Session 1: Chemical Risk Assessment in Japan; Current Status, Challenges and Opportunities

Risk Assessment Methodology for Chemicals and Contaminants in Foods

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Today’s topics

• CSAF & BMD methods
• TTC (Threshold of Toxicological Concern)
• QSAR/Category approach
• Needs of integrated risk assessment and more experts ...
Hazard Identification
Identification of adverse effects
- type of toxicity (in vivo & in vitro)
- how dose of exposure causes the effects
  (genotoxic carcinogenesis or estimating
  no observed adverse effects level (NOAEL))

Exposure Assessment
Levels of exposure by routes
- levels in substance in air, food
  or water etc.
- levels in human tissues
  (simulation models)

Hazard Characterization
Selection of critical data
- dose response characterization in
  human based on the toxic mechanism
  (setting TDI or VSD in some cases)

Risk Characterization
Integration of exposure and
hazard characterization

Problem formulation

Traditional Risk Assessment paradigm
Hazard characterization
Dose Response Assessment

Toxic effects with threshold

Non-threshold toxicity
(DNA-direct acting chemicals)

TDI/ADI approach
(UF approach)

Mathematical modeling
or MOE approach
Derivation of ADI: Acceptable Daily Intake or TDI: Tolerable Daily Intake

\[ \text{ADI} = \frac{\text{NOAEL}}{\text{SF}} \text{ (Safety factor)} \]
\[ \text{TDI} = \frac{\text{NOAEL}}{\text{UF}} \text{ (Uncertainty factor)} \]

[Construction of the UF or SF]

1. Inter species difference : 10, (or allometric adjusting)
2. Intra species difference : 10 to adjust scientifically
3. Short-term study : max. 10
4. LOAEL (NOAEL is not determined) : max. 10
5. Severity of toxicity : max. 10

(carcinogenicity, teratogenicity, neurotoxicity etc.)
Sub-division of UF, and replacement with CSAF

(CSAF: Chemical specific adjusting factor)

The UF could be divided by toxicokinetic and toxicodynamic factor. Each factor could be replaced with the specific factor, which is derived from scientific evidence.

CSAFs could be estimated from in vitro or in vivo studies, when toxicodynamic components such as target cell sensitivity has been delineated.

CSAFs could be estimated from comparison analysis such as blood concentration or AUC of the active moiety in the general circulation.

A – animal to human; H – human variability; D – toxicodynamics; K – toxicokinetics

AF - the adjustment factor calculated from chemical-specific data
• The benchmark dose is the effective dose (or its lower confidence limit) that produces a certain increase in incidence above control levels.
• The advantages of the benchmark dose are that it takes into account the slope of the dose-response curve, the size of the study groups and the variability in the data.
Risk Characterization for each chemical

• Direct comparison between TDI (ADI or VSD) and Daily Intake
  
  Whether is “TDI” > “Total daily Intake” (or Estimated Intake), or not?

• For derivation of guidance values (GV)
  (health based standards for foods, drinking water or air), the below equation is usually accepted.

  \[
  GV = TDI \times (\text{average body weight}) \times (\text{allocation factor}^*)
  \]

  \[\text{total daily intake of vehicle}\]

  (*: the ratio of contribution via the targeted vehicle among all exposure scenarios)

• Margin of Exposure or Margin of Safety

  \[
  \text{MOE or MOS} = \frac{\text{NOAEL}}{\text{Human Exposure level}}
  \]

  (The value of MOE may be used for chemical management prioritization or political decision etc.)
Hazard characterization

Dose Response Assessment

Toxic effects with threshold

TDI/ADI approach (UF approach)

Non-threshold toxicity (DNA-direct acting chemicals)

Mathematical modeling or MOE approach
Genotoxic carcinogen risk assessment by using BMD method

MOE = \frac{POD}{\text{human exposure level}}

Point of Departure (POD)

Curve fitting to experimental range by the BMD approach

If no biological model is available, the LNT approach would be applied.

BMD method is also used for derivation of the POD in the genotoxicity risk assessment.
Problem of the risk assessment of plastics for food container
(In case of very low level exposure and limited toxicity information)

• What is targets chemicals?
  Plastics as high molecular weight polymer could not be absorbed into the body. → no health concern.
  But, foods might be contaminated with eluted chemical from plastics
  → Plastics might contain additives, by-products, catalysts, monomer, impurities, degradation products, etc.

• How to assess safety for many kinds of chemicals in plastics?
  It is not realistic to assess fully the potential risks of all chemicals.
  Toxicological information for most of the chemicals are limited.
  → The toxicity testing schemas depending on migration levels are required. (Threshold of exposure level is necessary)
## Summary table of minimum required toxicity tests

<table>
<thead>
<tr>
<th>Levels of migrant (intake estimate at 3 kg of total diet in case of FDA)</th>
<th>U.S. FDA</th>
<th>EFSA</th>
</tr>
</thead>
</table>
| **≤0.5 ppb** (≤1.5 ug/day) | No safety studies are recommended; evaluation of structural similarity to known toxicants | • 3 genotoxicity studies in vitro:  
  i) A test for induction of gene mutations in bacteria  
  ii) A test for induction of gene mutations in mammalian cells in vitro (preferably the mouse lymphoma (ML) to assay)  
  iii) A test for induction of chromosomal aberrations in mammalian cells in vitro |
| 0.5 ~ 50 ppb (1.5 ~ 150 ug/day) | 2 genotoxicity studies in vitro:  
  i ) a test for gene mutations in bacteria and  
  ii ) an in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk± assay | First threshold of regulation (TOR) by FDA |
| 50 ppb ~ 1 ppm (150 ~ 3000 ug/day) | • Above 2 tests+an in vivo test for chromosomal damage using rodent hematopoietic cells  
  • 2 subchronic oral toxicity tests (a rodent and a non-rodent species). | • Above 3 mutagenicity tests  
  A 90-day oral toxicity study  
  Data to demonstrate the absence of potential for accumulation in man |
| >1 ppm ~5 ppm | food additive petition should be submitted | • Above tests  
  Studies on absorption, distribution, metabolism and excretion  
  Studies on reproduction in one species, and developmental toxicity, normally in two species  
  Studies on long-term toxicity/carcinogenicity, normally in two species |
| >5 ppm | | |
The first TTC of the TOR (Threshold of Regulation) in the U.S.FDA was developed by using the calculate VSD (Virtual Safety Dose) from $TD_{50}$ in the Carcinogenic Potency Database (CPDB).

The value of the VSD linearly extrapolated from $TD_{50}$ is more conservative than the value of the VSD calculated with the LMS (linearized multistage) model.
<table>
<thead>
<tr>
<th>Levels of Migrant (Intake Estimate at 3 kg of Total Diet in Case of FDA)</th>
<th>U.S. FDA</th>
<th>EFSA</th>
<th>Proposal</th>
<th>Estimated Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.5 ppb (≤1.5 ug/day)</td>
<td>No safety studies are recommended; evaluation of structural similarity to known toxicants</td>
<td>Studies in vitro: i) A test for induction of gene mutations in bacteria</td>
<td>No safety studies are recommended; evaluation of structural similarity to known toxicants</td>
<td>≤1.5 ug/day (0.5 ppb)</td>
</tr>
<tr>
<td>0.5 ~ 50 ppb (1.5 ~ 150 ug/day)</td>
<td>2 genotoxicity studies in vitro: i) a test for gene mutations in bacteria and ii) an in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk± assay</td>
<td>2 of 3 tests: i) Ames test ii) CA test in mammalian cells in vitro (preferably the mouse lymphoma (ML) assay) iii) ML assay</td>
<td>Above 3 tests: A 90-day oral toxicity study (except of organophosphate)</td>
<td>&gt;1.5 ~ 100 ug/day (50 ppb)</td>
</tr>
<tr>
<td>50 ppb ~ 1 ppm (150 ~ 3000 ug/day)</td>
<td>Above 2 tests + an in vivo test for chromosomal damage using rodent hematopoietic cells 2 subchronic oral toxicity tests (a rodent and a non-rodent species).</td>
<td>Above 3 mutagenicity tests: A 90-day oral toxicity study Data to demonstrate the absence of potential for accumulation in man</td>
<td>Above tests: Studies on reproduction in one species, and developmental toxicity, normally in two species</td>
<td>&gt;100 ~ 2000 ug/day (1 ppm)</td>
</tr>
<tr>
<td>&gt;1 ppm ~5 ppm</td>
<td>Food additive petition should be submitted</td>
<td>Adequate toxicity information for the compound specific risk assessment (usually all toxicity tests for food additive petition)</td>
<td>Above tests: Studies on reproduction in one species, and developmental toxicity, normally in two species</td>
<td>&gt;2000 ug/day (1 ppm)</td>
</tr>
<tr>
<td>&gt;5 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Threshold of concern for non-carcinogenic toxicity**

- ≤0.5 ppb (≤1.5 ug/day)
- 0.5 ~ 50 ppb (1.5 ~ 150 ug/day)
- 50 ppb ~ 1 ppm (150 ~ 3000 ug/day)
- >1 ppm ~5 ppm
- >5 ppm

**Threshold of concern for specific toxicities (ex. reproductive and developmental toxicity)**

- Studies on reproduction in one species, and developmental toxicity, normally in two species
- Studies on long-term toxicity/carcinogenicity, normally in two species
Discussion for application of the TTC concept

• The proposed thresholds for toxicity testing schema based on the TTC concept is considered to be similar to other authorities which were traditionally established.

• Development of genotoxicity QSAR system for helping TOR decision would be necessary

• In addition, more precise research on the advancing structural categorization, especially for repeated-dose or developmental toxicities categorization, for developing specific TTCs would be required in future.
Combination (Q)SAR approach
with three mutagenicity (Q)SAR models

In silico

<table>
<thead>
<tr>
<th>Ames test</th>
<th>&lt;++</th>
<th>&gt;--</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>19</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>-</td>
<td>23</td>
<td>147</td>
<td>170</td>
</tr>
</tbody>
</table>

Sensitivity: 73.1 %
Specificity: 86.5 %
Concordance: 84.7 %
Applicability: 95.1 % (196/206)

In silico

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<tr>
<th>Ames test</th>
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<th>Total</th>
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<tbody>
<tr>
<td>+</td>
<td>13</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>94</td>
<td>99</td>
</tr>
</tbody>
</table>

Sensitivity: 86.7 %
Specificity: 94.9 %
Concordance: 93.9 %
Applicability: 55.3% (114/206)

Acknowledgements: This work was supported by Health and Labour Sciences Research Grants of MHLW, Japan
A chemical category can be represented graphically as a two-dimensional matrix in which category members occupy different columns, and the category endpoints occupy different rows.

Data gaps may be filled by read-across from a tested to an untested chemical or by trend analysis.
The OECD QSAR Toolbox for Grouping Chemicals into Categories

**Category definition**
Side-bar of experimental data

1. Double-click on the cell with measured data to see detailed information on the data point.

In cooperation:

- OECD
- ECHA

Donation (Version 2.1) of database, profiler or QSAR from:

- U.S. EPA
- Istituto Superiore de Sanita, Italy
- European Commission
- Environment Canada
- Danish EPA
- RIVM, the Netherlands
- MOE, Japan
- MHLW, Japan
- METI, Japan
- NEDO, Japan
- Fraunhofer Institute, Germany
- LMC, Bulgaria
- BfR, Germany
- Istituto Superiore de Sanita, Italy;
- Office of Public Health, Switzerland
- University of Vienna, Austria
- University of Tennessee, Knoxville,
- ECETOC
- CEFIC
- RIFM
- International QSAR Foundation
- Multicase Inc.; ChemAxon;
- Exxon Mobil; Unilever;
- P&G; L’Oréal; Dow Chemical;

http://www.qsartoolbox.org
Development of Hazard Evaluation Support System (HESS) and the attached database (HESS DB)

Data (Collected by NIHS, NITE, Tohoku Univ., Bourgas Univ.)
- Toxicity test reports (545 reports for 515 chemicals, GLP standards)
  - 28d repeated dose toxicity (RDT) studies under Japan’s Chemical Substances Control Law (CSCL)
  - Combined RDT and reproductive/developmental toxicity studies under CSCL
  - 13w NTP studies etc.
- Toxicity profiles (530)
  - judged by the committee of Japan’s CSCL or created by the experts in toxicology
- Related references (ADME, toxicity mechanism)

HESS (Developed by LMC, Bourgas Univ.)
- Chemical
- Repeated dose toxicity DB
  - LOEL and NOEL for each endpoint
- Category library
- Metabolism map & simulator
- Compatible to OECD QSAR Toolbox

HESS DB (Developed by Fujitsu Limited)
- Chemical
- Toxicity test report DB
  - Toxicological profiles
  - Measured data
- ADME DB
- Toxicity mechanism DB
**HESS (Data structure)**

**Repeated dose toxicity data**

- NOEL (~500 endpoints)
- LOEL

**Category information**

- Structural boundary
- Adverse outcome pathway
- Training set

Currently 33 categories included
<table>
<thead>
<tr>
<th>Endpoint</th>
<th>LOEL (Target)</th>
<th>LOEL (Analogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>M: 15 mg/kg/day, 15 mg/kg/day</td>
<td>M: 15 mg/kg/day, 20 mg/kg/day</td>
</tr>
<tr>
<td>HGB</td>
<td>M: 15 mg/kg/day, 15 mg/kg/day</td>
<td>M: 15 mg/kg/day, 20 mg/kg/day</td>
</tr>
<tr>
<td>HTC</td>
<td>M: 50 mg/kg/day, 50 mg/kg/day</td>
<td>M: 50 mg/kg/day, 60 mg/kg/day</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>M: 50 mg/kg/day, 50 mg/kg/day</td>
<td>M: 50 mg/kg/day, 60 mg/kg/day</td>
</tr>
<tr>
<td>Methemoglobin</td>
<td>M: 5 mg/kg/day, 1E4 mg/L, 1E4 mg/L</td>
<td>M: 1E4 mg/L, 1E4 mg/L</td>
</tr>
<tr>
<td>Histopathological Findings</td>
<td>M: 15 mg/kg/day, 15 mg/kg/day</td>
<td>M: 15 mg/kg/day, 20 mg/kg/day</td>
</tr>
<tr>
<td>Organ Weights</td>
<td>M: 15 mg/kg/day, 5 mg/kg/day, 5 mg/kg/day</td>
<td>M: 15 mg/kg/day, 5 mg/kg/day, 5 mg/kg/day</td>
</tr>
<tr>
<td>NOEL</td>
<td>M: 15 mg/kg/day</td>
<td>M: 5E3 mg/L</td>
</tr>
</tbody>
</table>

LOEL for RGB: 22
How to assess the risk of the mixture exposure?

**WHO framework**

**Tiered Exposure Assessments**
- **Tier 0**: Simple semi-quantitative estimates of exposure
- **Tier 1**: Generic exposure scenarios using conservative point estimates
- **Tier 2**: Refined exposure assessment, increased use of actual measured data
- **Tier 3**: Probabilistic exposure estimates

**Tiered Hazard Assessments**
- **Tier 0**: Default dose addition for all components
- **Tier 1**: Refined potency based on individual POD, refinement of POD
- **Tier 2**: More refined potency (RFP) and grouping based on MOA
- **Tier 3**: PBPK or BBDR; probabilistic estimates of risk

**Is the margin of exposure adequate?**
- **Yes**, no further action required
- **No**, continue with iterative refinement as needed (i.e. more complex exposure & hazard models)
WHO framework

Tiered Exposure Assessments

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**In future**
Toxicology Recognition Task Force (TRTF) Matrix

As a result of 2009 and 2011 surveys and subsequent meetings with its Member Societies, IUTOX has learned that an area of high interest among many members is to identify the means with which to recognize the thousands of toxicologists working in every corner of the world. With a clear mandate from the Members Societies, the IUTOX Executive Committee established a Toxicology Recognition Task Force (TRTF) and named Dr. Lewis Smith as Chair of the TRTF.

Certified toxicologists as experts for hazard assessment

**DABT**: Diplomat of American Board of Toxicology (USA)
**DJSOT**: Diplomat of Japanese Society of Toxicology (Japan)
**ERT**: European Registered Toxicologist (EU)
**ATS**: Fellow of the American Toxicological Society (USA)
**DKBT**: Diploma, Korean Board of Toxicology (Korea)
**Expert in Toxicology, DGPT**: sponsored by the German Society of Experimental and Clinical Pharmacology and Toxicology (Germany)
**UK Register of Toxicologists**: sponsored by the Society of Biology and the British Toxicology Society (United Kingdom)
**DCST**: Diplomat of the Chinese Society of Toxicology (China)