WHY ANIMAL STUDIES ARE STILL BEING USED IN DRUG DEVELOPMENT

AN INNOVATION SYSTEM PERSPECTIVE

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AN INNOVATION SYSTEM PERSPECTIVE

Waarom dierproeven nog steeds worden gebruikt in medicijnontwikkeling
Een innovatiesysteem perspectief

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen

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1. INTRODUCTION

1.1 BACKGROUND

Animal studies have played a leading part in drug development[1] ever since modern medicine regulation started in the first half of the 20th century. At that time, governments experienced that more regulation was necessary to guard citizens from possible adverse effects of drugs as a consequence of the increasing role of drugs in everyday life and the Elixir Sulfanilamide[2] tragedy in 1937 (Rägo & Santoso, 2008; Hartung & Daston, 2009). As a result of this tragedy and the increasing role of drugs in society, the Federal Food, Drug and Cosmetic Act was implemented in the United States in 1938. This act required a premarket proof-of-safety for new drugs (Food and Drug Administration, 2012). The Thalidomide[3] disaster in the late 1950s influenced the development of medicines far more, as it totally reshaped regulatory systems for drugs. With the introduction of the Kefauver-Harris amendment in the United States in 1962, it became required that all new drugs had to be approved by the Food and Drug Administration (FDA) to gain market authorization. Approval could be gained based on proven efficacy and safety. Other countries followed and introduced similar acts such as Directive 65/65/EEC in the European Economic Community in 1965. In that way animal models became the gold standard to demonstrate safety and efficacy of drug for humans. As pharmaceutical markets became more globalized in the 1980s, animal studies became entrenched in international test guidelines of the Organisation for Economic Co-operation and Development (OECD) and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The Nuffield Council on Bioethics estimated that new drugs have to be tested in between 1500-3000 animals just for the safety assessment to gain market authorization (Nuffield Council on Bioethics, 2005).

Many of the animal studies required by the legislation today were developed under crisis conditions such as the Elixir Sulfanilamide and Thalidomide tragedies (Abbott, 2008). As a result, the value of these animal studies to predict human risk has never undergone rigorous validation (Abbott, 2008). Most animal studies in the drug development process have relied on face validity (empirical evidence based on

1 Drug development includes the safety, efficacy and quality assessment of drugs.
2 Nearly 100 people died after ingesting a drug called Elixir Sulfanilamide in 1937.
3 In the late 1950s, the sedative drug, Thalidomide, was used to cure morning sickness for pregnant women, among others. The drug was withdrawn from the market in 1961 because it caused severe birth defects.

observations that the experiment 'looks like' it is measuring what it is supposed to measure), but construct validity (whether it actually measures what we think it measures) has often not been determined (Della Pasqua, 2013). It is remarkable that the construct validity of animal studies is rarely rigorous assessed.

Animals are in many respects different from humans (McMaster, 1993; Kola & Landis, 2004; Hartung, 2009; Khanna, 2012; Gori, 2013a), especially considering that the results of animal studies in apparently closely related species, as for example rats and mice, often not correspond (Rowan, 2007; Gori, 2010; Hartung, 2009, 2010). Modeling the effect of drugs on humans in animals is usually a trial-and-error process. A priori it is unknown if a given model will faithfully reproduce the effect of drugs on humans (Editorial, 2008). The predictive value of animal studies for human risks becomes even more uncertain when conducted to assess drugs for human specific diseases or to assess drugs based on human specific targets and structures.

Around the start of the 21st century, the value of animal studies has gained increasing attention of researchers. Scientists at pharmaceutical companies and universities explored and criticized the value of animal studies in drug development (Igarashi et al., 1995; Olsen et al., 2000; Robinson et al., 2008; Hartung, 2008, 2009, 2011; Francia & Kerbel, 2010; Hunter et al., 2011; Begley & Ellis, 2012; Gori, 2013, 2013a; Van Meer et al., 2012, 2013; Rice, 2012; Laurijssens et al., 2013; Seok et al., 2013). The results of these studies support the presumption that animal experiments frequently have limited value in assessing the safety, efficacy and quality of drugs for humans. However, these studies to determine the value of animal experiments have limitations usually due to limited and/or biased datasets. More research is necessary to get a clear picture of the value of animal studies in drug development.

The limited value of animal studies to predict human outcomes is an incentive for pharmaceutical companies to search for methods with a higher predictive value. The limited predictive value of animal studies results in the loss of valuable drugs and makes animal studies a slippery slope to clinical trials as it provides a false sense of safety. The attrition rate during clinical trials, the most expensive and time consuming phases of drug development, is high. Almost nine out of ten new drugs that enter clinical trials fail despite often encouraging results in animals studies (DiMasi et al., 2003; Kola & Landis, 2004; Rang, 2006; Paul et al., 2010). The research and development (R&D) efficiency of the pharmaceutical industry would benefit from innovative methods that

are more predictive for the effect of the use of drugs in humans than animal studies (Kola & Landis, 2004; Lindner, 2007; Paul et al., 2010; Mittra et al., 2011; Pammolli et al., 2011; Elebring, 2012; Khanna, 2012; Moggs et al., 2012; Ekins et al., 2013; Moreno & Pearson, 2013; Muthas et al., 2013).

Furthermore, the opposition towards animal studies is as old as its use. Scientists and animal welfare organizations have attempted to create awareness about the limited value of animal studies to predict hazards for humans and about the ethical and economic issues related to the use of animal studies. The campaigns of animal welfare organizations that targeted at the use of animal studies in especially the development of cosmetics started to pay off in the European Union at the end of the 1970s (Salem & Rowan, 2001). Since then animal studies gained more public attention and were put on political agendas. In 1986, the European Union implemented Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. This Directive intended to protect laboratory animals and to reduce the number of animal studies. On the one hand, the Directive required that animal studies should not be performed when alternative methods exist and on the other hand, it encouraged the development of innovative animal-free methods and testing strategies to substitute animal studies (referred to as 'innovative methods' in the remainder of this thesis) (European Economic Community, 1986). Another outcome of this increased public attention was that the search for innovative methods transformed from a minor antivivisectionists' issue into a large-scale operation mainly financed by multi-million dollar cosmetic companies (Sina & Gautheron, 1998).

Since the implementation of Directive 86/609/EEC, it is estimated that the European Commission and its member states alone already invested more than 500 million in developing methods to Refine, Reduce and Replace (3R) animal studies (Hartung, 2011). Numerous innovative methods have been developed to substitute animal studies in drug development, such as the human Ether-à-go-go-Related Gene (hERG) assay to assess QT prolongation (Pollard et al., 2010), physiologically based pharmacokinetic (PBPK) modeling and various other in silico approaches (Clewell & Clewell, 2008; Modi et al., 2012; Przybylak & Cronin, 2012; Valerio, 2012; Bajorath, 2013), microdosing (Lappin & Garner, 2008; Lappin et al., 2013), methods for reproductive and developmental toxicity testing (Rogiers, 2007; Chapin et al., 2008; van der Laan et al., 2012; Theunissen, 2013), methods for immunotoxicity testing

(Galbiati et al., 2010), -omics[4] approaches (Gallagher et al., 2009), and integrated programs as Tox21[5] (Stephens et al., 2012).

Despite the requirement in Directive 86/609/EEC that animal experiments should not be conducted when innovative methods are available, animal studies are still broadly used. Various studies have been conducted and workshops have been organized to improve understanding about why these innovative methods are not being broadly used. Punt and colleagues provided an overview of research activities to develop innovative methods and identified research strategies to increase the impact and applicability of innovative methods in risk assessment practices (Punt et al., 2011). Schiffelers and colleagues identified in interviews with legislators, regulators, industry, science and animal welfare organizations technical, political/administrative and societal problems obstructing the implementation of the 3Rs in the regulatory process (Schiffelers et al., 2007). Furthermore, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) organized different workshops such as 'The three Rs: the way forward' (Balls et al., 1995), 'Practical Aspects of the Validation of Toxicity Test Procedures' (Balls et al., 1995a), 'Development and Validation of Non-animal Tests and Testing Strategies: the Identification of a Coordinated Response to the Challenge and the Opportunity Presented by the Sixth Amendment to the Cosmetics Directive (76/768/EEC)' (Balls et al., 1995b), 'Optimisation of the Post-validation Process' (Bottini et al., 2008) and 'Overcoming barriers to validation of non-animal partial replacement methods/integrated testing strategies' (Kinsner-Ovaskainen et al., 2009). The focus of all these studies and workshops was on the refinement, reduction and replacement of animal studies in research, education and development of cosmetics, chemicals and drugs or on specific barriers to the use of 3R methods.

However, so far no studies have been conducted that specifically focus on the mechanisms that influence the innovation process towards innovative methods in the drug development process. It is remarkable that so little attention has been paid to the substitution of animal studies in drug development. Animal studies have been used

4 -omics refer to fields of study in biology ending in -omics, such as (toxico)genomics, proteomics and metabolomics.

5 Tox 21 is a project wherein federal resources and expertise from Environmental Protection Agency, National Institutes of Environmental Health Sciences/National Toxicology Program, National Institutes of Health, National Institute of Health Center for Advancing Translational Sciences and the Food and Drug Administration are pooled to the development of innovative methods.

in drug development in rather the same way for 40-80 years (Hartung, 2011) and are thus very persistent. Furthermore, approximately 30% of all animal studies conducted in the European Union are done for the development of drugs (European Commission, 2010). This means that in the European Union alone roughly 3.6 million animals are annually used for the development of drugs. In fact, in the last three decades, only an increasing number of animal studies have been required by legislation for the assessment of new drug classes, such as biopharmaceuticals and gene therapy medicinal products, and newly identified risks related to drugs, such as the quality of cell based medicinal products and QT-prolongation. In the past decades, on average 5000 animals have annually been used for the development of cosmetics in the European Union (European Commission, 1994, 2003, 2005, 2007). Whereas much more attention is being paid to the substitution of animal studies in the development of cosmetics by animal welfare organizations and the European Union, in terms of absolute numbers, there is much more to gain by substituting animal studies in drug development. In this thesis, we therefore focus on substitution of animal studies in drug development.

The persistent use of animal studies in drug development is an innovation problem. Innovative methods have not substituted animal studies on a large scale yet. This lack of success of innovative methods in drug development can then be studied from an innovation perspective. Innovation is the successful implementation of an invention into practice. The main lesson from early innovation studies is that science and technology are only two of the numerous ingredients for innovation (Fagerberg, 2006). For turning an idea into an innovation, a network of actors is essential that unites the right knowledge, capabilities, skills and resources at the right time (Fagerberg, 2006). Innovation processes are complex and characterized by complicated feedback mechanisms and mutual interactions involving science, technology, production, policy and demand (Edquist 1997; Lundvall et al., 2002). Innovation is thus not an isolated process but comes about in interplay of actors in a specific context. This context is labeled the 'innovation system'. Freeman (1987) defined innovation systems as the network of institutions in public and private sectors whose activities and interactions initiate, import, modify and diffuse new technologies. Innovation systems can be delineated based on national or regional borders or on sectorial or technological characteristics. In this thesis, the delineation of the innovation system is based on the technological characteristics as we analyze why innovative methods to substitute animal studies are not successfully applied on a large scale.

The functions of the Technological Innovation System (TIS) approach can be used as an analytical tool to study the innovation process of emerging technologies from a system perspective. The performance of the TIS is assessed by analyzing seven processes that are crucial for the generation, maturation and utilization of emerging technologies. This approach is praised for its analytical power to study emerging technologies and has been used to analyze many innovation processes contributing to sustainability such as biomass, wind energy and waste water systems (Negro et al., 2007,2008; van Alphen et al., 2010; Suurs et al., 2009, 2010; Del Rio & Bleda, 2012; Köhler et al., 2013). In this thesis this approach is used to analyze the innovation process of emerging innovative methods to substitute animal studies.

To be used in drug development innovative methods have to be implemented in drug regulation, whereas established animal tests have to be removed from it. Therefore, changes in the regulation are necessary to substitute animal tests by innovative methods in drug development. Daemmrich and Krücken (2000) have already shown that the institutional context strongly influences change processes in drug regulation. They studied amongst others the impact of the Thalidomide tragedy on drug regulation in the United States and in Germany and they showed that differences in the institutional context in those countries resulted in different regulative changes. The TIS approach is criticized because it does not take into account processes and influences from outside the technological innovation system, such as the established institutional context. Although the success of emerging technologies depends as much on the generation, maturation and utilization of emerging technologies as on escaping practices embedded in the established institutional context, the success of innovations is regarded as a consequence of the functioning of the TIS itself. Due to this inward orientation, the TIS approach does not explicitly analyze the persistency of the established institutional context and its effect on the innovation process of emerging technologies (Markard & Truffer, 2008). To fully understand why animal studies are still being used in drug development, the persistency or lock-in of the use of animal studies in drug development and how that influences the innovation process also needs to be analyzed.

Lock-in of an established practice is the result of a process of technological and institutional co-evolution driven by path-dependency (Unruh, 2000). Institutional theory is used to analyze the lock-in of animal studies as it focuses on the resilience and change of the rules that structure and stabilize daily life (Seo & Creed 2002; Lawrence

& Suddaby, 2006; Scott, 2008; Kalantaridis & Fletcher, 2012;). Following institutional theory, established practices, such as animal studies are locked-in because they are embedded in a well aligned set of institutions that are taken for granted, normatively endorsed, backed up by regulatory authorities (Scott, 2008; Thornton et al., 2012). The sets of institutions that guide behavior of actors are referred to as institutional logics (Scott, 2008; Reay & Hinings, 2009). The concept institutional logic is used as a heuristic to study the institutional context that locks-in animal studies in this thesis.

1.2 OBJECTIVES AND RESEARCH QUESTION

The innovation process from animal studies towards innovative methods in drug development has been ongoing for more than three decades. Despite the investments of several actors in innovative methods, such as the European Union and pharmaceuticals companies, only a few animal studies have been substituted. Animal studies still play a key role in drug development as the innovation process towards innovative methods proceeds slowly. The objective of this thesis is to improve understanding of why this innovation process proceeds so slowly. This leads to the following main research question:

Which mechanisms explain the lock-in of animal studies in drug development?

Improved insight into why animal studies are still being used in drug development, contributes to escaping the lock-in of animal studies because it provides leads to accelerate the substitution process. Besides this societal contribution, the contribution of this dissertation to innovation theory is twofold.

In one respect, this thesis contributes to further theoretical saturation of the TIS approach. The TIS approach is well established for analyzing innovation processes in the field of sustainable development, but has hardly ever been applied to drug development processes. Drug development has specific characteristics. Firstly, the specific market of drugs complicates innovation (Tait, 2007). Drugs are not ordinary consumers' products. As drugs are prescribed by physicians and pharmacists, consumers are not in the position to make autonomous decisions about the use of drugs. Secondly, the pharmaceutical industry is more regulated than most other industries (Mittra et al., 2011). The Thalidomide tragedy made clear that even healthcare professionals do not have the capabilities to make informed decisions about all safety and quality aspects

related to drugs (McKelvey et al., 2004). Governments, therefore, established regulatory authorities to ensure the safety, efficacy and quality of drugs (Rägo & Santoso, 2008). Regulatory authorities have the responsibility to safeguard public health and they would rather be safe than sorry when it comes to changing established practices to assess the safety of drugs. Several innovation scholars concluded that innovation in the pharmaceutical industry is often hampered by regulation (McKelvey et al., 2004; Mittra et al., 2011; Tait, 2007). It is expected that these specific characteristics of the drug development process directly impact the dynamics in the key processes of the TIS.

The second contribution is that we put the functions of the TIS approach in a broader perspective. The conceptual basis to assess the effect of the wider system wherein established technologies are embedded is still rather weakly developed. By adding an analysis of the institutional context wherein the established practice is embedded to the TIS approach, the effect of the established institutional context on innovation processes can be assessed. The TIS approach is therefore complemented with an analysis of the institutional logic reinforcing the animal studies lock-in. This combined framework may tackle the criticism that the TIS approach is myopic as the success of innovations is no longer just regarded as a consequence of the performance of the TIS. Furthermore, it is assumed that this new framework will provide a richer understanding of the innovation process.

1.3 THESIS OUTLINE

The main text of this thesis covers the content of this dissertation from introduction to conclusions without individually describing the empirical studies on which this research is based. We selected this structure as it captures the content of the dissertation in one main text.

The following section lays out the general theoretical background of the thesis. Section 3 describes the research design. This dissertation concludes with the contributions to the understanding the lock-in of animal studies in drug development in section 4.1. Section 4.2 gives the (policy) recommendations for escaping the lock-in of animal studies. Section 4.3 briefly discusses the theoretical contribution of this dissertation. We continuously refer to the specific individual papers that are the basis of the conclusions in this thesis. Section 4.4 concludes with final considerations. Annexes I-VI provide the individual papers of this thesis.

2. THEORETICAL BACKGROUND

2.1 INSIGHTS FROM INNOVATION THEORY

Scholars in the field of innovation studies aim to explain how innovation occurs. For a long time, scientists considered explaining innovation as an impossible task as innovation was seen as a random phenomenon, 'manna from heaven.' Schumpeter was the first to object that innovation was just a random phenomenon (Fagerberg, 2006).

Innovations can range from incremental improvements to established practices to technologies that substitute the established practice. Schumpeter already made a distinction between path breaking innovations (Mark I) and path dependent innovations (Mark II) (Nelson & Winter, 1982). The innovation process of path dependent innovations is often easier as the innovation builds on the established practice. An example of a path dependent innovation is carbon-capture storage as this technology makes power plants more sustainable without changing the process of energy generation. Path breaking innovations step outside established paradigms and they therefore have wide social and economic implications (Tait, 2007). These innovations discontinue innovation pathways and require technological, organizational, economic, institutional, social-cultural and political change (Fagerberg, 2006). Solar energy is an example of a path breaking innovation as it has a different process of energy generation. In this thesis, we study innovative methods to substitute animal studies in drug development. These methods are not based on living systems and thereby discontinue the current pathway of using animal studies in developing drugs. That is why innovative methods are path breaking innovations (for the remainder of this dissertation, innovation therefore refers to path breaking innovation).

One of the main lessons from early innovation studies is that science is only among one of the several ingredients for innovation (Fagerberg, 2006). Science is an important source of invention, the first occurrence of an idea for a new product or process. Yet, innovation is the result of complicated feedback mechanisms and a complex interplay of actors in a specific context involving science, technology, production, institutions, policy and demand (Edquist, 1997; Lundvall et al., 2002). To study innovation processes Freeman (1987) and Lundvall (1992) introduced the concept of innovation systems. Innovation systems are networks of institutions in the public and private sectors, wherein activities and interactions initiate, import, modify, and diffuse new technologies (Freeman, 1987).

The innovation system framework explains innovation as a result of a complex set of relationships among actors and institutions in the system (Edquist, 1997). An important feature of systems is the strong complementarities that commonly exist between the components of and processes in a system. If, in a system, one critical component or process is lacking, this may block or slow down the performance of the entire system (Hekkert et al., 2007). A snapshot of the innovation system at a certain point in time provides insight into whether the essential elements for turning an invention into an innovation are available and connected. Missing elements and links can be identified and used as leads for actors aiming to influence innovation processes, such as companies and policymakers.

There are several approaches to delineate innovation systems. The delineation of the innovation system can be done on the basis of national or regional borders and sectors or technological characteristics (Edquist, 1997). The national or regional innovation system provides a snapshot of the state of affairs of innovation in a country or region. The sectorial innovation system approach gives an indication of the current situation concerning innovation in a sector. The technological innovation system approach focuses on emerging technologies and is used to gain understanding about the innovation process of these particular technologies. Markard and Truffer (2008, p. 611) defined TIS as *"…a set of networks of actors and institutions that jointly interact in a specific technological field and contribute to the generation, diffusion and utilization of variants of a new technology and/or a new product."* In this dissertation, the innovation system is delineated based on technological characteristics as the aim of this thesis is to gain insight into the generation, diffusion and utilization of emerging technologies that have the potential to substitute animal studies in drug development.

In the context of a specific innovation system, inventions become emerging technologies while they are developed and applied. In the TIS, an increasing knowledge base, new entrants, growing networks, and specific institutional arrangements contribute to the maturation and utilization of the technology and when the maturation and utilization of new technologies gets going they often reinforce themselves (Hekkert et al., 2007). However, when vital inputs or complementary factors for the generation, maturation and utilization of technologies are not available, this will hamper the innovation process. To understand innovation, it is thus important to study the different processes that are essential for the generation, maturation and utilization of technologies. In the innovation literature, several sets of key processes

are identified (Chaminade & Edquist, 2005; Hekkert et al., 2007; Bergek et al., 2008; Markard & Truffer, 2008). There is no common set of key processes, but the sets have much overlap and differences mostly reside in the way of clustering processes. The seven key processes as proposed by Hekkert and colleagues are used in this thesis (Hekkert et al., 2007) (table 1).

The TIS approach is praised for its analytical power to assess the performance of technological innovation systems. However, the focus of this approach is only on the processes that contribute to the generation, maturation and utilization of emerging technologies. This inward orientation makes the approach myopic for the impact of external influences on the innovation processes, such as the established institutional context and competing emerging technologies. However, the success of innovative methods depends as much on the generation, diffusion and utilization of innovative methods as on escaping animal studies in drug development. Established practices are often resistant to change (inertia) and create economic, technological, cognitive and social barriers for innovations (Kemp et al., 1998). Changes in the wider context of established practices are needed to enable innovation because the established structures of markets, patterns of consumer demand, institutional systems and inadequate infrastructures for change can hamper the innovation process (Smith et al., 2005). To understand how the innovation process is influenced by the institutional context wherein animal studies are embedded, this dissertation complements the functions of the TIS approach with an explicit outward focus on the institutional context of drug development.

2.2 LOCK-IN, INSTITUTIONAL THEORY AND INNOVATION

Drug development is a typical process as it is one of the most regulated product development processes. Animal studies are embedded as gold standard in drug regulation. Due to this embeddedness of animal studies in regulation, innovative methods cannot be just used instead of animal studies in drug development. Innovative methods have to substitute animal studies in the regulation to become broadly utilized. This is challenging as animal studies have been locked into drug development for many decades. We added an analysis focused on the institutional context of drug development to the TIS approach to increase insight into why animal studies are still being used in drug development.

TABLE 1: THE SEVEN KEY PROCESSES OF TIS	
1	Entrepreneurial activity
	Entrepreneurs are either new entrants that see opportunities in new markets or incumbent companies who diversify their business to take advantage of new developments (Hekkert et al., 2007). They are essential for a well-functioning innovation system because they turn the potential of new knowledge, networks and markets into concrete actions to generate and take advantage of business opportunities (Hekkert et al., 2007; Negro et al., 2007; Jacobsson & Bergek, 2011). By entrepreneurial experimenting, many forms of learning take place. More knowledge is necessary to deal with the uncertainties of emerging technologies. The presence of active entrepreneurs is a first and prime indication of the performance of an innovation system. When entrepreneurial activity lags behind, causes may be found in the other six functions (Hekkert et al., 2007; Negro et al., 2007).
2	Knowledge development
	Knowledge development is fundamental for every innovation. Knowledge is the basis of emerging technologies and is important to reduce uncertainty and improve the performance of new products and processes.
3	Knowledge diffusion
	Knowledge exchange is important in a strict R&D setting, but especially in a heterogeneous context where R&D meets government, competitors, and market. Policy decisions (standards, long term targets) should be consistent with the latest technological insights and, at the same time, R&D agendas should be affected by changing norms and values.
4	Guidance of the search
	Guidance of the search refers to those activities that can positively affect the visibility and clarity of specific wants among technology users (Hekkert et al., 2007; Negro et al., 2007). Since resources are almost always limited, it is important that, when various technological options exist, choices are made for further investments (Hekkert et al., 2007; Negro et al., 2007). Expectations are an important phenomenon when making these choices. Choices of actors are often initially driven by little more than a hunch (Hekkert et al., 2007; Negro et al., 2007).
5	Market creation
	Emerging technologies often have difficulties competing with incumbent technologies, because they are still badly adapted to many of the uses to which they will be put. Incumbent technologies enjoy increasing returns on investment whereas emerging technologies are expensive and often offer only very small advantages over previously established techniques (Hekkert et al., 2007). To be able to compete with the incumbent technologies, creation of competitive advantages for emerging technologies is often necessary.
6	Resource mobilization
	Resources, both financial and human capital, are necessary as a basic input for all activities within the innovation system (Hekkert et al., 2007).
7	Counteract resistance to change
	New technologies have to become part of an incumbent regime, or have to overthrow it. Stakeholders with vested interests will often oppose this force of "creative destruction." Advocacy coalitions can put a new technology on the agenda (function 4), lobby for resources (function 6) and favorable tax regimes (function 5), and by doing so create legitimacy for a new technological trajectory (Negro et al., 2007).

The institutional theory focuses on the rules that structure and stabilize daily life and considers the mechanisms by which these rule systems are created, diffused, adopted, adjusted and disused as authoritative guidelines for social behavior over space and time (Seo & Creed 2002; Lawrence & Suddaby, 2006; Scott, 2008; Kalantaridis & Fletcher, 2012). Scott defines institutions as follows: *"Institutions are comprised of regulative, normative and cultural-cognitive elements that, together with associated activities and resources, provide stability and meaning to social life"* (Scott, 2008, p 48). Scott distinguishes three elements of institutions that move from the conscious to the unconscious and from legally enforced to taken for granted (Scott, 2008): (i) regulative institutions define behavioral constraints in the form of rules and laws; (ii) normative institutions are norms and values; and (iii) cultural cognitive institutions are *"the shared conceptions that constitute the nature of social reality and the frames through which meaning is made"* (Scott, 2008, p 57).

Different sets of institutions guide daily behavior in different contexts. When driving to work, people stick to the traffic rules and the norms and values of driving. At work, the behavior of people is guided by norms, values and rules prevailing in the company by which they are employed. To describe the set of institutions guiding behavior, Alford and Friedland (1985) introduced the concept of institutional logic. Institutional logics comprise a set of institutions that independently contribute to a powerful structure that guides daily behavior of actors in specific contexts (Scott, 2008; Reay & Hinings, 2009). Thornton and Ocasio (1999, p 804) defined institutional logics as *"the socially constructed, historical patterns of material practices, assumptions, values, beliefs and rules by which individuals produce and reproduce their material subsistence, organize time and space and provide meaning to their social reality."* Institutional logics are enacted through institutionalized practices that are reproduced within a specific context (Berman, 2012). Institutional logics are reinforced when their institutions are aligned. Institutionalized practices are often locked-in because these practices are taken for granted, normatively endorsed, and backed up by regulatory authorities (Scott, 2008; Thornton et al., 2012). Institutional logics can be found at multiple levels such as the societal, organizational, market and industrial level (Thornton & Ocasio, 2008). In this thesis we study the lock-in of animal studies in the institutional logic of drug development at the industrial level.

Innovations deviating from established practices often have more difficulties breaking through because they are not regarded as legitimate, not taken for granted and

not supported by authorized powers. For these innovations, it is essential that the established institutional logic changes to accommodate the path breaking innovation, or that an alternative institutional logic supporting the innovation becomes dominant. Berman (2012, p 261) argues that *"When one logic is dominant, innovations based on alternative logics may have trouble gaining the resources they need to become more broadly institutionalized. But if a changing environment starts systematically to favor practices based on an alternative logic, that logic can become stronger even in the absence of a coherent project to promote it."* Changing institutions is often beyond the capacity of individual actors and therefore requires joint activities by a wide group of actors on the basis of mutual interests (Oliver, 1993; Wijen & Ansari, 2007). Collective inaction, as a result of a collective action problem, often hampers these change processes. Collective action problems can be the result of a lack of leadership, free-rider problems, or actor apathy (Heckathorn, 1996; Wijen & Ansari, 2007). The free-rider problem is created by a situation in which all actors benefit regardless of whether they contribute to the change process or not. Actor apathy originates from the perception of actors that their contribution to the problem is insignificant.

The framework used in this thesis combines the functions of the TIS approach to analyze the generation, maturation and utilization of innovative methods with an analysis of the institutional logic structuring drug development to improve understanding about why animal studies are still being used in drug development. This combination enables studying the key processes of the TIS, the institutional context that reinforces the lock-in of animal studies and the interaction between the key processes and the institutional context over time.

3. RESEARCH DESIGN

Animal studies will remain to be used in drug development as long as innovative methods are unavailable or not capable of escaping the animal studies lock-in. Innovative methods can escape the lock-in of animal studies via two routes. Firstly, innovative methods can *substitute* animal studies in *established* drug development processes (figure 1.1). Examples of innovative methods that substituted animal studies are the Isolated Chicken Eye (ICE) test and Bovine Corneal Opacity and Permeability (BCOP) test which replaced the Draize eye irritation test in rabbits. The Ames test using bacteria substituted, to a large extent, animal tests to assess mutagenicity and the limulus amebocyte lysate (LAL) test using aqueous extract of blood cells from the horseshoe crab replaced animal studies to detect and quantify bacterial endotoxins. Secondly, innovative methods can escape the animal studies lock-in by being *adopted* in *novel* drug development processes for new drug classes (figure 1.2). New drug classes provide opportunities for innovative methods because the drug development processes for new drug classes has to be established. These novel drug development processes are greenfields wherein innovative methods can be adopted without the need of substituting animal studies. Analyzing how innovative methods are successful and unsuccessful to escape the lock-in of animal studies in drug development via these two routes provides a comprehensive overview of mechanisms that influence the lock-in of animal studies in drug development.

3.1 SUBSTITUTING ANIMAL STUDIES IN ESTABLISHED DRUG DEVELOPMENT PROCESSES

Substitution of animal studies in the established drug development process by innovative methods is challenging because the animal studies are already embedded in the regulation. The significant amount of innovative methods that has not yet managed to be implemented in the established drug development process illustrates that substitution of animal studies is not self-evident. To escape the animal studies lock-in, improved understanding of the mechanisms that influence the substitution of established animal studies is essential. Therefore, two case studies were conducted. In these studies the TIS approach in combination with an analysis of the institutional logic was used to identify the mechanisms that can explain the lock-in of animal studies in drug development (see table 2).

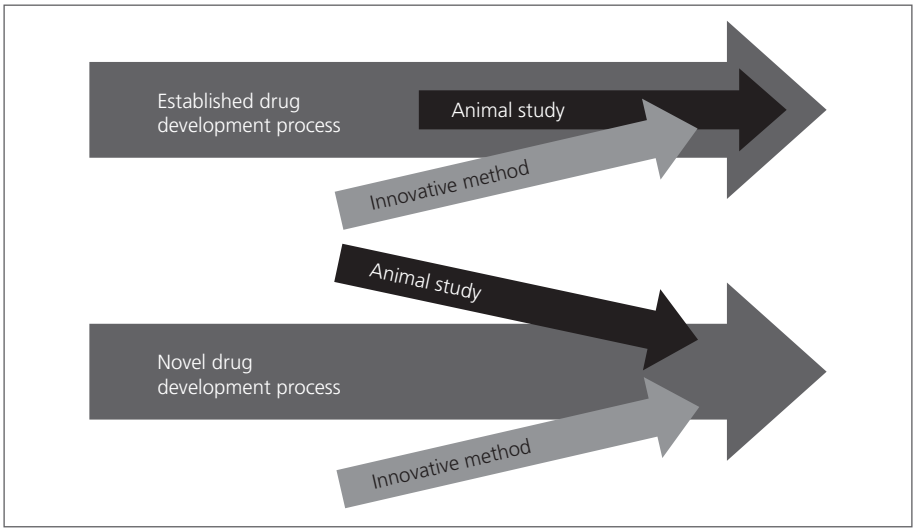

Figure 1: The routes to escape the animal studies lock-in in drug development

1. Innovative methods can substitute animal studies in the drug development process of established drug classes

2. Innovative methods can also escape the animal studies lock-in by being adopted in novel drug development processes of new drug classes

Case studies have the advantage that they allow a much richer and greater depth of data collection than other research designs. They therefore allow for the exploration of solutions for complex issues. The flipside of this study design is that findings cannot necessarily be generalized to the wider population. To get a comprehensive overview of the mechanisms influencing the substitution of animal studies in the established drug development process and to assess the generalizability of the case studies, two opposite cases were selected. In the first case study on erythropoietin (EPO) potency testing, the animal study was not substituted. Despite the fact that the adoption of innovative methods for EPO potency testing was called for by a range of actors since the 1990s and that innovative methods for EPO potency testing have been available for decades, doing animal studies to assess EPO potency is still required by regulation. In contrast, the Draize eye irritation case showed that escaping the animal study lock-in is possible. Two innovative methods were implemented in the OECD guidelines on eye irritation testing in 2009[6].

6 In 2013, the OECD test guidelines have been revised extending the applicability domain of two in vitro methods: the BCOP test and the ICE test. Both methods have been adopted for the purpose of eye irritation testing. The revision of two OECD Test Guidelines based on organotypic methods for eye irritation testing was adopted at the OECD meeting of the Working Group of National Coordinators of the Test Guideline Programme held in Paris on April 9 to 11, 2013 (ECVAM, 2013).

Furthermore, the selected cases concerned animal studies used in different aspects of drug development. Eye irritation testing is done to assess drug safety, whereas EPO potency testing is done to assess drug quality. Finally, the timelines of the cases are also different. The Draize test is an old test used since the 1950s and the EPO potency test was implemented in the assessment of EPO in 1999. The case studies are described in paper I and II (Annex I and II).

3.2 ADOPTING INNOVATIVE METHODS IN NOVEL DRUG DEVELOPMENT PROCESSES OF NEW DRUG CLASSES

The rapid developments at the end of the 20th century brought about radically new drug classes, such as biopharmaceuticals and advanced therapy medicinal products (ATMP)[7]. When these new drug classes were emerging, the risk profiles were still unknown and no established routes to the market were present yet. Such emerging drug classes provided windows of opportunity for innovative methods to be adopted in the novel drug development process of these drug classes.

In these nascent drug development processes, innovative methods can be adopted without substituting animal studies. This implies that, in theory, the method best predicting human outcomes should be adopted. Animal studies have a central role in guideline ICH S6 *"Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" (ICH, 1997)* and in the revised Annex I of Directive 2001/83/EC to accommodate the development of ATMPs. The adoption of animal studies in these regulations indicates that either animal studies are the best model to predict human outcomes of biopharmaceuticals and ATMPs, or that these nascent drug development processes were not so green after all. Four studies were conducted to gain insight into why the adoption of innovative methods in the novel drug development processes of new drug classes is not self-evident (see Table 2).

Two studies were conducted to assess the value of animal studies in the development of biopharmaceuticals. In paper III, we analyzed the value of studies in nonhuman primates in the development of monoclonal antibodies (a subclass of biopharmaceuticals). Animal studies only provide valuable results when conducted in relevant species that possess the target and intended mechanisms of action. We studied the value of

7 ATMPs include gene therapy medicinal products and cell based medicinal products

nonhuman primates because these species are often the only relevant species in the development of monoclonal antibodies. Paper IV followed up on paper III and studied whether immunogenicity of monoclonal antibodies could be predicted in nonhuman primates. Both papers showed that the value of studies in nonhuman primates is limited. If the value of animal studies is limited, why are animal studies then adopted in the guidelines? An analysis of the evolution of the guideline for the nonclinical development of biopharmaceuticals (paper V) was done to identify why animal studies were still adopted in this novel drug development process.

ATMPs are the cutting edge of innovative drugs. The guidelines concerning the development of ATMPs have only been adopted in the past decade. Animal studies have a key role in these guidelines. However, the guidelines and legislation concerning ATMPs development in Europe provide a window of opportunity for the use of innovative methods. The legislation in the European Union recommends the use of a risk-based approach in the development of ATMPs. The European Medicines Agency (EMA) defines the risk-based approach as *"a strategy aiming to determine the extent of quality, non-clinical and clinical data to be included in the Marketing Authorisation Application (MAA), in accordance with the scientific guidelines relating to the quality, safety and efficacy of medicinal products and to justify any deviation from technical requirements* (European Medicines Agency, 2013, p4). Paper VI analyzes whether the window of opportunity created by the risk-based approach contributes to escaping the lock-in of animal studies in drug development. The scientific advice letters[8] formulated by the EMA were used to analyze whether companies use the risk-based approach and to assess whether regulators accept risk-based arguments to omit animal studies. The advantage of analyzing the scientific advice letters is that they include the arguments of companies to omit animal studies and the arguments of the EMA to accept or not accept the proposal of the company to omit animal studies. However, the use of scientific advice letters also has limitations: (i) not all developers of ATMPs request scientific advice at the EMA and (ii) the discussed nonclinical drug development strategies in the scientific advice letters are only proposals and it is unknown whether these proposals will be actually used in practice.

8 Companies can apply for scientific advice at the Committee for Medicinal Products for Human Use (CHMP) of the EMA. In their scientific advice request, companies can pose questions related to quality, nonclinical and clinical development. The EMA answers the questions and composes a scientific advice letter including the information provided by the company, the questions posed by the company and the answers formulated by the EMA.

TABLE 2: METHODS AND DATA USED IN THE INDIVIDUAL PAPERS

		Title	Authors	Method	Analysis	Data source	Scope	Technological scope	Status	Appendix
Perspective 1: Substituting animal studies in established drug development processes	I.	How institutional logics hamper innovation: the case of animal testing	M. Kooijman, P.J.K. van Meer, E.H.M. Moors, H. Schellekens, M.P. Hekkert	Case study	Qualitative	Primary (semi-structured interviews) and secondary (literature and legislation) data	Europe	Innovative methods to substitute EPO potency testing in mice	Submitted to Technological Forecasting and Social Change	I
	II.	How institutional logics influence innovation: the case of animal testing	M. Kooijman, D. van der Zwan, P.J.K. van Meer, E.H.M. Moors, H. Schellekens, M.P. Hekkert	Case study	Qualitative	Primary (semi-structured interviews) and secondary (literature and legislation) data	Global	Innovative methods to substitute Draize eye irritation test	Submitted to Plos One	II
Perspective 2: Adopting innovative methods in novel drug development processes of new drug classes	III.	The value of non-human primates in the development of monoclonal antibodies	P.J.K van Meer M. Kooijman J.W. van der Laan E.H.M. Moors H. Schellekens	Drug registration file analysis	Quantification of mainly qualitative data	Primary data (drug registration files of the EMA)	Europe	Non-clinical drug development of monoclonal antibodies	Published in Nature Biotechnology, 2013, 31(10), 881-882	III
	IV.	Immunogenicity of mAbs in non-human primates during nonclinical safety assessment	P.J.K. van Meer, M. Kooijman, V. Brinks, C.C. Gispen-de Wied, B. Silva-Lima, E.H.M. Moors, H. Schellekens	Drug registration file analysis	Quantitative data and quantification of mainly qualitative data	Primary data (drug registration files of the EMA)	Europe	Non-clinical drug development of monoclonal antibodies	Published in mAbs, 2013, 5(5), 810-816	IV
	V.	30 years of preclinical safety evaluation of biopharmaceuticals: did scientific progress lead to appropriate regulatory guidance?	M. Kooijman P.J.K. van Meer E.H.M. Moors H. Schellekens	Review	Qualitative	Secondary (literature and proceedings ICH meetings) data	Global	Non-clinical drug development guideline of biopharmaceuticals	Published in Expert Opinion on Drug Safety, 2012, 11(5), 797-801	V
	VI.	Risk-based approach for the nonclinical development of ATMPs – useful strategy to realize science-driven regulatory drug testing?	M. Kooijman P.J.K. van Meer C.C. Gispen- de Wied E.H.M. Moors M.P. Hekkert H. Schellekens	Scientific advice letter analysis	Quantification of mainly qualitative data	Primary data (scientific advice reports of the EMA)	Europe	Non-clinical drug development of advanced therapy medicinal products	Published in Regulatory toxicology and pharmacology, 2013, 67(2), 221-225	VI

4. CONCLUSION & DISCUSSION

4.1 UNDERSTANDING THE LOCK-IN OF ANIMAL STUDIES IN DRUG DEVELOPMENT

Innovation in drug development only takes place when innovative methods are available and capable of substituting the locked-in practice. The substitution of animal studies in drug development fails to occur due to several mechanisms that reinforce the lock-in of animal studies and hamper the innovation process of innovative methods.

The institutional logic that structures drug development gained importance in the 20th century. The Elixir Sulfanilamide and Thalidomide tragedies made governments aware that the public needed to be protected for risks related to the use of drugs and other products such as cosmetics and chemicals. As a result, national authorities in the United States and the Europe Union required that the safety, quality and efficacy of new drugs needed to be demonstrated in order to obtain market approval. In these regulative institutions, animal studies received a central role. Historically, this is understandable because animal studies had been used as models for humans in scientific research for centuries. It also follows from the fact that animals are cognitively the closest model to humans compared to any other organism. The implementation of animal studies in drug regulation, in combination with the lack of commensurable tragedies induced by drugs in the period after implementation reinforced the belief that animal studies are a good model for humans and thereby locked animal studies into drug development.

Theoretical risks and new safety issues related to drug use extended the regulative institutions concerning drug development through the years. More and more animal studies became required for the market approval of drugs and other products. Due to this rise, animal studies gained attention from animal welfare organizations in the second half of the 20th century. Animal welfare organizations were very successful in creating public awareness about the ethics of the use of animal studies specifically in the development of cosmetics. The increased public concern about animal studies did not go unnoticed. Animal studies were put on the political agenda and this resulted in the implementation of Directive 86/609/EEC in the European Union. This regulative institution stimulated the development of innovative methods and required that innovative methods had to be used instead of animal studies when available.

The public concern about animal studies in combination with Directive 86/609/EEC created legitimacy for the development of innovative methods. The case studies in paper I and II concerning EPO potency testing and eye irritation testing showed that various innovative methods were developed to substitute animal studies. However, many innovative methods were prematurely shelved. The interviewed researchers indicated that they did not further develop their innovative method for various reasons. For instance, they ran out of resources and it was challenging to get additional funding, they got other interests, or they acquired a different position. Further developing and validating innovative methods is not interesting for many researchers because it is time consuming and does not provide many publications. Except for personal motives to reduce the use of animal experiments, there are often no incentives for researchers to undertake these efforts.

A comparison of the case studies in paper I and II made clear that the TIS of innovative methods for EPO potency testing and the TIS of innovative methods for eye irritation testing functioned very differently. For instance in the TIS of innovative methods for eye irritation testing, there was more counteracting resistance to change, there were more resources available, there was more guidance of the search, there was a market for cosmetics not tested on animals and there was more interaction between the cosmetic industry, regulators and researchers.

These differences in functioning can be related to a difference in public pressure. Actually, eye irritation testing is also used to assess the safety of cosmetics (paper II). As a consequence of the anti-Draize campaign, the public experienced the use of animal studies in the development of cosmetics more controversial than the use of animal studies in drug development. In fact, the Eurobarometer of 2008 concluded that according to the public opinion in the European Union it is legitimate to conduct animal studies to safeguard patients using drugs for potential health risks (European Commission, 2008). So, public pressure on the use of animal studies in the development of cosmetics created legitimacy for innovative methods and drove many key processes in the TIS of eye irritation (paper II). For instance, public resistance to the use of animal studies in the development of cosmetics created a market for "animal-free cosmetics." Furthermore, the use of animal studies in the development of cosmetics damaged the corporate image of cosmetics companies as the public no longer perceived the use of animal studies as legitimate. To control the damage, cosmetics companies mobilized resources and established a fund to finance the generation of innovative

methods. Finally, the large-scale public resistance resulted in effective guidance of the search, as a ban on the use of animal studies in the development of cosmetics was adopted in the European Union. This ban changed the cosmetics market because after the deadline of the ban, it would be no longer allowed to introduce cosmetics that had been tested on animals to the European market. So, the anti-Draize campaign successfully counteracted resistance to change. The public pressure to cease the use of animal studies in the development of cosmetics created urgency to substitute animal studies and consequently boosted the innovation process of innovative methods for eye irritation testing.

Furthermore, paper II showed that substituting a locked-in practice by a path breaking innovation is difficult. Innovative methods have to be implemented in the regulation. To be accepted in the regulation innovative methods need to fit in the existing institutional logic of drug development. However, the path breaking innovative methods have misfits with the assumptions, values, beliefs and rules about the use of and value of animal studies drug development. In order to enable innovation, change in the established institutional logic is required. The aim of the drug development process, to safeguard the public from the potential risks of drugs, further enhances the inertia in the institutional logic structuring drug development. There is always a risk when changing an established practice. Regulators are therefore very cautious when it concerns changing practices that have the purpose to safeguard public health. To facilitate the implementation of innovative methods and simultaneously limit the risks to public health, a validation process for innovative methods was introduced by the European Union (Rosholt, 2005). The validation process implies a structured multi-laboratory assessment of the value of innovative methods as compared to the animal study. Validated innovative methods should automatically substitute the animal study as Directive 86/609/EEC prescribes that animal studies cannot be used when innovative methods are available.

Both paper I and II showed that the validation process turned out to be counterproductive. This process severely hampered innovation. In the case study on EPO potency testing (paper I), the extensive multi-laboratory validation studies were never initiated. The interviewees indicated that there were no incentives for the involved actors to undertake the costly and time consuming validation studies. It is thus unlikely that researchers at universities, research institutes or national reference laboratories will undertake validation studies as it is laborious to obtain resources and

researchers do not gain from conducting this type of research as validation takes much time but does not result in many publications. For manufacturers, the only incentive to invest in validation is to reduce animal experimentation. However, there are more efficient ways to reduce the use of animal tests, as for example in drug discovery. Animal studies in drug discovery are more broadly applied than regulatory required animal studies as the EPO potency test. Furthermore, replacing animal studies in drug discovery requires fewer investments, because inter-laboratory validation studies are not necessary as these tests are not laid down in regulation. Finally, regulatory authorities refuse to implement patented methods into regulation. Without patenting the innovative method, it is not possible to recover costs of the generation and validation of the innovative method. The lack of incentives for researchers, pharmaceutical companies and regulatory authorities to conduct validation studies in combination with the costs of the validation studies and the fact that patented methods cannot be implemented in regulation induced collective inaction due to a free-rider problem. An actor does not individually profit from conducting validation studies. Once validated and implemented in regulation, all actors, including the actors that do not contribute to the validation studies, profit equally as they all have to use the innovative method according to Directive 86/609/EEC. Organizing collective action is challenging. This was illustrated by the three attempts of the European regulatory authority to realize collective action to validate innovative methods for EPO potency testing (paper I). None of the attempts succeeded because insufficient other actors joined and supported the attempts.

In the case study on eye irritation testing (paper II), collective inaction due to the free-rider problem was less prominent. The regulatory ban on the use of animal studies in the development of cosmetics increased the urgency of the validation of innovative methods for eye irritation testing. Different actor groups conducted in total six validation studies with innovative methods for eye irritation testing. However, none of these studies resulted in the validation of innovative methods. The results of animal studies were used as reference. Correlation between the results of innovative methods and the results of animal studies was an unrealistic endpoint. The results of animal studies are more variable than the results of innovative methods due to the systemic character of animal models. This difference reduced the correlation between the results of animal studies and innovative methods. Correlations were further reduced because the innovative methods were not able to capture the spectrum of eye irritation covered in animal studies.

Paper I and II demonstrated that innovative methods have misfits with the institutional logic of drug development as these methods are not systemic tests like animal studies. An important consequence of this misfit is that animal studies are not a good comparison to assess the value of innovative methods. The misfit is even more problematic for innovative methods based on human data and human mechanisms of action, because animals are in many ways different from humans. Animal studies are anything but a gold standard, because their relevance for humans is often unknown. It is often unclear to what extent the results of animal studies correlate with outcomes in humans and thus with the results of innovative methods based on human data and human mechanisms of action. It is therefore unlikely that innovative methods developed as models for humans will be validated as long as the results of animal studies are used as validation endpoint. This misalignment between innovative methods and the institutional logic structuring drug development need to be resolved to enable innovation.

In the case study on eye irritation testing (paper II), resolving the misfit between the institutions structuring drug development and innovative methods was inevitable as the ban on the use of animal studies in the development of cosmetics approached. Two innovative methods substituted the Draize test in rabbits for assessing serious eye irritation in the OECD test guidelines in 2009 and completely substituted the Draize test in 2013. The misfit was reduced by adapting two important institutions that structured the validation process. Firstly, the validation endpoint was adapted. It became the norm to use reference standards instead of random substances in the validation studies. Reference standards have a known degree of toxicity in animals. The use of reference standards makes it possible to control for the variability of the results of animal studies. Secondly, the assumption that just one individual innovative method should substitute the animal study was revised. It was acknowledged that catching all aspects evaluated in the Draize test in one innovative method was unfeasible. This insight enabled the validation of innovative methods for segments of eye irritation. The strategy was no longer full (one-to-one) substitution of the Draize test but a tiered-testing strategy. These changes made validation of innovative methods based on the results of six conducted validation studies possible.

Based on paper I and II, it can be concluded that substitution of animal studies that are constitutionalized in the regulation is challenging. Although, the public, regulators and pharmaceutical companies aspire to reduce animal studies, there is no necessity to

change the drug development practice for any of these actors. The public experiences the use of animal studies for drug development to be legitimate. Regulators aim to safeguard public health. They feel no urgency to change the drug development process as serious incidents with drugs are exceptional and the current drug development practice is perceived to be successful in safeguarding public health. Pharmaceutical companies aim to get their products on the market as soon as possible. The current regulation is experienced to be effective and is clear about what tests are necessary to gain market approval. As animal studies are requested by regulation, pharmaceutical companies will follow up on the request as that decreases the chance of delay of market approval. In economic terms: the cost of a delay is much higher than the cost of animal studies. This lack of urgency hampers the innovation process of innovative methods. It makes it more difficult to overcome barriers such as acquiring resources for maturation of innovative methods and the validation process. Without urgency, solving the collective action problem related to the validation process is also very challenging. Paper I and II showed that under the current conditions, it is unlikely that innovative methods will unlock animal studies in the established drug development process.

New drug classes provide opportunities for innovative methods because the drug development process for new drug classes still has to be established. This provides equal chances for animal studies and innovative methods to be adopted in the drug development because innovative methods do not have to substitute animal studies.

Since the 1980s, two new drug classes were introduced: the biopharmaceuticals and ATMPs. Both drug classes include drugs specifically based on human proteins, human mechanisms of action and/or human cells. Paper V elucidates that animal studies were not expected to be good models to assess these new drug classes because these drugs are often human specific. This is in favor of innovative methods as these methods are also based on human data, mechanisms of action and/or cells. Nevertheless, animal studies did get a key role in the development of these new drug classes. The adoption of animal studies in the development process of biopharmaceuticals and ATMPs could be explained by the fact that animal studies, contrary to expectations, are valuable as a model for humans in the development of biopharmaceuticals and ATMPs or that the preference for animal studies embedded in assumptions, values, beliefs and rules of drug development is so strong that it even influenced the design of the drug development process of new drug classes.

To elucidate whether animal models are valuable as model for humans in the development of biopharmaceuticals, we analyzed the studies in nonhuman primates conducted in the development of monoclonal antibodies, a sub-class of biopharmaceuticals in papers III and IV. Paper III on the use of nonhuman primates in the development of monoclonal antibodies showed that the value of studies in nonhuman primates is limited. There are two types of adverse effects induced by monoclonal antibodies. On the one hand, pharmacology mediated adverse effects that are highly predictable based on the mechanism of action of the monoclonal antibody. Animal studies do not provide new insights into these adverse effects; they only confirm what is expected. On the other hand, adverse effects can be mediated by immune responses against the monoclonal antibody. Due to differences in the immune systems between animals and humans, it was expected that these adverse effects are difficult to assess in animals.

Paper IV confirmed that adverse effects mediated by immune responses to the monoclonal antibody in nonhuman primates do not correlate with the effects in human and the paper showed that the value of studies in nonhuman primates is further limited due to immune responses whereby nonhuman primates form clearing and neutralizing antibodies to the monoclonal antibody. These immune responses bias the results of the study as they interfere with the pharmacological effect of the monoclonal antibody. Summarizing, papers III and IV indicate that animal models have limited value in the development of biopharmaceuticals.

In paper V we reviewed the evolution of nonclinical assessment of biopharmaceuticals to study how the lock-in of animal studies influenced the design of drug development process of new drug classes. When the first biopharmaceuticals were developed, the risks related to the use of biopharmaceuticals were unknown. It was therefore also unclear how to assess the risks of this new drug class. Regulators and pharmaceutical companies had to deal with this uncertainty. Paper V showed that to handle the uncertainty, regulators tend to fall back on animal studies for two reasons. Firstly, the deeply entrenched belief that animal studies provide useful results because animals are complex living systems similar to humans, made animal studies the first choice as model to assess unknown risks for humans. As regulators and scientists still have been trained that animal studies are the golden standard to assess the safety and quality of medicines for humans, this cognitive institution dominates current drug development. However, the complexities of living systems and their interspecies differences can

confound the results of animal studies in development of drugs based on human data, mechanisms of action and/or cells. Secondly, regulators tend to build on the established practice because the use of innovative methods seem to further increase the uncertainty as they do not have a track record in the safety assessment of drugs. However, experience with the use of animal studies in the development of traditional small molecule drugs does not tell anything about the value of animal studies in the development of biopharmaceuticals. After all biopharmaceuticals are human specific and significantly different from small molecule drugs. The value of animal studies is as uncertain as the value of innovative methods. So, building on the established practice does not decrease the uncertainty in comparison with the use of innovative methods.

Falling back on animal studies resulted in path dependent and inefficient drug development processes for new drug classes. Paper V showed that the initial use of animal studies in the development of biopharmaceuticals resulted in experience with and more knowledge about animal studies in the development of biopharmaceuticals. For example, the use of animal studies showed that the animal species traditionally used in drug development did often not provide valuable results for biopharmaceuticals. Studies only provided valuable results when conducted in relevant species wherein the target receptor and intended mechanisms of action were available. This resulted in an increased use of more unconventional species such as nonhuman primates.

Paper V also showed that cultural-cognitive and normative aspects of the use of animal studies in drug development are very strong. Even when no relevant animal model could be identified, a relevant animal model had to be created at any cost. To create a relevant animal model, the animal or the drug has to be adapted. Animals exhibiting the target receptor can be created using genetic modification techniques. Animal homologous can be produced in parallel with the biopharmaceutical. These alternative animal models do not contribute to safer drugs. These animal models even introduce more uncertainty in the drug development process as the value of the results can only be confirmed in clinical trials.

Due to the path dependent behavior of regulators and pharmaceutical companies to cope with the uncertainty related to new drug classes, the design of the drug development process of new drug classes is not science-driven but experience-driven. Consequently, the drug development process of new drug classes is built on the experience and knowledge obtained from animal studies making a key role for animal

studies inescapable. This reduces the opportunity for innovative methods and more importantly results in an inefficient drug development process for new drug classes[9].

Smart regulation[10], such as the risk-based approach recommended for the development of ATMPs, provides an escape to the animal studies lock-in. The risk-based approach is a strategy to determine the extent of quality, non-clinical and clinical data to be included in the MAA and to justify any deviation from technical requirements and the scientific guidelines relating to the quality, safety and efficacy of ATMPs. Its purpose is to create a framework in which it is encouraged to deviate from the established drug development practice and to take into account continuously evolving science and technology in order to design a tailor-made ATMP development program. Furthermore, it provides an opportunity to pharmaceutical companies to use innovative methods without going through the extensive validation procedures. In paper VI we studied whether the risk-based approach is used to omit animal studies in the development of ATMPs. An analysis of the scientific advice letters concerning the development of ATMPs showed that some pharmaceutical companies use the risk-based approach in the development of ATMPs. Of all the ATMP developing companies that requested scientific advice from 2009 to 2012, 45% used the risk-based approach to justify the omission of animal studies. The EMA accepted the majority of these proposals. Paper VI showed that the use of smart regulation in drug development, as the risk-based approach, can result in a decline of the use of animal studies.

Animal studies are still used in the development of established drugs and also still adopted in the development processes of new drug classes. In this thesis we aimed to contribute to the understanding of why animal studies are still being used in drug development. We conducted six studies to answer our main research question: *"Which mechanisms explain the lock-in of animal studies in drug development?"* Animal studies are still locked in drug development due to several mechanisms. Firstly, the lock-in of animal studies remained because several key processes to enable the generalization, maturation and utilization of innovative methods perform inadequate. Insufficient resources, moderate legitimacy, no legislative deadline and lack of incentives for researchers and investors hamper especially the maturation and utilization of innovative methods. The comparison between the two case studies showed that

9 Demortain (2013) observed similar processes in the formulation of guidelines to assess the safety of genetically modified foods.

10 Mittra and colleagues (2011) also suggested that smart regulation could contribute to innovation in the pharmaceutical industry.

the inadequate performance is caused by a lack of urgency to escape the lock-in of animal studies in drug development. For broad utilization innovative methods have to substitute animal studies in the regulation. The second mechanism that explains the lock-in of animal studies is the extensive validation process required in order to be considered for implementation in regulation. This requirement originates in the tendency to behave risk averse in drug development. Such a costly validation process in combination with the fact that patented innovative methods are not accepted by regulators hampers the innovation process as it causes collective inaction as a result of a free-rider problem. Furthermore, the rules, assumptions, values, and beliefs in the institutional logic of drug development are strongly biased in favor of animal studies. This bias towards animal studies is the foundation of several mechanisms that inhibit the innovation process, such as the selection of animal studies instead of human endpoints as reference methods and the assumption that animal studies should be replaced by just one innovative method. These assumptions, values, and beliefs are so resilient that the rules of drug development require the use of alternative animal models, such as the use of homologous drugs and transgenic animal models, when no animal model exists and provide animal studies a head start in the drug development process of new drug classes. The window of opportunity provided by smart regulation, such as the risk-based approach, to use innovative methods without implementation in the current drug development regulation can be promising. However, there is room for broader use of such opportunities by pharmaceuticals companies. Under the current conditions, it is likely that animal studies will continue to be used in drug development and introduced in new drug development processes.

4.2 ESCAPING THE LOCK-IN OF ANIMAL STUDIES IN DRUG DEVELOPMENT

Due to the present conditions it is unlikely that the lock-in of animal studies in drug development will be escaped in the near future. Based on the conclusions above, we formulated five recommendations that contribute to escaping the animal studies lock-in.

Firstly, creating incentives for actors to develop, diffuse and implement innovative methods will boost the innovation process of innovative methods. Paper I and II revealed that there are three actor groups in the TIS of innovative methods: research institutes (including universities) developing innovative methods, governments and

pharmaceutical companies. The power to stimulate the innovation process of research institutes developing innovative methods is limited. Research institutes have limited resources and their main interest in developing innovative methods is developing basic knowledge and not undertaking activities to further develop and validate animal studies. Pharmaceutical companies are the users of the animal studies. Creating incentives for pharmaceutical companies to use innovative methods instead of animal studies can stimulate these companies to participate in the TIS and positively impact the innovation process. The case on eye irritation testing (paper II), for example, showed that a ban on the use of animal studies creates incentives for companies to invest in innovative methods. A reward structure to decrease animal studies in drug development is also a potential incentive for pharmaceutical companies. Such reward structure can include extension of the data protection and/or a fast track for the market authorization procedure for drugs that have been development with limited use of animal studies. Governments are powerful actors that can create such incentives for the pharmaceutical industry. However, governments need public support to act. Unfortunately, public support to substitute animal studies in drug development is limited. Paper II showed that campaigns of animal welfare organization can increase public support. More research is necessary to identify the most effective incentives for pharmaceutical companies to use innovative methods instead of animal studies and to analyze whether there is sufficient public support to implement these incentives.

Secondly, permitting the implementation of patented innovative methods in regulation could make the development and validation of innovative methods more commercially attractive. Paper I elucidated that innovative methods under patent protection are not considered to be implemented in the regulation concerning quality control of drugs. Regulatory acceptance of patented innovative method can solve the free-rider problem as it creates an opportunity for investors in innovative methods to recover costs and maybe even to profit from their investments. More research is necessary to explore the support and effectiveness of regulatory acceptance of patented innovative methods.

Thirdly, revising the validation process can have an accelerating effect on innovation. Paper I and II showed that the validation process severely hampered the innovation process of innovative methods. In a revised validation process, human data should be used as a reference when possible. If no human data is available, human data should be created by using the innovative methods in parallel with the currently required

animal studies and clinical studies. The burden of proof for validation should be a shared effort between pharmaceutical companies and the regulatory authorities. In a revised validation process, the regulatory authorities should be able to require that pharmaceutical companies use innovative methods in parallel with animal studies. Pharmaceutical companies have to include results of innovative methods in the application dossier for marketing authorization. The regulatory authorities do not use the results of innovative methods for market approval but to build a database that can be used to evaluate the value of innovative methods in comparison to animal data and human data. This evaluation is then the basis for revising the drug development process.

Several initiatives already explore more flexible processes to realize regulatory acceptance of innovative methods than the validation process. The EMA, for example, introduced the qualification procedure for innovative methods (European Medicines Agency, 2012). In this procedure regulatory acceptance is not based on formal validation but on scientific validity. Furthermore, the EMA is currently working on a new guideline on the regulatory acceptance of 3R methods that follows on the qualification procedure. This new guideline aims to clarify the regulatory acceptance process of innovative methods in drug development and will presumably introduce a data collection period under the safe harbor concept to facilitate parallel testing of innovative methods (Beken, 2013). The safe harbor concept implies that the results of the innovative methods can be used to evaluate the value of innovative methods but cannot be used in the decision making process of market authorization of drugs. The ICH neither requires formal validation of innovative methods. The ICH Safety Topic Recommendation Working Group (STRWG), effective as of February 2013, introduced a three stage regulatory acceptance procedure (Beken, 2013). The STRWG identifies and evaluates innovative methods. Based on the evaluation, it provides recommendations to the ICH. The STRWG can recommend immediate implementation or it can advise to create a working group to gather more data.

Fourthly, as paper VI showed, smart regulation to facilitate science-driven drug development, such as the risk-based approach, creates an opening for pharmaceutical companies and regulators to omit animal studies and provides an opportunity to gain experience with innovative methods. Experience with innovative methods can result in substitution of animal studies in the long run. The degree of decline in the use of animal studies and the extent to which experience is gained with innovative methods

is dependent on pharmaceutical companies. The companies decide whether they make use of smart regulations or follow the guidelines and requirements in drug development. The results of paper VI indicate that there is room for broader use of the risk-based approach in the development of ATMPs. Incentives, such as extension of the data protection and/or a fast track for market authorization procedure, could stimulate pharmaceutical companies to use the openings provided by smart regulation to deviate from the standard drug development process. In further studies, effective incentives should be further identified.

These four solutions facilitate the innovation process of innovative methods and can induce step-by-step transformation of the drug development process. The last solution can delegitimize animal studies and thereby create space for innovative methods to be used in the drug development process. Research into the value of animal studies in drug development can have considerable impact on the lock-in of animal studies. The restricted number of studies conducted to assess the value of animal studies indicates that their value is constrained. However, these studies have limitations. More research is necessary to determine the value of animal studies in drug development. The Nuffield Council on Bioethics concluded that *"For if it were the case that harmful animal research provided no useful knowledge or application, it would be difficult to see how it could be morally justified"* (Nuffield Council on Bioethics, 2005, pXX). Concrete evidence that the animal studies are inadequate models for humans undermines foundational institutions reinforcing the animal studies lock-in. This is the grand escape on the lock-in of animal studies in drug development and will radically change the drug development process.

4.3 THEORETICAL CONTRIBUTION

In this dissertation we applied the functions of the TIS approach to a new field. We used the approach to understand innovation processes in drug development. We showed that in the distinct field of drug development the analysis of the key processes of the TIS provides insight into the progress of the innovation process. However, the dynamics of the key processes in the TIS of innovative methods to replace animal studies in drug development were particular in comparison to what is observed in previous cases studies on for example wind energy and biomass. As was anticipated, market creation was of no importance in the innovation process of innovative methods. Firstly, Directive 86/609/EEC requires that innovative methods

have to be used when they are available. Secondly, the drug market is not a free market. As drugs are prescribed by physicians and pharmacists, consumers are not in de position to decide when and how to use drugs, let alone that consumers can decide to use "animal-free drugs".

Furthermore, we combined the functions of the TIS approach with an analysis of institutional logics. Innovative methods have to substitute animal studies in regulation to become broadly utilized. Consequently, innovative methods have to be adopted in the established drug development process. The function of the TIS approach does not capture wider contexts of the innovation process such as the effect of established practices on the innovation process. Paper I and II showed that the concept of institutional logic is a powerful heuristic to understand the lock-in of established practices in drug development and the effect of this lock-in on the innovation process of innovative methods. Due to the strong alignment of assumptions, values, beliefs and rules about the use and value of animal studies in drug development, animal studies have retained their status as gold standard. Insight in the institutional logic wherein animal studies are embedded helped to understand the origin of several mechanisms that hampered the innovation process. These insights elucidated the mismatch between the institutional logic of drug development and path breaking innovative methods to substitute animal studies and thereby provided understanding about why innovative methods could not break through. Finally, the analysis of institutional logics of drug development elucidated that the cultural-cognitive and normative aspects of institutional logics can be so persistent that innovative methods lost out in the greenfield of drug development processes of new drugs classes. Based on our findings it can be concluded that the combined analysis of the functions of the TIS of innovative methods and the institutional logic structuring drug development enriched our understanding of the studied innovation process.

The studied cases may present extreme cases due to the highly regulated character of drug development, the specific perception of risks related to drugs, and a lack of normal market conditions. Yet, all emerging technologies compete in one way or another with established practices and the institutional logic wherein they are embedded. The mechanisms by which the institutional logic hampered innovation in these extreme cases may also explain slow innovation processes in other fields, such as the slow adoption of electric vehicles or solar panels and shed a different light on the emergence and success of new technological fields.

To conclude, combining the functions of the TIS approach with an analysis of the institutional logic structuring drug development can also be valuable in an early stage of the innovation process. Understanding of the institutional logic wherein animal studies are embedded in an early stage of the development of innovative methods would likely have facilitated the innovation process. It would enable involved actors to identify potential influences of the assumptions, values, beliefs and rules that reinforce animal studies, such as the requirement to conduct extensive validation studies, on the innovation process. In an early stage actors in the TIS can then anticipate on these influences, for example by interacting with regulators about the specifications of the validation process. Understanding of the institutional logic wherein the established practice is embedded is expected to accelerate innovation processes as it provides the opportunity to anticipate to barriers that will likely hamper innovation in a later stage of the innovation process.

4.4 FINAL CONSIDERATIONS

Innovation is a complex process that tends to take decades to occur. The substitution of animal studies in drug development with innovative methods is even more challenging because it concerns changing an established practice that safeguards the delicate issue of public health. In spite of this, animal studies have been substituted in the past three decades. This thesis showed that many lessons have been learned over this period. Important steps have been taken to resolve several mechanisms that lock-in animal studies in drug development such as the use of reference standards in validation studies and the introduction of the risk-based approach in the development of ATMPs. Furthermore, a growing number of studies criticize the value of animal studies as predictive models for humans in drug development. These criticisms can alter the assumptions, values and beliefs concerning animal studies in drug development and thereby change the conditions in favor of innovative methods. Consequently, these promising trends may result in a substantial reduction in the use of animal studies in drug development over the coming decades.

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5

REFERENCES

ANNEXES

ANNEX I

HOW INSTITUTIONAL LOGICS HAMPER INNOVATION: THE CASE OF ANIMAL TESTING

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ABSTRACT

A process of socio-technical transition is necessary in order to sustain economic welfare and at the same time to reduce its impacts on the natural environment. New technologies and changing the established practice are essential to realize these socio-technical transitions. Nevertheless, novelty is often at the center and only little attention is given to how the persistence of established technologies and underlying rules influence the creation of novelty in the transition literature. This paper aims to contribute to this gap by increasing the understanding about the persistence of established practices when confronted with new technologies and the effect of this persistence on the innovation process. We do this by using a framework that combines the functions of TIS approach with an analysis of the institutional logic reinforcing the established practice.

The studied case concerns the purposive change process to animal-free EPO potency testing. Despite the fact that the replacement of animal tests is called for by a range of actors since the 1980s and animal-free methods are available, regulation still require the use of animal tests to assess EPO potency.

This study shows that although the emergence of technologies is crucial for innovation, the main barriers hampering the development and utilization of animal-free methods for EPO potency testing were introduced into the TIS by the institutional logic of medicine regulation. Adding a systematic analysis of the institutional logic reinforcing the established practice to the TIS analysis enhances our understanding of innovation processes and can help to prevent and solve deadlocks in purposive change processes.

1. INTRODUCTION

In order to sustain economic welfare and at the same time to reduce its impacts on the natural environment socio-technological transitions are necessary. Socio-technical transitions are complex change processes including technical, organizational, economic, institutional, social-cultural and political changes (van den Bergh et al. 2011). The research field of transition studies tries to understand the mechanisms that underlie these complex change processes (Markard et al., 2012). The work of this research community resulted in frameworks to study the dynamics of transition processes as the Multi-Level Perspective (MLP) and the Technological Innovation System (TIS) approach. Both frameworks recognize that new technologies are key to realize societal transitions and that transitions do not easily occur because new technologies are often poorly aligned with established practices (Kemp et al., 1998; Geels, 2002; Hekkert et al, 2007). Nevertheless, novelty is at the center of attention in most transition studies. For example the TIS approach focuses on emerging technologies and the development of the innovation system supporting the emerging technology (Negro et al, 2008; Suurs et al., 2009; van Alphen et al. 2010). This approach regards the success of innovations mainly as a consequence of the performance of the innovation system while it pays less explicit attention to the broader context outside the TIS such as established practices (Markard & Truffer, 2008). The MLP framework does take the established practice and underlying rules into account in the regime. The regime, however, is often analyzed only as a barrier to be overcome or as creating windows of opportunity and not as a dynamic context continuously influencing the innovation process (Geels, 2005; Elzen et al., 2011; Yuan et al., 2012).

Thus, in the transition literature there is strong recognition that the success of emerging technologies depends as much on the development of emerging technology as on changing technical regimes, e.g. the context of the established technology (Kemp et al., 1998; Turnheim & Geels, 2012). Nevertheless, the transition literature gives only little attention to understanding how the persistence of established practices and underlying rules can impact the creation of novelty. In MLP wording: far less notice is taken of processes that stabilize the technical regime (Turnheim & Geels, 2012). And yet, a better understanding of this persistence is crucial to understand change processes. This paper aims to contribute to this gap in the transition debate by focusing on why established practices persist when confronted with emerging technologies.

This paper focuses on the persistency of established technologies by taking an institutional perspective on established technologies. The term institution refers to rules. Not just rules in the form of a set of commands and requirements but also rules in the sense of roles and practices that are being established and that are not easily dissolved (Kemp et al., 1998). Following the institutional theory literature stability and change can be understood in terms of institutional logics. Institutional logics refer to 'the belief systems and related practices that predominate in an organizational field' (Scott 2001, p 139). They provide the organizing principles for a field (Friedland & Alford, 1991). A poor fit of emerging innovations with the prevailing institutional logic hampers successful diffusion of innovations, because it creates economic, technological, cognitive and social barriers for new technologies (Kemp et al., 1998). Change in the institutional logic is then essential for innovation (Reay & Hinings, 2009).

The aim of this paper is to increase understanding about why established practices persist when confronted with emerging technologies. This is done by combining the TIS approach to analyze the innovation process of emerging technologies with an analysis of the institutional logic reinforcing the established practice. The reason for deploying the TIS approach in this study is that it is praised for its powerful analysis of the conditions that enable and hamper innovation processes (Markard & Truffer, 2008). We selected the change process towards animal-free testing in medicine regulation as empirical field. Despite the fact that the replacement of animal testing is called for by a range of actors since the 1980s, animal tests are still deeply embedded in medicine regulation. Although the change from animal-based drug testing to animal-free testing is too narrow to be labeled as a societal transition, due to the persistency of animal studies in medicine regulation, this empirical field is very useful to increase understanding about why established practices persist when confronted with emerging technologies. Furthermore, this desired change process shares characteristics with socio-technical transitions as for example low-carbon transitions. Turnheim & Geels (2012) describe low-carbon transitions as purposive transitions, which are deliberately pursued from the start to solve an explicit set of societal problems. Because private actors have limited incentives to address societal problems (because of market failures and free-rider problems), it is likely that social movements, public opinion, and policy makers play important roles in purposive transitions (Turnheim & Geels, 2012). Just like low-carbon transitions, the change process to animal-free methods in medicine regulation can be regarded as a purposive change process to solve the problem of the undesired use of animal tests in which private actors have limited incentives.

To explore the persistence of the institutional logic reinforcing animal testing and the effect of this persistence on emerging animal-free methods we study one particular case; the erythropoietin (EPO) potency test in mice. EPO[11] is a biotechnology-derived medicine developed to treat patients with anemia. This case is of particular interest because EPO received market authorization at the end of the 1980s just after European legislation was implemented that discouraged animal testing and promoted the use of animal-free methods. Despite the public resistance to the use of animal tests, and the availability of animal-free methods, European quality control regulation still requires that the potency of every batch of EPO is assessed in mice. To unravel why the animal test persisted and how the persistency of this practice hampered the development and use of innovative (animal-free) methods the EPO potency testing practice is studied from 1970-2010 in Europe.

2. THEORETICAL FRAMEWORK

In the mid-1980s innovation system approaches were developed as a policy concept. They were developed in reaction to perceived inadequacies to explain change processes of neoclassical economics (Sharif, 2006). The Technological Innovation System (TIS) approach is one of the innovation systems approaches and is used to conceptualize and analyze the complex process of the development, diffusion and use of new technologies (Bergek et al., 2008; Hekkert & Negro, 2009). The basic assumption of the TIS approach is that innovations do not develop in isolation, but that a socio-technical system, including policy and perceived legitimacy, enables the development, diffusion and use of technologies. An innovation system consists of actors that contribute to the innovation process in a wide variety of ways, for instance through knowledge development, supply of financial resources, standardization, and use of the innovation. These actors are constrained and enabled in their actions by the structure of the innovation system that consists of network characteristics, technological artifacts and institutional settings.

The functional analysis of the TIS focuses on the key processes that take place in the innovation system (see Table 1). These key processes are necessary to provide

11 EPO is a natural occurring hormone EPO controls the red blood cell production in humans. A lack of this hormone causes anemia. EPO is also known as a forbidden performance-enhancing drug in professional sports.

the ideal circumstances for actors to innovate. When an innovation system is in an emerging stage of development, these key processes contribute to the build-up of the innovation system's structure. When this structure is in place, innovation becomes easier. Important features of systems are the strong complementarities that commonly exist between the components of and processes in a system. If, in a system, one critical component or process is lacking, this may block or slow down the performance of the entire system (Hekkert et al., 2007). Thus, when one or more of these key processes do not take place sufficiently innovation can be hampered (Jacobsson & Bergek, 2011).

The powerful analysis of the performance of innovation systems is the key contribution of functions of the TIS approach to innovation studies (Markard & Truffer, 2008). The main critique on this approach is that it regards the success of innovations mainly as a consequence of the performance of the TIS and does not systematically take into account external influences such as the established practice and its underlying rules (Markard & Truffer, 2008). Using the strengths of one theory to address the limitation of another can provide an enriched understanding of the studied phenomenon. We therefore make use of the rich body of literature on institutional theory to conceptualize established practices.

Institutional theory studies the deeper and more resilient aspects of social structure (Scott, 2008). Scott defines institutions as follows: *"Institutions are comprised of regulative, normative and cultural-cognitive elements that, together with associated activities and resources provide stability and meaning to social life"* (Scott, 2008, p 48). Thus, institutions are the taken-for-granted rules (e.g. regulations, user practices, symbolic meanings) that structure and stabilize the practices of daily life (Seo & Creed, 2002; Kalantaridis & Fletcher, 2012). Institutions have distinctive properties. They are relatively resistant to change and they tend to be maintained and reproduced across generations (Scott, 2008). Institutions control and constrain behavior because they impose restrictions by defining legal, moral and cultural boundaries, setting off legitimate from illegitimate activities (Scott, 2008; Kalantaridis & Fletcher, 2012; Thornton et al., 2012). However, institutions also support and empower activities and actors (Lawrence & Suddaby, 2006; Thornton et al., 2012). Institutional scholars study how institutions are created, maintained, adapted and dismissed (Lawrence & Suddaby, 2006; Scott, 2008).

Thornton and Ocasio (1999, p 804) defined institutional logics as 'the socially constructed, historical patterns of material practices, assumptions, values, beliefs, and rules by which individuals produce and reproduce their material subsistence, organize time and space, and provide meaning to their social reality'. Institutional logics comprise of a set of institutions that are the basis of taken-for-granted rules guiding behavior of actors (Scott, 2008; Reay & Hinings, 2009). Institutional logics can be found at multiple levels such as the societal, organizational and industrial level (Thornton & Ocasio, 2008). In this manuscript we study the institutional logic of medicine regulation at the industrial level.

Institutional logics are reinforced because they are taken for granted, normatively endorsed and backed up by authorized powers (Scott, 2008; Thornton et al, 2012). These logics are enacted through institutionalized practices that are reproduced within the field (Berman, 2012). The alignment of underlying assumptions, norms, beliefs and rules is important for the stability of the institutional logics because they enforce each other (Scott, 2008). When these elements are not well aligned, this provides conditions that are likely to give rise to alternative institutional logics and can result in institutional change (Hoffman, 1997; Caronna, 2004).

According to the literature, change in institutional logics is often driven by outsiders mobilizing resources and using social skills to promote change successfully (Hardy and Maguire 2008; Thornton & Ocasio, 2008). Furthermore, change can also be induced by powerful insiders (Rao et al., 2003; Greenwood & Suddaby, 2006). Finally, social movements can play a role in triggering change as sources of new logics or acting to destabilize fields, such as the anti-genetics movement influencing the success of biotechnology products of pharmaceutical companies in Germany in 1980s (Weber et al., 2009) and the recycling movement enabling the rise of a for-profit recycling industry (Lounsbury et al., 2003)

Innovations strongly deviating from established institutional logic will have problems in breaking through. They will not be regarded as legitimate, will not be taken for granted and will not be supported by authorized powers. Actors that support the innovation therefore have to challenge the dominant institutional logic as it is essential for the success of innovations. This institutional change is often beyond the capacity of individual actors and therefore requires joint activities by a wide group of actors on the basis of mutual interests (Oliver, 1993; Wijen & Ansari, 2007). Collective inaction

is often a problem in these change processes (Heckathorn, 1996). Collective inaction can be the result of the free-rider problem, lack of leadership and actor apathy because actors feel their contribution to the problem is insignificant (Wijen & Ansari, 2007).

Studying the medicine regulation logic will provide insight in the persistency of the EPO potency test in mice and might show how this logic creates barriers in the innovation process. Many scholars used the concept of practice as a way to study how institutions are enacted as well as how individual action has the potential to reshape them or create new ones (Seo & Creed, 2002; Colyvas & Powell, 2006; Berk & Galvan, 2009; Owen-Smith, 2011; Berman, 2012). Berman (2012, p 261) argues that *"When one logic is dominant, innovations based on alternative logics may have trouble gaining the resources they need to become more broadly institutionalized. But if a changing environment starts systematically to favor practices based on an alternative logic, that logic can become stronger even in the absence of a coherent project to promote it"*.

The practice studied in this paper is EPO potency test in mice. To systematically identify the logic reinforcing the use of the EPO potency test in mice, we used the institutional framework of Scott (2008). He distinguishes three types of institutional elements, which are referred to as the regulative, normative and cultural-cognitive elements. The regulative elements are explicit, regulative rules, which constrain behavior and regulate interactions, for example, government regulations, which structure the economic process. Normative elements include values (the preferred or the desirable, together with the construction of standards to which existing structures or behaviors can be compared and assessed), norms (how things should be done), role expectations, duties, rights and responsibilities. Cultural-cognitive elements embrace the nature of reality and frames through which meaning or sense is made (Scott, 2008). These elements are used as heuristics to explore the institutional logic.

3. METHOD

The institutional logic reinforcing the EPO potency test in mice is analyzed in combination with the innovation process of innovative methods to increase understanding of how established practices persist when confronted with emerging technologies. To gain detailed insight we employed an explorative case study

methodology. The studied case, the EPO potency practice, is an exemplifying case of purposive change processes. In this way this study in the field of animal-testing could give valuable insights for transition studies in general.

A qualitative event history analysis was done to gain insight the dynamics of the innovation process of animal-free methods that could replace the EPO potency test in mice and the institutional logic reinforcing the EPO potency test in mice. In the event history analysis the three institutional elements and the seven key processes of the TIS are operationalized (Table 1 and 2). Subsequently events were identified and related to the institutional elements and key processes. An event can be defined as an instance of change with respect to the institutional elements and key processes of the TIS, which is the work of one or more actors and which carries some public importance with respect to the institutions and TIS under investigation. Examples of such events are scientific studies carried out, policy measures issued and norms that are changed.

Event history data are collected retrospectively making use of scientific literature, reports and websites (Table 3 gives an overview). The delineation of the study is Europe, because EPO potency testing in mice was implemented in the European quality control regulation. It is recognized that the innovation process of animal-free methods is influenced by activities worldwide. Some of these activities, such as the development of animal-free methods by researchers and manufacturers outside Europe, are also included in the analysis when they have a great influence. The data is collected over a 40 year period from the early development of EPO in 1970 until 2010.

The identification of events was an inductive exercise for which the conceptual framework of key processes (Table 1) and institutional elements (Table 2) were used as a heuristic. With the definitions of the key processes and institutional elements in mind it was possible to interpret particular reports as events. The data has been triangulated using 9 semi-structured interviews with experts from industry and regulation (Table 4). A storyline was constructed based on the events.

TABLE 1: KEY ACTIVITIES OF THE INNOVATION SYSTEM (BASED ON HEKKERT ET AL., 200; NEGRO ET AL., 2007)

	KEY PROCESSES	INDICATORS
1	Entrepreneurial activity	
	Entrepreneurs are either new entrants that see opportunities in new markets or incumbent companies who diversify their business to take advantage of new developments. They are essential for a well-functioning innovation system because they turn the potential of new knowledge, networks and markets into concrete actions to generate and take advantage of business opportunities. By entrepreneurial experimenting many forms of learning takes place. More knowledge is necessary to deal with the uncertainties of emerging technologies. The presence of active entrepreneurs is a first and prime indication of the performance of an innovation system. When entrepreneurial activity lags behind, causes may be found in the other six functions.	Experiments with the innovative methods
2	Knowledge development	
	Knowledge development in fundamental for every innovation. Knowledge is the basis of emerging technologies and is important to reduce uncertainty and improve the performance of new products and processes.	Articles published about innovative methods
3	Knowledge diffusion	
	Knowledge exchange is important in a strict R&D setting, but especially in a heterogeneous context where R&D meets government, competitors, and market. Policy decisions (standards, long term targets) should be consistent with the latest technological insights and, at the same time, R&D agendas should be affected by changing norms and values.	Conferences and workshops about innovative methods
4	Guidance of the search	
	Guidance of the search refers to those activities that can positively affect the visibility and clarity of specific wants among technology users. Since resources are almost always limited, it is important that, when various technological options exist, choices are made for further investments. Expectations are an important phenomenon when making these choices. Choices of actors are often initially driven by little more than a hunch.	Policies, legislation and expectation with regard to innovative methods
5	Market creation	
	Emerging technologies often have difficulties to compete with incumbent technologies, because they are still badly adapted to many of the uses to which they will be put. Incumbent enjoy increasing returns on investment whereas emerging technologies are expensive and often offer only very small advantages over previously existing techniques. To be able to compete with the incumbent technologies, creation of competitive advantages for emerging technologies is often necessary.	Niche markets for innovative methods

6	Resource mobilization	
	Resources, both financial and human capital, are necessary as a basic input for all activities within the innovation system.	Financial and human capital invested in innovative methods
7	Counteract resistance to change	
	New technologies have to become part of an incumbent regime, or have to overthrow it. Actors with vested interests will often oppose to this force of "creative destruction". Advocacy coalitions can put a new technology on the agenda (function 4), lobby for resources (function 6) and favorable tax regimes (function 5), and by doing so create legitimacy for a new technological trajectory.	Advocacy coalitions for innovative methods

TABLE 2: INSTITUTIONAL ELEMENTS (BASED ON SCOTT, 2008)

	ELEMENTS	INDICATORS
1	Regulative	Rules, laws and sanctions
	The regulative elements refer to explicit, formal rules, which constrain behavior and regulate interactions, for example, government regulations, which structure the economic process. It is about rewards and punishments backed up with sanctions.	
2	Normative	Norms and values
	Normative elements are often highlighted by traditional sociologists. These institutions confer values, norms, role expectations, duties, rights and responsibilities.	
3	Cultural-cognitive	Common beliefs and shared logics of action
	Cultural- cognitive elements constitute the nature of reality and frames through which meaning or sense is made. Symbols (words, concepts, myths, signs, and gestures) have their effect by shaping the meanings we attribute to objects and activities.	

TABLE 3: OVERVIEW OF USED DATA SOURCES AND SEARCH STRATEGIES

	SOURCE	APPLIED SEARCH STRATEGY
1	Scopus.com	Initial search using combination of the following keywords: physicochemical/bioassay/in vitro AND erythropoietin/EPO AND potency/potency test/bioactivity/quality. Relevant articles were selected. Additional publications were identified by snowballing the references and citations
2	European Pharmacopoeia	Monograph 1316 and knowledge base. The knowledge base includes the history of monograph 1316 until European Pharmacopoeia 6.0 (2007)
3	European Pharmacopoeia archives	The archives includes the history of monograph 1316 until first publication in European Pharmacopoeia 3.0 (1999)
4	Pharmeuropa archives	The archives include publications concerning proposed changes for monograph 1316 and other publications related to EPO potency testing.
5	Google.com	Initial search using combination of the following keywords: physicochemical/bioassay/in vitro AND erythropoietin/ EPO AND potency/potency test/bioactivity/quality. Relevant websites were selected. When possible additional websites were identified by snowballing the references and citations

TABLE 4: OVERVIEW OF INTERVIEWEES

	TYPE OF ACTOR	DATE OF INTERVIEW
1	Industry1	09-08-2011
2	Industry2	20-09-2011
3	Regulator1	29-07-2011
4	National research laboratory1 (NRL1)	07-01-2011
5	National research laboratory2 (NRL2)	21-06-2011
6	National research laboratory3 (NRL3)	13-07-2011
7	Researcher1	18-01-2011
8	Researcher2	02-02-2011
9	Researcher3	30-11-2011

4. THE STORY OF EPO POTENCY TESTING

The case study concerns the practice of EPO potency testing that, following the dominant logic of medicine regulation, had to be established in the 1990s. The narrative is elaborated in the following sections.

4.1 THE LOGIC OF MEDICINE REGULATION

In Europe, the regulation concerning the safety, efficacy and quality of medicines started in the 1960s with the implementation of Directive 65/65/EEC and came into existence as a result of the Thalidomide tragedy[12] (Rägo & Santoso, 2008). Medicines are not ordinary consumers' products, as consumers are not in the position to make decisions about when, and how to use medicines. The Thalidomide tragedy made clear that even healthcare professionals did not have the capabilities to take informed decisions about all safety and quality aspects related to medicines. Governments are responsible for the protection of their citizens in fields where the citizens themselves are not able to do so. Governments therefore, required that all new drugs had to be approved by regulatory authorities based on the proven safety, efficacy and quality to gain market authorization (Rägo & Santoso, 2008).

Animals had been successfully used as model for humans throughout the history in scientific research and education (Monamy, 2000). This success had created profound belief in animal tests and made the use of animal tests to study the safety, efficacy and quality of medicines an obvious choice. Due to the deep belief in the value of animal models to predict effects in humans and the broad use of these models in scientific research, the animal experiments were implemented in regulation even without thoroughly validating their ability to predict efficacy and safety in humans. Animal studies have been the golden standard in medicine regulation ever since. Even the scientific discussions on the predictive value of animal studies did not influence the belief in and the position of animal studies as the golden standard.

12 In the late 1950s, the sedative medicine, Thalidomide, was used to cure morning sickness amongst others for pregnant women. The medicine was withdrawn from the market in 1961because it caused severe birth defects.

Directive 2001/83/EC, lays down that the European Directorate for the Quality of Medicines & HealthCare (EDQM) is responsible for the quality control of medicines in the European Union (European Commission, 2001). The EDQM standards for quality control of medicines are prescribed in regulative institutions; the monographs of the European Pharmacopoeia. The General Notices[13] of the European Pharmacopoeia report that *"statements in monographs constitute mandatory requirements"* (EDQM, 2012, p 4453).

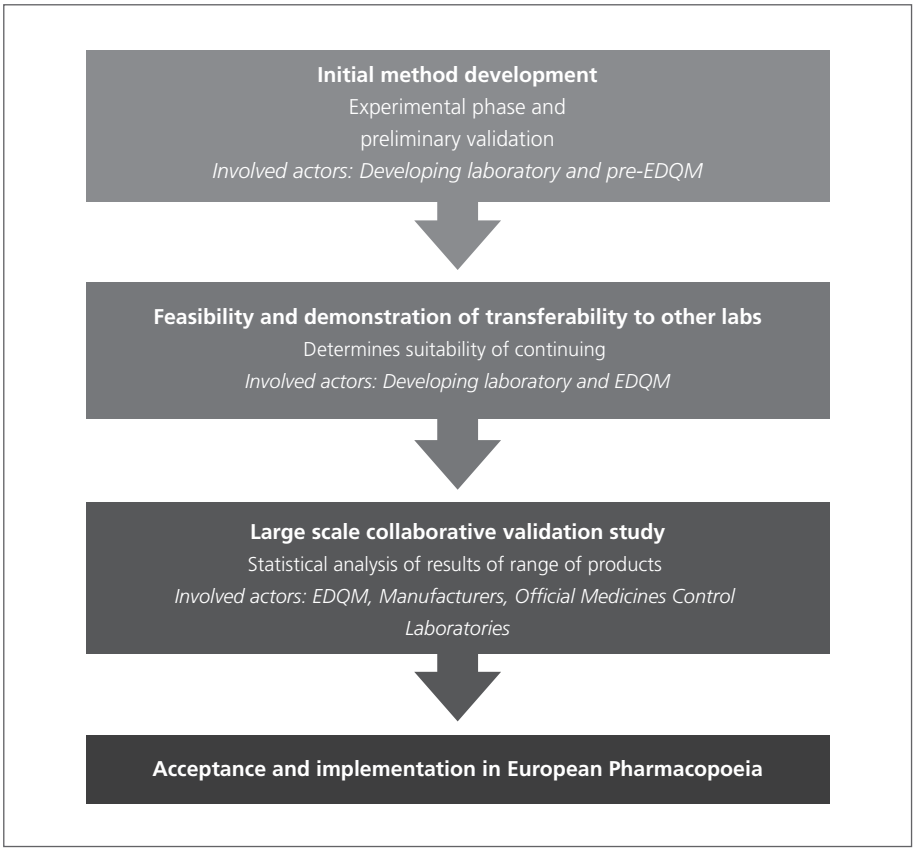

Figure 1: Basic summary of validation procedure (based on Coune, 2007)

The duty of the EDQM is protecting public health by ensuring the quality of medicines (EDQM, n.d.). In order to fulfill this duty the EDQM has to be intrinsically risk averse and conservative. This norm to act precautious is a major barrier to change. An interviewee stated *"the biggest problem is … just intrinsic conservatism. Once you have something*

13 General Notices 10000E of the European Pharmacopoeia apply to all the monographs

that works and you understand it, there is always resistance to change" (Industry2, 2011). As a result, an extensive validation procedure (see figure 1) and monograph revision process have to be followed to replace a test in the monographs of the European Pharmacopoeia (Coune, 2007). Furthermore, innovative methods can only be implemented in the European Pharmacopoeia when the member states of the EDQM unanimously accept the new test (Regulator1, 2011).

The aim of the monographs of the European Pharmacopoeia is to assess the quality of medicines for humans (EDQM1, n.d.). When validating a new quality test it would be most valuable to use the correlation between the effect of interest in humans and the results of the innovative test as validation endpoint. This is not the case. Instead, the correlation between the results of animal-free method and the effect in animals is used as reference (Industry1, 2011; NRL2, 2011; NRL3, 2011). This is not only strange but also problematic. The results of the animal tests are known to be variable and have limited sensitivity in many cases (Industry2, 2011). Thus, a non-ideal prediction model (animal test) for the effects on humans is used as a reference for the validation of animal-free methods. The rationale of using the animal data is that new methods are aimed at *replacing* that test. In general, regulators, manufacturers and scientists are still trained based on the notion that animal studies are the golden standard to assess the safety and quality of medicines for humans (Industry2, 2011; Researcher3, 2011). Furthermore, the experience with animal studies and belief in the value of animal tests support the choice for this reference as quality issues in humans have been uncommon under the current regulation (NRL1, 2011). Additionally, due to intrinsic conservatism the EDQM requires that the results of innovative methods show high correlation with the results of established animal tests. This is problematic when the animal test is variable and has limited sensitivity.

4.2 THE EMERGENCE OF ANIMAL-FREE METHODS FOR EPO POTENCY TESTING

Controversies have been surrounding animal experimentation throughout history (Ryder, 2000). The use of animal studies came under increasing pressure in the second half of the 20th century. Animal welfare organizations were set up and the predictive value of animal tests in drug development came under extensive scientific discussion (Igarashi et al., 1995; Olson et al, 2000; van Meer et al., 2012). The scientific debates and actions of animal welfare organizations led to increased public awareness and

resistance to animal experimentation. As a consequence, animal experimentation was put on political agendas and Directive 86/609/EEC and ETS 123 aiming to protect laboratory animals, discourage animal testing, facilitate the development of innovative methods and force the use of innovative methods were implemented in Europe in 1986 (European Economic Community, 1986; Council of Europe, 1986; Balls, 1994; Baumans, 2004; Kolar, 2006, European Commision, 2012)[14].

The reduced public acceptance of the use of animal experimentation created legitimacy to develop innovative methods. As a result, resources were increasingly invested in the development of animal-free methods to assess the safety, efficacy and quality of medicines, including animal-free methods for EPO potency testing.

At the end of the 1970s the amino acid sequence of EPO was identified and the corresponding gene isolated. Based on that gene Amgen developed the medicine EPO. EPO entered the market in the 1990s. Under influence of the medicine regulation logic quality control for EPO had to be formalized. EPO potency testing is a component of the quality control of EPO. Potency is the ability of medicines to exert their intended activity (Mire-Sluis, 2001). EPO potency is dependent on correct folding for binding affinity and signal transduction and on glycosylation to decelerate clearing from the body by the liver (Imai et al., 1990; Watson & Yao, 1993; Barth et al., 2007; Ferretto et al., 2009).

EPO potency testing is required because every produced batch of EPO has a different composition. EPO is produced in cells. This biotechnological production process is not fully controlled. The folding and glycosylation of EPO are less controlled processes because they are dependent on the conditions in the cell. Slightly different process conditions or starting materials can result in other end-products. This means that the folding and the glycosylation patterns can vary within and between batches. To safeguard acceptable and comparable dosing regimens for patients the potency of all EPO batches needs to be assessed.

The practice to assess the potency of this new medicine still needed to be developed. Before EPO received market approval in the 1990s, several studies had already been done to develop animal-free methods to assess EPO potency. It had taken decades

14 Directive 86/609/EEC was revised in 2010 to further foster the development of animal-free methods (European Commission, 2012a).

of research to correlate the potency of protein medicines in animals with the results of animal-free methods and developing animal-free methods for EPO potency testing presented additional difficulties (Barth et al., 2007). The potency of most protein medicines depends only on binding affinity and signal transduction, whereas for EPO potency there is also a strong, but not fully understood, correlation between glycosylation and potency (Imai et al. 1990; Watson & Yao 1993; Barth et al. 2007; Ferretto et al. 2009, Industry1, 2011). Various animal-free methods to assess EPO potency (e.g. quantitative Western method, radioimmunoassay (RIA), capillary zone electrophoresis (CZE) and iso-electric focusing (IEF)) were developed in the TIS (Hammerling et al., 1996; Goldwasser, Eliason & Sikkema 1975; Sherwood, Goldwasser 1979, Watson, Yao 1993, Bietlot, Girard 1997) by research laboratories, manufacturers and universities often funded internally or with public money (Researcher1, 2011; Researcher2, 2011; Researcher3, 2011). Nevertheless, it remained a challenge to assess glycosylation, the other factor influencing EPO potency.

4.3 INSTITUTIONALIZATION OF THE EPO POTENCY TESTING PRACTICE

Following the institutional logic of medicine regulation the EDQM formulated a draft monograph for EPO quality control in 1996. The actors in the TIS of animal-free EPO potency testing had been very successful in developing and experimenting with animal-free methods to assess folding and binding affinity. However, the TIS actors did not manage to develop a method to quantitatively measure glycosylation (Regulator1, 2011). Without being able to measure glycosylation it is not possible to predict the potency of EPO using animal-free methods (Garthoff, 1995; Bristow & Charton, 1999).

In 1996, the EDQM proposed to implement two animal tests in which the potency is mapped by directly measuring the increase in red blood cells in mice after injecting EPO (Textbox 1) (EDQM, 1996). To determine comparable dosage the increase in red blood cells is related to the increase of red blood cell induced by the reference product (Biological Reference Preparation (BRP)[15]). The relative potency measured in the animal test as measure for EPO potency in human was never extensively validated. The test was assumed to predict EPO potency in humans, even though it was recognized that the results of animal test were variable between animals and between experiments.

15 Biological reference preparations are medicine standards set by the World Health Organization

In 1999 monograph 1316 was implemented in the European Pharmacopoeia and it required that EPO potency had to be assessed, using one of the two prescribed animal tests, prior to product release (EDQM, 2012).

Influence by the logic of medicine regulation, the quality control of EPO had to be formalized soon after market introduction of the new medicine. The actors of the TIS could not deliver an animal-free method that was able to assess EPO potency and therefore the golden standard of medicine regulation, animal experiments, was implemented in the monograph.

TEXTBOX 1: EPO POTENCY TESTING METHODS DESCRIBED IN THE RECOMBINANT HUMAN ERYTHROPOIETIN MONOGRAPH 1316

Method A. In polycythaemic mice
The activity of the preparation in estimated by examining under given conditions, its effect in stimulating the incorporation of ^{59}Fe into circulating red blood cells of mice made polycythaemic by exposure to reduced atmospheric pressure.

Method B. In normacythaemic mice
The assay based on the measurement of stimulation of reticulocyte production in normocythaemic mice.

4.4 DEADLOCKED INNOVATION DUE TO COLLECTIVE INACTION

Only six months after the implementation of monograph 1316, it was concluded that a newly developed method using CZE could quantitatively assess glycosylation (Bristow & Charton, 1999). Of the in total eight animal-free methods for EPO potency testing[16] none could assess binding, signal transduction and glycosylation (see table 5). Only a combination of two methods could assess EPO potency. It was anticipated that a cultured cell assay in combination with this new CZE method could replace the EPO potency test in mice (Charton & Castle, 2001).

In 2010, a decade had passed and the EPO potency test in mice is still not replaced. With the implementation of EPO potency testing in monograph 1316 the EPO potency testing practice became institutionalized in the logic of medicine regulation. Thereby

16 Some methods developed by manufacturers might be missing in this overview, because they are not published and not shared in interviews for strategic reasons (Industry1, 2011; Industry2, 2011).

the TIS of animal-free methods became exposed to the institutional logic of medicine regulation and its constraining and enabling elements (see 4.1). This changed the rules for the TIS of animal-free methods and this change appeared to be unfavorable for the emerging technologies in the TIS.

The logic of medicine regulation restricted the market potential for animal-free methods, because only results of methods prescribed in the European Pharmacopoeia are accepted by the EDQM. For market formation it became essential to validate animal-free methods (Figure 1). To undertake this extra entrepreneurial activity significant resources needed to be mobilized. The aim of the TIS actors had to shift. Instead of developing just a method to assess EPO potency, a method to replace the animal study in the European Pharmacopoeia had to be developed.

Furthermore, the results of the validation process were expected to be disputable making unanimous acceptation by the member states of the EDQM unlikely, and thus implementation in the European Pharmacopoeia, uncertain. Using high correlation with the results of the EPO potency test in mice as a validation requirement is unrealistic.

TABLE 5: METHOD CHARACTERISTICS*

METHOD	BINDING	SIGNAL TRANSDUCTION	GLYCOSYLATION PATTERN	ACCURACY
Animal test	+	+	+	Direct
Radioimmunoassay (Egrie et al. 1987)	+	-	-	Indirect
Cultured cells (Krystal, Eaves & Eaves 1981)	+	+	-	Indirect
Western method (Hammerling et al. 1996)	+	-	-	Indirect
Sialylation-sensitive cell method (Liefooghe et al. 2005)	+	+	+/-	Indirect
RP-LC method (Barth et al. 2007)	-	-	+	Indirect
MALDI TOF MS method (Llop et al. 2008)	-	-	+	Indirect
CZE method (Zhang et al. 2009)	-	-	+	Indirect
cIEF method (Cifuentes et al. 1999)	-	-	+	Indirect

*This list of criteria is not exhaustive and not prioritized

Demonstrating high correlation between the animal test and the combined animal-free method is technically not possible for three reasons. Firstly, it is unclear how to correlate the results of the animal test, directly measuring the effect of EPO on red blood cell production, with the results of a combination of animal-free methods indirectly measuring the potency of EPO (Industry2, 2011; NRL2, 2011; NRL3, 2011). Secondly, the results of the animal test are variable while this is less the case for animal-free tests (EDQM, 2012; Jelkmann, 2009). Finally, experience with the animal tests showed that these tests are not very sensitive (EDQM, 2012; Jelkmann, 2009). An interviewee from industry stated: *"The reticulocyte assay (Method B) is … a very imprecise assay… it is very difficult to detect a 50% change of activity… The iron59 incorporation assay (Method A) can detect something like a 20-25% change in activity"* (Industry 2, 2011). Actor apathy hampered the validation process because it remained unclear how to show high correlation between one direct indicator and a combination of indirect indicators, whereof the direct indicator shows variability and has a lower sensitivity.

The uncertainty and additional requirements introduced into the TIS of animal-free methods for EPO potency reduced the legitimacy for investing in animal-free methods for EPO potency testing. Furthermore, the costly validation process in combination with the norm that patented practices are not accepted in the monographs of the European Pharmacopoeia (Regulator1, 2011) created a free-rider problem as there was no chance of recovering costs while other actors could directly profit from the investments if the animal-free methods would be implemented in the monograph.

The EDQM undertook three attempts to realize collective action. None of the attempts succeed. The EDQM attempted to realize collective data gathering by proposing to implement an animal-free method into monograph 1316 without replacing the mice test (EDQM, 2002; Regulator1, 2011). The results of the animal-free test provided by manufacturers would be used for the validation. However, the proposed revision was not unanimously accepted by the member states of the EDQM and therefore not adopted (Regulator1, 2011). The main objection was that the description of the animal-free methods, the 'in vitro activity assay', was not specified enough (Regulator1, 2011). The EDQM also attempted to organize collective action to gather data for validation in collaborative studies for the establishment of EPO BRP batch 2 and 3 in 2004 and 2007 (Behr-Gross et al., 2004; Behr-Gross et al., 2007). The EDQM included cultured cell methods and the CZE method into the study design to gather

data for the validation of these methods. In both studies only three of the participating laboratories followed up on that request. It was concluded that due to the limited amount of data, the results of the animal-free methods could not be compared with results obtained by animal tests (Behr-Gross et al., 2004; Behr-Gross et al., 2007).

The variable and limited sensitive EPO potency test in mice was implemented in the monograph of European Pharmacopoeia because the logic of medicine regulation required the formalization of the EPO potency testing practice at a moment when animal-free methods were not able to fully assess EPO potency. With the implementation in the monograph, the EPO potency testing practice became part of the logic of medicine regulation. Only six months after the implementation of the EPO potency test in mice in the monograph a combined animal-free method to assess EPO potency became available. The animal-free methods now had to replace the animal test. Animal studies have been the gold standard in medicine regulation for decades and its elements, as the belief in the value of animal studies and the norm to be precautious when it comes to changing established practices, introduced new barriers into the TIS of animal-free EPO potency testing. The extensive validation procedure required to be considered as replacement alternative by the EDQM deadlocked the innovation process as the procedure was never initiated due to collective inaction caused by a free-rider problem and actor apathy.

5. CONCLUSION

The aim of this article was to increase understanding about the persistence of established practices and the effect of this persistence on emerging innovations. We studied the EPO potency testing practice as exploratory case. By doing that we aimed to contribute to the theoretical understanding of how persisting practices can hamper purposive change processes.

The analysis of the medicine regulation logic provided insight in why the EPO potency test was implemented in regulation in the first place. Furthermore, analyzing the innovation process of animal-free methods for EPO potency testing with a framework that combines concepts of the TIS approach and institutional logics showed us that, although the emergence of technologies is crucial for change, the main barriers for the success of animal-free methods were introduced into the TIS by institutional logic

of medicine regulation. Changing the EPO potency test in mice was not realized because the elements of the medicine regulation logic were well aligned towards animal studies as model for humans. The analysis showed that the implementation of animal tests in new regulations should be prevented.

The lesson to be learned from this case is that the influence of the institutional logic reinforcing the established practice on the innovation process is often undervalued in the transition literature. For purposive change processes a better understanding of this institutional logic contributes to our understanding of the innovation process as it provides insight in the origin of barriers and helps to identify potential future barriers. This analysis provides leads to resolve barriers and makes it possible to anticipate to potential barriers of the future. Therefore, a systematic analysis of the institutional logic reinforcing the established practice will increase the chance for success of emerging technologies.

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ANNEX II

HOW INSTITUTIONAL LOGICS INFLUENCE INNOVATION: THE CASE OF ANIMAL TESTING

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ABSTRACT

Solving grand societal problems as climate change and ever increasing health care costs is challenging. It requires socio-technical transitions wherein technical, organizational, economic, institutional, social–cultural and political changes are combined.

The transition literature recognizes that the success of emerging technologies depends on two aspects: (i) development of emerging technologies and (ii) destabilization of established practices. Nevertheless, frameworks used in transition studies, such as the Multi Level Perspective and the functions of the Technological Innovation System (TIS) approach, often focus on emerging technologies leaving the destabilization of the established practice under analyzed. Institutional theory can be valuable in complementing the current approaches to study transitions, as it focuses on the resilience and change of the rules that structure and stabilize practices of daily life. The aim of this paper is to assess if adding an analysis of the institutional context of the established practice to the functions of the TIS approach provides a richer understanding of transition processes.

The replacement of Draize eye irritation test in rabbits by the animal-free Isolated Chicken Eye (ICE) and Bovine Cornea Opacity Permeability (BCOP) tests is used as a case study. The results show that analyzing complex change processes using either the TIS approach or an institutional theory perspective leads to underexposure of one of the two aspects enabling innovation and results in a biased understanding of the innovation process. Combining the TIS approach with an analysis of the relevant institutional logics provides an enhanced understanding of the processes that hampered and facilitated change, because it also allows gaining insight in the interaction between the emergence of technology and the destabilization of the established practice in the innovation process.

1. INTRODUCTION

Solving grand societal problems as climate change and ever increasing health care costs is challenging. It requires a combination of technical, organizational, economic, institutional, social–cultural and political changes (van den Bergh, Truffer, & Kallis, 2011). Such complex change processes are referred to as socio-technical transitions. Recently a new academic field has emerged that focuses specifically on understanding the dynamics of socio-technical transitions (Markard, Raven & Truffer, 2012).

The transition literature recognizes that socio-technical transitions depend as much on development of emerging technologies as on destabilizing established practices (Kemp, Schot, & Hoogma, 1998; Turnheim & Geels, 2012). Nevertheless, transition studies often focus on emerging technologies leaving the established practice under analyzed. The Technological Innovation System (TIS) approach and the Multi-Level Perspective (MLP) have emerged as important frameworks to analyze socio-technical transitions (Markard & Truffer, 2008). The TIS approach focuses on the prospects and dynamics of emerging technologies that have the potential to contribute to socio-technical transitions (Hekkert et al., 2007, Markard & Truffer 2008). This approach extensively studies the system around the emerging technology but it does not explicitly analyzes the counter part of innovation; the dynamics of the system wherein established practice are embedded (Markard & Truffer, 2008). Theoretically, the MLP has much better conceptualized established practices and the difficulties of replacing them. This system around the established practice is rightfully coined 'the regime'. Rip and Kemp (1998) defined regimes as *"…the rule-set or grammar embedded in a complex of engineering practices, production process technologies, product characteristics, skills and procedures, ways of handling relevant artifacts and persons, ways of defining problems; all of them embedded in institutions and infrastructures"* (Rip and Kemp, 1998, p. 340). Nevertheless, also in the empirical studies novelty is often at the center of attention and much less attention is paid to forces of inertia at the regime level. This is acknowledged by Turnheim and Geels (2012) who state that: *"The destabilisation of existing regimes is assumed to happen along the way and has received far less analytical attention"* (Turnheim & Geels, 2012, p 35).

Institutional theory can be valuable in complementing the current approaches to study transitions, as it focuses on the resilience and change of the rules that structure and stabilize established practices in daily life (Seo & Creed, 2002; Kalantaridis & Fletcher,

2012). Institutions are not just rules in the form of a set of commands and requirements, but also rules in the sense of roles and practices that are being established and that are not easily dissolved (Kemp et al., 1998). A poor fit of emerging technologies with the prevailing institutions hampers successful diffusion of innovations, because it creates economic, technological, cognitive and social barriers for new technologies (Kemp et al., 1998). Institutional change is then essential to destabilize the established practice and enable innovation.

In this study we combine the TIS approach with institutional theory for two reasons. Firstly, the TIS approach is praised for its powerful analysis of the conditions that hamper and stimulate the development of emerging technologies (Markard & Truffer, 2008). Secondly, this framework is explicitly criticized for not conceptualizing the wider environment wherein emerging technologies compete with the established practice. The TIS approach and institutional theory complement each other because jointly they conceptualize both the processes on which transitions depend; the emergence of technologies and the processes that reinforce or undermine established practices. Therefore, adding an analysis of the institutional context wherein the established practice is embedded to the TIS framework might be a solution to deal with the criticism on the functions of the TIS approach.

The aim of this study is to assess if adding an analysis of the institutional context of the established practice to the functions of the TIS approach provides a richer understanding of transition processes. The replacement of Draize eye irritation test in rabbits by the animal-free Isolated Chicken Eye (ICE) and Bovine Cornea Opacity Permeability (BCOP) tests is used as a case study. The use of animal studies has been the gold standard to assess the eye irritation potential of medicines, cosmetics and chemicals for over four decades. Since the 1980s a range of actors, among which scientists and animal welfare organizations, call for the reduction of animal testing for various reasons such as animal welfare concerns and scientific and economic inadequacies of animal studies (Piersma, 2006; National Research Council, 2007; Briggs, 2008; Bottini & Hartung, 2009). The Draize test was one of the first tests for which there was dedicated effort to develop new, innovative animal-free methods. Although it took almost three decades, it was also one of the few tests for which innovative methods actually have replaced an animal test in the guidelines of the Organisation for Economic Co-operation and Development (OECD) (Eskes, 2010).

The replacement of the Draize test by animal-free methods is selected as case study to assess the value of the combined framework because it is an extreme case. Firstly, the replacement of the Draize test by the animal-free methods came about very slow as it took three decades. Secondly, the Draize test is embedded in international regulation. Animal-free methods can only be used to assess the eye irritation potential of medicines, cosmetics and chemical if these methods replace the Draize test in regulation. The system around the Draize test is expected to influence the innovation process of animal-free methods since animal-free methods need to be implemented in this system.

Although the change from animal experimentation to animal-free testing is too narrow to be considered a socio-technical transition, it can provide useful insights into transition processes. The replacement process of the Draize test shares characteristics with socio-technical transitions as for example low-carbon transitions. The studied change process is also incentivized by a societal demand and dynamics of this change process are also the results of the interaction between technological, cultural, economic and political factors.

The remainder of the paper is organized as follows: The next section develops theoretical considerations about adding an institutional analysis to the TIS framework. Section 3 describes the methodology. Section 4 provides the storyline of the case study and the analysis is presented in section 5. We discuss the conclusions in section 6.

2. THEORETICAL FRAMEWORK

In reaction to perceived inadequacies of neoclassical economics, innovation system approaches were developed as a policy concept in the mid -1980s (Sharif, 2006). The Technological Innovation System (TIS) approach is one of these innovation system approaches and is used to conceptualize and analyze the complex process of the development, diffusion and use of new technologies (Bergek et al., 2008; Hekkert & Negro, 2009). The basic assumption of the TIS approach is that technologies do not emerge in isolation, but that the socio-technological context, including policy and perceived legitimacy, influence the innovation process. The actors in an innovation system contribute to the process of the development, diffusion and use of new technologies in a wide variety of ways such as by knowledge development,

experimentation with the new technology and supply of resources. The structure of the innovation system, consisting of institutional settings, technological artifacts and network characteristics, enable and constrain the actors in the TIS in their actions.

The functional analysis of the TIS focuses on the seven key processes that take place in the innovation system: entrepreneurial activities (F1), knowledge development (F2), knowledge diffusion (F3), guidance of the search (F4), market formation (F5), resource mobilization (F6) and creation of legitimacy (F7) (Hekkert et al. 2007). These key processes are necessary to provide the favorable circumstances for actors to innovate. When one or more of these key processes do not sufficiently take place innovation is hampered (Jacobsson & Bergek, 2011).

The key contribution of the TIS approach to innovation studies is its powerful analysis of the socio-technical system around emerging technologies (Markard & Truffer, 2008). While there is strong recognition in the transition literature that the success of emerging technologies depends as much on the development of emerging technology as on changing technical regimes (Kemp et al., 1998; Turnheim & Geels, 2012), the functional analysis of the TIS regards the success of innovations mainly as a consequence of the performance of the TIS and does not systematically take into account the influence of the system around the established practice (Markard & Truffer, 2008). Using the strength of one theory to address the limitation of another can provide an enriched understanding of the studied phenomenon. We therefore make use of the rich body of literature on institutional theory to conceptualize the system wherein established practices are embedded to complement the functions of the TIS approach.

Institutional theory studies the more resilient features of social structure (Scott, 2008). Institutions are the taken-for-granted rules (e.g. regulations, user practices, symbolic meanings) that stabilize and structure the daily life practices (Seo & Creed, 2002; Kalantaridis & Fletcher, 2012). They are relatively resistant to change and tend to be preserved and reproduced across generations (Scott, 2008). Institutions impose restrictions by defining legal, moral and cultural boundaries, setting off legitimate from illegitimate activities and thereby control and constrain behavior, but also support and empower activities (Lawrence & Suddaby, 2006; Scott, 2008; Kalantaridis & Fletcher, 2012; Thornton et al., 2012). Institutional scholars study how institutions are created, maintained, adapted and dismissed (Lawrence & Suddaby, 2006; Scott, 2008).

Alford and Friedland (1985) introduced the concept of institutional logics. Institutional logics comprise of a set of institutions that are the basis of taken-for-granted rules guiding behavior of actors in specific contexts (Scott, 2008; Reay & Hinings, 2009) and can be defined as *"the socially constructed, historical patterns of material practices, assumptions, values, beliefs, and rules by which individuals produce and reproduce their material subsistence, organize time and space, and provide meaning to their social reality"* (Thornton & Ocasio, 1999, p 804). Institutional logics can be found at multiple levels such as the societal, organizational, market and industrial level (Thornton & Ocasio, 2008). In this paper we study the institutional logic of safety regulation of medicines, cosmetics and chemicals at the industrial level.

Institutional logics are reinforced because their institutions are aligned which makes them taken for granted, normatively endorsed and backed up by authorized powers (Scott, 2008; Thornton et al, 2012). These logics are enacted through institutionalized practices that are reproduced within the field (Berman, 2012). In this article, for example, the Draize test is embedded in the institutional logic of safety regulation of medicines, cosmetics and chemicals.

For emerging technologies strongly deviating from the dominant institutional logic it is often more difficult to break through because they are not regarded as legitimate, not taken for granted and not supported by authorized powers. Then institutional change is essential for innovation. According to the literature, change in institutional logics is often driven by outsiders (institutional entrepreneurs) mobilizing resources and using social skills to promote a new logic successfully (Hardy and Maguire 2008; Thornton & Ocasio, 2008). However, institutional change can also be induced by powerful insiders (Rao et al., 2003; Greenwood & Suddaby, 2006) and social movements (Lounsbury et al., 2003; Weber et al., 2009). Institutional change is often beyond the capacity of individual actors and therefore requires joint activities by a wide group of actors on the basis of mutual interests (Oliver, 1993; Wijen & Ansari, 2007). Collective inaction, as a result of free-rider problems, lack of leadership or actor apathy (actors feel their contribution to the problem is insignificant), is often a problem in these change processes (Heckathorn, 1996; Wijen & Ansari, 2007).

Many scholars used the concept of practice as a way to study how institutions are enacted as well as how individual action has the potential to reshape them or create new ones (Seo & Creed 2002; Colyvas & Powell, 2006; Berk & Galvan, 2009; Owen-

Smith, 2011; Berman 2012). Berman (2012, p 261) argues that *"When one logic is dominant, innovations based on alternative logics may have trouble gaining the resources they need to become more broadly institutionalized. But if a changing environment starts systematically to favor practices based on an alternative logic, that logic can become stronger even in the absence of a coherent project to promote it"*. It is expected that studying the institutional logic wherein the Draize test is embedded in combination with the TIS approach analyzing the emergence of animal-free methods for eye irritation testing, will provide enriched insight in the influence of the system around the established practice on innovation processes.

To systematically identify the assumptions, values, beliefs, and rules of the institutional logic that reproduce the use of the Draize test, we used the institutional framework of Scott (2008). He distinguishes three types of institutional elements: regulative, normative and cultural-cognitive elements. The regulative elements refer to explicit, regulative rules, which constrain behavior and regulate interactions. The Directives of the European Commission are examples of regulative elements. The normative elements confer values (the preferred or the desirable, together with the construction of standards to which existing structures or behaviors can be compared and assessed), norms (how things should be done), role expectations, duties, rights and responsibilities. The cultural-cognitive elements constitute the nature of reality and frames through which meaning or sense is made (Scott, 2008). These elements are used as heuristics to explore the institutional logic.

3. METHODOLOGY

To study if adding an analysis of the institutional context of the established practice to the functions of the TIS approach provides a richer understanding of transition processes we employed an explorative case study methodology. The studied case, the replacement of the Draize test by the ICE and BCOP test, is an extreme as it concerns the substitution of highly regulated practice.

A qualitative event history analysis was done to gain insight in the changes in the institutional logic wherein the Draize test is embedded and the dynamics of the innovation process of animal-free methods that could replace the golden standard over time. In the event history analysis events are identified and related to the

institutional elements and the seven key processes of the TIS. An event can be defined as an instance of change with respect to the assumptions, norms, beliefs and rules reinforcing the use of the Draize test and the key processes of the TIS, which is the work of one or more actors and which carries some public importance with respect to the institutions and TIS under investigation. Examples of such events are scientific studies carried out, policy measures issued and norms that are challenged.

Event history data are collected retrospectively making use of scientific literature, reports and websites (Table 1 gives an overview). The delineation of the study is global, because eye irritation testing is embedded in international regulation. The data is collected over a 45 year period from the implementation of eye irritation testing in regulation in the Unites States in 1964 until adoption of the innovative ICE test and the BCOP test in the OECD test guidelines in 2009. Based on the identified events a narrative was composed.

The identification of events was an inductive exercise for which the conceptual framework of key processes and institutional elements were used as a heuristic. With the definitions of the key processes and institutional elements in mind it was possible to interpret particular reports as events. The data has been triangulated using 11 semi-structured interviews with experts from industry and regulation (Table 2).

TABLE 1: OVERVIEW OF USED DATA SOURCES AND SEARCH STRATEGIES

	SOURCE	APPLIED SEARCH STRATEGY
1	Scopus.com	Initial search using combination of the following keywords: physicochemical/bioassay/in vitro AND chemicals/cosmetics/drugs AND eye irritation/Draize. Relevant articles were selected. Additional publications were identified by snowballing the references and citations
2	OECD Guidelines for the Testing of Chemicals	Section 4, Health effects, Test No. 405, Test, No. 437, Test No. 438
3	ECVAM	Validation process
4	Google.com	Initial search using combination of the following keywords: physicochemical/bioassay/in vitro AND chemicals/cosmetics/drugs AND eye irritation/Draize. Relevant websites were selected. When possible additional websites were identified by snowballing the references and citations

TABLE 2: OVERVIEW OF INTERVIEWEES			
	TYPE OF ACTOR	DATE OF INTERVIEW	REFERENCE IN THE TEXT
1	Symposium of Research Community (SRC)	12-04-2011	(SRC, 2011)
2	Researcher 1	26-05-2011	(Researcher 1, 2011)
3	Researcher 2	25-05-2011	(Researcher 2, 2011)
4	Researcher 3	31-05-2011	(Researcher 3, 2011)
5	Industry 1	30-05-2011	(Industry 1, 2011)
6	Social Movement Organization (SMO)	01-06-2011	(SMO, 2011)
7	Social Movement Organization/ Regulator/Researcher (SMO/R/R)	19-05-2011	(SMO/R/R, 2011)
8	Industry 2	31-05-2011	(Industry 2, 2011)
9	Industry 3	27-05-2011	(Industry 3, 2011)
10	Industry 4	30-05-2011	(Industry 4, 2011)
11	Researchers/ Regulator (R/R)	02-06-2011	(R/R, 2011)
12	Regulator	25-05-2011	Regulator, 2011)

4. THE STORY OF THE EYE IRRITATION TESTING PRACTICE

The case study concerns the practice of eye irritation testing of cosmetics, chemicals and medicines. Following the dominant institutional logic of safety regulation of medicines, cosmetics and chemicals, assessment of the eye irritation potential has been required for new substances to gain market authorization since the late 1960s. In the 1980s, a TIS of animal-free methods for eye irritation testing emerged. The actors of this innovation system aimed to develop animal-free methods that could circumvent the use of animal tests for this eye irritation testing practice. The Draize test on rabbits persisted as golden standard until two innovative methods, the ICE test and the BCOP test, were finally accepted by the OECD to identify moderate to severe eye irritants in 2009. Adoption in the OECD test guidelines can be considered the highest available degree of acceptance (Regulator, 2011). The eye irritation testing narrative depicted in figure 1 is elaborated in the following sections.

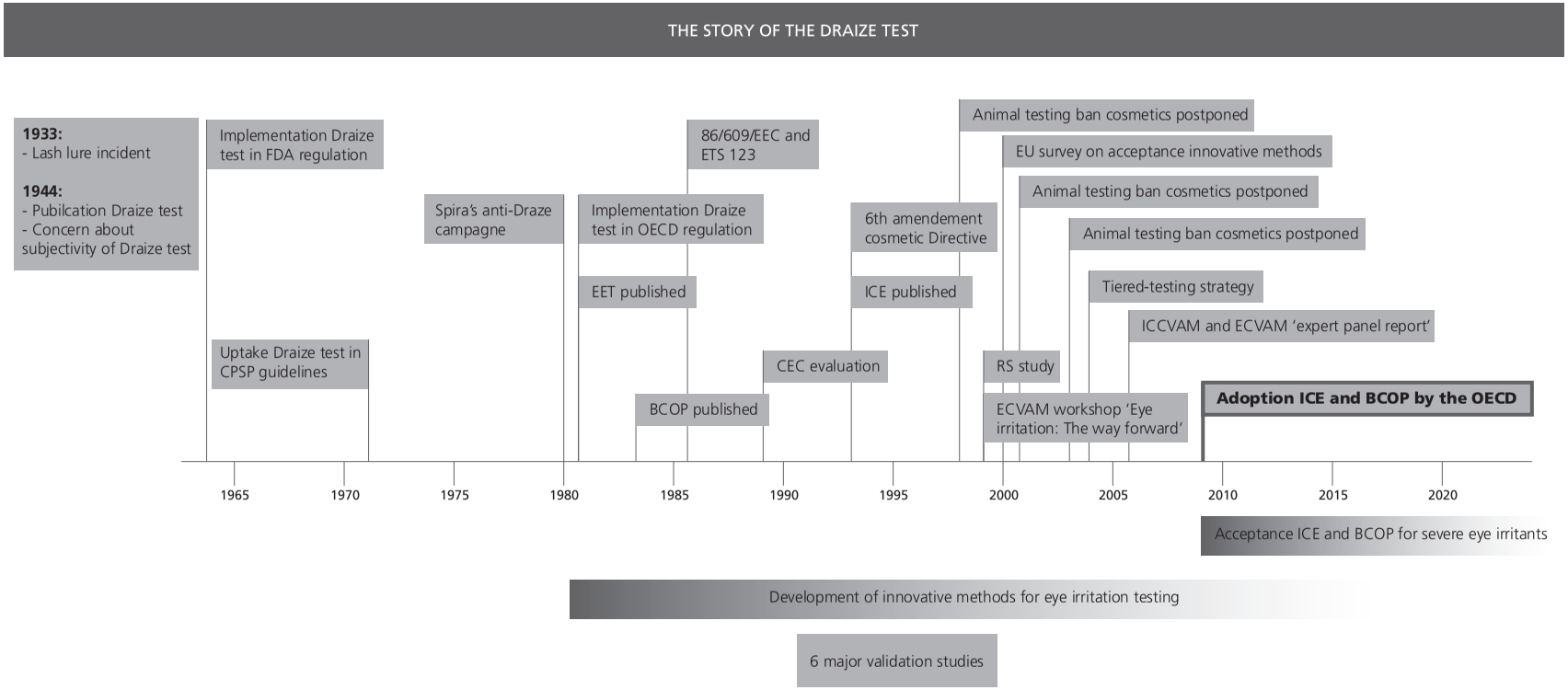

Figure 1: Timeline of the events influencing the transformation towards the ICE and BCOP tests

4.1 THE LOGIC OF SAFETY REGULATION

The institutional logic of safety regulation of cosmetics, chemicals and medicines emerged in the first half of the 20th century. At that time governments experienced that regulation was necessary to guard citizens from possible adverse effects of cosmetics, chemicals and medicines as a consequence of the increasing role of these type of products in everyday life the Lash-Lure[17] and Elixir Sulfanilamide[18] tragedies (Rägo & Santoso, 2008; Hartung & Daston, 2009). In the United States, the regulation concerning product safety started in 1938 with the implementation of Federal Food, Drug and Cosmetic Act (FDA, 2012). Other countries soon followed and introduced similar acts. These acts required that cosmetics, chemicals and medicine manufacturers had to demonstrate the safety of their products to regulatory authorities in order to gain market authorization.

Animals had been successfully used as model to study humans in scientific research and education throughout the history (Monamy, 2000). This success had created profound belief that animals are good models to predict the risks of drugs, cosmetics and chemicals for humans. In addition, animals are cognitively the closest model to humans compared to any other organism thus it was assumed that they would be the best models to predict human risks. These assumptions about and beliefs in animal studies as good models to predict human risk made the use of animals to assess the safety of cosmetics, chemicals and medicines an obvious choice. Animal studies were implemented in regulation without thoroughly validating their ability to predict efficacy and safety in humans. In regulatory safety testing, animal studies have been the gold standard ever since. Even the scientific discussions concerning the predictive value of animal studies (Igarashi et al., 1995; Olson et al. 2000) did not influence the belief in and the position of animal studies as the golden standard.

The aim of safety regulation of cosmetics, chemicals and medicines is protecting public health by ensuring the safety of these products. Due to this objective, the regulatory authorities responsible for the enforcements of these acts, such as the Food and Drug Administration (FDA) and OECD, are intrinsically risk averse and conservative in order to fulfill their task. The norm for regulatory authorities is to be precautious when it concerns changing established practices. This norm is a major barrier to

17 More than a dozen women were blinded and one woman died from using Lash-Lure, permanent mascara containing severely irritating ingredients, in 1933 (National Research Council, 2004).

18 Nearly 100 people died after ingesting a medicine called Elixir Sulfanilamide in 1937 (Berger & Berger, 2005)

change (SMO, 2011; R/R, 2011). As a result, an extensive validation procedure has to be followed to replace a test in the regulation.

4.2 INSTITUTIONALIZATION OF THE DRAIZE TEST

In the 1960s the Draize test was the only test to assess eye irritation. This test on rabbits to quantitatively determine the irritant and corrosive potential of substances on the eye was developed by Draize, head of the Dermal and Ocular Toxicity Branch of the FDA, in the 1940s (Draize *et al.,* 1944). In this test, substances were applied to the animal's eye, and subsequent physiological responses were scored by visual examination of different aspects of the eye. Although the Draize test had several shortcomings, as for example high variability and low reproducibility (Weil & Scala, 1971; Burton, 1972; Frazier, 1987; Lordo *et al.,* 1999; Ohno *et al.*, 1999), uncontrolled and non-standardized exposure conditions (Burton, 1972; Frazier, 1987; Prinsen, 2006) and difference in sensitivity to tested substances between rabbits and humans (Freeberg *et al.*, 1986), it was institutionalized as mandatory test by the FDA, regulatory authorities in European countries and the OECD regulation between the 1960s and early 1980s (Eskes, 2010). According to formal European regulation eye irritancy potential has to be assessed for every new cosmetic product (EEC, 1976). The labeling of chemicals containing harmful substances, including eye irritancy potential, was not regulated by the European Commission (EC), but was the responsibility of chemical companies. This situation changed with the implementation of REACH[19] in 2007. Eye irritation testing is not formally required for most medicinal products except for medicines using topical or ocular route of administration (Industry 1, 2011; Industry 4, 2011).

Despite the shortcomings, the test is experienced to be successful in preventing eye irritation in humans since tragedies like the Lash Lure incident did not happen again. This gave regulators (and society as a whole) confidence in the test and resulted in the assumption that the Draize test had a high predictive value, making it the gold standard. The regulative and normative institutionalization of the Draize test would turn out to be an important source of inertia to change this practice in following decades. Until 2009, the Draize test stayed the only formal method to evaluate eye irritation.

19 Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) is the European Community Regulation on chemicals and their safe use

4.3 EMERGENCE OF THE INNOVATION SYSTEM FOR ANIMAL-FREE EYE IRRITATION TESTING

Although controversies had been surrounding animal experimentation throughout history, the attention of animal welfare organizations put them under increasing pressure in the second half of the 20th century (Ryder, 2000). The implementation of the Draize test in regulation implied a substantial increase in the use of this test in the 1970s. This increase did not go unnoticed by animal welfare organizations and the media, and they fueled great public concern. While animal rights activists already had targeted the Draize test on ethical grounds in earlier years (Wilhelmus, 2001), at the end of 1970s Henri Spira, founder of the Animal Rights International, launched a campaign to abolish the Draize test in the United States.

After learning that cosmetics manufacturer Revlon used 2000 animals for eye irritation testing in 1978, Henri Spira began writing letters and questioning the company's chairman at stockholder meetings (Rowan & Loew, 1995). To create legitimacy, he brought together 407 animal welfare organizations under the 'Coalition to Stop Draize Rabbit Blinding Tests' (Spira, 1985). Spira succeeded in influencing the public opinion to demand substitution of the Draize test through the effective use of mass media (SMO, 2011). To create public awareness, Spira first ran a page size advertisement in the New York Times in 1980 and advertisements in other newspapers followed later (Spira, 1985). Furthermore, television attention and protesters handing out leaflets and stickers in department stores all over the world further spurred the public awareness. These societal actions decreased the legitimacy of the Draize test and animal testing for cosmetics in general. This delegitimization led to increased pressure to change this practice.

As a consequence legislations as the Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals were revised in the United States in 1985. Both Acts influenced the established norm by incorporating the requirement that innovative methods have to be considered before the start of research involving animals (Balls et al., 1999). In Europe, Directive 86/609/EEC and ETS 123 aiming to protect laboratory animals, discourage animal testing, facilitate the development of innovative methods and force the use of innovative methods, were implemented in 1986 (European Economic Community, 1986; Council of Europe, 1986; Balls, 1994; Baumans, 2004; Kolar, 2006, European Commision, 2012)[20]. By

20 The EU Directive was revised in 2010 to further foster the development of animal-free methods (European Commission, 2010).

requiring the use of innovative methods when available, these laws changed the norm that the use of the Draize test was self-evident.

The anti-Draize campaign of Spira effectively created legitimacy for animal-free methods (F7) and thereby marked the start of the TIS of animal-free methods for eye irritation testing. The campaign changed the normative institutions concerning the use of the Draize test. The use of the Draize test was no longer self-evidently accepted by the public. To repair brand and corporate image, Revlon, the company that was targeted in the advertisements, provided a $750.000 fund to the Rockefeller University to develop animal-free methods for eye irritation testing by the end of 1980 (F6) (Rowan & Loew, 1995; CAAT, 2012). More resources were mobilized when other cosmetics companies (F6) (i.e. Avon and Bristol-Meyer) provided a start-up grant to establish the John Hopkins Centre for Alternatives to Animal Testing (CAAT) (F2) (CAAT, 2012).

The change of normative institutions induced by the anti-Draize campaign not just created an incentive for cosmetic companies to invest in innovative methods, it also transformed the search for innovative methods from a minor antivivisectionists' issue into a large-scale operation supported by multi-million dollar corporations (Sina & Gautheron, 1998). Furthermore, the campaign set animal testing on the political agenda and resulted in legislation that facilitated the development of animal-free methods (F4). This social movement organized by Spira put pressure on animal experiments as golden standard of the safety regulation logic.

Burton, researcher at Unilever, was the first to develop and publish an innovative method intended to replace the Draize test (F2). Burton's Enucleated Eye Test (EET) used isolated eyes of deceased rabbits and was originally intended as a prescreen for the assessment of severe eye irritants (Burton et al., 1981). TNO[21] and Shell Research Ltd heard of the EET and decided to use it (F3) (SRC, 2011; Researcher 2). Both parties ran in-house validation studies (F1) using the Draize test as reference and concluded that; *'it is a reliable predictor of the potential to cause ocular injury of a wide variety of chemicals'* (Price & Andrews, 1985, p 314).

Despite the positive results, the board of TNO did not fund the further development of the EET (F6) because the board was holding the view that animal tests could

21 TNO is an independent research organization and also CRO in the Netherlands

not be replaced (SRC, 2011; Researcher 2; SMO/R/R, 2011). Nevertheless, individual researchers, at for example TNO and animal welfare organizations, continued experimenting (F1), often in their own time (Researcher 3, 2011; SMO/R/R, 2011). The main drivers for the experiments with the EET at this point were personal convictions of the involved researchers (Researcher 2, 2011; SMO/R/R, 2011; Industry 2, 2011). They were convinced that innovative methods not using living animals could produce similar results as the Draize test (F4).

In line with the ambitions in Directive 86/609/EEC, the Commission of the European Economic Community, the predecessor of the EC, provided the resources (F6) for an initial study on the evaluation of innovative methods that could replace the Draize test, including the EET method, in 1988 (F1). The conclusion of this study was that the test correctly predicted the irritancy grade of the compounds (Prinsen & Koeter, 1993). However, a practical problem with the use of rabbit eyes in the EET was identified; the relative scarceness of rabbit slaughterhouses could give proximity problems for other laboratories (Prinsen & Koeter, 1993). To overcome this problem, researchers at TNO and De Montfort University started to explore eye irritancy on the eyes of other animal species (F2) (SRC, 2011; Researchers 2, 2011).

Besides the scarceness of rabbit slaughterhouses, researchers at TNO also noticed that the process for slaughtering the rabbits made it difficult to obtain undamaged eyes (Prinsen & Koeter, 1993). The use of chicken eyes circumvented both problems, because they were less scarce and in the slaughter process chickens were decapitated first, leaving the eyes intact for the researcher to remove them (F2). The financial resources that made this research possible were made available by the Dutch Society for the Protection of Animals (F6) (SRC, 2011; Researcher 2; SMO/R/R, 2011). The result was the ICE test. Prinsen and Koeter concluded that the ICE test *"can be considered a sensitive, but not over-sensitive, means of predicting the eye irritancy potential of all types of compounds"* (Prinsen & Koeter, 1993, p 75). The method had only been tested with 21 reference compounds in-house, and further validation was still needed to realize formal validation. At this point, it was expected that within a timeframe of 5 to 10 years, complete validation of the ICE test was possible (SRC, 2011; Researcher 2; SMO/R/R, 2011).

Muir, researcher at De Montfort University in Leicester, developed a similar method in the 1980s (F2) (Muir, 1984). Instead of rabbit eyes he used bovine cornea (Muir,

1984, 1985). He selected bovine cornea because these were freely available from the local slaughterhouse and he believed that this technique was more sensitive than the EET (Muir, 1984). In 1988, this method, the BCOP test, gained the interest of Merck[22] (F3) (Gautheron et al., 1992; Industry 2, 2011). Merck intended to use the method to screen the toxicology of intermediate drug compounds for occupational hazard purposes (Industry 2, 2011). In this niche, it is not required to obtain safety data by using acknowledged methods (such as the Draize test) (Industry 2, 2011). Companies can use internal standards to evaluate innovative methods, without official validation studies and approval of regulatory authorities (Researcher 1, 2011; Industry 1, 2011; Industry 3, 2011; Industry 4, 2011; R/R, 2011). This allowed Merck to use internal validation criteria. The BCOP test was accepted for application in the niche of assessing occupational hazard (F5) (Gautheron et al., 1994).

In November 1991, the EC and the British Home Office (HO) arranged a meeting to review the recent progress of innovative methods (F3). The participating actors from industrial companies, industry associations, academia and animal welfare groups concluded that several innovative methods were mature enough to be tested in a formal validation study (Balls et al., 1995). The EC and HO organized such a validation study including nine innovative methods (F1) (Balls et al., 1995). It was expected that the validation study initiated by the EC/HO would decide upon the replacement for the Draize test (Researcher 1, 2011; Researcher 2; 2011; SMO/R/R; 2011; Industry 2, 2011; R/R, 2011; Regulator, 2011). However, the results showed that none of the tests were sufficiently correlated with eye irritancy in rabbits (Balls et al., 1995).

Numerous innovative methods were developed and tested on a small scale in the 1980s (F2). However, no innovative method was formally validated. Following the regulation, non-validated methods could only be used in niches e.g. for occupational hazard screening and as a pre-screen for an original Draize test (F5) (Researcher 2; 2011; Industry 2; 2011, Industry 4; 2011).

22 Being one of the largest pharmaceutical companies in the world, the company name has seen changed over the years. Formally the company is registered as 'Merck & Co., Inc' and outside the US also known as Merck Sharp & Dohme (MSD). The remainder of this report simply uses 'Merck' for clarity purposes.

4.4 REPLACING THE DRAIZE TEST

Whereas developing medicines for human disease and assuring their safety was experienced as a justifiable cause for animal testing, the use of animals for testing cosmetics and chemicals became increasingly controversial (European Commission, 2008). A major decision was made at the EC in 1993 that put the animal experimentation to assess the safety of cosmetics under increasing pressure (Rosholt, 2005). The 6th amendment to the Cosmetics Directive (93/35/EEC) was accepted, introducing a ban on selling cosmetic products containing ingredients that have been tested on animals as per 1998 (F4) (European Commission, 1993). Before this amendment, investing in innovative methods was often done to repair corporate image; now the approaching deadline of the ban became the incentive for further development and validation of innovative methods (Zuang, 2002).

Between 1990 and 1998 a total of six major validation studies were undertaken (F1). Around 30 methods were evaluated in an extensive number of laboratories. However, no single innovative method was validated (Balls et al. 1999). In retrospect, several factors that could account for the low predictions in the validation studies were identified, amongst which the subjectivity and the variability of the Draize test (Balls et al., 1999; Eskes, 2010, Researcher, 1, 2011; Researcher 2, 2011; SMO/R/R; 2011; Industry 3, 2011; Regulator; 2011).

Although high correlation with the eye irritation potential of substance in humans would be more appropriate to assess the predictive value of animal-free methods, the goal was to demonstrate high correlation between the results of the innovative methods and the golden standard, the Draize test. The rationale of using the results of the Draize test as reference was that innovative methods were aimed at replacing that test. In general, regulators, manufacturers and scientists are still trained that animal studies are the golden standard to assess the safety of cosmetics, chemicals and medicines (R/R, 2011). The belief in the predictive value of the Draize test for human risk was further enforced by the lack of commensurable tragedies as the Lash-Lure incident since the implementation of the Draize test in regulation (SMO, 2011). Additionally, due to intrinsic conservatism the regulators required that the results of innovative methods showed high correlation with the results of established animal tests. Nevertheless, showing high correlation is problematic because the Draize test has shortcomings regarding reproducibility and variability. The conservative validation endpoints complicated the implementation and use of innovative methods considerably. Although many experts agreed that less strict

requirements for validation would be scientifically favorable, they could not convince the regulators (Researcher 1, 2011; Industry 2; 2011; Regulator, 2011).

Meanwhile the deadline for the ban institutionalized in the 6th amendment to the cosmetics directive loomed. In 1996, although many innovative methods were available, the validation was not expected to be completed before the deadline of the marketing ban in 1998. It was concluded that *"Currently there are no validated alternative methods capable of replacing the OECD-405 in vivo eye irritancy test"* (European Commission, 1997, p30). The EC made use of the clause in the 6th amendment that stated that if there were no validated innovative methods, the Commission would be able to postpone the ban. The EC postponed the ban to June 30th 2000 (F4) (European Commission, 1997a).

In 1998, the European Centre for the Validation of Alternative Methods (ECVAM) organized a workshop named 'Eye Irritation testing: The Way Forward' (Balls et al., 1999) to thoroughly review the problems associated with the validation efforts (F3). The use of reference standards[23], to circumvent the variability of the Draize test, was proposed as a potential solution, but further research was necessary to evaluate the usefulness of reference standards (Researcher 1; 2011; Regulator, 2011). As a follow-up on the workshop, the ECVAM organized a study to assess the feasibility of using reference standards for validating five innovative tests in 1999-2000 (F2). It was concluded that scientific validation would be possible using reference standards (Zuang, 2002).

Despite the developments with regard to the validation of innovative methods, the Cosmetics Directive was amended again in 2001 (F4). The date of the ban on the marketing of cosmetic products containing ingredients, or combinations of ingredients that have been tested on animals was postponed to June 30th 2002 due to *'insufficient progress in developing satisfactory methods to replace animal testing'* (European Commission, 2000, p1). In 2003 the ban was postponed again. In view of the apparent failure of the 6th amendment to accelerate the introduction of innovative methods to replace animal testing in the cosmetic industry, the EC introduced the 7th amendment (F4) (Rosholt, 2005). In this amendment to the Cosmetics Directive, two bans were enforced effective from March 11th 2009 (European Commission, 2003): (i) testing ban

23 "Reference standard is a substance which has a known degree of toxicity in vivo, and which can be used in vitro to determine the degree of toxicity of test substances, whose effects are scaled relative to the RS." (Balls et al., 1999, p 61).

(the use of animals for testing of cosmetics or cosmetics ingredients is banned in the European Union) and (ii) marketing ban (a ban on the selling of products (containing ingredients) tested on animals, with the exception of tests for repeated-dose toxicity, which should be banned on the condition that animal-free methods are available). This 7th amendment is a unique regulative institution because it phases out essential safety tests before alternatives are formally validated, making postponement of the deadlines impossible (European Commission, 2003, recital 5). The message of the marketing ban in the 7th amendment was clear and provided a major stimulus to invest in the validation of innovative methods for eye irritation testing (Researcher 1, 2011; Researcher 3; 2011; SMO, 2011; Industry 2, 2011; Industry 3, 2011; Industry 4; 2011).

In 2004 a thorough review of the status of the innovative methods for eye irritation was carried out on behalf of the EC (F2) (Eskes et al., 2005). It was concluded that none of the methods could be validated because they did not meet the requirements regarding the correlation with results of the Draize test (regulative institution). Furthermore, it was noticed that innovative methods often had good correlation with the results of parts of the eye irritation spectrum assessed in the Draize test, i.e. some methods showed good correlation with the Draize test for the identification of intermediate to severe eye irritants, whereas other methods showed good correlation with the Draize test for the identification of mild to non-eye irritants. This led to a major shift in a cultural-cognitive element of the safety regulation logic: it became clear that under current conditions, it would be impossible to find a single replacement for the Draize test thus innovative methods should be validated to assess parts of the eye irritation spectrum (Eskes, 2010).

To further elaborate this new strategy to replace the Draize test, the ECVAM organized an expert meeting in 2005 (F2 and F3) (Eskes, 2010). A solution to the detection limits of individual innovative methods would be to make use of combined testing (tiered-testing) strategies that utilize strengths of individual methods to assess a specific range of chemical classes or eye irritation potential (Researcher 1, 2011; Industry 3, 2011; Regulator; 2011). A tiered-testing strategy would use multiple tests to replace the Draize test, e.g. one for the non to mild irritancy range and one for the moderate to severe irritancy range (figure 2). This eventually changed the validation process of innovative methods (Scott et al., 2010; Researcher 1, 2011; Researcher 3, 2011; Regulator, 2011).

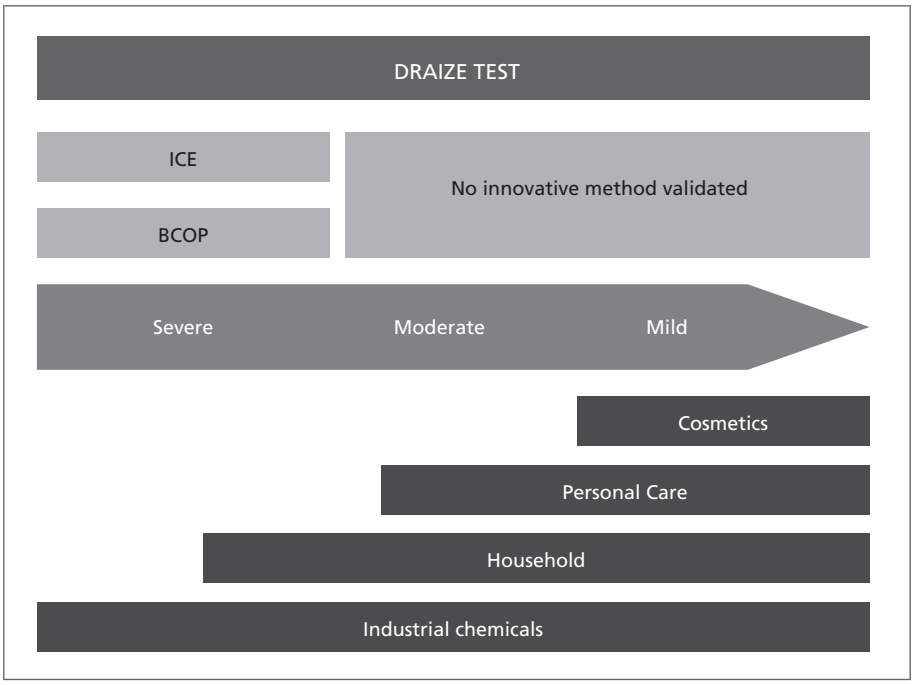

Figure 2: Tiered testing strategy

Validation of innovative methods for tiered-testing strategies was performed retrospectively, i.e. with data already available from previous studies, eliminating the need to perform additional tests (Researcher 1, 2011). The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), together with ECVAM, conducted such a study between 2003 and 2006 (F2) (Eskes, 2010). As part of the retrospective validation study, the ICCVAM compiled extensive reports (Background Review Documents (BRD)) on four innovative methods[24] and organized an international panel discussion to discuss the current validation status of these methods (F3). The conclusions of this expert panel report formed the basis for the acceptance of the ICE and BCOP tests by the regulatory authorities in the United States (NICEATM-ICCVAM, 2006). The ECVAM also based their recommendations on this review (ESAC, 2007). In 2009 the OECD adopted the ICE and BCOP tests for moderate and severe eye irritants in test guidelines 437 and 438 on the basis of the BRD and validation data (OECD, 2009, 2009a).

24 Bovine Corneal Opacity and Permeability (BCOP), Isolated Chicken Eye (ICE), Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) and Isolated Rabbit Eye (IRE)

5. ANALYSIS

The narrative showed that understanding of the institutional logic wherein the Draize test was embedded enriched the understanding of the transition for the Draize test to innovative methods. Although the emergence of innovative methods was crucial, the main barriers for the replacement of the Draize test did not originate in the technological innovation system, but in the institutional logic of safety regulation of medicines, cosmetics and chemicals.

The anti-Draize campaign delegitimized the use of the Draize test and thereby created legitimacy to develop innovative methods. As a result, resources to develop animal-free methods were made available by the cosmetic industry. This preluded the buildup of the TIS of animal-free methods for eye irritation testing. This innovation system was very successful in developing animal-free methods. The implementation of these animal-free methods was hampered, because innovative methods were not based on living systems. Innovative methods therefore deviated from the established practice and had a misfit with the institutional logic of safety regulation of medicines, cosmetics and chemicals.

Based on beliefs in and assumptions about the value of the Draize test to predict human risk, this test was taken for granted and backed up in regulation. This alignment of beliefs, assumptions, norms and rules in the logic of safety regulation of medicines, cosmetics and chemicals reinforces the use of the Draize test. The societal function of safety regulation further strengthened this logic, because regulators are risk averse when it comes to changing regulative practices that are established to safeguard public health. As a result of this norm to act precautious when it comes to changing established practices, the regulators required unfeasible validation endpoints; innovative methods should score as good as the Draize test. The unfeasible validation endpoints in combination with the prevailing assumption that the Draize test should be replaced by one single innovative method raised high barriers for the development and implementation of innovative methods. So, changes in the institutional logic of safety regulation were necessary to implement the innovative methods developed in the TIS.

The implementation and reinforcement of a ban on animal testing for the development of cosmetics in the European Union provide the momentum to achieve change in

the institutional logic of safety regulation. This ban created urgency to realize the replacement of the Draize test. Workshops bringing together the relevant actors were organized to resolve the misfits between innovative methods and the institutional logic that hampered validation. The workshops resulted in two changes in the safety regulation logic. As a result of the first workshop it was concluded that the use of reference standards provided better insight in the performance of animal-free methods than the results of the Draize test. During the second workshop the assumption that the Draize test had to be replaced by one single animal-free method was contested. This led to the tiered-testing strategy. These two changes made validation of the ICE test and BCOP possible and resulted in the implementation of both tests in the OECD test guidelines in 2009. Thus, the replacement of the Draize test was enabled by changes in the institutional logic of safety regulation.

6. CONCLUSION

This study analyzed the replacement of the Draize eye test by the animal-free ICE and BCOP test to assess if adding an analysis of the institutional logic, wherein the established practice is embedded, to the functions of the TIS approach enriches the understanding of innovation processes. In this way we aimed to contribute to the conceptualization of the system around established practice in transition studies.

The analysis of replacement of the Draize test by innovative methods showed that the dominant institutional logic induced barriers to innovation and that changes in this logic were crucial to overcome these barriers and enable innovation. Using only the functions of the TIS approach would have provided insight in the development and implementation process of animal-free methods and enabled us to identify the barriers that hampered this process. The results, however, would not have explained the origin of these barriers which is essential for resolving them. By using the TIS approach, for instance, validation would have been identified as a barrier that hampered the implementation of the ICE and BCOP test for two decades. Furthermore, it would be concluded that this barrier was overcome by a change in the validation strategy and endpoints. Nevertheless, it would have remained unclear why this barrier was there and what actions were necessary to realize these changes that enabled overcoming this barrier.

Only analyzing the dynamics of the institutional logic wherein the Draize test was embedded would have provided insight in how the institutional logic transformed this practice over time. Nevertheless, it would have taken the emergence of innovative methods to assess eye irritation as given. For instance, it would have remained unclear why the EET was not further developed. It would neither have enabled us to explain why changes in the logic, as for example the shift towards tiered-testing, were crucial to enable the success of the animal-free methods.

In this paper we concluded that by analyzing innovation processes using either the functions of the TIS approach or a perspective based on the institutional theory, leads to underexposure of one of two the aspects enabling innovation: (i) the emergence of technologies or (ii) escaping the established practice. This results in a biased understanding of the innovation process. Combining the functions of the TIS approach with an analysis of the institutional logic wherein the established practice is embedded, reduces this bias because it allows gaining in depth insight of both aspects that enable innovation. This combined approach thereby provides an improved understanding of the processes that hamper and facilitate innovation and transitions.

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ANNEX II

ANNEX III

THE VALUE OF NONHUMAN PRIMATES IN THE DEVELOPMENT OF MONOCLONAL ANTIBODIES

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ABSTRACT

Nonhuman primates (NHP) are often assumed to be the only relevant species to evaluate safety and efficacy of monoclonal antibodies (MAbs). However the scientific value of NHP in their development has never been established. Using drug registration files of all MAbs marketed in the European Union, we studied the value of using NHP to evaluate the safety and efficacy of these products. Inadequate justification of NHP use and ineffective study design led to a needless increase of NHP use. Immunogenicity further limited the value of studies in NHP. As a predictive model NHP do not stand out because MAbs only exert their expected pharmacological action. Nevertheless, their use continues to increase. Therefore, a reevaluation of the need for routine studies with NHP to develop MAbs is urgently required.

1. INTRODUCTION

In the 1980s the first recombinant biotech products started coming onto the market. From discussions on the new challenges these products posed for drug safety testing, it became clear that non-clinical safety programs used for small molecule therapeutics would not be appropriate (1,2). These discussions eventually led to the publication of a recently updated international guideline adopted by FDA, EMA and other regulatory bodies called 'ICH S6 - preclinical safety evaluation of biotechnology-derived pharmaceuticals'(3).

Non-clinical testing of small molecules is driven by an extensive set of guidelines which covers the non-clinical testing program. In contrast, ICH S6 offers a flexible, science based, and case-by-case approach to develop biotech products. For instance, animal studies to determine metabolism and genotoxicity are not required (therapeutic proteins are degraded to peptides and amino acids and it is not expected that therapeutic proteins interact with DNA). Carcinogenicity testing is only required if the mechanism of action of a therapeutic protein raises concerns. In the case of MAbs, most effects are highly species specific and often only nonhuman primates (NHP) possess the relevant target antigen. As a result, they are often considered the only relevant species for non-clinical studies. The use of NHP to assess the safety of monoclonal antibodies (MAbs) in drug development seems legitimate because they are often considered the only species sensitive to the adverse effects. However, the use of NHP poses ethical, practical and financial hurdles. But from a scientific point of view their use is also questionable (4).

Surprisingly few studies have been done to assess the value of NHP use in drug development and those have mainly focused on how to reduce their use (5-7). Some of these have suggested that, for small molecule therapeutics, NHP studies often under-predict serious toxicity and are less predictive for humans as assumed (8). This was also the case in the incident involving the phase I clinical trial with a CD28 superagonist monoclonal antibody, TGN-1412. There, six volunteers developed a severe immune reaction known as a 'cytokine storm' soon after receiving the first infusion. Because TGN-1412 does not act as a superagonist in NHP, this effect was not observed in safety studies and the product was considered safe in NHP (9).

Despite uncertainties over NHP use, their use in drug development has increased substantially has over the years because species specific MAbs with a broad range of indications are a fast growing class of products (10). Increasing regulatory demands are also believed to contribute to this rise (5). Scientific evidence is urgently needed to have an informed discussion on the use of NHP in the development of biotech products. We performed a comprehensive study on the value of NHP in the development of MAbs. To perform this study we had unique access to confidential drug registration files, which contains the results of all animal studies done to support marketing authorization, of all MAbs approved in the European Union. To our knowledge, this source has not been used for analysis before. This is also the first comprehensive study to include all MAbs since muronomab (Orthoclone-OKT3) was registered in the late eighties.

2. MONOCLONAL ANTIBODY AUTHORIZATIONS IN THE EU

33 MAbs have been approved in the EU. IgG1 was the most common isotype (n=19) and seven products were Fab' fragments (Figure 1). Antineoplastic (n=8) or immunomodulatory (n=13) indications were dominant for older products, although often extension of indication were sought for products later (for example, adalimumab, trastuzumab, infliximab). More recently, six indications other than antineoplastic, immunomodulatory or diagnostic agents have been registered (Figure 1). Early MAbs were predominantly murine and rodent-human chimera. Humanized MAbs, which are considered to have an improved immunogenicity profile, are currently the main form of therapeutic MAbs and fully human MAbs are a rapidly growing class of products. Six MAbs have been withdrawn from the market. For only one product (Efalizumab, Raptiva) this was related to safety issues which were identified after marketing authorization (11).

3. NHP USE IN NUMBERS

NHP were not used to assess the safety of six MAbs. Five of these were murine monoclonal antibodies, of which four have no target antigen in NHP because they are intended for diagnostic use in cancer. The fifth murine antibody is muronomab

that targets to the T3 antigen of CD3 positive T-cells and is indicated for graft rejection therapy. This antigen is only present in humans, chimpanzees and gorillas. In agreement with the FDA, toxicity studies were not requested. Japanese regulatory authorities requested toxicity studies in rat and mouse with oral, iv and sc exposure which did not result in evidence of toxicity. Finally, for the sixth MAb, Eculizumab (Soliris), a humanized monoclonal antibody that targets C5 complement, non-clinical efficacy and safety testing was done using a surrogate antibody in mice (without signs of toxicity) because this MAb did not cross-react with its target in a range of commonly used NHP and other species (12). 6,045 NHP were used in the non-clinical programs of the remaining 27 monoclonal antibodies (Table 1).

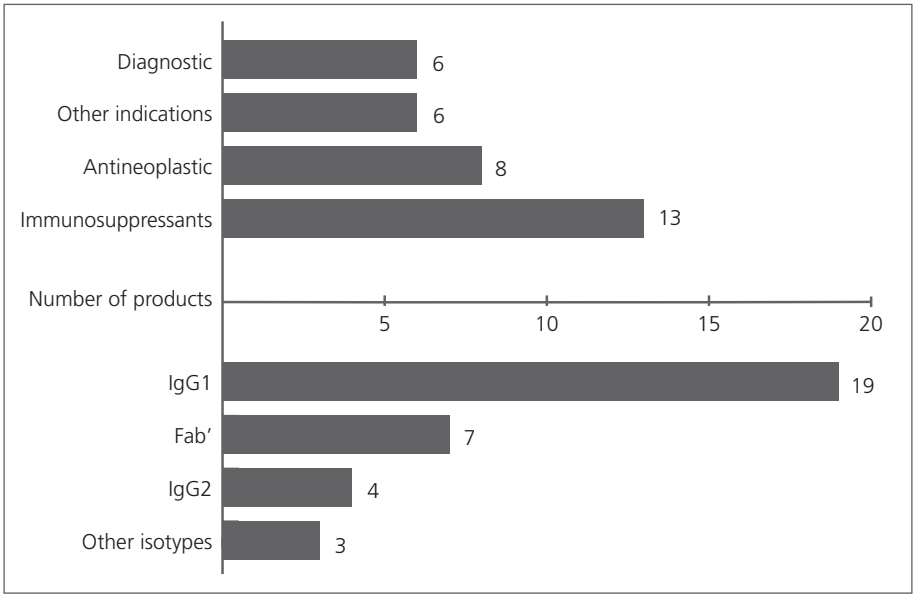

Figure 1: Number of products by indication and isotype (n=33).

Above x-axis: antineoplastic and immunomodulatory agents form over 63% of all monoclonal antibodies on the market today. Aside from diagnostic agents, other monoclonal antibodies include a radiotherapeutic, an ophthalmological, an anti-thrombotic, an anti-osteoporotic and an anti-infective agent and a monoclonal antibody against obstructive airway disease. Below x-axis: monoclonal antibodies of the IgG1-isotype have been the primary source for development of therapeutic antibodies.

On average, a non-clinical program used 224±212 NHP with a median of 164 NHP. In one program, one cynomolgus monkey was used to assess pharmacokinetics. The most NHP that were used in a non-clinical program was 755. Interestingly, the use of NHP increased as monoclonal antibodies became more human (Figure 2). There is a moderate correlation for increasing use of NHP in human MAb development over time (linear regression correlation coefficient r2=0.698 p=0.0098, data not shown). For murine, chimera and humanized products no temporal correlation was found. Finally, except for the indication of diagnostic agents, where few NHP are used, there is no difference between NHP use for immunomodulatory, antineoplastic and other indications (data not shown).

TABLE 1: THE MAJORITY OF NHP USED IN DRUG DEVELOPMENT IS CYNOMOLGUS (86%). IN ONLY THREE CASES (INFLIXIMAB, EFALIZUMAB AND OMALIZUMAB), CHIMPANZEE IS USED DURING THE NON-CLINICAL DEVELOPMENT PROGRAM.

SPECIES	TOTAL NUMBER OF NHP (PRODUCTS USING THIS SPECIES FOR DEVELOPMENT)
Cynomolgus	5171 (24)
Rhesus	391 (6)
Marmoset	388 (2)
Chimpanzee	68 (3)
Baboon	18 (1)
African Green	9 (1)
Total NHP	6045

4. SAFETY EVALUATION OF MABS IN NHP

15 MAbs (56%) were well tolerated in non-clinical studies using NHP, even when the animals were given high doses, and severe adverse effects were not noted. Other

than the intended pharmacology, effects that were observed were generally mild and related to (secondary) pharmacology or injection site reactions. The remaining 12 MAbs induced more severe pharmacology or immune mediated reactions including death. Developmental and reproductive toxicity studies have been conducted in NHP for the safety assessment of 12 MAbs. This included all human MAbs except for votumumab, a diagnostic agent. In all but three cases, exposure to MAbs did not result in any effects on fertility or embryofetal development. In these three cases the effects were the result of the primary or secondary pharmacological action of the MAb (Box 1).

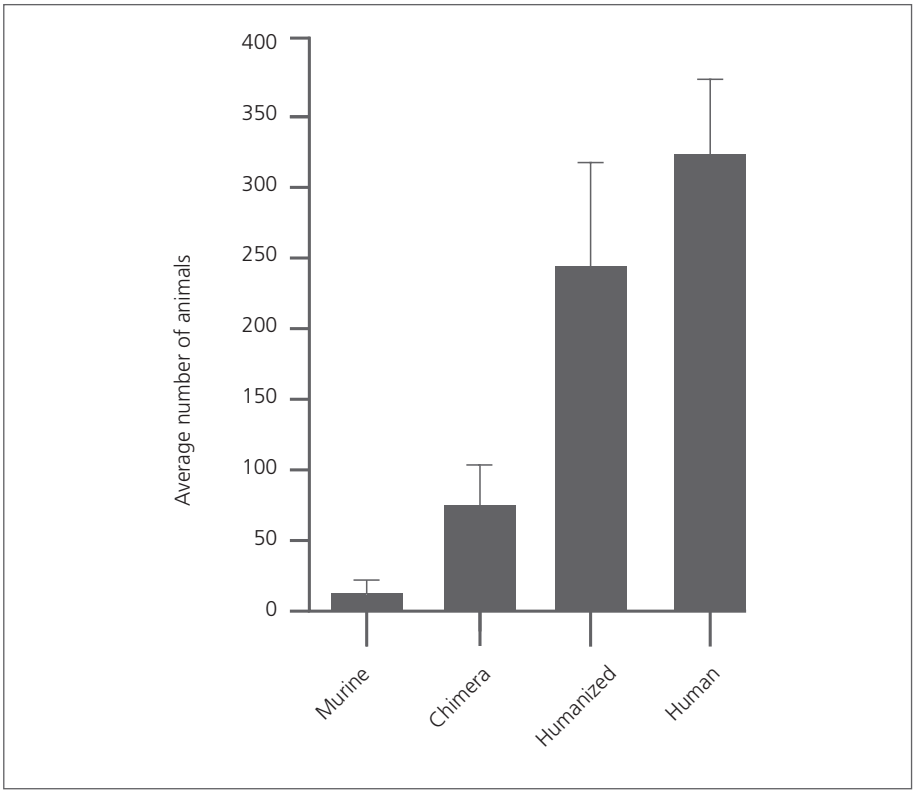

Figure 2: The average use of NHP per non-clinical programs

The average use of NHP per non-clinical programs increases as the MAb in development becomes more human ±SD. As humanization occurs more frequently in newer products, this increase may also reflect a temporal trend. The increase occurs irrespective of species specificity of the MAb. There is a significant difference between the group averages (non-parametric Kruskall Wallis test, p=0.001354. ANOVA with post-hoc Bonferroni comparison of means showed a significant difference between murine and human averages, p=0.01144)

BOX 1: REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF MABS ARE ALSO PHARMACOLOGICALLY MEDIATED

Panitumumab (Vectibix), an EGFR inhibiting antibody, increased the duration of the menstrual cycle (24). This effect may be pharmacological since EGFR is involved in endometrial growth and differentiation and may play a role in fetal implantation (25,26) but could also have been a secondary effect to weight loss in these animals since there was no effect on female reproductive organs. Similarly, treatment with Bevacizumab (Avastin), a VEGF inhibitor, resulted in reduced ovarian function in NHP (27). In the case of Natalizumab (Tysabri), a significantly increased abortion and stillbirth rate was observed (20). This may be related to involvement of integrins (the target of natalizumab) in placental and embryofetal development (28,29). However, in a recent study, cynomolgus monkeys exposed to Natalizumab until gestation day 100 did not exhibit these adverse developmental effects (30,31).

5. JUSTIFICATION OF NHP USE

For the non-clinical development of monoclonal antibodies choosing a relevant model species is paramount (13). Currently, ICH S6(R1) suggests the use of a species in which the product binds to its target and is pharmacologically active. A justification for the choice of species is also required if only one relevant species can be identified. The majority of study programs (78%, n=21) justified their choice for NHP by the ability of the MAb to bind to its target. But only in six cases the justification was also based on observed pharmacological activity following this binding.

In eight study programs (30%) the justification was either lacking, did not sufficiently take into account availability of other non-rodent species or NHP only provided a limited value. NHP were used in the case of Palivizumab (Synagis) because it was a commonly used species in monoclonal antibody development. In this case, NHP was used as a second, non-rodent, species next to rat and rabbit. The relevance of NHP use in this case was limited because Palivizumab does not have a target in NHP or human (14). In the case of votumumab (HumaSPECT), the non-clinical summary did not include a justification for the use of NHP. Reduced affinity for the target epitope (Alemtuzumab, MabCampath) (15) or reduced activity (Certolizumab, Cimzia) (16) limited the relevance of NHP as a model species in two non-clinical programs. However, other non-rodent models were not available and so, NHP were considered

as the only relevant option. Despite identifying marmosets as the only relevant NHP species for non-clinical studies with Canakinumab (Ilaris), one pharmacokinetic study was conducted in rhesus monkeys (17). Catumaxomab (Removab) does not bind to NHP tissue. However, one cynomolgus monkey was used in a single dose immunogenicity/tolerance study (18). The affinity of Ranibizumab (Lucentis) for its target, VEGF-A, in NHP was not determined, although sequencing demonstrated an almost identical protein sequence to humans and predicted a high homology with the human target (19). Natalizumab (Tysabri), a alfa-4-integrin inhibitor, binds to its target in several species; in addition to NHP, the dog, pig, ferret and guinea pig were all found to be a suitable model species with similar binding affinities for one target as humans. Dog (safety pharmacology) and guinea pig (multiple sclerosis model, reproductive toxicity) were accordingly used in non-clinical studies. Nevertheless, the non-clinical program included extensive testing in NHP in addition to studies in rodents. The latter were considered to have limited value because the MAb did not bind to rodent alfa-4-integrin (20). Although species specificity of the MAb or its target often justified the use of one species, only one MAb (Basiliximab, Simulect) has been developed using only NHP. In the majority of cases rodents were extensively used as the second species for safety assessment even though these were not always a relevant species. Similarly, non-rodent species other than NHP were also used although the MAb was not necessarily pharmacologically active in those species.

6. DIFFERENT SPECIES OF NHP IN MAB DEVELOPMENT

If NHP were considered as the primary model species, the non-clinical program generally followed or exceeded those studies outlined in the ICH S6 guideline. The NHP species in most non-clinical programs was the cynomolgus macaque. Other species such as the rhesus macaque and the marmoset were also used as the primary model species in some programs. Eight non-clinical programs used two or more NHP species. This included the use of NHP in routine non-clinical studies, biocomparability studies and as animal models of disease such as thrombosis, organ transplantation and choroidal neovascularization. In three non-clinical programs chimpanzees were used. In these cases poor study design or the low number of animals in the different study groups limited the value of the studies (Box 2).

BOX 2: USE OF CHIMPANZEES IN MAB DEVELOPMENT

In cases where great apes are used the utmost care should be taken to ensure that the experiments are of the highest quality. This was not the case for three products that used chimpanzees in non-clinical study programs initiated between 1991 and 2000. In two cases, chimpanzees were used as the primary test species due to the species specificity of the target antigen whereas in one case chimpanzees were used in one study alongside other NHP species to elucidate the mechanism of an adverse effect. A common feature of programs where chimpanzees were used as a primary species was an insufficient a priori justification of their use and a scientific study rationale. The subsequent problematic study design and conduct led to a limited value of their use to evaluate the safety of these products. Likely because of ethical considerations, cost and/or difficulties in handling chimpanzees, few animals were used in the non-clinical programs. Low number of chimpanzees study or per group within a study negatively influenced the scientific value due to lack of statistical power. Studies as small as one animal per study or one animal per group were recorded. However, such studies may still be useful in hazard identification. Even so, regulatory authorities considered one of the programs to be of marginal value for the human safety assessment due to uncertainties in the conduct of experiments and their study design (32).

7. IMMUNOGENICITY IN NHP STUDIES

Anti-drug-antibodies (ADAs) may be formed in NHP as an auto-immune response to exposure to therapeutic MAbs and the can influence the activity of these products in several ways; ADAs may neutralize the effect of the MAb by binding with high affinity to their active site; ADAs can affect pharmacokinetic behavior of the MAb by clearing it from the body; and ADAs can enhance the pharmacological effect of MAbs. MAbs were immunogenic in most non-clinical programs which used NHP (93% n=25). ADAs influenced pharmacokinetic or pharmacodynamic parameters of MAbs in the majority of (78%, n=21) the programs. For one of the products, immune responses led to anaphylactic shock. In twelve programs pharmacokinetic or pharmacodynamic profiles were changed to some extent due to formation of ADA. In nine cases a considerable number of monkeys were affected by immunogenicity leading to a limitated duration of long term toxicity studies.

8. OTHER FACTORS INFLUENCING THE VALUE OF NHP

Immunogenicity and species specificity were not the only factors which reduced the value of NHP studies. Wild caught monkeys were used as a model species in two non-clinical programs. But wild caught NHP may be inappropriate because their age, genetic background, exposure to pathogens, environmental and social conditions are not known. In both non-clinical programs, high inter-animal variability interfered with interpretation of study results.

Study design also limited the scientific quality of the data. Some studies were initiated that could be considered irrelevant a priori, for example because the route of administration in the study was oral, even though MAbs are not taken up by the gut; the MAb was known not to be active in NHP; or developmental and reproductive toxicity studies may not have been necessary because the MAb was developed as an antineoplastic intended for co-treatment with cytostatics. In a few cases, studies requested by regulatory authorities were not relevant.

All studies with NHP used necropsy to evaluate the effects of the MAb at a target organ level with the exception of the programs that used chimpanzees. Necropsy was performed in control and treatment groups before and after a recovery period to study the mechanism of action, effects at the tissue level and reversibility of adverse effects. In most cases, necropsy confirmed findings from non-invasive methods such as clinical observations and blood chemistry but did not reveal any new adverse effects.

9. DISCUSSION

One important limitation of our analysis is that we have only used data from MAbs which received a marketing authorization; we did not have access to data from MAbs that were never marketed. The number of NHP used per MAb and other metrics concerning NHP use are a conservative estimate since we could not always recover all the data for all studies.

When most companies started their non-clinical development phase, little was known about sustained effects of therapeutic MAbs in a complex organism such as NHPs. How MAbs mediate safety issues either through on-target and off-target pharmacology, toxicity, complement activation or immune responses was not as well understood as it is today. The use of a relevant NHP model to investigate the non-clinical fate of MAbs has led to the current body of work, spanning almost 30 years and a wide variety of antibodies and targets. This current state of the art continues to evolve and would not be as complete without NHP studies and for this reason alone their past use has not been altogether valueless.

The use of NHP in research is rising because of an increasing development of MAbs but a scientific basis for their increase is lacking. The use of NHP is primarily justified by the expression of the relevant target epitope with a comparable tissue distribution to human. NHP have been used even if they lack this attribute. The widespread use of rodent species in non-clinical programs is also surprising. ICH S6 explicitly allows for exclusion of multiple species testing in favor of testing in one scientifically relevant species if this can be justified. The choice for two species testing may be driven by risk averse behavior and uncertainty about regulatory demands. The strategy to simply follow the guidances rather than develop a limited but science based non-clinical program is further supported by the fact that most non-clinical programs generally followed or exceeded the studies outlined by the guidelines.

An increase in the use of NHP was observed as MAbs became more human, but increased humanization does not necessarily restrict the number of species which possess the relevant target antigen. Likely, the increase in their use occurred because NHP are perceived to better predict pharmacokinetic behavior and to show less immunogenicity due to the close relationship with human or because of increased affinity of the MAb in NHP. Nevertheless, most products were immunogenic in NHP. Immunogenicity further compromised the predictive value of NHP because it can lead to limitations in study duration and loss of dose groups that could not be evaluated because of clearing ADAs. In addition, sub-optimal study design and the use of NHP when it was not a relevant species limited the value of NHP studies.

The main reason NHP are of very limited value is because the adverse events induced by MAbs are highly predictable. Those effects are either mediated by the pharmacology of the MAb which may be exaggerated by dose or exposure or they are mediated by

immune responses mounted against the therapeutic MAb (21-23). This was confirmed in our analysis where we have also not found any likely off-target effects. This included findings from post-mortem and developmental and reproductive toxicity studies. However, there is still some remaining value for NHP use in MAb development. In science driven proof of concept studies they may be informative or they can be used in small confirmatory studies once the target pharmacology is characterized in vitro.

In conclusion, the value of extensive NHP use in routine safety and efficacy studies for the non-clinical assessment of MAbs is scientifically debatable and may, in that way, also miss a moral basis. Incentives for pharmaceutical companies to develop biotech products without the use of animals may help in facilitating the much needed innovation in non-clinical drug development. Dialogue between pharmaceutical industry and regulatory authorities on non-clinical requirements should be increased. More importantly however, an in depth reevaluation of the value of NHP in all stages of drug development, including products which did not reach the market, is long overdue.

10. METHODS

Monoclonal antibodies (MAbs), and immunoglobulin fragments, which received a marketing authorization in the EU or one of its member states up to 01-06-2011 were identified from websites of the EMA, Medicines Evaluation Board (CBG-MEB) and literature (33,34). For each MAb the drug registration file (common technical document, CTD) was located at the archives of the CBG-MEB in The Hague. For one MAb (Xolair, Omalizumab), the non-clinical overview was obtained from the EMA. The non-clinical overview and tabulated non-clinical summaries contained in the CTD were used as the primary source for this study. The total number of NHP per product is the sum NHP per study described in the tabulated summary and includes F_1-offspring generated in developmental and reproductive toxicity testing, if applicable. For studies providing ranges of animals used, the range average was used. If no accurate count could be obtained and individual study reports in module 4 of the CTD could not be accessed, counts were left blank. Qualitative data was extracted, summarized and categorized by the first author.

11. STATISTICS

Because some products did not use NHP, our samples contain 0 as an outcome. Therefore, we added 1 to all our NHP counts for statistical analysis. SPSS (version 16.0 for Windows, IBM, Armonk, NY, USA) was used to conduct the non-parametric Kruskall-Wallis test with a Bonferroni post-hoc test to identify variance between groups. Prism (Prism 4 for Windows, v.4.03, GraphPad Software Inc., La Jolla, CA, USA) was used for linear regression.

CONFLICT OF INTEREST

P.J.K. van Meer, M. Kooijman, JW van der Laan and E.H.M. Moors declare no conflict of interest. H. Schellekens participated in meetings and publications sponsored by Amgen, Johnson & Johnson, Roche, Sandoz and Hospira. Part of his research is directly or indirectly sponsored by Roche and Amgen.

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ANNEX IV

IMMUNOGENICITY OF MABS IN NON-HUMAN PRIMATES DURING NONCLINICAL SAFETY ASSESSMENT

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ABSTRACT

The immunogenicity of biopharmaceuticals used in clinical practice remains an unsolved challenge in drug development. Non-human primates (NHPs) are often the only relevant animal model for the development of monoclonal antibodies (mAbs), but the immune response of NHPs to therapeutic mAbs is not considered to be predictive of the response in humans because of species differences. In this study, we accessed the drug registration files of all mAbs registered in the European Union to establish the relative immunogenicity of mAbs in NHPs and humans. The incidence of formation of antidrug-antibodies in NHPs and patients was comparable in only 59% of the cases. In addition, the type of antidrug-antibody response was different in NHP and humans in 59% of the cases. Humanization did not necessarily reduce immunogenicity in humans. Immunogenicity interfered with the safety assessment during non-clinical drug development when clearing or neutralizing antibodies were formed. While important to interpret the study results, immunogenicity reduced the quality of NHP data in safety assessment. These findings confirm that the ability to compare relative immunogenicity of mAbs in NHPs and humans is low. Furthermore, immunogenicity limits the value of informative NHP studies.

1. INTRODUCTION

The advent of recombinant technology some 30 years ago led to revolutionary novel methods of drug development that allowed the production of any protein-based drug in cell culture. These protein-based biopharmaceuticals offer important advantages over classical small-molecule drugs, such as longer half-lives and very high specificity. In contrast to small molecules, which are metabolized, therapeutic proteins are degraded into their constituent amino acids. As a result, most adverse effects are a result of exaggerated pharmacodynamics.[1] An unsolved challenge in biopharmaceutical development is that these proteins ultimately become immunogenic in some patients, provoking an immune response. The immunogenicity of the agents is dependent on specific properties, such as protein folding, aggregation, post-translational modifications and the presence of B and T cell epitopes. In addition, the presence of impurities in the formulation, the route of administration, mode of action, patient population and treatment regimen may all affect immunogenicity.[2] In clinical practice, a drug-evoked immune response can lead to a host of side effects, such as serum sickness, hypersensitivity and injection site reactions or, in some rare cases, hazardous cross-reactivity with endogenous proteins.[3] More commonly, the immune response leads to a loss of drug efficacy because of the development of neutralizing or clearing antidrug antibodies (ADAs).[4]

The engineering of proteins may yield potentially marked reductions in immunogenicity of protein-based drugs.[5] It is, however, difficult to evaluate the immunogenicity of mAbs because there are few robust and predictive bioinformatics approaches or in vitro screens to measure and characterize the immune response. Bioinformatics approaches have been developed that can identify immunogenic T cell epitopes,[6] and removal of these T cell epitopes is suggested to reduce immunogenicity.[7] Harding et al. have shown that removal of $CD4^+$ T-helper cell epitopes from V-region peptides of the chimeric antibody cetuximab by humanizing these peptides results in a reduction in immunogenic potential.[8] T cell activation assays could also be used to measure the potential of protein drugs to evoke an immune response,[9,10] but, in non-clinical safety assessment, these studies are not required and laboratory animals are routinely used to evaluate immunogenicity. The predictive value of immunogenicity measured in common animal models such as rodents and dogs, however, is low because these models generally overpredict immunogenicity in humans.[11] Non-clinical immunotoxicity studies in animals are also considered inadequate to evaluate safety issues related

to immunotoxicity such as hypersensitivity and auto-immunity.[12] The shortcomings of animal studies are reflected in international and European immunogenicity guidelines.[13,14] Although the assessment of immunogenicity in non-clinical studies is not recommended as a way to estimate the response in humans, animals may be useful to study some aspects of immunogenicity, such as determining the relative immunogenicity of a biosimilar compared with its reference product[15] and to interpret the findings from animal studies.[16-18]

Besides the low predictive value of immunogenicity in animals, a major handicap is that assays used to assess the immunogenicity of therapeutic proteins are not standardized. A recent industry survey showed that several assays are being used that, although complying with general guidelines, often yield variable results that cannot be compared because of different assay formats. Moreover, the lack of a reference standard, among others, makes these assays semi-quantitative.[19] This makes direct comparisons of immunogenicity between products and species particularly challenging, if not impossible. The relative immunogenicity of mAbs in humans and animals has been assessed in the past.[11,17] Here, we provide an overview and comparison of the immunogenicity in NHPs and humans of all mAbs approved for use in the European Union (EU) through 2010. We also studied the influence of immunogenicity on the ability to interpret non-clinical study findings. For this study, we had access to the marketing authorization applications, which contain all animal studies done to support marketing authorization of mAbs approved in the EU.

2. RESULTS

2.1 IMMUNOGENICITY IN NHPS

Of 33 mAbs in our analysis (Table 1), the safety of 27 mAbs was evaluated in NHPs. MAb29 and MAb31 were not immunogenic in NHP, whereas the remaining 25 MAbs (93%) were. Moreover, the presence of ADAs led to changes in the pharmacokinetic or pharmacodynamic profiles of all but four mAbs: MAb8, MAb13, MAb14 and MAb17. For the remaining 21 products, immunogenicity influenced pharmacokinetics and pharmacodynamics with varying magnitude and severity (Table 2). Repeated dose studies for five MAbs (MAb7, MAb9, MAb14, MAb15 and MAb21) were limited in duration due to immunogenicity.

TABLE 1: MONOCLONAL ANTIBODIES APPROVED IN THE EUROPEAN UNION FROM 1988 TO 2010

INN	BRAND NAME	APPROVAL DATE
Abciximab	Reopro, CentoRx	1995**
Adalimumab	Humira, Trudexa	2003
Alemtuzumab	MabCampath	2001#
Anti-melanoma antibody fragments*	Tecnemab-K1	1996#
Arcitumomab*	CEA-scan	1996#
Basiliximab	Simulect	1998
Besilesomab*	Scintimun	2010
Bevacizumab	Avastin	2005
Canakinumab	Ilaris	2009
Catumaxomab	Removab	2009
Certolizumab pegol	Cimzia	2007
Cetuximab	Erbitux	2004
Daclizumab	Zenapax	1999#
Denosumab	Prolia	2010
Eculizumab	Soliris	2007
Efalizumab	Raptiva	2004#
Golimumab	Simponi	2009
Ibritumomab tiuxetan	Zevalin	2004
Igovomab*	Indimacis 125	1996#
Infliximab	Remicade	1999
Muromonab	Orthoclone-OKT3	1988**
Natalizumab	Tysabri	2006
Ofatumumab	Arzerra	2010
Omalizumab	Xolair	2005
Palivizumab	Synagis	1999
Panitumumab	Vectibix	2007
Ranibizumab	Lucentis	2007
Rituximab	Mabthera	1998
Sulesomab*	Leukoscan	1997
Tocilizumab	RoActemra	2009
Trastuzumab	Herceptin	2000
Ustekinumab	Stelara	2009
Votumumab*	Humaspect/ Oncospect CR	1998

INN: International nonproprietary name.

The infixes that immediately precede -mab indicate the sequence source: u, human; zu, humanized; xi, chimeric; o, mouse; axo, rat/mouse.

*Diagnostic/imaging agent.
**Country specific approval
#Withdrawn from use in the European Union.

TABLE 2: INCIDENCE AND RESPONSE LEVEL, EITHER CLEARING OR NEUTRALIZING, OF ANTI-DRUG ANTIBODIES IN NON-HUMAN PRIMATES

ADA INCIDENCE IN NHP	INCIDENCE OF CLEARING OR NEUTRALIZING ANTIBODIES IN NHP		
	Low	Intermediate	Majority
Low (0–6%)	MAb12 MAb14 MAb16 MAb17 MAb25 MAb29 MAb31	MAb21	MAb30
Intermediate (6–45%)	MAb9 MAb26 MAb23 MAb27	MAb7 MAb10 MAb24	MAb20 MAb22 MAb32 MAb33
High (< 45%)			MAb5 MAb8 MAb11 MAb13 MAb15 MAb18 MAb28

ADA: Anti-drug antibodies. NHP: Non-human primate

2.2 MURINE ANTIBODIES

Three murine mAbs were assessed in NHPs. MAb5 was highly immunogenic because all NHPs developed clearing antibodies by the end of a one-month repeated dose study. MAb7 was moderately immunogenic in NHPs, with repeated dose studies being restricted to two weeks because ADA development was expected to interfere with the safety assessment. MAb8 had immunogenic potential only because ADAs were detected after the last dose in an escalating repeated dose study and it was assessed in only one NHP.

2.3 CHIMERIC ANTIBODIES

The safety of five chimeric mAbs was assessed in NHPs. MAb13 had low immunogenic potential and a low-titer ADA response was measured in one control group chimpanzee

that was accidentally dosed with MAb13. MAb10 and MAb11 had moderate immunogenic potential. In a two-week repeated dose study with MAb11, the death of one animal with high titers of ADAs was attributed to thrombocytopenia. Two animals with high ADA titers that received a second MAb11 dose rapidly developed thrombocytopenia. MAb9, MAb12 were highly immunogenic. Repeated dose studies with MAb9 were limited to eight weeks because of the development of ADAs, which resulted in the rapid clearance and decreased pharmacodynamics. The considerable ADA response made it difficult to generate conclusive data on the effects of long-term treatment in NHPs.

2.4 HUMANIZED ANTIBODIES

Eleven of the 12 humanized mAbs included in this study were evaluated in NHP. MAb14, MAb16, MAb17, MAb21 and MAb25 had low immunogenicity in NHPs, whereas MAb20, MAb22, MAb23 and MAb24 had moderate immunogenicity. In the case of MAb21, the duration of meaningful repeated dose studies was limited to one month. Anti-MAb24 antibodies were only measured in repeated dose studies. Interestingly, serum concentrations of MAb24 were increased in NHPs positive for anti-Mab24 antibodies. In addition, perivascular sheathing in some NHPs was associated with high anti-Mab24 antibody titers. MAb15 and MAb18 were highly immunogenic. Antibodies to MAb15 developed in most NHPs within two weeks of single or multiple doses and increased clearance. Immunogenicity was reduced when the dose was increased. In repeated dose studies with MAb18, antibodies to MAb18 were always associated with rapid clearance of the drug. Reliable estimates of pharmacokinetic parameters could only be obtained after the first dose because the development of ADAs interfered with the distribution and pharmacokinetics.

2.5 HUMAN ANTIBODIES

NHPs did not develop an immune response to MAb29 and MAb31. MAb30 was poorly immunogenic and MAb26, MAb37, MAb32 and MAb33 were moderately immunogenic in NHPs. In the case of MAb30, clearing antibodies developed only in single dose, but not repeated dose, toxicity studies. Antibodies to MAb26 were detected after repeated dosing. In some cases, the presence of ADAs was associated with increased plasma clearance. Two NHPs developed clinical hemolytic anemia that may have been secondary to high antibody titers. It is likely that ADAs developed

more often in MAb26-treated animals because a direct Coombs' test, which is used to determine autoimmune hemolytic anemia, suggested that most positive animals were slowly developing anemia. The pharmacokinetic profile of MAb33 was affected by the development of ADAs after repeat dosing, leading to an inverse dose–response relationship. Similarly, clearing antibodies to MAb32 were detected more frequently in NHPs receiving low doses. There, the presence of ADAs was associated with an increased clearance and reduced half-life and anti-MAb32 antibodies developed in up to 97% of the animals after a single low dose. One human mAb, MAb28, was highly immunogenic in NHPs and led to the formation of binding and neutralizing antibodies, and higher doses were needed to maintain exposure. Antibodies to MAb28 were formed in more than 50% of animals tested.

2.6 COMPARISON OF IMMUNOGENICITY IN HUMANS AND NHPS

Data on the clinical immunogenicity of all mAbs was included in the SPC (29 products) or EPAR (4 products). The induction of ADAs to 20 mAbs affected clinical efficacy, altered pharmacokinetic profiles or caused adverse effects. The presence of ADA to nine mAbs did not have consequences, and 4 mAbs did not give rise to ADA development (Table 3). For seven mAbs the incidence of ADAs in NHPs overpredicted the induction of ADAs in humans, and for four mAbs the reverse, underprediction was the case. Sixteen mAbs had comparable ADA incidences in both NHPs and humans. The ADA response was similar for nine mAbs, including four mAbs that did not cause an ADA response in either NHPs or humans. Two mAbs (MAb15 and MAb22) induced clearing and neutralizing antibodies in NHPs, but neutralizing antibodies only in humans. In NHPs, ADAs were more often directed against the Fc-region, resulting in clearing antibodies (17 out of 27 cases) whereas in humans, ADAs were most often formed against the complementarity-determining region (CDR), resulting in neutralizing antibodies (10 out of 33 cases).

TABLE 3: INCIDENCE AND EFFECT OF ANTI-DRUG ANTIBODIES IN CLINICAL TRIALS IN COMPARISON WITH NON-CLINICAL DATA

Product	Clinical immuno-genicity	ADA response clinical	Non-clinical immunogenicity	ADA response non-clinical
MURINE ANTIBODIES				
MAb1	Marked	Reduced efficacy due to interference	Not available	Not available
MAb2	Negligible	Diminished efficacy and allergic or hypersensitivity reactions	Not available	Not available
MAb3	Negligible	None	Not available	Not available
MAb4	Negligible	Diminished efficacy	Not available	Not available
MAb5	Tolerable	Diminished efficacy possible	High	Clearing
MAb6	Marked	Neutralizing and hypersensitivity	Not available	Not available
MAb7	Tolerable	Unknown	Intermediate	Clearing
MAb8	Marked	Neutralizing	High	None
CHIMERIC ANTIBODIES				
MAb9	Tolerable	Allergic or infusion site reactions in few patients	High	Clearing
MAb10	Tolerable	Clearing (in few patients)	Intermediate	Clearing
MAb11	Tolerable	Thrombocytopenia	Intermediate	Thrombocytopenia
MAb12	Tolerable	Unknown	High	Clearing
MAb13	Marked	Neutralizing and hypersensitivity	Low	None
HUMANIZED ANTIBODIES				
MAb14	Negligible	None	Low	None
MAb15	Negligible	Positive Coombs' test, Neutralizing antibodies	High	Clearing and neutralizing
MAb16	Negligible	Allergic reaction in 1 patient	Low	Clearing
MAb17	Negligible	None	Low	None
MAb18	Tolerable	Neutralizing	High	Clearing
MAb19	Tolerable	None	Not available	Not available
MAb20	Tolerable	Clearing	Intermediate	Clearing
MAb21	Tolerable	None	Low	Neutralizing and anaphylaxis

MAb22	Negligible	Neutralizing and hypersensitivity	Intermediate	Clearing and neutralizing
MAb23	Tolerable	Clearing	Intermediate	Clearing
MAb24	Tolerable	Possible role in inflammation	Intermediate	Perivascular sheathing, increased exposure
MAb25	Negligible	None	Low	Clearing
HUMAN ANTIBODIES				
MAb26	Negligible	Unknown	Intermediate	Anemia
MAb27	Tolerable	Neutralizing and binding	Intermediate	Clearing
MAb28	Negligible	None	High	Neutralizing
MAb29	Negligible	None	Low	None
MAb30	Tolerable	Neutralizing	Low	Clearing
MAb31	Negligible	None	Low	None
MAb32	Tolerable	Neutralizing and infusion reactions	Intermediate	Clearing
MAb33	Tolerable	Neutralizing	Intermediate	Clearing

3. DISCUSSION

Minimizing immunogenicity remains a considerable challenge in the development of mAbs. While the humanization of mAbs has been successful in reducing the immunogenicity of some products, clinically relevant immunogenicity can still occur despite such modifications.[20-22] Most mAbs in the clinic can be categorized as negligibly or tolerably immunogenic. The onset of ADA formation in the clinic usually occurs after multiple injections that can cover months of treatment. For physicians, treatment management should include frequent monitoring for neutralizing ADA and, when these occur, treatment should be stopped or the patient should switch to a new treatment.[23] Immunogenicity of single-use products such as diagnostics is generally not an issue, but it should be considered that when ADA to the diagnostic agent develops, they can negatively influence the imaging. Immunogenicity can also result in profound adverse effects after only a few administrations. For example, thrombocytopenia caused by antibodies specific to the murine-derived CDR regions of abciximab is seen in 1% of patients treated with the product. The incidence of this effect could be increased 4-fold after a second administration of abciximab to patients.[24]

TABLE 4: IMMUNOGENICITY IN NON-HUMAN PRIMATES VERSUS IMMUNOGENICITY IN HUMAN

		IMMUNOGENICITY IN NHP			
		Not evaluated in NHP	**Low**	**Intermediate**	**High**
Immuno-genicity in clinical trials	Low	Murine Murine Murine	Humanized Humanized Humanized Humanized Human Human	Humanized Human	Humanized Human
	Inter-mediate	Humanized	Humanized Human	Murine Chimeric Chimeric Humanized Humanized Humanized Human Human Human	Murine Chimeric Chimeric Humanized
	High	Murine Murine	Chimeric		Murine

Interestingly, the level of humanization did not appear to influence the ADA incidence in humans (Table 4). This is surprising because the aim of humanization is to reduce immunogenicity. The merits of humanization have been questioned before.[25] An interesting hypothesis put forward by Clark suggests that the basic idea of humanization to create self-like-proteins is flawed because every B cell clone with a unique specificity also has a unique V-region sequence, and it not likely that tolerance to each clone exists for every new sequence. A complete converse immunological concept is that B cell clones provoke anti-idiotype responses forming an antibody network that regulates immune responses. An equilibrium of these opposites more reflects the real situation. Therefore, immunogenicity and humanization of the variable region are not necessarily correlated and decreasing immunogenicity is not a simple matter of increasing the sequence homology.[25,26] There also appeared to be little difference between the relative immunogenicity of chimeric, humanized and human mAbs in NHPs. This is not unexpected because mAbs are probably readily recognized as non-self in NHPs because of species differences in major histocompatibility complex classes and T cell subsets. Most ADA responses in NHPs were directed against the Fc-region (anti-isotype) of the mAbs, resulting in enhanced clearance. In some cases, loss of efficacy and adverse effects were reported after the induction of ADAs. Conversely,

in humans, antibodies were more often directed against the CDR (anti-idiotype), resulting in neutralization of the function of the antibody and loss of efficacy. This may occur because the CDR, which is a unique sequence, is the most foreign region of a mAb in humans, whereas both the CDR and Fc regions are foreign in NHPs.

Because it is difficult to compare directly the immunogenicity of different products in different species, their relative immunogenicity is used to make between-species and -product comparisons. Even with this type of normalization, immunogenicity in laboratory animals is not considered predictive of immunogenicity in humans.[18,27,28] This was confirmed by our analysis, with only 59% of the tested antibodies having comparable incidence of immunogenicity in NHPs and humans. While the incidence of ADAs was comparable in some cases, immunogenicity in NHPs over-predicted the immunogenicity of 30% of the mAbs and underpredicted the immunogenicity of 11% of the mAbs in humans. Bugelski and Treacy established immunogenic classes of recombinant therapeutic proteins based on their source.[11] Prokaryotic and mammalian protein would have very low homology to human protein and these are generally highly immunogenic. Other classes were novel constructs and chimeric, humanized or human antibodies. The homology of these proteins is high, although immunogenicity could be variable. And even NHP studies had limited ability to predict immunogenicity with a trend to over-predict, despite the perceived extensive identity of V-regions (93% at amino acid level for the VH-framework regions and 88–99% for V_K of cynomolgus monkeys to human antibody sequences). Similarly, comparison of macaque V-regions with that of humans revealed identity between 84% and 97%; however, here differences were considered to possibly result in increased immunogenic response.[29]

The presence of ADAs often interfered with the assay used to detect mAb concentrations in serum. Therefore, pharmacokinetic or safety data should be interpreted with caution. Improvements in assay design could partially overcome some of these difficulties;[30,31] however, proper validation of assays is impeded by the lack of relevant reference standards, and other animal species are often used as controls, which makes the assay less specific. Animals are also the source of antisera needed to develop and validate assays for antibodies to be used during clinical development.

Safety and dose-finding studies make use of laboratory animals, but the potential for immunogenicity complicates the interpretation of kinetic and toxicity data, especially

because the development of ADAs can alter or abolish exposure and in some cases result in loss of efficacy. Immunogenicity is not a problem if study groups are of sufficient size and a sufficient number of animals do not develop ADAs, but ethical constraints typically limit NHP studies to small sample sizes. This means that safety studies are particularly difficult to interpret if most or all of the NHPs develop a significant ADA response. For example, in the case of MAb18 the majority of NHPs developed clearing ADAs after the first dose, which affected the interpretability of study results. Therefore, the value of using NHPs during non-clinical drug development will be limited if there is a significant immune response to the test substance. Continuing the study beyond this point will not yield relevant data and subsequent long-term studies should be reconsidered.[32]

Our study had some limitations. Grouping the NHP data into three operative categories is a necessary over-simplification of immunogenicity. In addition, the various studies differed in their reporting of the rate and effect of ADA development. We used the scale established by Hwang and Foote to classify the immune response, and as the immunogenicity of mAbs is probably higher in animals (the mAbs are foreign) than in humans, we chose to increase the ranges 3-fold.[33] This choice could be considered arbitrary; however, higher or lower ranges would lead to either an under- or over-estimation of immunogenicity in NHPs, respectively. Even though our data set included all mAbs approved in the EU through 2010, there were not enough samples to perform statistical analyses. Therefore, we could only observe and describe trends. Lastly, we only investigated mAbs that received marketing authorization. Inclusion of mAbs that failed during drug development or regulatory review would have provided a larger study cohort, but sufficient immunogenicity data for these mAbs are not publically available.

In conclusion, the results of this study suggest that the immunogenic response in NHPs is poorly predictive of the response in humans, even when using broad categories of immunogenicity. The development of clearing or neutralizing antibodies against the test mAb in NHPs might limit exposure or the duration of repeated dose studies, which in turn can influence the reliability and interpretability of pharmacokinetic, pharmacodynamic and safety data. Lastly, it is difficult to compare the immunogenicity across products and species because of species differences and limits in assay technology. Therefore, NHPs may not be a suitable species for testing mAbs that are immunogenic in NHP, even if these are the only species available.

4. MATERIALS AND METHODS

The drug registration files of mAbs and immunoglobulin fragments approved in the EU through 2010 (Table 1) were accessed at the Dutch Medicines Evaluation Board. The non-clinical summary and overview, including the tabulated study reports, were used to evaluate the immunogenicity of the mAbs in NHPs. This evaluation was done by assessing the presence of antidrug antibodies in serum from mAb-treated NHPs. Reporting of immunogenicity in animal studies is not standardized in marketing authorization applications, which could be quantitative or qualitative. To normalize the data and to enable comparison between products and species, the incidence of ADAs was classified into three categories, namely, low, intermediate or high. The incidence of ADAs was scored regardless of titer, time of occurrence and persistence of the response. The incidence was scored as "Low" when no or fewer than 6% of animals were positive for ADAs, "Moderate" when between 6% and 45% of animals were positive for ADAs and "High" when more than 45% of the animals were positive for ADAs. These categories were based on those established by Hwang and Foote and were three times higher than similar categories in humans based on higher baseline immunogenicity of NHPs.[33] Three authors individually categorized immunogenicity of the mAbs. When opinions deviated, the mAbs were discussed to come to a final distribution. If changes in pharmacokinetic or pharmacodynamic profiles occurred, the type and incidence of these changes were recorded. Lastly, ADA incidence and the rate of pharmacokinetic or pharmacodynamic changes were aggregated in a 3x3 matrix, with ADA incidence in the rows and rate of pharmacokinetic or pharmacodynamic changes in the columns (Table 2).

The Summary of Product Characteristics (SPC) or the European Public Assessment Report (EPAR) was used to determine the immunogenicity of therapeutic mAbs in humans, defined as the proportion of patients in Phase 3 clinical trials that were positive for ADAs. When these could not be retrieved, other online sources such as company websites or web databases were used. The categories established by Hwang and Foote were used: negligible if immunogenicity was seen in 2% of patients or less, tolerable when the incidence was between 2% and 15% and marked if immunogenicity occurred in more than 15% of the patients. Changes in efficacy mentioned in the SPC or EPAR as a result of the development of ADAs were noted. Immunogenicity in NHPs was considered predictive if human and NHP immunogenicity fell into the same operative category. MAbs evaluated in NHP were anonymized.

CONFLICT OF INTEREST

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ANNEX V

THIRTY YEARS OF PRECLINICAL SAFETY EVALUATION OF BIOPHARMACEUTICALS: DID SCIENTIFIC PROGRESS LEAD TO APPROPRIATE REGULATORY GUIDANCE?

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ABSTRACT

Introduction: The first biopharmaceuticals were developed 30 years ago. Biopharmaceuticals differ significantly from small molecule therapeutics (SMTs). Because of such differences, it was expected that classical preclinical safety evaluation procedures applied to SMTs would not predict the adverse effects of biopharmaceuticals. Therefore, until sufficient experience was gained, the preclinical safety evaluation of biopharmaceuticals was carried out on a case-by-case basis. 30 years of experience has since expanded the knowledge base in this area, in the hope to design a preclinical safety evaluation procedure suited to biopharmaceuticals.

Areas covered: This review describes how the preclinical safety evaluation of biopharmaceuticals has evolved. It shows that, as result of the risk-averse behavior of regulators and industry, classical procedures were taken as starting point although state-of-the-art knowledge on biopharmaceuticals was directed towards creating a new procedure, driven by the specific properties of biopharmaceuticals.

Expert opinion: Current preclinical safety evaluation guidance of biopharmaceuticals is criticized because it employs a checkbox approach. The adverse effects induced by biopharmaceuticals are on-target or immune system–induced, therefore, the preclinical safety evaluation should not be standardized, but rather driven by product specific safety concerns.

1. INTRODUCTION

30 years ago the first biopharmaceuticals produced by recombinant DNA technology and other biotechnological methods were introduced. At that time it was already known that the classical preclinical safety evaluation procedure applied to small molecule therapeutics (SMTs) would not predict adverse effects of biopharmaceuticals [1]. The checkbox approaches used in the classical procedure were considered not appropriate [2,3]. It was suggested, *"when developing a biotechnology product, attention should be focused on the unique characteristics of the products itself, rather than on existing testing guidelines"* ([2], p 170).

It was clear that new safety evaluation procedures needed to be developed. However, more knowledge and experience was necessary to design a scientific and rational preclinical safety evaluation procedure for biopharmaceuticals [3,4]. To gain that experience, the safety evaluation of biopharmaceuticals should be done on a case-by-case basis [4,5].

Since then the knowledge base has expanded, experience has been gained, and several preclinical safety guidelines for biopharmaceuticals have been implemented, replaced, and updated. But did these advancements in science enable regulators to design a preclinical safety evaluation procedure suited to biopharmaceuticals?

2. THE EVOLUTION OF THE PRECLINICAL SAFETY GUIDELINE

Analysis of the evolution of the preclinical safety evaluation procedures of the last 30 years shows how the current guideline, ICH S6(R1), came about. When the first biopharmaceuticals received market approval many experts concluded that biopharmaceuticals have different safety concerns than SMTs and so the classical preclinical safety evaluation procedure would not provide useful results concerning the safety of biopharmaceuticals [1,2,4,5]. Due to the novelty and different safety concerns, scientific progress was necessary to enable the design of a scientific and rational preclinical safety evaluation procedure for biopharmaceuticals [3,4]. To gain experience, the preclinical safety evaluation would be conducted on a case-by-case basis focused on the unique characteristics of these biotechnology-derived products [4,5].

However, case-by-case approaches are difficult to apply in practice, because case-by-case approaches require a high level of expertise from pharmaceutical companies and regulators. The absence of standardized rules also contradicted the "safety-first principle" which is a basic principle of drug regulation. Driven by risk-averse behavior, national regulatory authorities requested, and industry used, the classical preclinical safety evaluation procedure to assess the safety of biopharmaceuticals. However, deviating from this standard procedure was possible if the company developing the biopharmaceutical could justify that a different approach was required [4,6,7].

In 1997, the national procedures were replaced by a harmonized guideline, S6 [8]. The S6 was designed by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) with the aim to harmonize the practice of the preclinical safety evaluation of biopharmaceuticals. But while there are differences, as for example that the use of one species can be sufficient and that genotoxicity is not required as a standard, in general this guideline resembled the classical safety evaluation program as this had become the common practice of the regulators and industry [7]. Although S6 provides opportunities for case-by-case flexibility, the checkbox approach remains dominant in S6. This is understandable because harmonization of guidelines is perpendicular to the flexibility needed for a case-by-case approach. The aim of harmonizing guidelines is to create a basic framework, recognized by all parties, that streamlines the regulatory assessment process and reduces the development times and resources for drug development. Flexibility (e.g. case-by-case approaches) may lead to differing interpretations and inconsistent opinions between regulatory agencies [9] and it therefore does not reduce the development time and does not contribute to a streamlined regulatory assessment process [9], which is the opposite of what ICH tries to achieve.

3. STATUS QUO

Revision of the S6 was considered to be necessary by the ICH because *"clarification (and sometimes amplification) of this guidance (S6) is needed as substantial experience and new information has been gained since Step 4 (the adoption of the guideline) (1997)"* ([10], p 1). According to the ICH preclinical safety experts clarification was required with regard to species selection, study design, reproductive and developmental toxicity, carcinogenicity and immunogenicity [8]. In June 2011 the

revised guideline S6(R1) concerning the preclinical safety evaluation of biotechnology-derived pharmaceuticals (biopharmaceuticals), was approved by the ICH steering committee [8]. Did the ICH design this revised guideline making use of the state-of-the-art knowledge.

4. STATE OF THE ART KNOWLEDGE ON BIOPHARMACEUTICALS

Experience with biopharmaceuticals has shown safety concerns of biopharmaceuticals to be different from SMTs. The adverse effects of biopharmaceuticals are either caused by exaggerated pharmacology, unintentional tissue cross-reactivity, or by immune system-mediated adverse effects [11-14]. These insights validate the proposed approach to design the preclinical safety evaluation procedure on a case-by-case basis, driven by product specific questions, such as the cause, mechanisms, and reversibility of adverse effects. Despite the fact that from the start of modern biotechnology in drug development experts have recommended a case-by-case approach [5], this approach was not used as basis for the preclinical safety evaluation guidelines. Instead a standardized checkbox approach with some case-by-case decisions has been dominant in the preclinical safety evaluation guidelines.

The recent update of the ICH S6 guideline was an opportunity to catch up with scientific progress and introduce preclinical safety testing driven by the specific properties of biopharmaceuticals. Instead this update only clarified and complemented the S6, thus still closely resembles the classical preclinical safety evaluation procedure and checkbox approach. S6(R1) is only an update of S6, whereas a total reform of S6 would have been more appropriate. Unfortunately, regulators missed the opportunity to catch up with scientific progress into the toxicity of biopharmaceuticals and design a new preclinical safety evaluation procedure suited to biopharmaceuticals.

5. THE RISK OF RISK AVERSION

30 years of experience with preclinical safety evaluation of biopharmaceuticals did not result in a science-based rational design of the preclinical safety evaluation procedure for biopharmaceuticals, but in outdated guidelines driven by risk-averse behavior. Risk

aversion induces behavior that prefers elaborating on the successful approaches of the past over new approaches. In other words, risk aversion leads to path dependency. Path dependency is the routine whereby the set of solutions is limited by knowledge and experiences gained in the past, even though past circumstances may no longer be relevant [15]. Consequently, suboptimal solutions for problems are adopted. This can be exemplified by the role of animal experimentation in the preclinical safety evaluation procedure of biopharmaceuticals.

When the first biopharmaceuticals entered the market, it was already clear that the value of animal experimentation in the preclinical safety evaluation of biopharmaceuticals was limited due to species specificity and immunogenicity [1,3,5,16]. At the same time, the European Union expressed its ambition to reduce animal experimentation in Directive 86/609/EEC. The recognized limited value of animal studies for the preclinical safety evaluation of biopharmaceuticals in combination with the implementation of Directive 86/609/EEC provided a window of opportunity to design a preclinical safety evaluation procedure whereby the role of animal experimentation could be limited. However, instead of actively exploring possibilities to use radically different techniques in the preclinical safety evaluation procedure, the regulatory authorities used the classical procedure, with animal studies playing a leading role. Using this classical procedure, studies in species wherein the biopharmaceutical is pharmacologically active were shown to provide insight in the potential adverse effects [9,17-19]. As a result, studies in Non-Human Primates (NHPs) became increasingly popular in preclinical safety evaluation testing of biopharmaceuticals [13]. Nevertheless, using NHPs only partly solved the problem; some biopharmaceuticals are human specific [20,21] and in addition, reproductive and developmental toxicity and carcinogenicity are not easily studied in NHPs [13]. Two new animal-based approaches were introduced to conduct studies for human specific biopharmaceuticals and reproductive and developmental toxicity and carcinogenicity studies: i.e., (1) adapting the animals to the human product by using transgenic animals, and (2) adapting the product to animals by the development of animal homologues. However the value of the results of safety evaluation studies using transgenic animal or animal homologues is uncertain [22], because *"the ultimate validation (of transgenic animals) will not occur until there are clinical data to compare with..."* ([20], p 233) and *"until the clinical candidate has been evaluated in humans, the extent to which the surrogate molecule (homologue) is truly homologous or analogues cannot be completely understood"* ([20], p 234).

The window of opportunity to realize the long-desired break with the classical animal testing paradigm was not effectively exploited. To overcome problems as species specificity and immunogenicity, the regulatory authorities and pharmaceuticals companies did not choose to develop new approaches, but they developed and adopted suboptimal new animal-based approaches. Despite the public and political pressure to reduce animal testing and the scientific discussion concerning the predictive value of animal testing, rigidity and risk-averse behavior of regulators and pharmaceutical companies alike have made it impossible to break through the path dependency.

The role of animal experimentation in the preclinical safety evaluation procedure of biopharmaceuticals is a perfect analogy for the evolution of this preclinical safety evaluation procedure in general. Instead of creating a new procedure that takes into account the differences in safety concerns between SMTs and biopharmaceuticals, the classical procedure was taken as starting point. So the result is a flawed preclinical safety evaluation procedure for biopharmaceuticals.

6. CONCLUDING REMARKS

Although scientific evidence has accumulated, regulators only used this knowledge to complement and clarify the preclinical safety evaluation guidance instead of using these insights to revise the procedure to a more flexible procedure driven by product specific questions. Regulation got behind scientific progress due to risk aversion.

Today, the regulatory authorities are confronted with comparable challenges. For instance, preclinical safety evaluation procedures have to be developed for innovative medicines, such as nanomedicines and advance therapies. To prevent the development of suboptimal preclinical safety evaluation procedures for innovative medicines, lessons should be learned from the development of the preclinical safety evaluation procedure for biopharmaceuticals. Pharmaceutical companies in collaboration with regulatory authorities need to explore the window of opportunity, when innovative medicines enter drug development, to design state-of-the-art procedures to guarantee goal-oriented preclinical safety procedures and patient safety. They should step out of their comfort zones and design a relevant preclinical safety evaluation procedure based on the characteristics and the safety concerns of the specific product under study and not on the procedures that have been successfully applied to incomparable products

in the past, because past performance of a procedure does not guarantee that this procedure will also be efficient in predicting the toxicity of incomparable products in the future.

7. EXPERT OPINION

The main critique on the ICH S6 is that it is a guidance inspired by standard procedures that are somehow adapted to be useful for the evaluation of biopharmaceuticals and that it does not operate a full case-by-case approach that is only driven by product specific concerns. That a case-by-case approach should be leading in the design of the preclinical safety evaluation of biopharmaceuticals was already suggested at the time of the introduction of the first biopharmaceuticals. The scientific evidence showing that adverse effects are target-induced or immune system-mediated also supports a case-by-case approach.

In practice a full case-by-case approach would mean that the preclinical safety evaluation should not be standardized but should be designed for every product in dialog with the regulators. Studies with homologues and transgenic animals should be discouraged, because studies should only be done when they provide insights that do not have to be confirmed in the first-in-human studies. When confirmation of results of animal studies is necessary then microdosing in humans would be appropriate.

However, microdosing is not popular because many companies and regulators do not feel comfortable about testing biopharmaceuticals in humans without having the product tested in animals. Even though companies and regulators know that the findings of animal studies only confirm what they already knew or are irrelevant, they feel more secure when they have done animal tests to verify whether the biopharmaceutical does not induce any unexpected toxicity. This extra sense of safety that the results of animal tests provide to regulators and companies is thus false but also exposes participants of standard first-in-human studies to a higher risk than participants in microdosing studies because results of animal tests often make it possible to start at higher doses than is allowed in microdosing studies. To reduce the use of irrelevant studies in the preclinical safety evaluation of biopharmaceuticals the misperception that animal testing provides more safety to clinical trial participant than the lower starting dose in microdosing should be cleared up.

Furthermore the authors would like to signal to regulators now working on guidance for the preclinical evaluation of advanced therapies, nanotechnology and other innovative drugs to take the product or class specific concerns and not the standard approaches as point of departure. Taking standard approaches as basis results in adaptations of these procedures and guidance documents including many studies often not relevant.

DECLARATION OF INTEREST

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ANNEX VI

THE RISK-BASED APPROACH TO ATMP DEVELOPMENT – GENERALLY ACCEPTED BY REGULATORS BUT INFREQUENTLY USED BY COMPANIES

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ABSTRACT

Advanced therapy medicinal products (ATMPs) are the cutting edge of drug innovation. ATMPs have different challenges than other drug classes. To accommodate these challenges and facilitate science-driven development, flexibility in the requirements to demonstrate the safety and efficacy of this rapidly evolving drug class is necessary. To create flexibility, the European Union introduced the risk-based approach. This approach provides the possibility of omitting guideline-based studies based on risk analyses. To gain insight into the effect of the risk-based approach on the non-clinical development of ATMPs, two questions are addressed in this paper. Firstly, "Do companies use a risk-based approach for the non-clinical development of ATMPs?" and, secondly, "Does the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) accept non-clinical development programs based on the risk-based approach?". Scientific advice letters formulated by the CHMP were analyzed. The risk-based approach was used to justify deviations from the guidelines in the majority (75%) of the cases. The CHMP accepted 40% of the proposals to omit studies and stated that additional data was necessary to make an informed decision for 35% of the proposals. This indicates that the risk-based approach facilitates the science-driven development of ATMPs.

1. INTRODUCTION

Advanced therapy medicinal products (ATMPs), the cutting edge of drug innovation, hold promise to offer a cure for a variety of diseases for which there are no satisfactory therapies, such as cancer, inherited monogenic diseases, cardiovascular disease, Parkinson's disease, diabetes and arthritis (Jekerle, 2010; Jilma, 2010; Klug et al., 2012; Maciulaitis et al., 2012; Schneider et al., 2010; Vamvakas, 2011). ATMPs are diverse in nature, comprising gene therapy medicinal products (GTMPs)[25] and cell-based medicinal products (CBMPs) (including cell therapy[26] and tissue engineering[27]). These products have different characteristics than small molecule and biotechnology-derived drugs. These differences entail that ATMPs also have a different risk profile and other challenges for safety evaluations, such as the effects of ineffective integration into to the patient's body systems, cells and genes and immune responses. Moreover, some ATMPs can stimulate tumor growth (Mavilio, 2012; Schneider et al., 2010).

To accommodate the development of this new class of pharmaceuticals, the European Union created regulation EC 1394/2007 on ATMPs and revised Directive 2001/83 on the community code relating to medicinal products for human use (Klug et al. 2012). As a general principle, ATMPs have to fulfill the same scientific and regulatory standards as other medicinal products (European Union, 2001, 2007; Jekerle, 2010; Klug et al., 2012; Mavilio, 2012). Nevertheless, regulation EC 1394/2007 together with the technical requirements contained in revised Annex I of Directive 2001/83/EC introduce specific requirements for ATMPs (European Union, 2001; Klug et al., 2012). These specific requirements are high level requirements because the type and amount of data necessary to demonstrate the quality, safety and efficacy of diverse of ATMPs is highly specific (Jekerle, 2010). Sufficient flexibility in the development

25 Definition of GTMPs: "Gene therapy products consist of recombinant nucleic acids administered to humans with a view to regulating, repairing, replacing, adding or deleting a genetic sequence, and whereby its effect relates directly to the recombinant sequence or the product of genetic expression of this sequence. Novel recombinant or vector-based vaccines against infectious diseases are also specifically excluded from the definition of gene therapy products" (Jekerle, 2010, p6).

26 Definition of cell therapy: "Cell therapy medicinal products contain or consist of substantially manipulated cells and have properties for treating, preventing, or diagnosing a disease through the pharmacological, immunological and metabolic action of the cells or tissues" (Jekerle, 2010, p6).

27 Definition of tissue engineering products: "It should be noted that it is the intended action (i.e., treating, preventing or diagnosis a disease for cell therapy medicinal products vs. regenerating, repairing, replacing a human tissue for tissue engineered products) that will differentiate a cell therapy medicinal product from a tissue engineered product" (Jekerle, 2010, p6).

of ATMPs is important to anticipate the rapid evolution of science and technology in the field of ATMPs (Jekerle, 2010; Schneider et al., 2010). The technical requirements are therefore further explored in several class-specific guidelines of the EMA (Jekerle, 2010; Schneider et al., 2010).

To create more flexibility, the legislation and guidelines concerning ATMPs recommend companies to use a risk-based approach during development (European Medicines Agency, 2013; Klug et al., 2012; Jekerle, 2010; Cohen-Haguenauer, 2013). The EMA defined the risk-based approach as *"a strategy aiming to determine the extent of quality, non-clinical and clinical data to be included in the Marketing Authorisation Application (MAA), in accordance with the scientific guidelines relating to the quality, safety and efficacy of medicinal products and to justify any deviation from technical requirement as defined in Annex I, part IV of Directive 2001/83/EC"* (European Medicines Agency, 2013, p4). The purpose of the risk-based approach is to obtain a profile of the risks associated with the use of a specific ATMP by identifying the various risks[28] associated with the clinical use and the risk factors[29] inherent to the ATMP with respect to quality, safety and efficacy.

The risk-based approach is an ongoing process whereby the manufacturer conducts risk analyses at the beginning and during product development (European Medicines Agency, 2013; Jekerle, 2010). The amount of data required for MAA depends on the level of risk, state of knowledge about the product under development and experience of the manufacturer with other ATMPs (Cohen-Haguenauer, 2013). The final risk profile will take shape as a result of the consolidation of the identified risk factors (Cohen-Haguenauer, 2013).

The purpose of the risk-based approach in the development of ATMPs is to create a framework wherein it is encouraged to take into account continuously evolving science and technology in order to design a tailor-made ATMP development program. The approach aims to provide the possibility of moving away from guideline-based

28 The EMA defines risks as "potential unfavourable effect that can be attributed to the clinical use of ATMP and is of concern to the patient and/or to other populations (e.g. caregivers and offspring)" (European Medicines Agency, 2013, p4). Examples of risks are: unwanted immunogenicity, disease transmission, tumor formation and treatment failure.

29 The EMA defines risk factors as "qualitative or quantitative characteristic that contributes to a specific risk following the handling and/or administration of an ATMP" (European Medicines Agency, 2013, p4). Risk factors are associated with the nature of the product, biodistribution and manufacturing issues.

ATMP development and to facilitate science-driven development strategies of ATMPs. The risk profile of an ATMP can point out that some, requirements prescribed in the EMA guidelines or in the Directive 2001/83/EC are redundant and that other strategies are more relevant to establish the quality, safety and efficacy of the product under study (European Medicines Agency, 2013).

The aim of this paper was to study whether the risk-based approach facilitates science-driven non-clinical drug development. To gain insight into the effect of the risk-based approach at the level of non-clinical development of ATMPs, two questions are addressed in this paper. Firstly, *"Do companies use a risk-based approach for the non-clinical development of ATMPs?"* and, secondly, *"Does the Committee for Medicinal Products for Human Use (CHMP) of the EMA accept non-clinical development programs based on the risk-based approach?".*

Only three ATMPs have received market approval in the EU. This sample is too limited for an analysis of the drug registration dossiers of those ATMPs. We therefore analyzed the scientific advice letters formulated by the CHMP in order to gain insight into the use and acceptance of the risk-based approach for the non-clinical development of currently developed ATMPs. Companies can apply for scientific advice of the CHMP at every stage of product development. In their scientific advice request, companies can pose questions related to quality, non-clinical and clinical development. The Scientific Advice Working Party of the (SAWP) answers the questions in close consultation with relevant working parties such as the Committee for Advanced Therapies (CAT), the Biologics Working Party (BWP) and the Safety Working Party (SWP) and prepares a scientific advice letter including the background on the disease and the product provided by the company, the questions posed by the company and the answers formulated by the SAWP and adopted by the CHMP. The questions posed by the manufactures indicate if and how often manufacturers use a risk-based approach. The answer provides insight into if and how often the CHMP accepts non-clinical development programs using the risk-based approach.

2. METHODOLOGY

The scientific advice letters of the CHMP concerning ATMPs between 2009 and 2012 were accessed at the Dutch Medicines Evaluation Board. The letters wherein the applicant sought advice about non-clinical development were selected for this analysis. The questions in the section *"Questions on Toxico-Pharmacological Development"* were used to evaluate whether companies proposed non-clinical development programs using a risk-based approach. The answers to these questions were used to assess to what extent the CHMP accepts innovative risk-based non-clinical programs for ATMP.

The aim of the risk-based approach is to determine the extent of data necessary for a marketing application and to justify any deviation from the requirements. Questions that could be related to deviating from the standard requirements were included and attributed to the following categories: 1) questions that proposed to do no animal studies; 2) questions that proposed to do one or two animal studies; 3) questions that proposed to not do repeat-dose toxicity studies in animals 4) questions that proposed to not do carcinogenicity studies in animals and 5) questions that proposed to not do reproduction toxicity studies in animals. These categories were selected because using a risk-based approach to justify the omission of chronic animal studies is most interesting since these studies are the most time-consuming and expensive of the regulatory required studies. Questions which could not be related to these categories were excluded. To determine whether companies used the risk-based approach to justify omitting a study, the line of argumentation used by the manufacturer was analyzed. The line of argumentation to not conduct animal studies was classified as 1) based on a risk analysis when companies argued that based on current knowledge and/or available data, the study would be redundant, 2) not based on a risk analysis when companies used no argument or other arguments, for example that the study was not feasible.

To evaluate whether the CHMP endorses risk-based non-clinical programs for ATMP, the answers of the CHMP to the questions classified as risk-based were analyzed and referred to the following categories: 1) proposal not accepted, 2) more information is necessary to confirm the provided line of argumentation, 3) proposal accepted and 4) no answer. The ATMPs and manufacturers were anonymized.

3. RESULTS

Between 2009 and 2012, pharmaceutical companies requested scientific advice 80 times for 66 different ATMPs of the CHMP. The scientific advice letters concerned 48 CBMP and 18 GTMP. Fifty letters (for 48 different products) included questions related to non-clinical development, 59 letters included questions related to clinical development and 45 included questions related to quality control (see Table 1).

TABLE 1: OVERVIEW OF ADVICE

ISSUES DISCUSSED IN SCIENTIFIC ADVICE LETTERS	
Non-clinical	9
Non-clinical and Quality	7
Non-clinical and Clinical	5
Non-clinical, Quality and Clinical	29
Quality	5
Quality and Clinical	4
Clinical	21
Total	80

Of the 50 scientific advice letters including questions related to non-clinical development, advice was requested for the non-clinical development of 14 GTMPs and 34 CBMPs (follow-up advice was requested for two CBMPs) (Table 2). This corresponds with earlier findings that three-quarters of the ATMPs under development are CBMPs (Maciulaitis et al., 2012). Sixteen products of these 50 were designated as an Orphan Medicinal Product. Eight advice letters concerned ATMPs developed by big pharmaceutical companies (in the top 100 based on total sales in 2011). The other 42 ATMPs were developed by other companies. This result supports the conclusion of Maciulaitis et al. that "*the vast majority of stakeholders developing ATMPs are academic institutions, charities, SMEs, or other small companies*" (Maciulaitis et al., 2012, p 479).

TABLE 2: OVERVIEW OF SCIENTIFIC ADVICE LETTERS WHEREIN NON-CLINICAL DEVELOPMENT WAS DISCUSSED

	PRODUCT CLASS			ORPHAN DRUG STATUS	
	CBMP (follow-up (FU) advice)	GTMP	Total (FU advice)	Yes (FU advice)	No (FU advice)
2009	8	2	10	4	6
2010	6 (1)	6	12 (1)	4	8 (1)
2011	9	4	13	5	8
2012	11 (1)	2	13 (1)	4 (1)	10
Total	34 (2)	14	48 (2)	16 (1)	32 (1)

In the fifty scientific advice letters related to the non-clinical development of ATMPs, companies proposed to deviate from the standard requirements in 29 scientific advice letters (60%). In these 29 letters 48 proposals to omit animal studies were done (Table 3). Six times companies proposed to do no (2) or only a few animal tests (4). In the other letters companies proposed to omit specific animal studies: repeat-dose toxicity testing (10), aspects of carcinogenicity testing (15) or aspects of reproductive toxicity testing (17).

3.1 NO ANIMAL STUDIES

Companies proposed to not conduct animal tests for two ATMPs. The companies argued that, due to a lack of adequate animal models, it was not relevant to do animal studies. In both cases, the CHMP agreed with the company's opinion that animal tests were not relevant. In one of these cases, the availability of *"clinical use for over 10 years (more than 200 patients treated) without any serious events being reported" was decisive "to support the safety of the product and that no additional animal experiments are called for".*

TABLE 3 – OVERVIEW OF COMPANIES REQUESTING TO DEVIATE FROM STANDARD REQUIREMENTS						
ATMP	NO ANIMAL STUDIES	FEW ANIMAL STUDIES	NO REPEAT DOSE TOXICITY STUDIES	NO CARCINO-GENICITY STUDIES	NO REPRODUCTIVE TOXICITY STUDIES	TOTAL
1			1	1	1	3
2				1	1	2
3	1					1
4			1		1	2
5				1	1	2
6					1	1
7			1	1		2
8			1		1	2
9				1		1
10		1				1
11					1	1
12			1	1	1	3
13				1	1	2
14					1	1
15				1		1
16		1				1
17				1		1
18		1				1
19		1				1
20					1	1
21	1					1
22			1	1	1	3
23				1		1
24				1	1	2
25			1			1
26			1	1	1	3
27			1		1	2
28			1	1	1	3
29				1	1	2
Total	2	4	10	15	17	48

3.2 FEW ANIMAL STUDIES

Performing only a few animal studies for the non-clinical development of ATMP was proposed for four products. In two cases, the companies argued that additional relevant animal studies were not possible due to the nature of the product. In one of these cases, the CHMP was not convinced by the argumentation that no other relevant studies were possible and stated that the company *"should justify their approach not to perform further studies using an animal equivalent"*. In the other case, the CHMP agreed with the proposed approach but argued that if (non)clinical data raised concerns, a homologous animal model might become necessary. In the other two scientific advice letters, the companies reasoned that the available clinical data in combination with the non-clinical pharmacology study made additional animal studies redundant. The CHMP agreed that no dedicated toxicology studies were necessary, but the CHMP encouraged one company to add toxicological endpoints to the planned non-clinical pharmacology study.

3.3 NO REPEAT-DOSE TOXICITY STUDIES IN ANIMALS

Ten pharmaceutical companies proposed to do no repeat-dose toxicity studies in animals. For eight ATMPs, multiple administrations in human patients were not anticipated, and the pharmaceutical companies therefore argued that there would be no need for repeat-dose toxicity studies. In six cases, the CHMP did not respond, while in two cases, the CHMP explicitly agreed that conducting a repeat-dose study would not be relevant. In one case, the company did not provide a rationale to omit repeat-dose toxicity testing. The CHMP emphasized in its answer to this company that a repeat-dose study would be necessary if readministration was anticipated in patients. In one case, the company argued that it would not be possible to assess repeat-dose toxicity because no relevant animal model was available. The CHMP did not agree with the company and advised the company to conduct repeat-dose toxicity studies in minipigs with a homologous product.

3.4 NO CARCINOGENICITY STUDIES IN ANIMALS

In fifteen scientific advice requests, the applicant proposed omitting carcinogenicity studies in animals. Thirteen times, companies argued that based on the available

data from in vitro or animal studies, it was concluded that the risk of carcinogenicity was negligible. In two requests, the companies also added that there was no animal model available to conduct carcinogenicity studies. In three cases, the CHMP advised companies to conduct additional (in vitro or in vivo) carcinogenicity tests to exclude potential risks. The CHMP concluded that more information was necessary to make an informed judgment in five cases. The CHMP agreed with the proposal in six cases. In four cases, the CHMP had the view that sufficient knowledge was available about the carcinogenic potential of the ATMP. In one case, the CHMP agreed with omitting the test because the product was developed for a life-threatening and debilitating disease, but added that carcinogenicity "may gain higher importance if the therapy is successful". In the sixth case, the CHMP agreed with the omission of an animal study because "at present no applicable in vivo test system exists". One company proposed omitting carcinogenicity testing but provided no argument. The CHMP advised the company to gather more information to increase the understanding about the potential carcinogenicity risk of the ATMP. Another company argued that carcinogenicity testing was not feasible. The CHMP did not agree with this company and advised the company to gain more knowledge about the carcinogenic potential of the ATMP because that knowledge is warranted to assess the benefit/risk ratio.

3.5 NO REPRODUCTIVE TOXICITY STUDIES IN ANIMALS

In seventeen scientific advice requests, companies proposed omitting reproductive toxicity studies in animals. In two cases, the scientific advice letter did not describe any argumentation to justify omitting reproductive toxicity studies in animals provided by the manufacturer. Nevertheless, the CHMP responded to one proposal by stating that more supporting evidence was necessary to judge whether the proposal would be acceptable for marketing authorization.

Only one manufacturer also used the lack of an animal model as an argument to omit reproductive toxicity studies in animals. The CHMP did not agree with this proposal because they held the opinion that the clinical data were too limited and that a relevant animal model was available. In the remaining advice letters, the manufacturers used the risk-based approach to support their proposed development program. These manufacturers argued that the risk of reproductive toxicity was negligible (based on the design of the product, based on the target population and/or because clinical

data were available) and/or that reproductive toxicity studies in animals were only necessary when the results of biodistribution studies provided reasons for concern. The CHMP did not give a reply in one case. In four scientific advice letters, the CHMP agreed with the approach proposed by the manufacturer, while in eight cases the CHMP stated that more testing or information was necessary to judge whether animal studies could be omitted. The CHMP disagreed once with the line of argumentation of the company and advised conducting reproductive toxicity testing.

3.6 APPLICATION AND ACCEPTANCE OF THE RISK-BASED APPROACH

Of the 48 proposals of ATMP developing companies to deviate from the standard requirements and omit animal studies, companies used the argument that no relevant animal model was available ten times (including five times in combination with arguments that the concerned risk was negligible) (Table 4). The analysis showed that 75% of the proposals (36) to omit non-clinical studies were based on arguments that it was not necessary to conduct the specific test because the risks (for the target population) were assumed to be negligible. Fourteen proposals were accepted by the CHMP and in thirteen cases the CHMP stated that additional data was necessary to make an informed decision (Table 5). The CHMP only rejected two proposals to omit animal studies. They rejected two proposals because they did not agree with the line of argumentation provided by the manufacturer. In both cases, the CHMP considered the animal study necessary to learn more about the potential risk.

4. DISCUSSION

In this study, scientific advice letters were analyzed to study whether the risk-based approach facilitates science-driven non-clinical drug development. The advantage of using scientific advice letters is that they reflect the current practices and discussions. The disadvantages of using scientific advice letters is that (i) not all companies request scientific advice at the CHMP during the development of ATMPs and (ii) the discussed non-clinical drug development strategies in the scientific advice letters are only proposals and it is unknown whether these proposals will be carried out until the company submits the Marketing Authorisation Application (MAA).

TABLE 4: OVERVIEW OF ARGUMENTATION TO OMIT ANIMAL STUDIES

	No animal studies	Few animal studies	No repeat-dose toxicity studies	No carcino-genicity studies	No reproductive toxicity studies	Total	%
Based on risk analyses							
Not necessary	0	2	8	7	14	31	65%
Not possible and not necessary	2	0	0	3	0	5	10%
Not based on risk analyses							
Not possible	0	2	1	1	1	5	10%
No argument	0	0	1	4	2	7	15%

TABLE 5: CLASSIFICATION CATEGORIES FOR THE ANSWERS OF THE CHMP

	No animal studies	Few animal studies	No repeat-dose toxicity studies	No carcino-genicity studies	No reproductive toxicity studies	Total
Proposal not accepted				1	1	2
More information is necessary to confirm the provided line of argumentation				5	8	13
Proposal accepted	2	2	2	4	4	14
No response			6		1	7
Total	2	2	8	11	14	36

Firstly, requesting scientific advice from the CHMP is optional in the drug development process. Only a portion of the companies developing ATMPs apply for scientific advice. The sample used in this study is likely biased toward complex ATMPs with specific challenges in non-clinical drug development or toward companies aspiring to deviate from the standard requirements. Therefore, this analysis only confirms the use of the risk-based approach in designing the development program of ATMPs and it does not provide information about the extent of the use of the risk-based approach.

Secondly, the development plans proposed by the companies in the scientific advice request might be adapted or completely revised over time. Thus, companies can aspire to apply a risk-based approach at the stage of the scientific advice request, but they can forgo this approach during development. Only when the companies submit the MAA, analyses of the drug registration files will reveal whether the company actually applied a risk-based approach. A follow-up study making use of drug registration files should be done to confirm the results of this study.

5. CONCLUSION

To accommodate the development of ATMPs and to facilitate the design of a science-driven development program for ATMPs, the CHMP recommends companies to use a risk-based approach. To gain insight into the effect of the risk-based approach on the non-clinical development of ATMPs two questions were addressed in this paper. Firstly *"Do companies use a risk-based approach in the development of ATMPs?"* and, secondly, *"Does the Committee for Medicinal Products for Human Use (CHMP) of the EMA accept non-clinical development programs based on the risk-based approach?"*.

Interestingly, the risk-based approach was used to justify deviations from the standard requirements in the majority (75%) of the proposals. This indicates that companies use the risk-based approach in the development of ATMPs. However, scientific advice for the non-clinical development was requested fifty times, but only 29 times (60%) companies proposed to deviate from the standard requirements using a risk-based approach. This result indicates that the manufacturers that apply for scientific advice generally do risk analyses and use the risk-based approach as an opportunity to deviate from the requirements as prescribed in Annex I of Directive 2001/83 and the

guidelines. However, there is room for broader use of the risk-based approach in the development of ATMPs.

The CHMP accepted 40% of the proposals directly, while for 35% of the proposals, the CHMP stated in their responses that additional data is necessary to make an informed decision about omitting a specific study. The CHMP rejected only two proposals to omit animal studies. These findings indicate that application of the risk-based approach for the design of the non-clinical development of ATMPs results in acceptable proposals for the CHMP.

CONFLICTS OF INTEREST

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VI

SUMMARY

Worldwide animal studies are the gold standard to assess the quality, safety and efficacy of drugs. Annually 3.6 million animals are used for the development of drugs in Europe alone (European Commission, 2010). Every new drug has been tested in 1500-3000 animals. Animals resemble humans more than any other organisms. Drugs are therefore first evaluated in animals before they are used in clinical trials. However, animal species differ in many ways from one another and from humans. As a result, experiments in closely related animals, such as rats and mice, often provide different results. The value of animal studies to assess the quality, safety and efficacy of drugs is never extensively validated. At present, more and more research indicates that the predictive value of animal studies in drug development is limited.

The limited predictive value of animal studies influences the drug development process in two ways. On the one hand, the development of drugs that induce serious adverse events in animals will be terminated before the clinical trials while these drugs could be safe and effective in humans. On the other hand, many drugs that showed promising results in animal studies do not reach the market because they were unsafe or ineffective in clinical trials. In other words, animal studies result in the loss of potentially valuable drugs and provide a false sense of safety and/or efficacy. Therefore, it seems desirable for both the regulatory authorities and the pharmaceutical companies to develop innovative methods that can replace animal studies in the development of drugs.

The development of innovative methods gained momentum at the end of the 1970s. The resistance to animal studies is as old as its use. Campaigns of animal welfare organizations against the use of animal studies in the development of cosmetics created public awareness and put the use of animal studies on political agendas. As a result, the Europe Economic Community implemented legislation to protect laboratory animals in 1986. Directive 86/609/EEC required that animal studies cannot be conducted when other methods are available and stimulated the development of innovative methods. Since 1986 different organizations, such as national governments in Europe and cosmetics companies, invested money in the development of innovative methods. These investments have led to a wide variety of innovative methods to assess drugs without the use of animals. However, the availability of these innovative methods has not resulted in large-scale replacement of animal testing in drug

development to date. We therefore aim to contribute to the understanding of why animal studies are still being used in drug development in this thesis. The research question we answer is "which mechanisms explain the lock-in of animal studies in drug development?" We conducted six studies to identify the mechanisms that can explain (i) why the replacement of animal studies in drug development proceeds slowly and (ii) why animal studies are still implemented in novel regulation regarding drug development of new drug classes.

The lack of success of innovative methods to replace animal studies is analyzed as an innovation problem in this thesis. For innovation it is crucial to develop new technologies and escape the established practice. The development of innovative methods is analyzed using the technological innovation system approach. New ideas become innovations in an innovation system. The development of innovative methods is a complex process involving not only knowledge development but also knowledge diffusion, mobilizing resources and creating legitimacy. Using the technological innovation system approach, it is possible to systematically analyze the processes essential for innovation over time and identify mechanisms that hamper the innovation process.

Escaping established technologies is often challenging because these technologies are embedded in a wide variety of institutions. Technologies can, for example, be embedded in legislation or regulation and the use of technologies seems self-evident due to tradition or experience. This embedding of technologies in a variety of institutions locks-in technologies and makes it difficult to escape the use of these technologies. The institutional context of wherein a technology is embedded is also referred to as an institutional logic. The institutional logic of animal studies in drug development is analyzed to understand the effect of this logic of the innovation process towards innovative methods.

In this thesis we combined the analysis of the technological innovation system of innovative methods with the analysis of the institutional logic of animal studies in drug development. New technologies are essential for innovation. It is equally important that innovative methods fit into the institutional logic of the established practice, or that the logic is modified or becomes obsolete. The used approach enabled us to elucidate a more complete overview of the different mechanisms that hampered the innovation process toward innovative methods in drug development. Especially

in the early stages of the innovation process this new approach is expected to be valuable because understanding the institutional logic of the established technology allows actors of an innovation system to identify the assumptions, values, beliefs and rules that lock-in the established technologies. These actors might then be able to anticipate on problems the institutional logic of the established practice can induce into the innovation process at an early stage.

The substitution of animal studies in established drug development processes is studied in the papers in appendices I and II. Paper I studies the innovation process of innovative methods for erythropoietin (EPO) potency testing. Although several innovative methods are available since the 1990s, EPO potency still has to be assessed in animals. Paper II concerns the innovation process of innovative methods to replace the Draize eye irritation test. In 2009, the Draize test has been replaced by two innovative methods after a bumpy innovation process that took over thirty years.

The results of the technological innovation system analysis presented in paper I show that there were several projects that aimed to development innovative methods for EPO potency testing. Following the institutional logic of drug development, the quality control of EPO had to be regulated when EPO gained market access. The first draft of the quality regulation of EPO was formulated in 1996, and in 1999 the guideline was implemented. As no innovative method could assess EPO potency yet, two EPO potency tests in mice were implemented in the regulation. In 1999, an innovative method was developed that could assess the potency of EPO. This innovative method is still not implemented in the regulation. Following the institutional logic of drug development, the innovative method has to substitute the animal studies in the regulation. Substitution is only possible when the innovative methods are validated in a multi-laboratory validation study. The validation study has not been initiated yet. The technological innovation system analysis indicates that there are no incentives for the actors of the innovation system of innovative methods to assess EPO potency to initiate the validation studies.

In paper II the technological innovation system analysis also showed that several innovative methods were developed to assess eye irritation. In line with the institutional logic of drug development, the innovative methods also needed to be validated in multi-laboratory studies. In total six validation studies were initiated with various innovative methods. No method was validated in these studies because none of

the innovative methods met the validation requirements. The main issue was that the results of the innovative methods did not sufficiently correlate with the results of animal studies. Eye irritation testing is also broadly used in the development of cosmetics. In 1993, the European Union introduced a ban on selling cosmetic products containing ingredients that have been tested on animals as per 1998. This ban created urgency for the validation of the innovative methods for eye irritation testing. As a result several multi-actor workshops were organized to tackle the validation problem. Based on the results of the workshops and an evaluation of the results of the different validation studies, the validation process was changed in two ways. On the one hand the use of reference standards, substances which have a known degree of toxicity in vivo, were used to circumvent the variability of the Draize test. On the other hand it was acknowledged that it would be impossible to find a single replacement for the Draize test. This acknowledgment was the basis for the tiered testing strategy whereby innovative methods would be validated to assess parts of the eye irritation spectrum. Validation of innovative methods for tiered-testing strategies was performed retrospectively. In 2009, the OECD adopted the Isolated Chicken Eye (ICE) test and Bovine Corneal Opacity and Permeability (BCOP) assay for moderate and severe eye irritants in test guidelines 437 and 438. Based on the results of paper I and II we identified the following mechanisms that hamper the innovation process towards innovative methods in drug development.

Firstly, the technological innovation system analyses of innovative methods for EPO potency testing and eye irritation testing show that several innovative methods to replace the animal study have been developed. However, the maturation and utilization of these innovative methods is hampered because several processes do not function properly. Many of the innovative methods never matured because there were insufficient financial resources, the researchers switched jobs or the researchers were not motivated to continue the development of the innovative method. Furthermore, there is no market for innovative methods as patented methods are not implemented in the regulation by the regulatory authorities. It is therefore not interesting for entrepreneurs to invest in the development of innovative methods. With the exception of personal motives to reduce the use of animal studies, there are often no incentives for the actors in the innovation system to futher develop innovative methods. A comparison of the results of paper I and II shows that the innovation process of innovative methods for eye irritation testing was driven by the public resistance to the use of animal studies in the development of cosmetics. This public resistance

boosted the innovation process in two ways. As result of this public resistance (i) the legitimacy of the use of animal severely decreased and (ii) guidance of the search was provided by a ban on cosmetics tested in animal in the European Union. The lack of urgency to replace animal studies and the lack of incentives to develop and implement innovative methods hamper the innovation process of innovative methods to replace animal studies in drug development.

Secondly, innovative methods have to replace animal studies in regulation. Thus, institutions have to be changed. Animal studies are not just part of regulations. They are locked-in drug development because they are taken for granted, normatively endorsed and backed up by authorized powers. To enable the replacement of animal studies regulatory authorities introduced the validation process. However, this process turned out to be counterproductive as it is based on the institutional logic of animal studies. In the validation process entrepreneurs have to show based on result from multiple laboratories that the innovative method is robust and provides the same results as the animal study. This validation process hampers innovation via two mechanisms. On the one hand, the outcome of validation studies is uncertain. Innovative methods do not measure the effect of a drug in full system, such as animals. Furthermore, innovative methods are often based of human mechanisms and human data. As a result, innovative methods and animal studies provide incommensurable results. On the other hand, the validations studies are time consuming and expensive. The lack of market, due to unwillingness of regulatory authorities to implement innovative methods into the regulation, makes recovering the costs of the validation studies unlikely. This causes collective inaction as the result of a free-rider problem. No actors want to invest because all actors benefit equally as the actor investing.

On basis of the results of paper I and II, it can be concluded that it is challenging to substitute animal studies in established drug development processes. Although the public, regulators and pharmaceutical companies have the ambition to reduce animal testing, there is no urgency to push this change process in drug development. Urgency seems necessary to advance the innovation process towards the use of innovative methods in drug development. Urgency to substitute animal studies makes it easier to overcome barriers in the innovation process, such as mobilizing resources, gaining legitimacy and overcoming collective inaction and validation issues. Paper I and II show that under the current circumstances, it is unlikely that innovative methods will replace animal studies in drug development.

The papers in appendices III, IV, V and VI study why animal studies are still adopted in novel regulation regarding the development of drugs of new drug classes. The development process of new drug classes still needs to be established. New drug classes are therefore an excellent opportunity to use innovative methods instead of animal studies, because innovative methods do not have to replace animal studies. Yet, animal studies play a leading role in the guidelines for the development of drugs that belong to the new drug classes that are developed in the last three decades, such as biopharmaceuticals and gene therapy. This central role of animal studies seems to indicate that animals are a better model to assess the quality, safety and efficacy of biopharmaceuticals than innovative methods. To test this hypothesis we studied the value of non-human primates studies in the development of monoclonal antibodies, a subclass of biopharmaceuticals, in paper III and IV. The results of both studies show that the value of studies in non-human primates is limited in the development of monoclonal antibodies. In paper III we conclude that adverse effects of monoclonal antibodies are either due to exaggerated pharmacology or immune system mediated. Exaggerated pharmacology can be predicted based on the mechanism of action and immune system mediated effects cannot be predicted in animals. Studies in primates provide no new knowledge concerning the safety of monoclonal antibodies for humans. Paper IV assessed the immunogenicity of monoclonal antibodies in non-human primates. The results of this study showed that immunogenicity in non-human primates is not predictive for immunogenicity in humans. Immunogenicity even decreases the value of animal studies, because the formation of clearing or neutralizing antibodies can make the results of non-human primate studies difficult to interpret.

Based on the results of paper III and IV it can be concluded that animal studies have been unfairly preferred over innovative methods. Why were animal studies preferred over innovative methods in the development of monoclonal antibodies? We tried to answer this question in paper V on the evolution of the guideline on the nonclinical development of biopharmaceuticals.

Paper V shows that the development of drugs that belong to a new drug class is associated with much uncertainty for both regulators and pharmaceuticals companies. These actors try to reduce uncertainty by building on technologies that proved their value in the past. The development process of new drug classes is not driven by science but by experience due to this path dependent behavior. As a result of the use of animal

studies, the experience with and knowledge about animal studies in the development process of biopharmaceuticals increased and the leading role for animal studies in the development process of biopharmaceuticals became inescapable. However, the value of animal studies in the development of traditional small molecule drugs does not guarantee the value of animal studies in the development of biopharmaceuticals. Biopharmaceuticals have a different structure and are often human-specific. The value of animal studies in the development of biopharmaceuticals is just as uncertain as the value of innovative methods. Thus, building on animal studies does not reduce the uncertainty, but it does decrease the likelihood that innovative methods are used.

Advanced Therapy Medicinal Products (ATMPs), including gene therapy and cell therapy, is the most innovative drug class. The first guideline on the development of those drugs was implemented in 2001 in Europe. Animal studies also play an important role in the guidelines for the development of ATMPs. European legislation recommends pharmaceutical companies to take a risk-based approach instead of just following the guidelines in the development of ATMPs. The risk-based approach enables companies to design a tailor-made drug development program in which they can use innovative methods to identify and evaluate the risks of ATMPs. Based on the results of innovative methods they can decide whether animal studies are valuable or can be omitted. In this way science-driven ATMP development is facilitated and innovative methods can be used without being formally validated. In the paper in appendix VI we studied scientific advice letters formulated by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) to analyze if pharmaceutical companies use the risk-based approach in the development of ATMPs. The results of this study show that the risk-based approach was used to justify deviations from the guidelines in 75% of the studied letters. The results of this study indicate that the risk-based approach facilitates the science-driven development of ATMPs.

Based on the studies in this thesis, it can be concluded that it is challenging to substitute animal studies in established drug development processes and that animal studies are still being adopted in novel drug development processes because animal studies get the benefit of the doubt over innovative methods. The institutional logic of drug development is, in general, at the basis of the barriers that hamper the implementation of innovative methods. Animal studies are locked-in into drug development because they are taken for granted as the most relevant model for humans, normatively

endorsed as they seem to be very successful in preventing tragedies with drugs, and they are embedded in legislation and regulation. This institutional logic is so strong that animal studies still get the benefit of the doubt over innovative methods. Smart regulation that aims at more science-driven drug development, such as the risk-based approach, can facilitate innovation as this provides an opportunity to gain experience with innovative methods in drug development without going through the formal validation studies.

We formulated five recommendations to facilitate the innovation process based on the findings of the studies in this thesis. Firstly, governments should create incentives for pharmaceutical companies to develop, validate and implement innovative methods. Incentives can be created by prohibiting or penalizing the use of animal studies. The innovation process of innovative methods can also be stimulated by rewarding the reduction of animal studies. Companies can be rewarded by an extension of the data protection or an accelerated procedure to obtain market access. Secondly, the innovation process can be facilitated by allowing patented innovative methods into the guidelines. The acceptation of patented innovative methods enable companies that invested in innovative methods to recover their costs. Thirdly, the validation process of innovative methods should be revised. In the revised process human data should be used as validation endpoint. If no human data is available then human data should be gathered by parallel testing with the established drug development process. Fourthly, smart regulation that stimulates science-driven drug development, such as the risk-based approach, can stimulate the innovation process towards innovative methods. Smart regulation should facilitate the use of innovative methods to identify and evaluate the efficacy and risk profile of new drugs. Based on results obtained with innovative methods informed choices can be made about the use and omission of animal studies. Such approaches will not only reduce animal studies but also enable pharmaceutical companies and regulatory authorities to gain experience with innovative methods. Finally, the innovation process towards innovative methods in drug development can be facilitated by delegitimizing the use of animal studies in drug development. Studies on the value of animal studies in drug development can delegitimize animal studies. When results of these studies show that the value is limited, this will influence the public acceptation of the use of animal studies in drug development.

SAMENVATTING

Dierproeven zijn wereldwijd de gouden standaard voor de evaluatie van de kwaliteit, werkzaamheid en veiligheid van medicijnen. In Europa worden alleen al 3.6 miljoen dieren per jaar gebruikt voor de ontwikkeling van medicijnen (European Commission, 2010). Elk nieuw medicijn wordt op 1500-3000 dieren getest. Dieren lijken van alle organismen het meest op mensen. Medicijnen worden daarom eerst geëvalueerd in dieren voordat ze getest worden op mensen. Echter, de diverse diersoorten verschillen onderling in vele opzichten van elkaar en van mensen. Daardoor leveren experimenten met nauw verwante dieren, zoals ratten en muizen, vaak verschillende resultaten op. De relevantie van dierproeven voor de evaluatie van de kwaliteit, werkzaamheid en veiligheid van medicijnen is nooit uitgebreid vastgesteld. Echter steeds meer onderzoek wijst uit dat de voorspellende waarde van dierproeven voor mensen beperkt is.

De beperkte voorspellende waarde van dierproeven beïnvloedt het ontwikkelingsproces van medicijnen op twee manieren. Ten eerste wordt de ontwikkeling van medicijnen die ongewenste effecten hebben op dieren beëindigd voor de start van klinische studies. Terwijl deze producten wellicht veilig en werkzaam voor mensen kunnen zijn. Ten tweede blijken veel medicijnen die veelbelovende resultaten opleveren in dierproeven alsnog onveilig of onvoldoende werkzaam te zijn voor de mens. Met andere woorden: dierproeven leiden enerzijds tot een onnodig verlies van mogelijk waardevolle producten en anderzijds geven deze experimenten een onterecht gevoel van veiligheid en/of werkzaamheid. Voor zowel regelgevende instanties als bedrijven lijkt het daarom wenselijk om innovatieve methoden te ontwikkelen om de kwaliteit, werkzaamheid en veiligheid van medicijnen beter te borgen.

De ontwikkeling van methoden om dierproeven te vervangen (in het vervolg van deze samenvatting wordt naar deze methoden verwezen als 'innovatieve methoden') kreeg een impuls aan het eind van de jaren zeventig van de vorige eeuw. De weerstand tegen dierproeven is zo oud als het gebruik er van. Campagnes tegen het gebruik van dierproeven voor de ontwikkeling van cosmetica, georganiseerd door dierenwelzijnsorganisaties creëerden publiekbewustzijn en zetten het gebruik van dierproeven op politieke agenda's. In 1986 leidde dit in Europa tot de invoering van wetgeving (Directive 86/609/EEC) die eiste dat innovatieve methoden altijd boven dierproeven moeten worden verkozen. Tevens werd de ontwikkeling van innovatieve methoden die dierproeven kunnen vervangen aangemoedigd. Sinds de invoering

van Directive 86/609/EEC is er veel geïnvesteerd in innovatieve methoden. Deze investeringen hebben geleid tot een grote diversiteit aan innovatieve methoden om de kwaliteit, werkzaamheid en veiligheid van medicijnen te testen zonder het gebruik van dieren. Helaas heeft de beschikbaarheid van deze innovatieve methoden tot op heden nog niet geleid tot grootschalige vervanging van dierproeven bij de medicijnontwikkeling. Het doel van dit proefschrift is dan ook om bij te dragen aan het begrip van waarom dierproeven nog steeds worden gebruikt in de ontwikkeling van medicijnen. De hoofdvraag van dit proefschrift is 'welke mechanismen kunnen de lock-in van dierproeven in medicijnontwikkeling verklaren?' Om deze mechanismen te identificeren hebben we in zes studies bestudeerd waarom het vervangen van dierproeven in de bestaande regelgeving traag verloopt en hebben we gekeken waarom dierproeven nog steeds worden opgenomen in de regelgeving voor de ontwikkeling van medicijnen in nieuwe geneesmiddelenklassen.

Het gebrek aan succes van innovatieve methoden om dierproeven te vervangen is geanalyseerd als een innovatieprobleem in dit proefschrift. In innovatieprocessen is het cruciaal dat er nieuwe technologieën worden ontwikkeld maar ook dat er wordt gebroken met de gevestigde technologie. Het ontwikkelen van innovatieve methoden is een complex proces waarbij het niet alleen draait om de ontwikkeling van kennis. De ontwikkeling van innovatieve methoden vindt plaats in een innovatiesysteem, waarin het ook belangrijk is om nieuwe technologieën zichtbaar te maken, middelen te mobiliseren en legitimiteit voor nieuwe technologieën te creëren. Om de ontwikkeling van innovatieve methoden te analyseren hebben we het analytisch kader van het technologische innovatiesysteem gebruikt. Met het raamwerk van het technologische innovatiesysteem is het mogelijk de belangrijkste processen van het innovatieproces systematisch te analyseren over de tijd. Op basis van de resultaten van deze analyse kan geïdentificeerd worden waardoor de ontwikkeling en toepassing van innovatieve methoden langzaam verloopt en hoe dit innovatieproces gestimuleerd zou kunnen worden.

Het is moeilijk om te breken met gevestigde technologieën omdat ze op verschillende manieren zijn geïnstitutionaliseerd. Technologieën kunnen bijvoorbeeld opgenomen zijn in de wet- en regelgeving, ook kan het gebruik van een technologie door traditie en ervaring vanzelfsprekend zijn geworden. Doordat technologieën op verschillende manieren zijn geïnstitutionaliseerd, raken technologieën ingelocked en zijn deze moeilijk te vervangen. De institutionele context waarin een technologie is ingelocked wordt ook wel de institutionele logica genoemd. De institutionele logica rondom het

gebruik van dierproeven in medicijnontwikkeling is geanalyseerd om te onderzoeken op welke manier deze logica het innovatieproces van innovatieve methoden beïnvloedt.

In dit proefschrift hebben we de analyse van het technologische innovatiesysteem van innovatieve methoden gecombineerd met een analyse van de institutionele logica waarin dierproeven zijn ingebed. Belangrijk is dat de innovatieve methoden passen in de institutionele logica rondom de gevestigde technologie, of dat deze logica wordt aangepast of in onbruik raakt. De gebruikte gecombineerde aanpak stelde ons in staat om een volledigere overzicht van de barrières in dit innovatieproces te identificeren. Vooral in vroege stadia van het innovatieproces lijkt dit nieuwe gecombineerde kader waardevol, omdat inzicht in de institutionele logica actoren de mogelijkheid biedt om de aannames, waarden, overtuigingen en regels die het gebruik van de gevestigde technologie vanzelfsprekend maken te identificeren. Deze actoren kunnen dan al in een vroeg stadium van het innovatieproces anticiperen op de mogelijke problemen die de institutionele logica kan veroorzaken.

Waarom het vervangen van dierproeven door innovatieve methoden in de bestaande regelgeving traag verloopt, is bestudeerd in twee studies beschreven in appendices I en II. Beide papers betreffen een case studie. Paper I bestudeert het innovatieproces van innovatieve methoden om de effectiviteit van erytropoëtine (EPO) te evalueren. Ondanks dat verschillende innovatieve methoden beschikbaar zijn sinds de jaren negentig, is de dierproef nog steeds niet vervangen. Paper II bestudeert het innovatieproces van innovatieve methoden die de Draize oogirritatie test kunnen vervangen. In 2009 is de Draize test na een traag innovatieproces van ruim dertig jaar vervangen door twee innovatieve methoden.

De analyse van het technologische innovatiesysteem in paper I laat zien dat er verschillende projecten zijn geweest die als doel hadden om innovatieve methoden te ontwikkelen om de effectiviteit van EPO te evalueren. De kwaliteitscontrole van EPO moest in navolging van de institutionele logica van medicijnontwikkeling worden gereguleerd. De concept richtlijn voor de kwaliteitscontrole van EPO werd in 1996 geformuleerd en de richtlijn werd in 1999 geïmplementeerd. Op dat moment waren er nog geen innovatieve methoden waarmee de effectiviteit van EPO geëvalueerd kon worden. Daarom werden twee testen op muizen in de richtlijn opgenomen. In datzelfde jaar werd er een innovatieve methode ontwikkeld waarmee de effectiviteit van EPO wel beoordeeld kon worden. Deze methode heeft nog steeds de muistest niet vervangen

in de richtlijn. In navolging van de institutionele logica van medicijnontwikkeling moeten innovatieve methoden om opgenomen te kunnen worden in de richtlijn eerste gevalideerd worden in verschillende laboratoria. Dit validatieproces is nog niet geïnitieerd. De resultaten van de technologische innovatiesysteem analyse laten zien dat er voor de actoren in het innovatiesysteem onvoldoende prikkels zijn om het validatieproces te initiëren.

Paper II laat middels de analyse van het technologische innovatiesysteem zien hoe het innovatieproces van verschillende innovatieve methoden om het oogirritatie potentieel van medicijnen te meten is verlopen. Ook deze methoden moeten eerst gevalideerd worden voordat de dierproef vervangen kan worden in de regelgeving. In totaal zijn er zes validatiestudies uitgevoerd. Geen van deze studies heeft geleid tot de beoogde validatie. Het grootste probleem was dat de resultaten van de methoden niet voldoende correleerden met de resultaten van de Draize test. De Draize test werd ook gebruikt in de ontwikkeling van cosmetica. In 1993 kondigde de Europese Unie een verbod aan op de verkoop van cosmetica die ingrediënten bevatten die zijn getest in dieren. Dit verbod zou in 1998 van kracht worden. Dit verbod maakte de validatie van innovatieve methoden urgent. Op basis van de conclusies van een aantal workshops en een analyse van de resultaten van de verschillende validatiestudies werden twee veranderingen aangebracht in het validatieproces. Enerzijds werden de resultaten van de innovatieve methoden gecorreleerd aan referentie standaarden, stoffen waarvan het oogirritatie potentieel bekend is, om de variabiliteit van de Draize test te ondervangen. Anderzijds werd erkend dat het onmogelijk zou zijn om één methode te ontwikkelen die de Draize test zou kunnen vervangen. Daarom werd een stapsgewijze teststrategie voorgesteld waarin innovatieve methoden kunnen worden gevalideerd voor het beoordelen van een deel van het oogirritatie spectrum. De validatie voor delen van het oogirritatie spectrum werd gedaan op basis van de data uit de reeds uitgevoerde validatiestudies. In 2009 heeft de OESO de Isolated Chicken Eye test (ICE) test en de Bovine Corneal Opacity and Permeability (BCOP) assay in de testrichtlijnen 437 en 438 ingevoerd voor het classificeren van matige en ernstige ogen irriterende stoffen. Op basis van de resultaten van paper I en II identificeerden we de volgende mechanismen die het innovatieproces naar innovatieve methoden in de ontwikkeling van geneesmiddelen belemmeren.

De analyse van het technologische innovatiesysteem van de innovatieve methoden voor het bepalen van de effectiviteit van EPO en het oogirritatie potentieel laten

zien dat er vaak verscheidene innovatieve methoden zijn ontwikkeld die de potentie hebben om de gevestigde dierproef in regelgeving te vervangen. De doorontwikkeling en het gebruik van deze methoden worden echter belemmerd doordat verschillende processen in het innovatiesysteem onvoldoende functioneren. Oorzaken voor het niet doorontwikkelen zijn het ontbreken van middelen, de onderzoekers een andere baan hadden verworven of omdat de onderzoekers het doorontwikkelen van innovatieve methoden niet interessant vonden. Daarnaast is er ook geen markt voor innovatie methoden omdat de regelgevende instanties de gepatenteerde innovatieve methoden niet willen implementeren in de richtlijnen. Het is daardoor niet mogelijk om investeringen in innovatieve methoden terug te verdienen. Met uitzondering van persoonlijke motieven om het gebruik van dierproeven te verminderen, zijn er vaak geen prikkels voor de actoren in het innovatiesysteem om innovatieve methoden door te ontwikkelen. De vergelijking tussen de twee case studies toont aan dat het innovatieproces van methoden om de Draize test te vervangen werd gedreven door de publieke weerstand tegen het gebruik van dierproeven voor de ontwikkeling van cosmetica. De publieke weerstand stimuleerde het innovatieproces op twee manieren. Enerzijds nam de legitimiteit voor het doen van dierproeven af en anderzijds werd er sturing aan het innovatieproces gegeven door het verbod van de Europese Unie op de verkoop van cosmetica die ingrediënten bevatten die zijn getest in dieren. Het gebrek aan urgentie om dierproeven te vervangen en het gebrek aan stimulansen om innovatieve methoden te ontwikkelen en implementeren belemmeren het innovatieproces van methoden om dierproeven te vervangen in de ontwikkeling van geneesmiddelen.

Om dierproeven te vervangen moeten innovatieve methoden worden geïmplementeerd in de regelgeving. Het vervangen van dierproeven in de regelgeving vereist veranderingen in instituties. Dierproeven zijn niet enkel onderdeel van de regelgeving. Dierproeven zijn ingelocked in medicijnontwikkeling omdat ze als vanzelfsprekend worden beschouwd, normatief zijn bekrachtigd en worden ondersteund door de regelgevende instanties. Om de vervanging van dierproeven te faciliteren werd het validatieproces geïntroduceerd. Het validatieproces bleek contraproductief omdat het gebaseerd is op de institutionele logica waarin dierproeven de gouden standaard zijn.

Dit implementatieproces ligt ten grondslag aan het tweede mechanisme dat het innovatieproces belemmert. De regelgeving is onderdeel van de institutionele

logica waarin dierproeven ingebed zijn. Innovatieve methoden worden alleen in de regelgeving opgenomen als de methode gevalideerd is. Bij het validatieproces moet in verschillende laboratoria worden aangetoond dat de innovatieve methode robuust is en dezelfde resultaten oplevert als de dierproef. De institutionele logica waarin dierproeven ingebed zijn is dus tegelijkertijd de selectieomgeving voor innovatieve methoden. Het validatieproces belemmert het innovatieproces via twee mechanismen. Enerzijds is het resultaat van validatiestudies onzeker. Innovatieve methoden meten de reactie op het medicijn niet in een volledig systeem, zoals wel het geval is bij dierproeven. Daarnaast zijn innovatieve methoden vaak gebaseerd op de situatie in de mens en niet op de situatie in dieren. Hierdoor zijn resultaten van innovatieve methoden vaak niet te vergelijken met de resultaten van dierproeven. Anderzijds zijn validatiestudies duur en tijdrovend. Doordat regelgevende instanties geen gepatenteerde innovatieve methoden willen opnemen in de regelgeving is het niet mogelijk de kosten van de validatiestudies terug te verdienen. Hierdoor ontstaat collectieve inactiviteit als gevolg van een free-rider probleem. Geen van de actoren wil investeren in de validatiestudies omdat de andere partijen die de validatiestudies niet uitvoeren, even veel profiteren als actoren die wel investeren.

Op basis van paper I en II kan geconcludeerd worden dat vervanging van dierproeven die vastgelegd zijn in de regelgeving een uitdaging is. Hoewel, het publiek, de regelgevende instanties en farmaceutische bedrijven streven naar het verminderen van dierproeven, is er voor geen van deze actoren noodzaak om de rol van dierproeven in de ontwikkeling van geneesmiddelen te veranderen. Dit gebrek aan urgentie belemmert het innovatieproces van innovatieve methoden. Het maakt het moeilijker om obstakels, zoals het verwerven van middelen voor het doorontwikkelen van innovatieve methoden en het validatieproces, te overwinnen. Zonder urgentie is ook het oplossen van collectieve inactiviteit met betrekking tot het validatieproces zeer uitdagend. Paper I en II laten zien dat onder deze omstandigheden, het onwaarschijnlijk is dat innovatieve methoden de ingelockte dierproeven zullen vervangen in het geneesmiddelenontwikkelingsproces.

Paper III, IV, en V bestuderen de vraag waarom dierproeven nog steeds worden geïmplementeerd in regelgeving voor nieuwe geneesmiddelenklassen. Nieuwe klassen van medicijnen bieden mogelijkheden voor innovatieve methoden, omdat het ontwikkelingsproces voor nieuwe geneesmiddelenklassen nog moet worden vastgesteld. Nieuwe geneesmiddelenklassen zijn dus een uitgelezen kans om

innovatieve methoden te gebruiken in plaats van dierproeven, omdat innovatieve methoden niet een in richtlijnen ingebedde dierproef hoeven te vervangen. Toch spelen dierproeven een hoofdrol in de richtlijnen voor de ontwikkeling van medicijnen die behoren tot de nieuwe geneesmiddelenklassen die de afgelopen drie decennia ontwikkeld zijn, zoals biotechnologische medicijnen en gentherapie. Dit lijkt er op te wijzen dat dierproeven de effecten van bijvoorbeeld biotechnologische medicijnen beter voorspellen dan innovatieve methoden. Om deze hypothese te testen is in paper III en IV onderzocht wat de meerwaarde van proeven op apen is in de ontwikkeling van monoklonale antilichamen, een subklasse van de biotechnologische geneesmiddelen. In beide studies laten de resultaten zien dat de meerwaarde van dierproeven in de ontwikkeling van deze medicijnen beperkt is. Op basis hiervan kan worden geconcludeerd dat dierproeven onterecht de voorkeur hebben gekregen boven innovatieve methoden. Maar waarom spelen dierproeven dan toch een belangrijke rol in de richtlijn voor het ontwikkelen van monoklonale antilichamen? Om deze vraag te beantwoorden is in paper V de evolutie van de richtlijn voor de ontwikkeling van biotechnologische medicijnen geanalyseerd.

Paper V laat zien dat de ontwikkeling van medicijnen die behoren tot een nieuwe geneesmiddelenklasse gepaard gaat met veel onzekerheid voor zowel regelgevende instanties als farmaceutische bedrijven. Deze actoren proberen de onzekerheid te verminderen door gebruik te maken van methoden die hun waarde hebben bewezen in het verleden. Door dit padafhankelijke gedrag van de regelgevende instanties en farmaceutische bedrijven wordt het ontwerp van het ontwikkelingsproces voor nieuwe geneesmiddelenklassen niet gedreven door de wetenschap maar door ervaring. Het gevolg hiervan is dat het ontwikkelingsproces van deze medicijnen is gebaseerd op de ervaring en kennis verkregen uit dierstudies en dat maakt de belangrijke rol voor dierproeven onontkoombaar. Helaas zegt de ervaring met dierproeven in de ontwikkeling van de klassieke medicijnen (kleine chemisch vervaardigde moleculen) niets over de waarde van dierproeven in de ontwikkeling van biotechnologische medicijnen. Biotechnologische medicijnen hebben immers een andere structuur en zijn vaak mensspecifiek. Hierdoor is de waarde van dierstudies in de ontwikkeling van biotechnologische medicijnen net zo onzeker als de waarde van innovatieve methoden. Dus, voortbouwen op de dierproeven doet de onzekerheid in vergelijking met het gebruik van innovatieve methoden niet afnemen, maar het vermindert wel de kans dat innovatieve methoden worden gebruikt.

Gen- en celtherapie vormen samen de meeste innovatieve geneesmiddelenklasse. Voor deze medicijnen zijn de eerste Europese richtlijnen pas in 2001 geïmplementeerd. Ook in de richtlijnen voor de ontwikkeling van deze producten spelen dierproeven een belangrijke rol. Echter raadt de wetgeving in Europa bedrijven die deze producten ontwikkelen aan om de richtlijnen niet klakkeloos te volgen, maar om de ontwikkeling te baseren op de mogelijke risico's van het product onder ontwikkeling. Op deze manier wordt wetenschapsgedreven medicijnontwikkeling gestimuleerd en kunnen bedrijven dierproeven overbodig maken door de risico's van producten op voorhand vast te stellen. Het is dan mogelijk om een op maat gemaakt ontwikkelingsprogramma te ontwerpen waarbij gebruik gemaakt kan worden van innovatieve methoden en afgeweken kan worden van vereiste studies. De innovatieve methoden kunnen dan worden ingezet zonder het doen van de eerder besproken validatiestudies. In de paper in appendix VI zijn de wetenschappelijk advies brieven geformuleerd door de Committee for Medicinal Products for Human Use (CHMP) van de European Medicines Agency (EMA) bestudeerd om te analyseren of bedrijven de ontwikkeling van medicijnen baseren op de risico's van deze producten. Deze studie laat zien dat in 75% van de geanalyseerde wetenschappelijk advies brieven farmaceutische bedrijven het risicoprofiel van producten gebruikte als argument om af te wijken van de richtlijn. De resultaten van deze studie geven aan dat het risico gestuurde medicijnontwikkeling een wetenschapsgedreven medicijnontwikkelingsproces faciliteert.

Op basis van de studies in dit proefschrift kan geconcludeerd worden dat het een uitdaging is om dierproeven in bestaande medicijnontwikkelingsprocessen te vervangen. In het innovatieproces is het validatieproces de grootste barrière. Door een gebrek aan urgentie worden validatiestudies vaak niet geïnitieerd. Dierproeven worden nog altijd opgenomen in nieuwe medicijnontwikkelingsprocessen omdat dierproeven het voordeel van de twijfel krijgen ten opzichte van innovatieve methoden. De institutionele logica van medicijnontwikkeling ligt over het algemeen aan de basis van de mechanismen die het innovatieproces van innovatieve methoden belemmeren. Dierproeven zijn ingelocked in medicijnontwikkeling omdat ze als vanzelfsprekend worden gezien als het meest relevante model voor mensen, normatief zijn bekrachtigd door hun succes in het verleden en omdat ze zijn vastgelegd in vele richtlijnen en wetten. Deze institutionele logica is zo sterk dat dierproeven over het algemeen de voorkeur krijgen in de ontwikkeling van medicijnen zelfs als er nog geen ontwikkelingsproces bestaat. Slimme regelgeving gericht op wetenschapsgedreven medicijnontwikkeling, zoals geïmplementeerd voor de ontwikkeling van gen- en

celtherapie, kan het innovatieproces van methoden om dierproeven te vervangen faciliteren omdat dergelijke regelgeving het mogelijk maakt ervaring op te doen met innovatieve methoden zonder dat ze formeel gevalideerd hoeven te zijn.

Gebaseerd op de gevonden resultaten hebben we vijf aanbevelingen geformuleerd die het innovatieproces kunnen versnellen. Ten eerste kan de overheid prikkels creëren voor farmaceutische bedrijven om innovatieve methoden te ontwikkelen, valideren en implementeren. Dit kan door middel van het verbieden of bestraffen van het gebruik van dierstudies. Verder zou het gebruik van innovatieve methoden of het uitvoeren van minder dierproeven beloond kunnen worden door verlenging van de databescherming van het te ontwikkelen medicijn of door een versnelde procedure om toegang te krijgen tot de markt. Ten tweede kan het innovatieproces gestimuleerd worden door gepatenteerde innovatieve methoden toe te laten in de regelgeving. Op deze manier kunnen bedrijven die geïnvesteerd hebben in de ontwikkeling en validatie van innovatieve methoden de investeringen terugverdienen. Ten derde kan het herzien van het validatieproces het innovatieproces stimuleren. In de herziene procedure moet humane data als validatie-eindpunt gebruikt worden. Wanneer geen humane data beschikbaar is moet deze data gecreëerd worden in parallel met het gevestigde medicijnontwikkelingsproces. Ten vierde kan 'slimme regelgeving' bijdragen aan het opdoen van ervaring met innovatieve methoden en het verminderen van dierproeven. Ten slotte kan het delegitimeren van dierproeven het innovatieproces versnellen. Onderzoek naar de relevantie van dierproeven in het ontwikkelingsproces van medicijnen kan, wanneer blijkt dat de meerwaarde beperkt is, grote invloed hebben op de publieke acceptatie voor het gebruik van dierproeven.

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ABOUT THE AUTHOR

Marlous Kooijman was born in Culemborg (1984). In 2002 she started her studies at Utrecht University. In the autumn of 2005 Marlous finished her bachelor degree in Science and Innovation Management and also concluded a year in the board of study society NWSV Helix. During her master Drug Innovation Marlous was an intern at the Dutch Vaccine Institute (NVI). In 2006 she started a second master, Science and Innovation Management. As part of this program Marlous studied a semester at University College Cork in Ireland. In 2009, after a research project at Roche pharmaceuticals and the Diakonessenhuis Marlous, obtained a Master's degree in Drug Innovation and a Master's degree in Science and Innovation Management (cum laude).

From 2009 until 2013 Marlous worked on the PhD-project presented in this thesis at the Innovation Studies Group, Utrecht University. This project was part of Top Institute Pharma (TIPharma). Marlous completed the TIPharma education program. From 2010 until 2013, she was the PhD representative in the board of studies of the Geoscience faculty and she founded Utrecht Geo Graduates (UGG), the PhD council of the Geosciences faculty. Besides her research, she supervised master and bachelor students, provided tutorials in research methods and innovation projects and provided lectures in innovation system theory, sustainable drug development and management of biopharmaceuticals. Furthermore, she organized the Utrecht Innovation Colloquium, a series of guest lectures, from 2010 until 2011. Marlous presented her work at numerous international conferences.

ABBREVIATIONS

3Rs	Refine, Reduce and Replace
ATMP	Advanced Therapy Medicinal Product
BCOP test	Bovine Corneal Opacity and Permeability test
CHMP	Committee for Medicinal Products for Human Use
EMA	European Medicines Agency
EPO	Erythropoietin
EURL ECVAM	European Union Reference Laboratory for alternatives to animal testing
FDA	Food and Drug Administration
hERG	human Ether-à-go-go-Related Gene
ICE test	Isolated Chicken Eye test
ICH	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use
LAL test	Limulus Amebocyte Lysate test
MAA	Marketing Authorization Application
OECD	Organisation for Economic Co-operation and Development
PBPK modeling	Physiologically Based Pharmacokinetic modeling
R&D	Research and Development
STRWG	Safety Topic Recommendation Working Group
TIS	Technological Innovation System

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