Alternatives to Animal Testing:

Opportunities and Limitations in the Regulatory Framework

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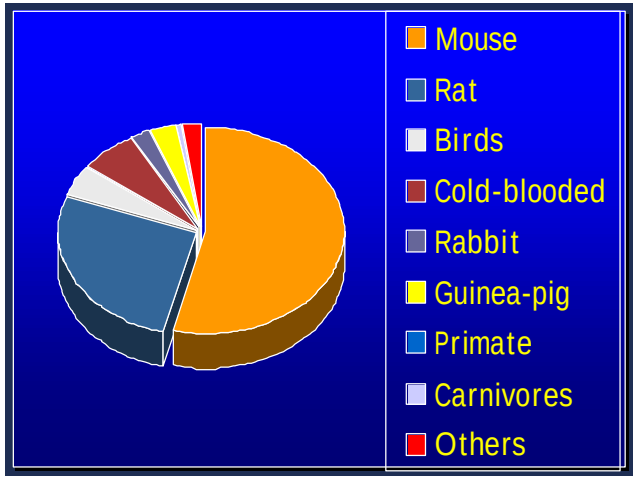
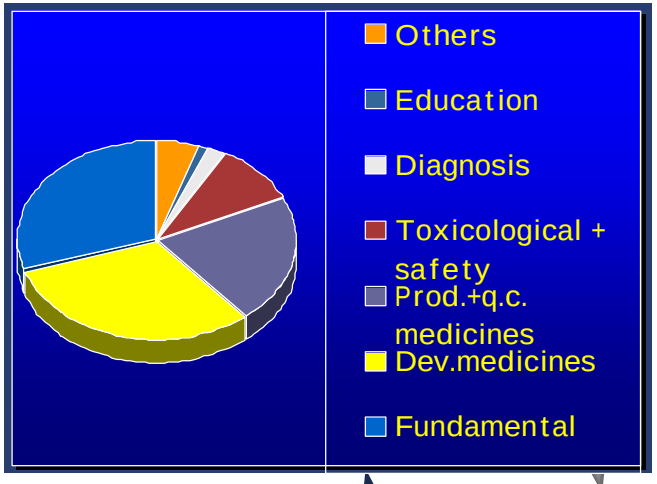
What I will discuss today

- For what purposes are animals being used and what are the numbers?
- Which % of these animals are used for regulatory purposes and what are the characteristics of regulatory testing?
- What exactly are ‘Alternatives to Animal Testing’ and why are these methods needed?
- What has been the result of our search for alternatives in the regulatory framework?
- What are the obstacles in the development, acceptance and implementation of alternatives in the regulatory framework?
- Which recommendations can be given?

Statistics on the use of animals in the MS of the European Union: specification for purposes and classes of animals (1999)

Council Directive 86/609/EEC on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes (1986)





Total use of animals 1996 : 11,646,130

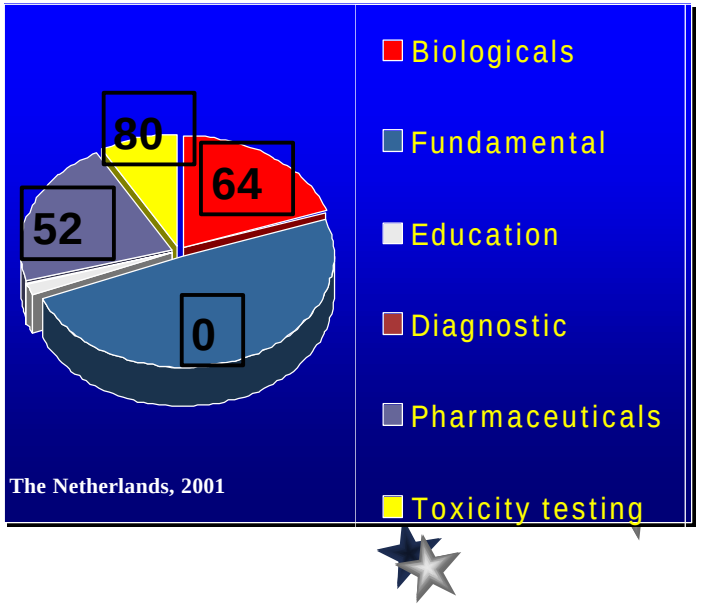
1999 : 9,814,171

Statistics on the use of laboratory animals in The Netherlands: % of use for regulatory testing

Tests performed for regulatory purposes (registration, batch release, etc.)

- Pharmaceuticals
- Toxicity testing (e.g. foodadditives, agrochemicals,cosmetics, etc.)
- Medical devices
- Biologicals (Vaccines, hormones, blood products, monoclonal antibodies, etc.)

Guidelines for regulatory testing provided by organisations such as OECD, Ph.Eur., WHO, EMEA, FDA, etc.



Animal testing for regulatory purposes : Characteristics

Characteristics of animal tests for regulatory purposes

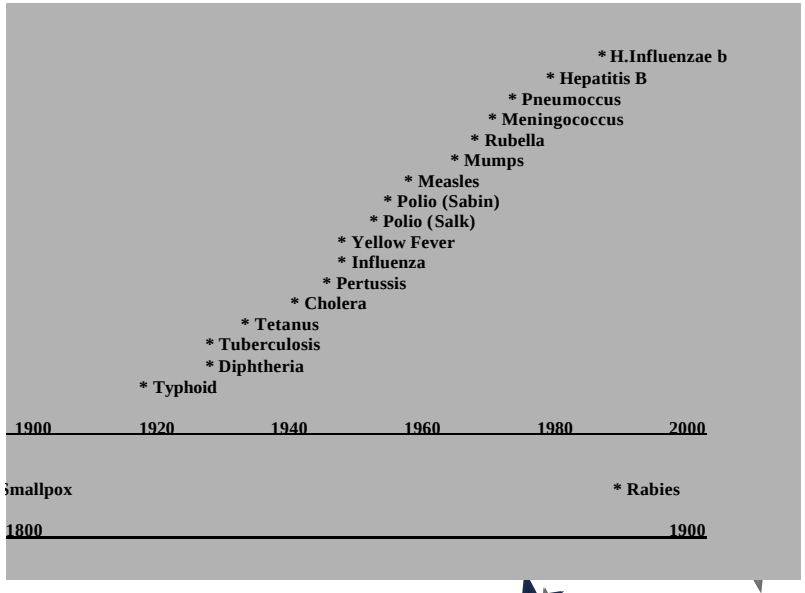
- Tests are performed routinely and quite often large numbers of animals are used per test
- Test guidelines are based on consensus and on strict protocols (the 'Cooking book scenario')
- High level of pain and distress
- Test guidelines have the tendency to expand and new research areas are added

% of experiments with severe pain & distress

Pharmaceuticals	**: 3%**
Toxicity testing	**: 10%**
Diagnostic testing	**: 0%**
Education & Training	**: 0%**
Fundamental research	**: 5.4%**
Biological products	**: 17%**

EU Chemicals policy (REACH), endocrine disrupters, new vaccines, etc

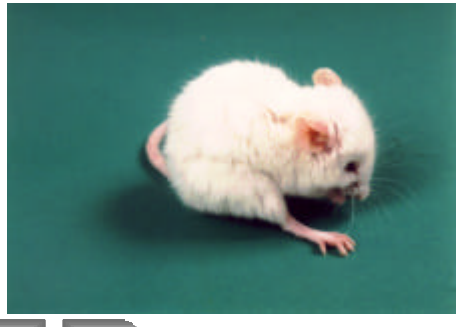
Has the use of laboratory animals been beneficial?



(Van der Zeijst, 1988)

Intrinsic problems of animal experiments

- Economic (time and cost)
- Scientific (standardisation, extrapolation, reproducibility)
- Ethical (what right do we have?)

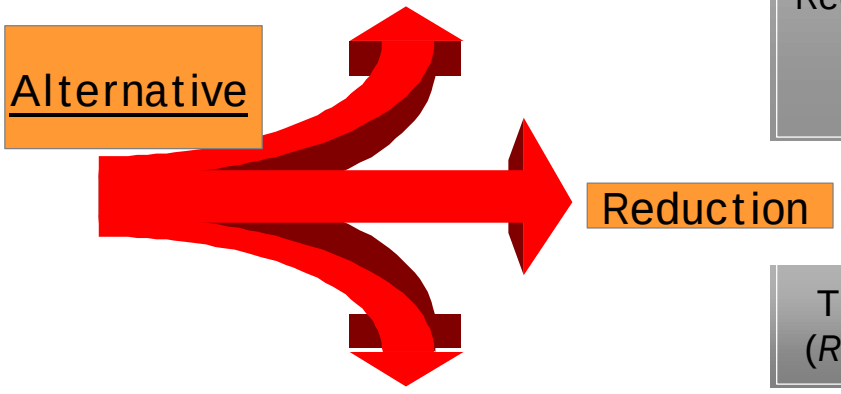


Influence of mouse strain on assayed T potency

Mouse strain	Assayed potency (IU/ml)
NIH	223
CFW	185
CDF1	142
BALB/c	105

Hardegree et al. (1972)

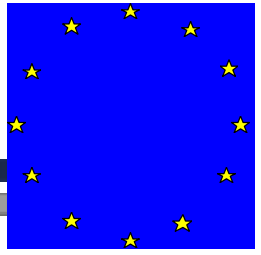
The Principle of the Three Rs



An Alternative is any method that leads to a Replacement, Reduction and Refinement of the use of animals (*Smyth, 1978)*

The Three R principle (*Russell & Burch, 1959*)

Council Directive 86/609/EEC



Council Directive 86/609/EEC on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes (1986)

Art. 7 (2)

An experiment shall not be performed if another scientifically satisfactory method of obtaining the results sought, not entailing the use of an animal, is reasonably and practically available

Art. 7 (3)

In a choice between experiments, those which use the minimum number of animals, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected

Art. 23 (1)

The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field

3Rs support by the regulatory and scientific community

'Testing in animals cannot be eliminated at present, but every effort should be made to discover, develop and validate alternative testing systems' *(OECD, 1982)*

'The European Pharmacopoeia has developed a policy for promoting animal welfare when preparing and revising pharmacopoeial control methods' *(Ph.Eur., 1999)*

'….ESF strongly endorses the principles of the "Three Rs".

European Science Foundation (2000): Policy on animals in research.

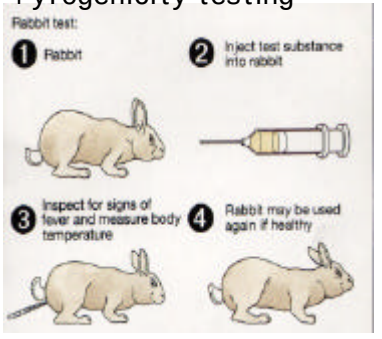
Establishment of the European Centre for the Validation of Alternative Methods (ECVAM) by the European Commission (1992)

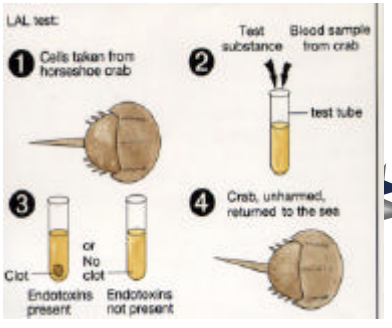
Various statements in the White Paper "Strategy for a Future Chemicals Policy that endorse a 3Rs approach. European Commission (2001)

Alternatives : the 3Rs Approach

- **Replacement alternatives:**
  - Tissue culture techniques (= in vitro method)
  - Physico-chemical & immunochemical methods
  - Computer models
  - Use invertebrate organisms
  - Human volunteers
- **Reduction alternatives:**
  - Test optimalisation and standardisation,
  - Improved statistical methods
- **Refinement alternatives:**
  - Anesthesia and analgesia
  - Humane endpoints
  - Improved housing (environmental enrichment)

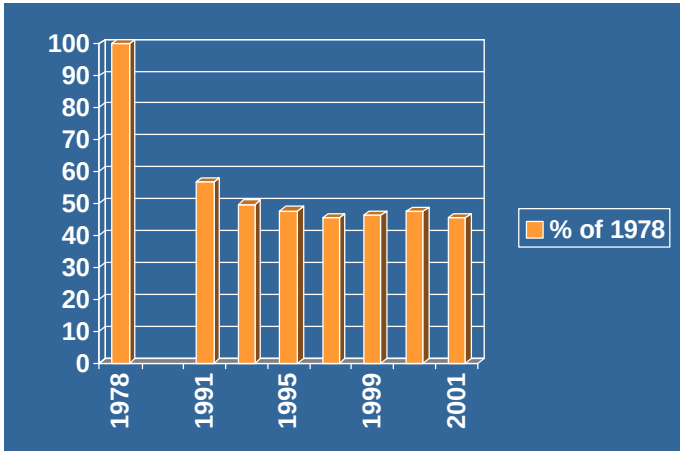
Pyrogenicity testing





The use of laboratory animals in the EU/the Netherlands

The use of animals in the Netherlands 1978 - 2001



NL: Total use of laboratory animals in 2001: 714.449

Total use of animals in the EU:

1996 : 11,646,130

1999 : 9,814,171

What has been the result of our search for alternatives in the regulatory framework?

“….so little progress has been made in replacing experiments on animals ….with alternative methods, which calls into question whether all reasonable endeavours have been made…..”

(European Parliament)

The Frustration: Cosmetic Testing

- Draize eye irritancy test in rabbits : target of animal welfare organisations for more than 20 years
- European Parliament resolution adoption calling for an end to animal testing for cosmetics.
- 6th Amendment to Cosmetics Directive (1993): ban on the sale of cosmetics tested on animals as from 1 January 1998, on the condition of scientific validation.
- Several collaborative studies on in vitro alternatives to Draize eye test : studies did not result in a validated method.
- Two postponements (2000 and 2002) of date of ban.
- 7th Amendment to Cosmetic Directive (Council Dir. 76/768/EEC). Sales ban and animal testing ban not until 2009. Sales ban two parts.

3Rs successes in Regulatory testing

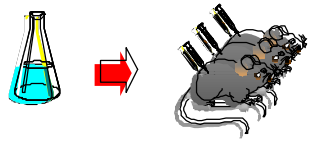
Product	Test	Alternative
Chemicals	Corrosivity testing	EpiDerm, EPISKIN, TER
	Photoirritation	3T3 NRU assay
	sensitisation	LLNA test
	LD50 test	FDP, UDP, ATC

Product		Test	Alternative
Hormones :	Insulin	Mouse convulsion test (P)	HPLC
	HGH	Rat Tibia test (P)	HPLC
	Oxytocin	Rat Uterus test (P)	HPLC
	Calcitonin	Rat serum test (P)	HPLC
	All products	Rabbit Pyrogenicity test (S)	LAL test
	All products	Test for depressor subst. (S)	deleted
	etc.		
Vaccines :	All human	Abnormal tox. Test (S)	deleted
	All products	Lethal endpoint tests	humane endpoint
	Diphtheria	G-P. safety test	cell culture test
	Tetanus	Mouse/G-P challenge test (P)	ELISA/ToBI (1)
	Erysipelas	Mouse challenge test (P)	ELISA
	Rabies	Mouse challenge test (P)	ELISA
	Hepatitis B	challenge test (P)	ELISA
	All products	Rabbit pyrogenicity test (S)	LAL test

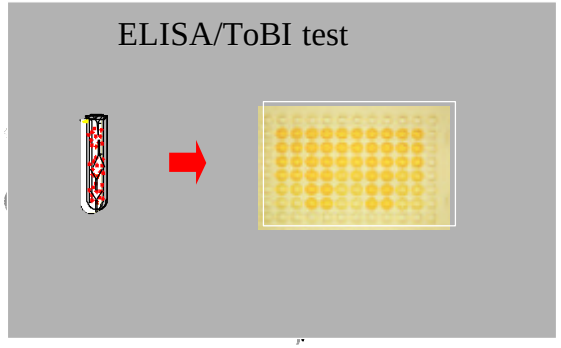
(1). Monograph in preparation

Reduction/Refinement in vaccine potency testing: Serological methods

Classical potency test



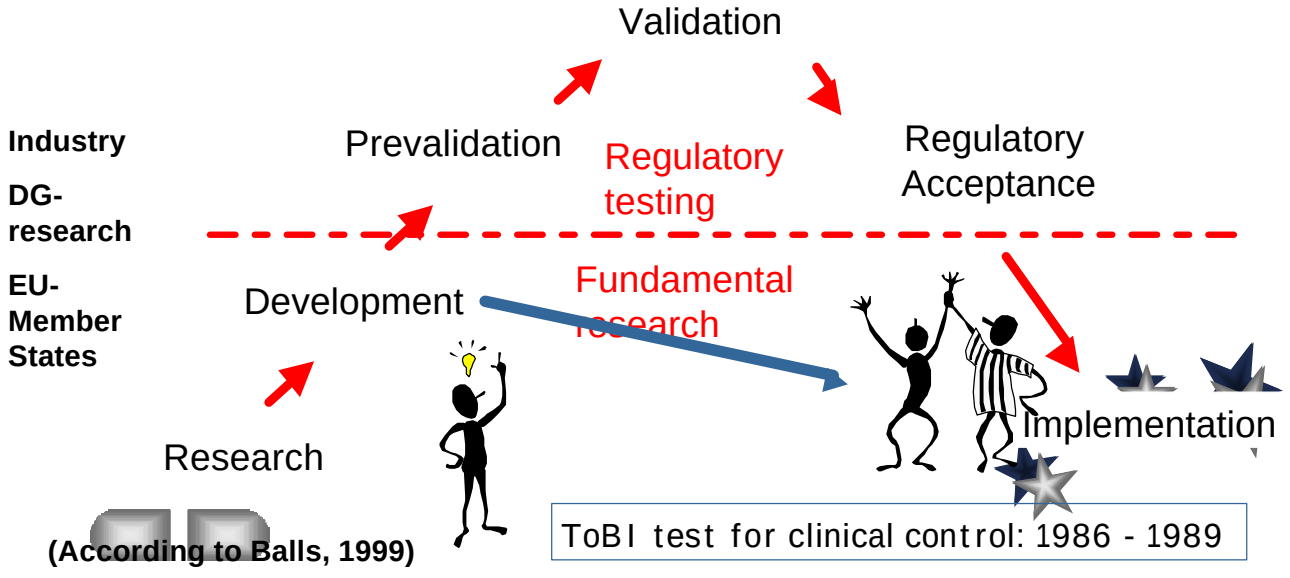
vaccine immunisation



Study started in 1986. Acceptance by European Pharmacopoeia Commission to be expected in 2004.

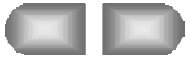
Regulatory testing and Three Rs: from development to implementation. Key steps

ToBI test for tetanus vaccine potency testing: 1986 - > 2003



From test development to test implementation in regulatory testing; The tetanus case study (1)

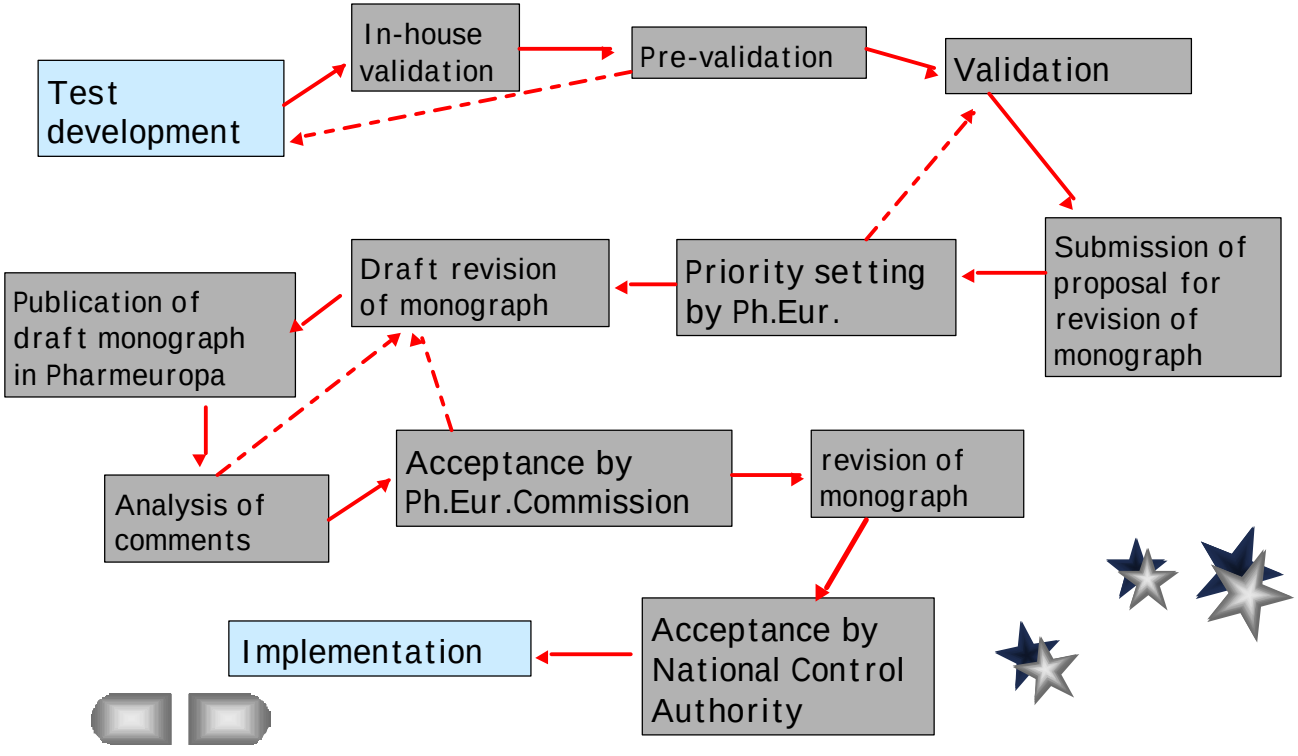
Analysis of the process	Year (total number)
a) Test development	1986 (0)
b) In-house validation	1989 (3)
c) Pre-validation (test optimisation, SOPs, technology tranfer)	1996 (10)
d) Formal validation	1996 (10)
e) submission of proposal for revision of monograph	2001 (15)



From test development to test implementation in regulatory testing; The tetanus case study(2)

Analysis of the process	Year (total number)
f) Priority setting	2001 (15)
g) Draft revision of monograph	2002 (16)
h) Publication in Pharmeurope	2002 (16)
i) Analysis of comments	2003 (17)
j) Acceptance by Ph.Eur.Commission	2004 (?)
k) Acceptance by National Control Authoruty	200? (??)
l) Implementation	200? (??)

Ph.Eur. Process from Test development to test implementation

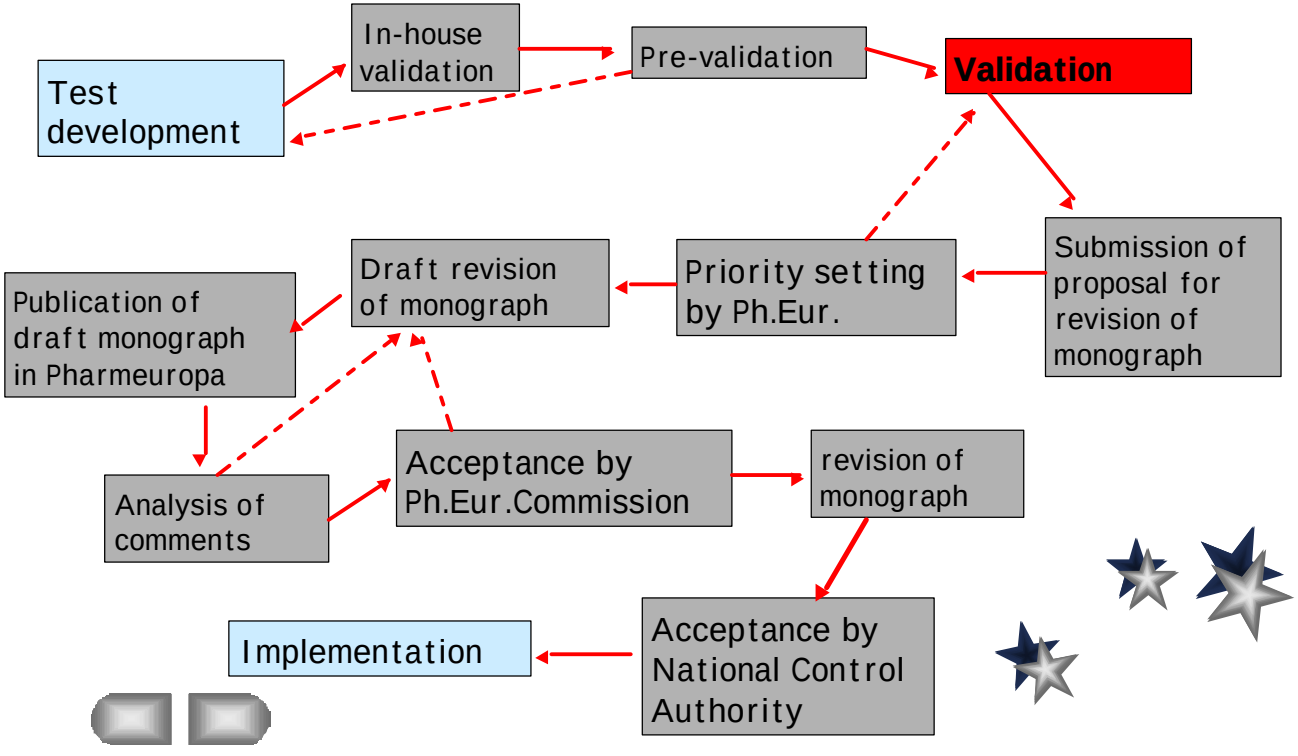


Obstacles: Development

- No scientific tools available
- Financial limitations
- Low priority in institutional key activity programs
- negative cost-benefit balance (e.g. industry)

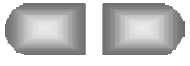
YearNo.	Budget	Budget available
2001:pre-appl. 43	€ 5.023.396	€ 900.000
full appl. 12	€ 1.532.111	€ 900.000
2002:pre-appl. 36	€ 7.268.421	€ 900.000
full appl. 13	€ 2.095.550	€ 900.000
2003: pre-appl. 39	€ 5.933.781	€ 900.000
full appl. 17 (?)		

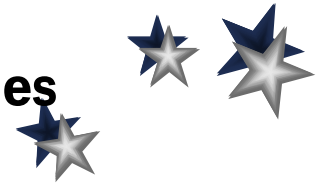
Ph.Eur. Process from Test development to test implementation



Obstacles: Validation

- **The animal model as the 'Gold standard'**
  (rabies vaccine potency testing, Draize eye test, pertussis vaccine potency testing, etc)
- **Costs of validation studies**
- **Logistics of validation studies**
- **Communication to regulatory bodies**





Eur.Phar./ECVAM Collaborative Study to the Use of in-vitro Serological Test Systems for Potency Testing of Tetanus Toxoid Vaccines for Human Use

DESIGN STUDY

Management: 4 partners/2 bio-statisticians

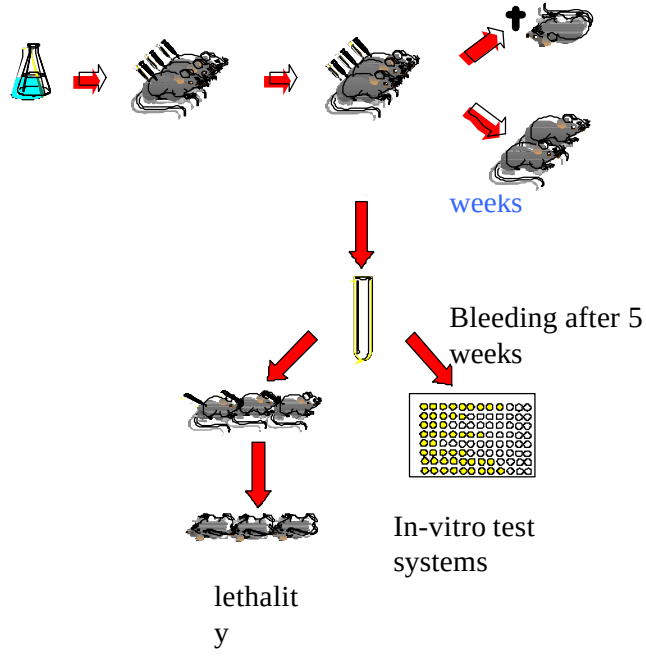
Study was divided in 4 phases

Pre-validation	: 4 laboratories
Phase 1	: 3 laboratories
Phase 2	: 3 laboratories
Phase 2b	: 2 laboratories
Phase 3	: 26 laboratories

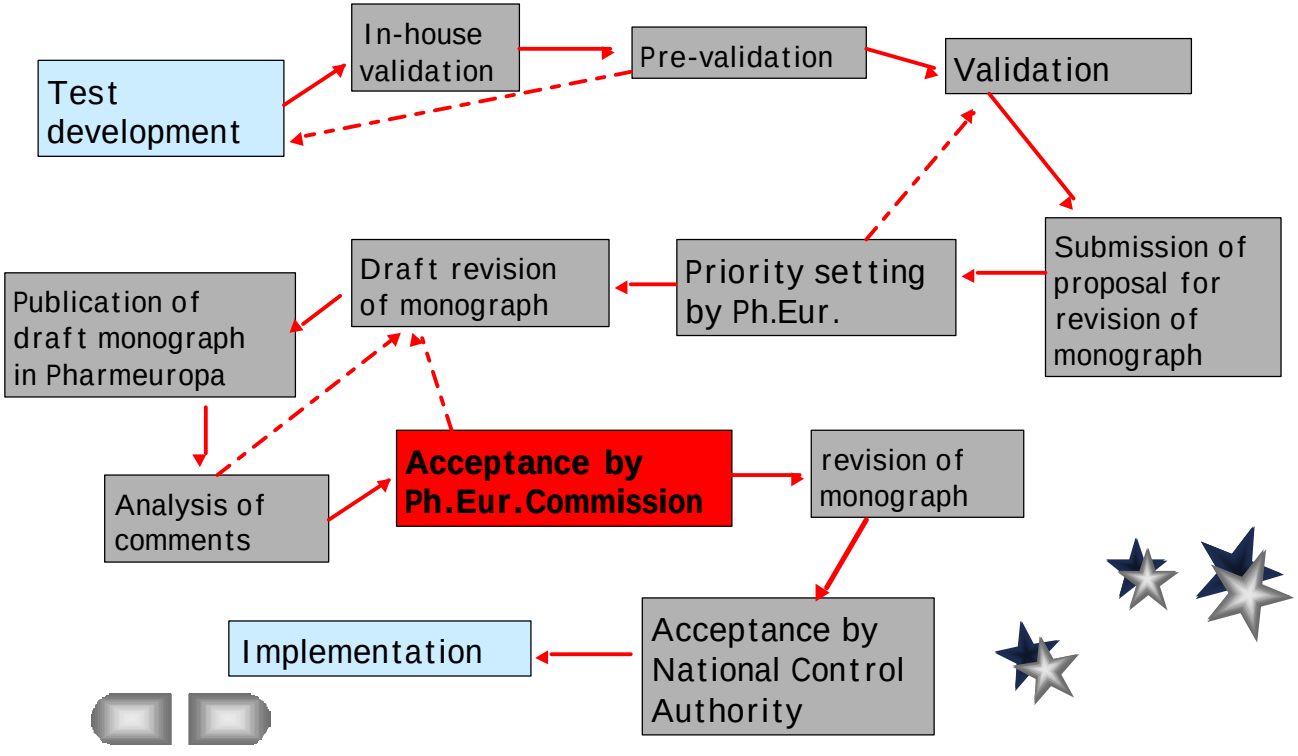
Time required : approx. 4 years

Costs : approx. 1 million EURO

POTENCY TEST

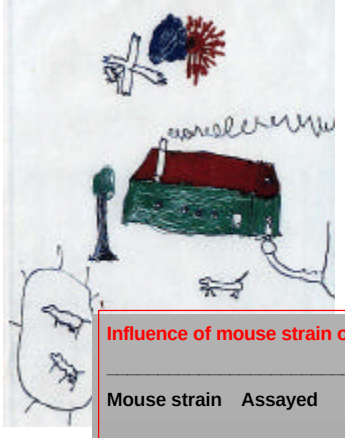


Ph.Eur. Process from Test development to test implementation



Obstacles: Acceptance

- Acceptance as a scietific and political process
- Meeting frequency of experts groups, commissions, etc.
- Psychological barrier of accepting data of in vitro tests



Influence of mouse strain on assayed T potency

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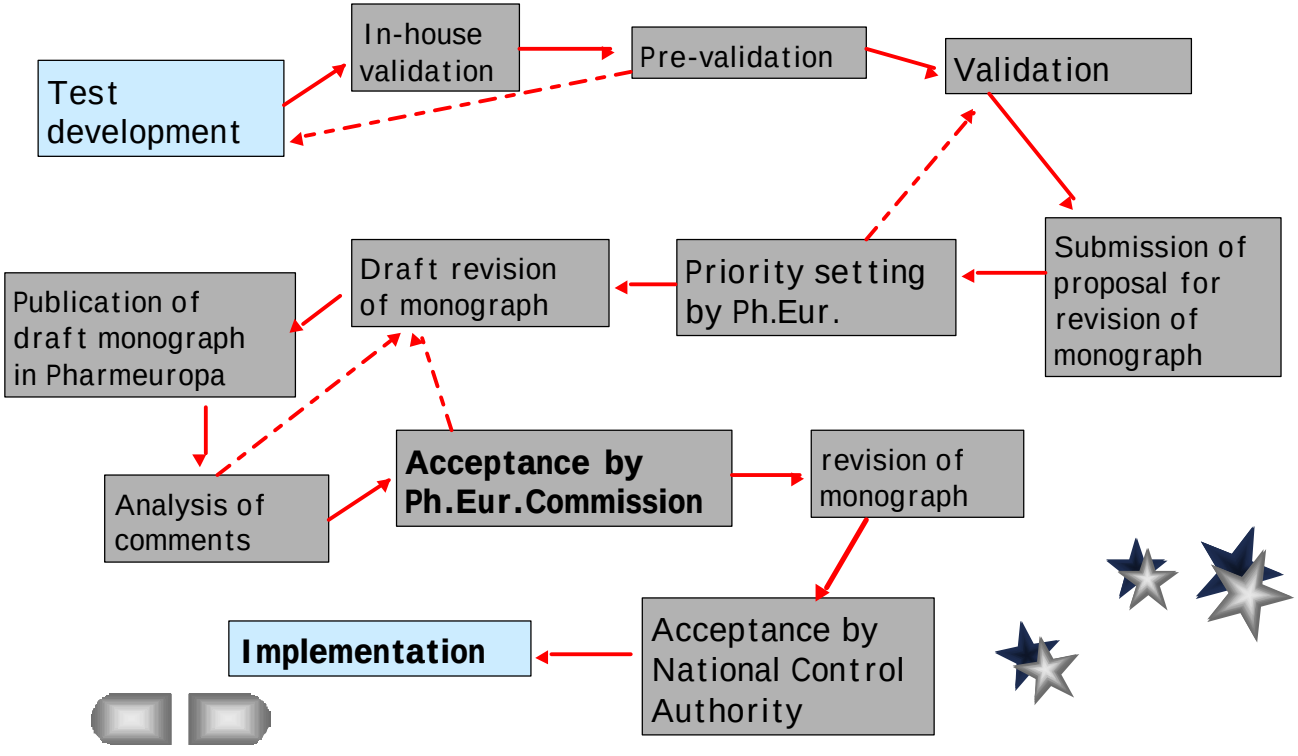
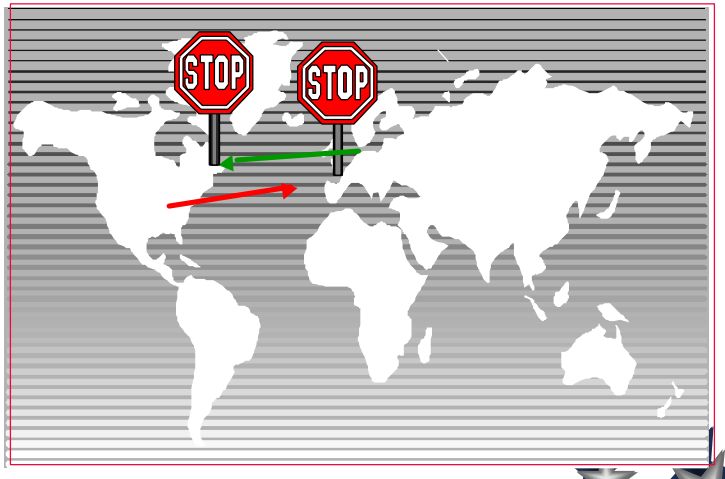
Hardegree et al. (1972)

Ph.Eur. Process from Test development to test implementation



Obstacles: Implementation

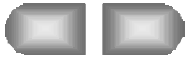
- Lack of training (www.vaccinetraining.com)
- Need for specific reagents (manufacturer dependency, patenting problems, etc.)
- Financial consequences
- Lack of harmonisation

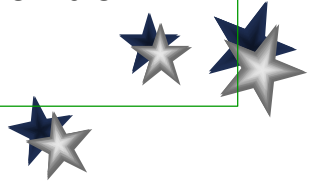


Conclusions

The process from test development to test implementation will continue to be frustrating, tedious and ineffective if in the future we continue to :

- **provide limited financial resources for 3Rs research and validation**
- **consider test harmonisation the only way forward**
- **validate our new 3Rs methods according to inflexible guidelines**
- **stick to our rigid testing strategies**





The rigid testing strategy

Chemicals

a) Base set testing

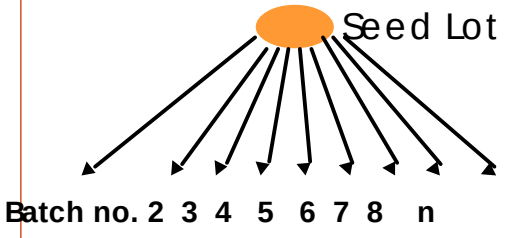
b) Base set testing + Level 1 testing

c) Base set testing + Level 2 testing

Procedure depending on Volume (tonnes per annum)

Council Directive 75/318/EEC

Vaccines



Each batch of vaccine produced is considered to be unique

each batch: animal test for potency & safety

Council Directive 67/548/EEC

The rationale testing strategy

Chemicals: the tiered testing approach

Testing is not based on a fixed set of guidelines but is dependant of the type of the chemical, its use and information already available.

Increased attention for:

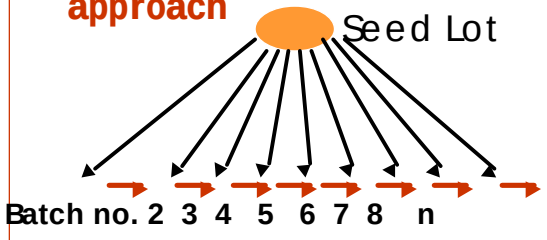
physico-chemical tests

***in silico* (computer) modelling**

cell culture assays, etc.

(ATLA (2002), 30, suppl.1, 1-125)

Vaccines : the consistency approach



Each batch of vaccine produced is part of a continuous process of production:

Testing for consistency using in vitro tests to 'fingerprint' the product

Testing Strategy : Skin and Eye Effects (OECD TG404, TG405)

(adopted 24 April 2002)

Existing human experience

↓

(Q)SAR
(eye irritation/corrosion)

↓

(Q)SAR
(skin corrosion)

↓

pH and buffering capacity

↓

evaluate dermal route toxicity data

↓

In vitro Test
(eye irritation/corrosion)

↓

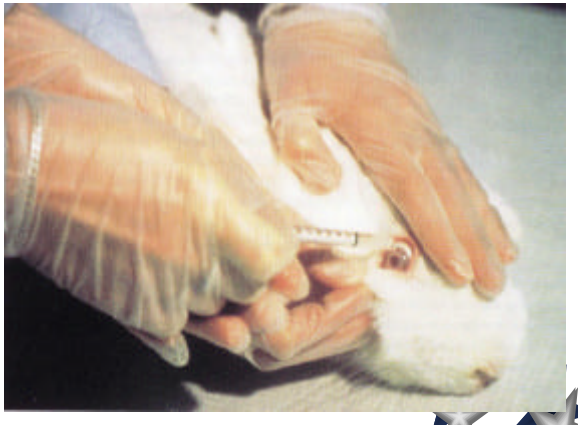
In vitro Test
(skin irritation/corrosion)

↓

In vivo 1 Rabbit

↓

In vivo 2 Rabbits



Reduces by 90% that eye corrosives damage a rabbit eye

(According to Spielmann)

Recommendations (1)

Animal experimentation an the 3Rs are 'an end of the day' issue. Get in higher on the (political) agenda.

Support and adopt policies that stimulate 3Rs development:

- **Framework Programs**
- **ECVAM**
- **Organisations such as ECOPA**
- **Taking away financial barriers**
- **Etc.**

Recommendations (2)

Fully implement Council Directive 86/609/EEC: at the EC level and at the level of the EU-MS

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An experiment shall not be performed if another scientifically satisfactory method of obtaining the results sought, not entailing the use of an animal, is reasonably and practically available

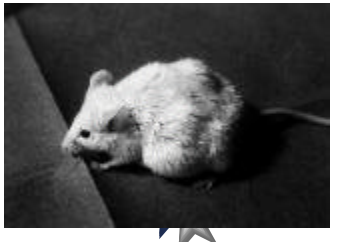
Art. 7 (3)

In a choice between experiments, those which use the minimum number of animals, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected.

Examples:

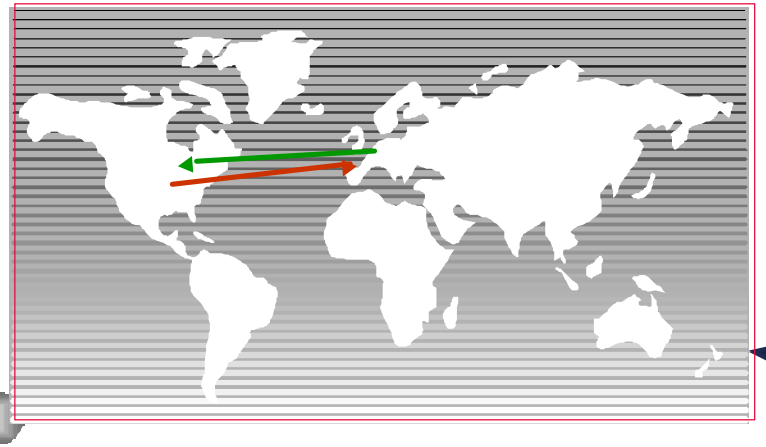
- tetanus potency testing: 2 different guidelines for potency testing, differing in the level of suffering

- Production of monoclonal antibodies



Recommendations (3)

Improve the exchange of test by harmonization of guidelines or by Mutual recognition of test data.



Recommendations (4)

Support the new testing strategies for testing of chemicals and biologicals that are more intelligent, more flexible and less burocratic.

Recommendations (5)

Consider the 3Rs of equal importance

Finally

Less animals make more science

and

more science makes better regulations