Proposal of *in vitro* assays useful for predicting repeated-dose toxicity of chemical substances

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HESS

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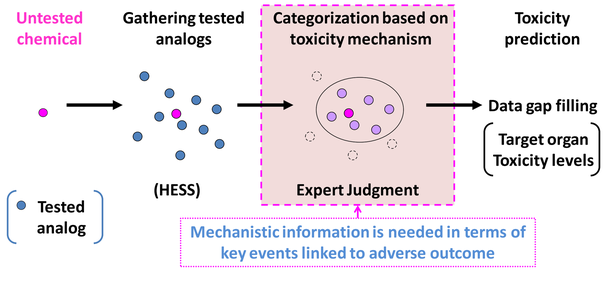
Abstract

Repeated-dose toxicity (RDT) is one of the key regulatory endpoints in hazard assessment of chemical substances. We have recently released Hazard Evaluation Support System (HESS) for RDT of chemical substances. HESS supports to form a toxicological category *in silico* by grouping structural analogs with RDT data. In many cases, however, it is difficult to build mechanism-based category using only structural information. Here, we evaluated adverse outcome pathways (AOPs) in HESS to identify mechanistic key events linked to *in vivo* toxicity. Based on the results, we propose *in vitro* assays useful for categorizing untested chemicals. Various AOPs in HESS were evaluated using papers and reviews of peer-reviewed journals and toxicology textbooks. Then, measurable key events linked to adverse outcome were identified based on weight of evidence analysis. Moreover, Japanese MITI chemical inventory was screened with OECD QSAR Toolbox to obtain candidate chemicals to be assayed. As a result, we identified several key events and found untested structural analogs; direct oxidation of hemoglobin and ROS generation for hemolysis of hydrazines, formation of phosgene for toxicity in liver and kidney of trihalomethanes, carbonic anhydrase inhibition for toxicity of urinary systems of sulfonamides and alkoxyacetic acid formation in liver for testicular toxicity of EGAEs etc. The *in vitro* assays to measure these key events could be useful, as a part of integrated testing strategy, to categorize untested chemicals and to predict the primary toxicity *in vivo*.

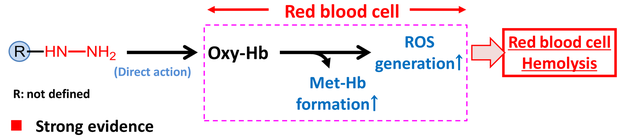
Introduction

- Repeated-dose toxicity
  - One of the key regulatory endpoints in hazard assessment
  - Testing: costly and time-consuming
- Category approach

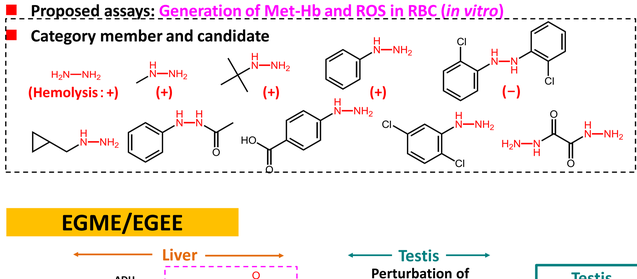


Result (proposed *in vitro* assays)

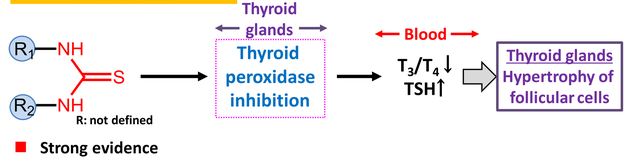
Hydrazines



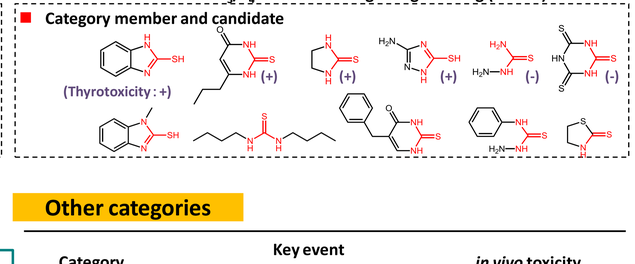
- Strong evidence
  - Administration of parent chemical → Met-Hb↑ + hemolytic anemia (*in vivo*)
  - RBC exposed to the parent → Met-Hb↑, ROS generation↑, cell damage↑ (*in vitro*)
  - + N-acetylcysteine → Cell damage↓ (*in vitro*)
- Proposed assays: Generation of Met-Hb and ROS in RBC (*in vitro*)
- Category member and candidate



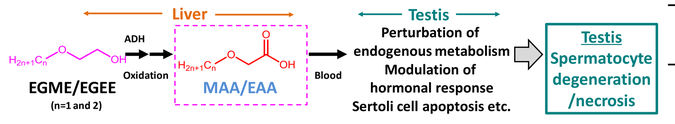
Thioureas



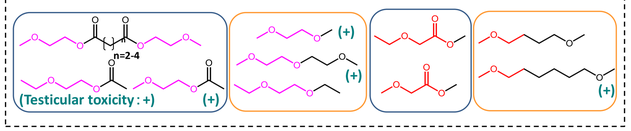
- Strong evidence
  - Administration of parent chemical → Blood T_3/T_4↓, TSH ↑ (*in vivo*)
  - Parent chemical → Inhibition of peroxidase activity (*in vitro*)
- Proposed assays : Inhibition of (thyroid) peroxidase activity (*in vitro*)
  Blood T_3/T_4 levels following a single dosing (*in vivo*)
- Category member and candidate



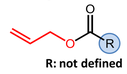
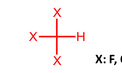
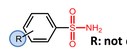
EGME/EGEE



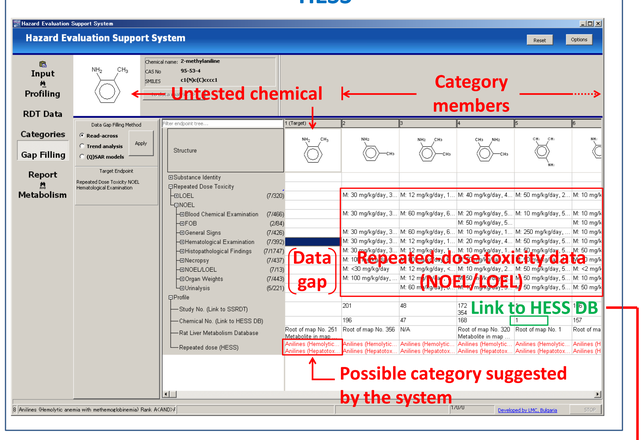
- Strong evidence
  - Pretreatment with ADH inhibitor→Urinary MAA↓, testicular toxicity↓ (*in vivo*)
  - Administration of MAA → Spermatocyte degeneration (*in vivo*)
  - Primary testicular culture exposed to MAA→ Cytotoxicity↑ (*in vitro*)
- Proposed assay: Metabolic formation of MAA/EAA in hepatocytes (*in vitro*)
- Category member and candidate



Other categories

Category	Key event (Proposed as *in vitro* assays)	*in vivo* toxicity
Allyl esters  R: not defined	Metabolic formation of allyl alcohol (intestine, liver) Oxidative stress (liver) Covalent binding to macromolecules (liver)	Liver: Hepatocyte necrosis (periportal) Liver: Bile duct hyperplasia
Trihalomethanes  X: F, Cl, Br	Formation of phosgene GSH depletion Covalent binding to macromolecules	Liver: Hepatocyte necrosis (centrilobular) Kidney: degeneration of proximal tubules
Sulfonamides  R: not defined	Inhibition of carbonic anhydrase activity Urinary calculi formation	Urinary bladder: Thickened mucous epithelium

HESS



HESS (Hazard Evaluation Support System):

- Software to support category approach *in silico*
- Compatible to OECD QSAR Toolbox
- Current status: 530 chemicals, 600 repeated-dose toxicity studies (GLP standard), 1000 metabolic map (*in vitro*/*in vivo*), 30 categories
- Linked to HESS DB (detailed dose-response data included)

✓ Often difficult to perform mechanism-based categorization based on only structural information

➢ This study identified key events measurable *in vitro* for several AOPs based on weight of evidence analysis using HESS/HESS DB.

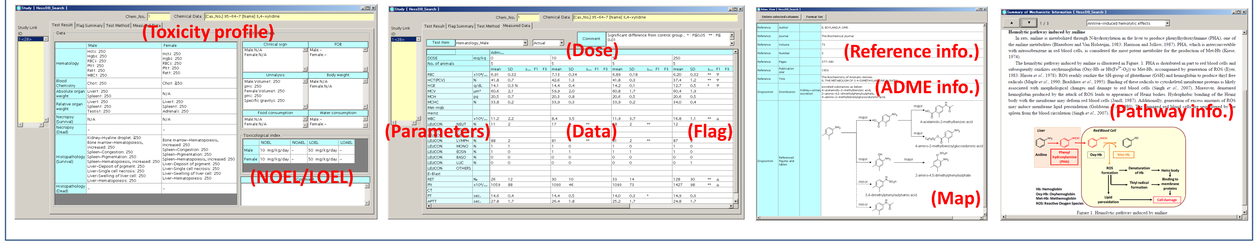
HESS DB

Toxicity profile
- Evaluated by committee of Japan's Chemical Substance Control Law or by toxicology experts of NIHS and NITE, Japan

Dose-response data
- Hematology
- Blood chemistry
- Organ weight
- Histopathology etc.

ADME
- Reference information
- Major/minor pathways
- Major/minor metabolites
- Metabolic enzymes etc.

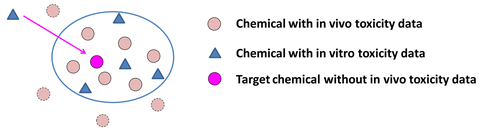
Toxicity mechanism
- Reference information
- Possible toxicant etc.
- Key events link to toxic effects
- Summary of pathway



Summary

- This study proposed key events linked to repeated-dose toxicity and measurable in *in vitro* based on information on HESS/HESS DB.
- *In vitro* assays could be useful for categorizing analogs without *in vivo* toxicity data and for refining chemical space of a category.

Mechanism-based toxicological category



Let's try HESS!

- Free software
- Developed with financial support of NEDO/METI, Japan, 2007-2012
- Downloadable from the website of NITE (searching words "HESS, NITE") http://www.safe.nite.go.jp/english/kasinn/qsar/hess-e.html
- Upgrade version with new data will be released in March 2014.
- Booth exhibition for the system will be held at SOT2014 in Phoenix.
- References

- Yamada et. al., A category approach to predicting the repeated-dose hepatotoxicity of allyl esters. Regul Toxicol Pharmacol. 2013; 65(2):189-95.
- Sakuratani et al., Hazard Evaluation Support System (HESS) for predicting repeated dose toxicity using toxicological categories. SAR QSAR Environ Res. 2013;24(5):617-29.