides
- retinoids (ATRA, retinol), rexinoids (bexarotene) and other reference compounds

SOURCE: updated from Baker et al. 2018

Physiologically Based Kinetic (PBK) models

A general term PBK is used. Noting that PBK, PBPK, PBBK and PBTK are synonyms.



Typical PBK models	New Generation PBK models
Calibration and evaluation of the model rely on in vivo data	Development of the model rely on in vitro or in silico methods
Model structure reflects a balance between the principles of parsimony and plausibility	Model structure reflects mechanistic understanding of biology and biochemistry
"Familiar uncertainty"	"Unfamiliar Uncertainty"

The aim of this document is to provide guidance on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models intended for use in the regulatory assessment of chemicals in cases where no *in vivo* kinetic data are available for model validation.

**Builds on existing guidance (EPA 2006, WHO 2010, EFSA 2014, CEN2015, FDA-EMA2019)

DRAFT GUIDANCE DOCUMENT ON THE CHARACTERISATION, VALIDATION AND REPORTING OF PBK MODELS FOR REGULATORY PURPOSES

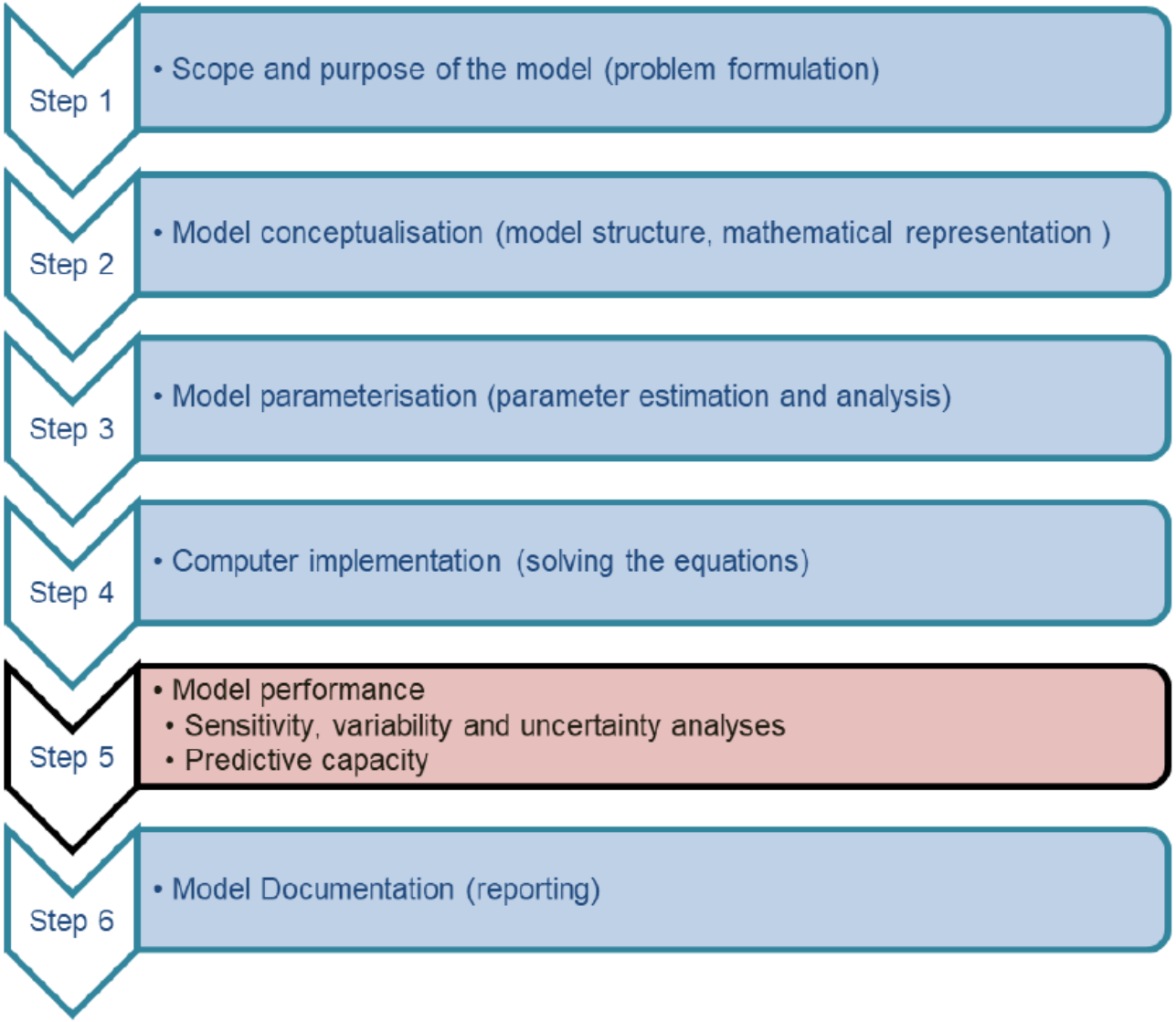


Figure 1. Workflow for PBK model development, validation, reporting and dissemination

Guidance Document for Consistent Reporting of 'Omics Data From Various Sources

-Transcriptomics Reporting Framework (TRF)
-Metabolomics Reporting Framework (MRF)

WNT meeting
April 2020

OECD
BETTER POLICIES FOR BETTER LIVES

TRF Document Structure

Toxicology Experiment Reporting Module

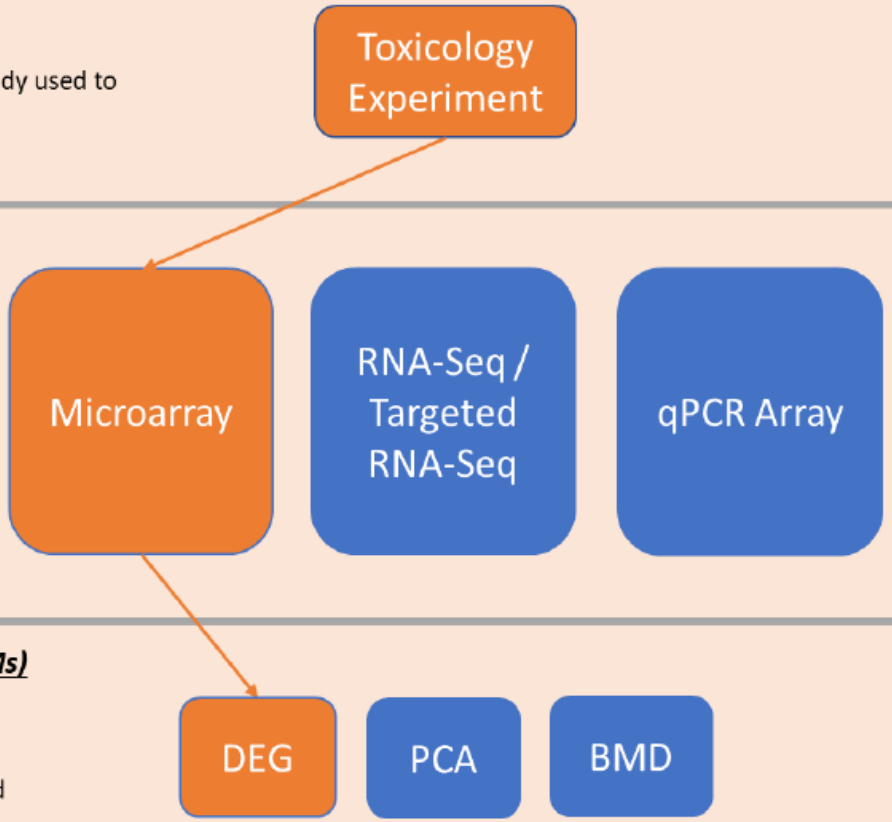
- Details the specifics of a laboratory-based toxicology study used to generate biological samples for transcriptomic analyses.

Technology-Specific Reporting Modules

- Details technology-specific aspects of data generation and initial processing of transcriptomic data, including:
  - Transcriptomic Technology Description
  - Transcriptomics Experimental Design
  - Specification of Raw Data
  - Data Normalization
  - Data Filtering
  - Data QC

Downstream Analysis Reporting Modules (DARMs)

- Details the steps and resources necessary to reproduce computational analyses of transcriptomic data.
- Intended to be coupled with upstream experimental and technology-specific reporting modules.



Detailed Review Paper

In vitro immunotoxicity testing

Contractor:

Hajime Kojima

Japanese Center for the Validation of Alternative Methods (JaCVAM)

National Institute of Health Sciences

3-25-26, Tonomachi, Kawasaki-ku, Kawasaki, JAPAN

Organisation for Economic Co-operation and Development

ENV/JM/HA(2020)13

For Official Use

English - Or. English

27 May 2020

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Working Party on Hazard Assessment

Addressing the Mutual Acceptance of Data from Computational Methods within OECD Test Guidelines

Towards a Good Computational Methods Practice

ICHの動向

厚生労働省医薬食品局審査管理課長

医薬品の遺伝毒性試験及び解釈に関するガイダンスについて

２．新ガイダンスの要点

(1)　S2A ガイダンス及び S2B ガイダンスを一つにまとめたこと。

(2)　遺伝毒性試験の標準的組合せについて、２つのオプションを提示したこと（*in vitro* ほ乳類細胞試験を含むものと、含まないもの）。

(3)　*in vitro* ほ乳類細胞試験において、最高濃度の上限を１mM 又は 0.5 mg/mL のいずれか低い濃度としたこと。

(4)　*in vitro* ほ乳類細胞試験として *in vitro* 小核試験の利用を認めたこと。

(5)　条件によっては、*in vivo* 遺伝毒性試験を反復投与毒性試験に組み込んでもよいこととしたこと。

(6)　第２の *in vivo* 試験として DNA 傷害性を評価できるコメット試験を推奨するとしたこと。

(7)　条件によっては、細菌を用いる復帰突然変異試験を１試験だけでもよいこととしたこと。

医薬品の光安全性評価ガイドラインについて

日米 EU 医薬品規制調和国際会議（以下「ICH」という。）が組織され、品質、安全性及び有効性の各分野で、ハーモナイゼーションの促進を図るための活動が行われているところである。

今般、医薬品の製造販売承認に際して添付すべき非臨床における安全性試験の資料に関し、ICH における三極の合意事項として、新たに「医薬品の光安全性評価ガイドライン」を別添のとおり定めましたので、下記事項を御了知の上、貴管内関係業者等に対し周知方御配慮願います。

記

1. 本ガイドラインの要点

(1) 本ガイドラインは、医薬品の光毒性及び光アレルギー検出のために行われる安全性評価の望ましい実施方法を示すものであり、従来の非臨床試験に係るガイドラインを補完するものである。

(2) 本ガイドラインは、新規医薬品有効成分、新規添加剤、経皮投与用臨床製剤（皮膚貼付剤等）、及び光線力学療法用製剤に適用される。

(3) 本ガイドラインで取り扱う内容は、光毒性（光刺激性）及び光アレルギーであり、光遺伝毒性及び光がん原性については取り扱われない。

(4) 光安全性評価の実施については、薬剤開発者に委ねられているが、外来での臨床試験を行う前に、光毒性の初期評価（UV～可視光領域の吸収スペクトルの評価等）の実施が提案・推奨されるほか、必要に応じ、実験的評価（*in vitro* 又は *in vivo* 試験）を実施すべきとされている。

(5) 光安全性評価の方法はフレキシブルであり、様々なアプローチが選択し得る。

「医薬品の臨床試験及び製造販売承認申請のための非臨床安全性試験の実施についてのガイダンス」について

１．背景

優れた医薬品の国際的な研究開発の促進及び患者への迅速な提供を図るため、承認審査資料の国際的なハーモナイゼーション推進の必要性が指摘されている。このような要請に応えるためＩＣＨが組織され、その合意に基づき、本ガイドラインが改正された。

２．改正の要点

動物実験の３Ｒ（使用動物数の削減／苦痛の軽減／代替法の利用）の原則に従って、各非臨床試験に関する見直しを行うとともに、新たに、一般毒性試験のための高用量の選択、早期探索的臨床試験のための非臨床試験、免疫毒性、光安全性試験、薬物乱用に関する非臨床試験及び配合剤のための非臨床試験等の考え方についての指針を示した。

1. 緒言

1.1 ガイダンスの目的

本文書の目的は、ヒト臨床試験の範囲と期間に応じて、また、製造販売承認を得るために推奨される医薬品の非臨床安全性試験についての国際的な基準を勧告し、そのハーモナイゼーションを促進することである。

各種非臨床安全性試験のガイダンスのハーモナイゼーションによって、現在の要求事項が明らかにされ、実質的な相違が各地域間に存在する可能性が減少すると期待される。

このガイダンスは、臨床試験の実施時期を適正化し、3R（使用動物数の削減／苦痛の軽減／代替法の利用）の原則に従って動物の使用を抑え、医薬品開発のための資源の有効利用に資するであろう。本ガイダンスでは論じていないが、安全性評価のための新しいインビトロ代替法の利用について考慮すべきである。これらの代替法は、バリデーションが完了し、全てのICH規制当局によって認められれば、現在の標準試験法の代わりに利用可能である。

本ガイダンスによって、医薬品の安全で倫理にかなった開発が促進され、新医薬品を一層早く利用できるようになるであろう。

ICH S5 (R3) guideline on reproductive toxicology: Detection of Toxicity to Reproduction for Human Pharmaceuticals

Step 5

4.2.2　胚胎児発生（EFD)リスクのため毒性作用の理解の代替法

- **創薬スクリーニングとして使用され、に役立つ。**
- **非臨床データを人間のリスクに変換するのに役立つ。**
- **適正があれば、代替法は従来の*in vivo*試験を延期または置き換える可能性がある。**
- **動物の使用を減らす可能性がある。**
- **代替法を組み込むアプローチは、現在の試験で提供されているものと少なくとも同等のヒト安全保証にある程度の信頼性が必要である。**
- **科学的発展の方向性に基づいて、規制の目的で、段階的アプローチまたは組み合わせプローチ内で、複数の代替法が使用されることが予想される。**
- **これらの試験戦略は、試験法の化学的および生物学的適用範囲内で適正とされる。**

ICH S5 (R3) guideline on reproductive toxicology: Detection of Toxicity to Reproduction for Human Pharmaceuticals

Step 5

補遺２　動物実験代替法

単独、または1つ以上の*in vivo*試験と組み合わせて実施された、適性な代替法から集められたデータは、限られた状況下での有害性の同定とリスク評価に利用できる。

- 胚胎児発生への悪影響を示唆する証拠がある状況（例：発生生物学の基本的な経路に影響を与える作用機序、遺伝子組み換え動物の表現型データ、クラス効果）
- 動物種の毒性により、使用条件下でのヒトへの暴露に関連する全身への暴露が得られない
- 動物実験であいまいな所見があった場合の証拠評価の重み付けのサポートとして
- 最大3か月間の最大150の妊娠可能な女性を含む臨床試験の部分的なサポートとして
- 特定の重度の衰弱性または生命を脅かす疾患または晩年発症疾患のために開発されている医薬品

ICH S5 (R3) guideline on reproductive toxicology: Detection of Toxicity to Reproduction for Human Pharmaceuticals

Step 5

奇形/胚/胎児毒性を適正に予測するNAMの基準

- **予測指標を含む、予測モデルの正当性**
- **胚胎児発生のメカニズムの説明を含むモデルの生物学的妥当性**
- **陽性基準（陰性か陽性の判断）**
- **奇形または胎児の致死を予測する分子および代謝マーカーの閾値と正当性**
- **決定樹のアルゴリズム**
- **トレーニングセットとテストセットの参照化合物のリスト**
- 参照化合物リストから取得されていない場合、in vivo曝露および奇形または胎児胎児の致死データのデータソース
- **代替法の生物学的および化学的適用範囲**
- **in vivoでの発生結果を予測するための感度、特異性、および再現性**
- 複数のアッセイが実施される場合、予測モデルに使用される統合評価に加えて、各アッセイの性能
- **陽性対照を含む、アッセイの開発と使用に関する履歴データ**

In vitro methods for reproductive toxicity

More than 30 different culture systems have been proposed

1) Tests on non-vertebrate species (*Hydra*, *Drosophila* etc.)
2) Tests on lower vertebrate embryo or embryonic aggregates (fish, birds etc.)
3) Tests on whole mammalian embryos
4) Tests on micromass cultures from mammalian embryos
5) Tests on embryonic stem cells (ES cells)
6) Tests on other mammalian cell lines (neuroblastoma cells, teratocarcinoma cells etc.)

(Food Chem. Toxicol.,2002 ,40,193)

No tests gain regulatory acceptance and use.

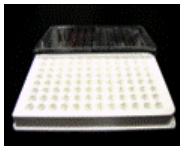
The research on alternative methods for detection of embryotoxicity is very challenging!

Hand1 gene related pathways

Day 0

Hand1-ES (KOB1)cells

Preparation of test compound and Establishment of the maximum dose



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120 Hr (Day 5)

- Cell viability assay (CellTiter Fluor) → ES-IC_{50} (cytotoxicity)
- Luc-activity (Steady-Glo) → ES-ID_{50} (Differentiation toxicity)

Data analysis

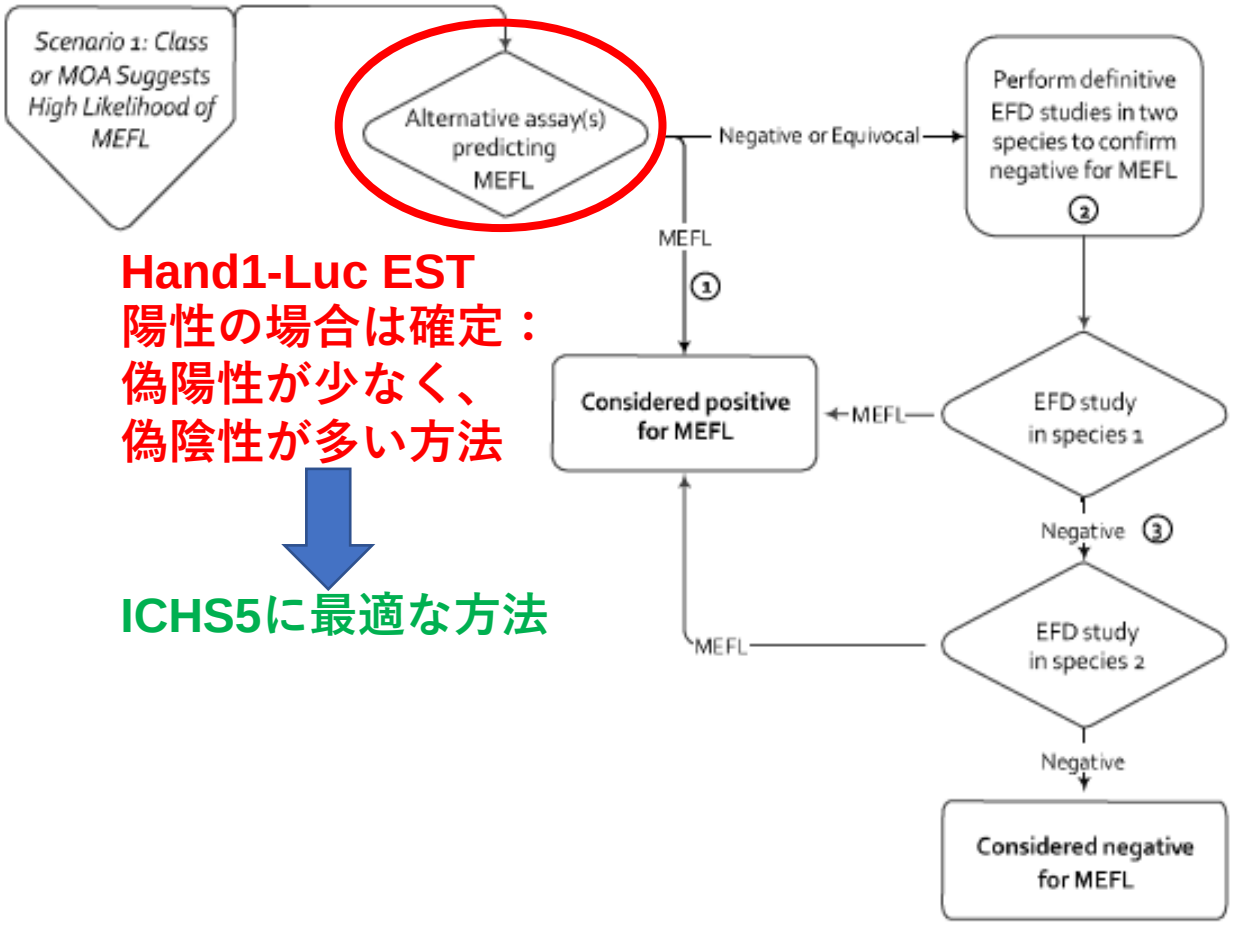
Hand1-Luc EST is an easy and inexpensive protocol

Predictive capacity of the Hand1-Luc EST with the most recent prediction model

		Hand1-Luc EST	
		Positive	Negative
In vivo	Positive (44)	21	23
	Negative (27)	5	22
		(26)	(45)

Predictivity parameter	Value
Sensitivity	47.7% (21/44)
Specificity	**81.5%** (22/27)
Positive predicted value	**80.8%** (21/26)
Negative predicted value	48.9% (22/45)
Accuracy	60.6% (43/71)

Figure 1: Use of Alternative Assays for Pharmaceuticals Expected to be EFD Toxicants



Hand1-Luc EST
陽性の場合は確定：
偽陽性が少なく、
偽陰性が多い方法

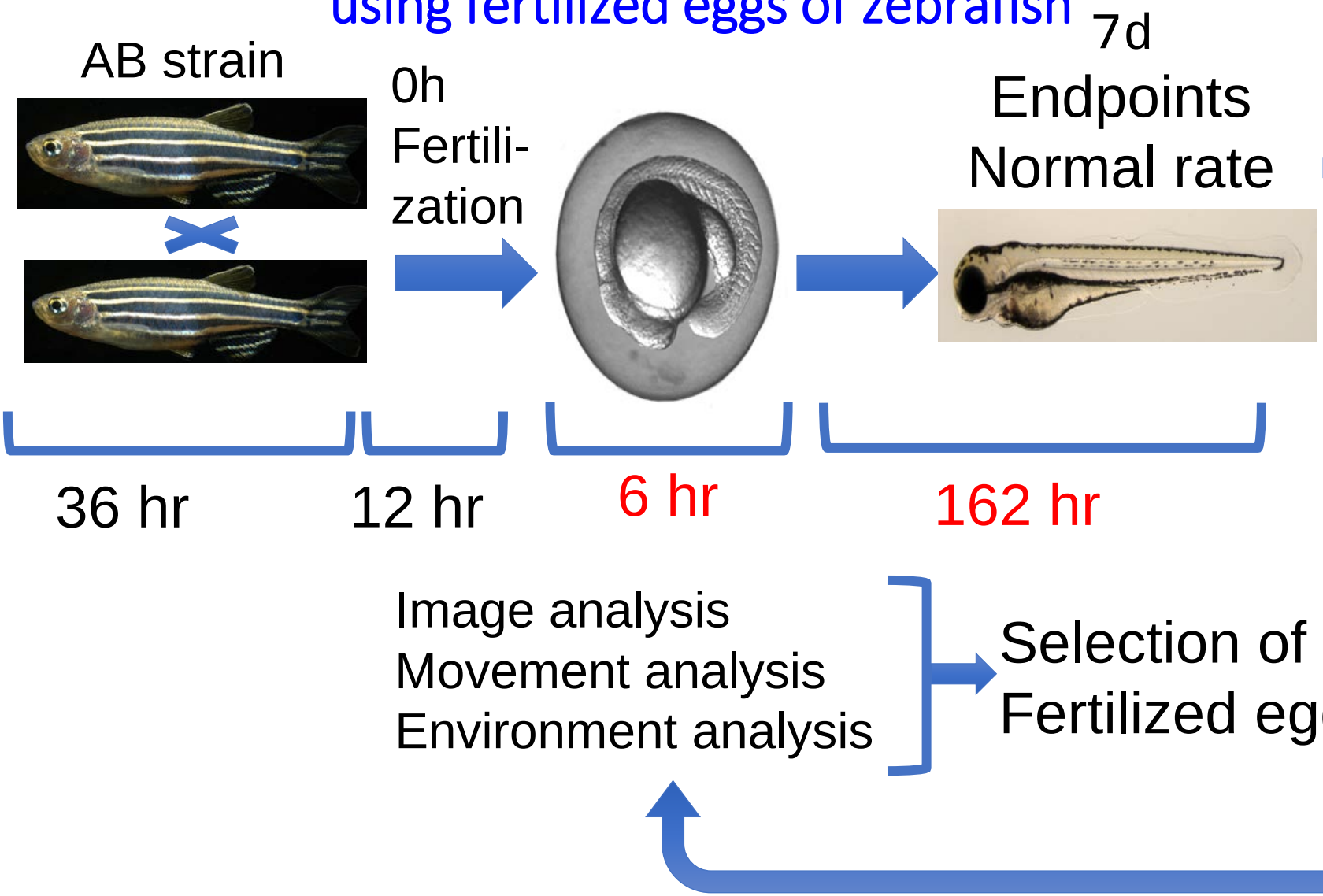
ICHS5に最適な方法

1) No additional assessment is warranted if unequivocal MEFL signal is observed at clinically relevant extrapolated exposures.

2) Alternatively, pEFD studies can be used; however, negative results should be confirmed by a definitive study in the relevant species

3) Conducting *in vivo* EFD studies in series, as shown, can permit reduction in animal use, as 2nd *in vivo* assay is not warranted if the first study is positive.

Development of protocol for embryotoxicity testing using fertilized eggs of zebrafish



Tanaka, T., et al., MIE Univ.

ICCRの動向

ICCR (International Cooperation on Cosmetics Regulations:
化粧品協力規制国際会議)と
ICATM (International Cooperation on Alternative Test Methods：
代替試験法国際協力会議)

Computational Toxicology 7 (2018) 20–26

Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.elsevier.com/locate/comtox

Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

Matthew Dent[a,*], Renata Teixeira Amaral[b], Pedro Amores Da Silva[b], Jay Ansell[c], Fanny Boisleve[d], Masato Hatao[e], Akihiko Hirose[f], Yutaka Kasai[g], Petra Kern[h], Reinhard Kreiling[i], Stanley Milstein[j], Beta Montemayor[k], Julcemara Oliveira[l], Andrea Richarz[m], Rob Taalman[n], Eric Vaillancourt[o], Rajeshwar Verma[j], Nashira Vieira O'Reilly Cabral Posada[l], Craig Weiss[p], Hajime Kojima[f]

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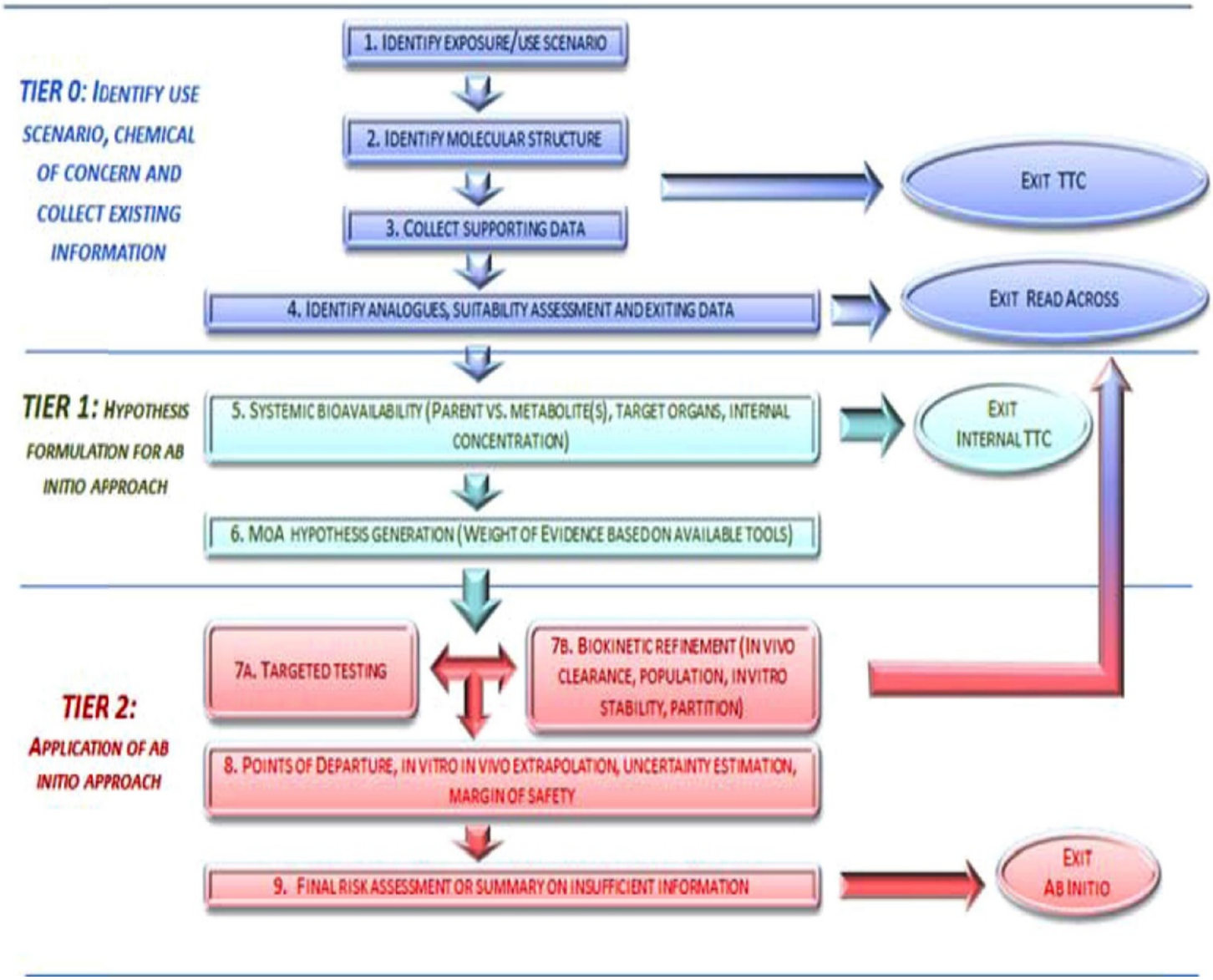
[b] ABIHPEC – Association of the Cosmetic, Toiletry and Fragrance Industry (ABIHPEC), Av. Paulista, 1313 Conjunto 62nd, São Paulo, SP 01311-000, Brazil
[c] US Personal Care Products Council (PCPC), 1620 I St. NW, Suite 1200, Washington, D.C. 20036, USA
[d] Johnson & Johnson Santé Beauté France, Domaine de Maigremont, CS 10615, F-27106 VAL DE REUIL Cedex, France
[e] Japan Cosmetic Industry Association (JCIA), Metro City Kamiyacho 6F, 5-1-5, Toranomon, Minato-ku, Tokyo 105-0001 Japan
[f] National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, 158-8501 Tokyo, Japan
[g] Kao Corporation, External Relations & Government Affairs 2-1-3, Bunka, Sumida-Ku, Tokyo 131-8501 Japan
[h] Procter and Gamble Services Company NV, Temselaan 100, B-1853 Strombeek-Bever, Belgium
[i] Clariant Produkte (D) GmbH, Global Toxicology and Ecotoxicology, Am Unisys-Park 1, 65843 Sulzbach, Germany
[j] US Food and Drug Administration (US FDA), Office of Cosmetics and Colors (OCAC), Center for Food Safety and Applied Nutrition (CFSAN), 5001 Campus Drive, College Park, MD 20740, USA
[k] Cosmetics Alliance Canada, 420 Britannia Road East Suite 102, Mississauga, ON L4Z 3L5, Canada
[l] Brazilian Health Regulatory Agency (ANVISA), Gerência de Produtos de Higiene, Perfumes, Cosméticos e Saneantes, SIA Trecho 5, lote 200, Área Especial 57 – CEP 71205-050, Brazil
[m] European Commission, Joint Research Centre (JRC), Directorate for Health, Consumers and Reference Materials, Chemical Safety and Alternative Methods Unit, Via E. Fermi 2749, 21027 Ispra, VA, Italy
[n] Cosmetics Europe, Avenue Herrmann-Debroux 40, 1160 Auderghem, Belgium
[o] Health Canada (HC), Consumer Product Safety Directorate, Healthy Environments and Consumer Safety Branch, 269 Laurier Ave. W., Ottawa, ON K1A 0K9, Canada
[p] Independent Cosmetic Manufacturing and Distributors (ICMAD), 21925 Field Parkway, Suite 205, Deer Park, IL 60010, USA

Part 2 Report: SEURAT-1 workflow as a basis



化粧品原料の安全性評価に使われる選抜された新規試験法の現状 (Amaral et al., 2018)

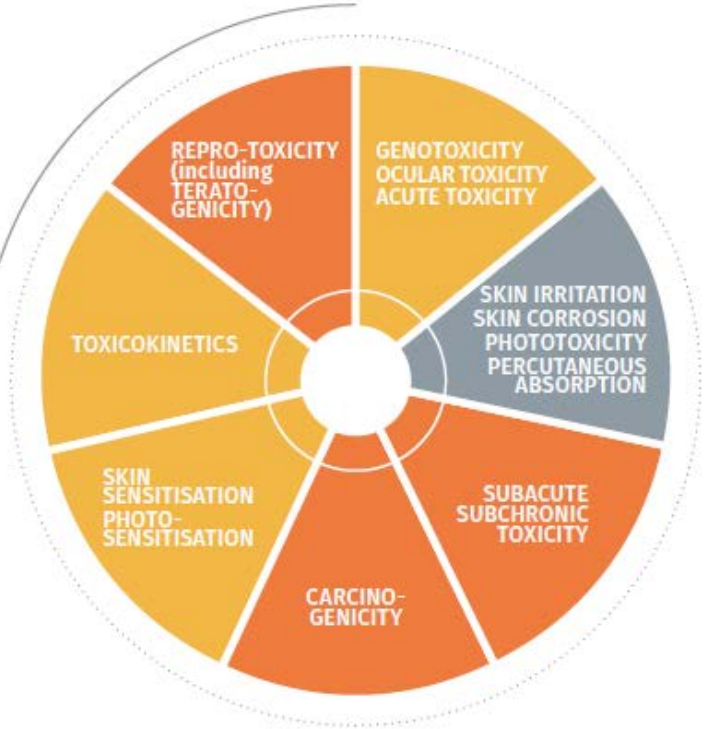
すでに使用中	未成熟な状況	不十分な状況
Read across (カテゴリー分類を含む)	‘Omics (特に transcriptomics)	Organ-on-chip
曝露回避	In vitro薬理学的プロファイリング	ゼブラフィッシュ受精卵試験
In silicoツール	経路モデル	
代謝と代謝物同定	3D培養システム (全身影響)	
薬物動態モデル		
In chemico 試験		
レポーター遺伝子試験		
3D 培養システム (局所および遺伝毒性)		
ヒト試験		

Status of AAT science: prospects by 2020

RESEARCH

ADVANCED

COMPLETED



CASE STUDIES

Test applicability in safety assessment

REGULATORY ACCEPTANCE

Systematic, stepwise integration of new safety assessment approaches in daily practice

Guidance on safety assessment capability gaps

Verification of results in safety assessment practice

DELIVER NEW SCIENCE

Develop new approaches to safety assessment (tools, methods, strategies)

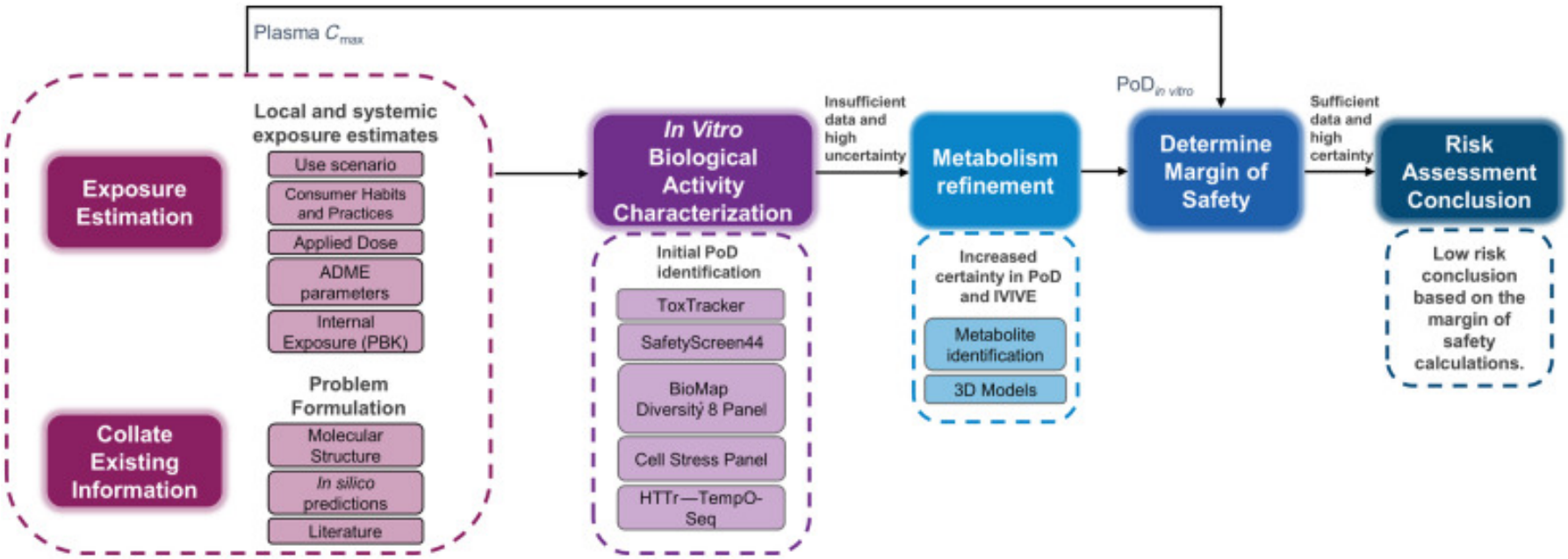
Toxicodynamics, toxicokinetics

Cosmetics Europe

the personal care association

OECD Fifth and Sixth meeting of IATA CS Project

1. Discussion of the case studies
    1. Case Study on use of an Integrated Approach to Testing and Assessment (IATA) and New Approach Methods to Inform a Theoretical Read-Across for Dermal Exposure to Propylparaben from Cosmetics [BIAC (Cosmetics Europe)]
    2. Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to Caffeine [BIAC (Cosmetics Europe)]
    3. Case Study on the use of Integrated Approaches for Testing and Assessment for the Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion [BIAC (Cosmetics Europe)]



A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products Maria T Baltazar, et al. Toxicol Sci. 2020 Jul; 176(1): 236–252.

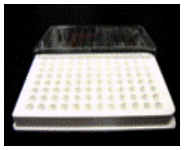
我が国におけるNAMの開発状況について

Hand1 gene related pathways

Day 0

Hand1-ES (KOB1)cells

Preparation of test compound and Establishment of the maximum dose



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- Luc-activity (Steady-Glo) → ES-ID_{50} (Differentiation toxicity)

Data analysis

Hand1-Luc EST is an easy and inexpensive protocol

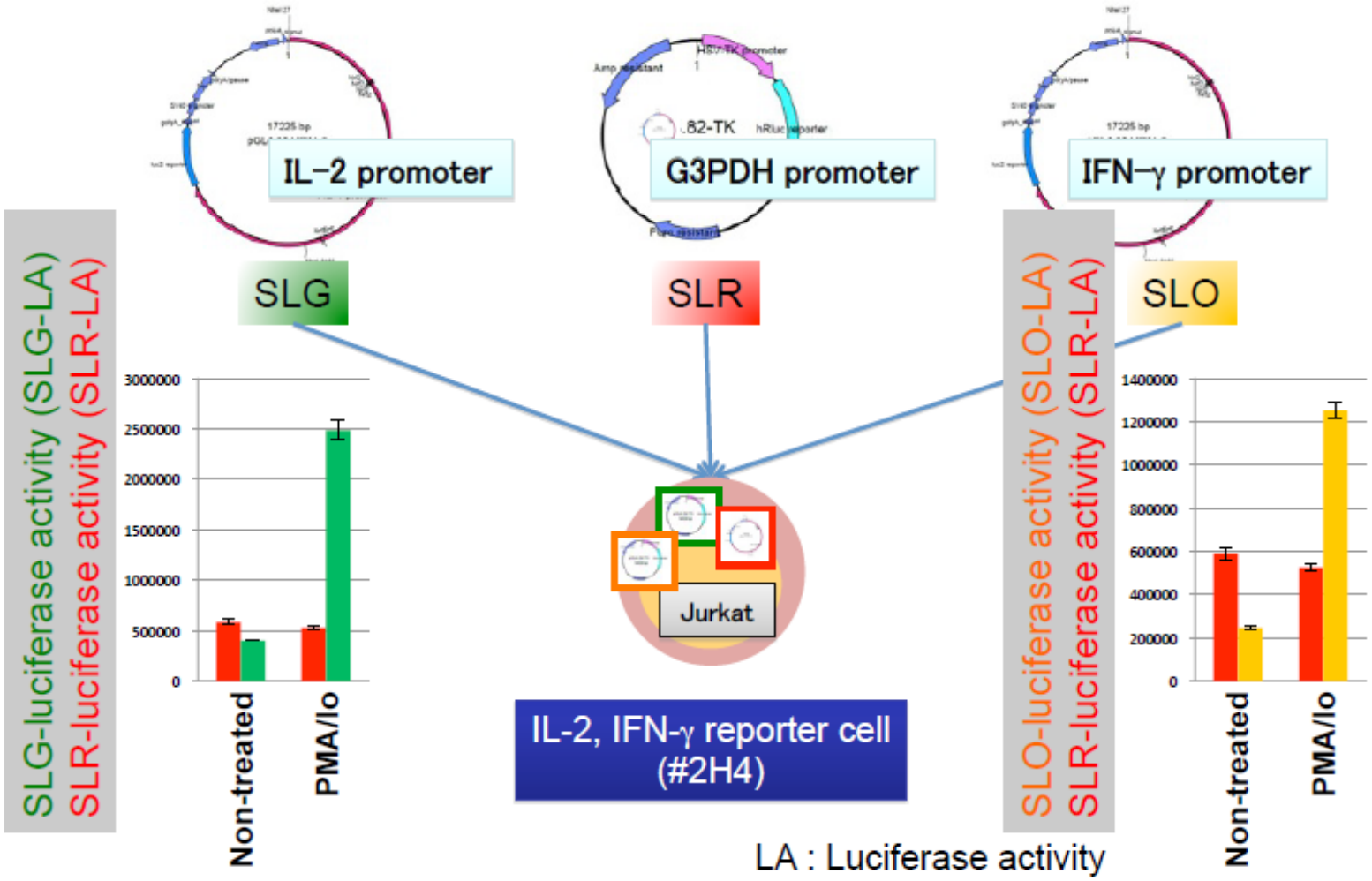
Test Guidelines Programme

DRAFT UPDATED WORK PLAN OF THE TEST GUIDELINES PROGRAMME

30th Meeting of the Working Group of the National Coordinators of the Test Guidelines Programme

Project 4.123: Review and feasibility of an Embryonic Stem Cell Test: In vitro assay detecting disruption to differentiation of rodent embryonic stem cells into cardiomyocytes using the Hand1 gene	
Can you pls insert timelines if known. Lead: Inclusion in work plan: Project status and milestones:	Japan 2017
• 1st step: Detailed Review Paper of available methods and evaluation of utility and application (Q1-Q4 2019); internal project meeting of experts selected by Japan for the drafting in Q1 2019 in Japan; • 2nd step: feasibility study of the development of a Test Guideline, (timelines are not provided yet).	
Subsidiary body of the JM	WNT
Expert group	

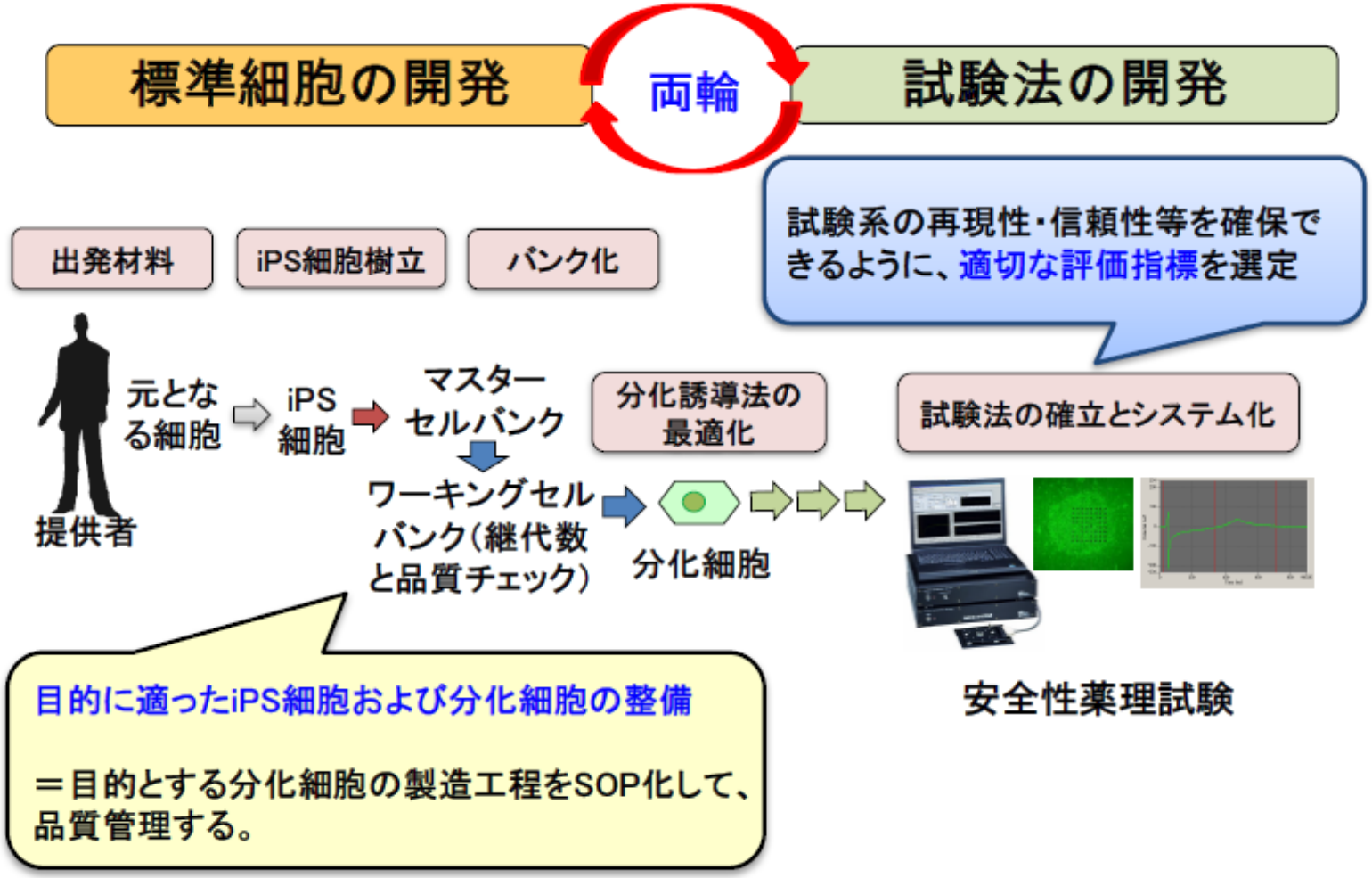
IL-2, IFN-γ reporter cell (#2H4)



Aiba, S., et al, Tohoku Univ.

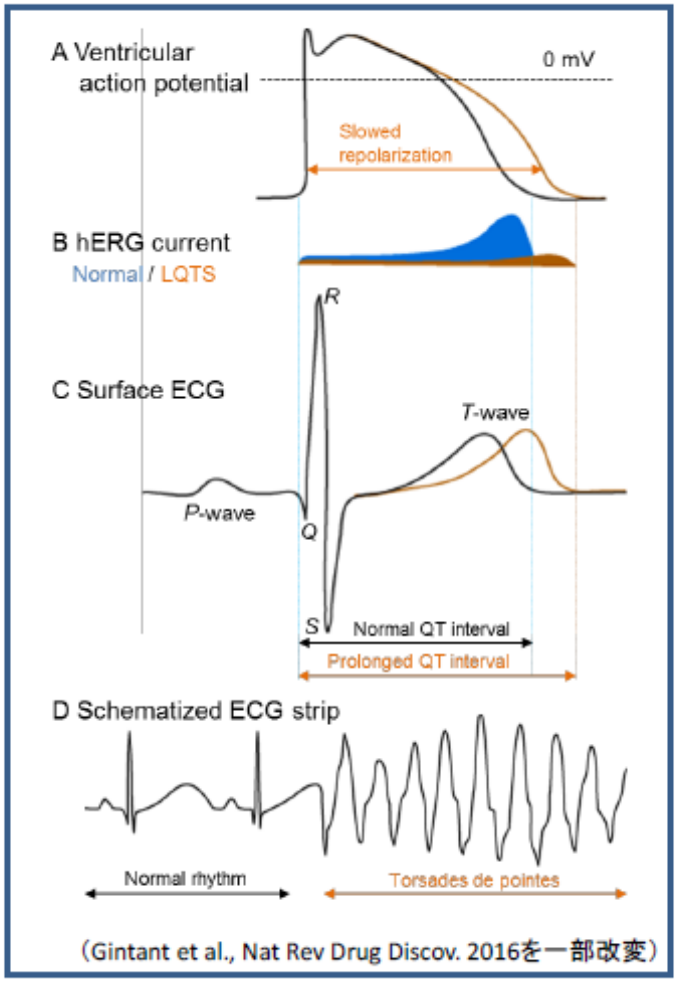
Project 4.134: Detailed Review Paper on application and interpretation of in vitro immune-toxicity assays and definition of a tiered approach to testing and assessment	
Lead: Inclusion in work plan: Project status and milestones:	Japan 2019
2019-2020: • Establish an Ad hoc Expert Group; • tele-conferences (at least 6) plus face to face meeting(s) to address issues identified after the first commenting round; • Discuss the regulatory needs in OECD countries as well as scope and outline of a document for integration of in vivo and in vitro methods for immunotoxicity testing; • Discuss i) which assays are ready-to-use depending on the problem formulation, ii) issues to be addressed upon application to regulation and iii) which assay should be included in the document; • Discuss how the data produced by the assays should be interpreted/used for a tiered approach. And draft the document; • Discuss a tiered approach for a testing strategy in DRP.	
Subsidiary body of the JM	WNT
Expert group	Ad hoc Expert Group on Immunotoxicity (to be established)

ヒトiPS細胞を用いた薬理試験法を実現するために



関野祐子先生（国立衛研・元薬理部長）より提供

hERG試験の問題点およびiPS心筋細胞の利点



現状の問題点

- QT延長作用のある薬剤が必ずTdPを誘発するわけではない
- hERGチャネル阻害試験の偽陽性が多い

より正確に薬剤の催不整脈作用を予測できる試験系が望まれている。
（ヒト、複数のイオンチャネルなど）

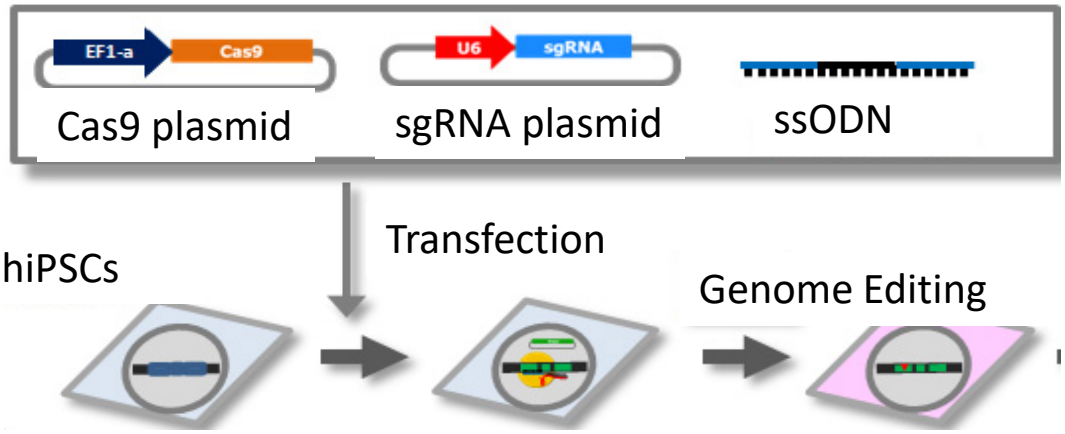
ヒトiPS細胞由来心筋細胞の利用が有用である。

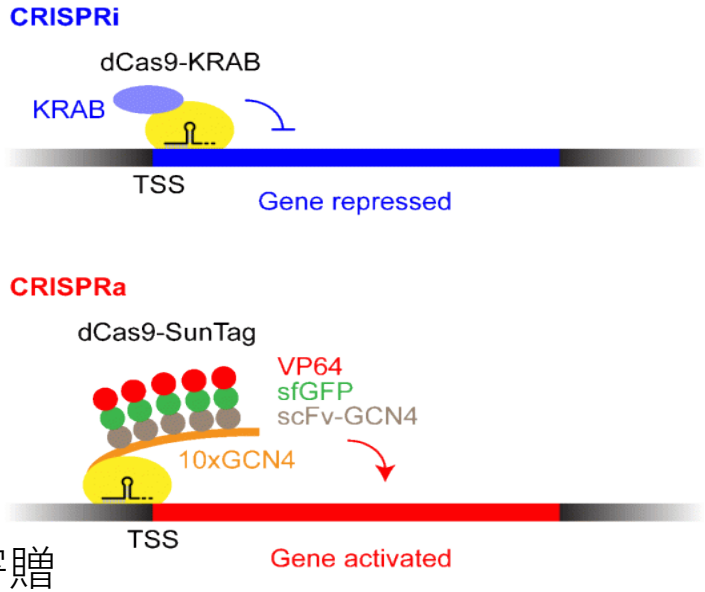
関野祐子先生（国立衛研・元薬理部長）より提供

Basic platforms to characterize disease-specific iPSCs and to develop genome-edited iPSCs

Basic platform of characterization and development of iPSCs

1. Evaluating iPSC lines	2. Identify mutations / genomic Information	3. Make isogenic corrected iPSCs	4. Differentiate into target cell types	5. Identify the disease-related phenotype	6. Make high-through put assays	7. Screening (genes or chemicals)
Self-renewal, pluripotency, vector removal, and karyotype	・Known responsible genes or mutations → Target Sequence ・Unknown disease → Whole genome sequence	Using genome editing technology Option：Artificial mutant iPSCs from healthy donor iPSCs	In order to make disease models depending on the symptoms of diseases Option：Make knock-in fluorescent reporter iPSCs	Compare with healthy-donor or isogenic corrected iPSCs Option: Make humanized animal models	Make 24-well -> 96-well -> 384-well plate formats	Drug development: chemical libraries Genes: CRISPRi & CRISPRa screening systems

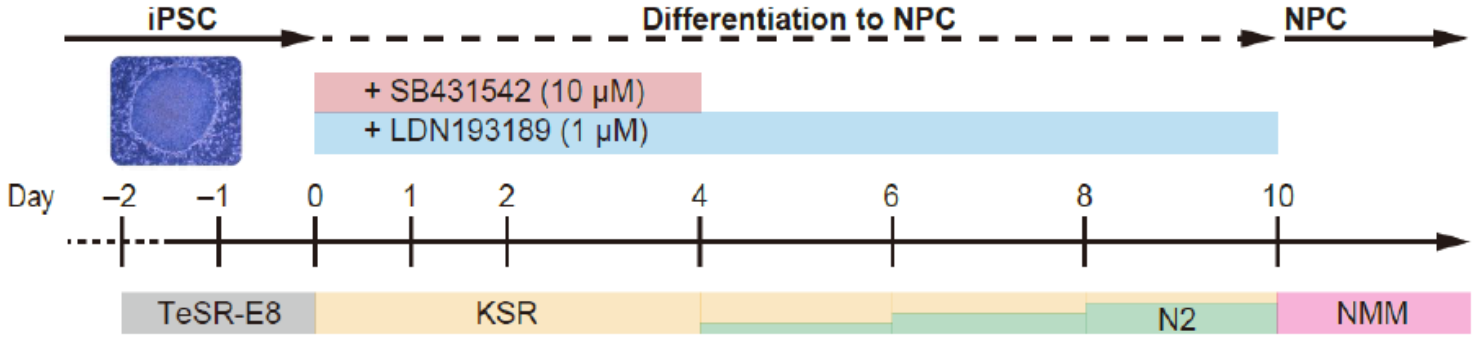




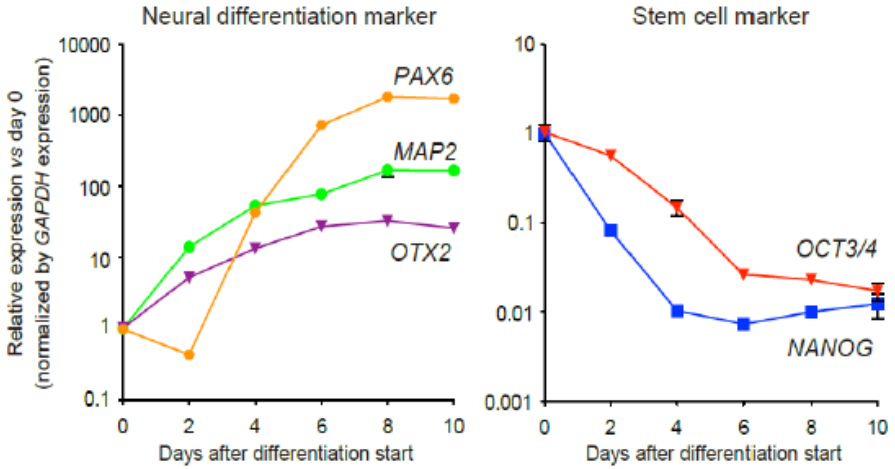
理化学研究所　林先生より寄贈

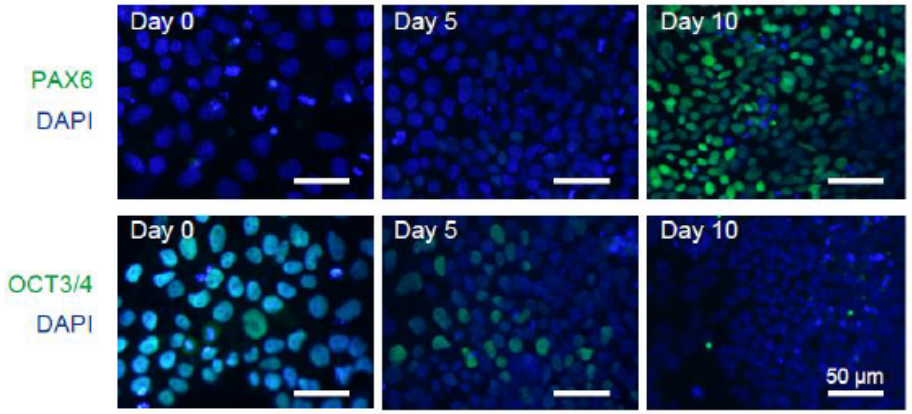
Neural differentiation protocol in iPS cells (dual SMAD inhibition)

Protocol



Expression of differentiation markers





Yamada et al, Tox in Vitro, 2016; Yamada et al, Sci Rep, 2017; Yamada et al, J Tox Sci, 2018

(7) LRI第８期委託研究課題

LRI第８期は、以下の研究分野において、新たに採択した６件の研究課題を含む、13件の研究委託を実施しています。

（敬称略）

研究分野	課題番号	研究課題	氏　名	所　属
1 新規リスク評価手法の開発、評価 ・簡便なばく露評価手法、 ・実験動物代替試験法 （含 *in vitro, in silico*）	18_S01-01	再構築皮膚モデルを用いた *in vitro* 皮膚感作性試験法 EpiSensA（Epidermal Sensitization Assay）のバリデーション研究	宮澤　正明	花王株式会社
	19_R01-01	ヒト幹細胞試験による迅速・正確・低コストの化学物質ハザード AI 評価法の開発	藤渕　航	京都大学 iPS 細胞研究所
	20-1-11	学習記憶障害をもたらすグルタミン酸受容体結合化合物の発達神経毒性・神経毒性を評価するインビトロ試験法の構築	關野　祐子	東京大学 大学院薬学系研究科
3 小児、高齢者、遺伝子疾患などにおける化学物質の影響に関する研究	19_R03-01	ヒトＴ細胞の活性化・分化誘導（Key event 4）を指標に感作性・アレルギー誘発性を評価する新規代替法の開発	善本　隆之	東京医科大学 医学総合研究所 免疫制御研究部門
	20-3-02	発達神経毒性ポテンシャルのスクリーニングとしての短期 in vivo 甲状腺ホルモン影響評価法の開発	山田　智也	住友化学株式会社
	20-3-06	発達期神経評価指標を用いた化学物質毒性評価法の確立	古武弥一郎	広島大学 大学院医系科学研究科
	20-3-08	発達神経毒性の AOP 解明に資する神経炎症評価法の確立	西村　有平	三重大学 大学院医学系研究科
	20-3-10	化学物質誘導性甲状腺機能低下症の発達神経毒性評価に資する Adverse Outcome Pathway の構築	中西　剛	岐阜薬科大学 衛生学研究室

幹細胞を用いた
化学物質リスク情報共有化コンソーシアム

JP EN Google カスタム検索 お問い合わせ

ホーム | 活動概要 | 活動報告 | 参加団体 | お知らせ | 会員専用ページ |

お知らせ

ホーム > お知らせ > 拡大コンソーシアム説明会

「幹細胞を用いた化学物質リスク情報共有化」拡大コンソーシアム説明会

日時：2017年10月27日（金）13:05-17:30（受付開始　12:30）
場所：京都大学iPS細胞研究所　1期棟1階講堂

内容：化合物動物実験禁止の流れからヒト細胞を用いた代替リスク試験法の必要性が高まっています。2009年より国の補助を得て、未分化なヒトES細胞の遺伝子発現量を用いた毒性予測が神経毒、遺伝的・非遺伝的発がん毒で95－100%に達しました。未分化細胞のため試験期間は数日のみで行うことができ、応答反応が出る化合物については将来iPS細胞を用いた有効な代替リスク試験法になる可能性を秘めています。
　本コンソーシアムでは、厚労省が連携し、日本人からの「未分化幹細胞」及び「品質が安定な分化細胞」を用いて化合物の反応データベースを構築し、今後の企業や研究の現場でヒト細胞への化合物リスク試験において評価情報の基盤を構築することを目指します。
　尚、本コンソーシアムの会費は当面は無料とします。ご興味ある皆様はぜひこの機会に本説明会にご参加下さい。

プログラム：
13:05-13:10　挨拶：藤渕　航（京大CiRA）
13:10-13:30　コンソーシアム開催趣旨説明：鈴木　譲（協和キリン）
13:30-14:00　新しいヒトES細胞を用いた化合物反応データベースとiPS細胞による毒性試験システムの概要：藤渕　航（京大CiRA）
14:00-14:30　製薬企業での毒性試験の現状と期待：棒木　基治（エーザイ）
14:30-15:00　化学企業での毒性試験の現状と期待：竹内　和由（日産化学）

【15:00-15:15　休憩（ドリンクを用意しています）】

15:15-15:45　iPS細胞とNGSを用いた毒性解析の実際：山根　陽子・倉原　一徳（京大CiRA）
15:45-16:15　食品企業での毒性試験の現状と期待：堀　絵生子（サントリー）
16:15-16:45　【特別講演】データベースに用いる化合物リストについて：鈴木　希子（国立環境研）

—Symposium Review—

ヒト ES 細胞を用いた高精度の化合物毒性予測システムの構築

山根順子,[*,a,b] 油谷幸代,[c] 今西　哲,[b] 赤沼宏美,[d] 永野麗子,[d]
加藤　毅,[e] 曽根秀子,[d] 大迫誠一郎,[b] 藤渕　航[a,c]

Construction of a High-precision Chemical Prediction System Using Human ESCs

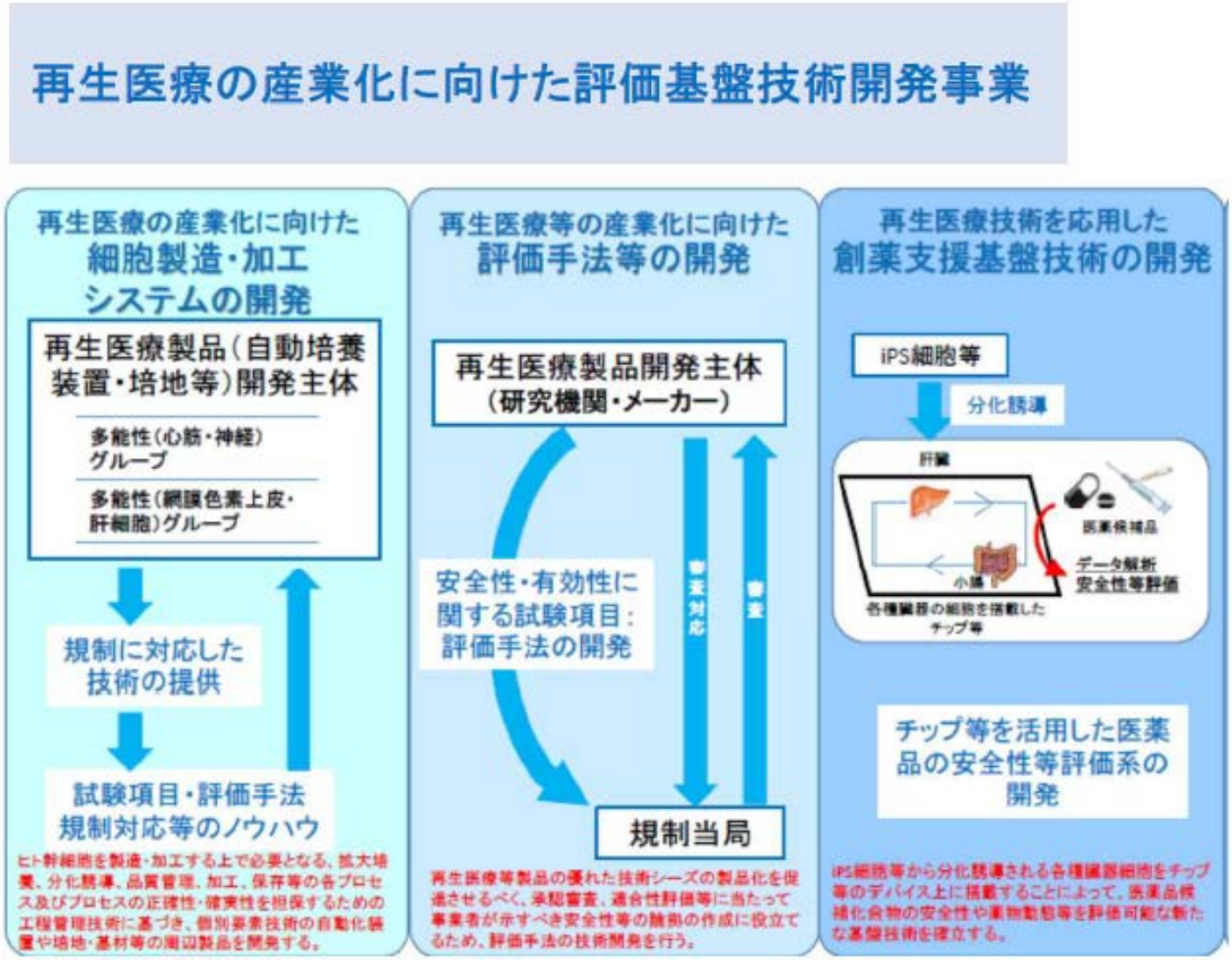
Junko Yamane,[*,a,b] Sachiyo Aburatani,[c] Satoshi Imanishi,[b] Hiromi Akanuma,[d] Reiko Nagano,[d]
Tsuyoshi Kato,[e] Hideko Sone,[d] Seiichiroh Ohsako,[b] and Wataru Fujibuchi[a,c]

[a]Kyoto University; 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606–8507, Japan: [b]The University of Tokyo; 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan: [c]National Institute of Advanced Industrial Science and Technology (AIST); 2–4–7 Aomi, Koto-ku, Tokyo 135–0064, Japan: [d]National Institute for Environmental Studies (NIES); 16–2 Onogawa, Tsukuba, Ibaraki 305–8506, Japan: and [e]Gunma University; 1–5–1 Tenjin-cho, Kiryu, Gunma 376–8515, Japan.

(Received October 11, 2017)

Toxicity prediction based on stem cells and tissue derived from stem cells plays a very important role in the fields of biomedicine and pharmacology. Here we report on qRT-PCR data obtained by exposing 20 compounds to human embryonic stem (ES) cells. The data are intended to improve toxicity prediction, per category, of various compounds through the use of support vector machines, and by applying gene networks. The accuracy of our system was 97.5–100% in three toxicity categories: neurotoxins (NTs), genotoxic carcinogens (GCs), and non-genotoxic carcinogens (NGCs). We predicted that two uncategorized compounds (bisphenol-A and permethrin) should be classified as follows: bisphenol-A as a non-genotoxic carcinogen, and permethrin as a neurotoxin. These predictions are supported by recent reports, and as such constitute a good outcome. Our results include two important features: 1) The accuracy of prediction was higher when machine learning was carried out using gene networks and activity, rather than the normal quantitative structure-activity relationship (QSAR); and 2) By using undifferentiated ES cells, the late effect of chemical substances was predicted. From these results, we succeeded in constructing a highly effective and highly accurate system to predict the toxicity of compounds using stem cells.

Physiologically-based kinetic modelling

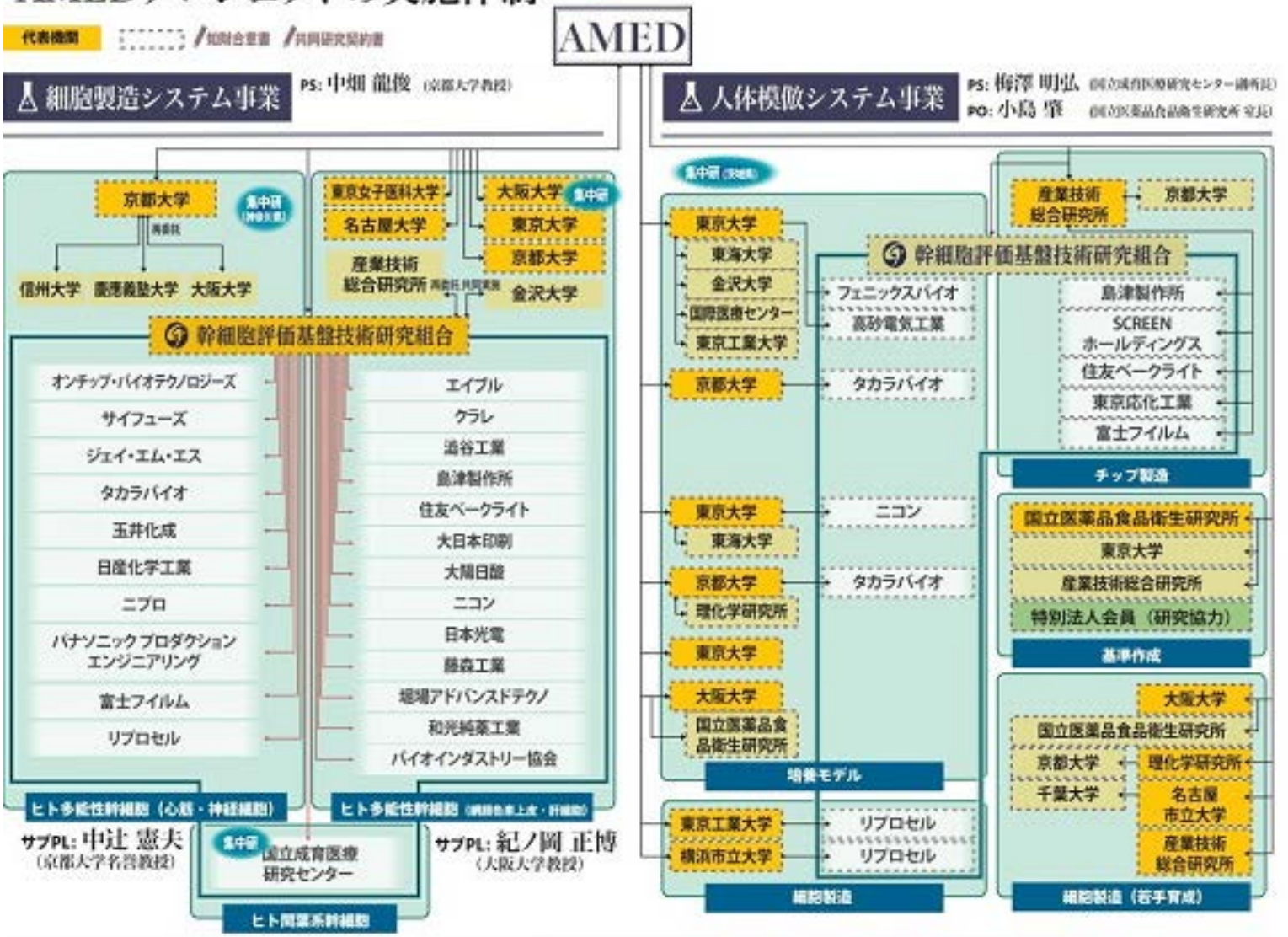


Microphysiological System(*MPS*)

SCA 幹細胞評価基盤技術研究組合
Stem Cell Evaluation Technology Research Association

AMED

AMEDプロジェクトの実施体制

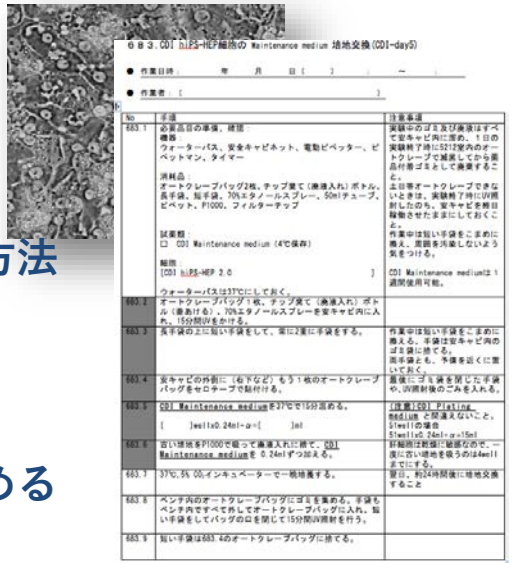


研究開発の流れ

Physiologically-based kinetic modelling

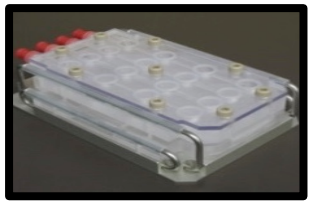
細胞の基準

・ユーザー（製薬企業）が臓器細胞に求める細胞性能の業界標準を整備。
・評価法プロトコル、使用細胞や実験の記録方法のプロジェクト内での共通化



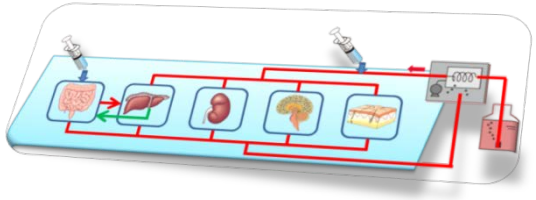
臓器ユニットの性能基準設定

・ユーザー（製薬企業）が臓器ユニットに求める細胞を実装したMPSの業界標準を整備。
・MPSに搭載しても、各臓器細胞の「基準」を満たすことが開発上クリアすべき条件。



多臓器モデルの性能基準設定

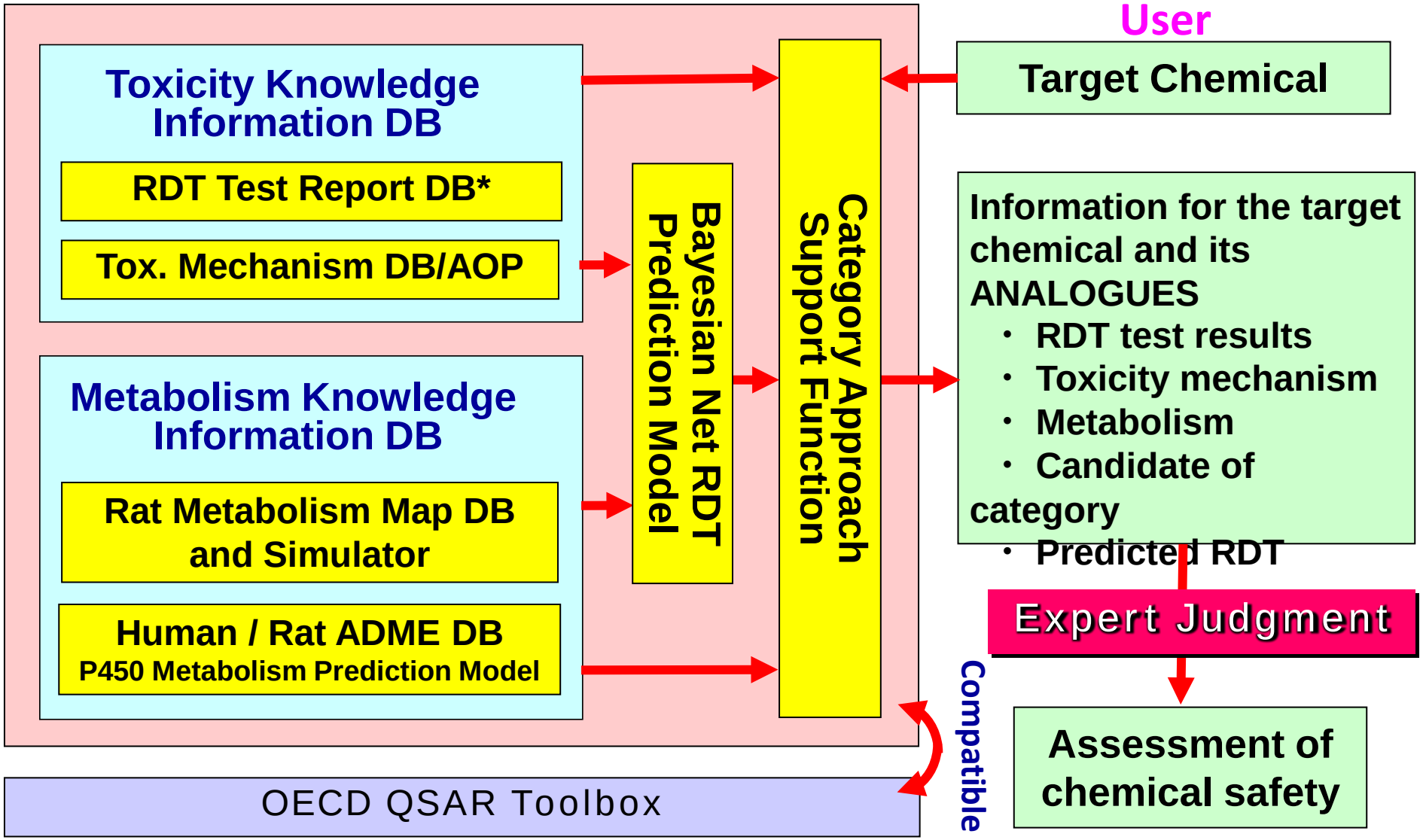
・多臓器化してもMPSの性能基準を満たすことを開発上クリアすべき条件に。



新薬開発のunmet needs

石田誠一先生（国立衛研・元薬理部）より提供

Hazard Evaluation Support System (HESS)
Integrated Platform for Repeated Dose Toxicity



*Core data are from 28-day repeated dose GLP tests on existing chemicals auspices of MHLW/NIHS, Japan

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

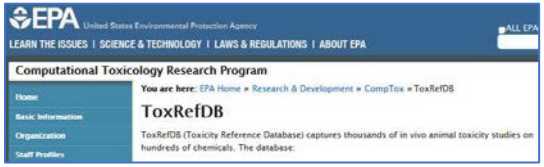
*In silico*開発のためのデータベース

Toxicity profile of new chemicals (not opened now)

MHLW: Toxicity test reports of existing chemicals
OECD SIDS (HPV chemicals)

added

HESS RDT DB (Core DB)



Sharing

HESS

DB/category provided

OECD
BETTER POLICIES FOR BETTER LIVES

QSAR TOOLBOX

COSMOS
Integrated *In Silico* Models for the Prediction of Human Repeated Dose Toxicity of COSMetics to Optimise Safety

Toxicity test data set provided

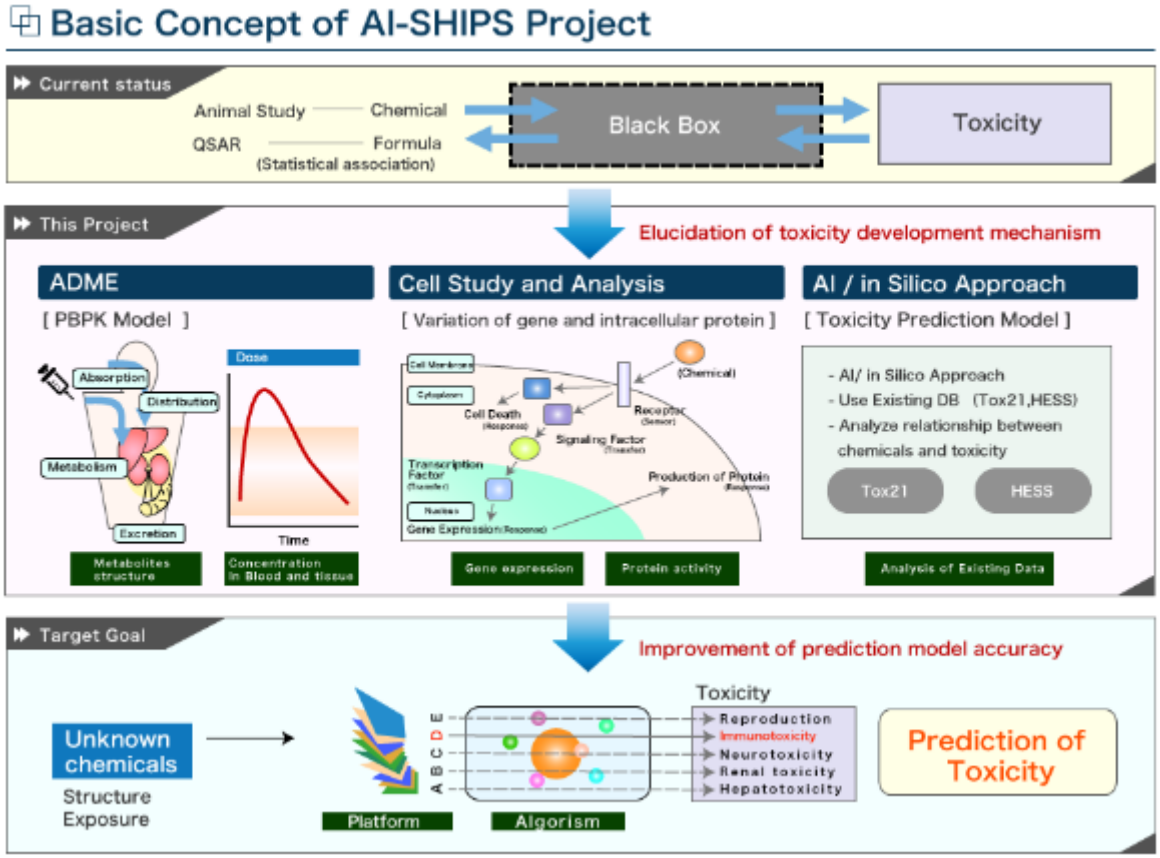
RepDose

MHLW Toxicogenomics Project/
Toxicogenomics Informatics Project
METI ToxOmics

Development of in silico hazard prediction system using AI technology

METI (Ministry of Economy, Trade and Industry) consignment project *"Development of AI based next generation safety prediction system using related Big data"*

AI-based
Substances
Hazardous
Integrated
Prediction
System project



Development policy and features of AI-SHIPS

- Unlike existing QSAR approach and other related prediction systems concept, we aim to develop the system that can predict chemical safety **based on mode of action i.e. AOP basis** and information of structural characteristics, physical properties of chemical substances.
- Conventionally, **ADME and PBPK** prediction methods that were not used in the such as prediction system can be drastically introduced, and to develop higher prediction accuracy system.
- By building a platform that unifies the DB of the existing chemical safety research and knowledge, we aim to develop the system **based on state-of-the-art artificial intelligence technology such as deep learning**
- For the elucidation of complicated and/or unique toxicity expression mechanism involving signal transduction mechanism etc., we will improve the system accuracy by reflecting the information that were obtained from comparison analysis of the data from **"Toxico-genomix"** approach with related toxicological end points.

Basic strategy for Develop a predictable system of the 28-day Repeated Dose Toxicity Test

1st STEP : To develop the prediction system of **Hepatotoxicity** (cytotoxicity, lipid abnormality, cholangiopathy and hypertrophy etc.) .
Hazardous and risk (NOEL)

2nd STEP : To develop **Hemato and Renal toxicity**.

- Industrial chemicals are not originally pursuing physiologically activity.
- Chemical structure is diverse e.g. the aliphatic chain to hetero ring, metal complex etc.
- Deepening the serious ecological health effects due to long-term exposure in trace amounts like PCB/TCDD, Organic Hg. Etc.

AI-SHIPS Research subjects HESS-DB listed substances

In this PJ, for the time being, we will cover substances listed in HESS * DB as substances to be studied or used. Detailed data on physical property information, Toxicity related data (blood chemistry, pathological data etc.) of repeated dose toxicity test (GLP etc.) are available.

①28－35days Repeated Dose 28-Day Oral Toxicity Study in Rodents （OECD TG407, METI STD)

235 substances

②Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test（OECD TG422） **263 substances**

③US NTP Data base: Repeated Dose 90-Day Oral Toxicity Study in Rodents (OECD TG408 etc.)

200 substances

Total 730 substances

*: Hazard Evaluation Support System Integrated Platform (HESS) “Toxicity knowledge information accumulating repetitive dose toxicity test data on chemical substances targeted for rats and action mechanism information related to toxicity System with two databases (HESS DB) of database and knowledge information database composed of metabolism information of chemical substances in mammals such as rats and humans System New Energy and Industrial Technology Development Organization (METI 2007—2010) and economy Commissioned work from the Ministry of Industry (METI 2011) ”Development of hazard assessment method by structure activity correlation method“ (Project Leader: Dr.M. Hayashi)

まとめ

　全身毒性試験代替法の中でも、特に発達神経毒性、MPS、AIの開発が世界的に進んでいる。我が国においても、適切な予算がついて研究が進んでおり、画期的な成果を期待したい。


ご静聴ありがとうございました