



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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to animal testing: do current EU
regulations and guidelines for regulatory
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RIVM Letter report 2015-0084
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Colophon

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Wettelijke beperkingen voor het gebruik van alternatieven voor dierproeven bij de beoordeling van geneesmiddelen

Bij de beoordeling van geneesmiddelen (veiligheid, kwaliteit en werkzaamheid) worden dierproeven ingezet. De overheid streeft ernaar hiervoor zo min mogelijk dieren te gebruiken. Het RIVM heeft onderzocht of er binnen de bestaande Europese en Nederlandse wetgeving voldoende mogelijkheden zijn om alternatieven voor dierproeven te gebruiken bij de beoordeling van geneesmiddelen. Uit dit onderzoek blijkt dat de huidige geneesmiddelenwetgeving het gebruik van alternatieve mogelijkheden voor dierproeven in strikt juridische zin niet belemmert, maar ook nauwelijks actief stimuleert. Het is toegestaan om alternatieven in te zetten, maar dan moet wel worden aangetoond dat ze dezelfde voorspellende waarde hebben als de dierproef. Deze validaties zijn in de praktijk ingewikkeld, kostbaar en tijdrovend.

Vooraf andere zaken dan de wet- en regelgeving zelf blijken de inzet van alternatieven voor dierproeven te belemmeren. Voor de beoordeling van geneesmiddelen gelden wetenschappelijke richtlijnen. Deze richtlijnen zijn niet wettelijk bindend, maar zijn wel doorslaggevend voor de beslissing of een geneesmiddel uiteindelijk op de markt mag worden gebracht. Hoewel vermindering en verfijning van dierproeven in richtlijnen veel aandacht krijgen, wordt in diezelfde richtlijnen nog regelmatig naar dierproeven verwezen. In de richtlijnen staan ook de voorwaarden vermeld waaraan alternatieven voor dierproeven moeten voldoen.

Andere belemmerende factoren zijn het gebrek aan alternatieve methoden die dierproeven in zijn geheel kunnen vervangen. Ook worden nieuwe alternatieve methodes niet altijd in alle landen geaccepteerd door registratieautoriteiten. Geneesmiddelen worden meestal voor meerdere landen gemaakt en in sommige landen worden dierproeven nog als de gouden standaard daarvoor gezien. Daardoor kiest een fabrikant soms voor dierproeven om tegemoet te komen aan de eisen van de verschillende autoriteiten.

Om de ontwikkeling van alternatieven te stimuleren is continue internationale afstemming nodig over de criteria waaraan ze moeten voldoen tussen registratieautoriteiten, wetenschappers en farmaceutische bedrijven. Aanbevolen wordt het onderzoek naar geschikte alternatieve methoden en de implementatie daarvan in richtlijnen voortdurend te stimuleren.

Kernwoorden: alternatieven voor dierproeven, wettelijke belemmeringen, risicobeoordeling, geneesmiddelen.

Synopsis

Legal barriers for the use of alternatives to animal testing: do current EU regulations and guidelines for regulatory acceptance of medicinal products pose legal barriers?

Animal tests are used to evaluate the safety, quality and efficacy of medicines. The Dutch government wishes to minimize the number of animals used for this purpose. The Dutch National Institute for Public Health and the Environment (RIVM) has investigated whether European and Dutch legislation offers sufficient scope for using alternatives to animal testing for the evaluation of (new) medicines. The investigation showed that existing pharmaceutical legislation does not impose any legal constraints on the use of alternatives to animal testing, but neither does it actively encourage the use of these alternatives. Alternative methods are permitted, but it must be demonstrated that these have the same predictive value as animal testing. In practice, the required validation procedure is often complicated, costly and time-consuming.

There are mainly other factors that discourage the use of alternatives to animal testing. For instance, medicines must be evaluated in accordance with strict scientific guidelines. These guidelines are not legally binding, but they do determine whether marketing authorization is eventually granted. Although the guidelines devote extensive attention to reducing animal testing, they make frequent references to such tests and contain requirements that any alternatives to animal testing must meet.

Other obstacles include the lack of alternative methods that can fully replace animal testing. In addition, new alternative methods are not always accepted by the registration authorities in all countries. Medicines are usually intended to be marketed in multiple countries, and animal testing is still regarded as the 'gold standard' in some countries. Therefore, manufacturers sometimes opt for animal testing to concede to the demands of the various authorities.

To encourage the development of alternative methods, regulatory authorities, researchers and pharmaceutical companies must engage in ongoing consultation at the international level concerning the criteria applicable to such alternatives. RIVM recommends the continued promotion of research into suitable alternative methods and their implementation in guidelines.

Keywords: alternatives for animal testing, legal barriers, risk assessment, medicinal products

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Summary

The Dutch Parliament wants to be informed on the presence of legal barriers to apply alternatives to animal testing regarding the regulatory acceptance of medicinal products. To this end, the ministry of Health, Welfare and Sport and the ministry of Economic Affairs have commissioned RIVM to investigate whether such legal barriers exist in the legislation concerning the development, production and/or release onto the market of medicinal products for human use in the Netherlands and Europe.

All relevant legislation that require data on safety, efficacy and quality of medicinal products for the evaluation (and authorisation) of medicinal products for human use were analysed for possibilities and barriers for the use of alternatives to animal testing. A distinction was made between the legally binding texts of EU Regulations and Directives and the European Pharmacopoeia (hard law) and the texts in ICH, EMA and OCABR guidelines (which do not have any legally binding force) that can be defined as 'soft law'.

In a strict legal sense, the legislative corpus of the EU and the Netherlands concerning medicinal products does not contain barriers for alternatives to animal testing. All tests on animals can be replaced by validated *in vitro* tests or more refined animal tests provided that the test results are of comparable quality and usefulness for the purpose of safety evaluation. At the same time, the extant legislative corpus does not offer many incentives to use alternative methods either.

Furthermore, EU-law, the European Pharmacopoeia and the guidelines issued by the ICH, the EMA and in the context of OCABR still refer to, require and/or set the standards for animal testing in pharmaceutical research and development or the batch release of biologicals. In this sense, there may be no formal legal barrier, but the informal barrier of soft law guidelines is almost as strong as formal law.

The development and validation of alternative methods and the acceptance of validation/evaluation results by authorities seem to be the real Achilles-heel in the implementation of alternative methods. While progress has been made in the field of reduction and refinement of animal tests, there are not many *in vitro* alternatives available that can fully replace an animal test.

Additionally, medicinal products are made for a global market. This means that the most 'cautious' regulatory authority determines the level of 'risk' that can be taken. In order to avoid the risk of a delay or a rejection, manufacturers try to fulfill the requirements that apply in most countries and therefore tend to stick to animal-based models.

While the major barriers for the uptake of alternative methods lie outside the realm of law, it might be worth considering to incorporate more 'active' incentives within the extant legislative framework. Moreover, increasing the use of alternative methods to animal tests

requires increasing harmonization of regulatory standards on a global level. It is also worth considering creating more and stronger incentives for science, industry and government to enter the process of validation or scientific evaluation after an alternative test strategy has been developed.

1 Introduction¹

On 22 September 2010, Directive² 2010/63/EU on the protection of animals used for scientific purposes of the European Union (EU) was published, which obliged the Netherlands to change its *Wet op de Dierproeven* (WoD, 2015) accordingly. The European Directive is based on the principle that animals have an intrinsic value which must be respected and that the use of animals for scientific and educational purposes should only be considered in case no alternative for the animal test is available. It aims to offer a better protection of animals used for scientific and educational purposes, e.g. by using 3R alternatives – tests that *refine*, *reduce* or *replace* animal tests and to strengthen the competitiveness of both science and industry in the European Union. A few requirements from the European Directive lead to changes in the Dutch *Wet op de Dierproeven*, such as:

- Projects for animal tests must be approved beforehand by an authorized body, resulting in a license for the project.
- Member states must install a national committee for the protection of animals that are used for scientific purposes.
- Every breeder, supplier and user must have sufficient employees that are competent and skilled in their tasks regarding the animals.
- Every breeder, supplier and user must install a body for animal welfare.
- Reports should include non-technical summaries.

The draft of the amended WoD was accepted by the Dutch Parliament on December 10, 2013. The discussion in Parliament resulted in the motion Heerema/Ouwehand (Heerema & Ouwehand, 2013) requesting the Dutch government to investigate which legal barriers exist for the application of alternatives to animal tests and to adapt laws and regulations to facilitate the use of alternatives by the industry if necessary.

On February 28, 2014, the Dutch Ministry of Economic Affairs presented an action plan to the Parliament. This plan states that in 2014 an assessment of the extent in which the legal framework contains barriers for the use of alternatives for animal tests will be undertaken for the relevant domains of the Ministries of Economic Affairs, of Health, Welfare and Sport and of Infrastructure and the Environment. The Ministry of Economic Affairs and the Ministry of Health, Welfare and Sport subsequently asked the RIVM to carry out this assessment for medicinal products (i.e. pharmaceuticals and vaccines), which is described in this report. A similar assessment has been performed for chemical substances (Heringa et al, 2014).

¹ The introduction is based on Heringa et al, 2014.

² An EU Directive is a form of legislation that is "directed" at the Member States. It will set out the objective or policy which needs to be attained. The Member States must then pass the relevant domestic legislation to give effect to the terms of the Directive within a time frame set in the directive, usually two years. (Source: *European Law Monitor*).

2 Background

Today, medicinal products are one of the most regulated products on the market. Their safety, efficacy and quality are under permanent surveillance. Before new medicinal products can be put on the market, they have to undergo an authorization procedure. After they have gained access to the market, (new) medicinal products are monitored for unknown adverse effects and consistent quality. Furthermore, most European countries have extensive legislation concerning the prices, labels and promotion of medicinal products. The European Pharmacopoeia (Ph. Eur) guards the quality and standardization of pharmaceutical products, their formulas, production and control method (Hoebert et al, 2014; Krapohl, 2008).

In order to identify legal barriers for the use of alternatives to animal testing, it is helpful to describe the process of drug development and to clarify the difference between the various forms of legislation that concern both animal testing and medicinal products first.

2.1 Different stages in the development of medicinal products

The development of almost every new medicinal product, whether it is a small molecule pharmaceutical or biopharmaceutical, e.g. a vaccine, starts with fundamental research. It is this first step in the development process of medicinal products that has the highest attrition rate. According to the European Federation of Pharmaceutical Industries and Associations (EFPIA), only one or two in every 10,000 substances synthesized in laboratories will, on average, successfully pass all stages needed for market authorization (EFPIA, 2013).

The route a medicinal product has to go to get from *scientific development* to actual use can be divided into two more or less 'separate' stages. The phase before a synthesized substance is allowed to enter the market as a medicinal product is called the pre-registration phase; the period after registration is usually referred to as the post-registration phase (or post-marketing) of development. The actual moment of registration (also called: marketing authorization) is a critical step in the life-cycle of a medicinal product. The legislation and requirements that govern the registration procedure, for a large part determine the choices that are being made during the development of a medicinal product in the pre-registration phase.

The pre-registration development phase itself can also be divided into two stages: a stage of non-clinical research and a stage of clinical research. The non-clinical stage of development is characterized by experimental research to test the pharmacological activity and toxicity of a synthesized substance, usually in laboratory animals. During the clinical stage of development the synthesized substance is also tested on human beings. Initially the product is tested in a small group of healthy volunteers or patients. This kind of research is usually referred to as 'Phase-I trials' -, after which the product is tested in a specifically selected group of patients (Phase-II trials). Phase -I and -II trials are

not strictly separated. After the Phase-I and -II trials are performed successfully, larger Randomized Controlled Trials (Phase-III trials) are set up and carried out.

It is possible that after the registration procedure – during the post-marketing phase – clinical research is being continued. This research (Phase IV-trials) is meant ‘to monitor the product used in public’. The product is being followed during a longer period of time in large patient groups in order to obtain broader clinical experience and detect/identify rare adverse effects.

Though we have presented the process of development of a medicinal product as a linear model (Figure 1), this process is not as linear in daily practice. For example, knowledge gained during the clinical or post-marketing trials sometimes lead to new non-clinical research and vice versa. Furthermore, chronic toxicity testing in animals usually runs parallel to Phase-II trials in human subjects.

After the stage of fundamental research, *scientific development* and *pharmaceutical development* run parallel with each other. During this latter process it is determined how to manufacture the active ingredients, how to make an actual dosage form (tablet, capsule or injection), how to test it for purity and how to ensure that the dosage form consistently delivers safe and effective drug levels. This process does not stop after registration and allows the producer of a certain medicinal product to apply for authorization of new manufacturing processes, new dosages or dosage forms, new ways to preserve the product or new therapeutic indications.

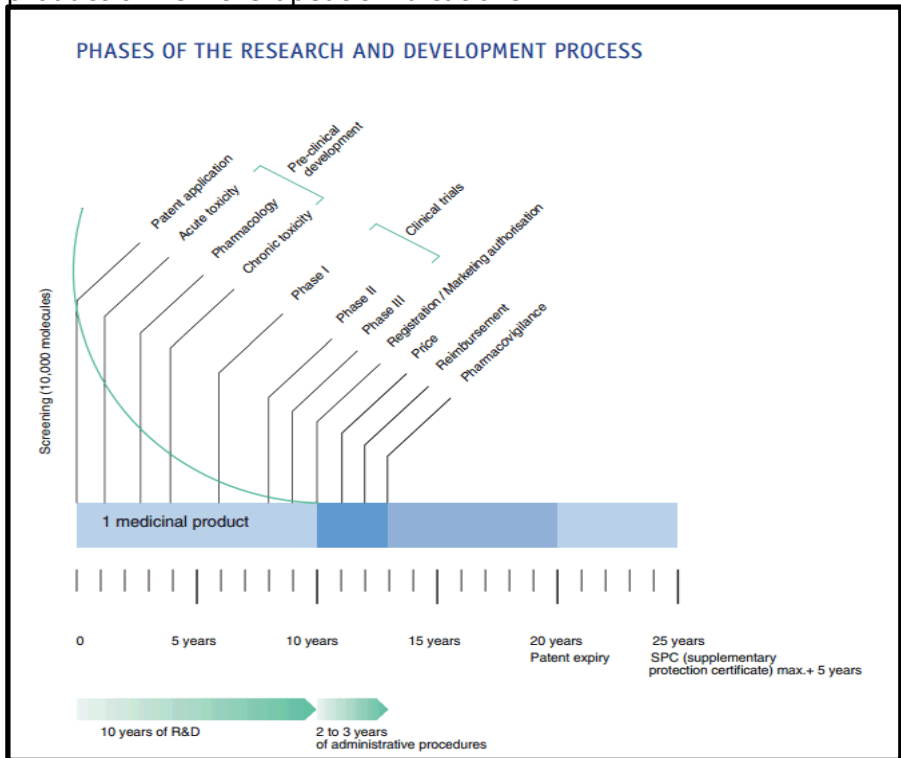


Figure 1. Phases of the research and development process for medicinal products. Source: EFPIA, 2013.

2.2 Different levels of legislation

The legislation governing the development, production, distribution and use of medicinal products is both extensive and complex. In order to provide a clear view on the legislative framework concerning animal testing within the domain of medicinal products, we follow the example of Schiffelers et al. (2005) and make a distinction between two categories of legislation:

- a. 'Horizontal legislation': laws, requirements and/or international treaties, which are specifically aimed at (the reduction of) animal testing and are intrinsically cross-sectoral in nature.
- b. 'Vertical legislation': laws, requirements and/or international treaties which are specifically aimed at a single sector, such as medicinal products or chemical substances. This kind of legislation generally touches the topic of animal testing in a more indirect way.

On the whole, horizontal legislation – in this case the legislation concerning animal testing – has prevalence over vertical legislation, such as the legislation concerning medicinal products. This means that legislation concerning medicinal products has to adhere to the legal requirements concerning animal testing laid down in the WoD and/or Directive 2010/63/EU concerning the protection of animals used for scientific purposes. The relation between both kinds of legislation is illustrated in the figure below (Schiffelers et al, 2005).

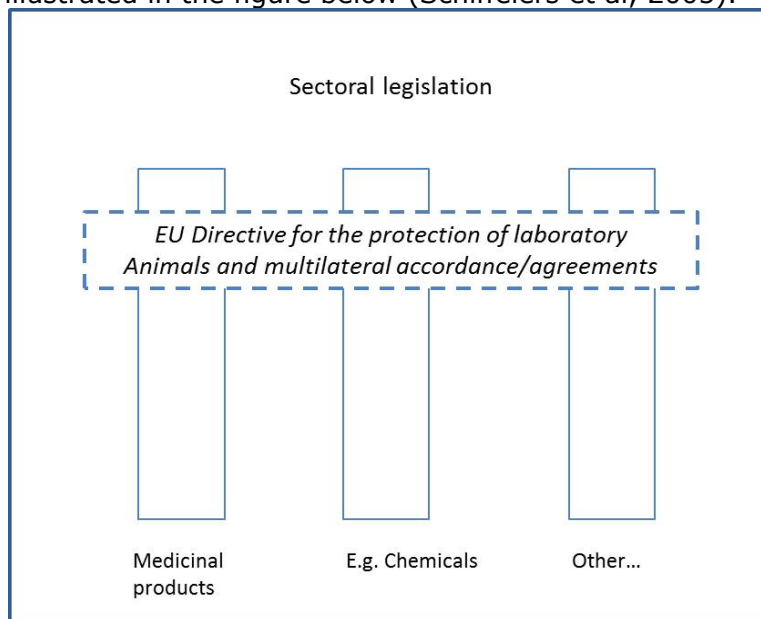


Figure 2. Relationship between horizontal and vertical legislation. Source: Schiffelers et al, 2005.

2.2.1 'Horizontal legislation': animal testing

The core of legislation concerning medicinal products and animal testing relevant for the Dutch situation is issued by the European Union. Virtually all EU-legislation concerning animal testing is based on the principles laid down in the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes*,

issued in 1985 by the Council of Europe³.

The convention recognizes that mankind has a moral obligation to respect all animals because of their capacity for suffering and memory. It furthermore propagates to limit the use of animals for experimental and other scientific purposes, with the aim of replacing such use wherever practical, in particular by seeking alternative measures and encouraging the use of these alternative measures (Council of Europe, 1986).

For the European Union, these principles have been codified in Directive 2010/63/EU, as was mentioned in the introduction of this report. Directives require member states to achieve a particular result without dictating the means of achieving that result, in this case a better protection of laboratory animals and the active promotion of the 3R-principles (replace, reduce, refine). The Dutch *Wet op de Dierproeven* was changed accordingly in 2013.

On a more general level, the OECD/EU-guidelines for Good Laboratory Practices and the Mutual Acceptance of Data also apply for research and production of medicinal products. Similarly, EU Regulation 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (2006) applies for all chemicals used as raw materials for medicinal products. Possible legal barriers for the use of alternative methods for animal testing in the field of chemical substances are investigated in a separate RIVM study (Heringa et al, 2014).

2.2.2 'Vertical legislation': medicinal products

A similar kind of multi-stage international interrelationship can be discerned with regard to pharmaceutical legislation. On a global level, the requirements for registration of medicinal products are coordinated by the *International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH), a cooperative platform of the European Union, the United States of America and Japan. The ICH issues guidelines, aimed at guaranteeing the safety, efficacy and quality of medicinal products. These technical guidelines are in turn referred to by European legislation, like Directive 2001/83/EC. This Directive is in turn implemented in the Netherlands through the *Geneesmiddelenwet*, the Medicines Act, (Gw, 2105).

Parallel to the rules and requirements for marketing authorization of medicinal products, there is specific legislation governing the quality of pharmaceutical products. An important legal keystone is the European Pharmacopoeia (Ph. Eur.) issued by the Council of Europe. The European Pharmacopoeia contains quality standards and control methods for medicinal products. Medicinal products in turn have to adhere to the requirements laid down in the European Pharmacopoeia at the moment of marketing authorization (Kubbinga et al, 2009).

³ The **Council of Europe** is an international organization promoting cooperation between all countries of Europe in the areas of legal standards, human rights, democratic development, the rule of law and cultural cooperation. It was founded in 1949. Not to be mistaken with the European Council/Council of the European Union.

The Ph. Eur. Monographs generally concern pharmaceutical substances intended for the manufacture of medicinal products, but can also concern quality requirements for intermediates, bulk products and final products, especially in the case of vaccines. Furthermore, the Ph.Eur. prescribes control methods and contains general quality requirements for formulated final products, such as tablets, capsules, etcetera. The Ph. Eur. is issued and managed by the *European Directorate for the Quality of Medicines* (EDQM) of the Council of Europe. The EDQM, national control authorities and the European Medicines Agency closely work together with regard to the sampling and testing of medicinal products through a European network of Official Medicines Control Laboratories (OMCLs).

Both the legislation concerning marketing authorization and the legislation concerning the production of medicinal products are closely intertwined.

2.3 Official Control Authority Batch Release of immunological medicinal products and medicinal products derived from human blood and plasma

While immunological medicinal products (i.e. vaccines) and medicinal products derived from human blood and plasma go through the same development process and the production, distribution and use are governed by the same legislation as other medicinal products, there's a specific additional requirement for these specific product groups: the Official Control Authority Batch Release (OCABR).

OCABR is the process of evaluating each individual batch of an already licensed vaccine or other biological by an Official Medicines Control Laboratory (OMCL) before giving approval for its release onto the market. Vaccines are made of (living) biological material, which could cause inconsistency in the production and control of these products. Moreover, vaccines are often used in healthy, often (very) young, individuals. Therefore, OCABR is an extra safeguard on product quality to guarantee to guarantee the efficacy and safety of each vaccine batch. Samples of a batch to be released have to be sent to an OMCL in the EU, along with production and control protocols. The OMCL reviews the manufacturer's production and quality control data and performs several quality control tests on the batch. (OCABR, 2015).

If the results are satisfactory, an 'Official Control Authority Batch Release Certificate' is issued. This certificate means that the batch has been examined and tested by an OMCL in accordance with the *Official Control Authority Batch Release for Human Biologicals: vaccines, blood and plasma derivatives* and is in compliance with the approved specifications laid down in the relevant monographs of the European Pharmacopoeia and in the relevant marketing authorization requirements.

OCABR is an activity within the General European OMCL Network, thus subject to its operating rules and involves countries from the European Union (EU) and the European Economic Area (EEA) only. It is supervised by an elected advisory group consisting of six representatives (three for

blood and three for vaccines) from different involved countries (EDQM, 2015a).

3 Methods

3.1 Aim

This report addresses the question 'Which legal barriers exist for the use of alternatives for animal tests in the development, production and/or release onto the market of medicinal products for human use in the Netherlands and Europe?'

In this study, medicinal products refer to traditional, small molecule pharmaceuticals, usually derived from chemical synthesis, and biopharmaceuticals, which include recombinant proteins, vaccines, blood products used therapeutically (such as IVIG), gene therapy, and cell therapy (for instance, stem cell therapies).

All relevant legislation that require data on safety, efficacy and quality of medicinal products for the evaluation (and authorisation) of medicinal products for human use were analysed for possibilities and barriers for the use of alternatives. This study is similar in its approach to the analysis for legal barriers for the use of alternative methods for animal tests for chemical substances (Heringa et al, 2014).

3.2 Definitions of 'alternatives to animal tests'

In accordance to Heringa et al. (2014) we define 'Alternatives to animal tests' as methods in a broad sense that lead to Refinement, Reduction or Replacement (3Rs) of animal tests (Heringa et al, 2014). For a definition of animal tests, for which alternatives are needed, Directive 2010/63/EU on the protection of animals used for scientific purposes gives a description:

- any use, of an animal for experimental or other scientific purposes, or educational purposes, "which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice." "The elimination of pain, suffering, distress or lasting harm by the successful use of anaesthesia, analgesia or other methods" does not exclude such a procedure from being an animal test. The killing of animals solely for the use of their organs or tissues is excluded.
- with the following animals:
 - a) non-human vertebrate animals, including:
 - i. independently feeding larval forms; and
 - ii. foetal forms of mammals as from the last third of their normal development;
 - b) live cephalopods ('ink fish').
where a non-human vertebrate animal at an earlier developmental stage than described above, which is "allowed to live beyond that stage of development and, as a result of the procedures performed, is likely to experience pain, suffering, distress or lasting harm after it has reached that stage of development" is included as well.
- excluding normal veterinary practices, agriculture and animal husbandry.

3.3 Scope

A distinction is made between the legally binding texts of regulations and directives (hard law) and the texts in ICH and EMA guidelines which can be defined as 'soft law'. Our analysis of hard law - Table 1 shows 'hard laws' included in this study - is based (if possible) on the 'consolidated version' of the Directives and Regulation which includes all amendments and corrections that were made over time.

The status of soft law, such as guidelines, is clarified by the EMA as follows (EMA, 2008):

Within the framework of the pharmaceutical legislation, scientific guidelines do not have legal force and the definitive legal requirements are those outlined in the relevant Community legislative framework (Directives, Regulations, Decisions etc.) as well as appropriate national rules. However, scientific guidelines are to be considered as a harmonised Community position, which if they are followed by relevant parties such as the applicants, marketing authorisation holders, sponsors, manufacturers and regulators will facilitate assessment, approval and control of medicinal products in the European Union.

Barriers found in guidelines can therefore not be considered as formal legal barriers, as guidelines - as a form of a 'code of conduct' - do not have any legally binding force (i.e. 'force of law') in themselves, but only gain formal authority through other legislation and/or daily practice.

The study touched upon, but did not include a full-scope systematic review of, possible barriers other than barriers in hard and soft law in the use of alternatives, such as the availability of or access to alternative methods. The question whether alternative methods are possibly better predictive for humans is not addressed either, because this falls outside the scope of the commissioned project. Also the question whether certain information requirements can be waived if other information (e.g. from alternatives) is available, is not addressed. These are in-depth scientific questions in the field of 'intelligent/integrated testing strategies' and these are outside of the scope of the current study.

Table 1. List of legislation ('hard laws') evaluated for legal barriers for the use of alternative methods for animal testing

Horizontal legislation		
1	Directive on the protection of animals used for scientific purposes	Directive 2010/63/EU
Vertical legislation		
2	Community Code relating to medicinal products for human use	Directive 2001/83/EU (consolidated version)
3	Clinical Trials on medicinal products for human use	Directive 2001/20/EC (consolidated version)
4	Regulation on clinical trials on medicinal products for human use	Regulation 536/2014
5	European Pharmacopoeia (8 th ed.)	Ph. Eur.

4 Barriers in 'hard law': EU Directives and Regulations

4.1 **Directive 2010/63/EU on the protection of animals used for scientific purposes**

Directive 2010/63/EU, issued on 22 September 2010, includes the minimum requirements to which national legislation on animal testing of Member States of the European Union has to adhere (EC, 2010). This Directive replaced Directive 86/609/EEC issued in 1986.

The Directive's final goal is the full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so. To that end, it seeks to facilitate and promote the advancement of alternative approaches. It also seeks to ensure a high level of protection for animals that still need to be used for animal tests. As is stated in the preambles of the Directive, animals have an intrinsic value which has to be respected (EC, 2010):

Animals should always be treated as sentient creatures and their use in procedures should be restricted to areas which may ultimately benefit human or animal health, or the environment. The use of animals for scientific or educational purposes should therefore only be considered where a non-animal alternative is unavailable.

Therefore, the Directive requires Member States to stimulate the use of 3R-principles (*replace, reduce, refine*) in scientific research as far as possible. Article 4, paragraph 1, requires Member States to ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals shall be used instead of an animal test. In article 13, paragraph 1, this principle is once more accentuated (EC, 2010):

Without prejudice to national legislation prohibiting certain types of methods, Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union.

Nevertheless, the Directive states that the use of animals for scientific tests remains necessary in certain occasions. To manage risks to human and animal health and the environment, some substances and products (i.e. medicinal products and chemical substances) can be marketed only after appropriate safety and efficacy data have been submitted. Some of those requirements can be fulfilled only by resorting to animal testing (regulatory testing).

In these cases, the Directive aims to enhance animal welfare by laying down minimum requirements for housing and care and regular checks on pain, suffering and distress and lasting harm of laboratory animals. Still, the main goal is to increase the use of alternative approaches and to eliminate unnecessary duplication of regulatory testing.

4.2 Directive 2001/83/EC on the Community code relating to medicinal products for human use

Directive 2001/83/EC, issued on 6 November 2001, contains the rules, requirements and procedures surrounding the authorization of medicinal products for human use for the European market and the rules and procedures governing the supervision and control of medicinal products that have gained market authorization. It integrates (and replaces) a large number of previously issued directives, such as Directive 89/342/EEC concerning provisions for immunological medicinal products consisting of vaccines, toxins or serums and allergens (EC, 2012).

Directive 2001/83/EC emphasizes that the essential aim of any rules governing the production, distribution and use of medicinal products must be to safeguard public health. At the same time, this objective must be attained by means which will not hinder the development of the pharmaceutical industry or trade in medicinal products within the EU.

In order to gain marketing authorization, the producers of medicinal products need to adhere to several criteria. One of these criteria is the submission of a dossier containing the results of all pharmaceutical, non-clinical and clinical tests that have been conducted for the medicinal product to be registered. In assembling this registration dossier for application for marketing authorization, applicants have to take into account the *Community guidelines* relating to the quality, safety and efficacy of medicinal products published by the European Commission in *The rules governing medicinal products in the European Community*⁴, Volume III and its supplements: *Guidelines on the quality, safety and efficacy of medicinal products for human use*. With regard to the practical implementation of the various tests that have to be conducted, Directive 2001/83/EC refers to the guidelines of both the ICH and the EMA and the Ph. Eur.

The analytical, pharmacotoxicological and clinical standards and protocols regarding the testing of medicinal products are laid down in Annex I of Directive 2001/83/EC. The introduction of this Annex states that (EC, 2012):

Member States shall (...) ensure that all tests on animals are conducted in accordance with Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulation and administrative provisions of the Member States regarding the protection of animals for experimental and other scientific purposes.

As was mentioned earlier, Directive 2010/63/EU has replaced Directive 86/609/EEC in 2010.

Nevertheless, animals test are explicitly mentioned when the tests are described that are necessary in order to apply for marketing authorization. This is especially the case for the toxicological and

⁴ See: http://ec.europa.eu/health/documents/eudralex/index_en.htm

pharmacological tests, as can be illustrated by some of the following examples:

1. In Annex I, part I, module 4, paragraph 4.2.3e concerning reproductive and developmental toxicity it is stated that (EC, 2012): *embryo/foetal toxicity studies shall normally be conducted on two mammalian species, one of which should be other than a rodent. Peri- and postnatal studies shall be conducted in at least one species.*

2. In Annex I, part I, module 5, paragraph 5.2b concerning the basic requirements and principles for clinical research it is stated that (EC, 2012): *clinical trials must always be preceded by adequate pharmacological and toxicological tests, carried out on animals in accordance with the requirements of Module 4 of this Annex.*

Even though animal tests are explicitly mentioned in Directive 2001/83/EC, the Directive does not contain any formal legal barriers for the use of alternative methods for animal tests. Once an alternative method is validated, there is no regulatory barrier to use it, as can for example be discerned from Annex I, part I, module 4, paragraph 4.2.3f on local tolerance studies (EC, 2012): *studies in animals can be substituted by validated in vitro tests provided that the test results are of comparable quality and usefulness for the purpose of safety evaluation.*

With regard to the ongoing scientific debate on the predictive value of animal test for identifying human risks (Hooijmans & Ritskes-Hoitinga, 2013; Greek & Menache, 2013; Perel et al, 2007), one can argue the usefulness of requiring an alternative *in vitro*-test to be of comparable quality to an animal test. In this respect, such a requirement can be considered as a barrier.

However, the question whether animal models are good predictors for human responses to medicinal products, lies outside the direct scope of this report and requires a more systematic review of the available scientific literature. It should furthermore be mentioned that, with regard to the actual decision whether or not to grant a specific product market authorisation, it is not the animal tests that are the decisive factor, but the results of studies with human subjects.

4.3 Directive 2001/20/EC and Regulation 536/2014

Directive 2001/20/EC on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use (also known as the 'Clinical Trials Directive'), issued on 4 April 2001, encompasses all rules and requirements for setting up and conducting clinical trials on medicinal products for human use. As is stated in the preambles of this Directive (EC, 2009):

The accepted basis for the conduct of clinical trials in humans is founded in the protection of human rights and the dignity of the human being with regard to the application of biology and medicine, (...). The clinical trial subject's protection is safeguarded through risk assessment based on the results of toxicological experiments prior to any clinical trial, screening by ethics

committees and Member States' competent authorities, and rules on the protection of personal data.

The legal emphasis with regard to toxicological experiments lies strongly on animal experiments, as we have seen in Directive 2001/83/EC, though in a strictly legal sense, there is no barrier for the use of alternative methods.

On 16 April 2014 this Directive was in part replaced by Regulation⁵ 536/2014 (EC, 2014). It allows for the speeding up of the process for authorizing new clinical trials and reduction of the administrative burden for applicants. Regulation 536/2014 relies on the existence of an EU portal and database. The EMA is currently starting up the portal and associated database. As a consequence, the regulation is expected to come into full force from 2016 onwards.

With regard to non-clinical research, Regulation 536/2014, does not differ from Directive 2001/20/EC. The application dossier: *shall also contain summaries of non-clinical pharmacology and toxicology data for any investigational medicinal product used in the clinical trial in accordance with international guidance* (EC, 2014).

4.4 European Pharmacopoeia 8th Edition

In the past, animal testing was common feature for several types of medicinal products, such as vaccines (both for human use and for veterinary use); blood products; antibiotics; hormones; other biological and biotechnological products. The European Pharmacopoeia (Ph.Eur) monographs for these products often contained animal tests and since the Ph. Eur. is a legal basis for the quality control of medicinal products during development, production and marketing processes, these tests were obligatory for their quality control.

Since 1986, the Ph. Eur. is committed to the reduction of animal use according to the Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes. At several levels, this commitment is expressed in the Ph. Eur.

First, the introduction of the Ph. Eur. clearly states this commitment to the Convention and the principles in the EU Directive 2010/63/EU. The European Pharmacopoeia Commission intensified activities to realize the 3Rs directly in 1986. (Castle, 2007)

Second, the chapter 'General Notices' indicates the option to use validated alternative test methods if they are not yet included in the monographs and general chapters (Milne and Buchheit, 2012). However, the condition has to be met that the alternative test method is able to show equivalent compliance to the monograph standards which has to be approved by the competent authority (i.e. the medicines agency). In practice, this condition is resource demanding and can be difficult to meet and competent authorities in different countries may differently assess the alternative method. For marketing authorization holders, a harmonized opinion is often a precondition to introduce an alternative

⁵ **Regulations** are legal instruments that are binding in their entirety and directly applicable in all Member States, thus reducing the potential differences between national legislations.

test method for the quality control of their product, since the registration dossier is applicable to several countries within the EU or even globally.

Third, general monograph 0153 Vaccines for human use requires that tests must use the lowest possible number of animals and cause the least pain, suffering, distress or lasting harm. This requirement should enhance reduction of animal use.

Fourth, an alternative test method can only become a pharmacopoeial method after exhaustive validation, often performed in international standardization studies. This route is time consuming, expensive and may require extra use of animals for validation, but it will result in a harmonized requirement. An example is the replacement of the rabbit pyrogen test by the bacterial endotoxin test for biological and biotechnological products. In several cases, Ph. Eur. still considers the traditional animal test as the 'golden standard' that has to be used in case of production changes or other inconsistencies, e.g. the challenge potency assay for Tetanus vaccine. Nevertheless, Ph. Eur. indicates that in the interests of animal welfare, the refined methods are used for routine batch release wherever possible.

Fifth, several animal tests in monographs have been deleted after review of historical data. In the past, production conditions for vaccines and other biologicals were less standardized and safety tests in animals were used to detect abnormal and specific toxicity. Since the introduction and refinement of GMP, several animal tests could be abolished or replaced, such as the test on abnormal toxicity (Charton, 2008).

The Ph. Eur. still includes animal tests, nevertheless the use of validated alternative methods to replace these tests is in principle possible under certain conditions. In the past 30 years, progress in the 3Rs has been made although alternative methods often have a long way to go. For the control of medicinal products derived from human blood and plasma as well as for many hormone preparations, such as insulin, growth hormone, calcitonin, the Ph. Eur. does not prescribe animal test methods anymore. For vaccines for human use, in several cases in vivo testing has been replaced by in vitro testing. For remaining animal tests, reduction and refinement have been achieved, such as the use of fewer animals in single dilution tests (reduction) or the replacement of cumbersome challenge tests by serological tests (refinement). (EDQM, 2015b).

For the quality control of newly developed medicinal products it is common to use in vitro assays instead of animal tests.

5 Barriers in 'soft law': ICH/EMA and OCABR guidelines

5.1 ICH guidelines: scope and structure

The purpose of the *International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use* (ICH; see background) is to make recommendations on ways to achieve greater global harmonization on the interpretation and application of technical guidelines and requirements for product registration in order to reduce or obviate the need to duplicate the testing carried out during the research and development of new medicinal products. Within ICH, both regulatory authorities and pharmaceutical industry collaborate and contribute to the harmonization process between Europe, Japan and the United States of America. The ICH prepares harmonized guidelines or internationally accepted common texts for the development of new pharmaceuticals (Van der Laan & DeGeorge, 2013).

The ICH guidelines can be grouped into four main categories (Table 2). The Quality guidelines cover aspects such as the conduct of stability studies and impurities. The Safety guidelines focus on uncovering potential risks like carcinogenicity, genotoxicity and reprotoxicity and QT interval prolongation⁶. Efficacy guidelines are in turn concerned with the design, conduct, safety and reporting of clinical trials, while the Multidisciplinary guidelines cover topics which do not fit uniquely into one categories (ICH, 2014; Van der Laan & DeGeorge, 2013).

Table 2. Categories and number of ICH-guidelines

Category	Number of guidelines
Quality	24
Safety	15
Efficacy	22
Multidisciplinary	8
Total	79

Source: ICH, 2014.

5.2 EMA guidelines: scope and structure

The ICH-guidelines are in turn incorporated by the European Medicines Agency in its own corpus of scientific guidelines. Yet, the EMA also issues its own scientific guidelines through the Committee for Medicinal Products for Human Use (CHMP). In comparison with the ICH process, the pharmaceutical industry is not directly involved in the development of these guidelines, although every party has the opportunity to respond to public consultation calls. Both the ICH and the EMA guidelines are meant to guide applicants in preparing marketing-authorization applications for human medicinal products, especially with regard to the demonstration of quality, safety and efficacy. The EMA strongly

⁶ QT time is the time between start of the Q wave and end of the T wave in the heart's electrical cycle. Prolongation of this interval is associated with deadly rhythm.

encourages applicants to follow these guidelines. Applicants need to justify deviations from guidelines fully in their applications at the time of submission (EMA, 2009a).

The scientific guidelines issued by the EMA vastly outnumber the ICH guidelines and are structured in different categories, i.e: Quality, Biological medicines, Non-Clinical, Clinical Safety and Efficacy and Multidisciplinary. Each of these categories is further separated into 2 to 18 subcategories under which a variable amount of guidelines can be found, ranging from 1 to 25+ guidelines. There is no clear indication how many EMA-guidelines there actually are, but by estimation, the total corpus of EMA-guidelines should amount to 650 to 700 guidelines in total, of which (roughly) 350 concern non-clinical and safety testing.

This high number of ICH/EMA-guidelines, makes a systematic review of all the existent guidelines with regard to animal tests unfeasible in the timespan of this study.

5.3 Barriers for the use of alternative methods in ICH and EMA guidelines

In a strict legal sense, both the EMA and ICH guidelines offer no formal barriers to replace animal models by *in vitro* tests. In 1997 the CPMP (the predecessor of the earlier mentioned CHMP) published a position paper in which the committee clearly stated its intent to implement Directive 86/609/EEC (now 2010/63/EU). According to the paper, the official position of the CPMP, and therefore of the EMA, is that studies on animals can be substituted by validated *in vitro* tests. It furthermore urges applicants to take into consideration all developments in alternative testing methods when compiling their dossier (CPMP, 1997).

The same attitude is voiced by the ICH in its guidance on *Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals* (EMA, 2009b):

This guidance should facilitate the timely conduct of clinical trials, reduce the use of animals in accordance with the 3R (reduce/refine/replace) principles and reduce the use of other drug development resources. Although not discussed in this guidance, consideration should be given to use of new in vitro alternative methods for safety evaluation. These methods, if validated and accepted by all ICH regulatory authorities, can be used to replace current standard method.

Both the ICH and the EMA facilitate and encourage the replacement of animal models with alternative methods, the reduction of the amount of required animals test and implementation of more refined testing tests (Ohno, 2002). This can, for example, be seen in the revised versions of the ICH-guidelines concerning carcinogenicity (S1), reprotoxicity (S5) and juvenile toxicity (S11) which have a strong focus on the reduction and refinement of animals tests. Illustrative for the focus on reduction and refinement is the ICH guideline concerning the *preclinical safety evaluation of biotechnology-derived pharmaceuticals* (S6-R1). This guideline states (ICH, 2011):

Safety evaluation programs should normally include two relevant species. However, in certain justified cases one relevant species may suffice (...). In addition even where two species may be necessary to characterise toxicity in short term studies, it may be possible to justify the use of only one species for subsequent long term toxicity studies (...) Toxicity studies in non-relevant species may be misleading and are discouraged.

Despite successful efforts to reduce and/or refine animal tests both the ICH and EMA guidelines still frequently refer to, require and/or set standards for animal tests, especially with regard to tests aimed to uncover potential risks like carcinogenicity, genotoxicity and reprotoxicity and in quality batch control. The EMA has recently acknowledged this by stating that (EMA, 2014):

*In the context of drug development and production, laboratory animal studies are mainly used for two purposes: (1) for non-clinical/safety testing during development of new human/veterinary medicinal products and (2) for quality batch control as part of the manufacturing process. **While animal tests are still required, some progress has been made in implementing 3Rs** [author's emphasis].*

From this we can conclude that – while there is no 'official' barrier to use alternative methods – the use of animal tests is still firmly rooted within the corpus of guidelines concerning the development, manufacturing and marketing authorizing process of medicinal products. The EMA underlines that the replacement of animal test with alternative methods is not going as fast as wanted. Existing regulatory requirement (i.e. scientific guidelines) can be seen as partially hampering this process, but at the same time both the industry and the scientific community have been unable to develop *in vitro* alternatives that can fully replace animal test. Replacement seems to be more complex than initially thought (see: chapter 6).

In order to take a more active stance, the EMA has recently installed an *ad hoc* expert group on the application of the 3Rs in regulatory testing of medicinal products (EMA, 2015). Furthermore, the EMA is presently working on a tabulated overview of the current regulatory testing requirements for human medicinal products and opportunities for implementation of the 3Rs (EMA, 2014). Unfortunately, this overview is not yet available.

The EMA claims that regulatory safety studies for human and veterinary medicinal products account for only a relatively low number of all animal test: ca. 4.4% of the total number of experimental animals used. Animal use for quality batch control testing of human medicinal products – both by manufacturers and regulators (see: §5.4) – accounts for 10.9% experimental animals (EMA, 2014).

5.4 Barriers for the use of alternative methods in OCABR guidelines

In order to facilitate mutual recognition and to ensure a common approach of all OMCLs in Europe with regard to the batch release of

vaccines and other biologicals (see: background), both a single procedure and a set of product specific guidelines have been established by the network of OMCLs and the EDQM.

The product specific guidelines cover all tests that have to be conducted by an OMCL before a vaccine, a combination of vaccines and/or a blood derived medicinal products can be released onto the European market while having a marketing authorization. The choice of tests that have to be conducted on a certain type of product is based on the registration dossier for market authorization in which manufacturers have recorded their specifications and test methods which have to comply with the relevant monographs in the Ph. Eur. Table 3, for example, gives an overview of vaccines for which the OCABR-guidelines require animal test before batch release (OCABR, 2015).

<i>Table 3. Vaccines for which OCABR guidelines require animal tests</i>
<i>BCG-vaccine (tuberculosis) (new working seed lot)</i>
<i>Diphtheria vaccine (final bulk)</i>
<i>Tetanus vaccine (final bulk)</i>
<i>Pertussis vaccine (whole cell) (final bulk)</i>
<i>Pertussis vaccine (acellular) (final bulk)</i>
<i>Japanese encephalitis vaccine (final bulk)</i>
<i>Polio vaccine (inactivated) (final bulk)</i>
<i>Polio vaccine (oral)</i>
<i>Rabies vaccine (every final lot)</i>
<i>Tick borne encephalitis vaccine (final bulk)</i>
<i>All combinations of vaccines mentioned above (final bulk)</i>

On the batch release certificate, the OMCL has to declare that the OCABR guidelines were followed for testing and that the product complies with the marketing authorization. To omit or reduce an animal test, a revision of the guideline has to be adopted in consensus by the OMCL meeting. In some instances, the guidelines already offer the possibility of reducing animal tests at an OMCL if sufficient data is available and the other OMCLs agree with the reduction. In OCABR context, the challenge is to reach consensus in the OMCL group to reduce testing. On the other hand, an alternative method will automatically be implemented for OCABR if it is accepted for the marketing authorization or in the Ph. Eur.

6 Barriers outside the legal framework

The previous chapters have shown that current EU regulations and guidelines do not pose legal barriers to the use of alternative methods for animal testing in the pharmaceutical sector. More important obstacles to greater use of the 3Rs as identified in literature are practical factors which in themselves have little to do with legislation. The most important of those barriers are described below.

6.1 Scientific limitations in the development and validation of alternative methods

Arguably the most important barrier for implementation of the 3Rs is the current state of scientific knowledge. Most available alternative test methods are designed to monitor a single toxic effect and not for the more complex systemic effects. In contrast to *in vitro* tests, animal tests enable the measurement of several parameters simultaneously, which gives indications on the effect of a medicinal product in a full, living biological system.

The full information provided by an animal test result cannot be given by a single alternative method. For that reason, animal tests cannot (as of yet) be replaced by a single alternative method. To be able to replace or reduce animal tests, there is a need to combine different alternative methods in integrated test strategies. In these strategies, animal tests may be included as well, if information from alternative methods is insufficient to make a decision on the safety of a substance (Schiffelers et al, 2014; Vandebriel & Opperhuizen, 2011).

In order to stimulate the development of alternative methods, databases are made available to exchange knowledge (EURL-ECVAM, 2014). However, there is still uncertainty about the performance of 3R models, mainly caused by uncertain predictability of 3R models and the challenging *in vitro-in vivo* extrapolation (Schiffelers et al, 2014).

Alternative methods have to be validated first before they can be used to replace animal studies in the market authorization process, (Schiffelers et al, 2007; Kooijman, 2013). This validation process has two aspects: a. the technical validation, which deals with questions whether a test is reproducible in a laboratory setting and between different laboratories; b. the practical validation, which deals with the question how to use a new method in a test strategy (Kooijman, 2013).

The validation or (scientific) evaluation of alternative methods is not necessarily a difficult process, but it is time-consuming, requires large investments by manufacturers and/or the scientific community against uncertain results. Additionally, its scientific output is not rated highly by the scientific community (Schiffelers et al, 2007). Furthermore, the lack of a market for alternative methods – in part due to reluctance of regulatory authorities to accept data gained through alternative test methods – makes recovering the costs of the validation studies unlikely

(Kooijman, 2013). These barriers strongly impede the implementation of alternative methods.

6.2 **Animal tests: a golden standard, but rightly so?**

Still, the use of animal tests in the domain of medicinal products is not only based on the lack of validated alternative methods. Worldwide, animal studies can still be a common scientific standard to assess the quality and safety of medicinal products for regulatory acceptance. According to Kooijman (2013), animal studies are locked-in the development of medicinal products, because they are taken for granted, normatively endorsed and backed up by regulatory authorities.

The majority of the scientific community still regards the value of animal test as 'self-evident'. While it is impossible to tar all animal tests with the same brush, the idea that animal research, particularly research relating to medicinal products and environmental agents, may be a poor predictor for human risk is gaining more scientific support (Hooijmans & Ritskes-Hoitinga, 2013; Greek & Menache, 2013; Perel et al, 2007). More attention could be paid to the value of a specific animal test to gain insight into certain risks. Some of the key problems with animal tests – as identified in the scientific literature – have been summarized by Pound et al. (2004):

- Disparate animal species and strains, with a variety of metabolic pathways and drug metabolites, leading to variation in efficacy and toxicity;
- Different models for inducing illness or injury, with varying similarity to the human condition;
- Variations in drug dosing schedules and regimens of uncertain relevance to the human condition;
- Variability in animals for study, methods of randomization, choice of comparison therapy (none, placebo, vehicle);
- Small experimental groups with inadequate statistical power; simple statistical analyses that do not account for confounding; and failure to follow intention-to-treat principles;
- Nuances in laboratory technique that may influence results, for example, methods for blinding investigators, being neither recognized nor reported;
- Selection of outcome measures, which being surrogates or precursors of disease, of uncertain relevance to the human clinical condition;
- Variable duration of follow up, which may not correspond to disease latency in humans.

6.3 **Risk, reluctance and cautious regulators**

Despite the existing doubts about the relevance of basic animal research in predicting the risk of medicinal products in humans, they are still widely used. This can partially be explained by an increasing risk aversion, both in society and among regulators. During the last decade, the regulatory system for medicinal products has suffered from an apparent decrease in public trust in its ability to guarantee the safety of pharmaceuticals (Hoebert et al, 2014).

Public accountability seems to have shifted from the manufacturer of a 'faulty product' and physicians who use the 'wrong product' to the regulator who has granted market authorization to 'dangerous products'. As a result, regulators have become more cautious (Scannel et al, 2012), both concerning medicinal products and 'new' test methods and favor familiar routines. This is not always deliberate. Regulators are relatively unfamiliar with the properties and scientific qualities of relevant, but new, alternative test methods. While the manufacturers of medicinal products do not always know what kind of information regulators need to make a comprehensive assessment of the risk-benefit balance of a specific product. Both sides therefore tend to adhere to classic (animal) models (Schiffelers et al, 2014; Van den Berg, 2011; Schiffelers et al, 2007).

However, during the past few years this attitude – especially with regard to alternative methods for animal tests – seems to be changing. Regulators are being more involved in the early stages of the development of new medicinal products. The EMA and national regulatory authorities have opened the possibility for companies to ask scientific advice during the early stage of the drug development process. Scientific advice helps the company to make sure that it performs the appropriate tests and studies, so that no major objections regarding the design of the tests are likely to be raised during evaluation of the marketing-authorisation application (EMA, 2015b). It is expected that this will have a beneficial effect on the use of 3R-alternatives for animal tests.

An illustrative case of this new development is a regulatory experiment that was started in 2013 by the ICH. From the retrospective analysis of the various datasets it was concluded that based on pharmacology, genotoxicity, and chronic toxicity data (usually present at the end of Phase-II research in the development of a new pharmaceutical) the outcome of the 2-year rat carcinogenicity study can be predicted with reasonable assurance. This means that for certain pharmaceuticals, carcinogenicity assessment could be completed without the need to conduct a 2-year rat carcinogenicity study. Based on these results, the ICH has advised regulators to open the possibility for companies to apply for a waiver for compulsory 2-year rat carcinogenicity studies (ICH 2013).

6.4 Global regulatory harmonization: a successful but slow process

Since medicinal products are usually made for a global market, the most 'cautious' regulatory authority determines the level of 'risk' that can be taken. In order to avoid the risk of a delay or a rejection, manufacturers try to fulfill the requirements that apply in most countries and therefore tend to stick to animal-based models, even when alternatives are available or when alternatives are stimulated by specific regulators, like, e.g., the EMA.

This barrier can only be lifted through the global harmonization of regulatory requirements. The ICH has put the promotion of 3R-initiatives high on its agenda, but harmonization is an inherently slow process. Still, progress is made, as can be illustrated by the current discussion

about new methods of *in vitro* testing of reproductive toxicity. This discussion, initiated by the EMA, started in 2010 at a ICH-conference in Tallin (Van der Laan et al, 2012). In June 2015 the ICH Expert Working Group will start to revise the ICH S5 Guideline in Fukuoka, Japan. The EU delegation has the intention to strongly promote the use of the *in vitro* alternatives, and will try to come to reduce the number of *in vivo* species from 2 to 1 as a start. The RIVM and CBG (the Dutch Medicines Evaluation Board) have contributed to this process by evaluation of the contribution of rat and rabbit-studies to the scientific evidence for reproductive safety of pharmaceuticals.

Despite these efforts to improve harmonization, different interpretations at national level can still lead to different requirements for regulatory acceptance between countries. The European urge to implement the 3R-strategies as widely as possible is not always shared by other regulators, like the American FDA or the Japanese PMDA/MHLW (Schiffelers et al, 2014; van den Berg, 2011; Vandebriel & Opperhuizen, 2011; Schiffelers et al, 2007).

7 Discussion and conclusion

The purpose of this study was to determine whether there are any legal barriers for the use of alternatives to animal tests in the development, production and/or release onto the market of medicinal products for human use in the Netherlands and Europe. This report intends to give insight into areas where action is needed to remove such barriers in order to clear the way for alternative methods.

7.1 No formal barriers in 'hard law', yet no incentives either

This study shows that the ultimate goal of Directive 2010/63/EU on the protection of animals used for scientific purposes is the full replacement of procedures on live animals for scientific and educational purposes as soon as it is scientifically possible to do so. To that end, it aims to facilitate and promote the advancement of alternative approaches. It also seeks to ensure a high level of protection for animals that still need to be used for animal tests.

Nevertheless, Directive 2010/63/EU also acknowledges that the use of animals for scientific tests remains necessary in certain occasions. To manage risks to human health, medicinal products can be marketed only after appropriate safety and efficacy data have been submitted: this phenomenon is also known as 'regulatory testing'. Some of those requirements can currently only be fulfilled by resorting to animal testing.

As we have seen, the legislative corpus of the EU concerning medicinal products (Directive 2001/83/EC; Directive 2001/20/EC and Regulation 536/2014) subscribes to the viewpoint that all tests on animals have to be conducted in accordance with Directive 2010/63/EU. Furthermore, EU-law allows for the replacement of *in vivo* test by *in vitro* test. Studies in animals can be replaced by validated *in vitro* tests provided that the test results are of comparable quality and usefulness for the purpose of safety evaluation. This leads to the conclusion that, in a strict legal sense, EU pharmaceutical law does not contain barriers for alternatives to animal testing.

On the other hand, the legislative corpus of the EU concerning medicinal products does not offer many incentives to use alternative methods either. The wording is cautious: it is, for example, 'allowed to use' alternative methods, instead of 'preferred' or 'obliged'. At the same time, legislation, such as Directive 2001/83/EC, sends out mixed signals. On the one hand, it endorses 3R-principles, while at the same time it explicitly refers to animals tests, in one case even stating the kind of animal (e.g. rodent/non-rodent) that should be used.

Yet, it is doubtful whether animal tests can be replaced on a one-to-one ratio with an *in vitro*-test, as the current wording of the Directive seems to imply. As mentioned in chapter 6 animal tests enable the measurement of several parameters simultaneously. At the moment, Integrated Testing Strategies (ITS), combinations of *in vivo*- and *in*

vitro-tests, are better suited to replace the traditional animal test (Vermeire et al, 2013). It's encouraging to see that Integrated Testing Strategies have been incorporated in the revised S1 and S5 safety-guidelines of the ICH.

7.2 **Strong barriers: tradition, reluctance and the state of science**

Although the replacement of animal test with alternative methods is formally allowed, this report shows that both Directive 2001/83/EC, the Ph. Eur., and the guidelines issued by the ICH, the EMA and in the context of OCABR still refer to, require and/or set the standards for animal testing in pharmaceutical research and development or the batch release of biologicals. The European Medicines Agency claims that animal tests are still commonly used, especially for non-clinical/safety testing during development of new medicinal products for human use and for quality batch control as part of the manufacturing process before release onto the market (EMA, 2014).

In this sense, there may be no formal legal barrier, but the informal barrier of 'tradition', 'cautious regulators' and (soft law) guidelines is almost as strong as formal law. Notwithstanding the fact that much progress has been made with the reduction and refinement of animal tests, animal tests often still have the position of 'golden standard' as accepted by the scientific and regulatory community - to which all alternative tests have to adhere.

Ironically, it is the validation of alternative methods that seems to be the real Achilles-heel in the uptake of alternative methods. While the development of alternative methods can rely on generous funding and yields a lot scientific prestige, the validation is a time-consuming process that requires large investments by the manufacturers against uncertain results, and additionally only results in minor scientific credits. Furthermore, validation is sometimes an effort scarcely worth making because of the extra animal testing needed to acquire sufficient data for the specific context in which the alternative method will be used.

To avoid the risk of a rejection or a delay, manufacturers, scientists and regulators tend to stick to animal-based models, even when alternatives are available.

To conclude, the application of alternatives to animal tests in the development, production and/or release of medicinal products is not hindered by legislation per se. As a matter of fact, the EU, is a frontrunner when it comes to 3R-principles. Yet, as we have seen, a system that seems to work on paper can be hindered by very actual barriers in daily practice.

7.3 **Recommendations**

Based on the results of this report, the following recommendations can be given:

- The major barriers for the uptake of alternative methods seem to lie outside the realm of law. On the other hand, it might be worth

considering to incorporate more 'active' incentives for the use of alternatives within the extant legislative framework.

- Considering the above, both the legislative framework concerning medicinal products and the market for medicinal products are globalized entities. The pharmaceutical industry does not make products for the European market alone. Increasing the use of alternative methods to animal tests within the domain of medicinal products requires that all parties involved share the same sense of urgency. This requires an increased effort and support of and consensus among European actors to place 3R-initiatives on the global regulatory agenda. Still, it not only depends on an increased effort alone. Medicinal products (especially vaccines) are also manufactured in developing countries, which do not always have the ability to implement high-tech alternative methods.
- Time and effort is required in order to create consensus among all parties involved. It would be beneficial to increase insight in the regulatory landscape of institutions involved in validation, acceptance and implementation of alternatives to animal testing, both at national level as at EU level.
- Our evaluation demonstrated that the scientific guidelines of the EMA, OCABR and the ICH and Ph. Eur. monographs still regularly refer to or require animal test. In order to increase the use of alternative methods, three interlinked efforts should be considered:
 - a. In order to facilitate a more comprehensive implementation of 3R-alternatives there is a need for a clear overview on which and how many guidelines actually require or refer to animal tests, including an overview of possible alternatives that can replace, reduce or refine these animal tests.
 - b. This (a.) will facilitate the inclusion of a standard re-evaluation of the need of animal tests and the opportunities to implement 3R-principles when updating ICH/OCABR/EMA-guidelines and/or revising monographs of the European Pharmacopoeia.
 - c. In order to facilitate this re-evaluation process (b.) more knowledge and discussion is needed on the exact predictive value of (a specific) animal test(s) and the necessity of requiring an alternative *in vitro*-test to be comparable to an animal test.
- As the validation of alternative methods seems to be the real Achilles-heel, it might be worth considering creating more and stronger incentives for science, industry and government to enter the process of validation after an alternative test strategy has been developed. This can be achieved, for example, by earmarking a portion of the public funding of research projects aimed at developing *in vitro*-alternatives to animal tests for validation purposes only.

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9 List of abbreviations

CHMP	Committee for Human Medicinal Products
EEA	European Economic Area
EC	European Commission
EDQM	European Directorate for the Quality of Medicines
EFPIA	European Federation of Pharmaceutical Industries and Associates
EMA	European Medicines Agency
EPAR	European public assessment report
EU	European Union
Gw	Geneesmiddelenwet
ICH	International Conference on Harmonisation
ITS	Integrated Testing Strategies
NCL	National control laboratory
OCABR	Official Control Authority Batch Release for Human Biologicals
OMCL	Official Medicines Control Laboratories
Ph. Eur	European Pharmacopoeia
WoD	Wet op de Dierproeven

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