Use of Risk Assessment to Effect Replacements of Animal-Based Toxicity Tests for Vaccines by In Vitro Tests

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Biosafety Risk Management
Assigning a risk class to a contained use: Model

I. CLASSIFY INTO HAZARD GROUP
   - Micro-organisms: (Lists of Royal decree 99/04/29 and AGW 2002/07/04)
   - Cell Culture
   - GMOs:

II. Characterize
   - Potential Harmful effects
   - Mode of transmission
   - Biological stability
   - Inactivation / decontamination

III. Analyse the contained use to assess the exposure
   - Co-Factors that modulate the risk:
     - Activity (lab - manufacture...)
     - Scale level
     - Process
     - Facilities / Equipment

IV. Assess the risk
    Classify the contain use
    - Null or negligible: classe 1
    - Low: classe 2
    - Moderate: classe 3
    - High: classe 4
Biosafety Risk Management
Assigning a risk class to a contained use model

I. Hepatitis A Virus

II. Characterise

III. Analyse the contained use to assess the exposure

IV. Assess the risk
   Classify the use
   Propose to the Belgian Experts

Risk Group 2

Potential Harmful effect: infection is often asymptomatic, sometimes an abrupt onset is observed followed by mild illness (1-2 weeks), or severely disabling illness (several months)

Mode of transmission: faecal-oral route

Survival outside the host: survive in water and sewage for long periods:

Physical inactivation: 4 min at 70°C, immediately at 85°C

Vaccine available (HAVRIX)

Cofactors affecting exposure
Scale: large scale
Process: GMP compliance, semi-closed system, no equipment which can increase the contact at the port of entry

Low: class 2
ICHQ9 Risk Management Process
Applicable on 3Rs? Specifically on replacement of in vivo testing?

ICH guidelines

Q8: Pharmaceutical Development (2009)
Q9: Quality Risk Management (2005)
Biosafety tool: Bowtie
CAUSES & CONSEQUENCES

Likelihood

Cause 1
Cause 2
Cause 3
Cause n

Barriers to AVOID risk from happening

Severity

Consequences

Risk description

Barriers to REDUCE consequences if risk has happened

Likelihood

Severity
Worked example of replacement
In vivo toxicity test Histamine sensitization assay for aP containing vaccine

Reference: Overview: International Working Group and the Collaborative Study for Alternatives to HIST (BSP114)
Richard Isbrucker, Ph.D. Health Canada Centre for Biologics Evaluation Bacterial & Combination Vaccines Division
There is a **risk of intoxication** of the patient that receives vaccine due to the **failure of detection** of residual toxin or reversion of toxoid to toxin because of inappropriate detoxification **process** beyond validated frame AND because of replacement of an in vivo method by an in vitro method or due to the **waiving** of in vivo assay from the control strategy.
Risk analysis & evaluation: 1) Causes
Apply model to in vivo toxicity testing replacement

• Cause 1: Alternative test failed to address in vivo toxic mechanism of action.

• Cause 2: Sufficient amount of toxin (to provoke symptoms) has not been fully detoxified AND would have been detected by in vivo method.

• Cause 3: Sufficient amount of toxin (to provoke symptoms) has reverted to toxicity within product shelf-life AND would have been detected by in vivo method.

• (Individual Cause 4: Subject stands, in vaccination schedule, in a position where he / she has not enough antibody to avoid intoxication.)
Risk analysis & evaluation: 2) consequences
Biosafety model

– Minimum dose of native biologically active toxin able to provoke intoxication symptoms in human?

– Risk of immediate harm to the patient?

– Risk of delayed harm to the patient?
Risk analysis & evaluation: 2) consequences

Minimum **dose** of native biologically active toxin able to provoke intoxication symptoms in human?
Risk analysis & evaluation: 2) consequences
Example of Pertussis Toxin

• Immediate harm

Intoxication transient symptoms

wP vaccine contains up to 300µg / dose (1) of PTx yet is considered as safe and effective (2)

(1) Ashworth (1983); Sato (1990); Redhead (1996)
(2) WHO Position paper (2005)

• Delayed harm

Acellular Pertussis vaccine does not contain the whole cells. There is no chance for bacterial infection causing delayed harm.

Aftermath?

No increased risk for invasive bacterial infection found following diphtheria-tetanus-pertussis immunization
Risk Reduction
Apply model to in vivo toxicity testing replacement

• Cause 1: Alternative test failed to address in vivo toxic mechanism of action.

• Evaluation of cause 1 worked example on Histamine Sensitization Assay replaced by CHO on formulated product

Deep dive on comparison of analytical validation

<table>
<thead>
<tr>
<th></th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection</td>
<td>Approx. 2 IU/ml</td>
<td>Approx. 0.006 IU/ml</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Intoxication (animal)</td>
<td>Intoxication (cell)</td>
</tr>
<tr>
<td>Production step (test sample)</td>
<td>Formulated final bulk</td>
<td>Formulated final bulk + other prod step</td>
</tr>
<tr>
<td>Does validation implies testing of « good » &amp; « bad » lots or spike ?</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Validation conditions (@4°C &amp; @ 37°C after 4 weeks)?</td>
<td>yes</td>
<td>yes</td>
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Risk Reduction
Apply model to in vivo toxicity testing replacement

- Detoxification process Design (strategic approach: detoxified purified PTx (+ process), genetically modified toxin, whole cell vaccine?)
- Detoxification process qualification (aldehyde(s) type(s) used, concentration, reaction time, …other Critical Quality Attributes (CQA))
- Historic (new process VS process with significant proven experience of safe product distributed and delivered? + size of data base history.)
- Stability data (@ 4°C & @ 37°C; up to end of shelf live)
- Robustness data
- Dilution factor (e.g. in process testing done on up stream concentrated purified bulk (Diphtheria / tetanus) assure appropriate detox. hundreds of final container doses)
Detoxification process strategy
Performance and stability of detoxification

Example of Pertussis Toxin Detoxification process.

- Carbohydrate binding activity as a marker of detoxification.
- Comparison of detoxification with Formaldehyde and /or Glutaraldehyde treatment.
  - At 4°C filled circles
  - At 37°C open circles

The sequential treatment with both denaturants had more drastic effect on the ADP-ribosyltransferase and the carbohydrate binding activities of PTx.

Characterization of the carbohydrate binding and ADP-ribosyltransferase activities of chemically detoxified pertussis toxins
H Oh, BG Kim, KT Nam, SH Hong, DH Ahn - Vaccine, 2013 - Elsevier
Risk Reduction: QbD
Consistency approach

• The consistency approach is a new paradigm for improved quality control of vaccines which moves away from the current focus on quality control of the final product and high reliance on in vivo models, to an integrated in-process quality monitoring programme during vaccine lot production using non-animal methods (in line with 3Rs principle and European Directive 2010/63).

• Part of an integrated approach covering all the production process

Advantages:

– Better characterization of the product (at all stages)
– Improved standardization and optimization of the production process
– Tight in-process control. Product monitoring with new and improved testing tools
– Use of Quality Systems to guarantee consistency (GMP, QA, Pharmacovigilance)
Risk Reduction
Apply model to in vivo toxicity testing replacement

– Control strategy (e.g. CHO test BEFORE detoxification (idea of PTx load to be detoxified, CHO test AFTER detox. monitors detoxification performance)
Process Characterization data

– Quality system (e.g. Process trend analysis: monthly follow up of trending, setting of action limit / alert limit, Semestrial meeting, Product Quality Annual Review, Deviation review, CAPAs, process change impact, product complaint,…)

– Quality By Design (e.g. built in quality, robust process under control, explored Proven Acceptance Range beyond Normal Operating Range)
Risk Acceptance
GSK Matrix used in risk assessment

Risk magnitude: low, medium, high

<table>
<thead>
<tr>
<th>Likelihood</th>
<th>Severity</th>
<th>Insignificant 1</th>
<th>Minor 2</th>
<th>Moderate 3</th>
<th>Major 4</th>
<th>Catastrophic 5</th>
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<tbody>
<tr>
<td>Rare 1</td>
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<td>1</td>
<td>2</td>
<td>3</td>
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<td>25</td>
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</table>

Risk Index Value = Severity score multiplied by Likelihood score.

- RIV = (S x L)
- RIV ➔ Color code
- Green = Low
- Yellow = Medium
- Red = High
- Orange = Stop activity!

Stop activity immediately! Implement additional control measures before restarting!
Model applied on pertussis toxin activity test
Scoring proposition (for example purpose only)

Likelihood

1: alternative ≠ toxic mechanism of action.
2: residual toxin AND detected by in vivo
3: revertant toxin AND detected by in vivo
4: not enough antibody to avoid symptoms.

Production process:
- Detoxification process strategy
- dPT dose in vaccine (25µg/dose)

Process validation
- security margin

Choice of alternative test
- Cell intoxication based

Test validation
- Accuracy, sensitivity

Control strategy:
- CHO before & after detox
- characterization data

Severity

QbD
- Antigen target profile (accelerated) by nature avoid delayed harm

Quality system
- Good storage conditions
- Trending

Control strategy:
- IPC / stability plan
- characterization data

No prolonged harm.
Far less than 300µg/dose of wP LD50 10x less than diphtheria and 1000 x less than Tetanus

Early detection by trending with sensitive tests

Likelihood

Severity

Consequence / severity overall score = 1

Cause / probability overall score = 1 or 2

![Risk description](image)
Risk review

Risk Event

- To loop-back to risk evaluation
- As part of Product Life Cycle monitoring on a regular basis check for:
  - Pharmacovigilance / safety signal evaluation
  - New clinical data
  - Periodic Quality Review
  - Deviation (recurrence)
  - Change control impact evaluation
  - Continued process verification (after process design and process qualification)
Risk review applied to testing replacement
Part of quality system

• Risk review
  – Product History (Take into consideration amount of doses distributed for which risk identified has not happened)
  – Trending (Analyse test trending addressing the CQA (and others) in Product Quality Reviews / Annual Product Reviews)
  – Complaint (Analyse number of recall / complaint consecutive to symptom of Diphtheria or Pertussis intoxication)
  – Adverse event (Analyse number of PSUR relative to symptom of Diphtheria or Pertussis intoxication)
  – Incident Management (Analyse number of product incident and recall relative to symptom of Diphtheria or Pertussis intoxication)
Consider Risk Assessment model in the application of 3Rs acceptance

- Broadening the scope permits to step back and to take a holistic view on a specific request.

- Adapt, create and apply a risk assessment model to include pertinent parameters to consider.

- Systematic approach (vs individual sensitivity)

- Put in perspective technical discussions on level of detection / sensitivity to reach for an alternative.

- May help building adapted / differentiated decision making process.

- When agreed, each reducing risk item / reducing severity item can be scored individually.

- Items can be affected by an agreed specific weight (e.g. additional weight on detoxification process vs storage condition)