How does the industry address the 3 R's Reduce – Replace – Refine

Harmonisation of the Care and Use of Fish in Research

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PHARMAQ AS

May 25. 2005

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Limitations

- **PHARMAQ AS presents its view as a representative of the fish vaccine industry.**
- **Fish vaccines are veterinary medicinal products, which are licensed through a strict regulatory framework**
- **The presentation and discussion are thus limited to use of experimental animals related to the requirements for documentation, development, release and maintenance of fish vaccines in Europe**

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Content of the presentation

- Regulatory framework
- Development and documentation process
- Fish used for
  - Development
  - Documentation
  - Field tests
- Fish used for batch release
- Reduce, Refine and Replace
- Conclusion

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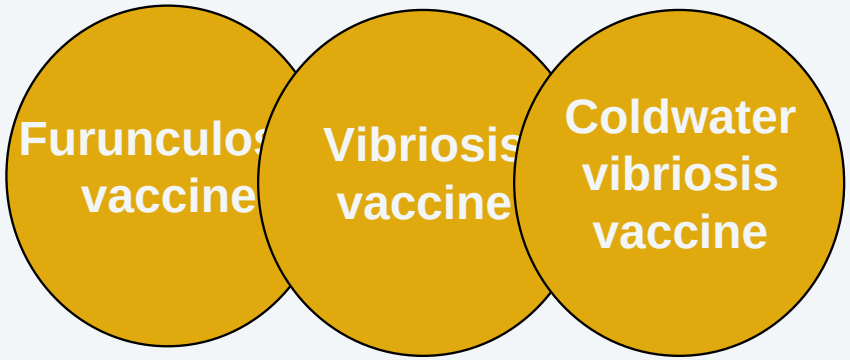
Regulatory framework

Licensing documentation

- **European Monographs**
  - **Mandatory**
  - **Must be implemented for all <u>new and existing</u> products**
- **Guidelines and Position papers**
  - **Neither mandatory for the industry nor the authorities**



- **Production and Control**
- **Safety**
- **Efficacy**

The framework sets the standard the industry applies

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Regulatory framework

Pharmacopoeia

- **Evaluation of safety of veterinary vaccines (Ph. Eur. 5.2.6)**
- **Evaluation of efficacy of veterinary vaccines (Ph. Eur. 5.2.7)**
- **Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids (Ph. Eur. 1521)**
- **Vibriosis (Cold water) vaccine (Inactivated) for salmonids (Ph. Eur. 1580)**
- **Vibriosis vaccine (inactivated) for salmonids (Ph. Eur. 1581)**

Mandatory for the industry

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Regulatory framework

Guidelines and Position Papers

- **Guideline on good clinical practice (CVMP/VICH/595/98)**
- **Good Laboratory Practice**
- **The general requirement for the production and control of live and inactivated vaccines intended for fish (81/852/EEC)**
- **Data requirement for removing the target animal safety test for immunological veterinary medicinal products in EU (EMEA/CVMP/865/03 Final)**

Guidelines may be deviated, when thoroughly justified

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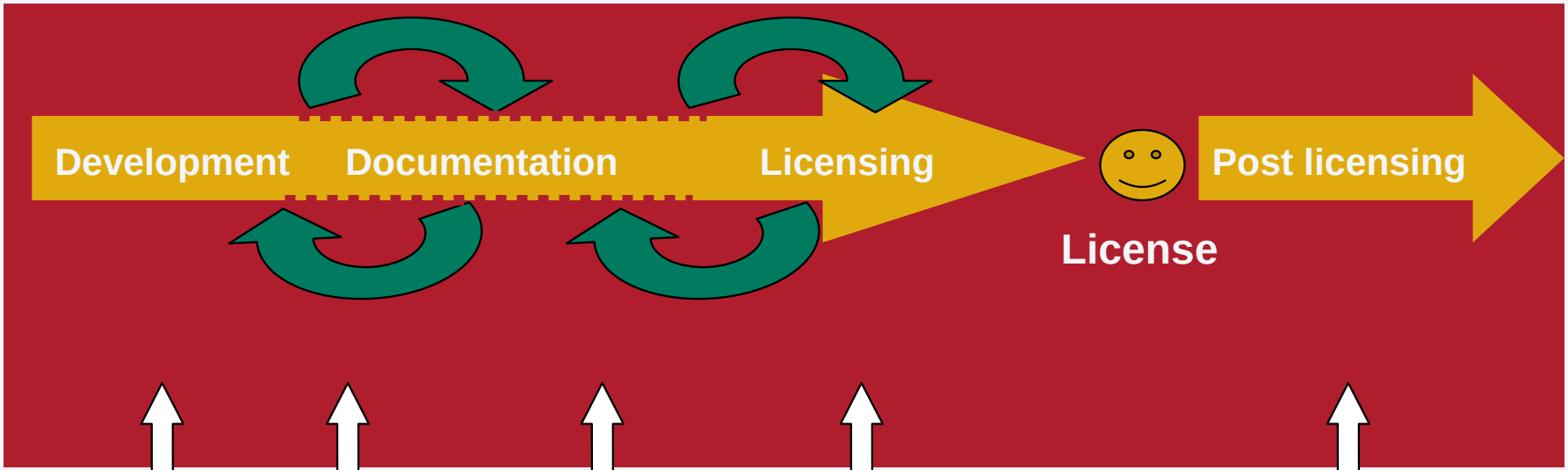
Development and documentation process

From R&D to market



Lab.studies challenges

Lab. studies of safety and efficacy

Field studies of safety and efficacy

Field studies, commercial scale

•Comparative field studies
•Batch testing
•Stability testing

The development and documentation process include fish-studies

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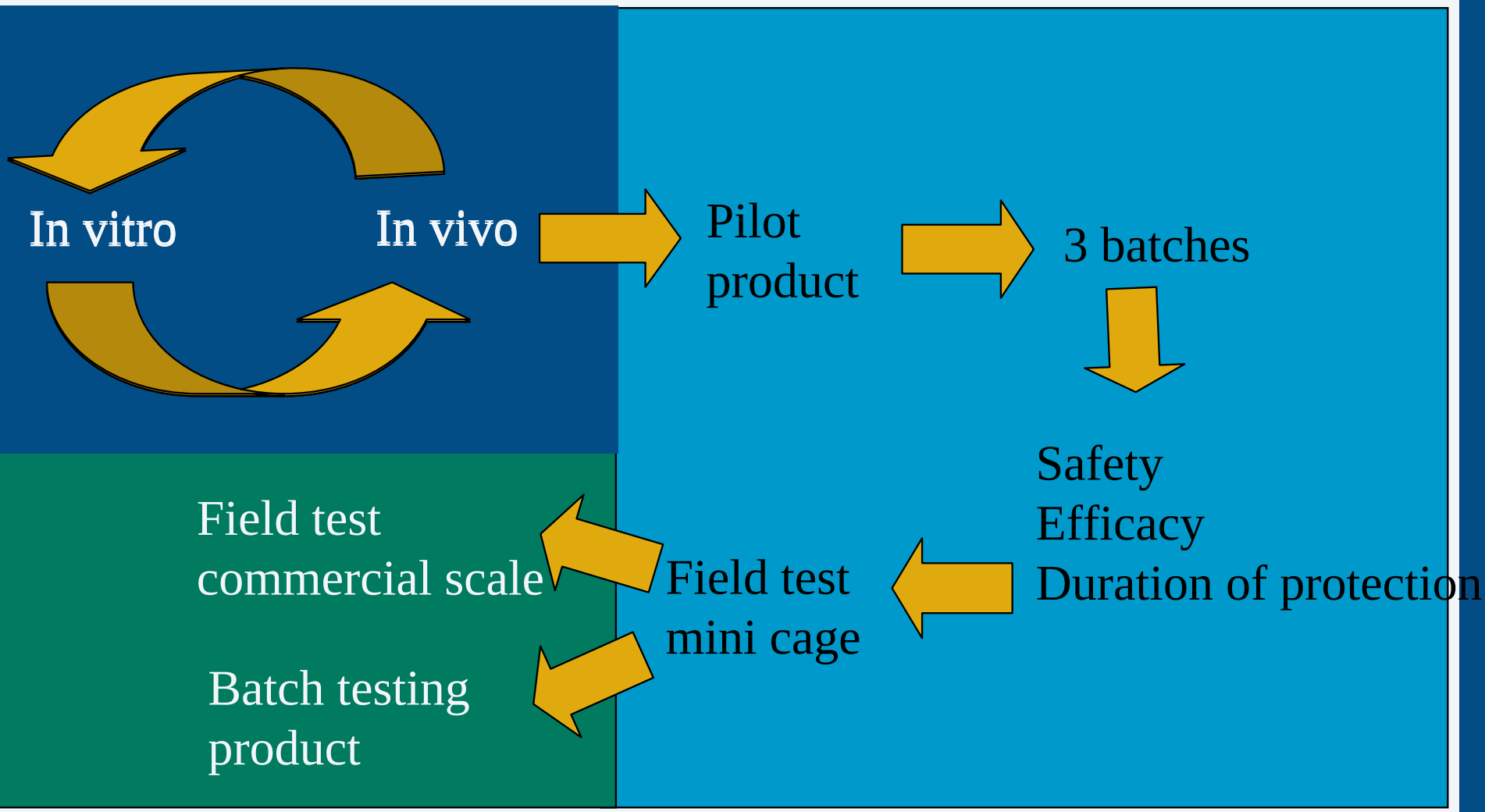
Development and documentation process

From R&D to market



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Fish currently used

General methods used in fish

- **Administration of vaccines i.p. or i.m., orally, by immersion or by bath**
- **Anaesthesia (MS222, benzokain, phenoxyethanol)**
  - **always used prior to i.p. or i.m. vaccination**
- **Blood-sampling**
- **Marking of fish by fin clipping, fluorescent dye, implant or others**
- **Exposing the fish for live bacteria or virus for challenge**
- **Euthanised for sampling**

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Fish currently used

Clinical development and documentation

- **Studies must be valid, using sufficient numbers of animals to obtain true differences between groups**
  - **Statistical design and methods must be used**
- **Tests and methods must be repeatable and reproducible**
- **Clinical laboratory and mini cage studies should give a real answer, thus mimic the situation in field**

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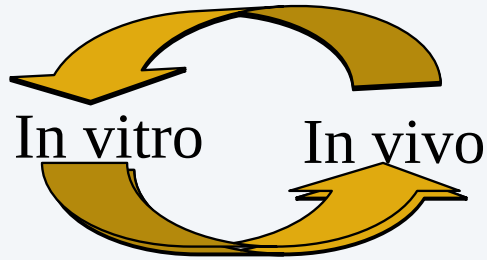
Fish currently used

Clinical development phase



- **Virulence testing by exposing fish to the disease agent**
  - **800 fish pr. study (4 strains * 2 adm. methods * 50 fish * 2 reps)**
- **Development of challenge models**
  - **800 fish pr. study (4 adm. methods * 2 groups * 50 fish * 2 reps)**
- **Cross protection studies in target species**
  - **2000 fish pr. study (2 groups * 100 fish * 5 challenge strains * 2 reps)**
- **Dose titration studies including challenge**
  - **2000 fish pr. study (5 doses * 100 fish * 2 groups * 2 reps)**

The number of fish sacrificed are dependent on the success rate

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Fish currently used

Documentation of safety - lab. (GLP)

- Secure that the product is safe to use (not toxic)
- Documentation of 3 batches
- Fish blood sampled prior to vaccination
- Marked by fin clipping
- Injected double dose of vaccine and observed for 21 days

Test	Guideline	# fish /batch	# fish (total)	Observation
Double dose safety	Ph Eur.	50	200	21 days
Field trials	Ph. Eur.	Not defined	Not defined	Until slaughter

Safety test is important, value of a 3 weeks test may be questioned

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Fish currently used

Documentation of efficacy –lab.

- Documentation of three batches of final product
- Show consistency between batches
- Discriminate between batches of optimal and sub-optimal p
- One dose of vaccine injected
- Fish marked by fin clipping
- Challenge i.p. 4-6 weeks post vaccination
- Control mortality ≥ 60%
- Mortality observed until 21 days after the first death of fish

Controversial:
Ph. Eur. method is not always the best tool to discriminate between batches

Test	Guideline	# fish / batch and antigen	# fish (total)	Observation
Efficacy	Ph Eur.	100		21 days after the first death
Monovalent			400	
Hexavalent			2400	

Efficacy test is important, numbers of fish statistically applicable

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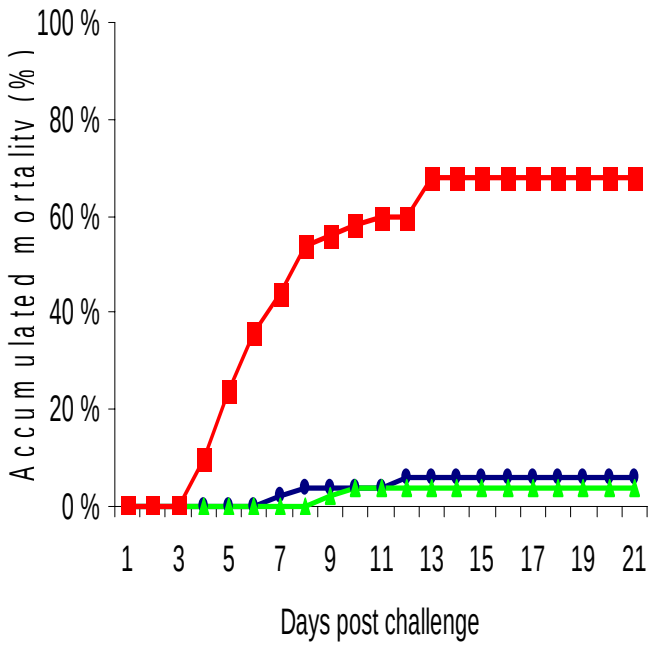
Fish currently used
Challenge studies

Results from laboratory challenge test



Salmon vaccinated with 2 commercial vaccines
Challenged 5 weeks post vaccination

Questions to be raised:

- **Stop the challenge earlier?**
- **Sample moribund fish**
  - **reduce suffering – more humane endpoint?**

Efficacy test is important, mortality vs morbidity may be discussed

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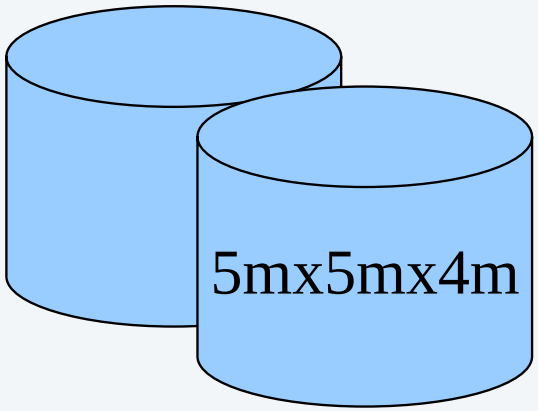
Fish currently used

Field documentation efficacy (GCP)

Trial in mini cages



Design

- Two replicate cages
- 1000 – 3000 fish per cage
- 6-8 groups per cage
- Groups are marked and mixed
- Two premises ran in parallel

Advantages

- Frequent sampling
- Eliminate cage variation
- May be exposed to natural challenge
- Use a limited number of fish

Disadvantages

- Outbreak of disease rarely occurs
- Does not equal production cages
- Growth

The mini cage studies give good and reliable documentation

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Fish currently used

Field documentation efficacy GPC

Com Field

Batch testing

Trial in production cages

20mx20m20m

Design

- One cages
- 100 000 fish per cage
- 10-50% fish marked
- ~~Fish used for consumption~~
- Often done with two licensed products

Advantages

- Production conditions
- Self experience
- May be exposed to natural challenge

Disadvantages

- Outbreak of disease rarely occurs
- Replicates more difficult
- Difficult to do proper sampling

Are fish vaccinated with licensed vaccines, under standard conditions experimental animals?

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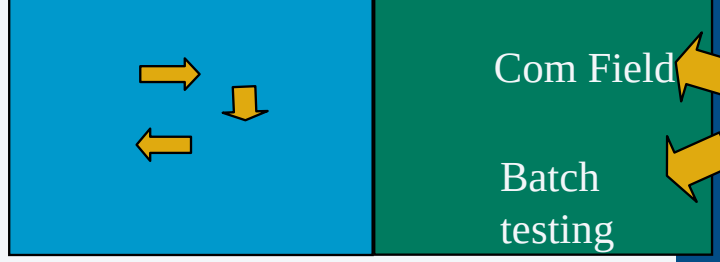
Fish currently used

Duration of protection



- **Mini cage trials suitable for field safety documentation**
- **Commercial scale trials useful for monitoring growth of vaccinated fish**
- **Field trials are not suitable for documentation of duration of protection**
  - Outbreak of disease rarely occur,
  - Antibody analysis?

- **Field duration of protection studies, has been <u>replaced</u> by: Laboratory duration of protection studies**
  - **The number of animals has been <u>reduced</u>**

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Fish currently used

Duration of protection

- Injected one dose of vaccine
- Blood sampled and marked fish
- Challenged at different time points post vaccination
- Mortality observed until 21 days after the first death of fish

Test	Guideline	Chall. time	# fish (total)	Observation
Efficacy	Ph Eur.			21 days after the first death
Monovalent		6 m.	400	
Hexavalent		6 m.	2400	
		12 m.	2400	

The test is essential for product documentation

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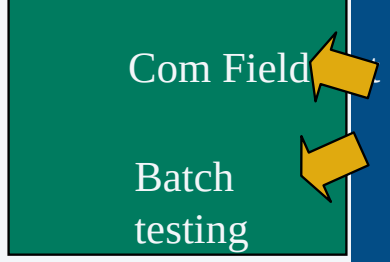
Fish for batch release

Current batch testing of product



- **Every batch must be tested for potency and safety (Ph. Eur.)**
- **Safety: 10 fish injected double dose per batch, 21 days observation**
- **Potency: minimum 30 fish vaccinated and challenge-tested per antigen per batch**
  - **70 fish for monovalent vaccine**
  - **420 fish for hexavalent vaccine**
  - **every test, includes challenge and takes approx. 3 months**

Batch testing is mandatory, currently fish challenge is used

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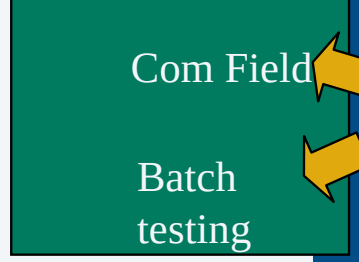

Fish for batch release

PHARMAQ numbers, 2004

- **Produced 40 batches of vaccine, released according to Ph. Eur.**
- **Stability tested 10 batches of vaccine**
  - **Fish sacrificed for standard safety testing: 1000**
  - **Fish sacrificed for potency testing: 11250**

Numbers include batch-testing vaccines for Canada, Chile, Denmark, Faeroe Islands, Finland, Greece, Iceland, Ireland, Norway, Sweden, Turkey and United Kingdom

Could these tests on a final product be reduced or replaced?

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Fish currently used - overall

The major use in the fish vaccine industry

- **Development and documentation**
  - **<20 000 fish per product (dependant upon success)**
- **Batch release of final products**
  - **< 15 000 fish per year**
- **Clinical field trials commercial scale, with licensed products**
  - **Several 100 thousands in one study**

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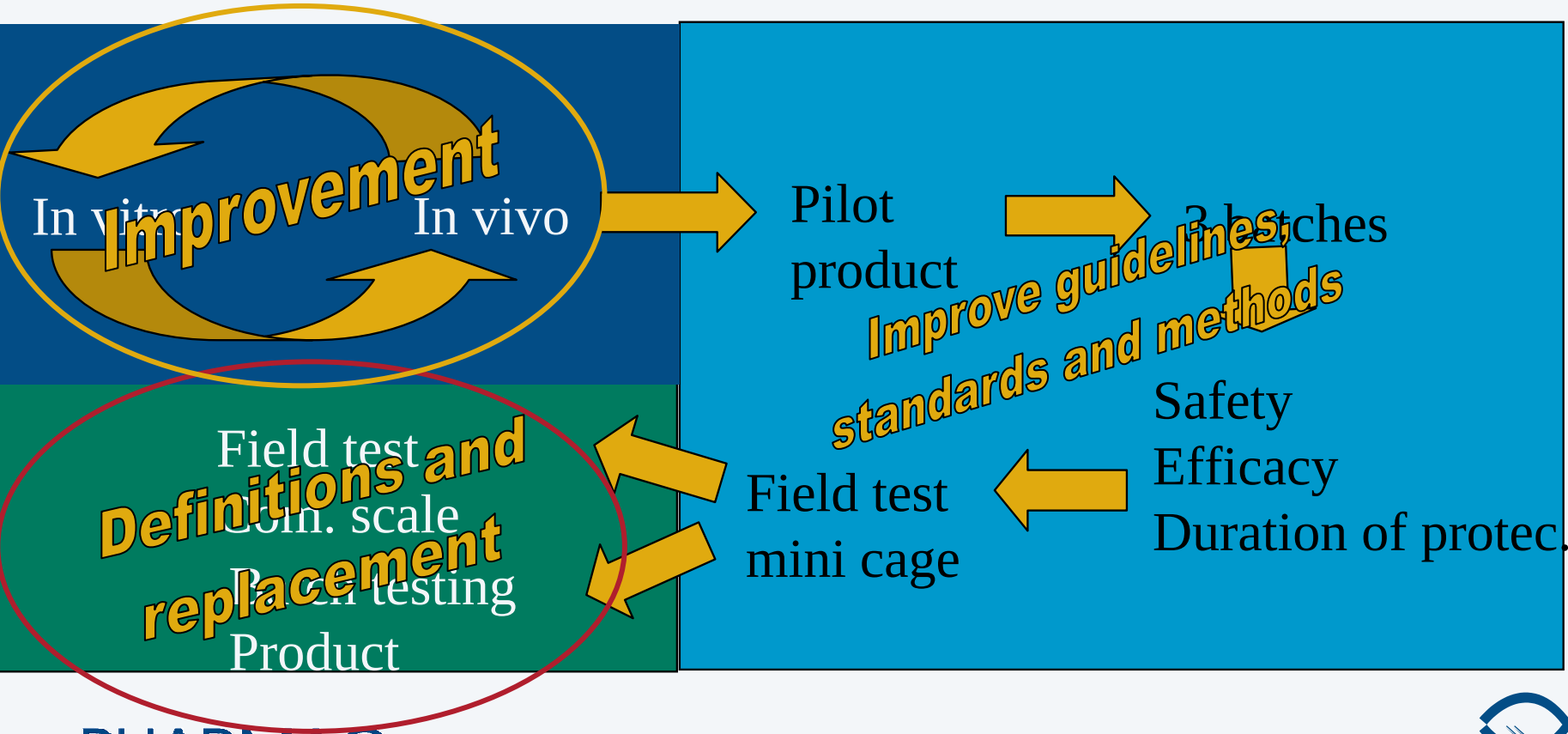
Fish currently used - overall

Main points for improvement

- **Secure quality of the products by *in vitro* quality control and -assurance prior to clinical trials**



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Reduce Refine Replace

Reduce batch safety and potency tests

- Good Manufacturing Practice ensures safety and efficacy
  - Production in consistence and suitable manner
  - Extensive In Process testing and control
  - Securing quality, reproducibility and quality at every step of production by validated *in vitro* tests
- Only inactivated fish vaccines are licensed (ex. Chile)
- Relevance of safety and potency tests can be questioned
  - Safety test is a toxicity test
  - Potency test does not always discriminate properly

Reduce Refine Replace

Reduce no. of fish in batch safety test

Position paper EMEA/CVMP/865/03 Final

- **Final bulk -> several batches -> one test**
  - **If several batches are prepared from same Final bulk, the safety test is carried out on the first batch and then omitted.**
- **Position paper suggests to reduce the frequency of safety test provided:**
  - **Full batch protocols on minimum 10 batches**
  - **Satisfactory pharmacovigilance system and pharmacovigilance data**

The frequency of the batch safety test may be reduced

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Reduce Refine Replace

Reduce no. of fish in batch potency test

- **Potency testing on final product is mandatory**
  - **Within the current framework, the methods may be refined from challenge to antibody measure**
  - **The monograph should be revisited, and *in vitro* test included**

New efficient potency tests should be developed and validated

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Reduce Refine Replace

Replace clinical potency by antibody measure

- **Potency test by antibody measure**
  - Ph. Eur. opens for antibody measures as potency test
  - Correlation between efficacy and titre must be demonstrated
  - The test must be validated
  - exist
  - nitiated
  - riosis

Paradoxical:
Number of fish to be used defined in Ph. Eur. prior the method has been developed.

- **Advantage:**
  - Reduced number of fish
    - From 420 to 35 fish for a hexavalent vaccine
  - Reduced suffering – no challenge
- **Implementation**
  - Every vaccine manufacturer must validate tests for its own products
  - Variation application (Type II) must be approved by the authorities prior to implementation.

Development -> method -> validated method -> approved method

1-2 yrs

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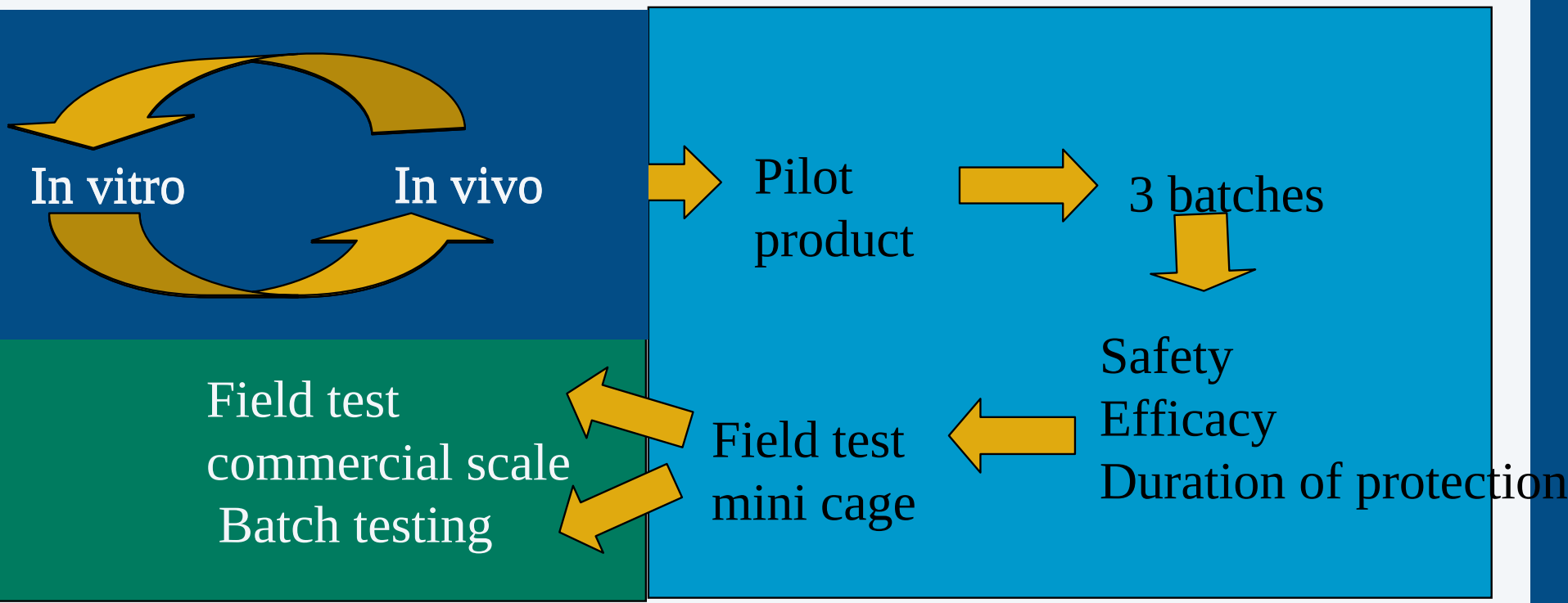
Conclusion

- **The industry should keep improving the in vitro quality assurance in order to test well defined during proof of concept**
- **The industry, the scientific community and regulatory authorities is and should be working to reduce and refine models**
- **The definition of experimental animals should be refined**



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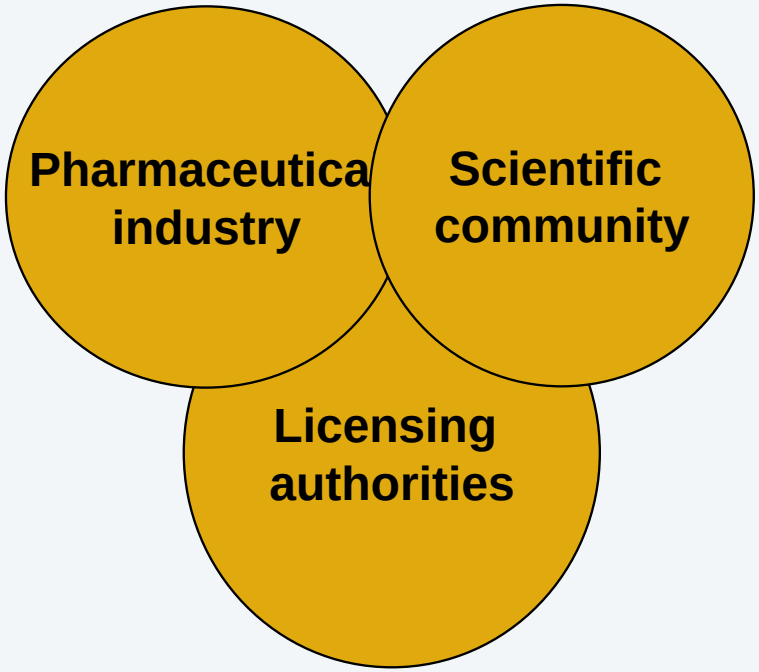
Future



Within 5-10 years

- **Reduced frequency of batch-safety tests**
- **Refined the potency method**
  - **Challenge replaced by antibody measure.**
  - **Reduced number of animals**

Within 10-15 years

- **Replaced batch potency by *In vitro* model**

Refine the definition on research animal:

- **Discriminate between fish animals that suffer (i.e. challenge) and animals that are handled by standard procedures used in the industry.**

Thank you for your attention

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