Critical analysis of refinement methods

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What will be discussed

✓ Introduction to the topic
✓ Refinement: some definitions and examples of refinement
✓ Why refinement is more than just an animal welfare concept
✓ Future refinement challenges
✓ Obstacles in development and use
✓ Conclusions and recommendations
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Problematic human – animal relationships

- Laboratory animals: (609.313)
- Farm animals: > 100 million
- Companion animals: approx. 20 million
- Circus animals: ??
- Pest animals: ??

(Data from the Netherlands)
Public acceptability of animal experiments

Survey Statement: Scientists should be allowed to do research that causes pain and injury to animals* if it produces new information about human health problems.

<table>
<thead>
<tr>
<th>Public Attitudes to Animal Research</th>
<th>1985</th>
<th>2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support</td>
<td>63</td>
<td>44</td>
</tr>
<tr>
<td>Oppose</td>
<td>30</td>
<td>52</td>
</tr>
<tr>
<td>Don’t know</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>


*N.B. according to US laws rodents are not counted as laboratory animals.
Replacement: the solution?

Production of monoclonal antibodies

Pyrogenicity testing

Safety test Diphtheria Vaccine

**Replacement**

**Reduction**

**Refinement**
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CCAC National Workshop, November 1, 2007
Refinement: definitions

Refinement: any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used
(Russell & Burch, 1959)

Refinement refers to any approach which avoids or minimises the actual or potential pain, distress and other adverse effects suffered at any time during the life of the animals involved, and which enhances their well-being
(Buchanan-Smith, Animal welfare 2005)
Examples of Refinement methods

Direct Refinement
- Pain relief and anesthesia
- Environmental enrichment
- Application of humane endpoints
- Group housing of social animals
- Use of less stressful procedures
- Etc.

Indirect Refinement
- Education & training
- Health welfare monitoring system
- Development of Codes of best Practice
- Culture of care in the institute
- Etc.

Percentage of total number of experiments involving > moderate/severe pain and distress: data from the Netherlands 1998 - 2005
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Why Refinement?

◆ Legal:

‘All experiments shall be designed to avoid distress and unnecessary suffering to the experimental animals’
(Europe: Council Directive 86/609/EEC, art.7.4)

‘Animals must not be subjected to unnecessary pain or distress. The experimental design must offer them every practicable safeguard, whether in research, in teaching, or in testing procedures’
(CCAC, Ethics of Animal Investigation, 1989)

◆ Moral/social:

- Animal experiments are less acceptable when these involve severe pain/distress
- Use of less refined methods when refined methods are available is unethical

◆ Scientific:

Public acceptance (%) of animal experiments for specific purposes with and without pain

(Aldhous et al. 1999)
Application of humane endpoints: possibilities

‘In experiments involving animals, any actual or potential pain, distress or discomfort should be minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research’  
*(CCAC, 1998)*

Types of humane endpoints  *(Olfert & Godson, 2000)*

**Biochemical/Hormonal:** e.g. acute phase proteins, prolactin, cathecolamines, corticosteroids

**Pathophysiological:** e.g. respiratory rate, body weight, dehydration, blood count

**Clinical/behavioral:** e.g. activity, response to handling, posture, vocalization
Humane Endpoints in wcP potency testing: surrogate endpoints evaluated

Clinical signs:
- Video recording
- Observation (twice a day)

Body weight:
- Daily

Body temperature:
- Transponder (twice a day)

Evaluation of humane endpoints

Selection of endpoints:
- Type of experiment
- Practical objectives
Humane endpoints: future challenges

‘In experiments involving animals, any actual or potential pain, distress or discomfort should be minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research’

(CCAC, 1998)

Adapted from Richmond, 1999
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- **Moral/social:**

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- **Scientific:**

  - Happy animals make good Science (T.Poole)
Refinement and optimisation of science: an example

wcP (whole cell pertussis vaccine) causes fever in infants

- Product improvement (decreasing biomass pertussis toxin)
- Rabbit animal model (parameter: increase in body temperature)
- Single cage housing, no habituation to handling

Results; no vaccine dose-dependent response

- High variation in experimental data
Effect blood sampling and immunisation in the non-optimized model

(Verwer et al, submitted)
Circadian body temperature

(Verwer et al, submitted)
Modifications in experimental conditions

**Housing**

- Group housing

**Handling procedure**

- Birth – weaning: weighing procedure
- Experiment: 3x p. week stroking on lap (5 min.)

(CCAC National Workshop, November 1, 2007)
**Effect blood sampling and immunisation**

![Graph showing blood sampling and immunisation response in °C for Modified laboratory conditions; Non-Handled rabbits and Handled rabbits.](image)

Modified laboratory conditions; Non-Handled rabbits

- Blood sampling
- Immunisation

Modified laboratory conditions; Handled rabbits

- Blood sampling
- Immunisation

Response in °C

Time of day (0 = midnight)

**Saline**

- 8 IOU

**16 IOU**

(Verwer et al, submitted)
Circadian body temperature

(Verwer et al, submitted)
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Future developments in laboratory animal science: Reduction and Refinement

Central line
Getting more and better data from less animals and with less pain and distress for the animals still involved

- Undisturbed behavioural monitoring systems in home cages
- Use of real time and life-time monitoring systems; e.g. telemetry
- Use of non-invasive technologies; e.g. NMR, biophotonic monitoring, -omics technology

Biophotonic imaging: makes use of the firefly gene (Luceferase)

Real-time monitoring will allow for:
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Obstacles in development and use

- Refinement is the Cinderella of the 3Rs
- Ethical dilemma (castration, conflict between refinement and reduction)
- Methods are not being used
- Regulatory constraints
Obstacles in development and use

- **Refinement is the Cinderella of the 3Rs**

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Refinement is the Cinderella of the 3Rs: Consequences:

- Difficult to get funding. Priority given to Replacement and Reduction

- Publication in scientific journals with a low impact factor

- Research is not always of high scientific quality

- Image problem: ‘soft’ science, not very ‘sexy’ for your c.v.

- Difficult to be used by policy makers (not quantifiable)
Obstacles in development and use

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- **Ethical dilemma (castration, conflict between refinement and reduction)**
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castration
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Conflict reduction vs. Refinement: examples

- Use of transgenic animals
- Biophotonic imaging
- Re-use of animals
- Use of telemetric devices
Radio telemetry

Scientific advantages of Radio telemetry

- Data can be obtained without stressful and physiological disturbing interventions
- Can be used to conduct studies that would previously not have been feasible (e.g. blood pressure, body temp.)
Radio telemetry: Reduction vs Refinement

Reduction

- Animals not necessarily need to be sacrificed at the end of the experiment and can be re-used
- Possible reductions in numbers of animals
- Animals can behave as their own controls

Animal welfare consequences

- Consequences of surgery
- Post-surgery pain and distress
- Transmitter in abdominal cavity
- Risk for complications due to surgery
- Individual housing
- Re-use of animals
Radio telemetry: Reduction vs Refinement

Refinement
- Reduced stress during monitoring (animals in home cage)
- Animal can behave and move normally
- On line recording directly informs the researcher about the welfare of the animal (humane endpoints)

Animal welfare consequences
- Consequences of surgery
- Post-surgery pain and distress
- Transmitter in abdominal cavity
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- **Methods are not being used**
- Regulatory constraints
Potential obstacles in the implementation of humane endpoints

Potential obstacles

- Lack of motivation
- Economical and practical obstacles: e.g. frequent monitoring or expensive equipment required
- Validation might be needed
- Harmonisation
- In case of clinical signs:
- Scientific obstacles: increased variability, objectives study, etc.

‘.....indication of a positive result is obtained the animal in question shall be humanely destroyed’.

European Pharmacopoeia

‘All experiments shall be designed to avoid distress and unnecessary suffering to the experimental animals’

(Council Directive 86/609/EEC, art.7.4)

Informal questionnaire on the use of humane endpoints, send to vaccine manufacturing companies in Europe

- death was frequently used as endpoint
- no analgesics were given
- few studied the use of humane endpoints
CD-ROM on Humane Endpoints for training purposes

For receiving a free copy of the cd-rom: mail to
i.boumans@uu.nl
Obstacles in development and use

- Refinement is the Cinderalla of the 3Rs
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Differences between injection volume specifications in the Pharmacopoeia European monographs for vaccines and reference volumes given in handbooks

<table>
<thead>
<tr>
<th>Ph.Eur. Monograph</th>
<th>Test</th>
<th>Animal species (weight)</th>
<th>Injection route</th>
<th>Ph.Eur. injection volume (ml)</th>
<th>Injection volume range from handbooks (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria &amp; Tetanus vaccines</td>
<td>Spec.tox.</td>
<td>Guinea-pig 250-350 g.</td>
<td>s.c.</td>
<td>5</td>
<td>1-3</td>
</tr>
<tr>
<td>Pertussis vaccine (acellular, adsorbed)</td>
<td>Absence</td>
<td>Mouse 18 – 26 g</td>
<td>i.v.</td>
<td>&lt; 0.5</td>
<td>0.125 – 0.3</td>
</tr>
<tr>
<td>IPV vaccine</td>
<td>Potency</td>
<td>Guinea-pig 250 – 350 g</td>
<td>i.m.</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>IPV vaccine</td>
<td>Potency</td>
<td>Rat 175-250 g</td>
<td>i.m.</td>
<td>0.5 (2 x 0.25)</td>
<td>0.1</td>
</tr>
<tr>
<td>Test for extraneous agents in viral vaccines</td>
<td>Test of virus seed lot</td>
<td>Mouse 15 – 20 g</td>
<td>i.c.</td>
<td>0.03</td>
<td>0.02</td>
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Conclusions & Recommendations

- Refinement will be one of the foremost future challenges to Laboratory Animal Sciences.
- Consider optimising welfare as the target rather than reducing pain and distress.
- Refinement is not an animal welfare concept alone, but a scientific concept as well: models might become more robust, better reproducible and more relevant. This should be communicated more loud and clear to the scientific community.
- An up-grade of refinement activities is needed in terms of funding priorities, publishing policies, scientific quality and scientific recognition.
- Progress in refinement is a worthwhile goal, although not a goal without obstacles.

Refinement: for the benefit of the animals AND for the benefit of science.
Thanks for your attention!