An OECD Harmonised Template (OHT) to Report NAM Results in Regulatory Environments: Principles and Practical Use

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European Commission – Joint Research Centre (JRC)

EPA NAM Conference 2020: State of the Sciences on Development and Use of NAMs for Chemical Safety Testing
What to expect from this presentation

- Standardizing hazard reporting - The OECD Harmonised Templates for Reporting Chemical Test Summaries (OHTs)
- The odd one out - OHT 201 - Intermediate Effects – Mechanistic Information
- How it all fits together - The triangle of chemical safety
- Sanity check - OHT 201 in real life
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Template? What template?...
One template to rule them all…
Apical vs Mechanistic Knowledge

- **Apical Knowledge**: Knowledge about traditional, directly measured whole-organism outcomes of exposure in *in-vivo* tests, generally death, reproductive failure, tumour formation, skin/eye irritation, skin/respiratory sensitisation or developmental dysfunction.

  *One in-vivo test tells us whether an adverse outcome has been observed or not.*

- **Mechanistic Knowledge**: Knowledge about the sequence of events leading from the exposure to an effective dose of a chemical to the production of a specific biological response in the target organ, in most cases measured in *non-in-vivo* tests.

  *A series of tests, mainly non-animal, tells us why an adverse outcome is likely to manifest itself or not.*
The OECD Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology establishes the **OECD Expert Group on Harmonising Templates**.

More **apical** templates over time.

The first 86 OECD **apical** Harmonised Templates (OHTs) are published around 2012.

The OECD Workshop on Electronic Tools for Data Submission, Evaluation and Exchange for the Regulation of New and Existing Industrial Chemicals, Agricultural Pesticides and Biocides recommends for the OECD to **support harmonisation where possible in the reporting of chemical test results, by the use of templates and XML tags**.

We need a **mechanistic** template!

OHT 201 developed.

OHT 201 refinement.

OHT 201 published!

New OHT 201 published!
OECD Harmonised Templates

- OHTs as such are only descriptions, not an ICT application
- OHTs can be implemented by anyone in their local ICT environments
- Most popular OHTs implementation
  - IUCLID development is managed by the OECD
  - IUCLID is free and can be installed in any ICT environment
OHTs (and IUCLID) are used in more and more legislations around the world, among them...

- Office of Chemical Safety and Pollution Prevention (OCSPP), Office of Pollution Prevention and Toxics (OPPT):
  - ATAEPI - Analysis of TSCA Available, Expected, and Potentially Useful Information

- NCCT and ECHA share data resources from ToxRefDB and IUCLID to provide a comprehensive public resource to estimate anticipated “spread” of repeat dose toxicity POD values.

- US EPA’s Responsible Appliance Disposal (RAD)
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Why OHT 201?

- All other OHTs are **apical**.
- Apical = referring to **one single endpoint** of regulatory concern
  - Ecotox: Fish toxicity, bird toxicity, …
  - Human health: skin sensitisation, carcinogenicity, mutagenicity, reprotoxicity, acute toxicity, …
- Mechanistic data are **not intrinsically linked** to an apical endpoint!
- They can be used to **underpin mechanistic explanations** of toxicity – across and beyond apical endpoints
Reporting paradigm using OHT 201

Chemical → Test Method → Measurement 1 → Measurement 2 → ... → Measurement n → Conclusion

Process → Object → Action → Intermediate Effect → Effect!

No effect!
OHT 201 fits all classes of methods

Chemical X

OHT 201

Intermediate Effect Identification:  
*Process – Object - Action*

Evidence independent from *Class of Method*

Chemical is involved in effect  
*Yes or No*

- OHT 201 *links* a chemical to an intermediate (mechanistic) effect, identified by a *Process-Object-Action* ontology
- OHT 201 is by nature *completely independent* from the *class of method* (*in-vitro*, QSAR, PBK, ‘omics, …) used to underpin the link
Intermediate Effect Naming

In OHT 201, effects are named using the same ontology as the AOP Framework

AOP Key Event ≈ OHT 201 Intermediate Effect
Sample Process – Object – Action names
Real life OHT 201 today

Chemical X

OHT 201

Intermediate Effect Identification: 
*Process – Object - Action*

- Chemical is Involved in Effect: Yes or No

- In order to increase its usefulness in *certain* environments, OHT 201 features **structured fields** to accommodate *certain* technologies

- Findings derived from *other technologies* can still be reported!

- Using, weblinks, PDF attachments etc.
# OECD Test Guidelines supported

<table>
<thead>
<tr>
<th>Guideline</th>
<th>Test Method</th>
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</thead>
<tbody>
<tr>
<td>TG442C</td>
<td>- DPRA</td>
</tr>
<tr>
<td></td>
<td>- ADRA</td>
</tr>
<tr>
<td>TG442D</td>
<td>- Keratinosens</td>
</tr>
<tr>
<td></td>
<td>- LuSens</td>
</tr>
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<td>- h-CLAT</td>
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<tr>
<td></td>
<td>- U-SENS</td>
</tr>
<tr>
<td></td>
<td>- IL-8 LUC assay</td>
</tr>
<tr>
<td>TG455 (including former TG457)</td>
<td>- ERTA STTA</td>
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<tr>
<td></td>
<td>- ERTA VM7Luc</td>
</tr>
<tr>
<td></td>
<td>- ERTA ERα CALUX</td>
</tr>
<tr>
<td>TG456</td>
<td>- H295R Steroidogenesis Assay</td>
</tr>
<tr>
<td>TG458</td>
<td>- ARTA STTA</td>
</tr>
<tr>
<td></td>
<td>- ARTA AR-CALUX</td>
</tr>
<tr>
<td>TG493</td>
<td>- hrER binding FW assay</td>
</tr>
<tr>
<td></td>
<td>- hrER binding CERI assay</td>
</tr>
</tbody>
</table>

If NAM **follows an OECD Test Guideline:**
Many fields are pre-filled

If NAM **does not follow an OECD Guideline:**
More manual work needed
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Stressors - Test Methods - AOPs

Adverse Outcome Pathways
Stressors - Test Methods - AOPs

- **In-Vivo Test Method**
- **In-Vitro Test Method**

**Adverse Outcome Pathways**

- **Stressor**
  - Undergoes Test

- **OHT 201**
- **OHT 201**
- **other OHTs**

- **IUCLID 6**

- **Molecular Interaction** → **Organelle responses** → **Cellular responses** → **Tissue responses** → **Organ responses** → **Organism responses** → **Population responses**

- **Inconclusive**
- **Does not trigger**
- **Triggers**
- **Does not trigger**
Stressors - Test Methods - AOPs

To be published in late 2020

What to expect from this presentation

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• The odd one out - OHT 201 - Intermediate Effects – Mechanistic Information

• How it all fits together - The triangle of chemical safety

• Sanity check - OHT 201 in real life
OHT 201 in EASIS

EASIS = Endocrine Active Substances Information System

6993 Health effects
550 (100 substances) Intermediate effects
2678 Effects on biotic systems

10221 study entries (629 substances)

To be published in the coming weeks!
<table>
<thead>
<tr>
<th>Subject name</th>
<th>Submission type</th>
<th>Dossier UUID</th>
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<tbody>
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<td>OECD harmonised templates</td>
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</table>

Dashboard > Substances

Advanced search

629 results found

Sort by Name (A-Z)
tris(2-chloroethyl) phosphate (TCEP)

General information
A Physico-chemical properties
B Degradation and accumulation
C Effects on biotic systems
D Health Effects
E Analytical methods
F Pesticide residue chemistry
G Efficacy
H Emissions from treated articles
I Intermediate effects

Dossier Submission Type

Dossier name (given by user)
tris(2-chloroethyl) phosphate (TCEP)

Version
oeCD 4.0

Submission Type
OECD harmonised templates

Dossier Subject

Dossier Subject
tris(2-chloroethyl) phosphate (TCEP) | tris(2-chloroethyl) phosphate | tris(2-chloroethyl) phosphate | 115-96-8

Dossier creation date/time
2019-12-13T16:30:12
**tris(2-chloroethyl) phosphate (TCEP)**

**Intermediate effects**

- BPI: Study title: Comprehensive analysis of antagonistic endocrine activity during ozone treatment of hospital wastewater
- BPI: Study title (b): TCEP binding to nuclear receptors of zebrafish
- BPI: Study title (c): TIP and TCEP induce oxidative stress and alter steroidogenesis in TM3 Leydig cells
- BPI: Study title (a): Investigation of cytotoxic, genotoxic,

**UUID:** eee9c20b-8b1b-4920-a17e-eea1d575187

**Administrative data**

**Endpoint**
- Intermediate effects, other
- TCEP binding to nuclear receptors

**Type of information**
- Calculation (if not (Q)SAR)

**Cross-reference**

<table>
<thead>
<tr>
<th>#</th>
<th>Reason / purpose</th>
<th>Related information</th>
<th>Remarks</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>reference to same study</td>
<td>experimental study</td>
<td>Danio rerio</td>
<td></td>
</tr>
<tr>
<td></td>
<td>previous name: Brachydanio rerio</td>
<td>static</td>
<td>embryo-rearing water (60 mg/L)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>instant ocean salts ...</td>
<td>120 h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Data source**
**tris(2-chloroethyl) phosphate (TCEP)**

**Intermediate effects**

- **BPI Study title**: Comprehensive analysis of antagonistic endocrine activity during ozone treatment of hospital wastewater

- **BPI Study title (b)**: The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish

- **BPI Study title (c)**: TPP and TCEP induce oxidative stress and alter steroidogenesis in TM3 Leydig cells

**Administrative data**

**Endpoint**
- short-term toxicity to fish
- Evaluation of the endocrine disrupting effects of TCEP on zebrafish

**Type of information**
- experimental study

**Data source**

**Reference**
- publication | The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris (2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish

**Data access**
- data published

**Materials and methods**

**Principles of method if other than guideline**
**Materials and methods**

**Principles of method if other than guideline**
AB wild-type embryos were exposed from 3 to 120hpf. Twenty-four embryos were randomly selected and placed in three beakers (25 mL). Each beaker contained 10 mL TCEP solution, and the vehicle control group and test groups were performed in triplicate. Exposures were conducted in an incubator maintained in a stable environment during the experiment (photoperiod: 14/10 h light/dark; static; temperature: 27 ± 1 °C). The experiment was terminated at 120 hpf. To avoid evaporation of the test solution, beakers were covered with a breathable membrane.

**Test material**
Specific details on test material used for the study
Tris(2-chloroethyl) phosphate (TCEP) was purchased from AccuStandard.

**Sampling and analysis**

**Analytical monitoring**

yes

Details on sampling
**tris(2-chloroethyl) phosphate (TCEP)**

**Intermediate effects**

- **BPI, Study title:** Comprehensive analysis of antagonistic endocrine activity during ozone treatment of hospital wastewater
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- **BPI, Study title:** TPP and TCEP induce oxidative stress and alter steroidogenesis in TM3 Leydig cells
- **BPI, Study title:** Investigation of cytotoxic, genotoxic,

**Details on analytical methods**

Concentrations of TCEP in exposure solutions were confirmed by LC-MS/MS. No statistically significant difference (t-test, p > 0.01) was observed between measured and nominal concentrations of TCEP, suggesting minimal error from solvent configuration or adsorption of the container wall.

**Test solutions**

**Vehicle**

yes

**Dimethyl sulfoxide (DMSO)**

**Details on test solutions**

A stock solution of TCEP was prepared in dimethyl sulfoxide (DMSO), stored at -20 °C and diluted with embryo-rearing water (60 mg/L instant ocean salts in aerated distilled water) to final concentrations immediately before use. The final concentration of solvent (DMSO) in test solutions was less than 0.1%.

**Test organisms**

Test organisms (species)
**Intermediate effects**

- **BPI**: Study title: Comprehensive analysis of estrogenic endocrine activities during ozone treatment of hospital wastewater
- **BPI**: Study title (b): The combination of in situ and in vivo approaches for the investigation of disrupting effects of tris(2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish
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- **BPI**: Study title (a): Investigation of cytotoxic, genotoxic,

**Details on test organisms**
AB wild-type zebrafish embryos were used for the toxicity test.

**Study design**
**Test type**
static

**Water media type**
other: embryo-rearing water (60 mg/L instant ocean salts in aerated distilled water)

**Limit test**
no

**Total exposure duration**
120 h

**Test conditions**
**Test temperature**
Temperature: 27 ± 1 °C

**Nominal and measured concentrations**
- Measured (call for data): 25 ± 5, 50 ± 15, 100 ± 20, 250 ± 50, 500 ± 100, 1000 ± 200
I. Intermediate effects

1. BPI, Study title: Comprehensive analysis of antagonistic endocrine activity during ozone treatment of hospital wastewater

2. BPI, Study title (b): The combination of in silico and in vivo approaches for the investigation of disrupting effects of tris(2-chloroethyl) phosphate (TCEP) toward core receptors of zebrafish

3. BPI, Study title (c): TPP and TCEP induce oxidative stress and alter steroidogenesis in TM3 Leydig cells

4. BPI, Study title (d): Investigation of cytotoxic, genotoxic,

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**Reported statistics and error estimates**

The data from MD simulations was analyzed in GROMACS 4. The root mean square deviation (RMSD), which is the measure of the average distance between the atoms of superimposed proteins, was calculated. Origin 8 was used to analyze the RMSD fluctuations. GraphPad was primarily used for statistical processing. One-way analysis of variance (ANOVA) and Tukey’s test were used to determine significant differences between experimental and control groups. The resulting network genes (nodes) were colored by the Enhanced Graphics application within Cytoscape v3.1.1 according to the significant changes in gene expression in the respective treatments.

**Expression of genes associated with several NR signaling pathways:**

- Exposure: 2.85, 28.5 and 285 μg TCEP/L resulted in changes in the mRNA expression of genes associated with several NR signaling pathways.

**Nominal test mat:**

- Effect size
- Sample size
- p-value
tris(2-chloroethyl) phosphate (TCEP)

201 intermediate effects

- BPI: Study title: Comprehensive analysis of antagonistic endocrine activity during ozone treatment of hospital wastewater

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Observations

**Level of biological organisation observed**
- molecular

**Concentration range tested**
- >= 100 <= 300 ng/L

**Type of dose metric**
- effective concentration
  - with or without hCG

**Effect concentration**
- >= 100 <= 300 ng/L

**Remarks on result**
- Significant decrease at 100 and 300 ug/mL when compared to controls (no hCG) Significant decrease at 300 ug/mL when compared to hCG controls (hCG + TCEP mix).
Take-home messages

- OHT 201 facilitates **reporting of NAM study results** in an internationally agreed format

- OHT 201 supports the chemical angle of the “Stressor – Method – AOP” triangle

- OHT 201 is available in a **free ICT** application

- OHT 201 supports **all classes of NAMs**, especially *in-vitro* methods

- OHT 201 reports (per chemical and intermediate effect) one or more objective measurements and **one subjective conclusion**
OECD Harmonised Template 201 is explicitly mentioned in the EPA NAMs workplan.

Develop robust reporting templates for NAMs

Studies are submitted to regulatory programs with specific reporting requirements to aid in evaluation and interpretation. To promote consistency, the OECD has general reporting:

Deliverable: Reporting templates which may be used by EPA and stakeholders that capture the range of specific NAMs used for Agency decisions. The reporting templates will be delivered in the fourth quarter (Q4) of 2022.

templates that may be used by different regulatory jurisdictions. The templates include standard elements that should be included in methods descriptions for individual test assays, batteries of assays, and algorithms for evaluating sets of assay results. Although the reporting templates for NAMs are still evolving, the OECD has developed guidance to help standardize in vitro methods suitable for regulatory purposes as well as a reporting template for in vitro tests describing molecular and cellular observations that can be relevant to the hazard assessment. To accommodate mutual acceptance of data, the EPA will build on these established templates while providing additional templates that capture the range of specific NAMs used for Agency decisions.

OECD. OECD Harmonised Template 201: Intermediate effects
Links

OECD Harmonised Templates
http://www.oecd.org/ehs/templates/

OHT 201
http://www.oecd.org/ehs/templates/harmonised-templates-intermediate-effects.htm

IUCLID

EASIS
Stay in touch

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YouTube: EU Science Hub