

3Rs Symposium

SESSION II

Implementation of the 3Rs:
Regulatory Aspects
Applying the 3Rs within the
regulatory system: obligations and
opportunities

Commission policy

- “... the Commission is committed to the reduction of animal usage, wherever possible, in pharmacopoeia testing and encourages those associated with its work to seek alternative procedures. An alternative or modified method is adopted by the Commission once it has been clearly demonstrated that it offers satisfactory control for pharmacopoeial purposes”
(Introduction to the 4th Edn)

Peter Castle, EDQM

2

Progress with 3Rs in Ph Eur

- Replacement: introduction of in vitro methods, elimination of unnecessary tests
- Reduction: transfer of tests upstream, deletion of requirements for minimum numbers of animals, one-dilution assays
- Refinement: introduction of serological assays
- Grey zones: some monographs allow application of 3Rs but keep animal test as reference method

Peter Castle, EDQM

3

Replacement

- *In vitro* test for residual diphtheria toxin
- Animal immunogenicity assay of haemophilus vaccine
- *In vitro* assays for hepatitis A and hepatitis B vaccines

Peter Castle, EDDM

4

Reduction

- Abnormal toxicity test as a validation requirement rather than for routine use
- Minimum numbers of animals deleted from assays for diphtheria and tetanus vaccines (rely on statistical validity)
- One-dilution assays mentioned to encourage their use (diphtheria, rabies, tetanus vaccines)

Peter Castle, EDDM

5

Refinement

- Serological assays for clostridial vaccines
- Other serological assays will be introduced (diphtheria, swine erysipelas vaccines)

Peter Castle, EDDM

6

Grey zones

- Monographs allow explicitly or implicitly the application of 3Rs methods
- Probably need validation for each product
- Competent authority has to approve the change on the basis of data

Peter Castle, EDQM

7

Alternative methods

- “ ... does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia ... The manufacturer may [use] data derived, for example, from validation studies of the manufacturing process and from in-process controls” (*General Notices*)

Peter Castle, EDQM

8

Vaccines for Veterinary use

- “...The identification test can often be conveniently combined with the batch potency test to avoid unnecessary use of animals. For a given vaccine, a validated *in vitro* test can be used to avoid the unnecessary use of animals.”

Peter Castle, EDQM

9

Humane endpoints

- Vaccines for Human/Veterinary Use
- “... tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. The criteria for judging tests in monographs must be applied in the light of this”

Peter Castle, EDQM

10

Humane endpoints (2)

- Use of humane endpoints is part of the application of the 3Rs and has to be considered by anyone carrying out testing according to Ph Eur
- Some monographs have alternative methods which for historical reasons are not equivalent for humane endpoints - choose the more humane endpoint!

Peter Castle, EDQM

11

Batch safety test for veterinary vaccines

- “...for an established vaccine the routine application of the safety test will be waived by the competent authority in the interests of animal welfare when a sufficient number of consecutive production batches have been produced and found to comply with the test, thus demonstrating consistency of the manufacturing process” (*Vaccines for Veterinary Use*, in press)

Peter Castle, EDQM

12

Batch safety test for veterinary vaccines (2)

- Waiving of test depends on decision of competent authority
- If animals are used for another test, e.g. extraneous agents, test cannot be discontinued, *unless* a validated in vitro test for extraneous agents can be used
- Drafting of a guideline on application of new measures proposed to CVMP

Peter Castle, EDQM

13

Replacement of a test within a monograph

- Residual toxin in diphtheria toxoid: guinea-pig test is no longer included in the monograph
- Method has internal validation with reference toxin BRP
- Change to new method is by variation to comply with the Ph Eur monograph

Peter Castle, EDQM

14

Immunochemical assay of tetanus immunoglobulin

- International collaborative study carried out by Ph Eur and WHO for establishment of BRP/IS and comparison of immunochemical assay with the mouse neutralisation test (MNT)
- For each product, satisfactory correlation with MNT has to be demonstrated for immunoassay, since the latter determines binding, not neutralisation
- Routine method in monograph is immunoassay
- Similar testing schemes will be introduced for veterinary clostridial sera (Pharmeuropa 14.4)

Peter Castle, EDQM

15

Abnormal toxicity test

- Historical study carried out by Paul-Ehrlich Institut
- Test moved to Production section as a validation requirement
- Data have to be submitted to licensing authority on a sufficient number of batches
- Or for existing vaccines, data on previously manufactured batches can be used

Peter Castle, EDDM

16

Waiving of tests by licensing authorities

- Is compatible with Ph Eur requirements (see General Notices)
- Examples:
 - Residual pertussis toxin test for acellular vaccine, *when consistency established*
 - Poliomyelitis vaccine (inactivated) in vivo assay, *when consistency established*

Peter Castle, EDDM

17

The role of EU guidelines in reducing animal usage in the testing of veterinary vaccines

David Mackay
Veterinary Medicines
Directorate UK



Content of the talk

- Role of guidelines in EU regulatory system
- Examples of
 - Reduction
 - Refinement
 - Replacement
- Current work
- Future plans



Regulatory 'Pyramid' for veterinary vaccines

Directive 2001/82/EC

European or national monographs

CVMP general and specific guidelines

Other regulatory guidance documents (OIE, FAO)



VICH*

- **EU/USA/Japan**
- **Observer countries**
 - Australia, New Zealand, others
- **Harmonisation of regulatory requirements**
 - Global market for veterinary products
 - Global programs of product development

* International Conference on Harmonisation of Technical Requirements
For the Registration of Veterinary Pharmaceutical Products



Role of CVMP guidelines

- **Clarification of EU legislative requirements**
- **Harmonisation of requirements between Member States of the EU**
- **Supplement requirements of the European Pharmacopoeia**
- **Promote 'best practice' for regulators and industry and thereby minimise animal usage by reducing**
 - Duplication
 - Redundancy



Reduction/Refinement

- **Removal of the abnormal toxicity test in mice**
- **Clarifying the requirements for demonstrating concurrent use of two vaccines**
- **Reduced requirement for safety testing for acceptance of a new strain of equine influenza virus**
- **Establishing criteria for acceptance of serology as an indicator of efficacy in duration of immunity trials**



Replacement

- Establishing criteria for replacement of *in vivo* batch potency tests for inactivated vaccines with *in vitro* antigen quantification tests
- Replacement of challenge with serology for equine influenza vaccines



Current work

- Replacement of challenge tests with serology for Foot-and-Mouth Disease vaccines
- Criteria to be met for a manufacturer to stop performing the target animal safety test on every batch



Future work

- Development of guidelines for harmonised 'Official Medicines Control Authority Batch Release' of immunological veterinary medicinal products
- OMCA Network established and run by EDQM
- Promoting mutual recognition of batch release by Member States OMCA's



Current proposals for OMCABR

- OMCABR Batch Release conducted on every batch
- OMCABR Batch Release must include product re-testing
- Harmonised testing reduces the potential for duplication of re-testing in several Member States
- No option for targeted re-testing
- No option for batch release based on documentary inspection by OMCABR
- Potential for overall increase in animal usage due to increased re-testing by OMCABRs



Conclusions

- Why is change so slow?
 - Reluctance
 - Habit and custom
 - 'Comfort factor'
 - Cost
 - Revalidation
 - Time
 - Cost recovery
 - Regulations
 - 'Catch 22' situation



Conclusions

- Working parties should be proactive in considering how best to implement the 3R's in terms of preparing new guidelines and revising old ones
- A full 'cost:benefit' assessment in terms of animal welfare should be conducted on each occasion that a requirement for animal testing is introduced or reviewed
- New guidelines or requirements for testing should only be introduced where the benefits, normally in terms of added safety, clearly outweigh the costs



3Rs Symposium
SESSION II
Implementation of the 3Rs:
Regulatory Aspects

29/11/02 1

The role of OMCLs in the
implementation of 3R
The special case of vaccines

Roland Dobbelaer, Dr. Sc.,
Head Biological Standardisation
Scientific Institute of Public Health
Federal Ministry of Public Health
Brussels

Scientific Institute of Public Health - Louis Pasteur
Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
Institut Scientifique de Santé Publique - Louis Pasteur
BIOLOGICAL STANDARDISATION
Copyright, 1996 © Dale Carnegie & Associates, Inc.

2

In-vivo tests may be used at all stages of the
life-span of a medicinal product

R&D

Licensing

IPC & QC

Quality Monitoring

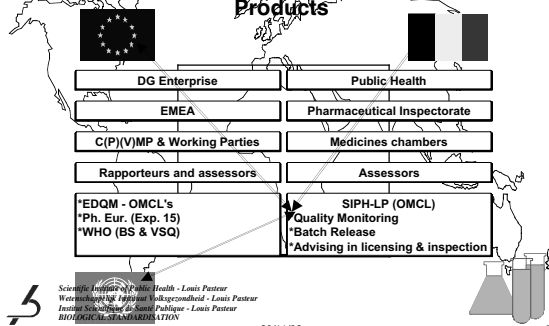
Scientific Institute of Public Health - Louis Pasteur
Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
Institut Scientifique de Santé Publique - Louis Pasteur
BIOLOGICAL STANDARDISATION
29/11/02

3

Biological medicinal products are special with respect to the use of laboratory animals

- Physico-chemical characterisation insufficient
- *In-vivo* testing may be required for the release of every batch
- Vaccines & plasma derivatives are submitted to OMCL Batch Release

Quality Surveillance of Biological Medicinal Products




CONTROL AUTHORITY BATCH RELEASE

- ◆ PRODUCER-INDEPENDENT OMCL PRE-MARKETING
- ◆ RE-testing and/or RE-evaluation of production & control protocol
- ◆ to verify & monitor conformity with MAA, Ph. Eur. & WHO

Main EU OMCL Batch Release tests on Bacterial Vaccines (1)


Vaccine Component	Tests (all in-vivo)
Diphtheria	<ul style="list-style-type: none"> • <i>Potency & identity on final bulk</i>: single or multiple dilution, toxin challenge, test on guinea-pigs versus in-house reference.
Tetanus	<ul style="list-style-type: none"> • <i>Potency & identity on final bulk</i>: single or multiple dilution, toxin challenge, test on mice versus in-house reference.
Pertussis (whole cell)	<ul style="list-style-type: none"> • <i>Potency & identity on final bulk</i>: multiple dilution, B. pertussis intracerebral challenge test (Kendrick test) on mice versus in-house reference.
Pertussis (acellular)	<ul style="list-style-type: none"> • <i>Potency & identity on final bulk</i>: multiple dilution, ELISA serological test on mice for PT, FHA & 69K components, versus in-house reference. • <i>Residual pertussis toxin on final bulk</i>: Histamin Sensitising Activity in mice • Endotoxin content (LAL)


 Scientific Institute of Public Health - Louis Pasteur
 Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
 Institut Scientifique de Santé Publique - Louis Pasteur
 BIOLOGICAL STANDARDISATION

29/11/02 7

Main EU OMCL Batch Release tests on Bacterial Vaccines (2)


Vaccine Component	Tests (all in-vitro)
Meningococcus	<ul style="list-style-type: none"> • <i>Identification & assay for each polysaccharide</i> by immuno rate nephelometry
Pneumococcus	<ul style="list-style-type: none"> • <i>Identification & assay for each polysaccharide</i> by immuno rate nephelometry
Haemophilus influenzae type B	<ul style="list-style-type: none"> • <i>Identity and molecular size distribution on PRR P and bulk conjugate</i> by HPLC • <i>Free polysaccharide content</i> by ELISA • <i>Total polysaccharide content</i> by rate nephelometry • <i>Molecular size distribution on final container</i> by HPLC • Endotoxin content (LAL)


 Scientific Institute of Public Health - Louis Pasteur
 Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
 Institut Scientifique de Santé Publique - Louis Pasteur
 BIOLOGICAL STANDARDISATION

29/11/02 8

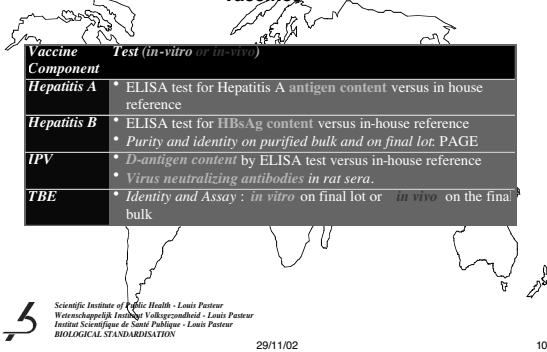
Main EU OMCL Batch Release tests on Bacterial Vaccines (3)

Vaccine Component	Tests (in-vitro and in-vivo)
Live typhoid (Ty21a)	<ul style="list-style-type: none"> • Identity and stability (absence of reversion) • Number of live bacteria, count of viable units (potency assay) • Serological characteristics
Typhoid PS	<ul style="list-style-type: none"> • Molecular size PS • O-acetyl PS • Endotoxin PS • PS content in final lot • O-acetyl in final lot • Endotoxin in final lot
BCG	<ul style="list-style-type: none"> • Identity • Viable count • Virulent Mycobacteria on seedlot • Excessive dermal reactivity on seedlot



 Scientific Institute of Public Health - Louis Pasteur
 Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
 Institut Scientifique de Santé Publique - Louis Pasteur
 BIOLOGICAL STANDARDISATION

29/11/02 9

Main EU OMCL Batch Release tests on non-live viral vaccines

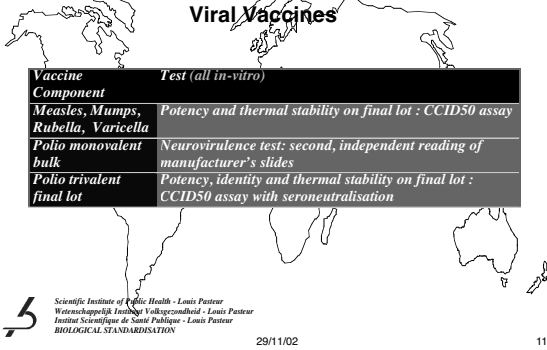


Vaccine Component	Test (in-vitro or in-vivo)
Hepatitis A	<ul style="list-style-type: none"> ELISA test for Hepatitis A antigen content versus in-house reference
Hepatitis B	<ul style="list-style-type: none"> ELISA test for HBsAg content versus in-house reference Purity and identity on purified bulk and on final lot. PAGE
IPV	<ul style="list-style-type: none"> D-antigen content by ELISA test versus in-house reference Virus neutralizing antibodies in rat sera.
TBE	<ul style="list-style-type: none"> Identity and Assay : in vitro on final lot or in vivo on the final bulk


 Scientific Institute of Public Health - Louis Pasteur
 Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
 Institut Scientifique de Santé Publique - Louis Pasteur
 BIOLOGICAL STANDARDISATION

29/11/02 10

Main EU OMCL Batch Release tests on Live Viral Vaccines




Vaccine Component	Test (all in-vitro)
Measles, Mumps, Rubella, Varicella	Potency and thermal stability on final lot : CCID50 assay
Polio monovalent bulk	Neurovirulence test: second, independent reading of manufacturer's slides
Polio trivalent final lot	Potency, identity and thermal stability on final lot : CCID50 assay with seroneutralisation

 Scientific Institute of Public Health - Louis Pasteur
 Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
 Institut Scientifique de Santé Publique - Louis Pasteur
 BIOLOGICAL STANDARDISATION

29/11/02 11

Main EU OMCL Batch Release tests on Plasma Derivatives

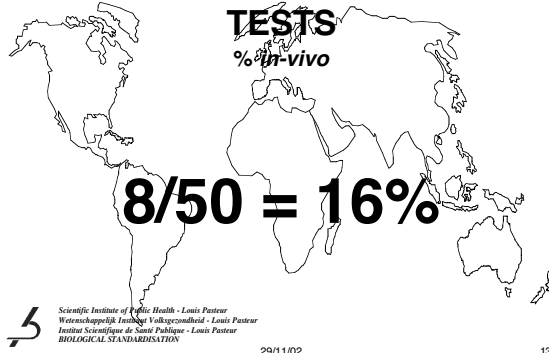


Product	Tests (all in-vitro)
Plasma pool	<ul style="list-style-type: none"> Viral markers <ul style="list-style-type: none"> Anti-HIV: ELISA HBsAg: ELISA Anti-HCV: ELISA HCV RNA: NAT
Albumin	<ul style="list-style-type: none"> Molecular Size Distribution: SEC-HPLC Pre-Kallikrein Activator: micro-colorimetry Appearance
Immunoglobulins	<ul style="list-style-type: none"> Molecular Size Distribution: SEC-HPLC Total Protein: colorimetry Protein composition: electrophoresis Appearance and solubility Potency (Hep A)
Clotting Factors	<ul style="list-style-type: none"> Appearance and solubility Potency Product-specific tests

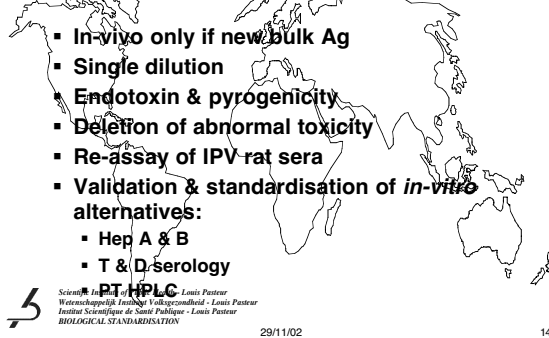
 Scientific Institute of Public Health - Louis Pasteur
 Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
 Institut Scientifique de Santé Publique - Louis Pasteur
 BIOLOGICAL STANDARDISATION

29/11/02 12

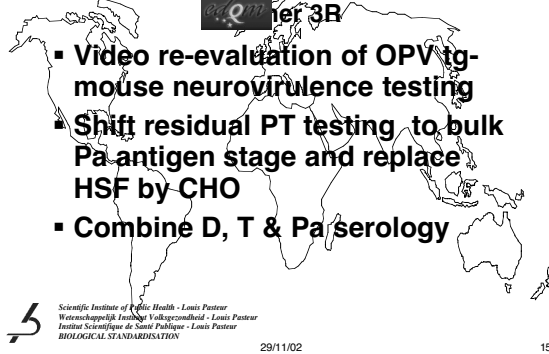
EU OMCL BATCH RELEASE




Initiatives by OMCL's/ in further 3R




Think-over initiatives by OMCL's/ in further 3R



Conclusions

- OMCLs  have played, play and will play an important role in implementing 3R
- Own testing
- Regulatory acceptance of alternative tests through validation, standardisation & involvement in licensing

 Scientific Institute of Public Health - Louis Pasteur
Wetenschappelijk Instituut Volksgezondheid - Louis Pasteur
Institut Scientifique de Santé Publique - Louis Pasteur
BIOLOGICAL STANDARDISATION

29/11/02 16

3Rs Symposium SESSION II

Implementation of the 3Rs: Regulatory Aspects

29/11/02 17

The 3Rs concept in candidate country

Vítková Eva
State Institute For Drug Control
Prague

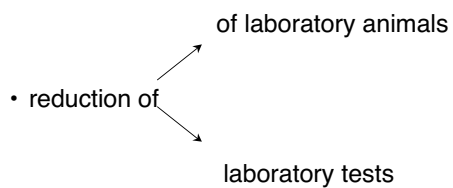
Vactrain - November 2002
Vítková SUKL

Concept 3Rs x Institute

- Knowledge
- Informations
- Implementation
- Future

Vactrain - November 2002
Vítková SUKL

Reduction

- reduction of 
 - of laboratory animals
 - laboratory tests

Vactrain - November 2002
Vítková SUKL

Refinement

- conditions of animal care
- conditions of laboratory testing
- trained staff

Vactrain - November 2002
Vitkova SUKL

Replacement

- general test - pyrogenicity x LAL
- vaccines - use of tissue cultures
ELISA
CHO, LPF

Vactrain - November 2002
Vitkova SUKL

Projects

- ELISA - control of Te, Di vaccines
HA, HB vaccines
- LAL - endotoxin content in
polysaccharide
vaccines
- specific toxicity wc pertussis

Vactrain - November 2002
Vitkova SUKL

Perspective

- Positive factors: reconstructed department
OMCL network support

- Negative factors: personnel work
high volume of paper
no accreditation

Vacrain - November 2002
Vikova SUKL

3Rs Symposium

SESSION II

**Implementation of the 3Rs:
Regulatory Aspects**

**THE 3 Rs CONCEPT IN THE
VETERINARY VACCINE CONTROL OF
THE CANDIDATE COUNTRIES**

Gábor Kulcsár and Tibor Soós

**Institute for Veterinary Medicinal Products
Budapest
Hungary**

Strasbourg, 7 November 2002

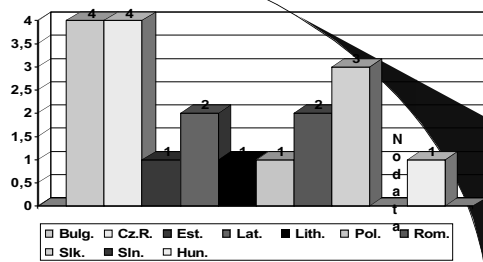
- **Veterinary vaccines in the candidate countries**
- **Vaccine control**
- **Animal tests and *in vitro* methods**

Strasbourg, 7 November 2002

Veterinary vaccines in the candidate countries

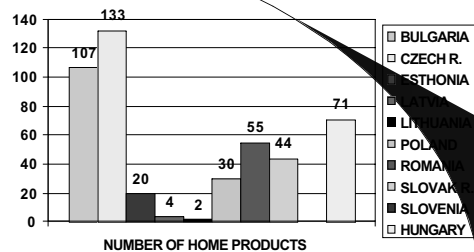
Strasbourg, 7 November 2002

NUMBER OF MANUFACTURERS: 19 COMPANIES IN THE REGION



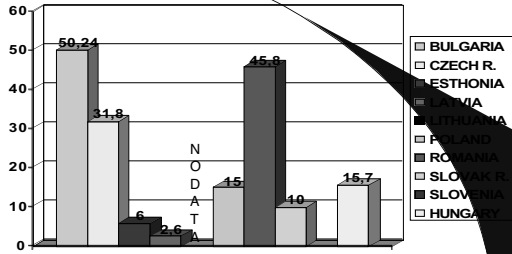
Strasbourg, 7 November 2002

NUMBER OF HOME PRODUCTS



Strasbourg, 7 November

PERCENTAGE OF HOME PRODUCTS IN THE MARKET



Strasbourg, 7 November 2002

- There are 100-450 veterinary vaccines in the different candidate countries.
- These vaccines are mostly imported from the EU.
- There is also import from the USA and other countries.
- In some candidate countries there are a lot of home products.

Strasbourg, 7 November 2002

Control of the veterinary vaccines in the candidate countries

Strasbourg, 7 November 2002

When do we control?

- control of registration samples before the marketing authorisation
- control of vaccines after granting the marketing authorisation

Strasbourg, 7 November 2002

- All the candidate countries require registration samples for testing during the marketing authorisation procedure of the veterinary vaccines

Strasbourg, 7 November 2002

BATCH RELEASE SYSTEM

CERT. OF QUAL.P. OF MANUF. STATE CONTROL

Bulgaria	yes	yes
Czech R.	yes	yes
Estonia	no	yes
Latvia	yes	yes
Lithuania	yes	no
Poland	yes	yes
Romania	no	yes
Slovakia	no	yes
Slovenia	No data	
Hungary	yes	yes

Strasbourg, 7 November 2002

SYSTEM OF STATE CONTROL

	Batch to batch manuf.	Random	Cont. of cert.of
Bulg.	yes	yes	yes
Cz.R.		yes	
Est.			yes
La.		yes	yes
Lith.	No state control		
Pol.	yes		
Rom.	home pr.	imported	all product
Slk.		yes	yes
Hung.	rabies, ND	yes	yes

Strasbourg, 7 November 2002

What do we control?

- quality
- safety
- potency

- Safety and potency tests require animal experiments!

Strasbourg, 7 November 2002

- 400 batches of veterinary vaccines are controlled in Hungary in every year

Strasbourg, 7 November 2002

How do we control?

Strasbourg, 7 November 2002

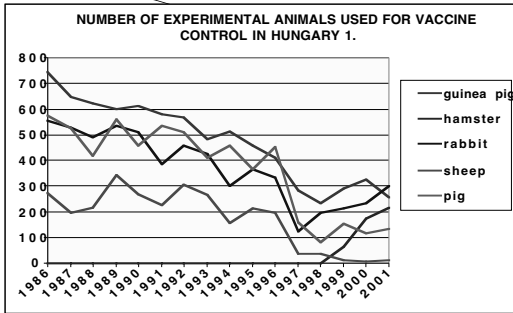
APPLIED REQUIREMENTS

<u>COUNTRIES</u>	<u>OIE</u>	<u>Ph.Eur.</u>	<u>96ER</u>	<u>National</u>	<u>Manufacturer?</u>
BULGARIA	YES	YES	NO	YES	YES
CZECH R.	YES	YES	NO	YES	YES
ESTONIA	NO	YES	NO	NO	YES
LATVIA		YES			YES
LITHUANIA		YES		YES	YES
POLAND	YES	YES	YES	YES	YES
ROMANIA	YES	YES	YES	YES	YES
SLOVAK R.	YES	YES	YES	YES	YES
SLOVENIA			No data		
HUNGARY	YES	YES	YES	YES	YES

Strasbourg, 7 November 2002

Animal tests or *in vitro* methods?

Strasbourg, 7 November 2002



Strasbourg, 7 November 2002

Number of experimental animals used for vaccine control in Hungary 2.

- mouse: approx. 10.000/year
- dog: 40/year
- cattle: 30/year
- poultry: 4000/year

Strasbourg, 7 November 2002

Number of experimental animals used for vaccine control in the candidate countries in 2001

	Czech R.	Slovakia	Bulgaria
dog	64	13	
cat	11	0	5
cattle	46	0	0
horse	98	0	0
pig	1701	0	15
sheep	0	0	30
poultry	110148	715	1000

Strasbourg, 7 November 2002

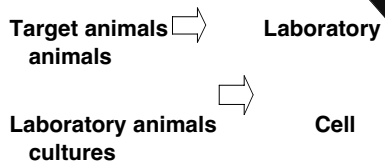
What can we do?

Strasbourg, 7 November 2002

- National authorities are in a difficult situation as they have to follow the international requirements and the methods of the manufacturers.

Strasbourg, 7 November 2002

Minimize pain and distress



Strasbourg, 7 November 2002

A. AUJESZKY



THANK YOU!

Strasbourg, 7 November 2002

3Rs Symposium

SESSION II

**Implementation of the 3Rs:
Regulatory Aspects**

Existing 3Rs methods Are they really used ?

- Remove
- Refine
- Reduce
- Replace
- Hints for the introduction
- How to improve the present situation

Nov-02



Remove

- Abnormal toxicity test
 - Waived
 - for products for veterinary use

Test is continued
- Moved upstream in production
 - for products for human use

Test is continued in the final product

Nov-02



Refine

- Vaccines for human use
 - Animals shall be humanely killed, as soon as sufficient indication of a positive result is obtained.

Usually protocols do not distinguish between animals, which have been humanely killed and those which died from infection

Nov-02



Reduce

- Swine erysipelas vaccine (inactivated)
 - Potency test
multi-dose test ▷ single dose test

The multi-dose test is continued

Nov-02



Replace

- Rabies vaccine inactivated for veterinary use
 - Determination of antigen content

Antigen content determination is not introduced to regular batch testing

Nov-02



Hints for the introduction

- Lack of harmonization
- Costs
- Validation
- Lack of knowledge

Nov-02