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Non-human primates in biomedical research

Frouke Pieters

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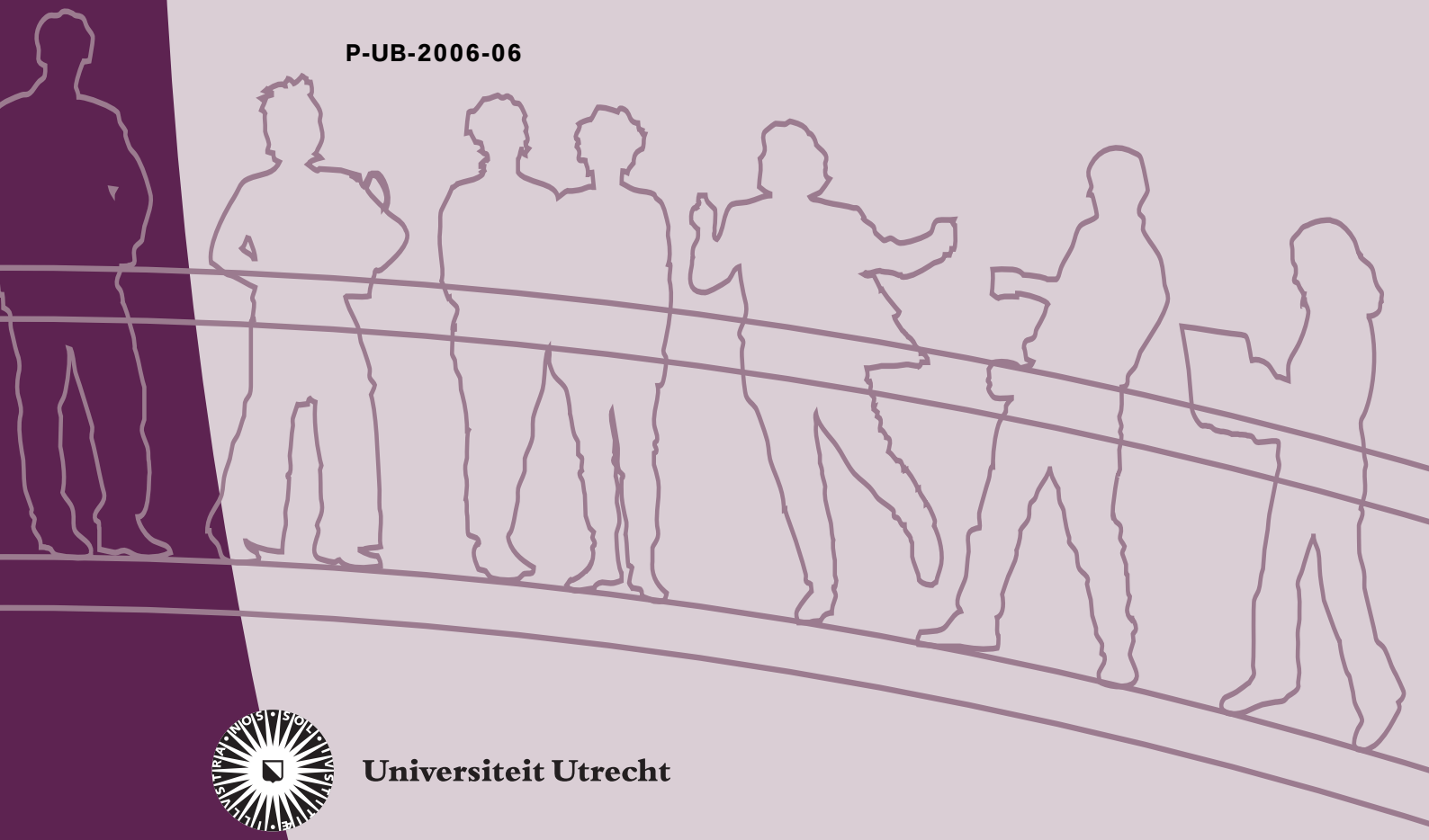

Universiteit Utrecht

Non-human primates in biomedical research

Reasons and alternatives for their use

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Contents

Preface

This report is the final thesis for my Masters in Biology at Utrecht University, the Netherlands. The study was conducted over a nine month period as a project at the Netherlands Centre Alternatives to animal use (NCA). Professor Coenraad Hendriksen was responsible for daily supervision of my research. Additional supervision was also given by Janne Kuil on behalf of the initiating working party 'Primate research in the Netherlands'. Furthermore, Manon Vaal of the Science Shop for Biology, Utrecht University acted as the intermediary supervisor between this working party and the student-researcher.

Naturally, it goes without saying that the study has been conducted from a scientific perspective; views and personal opinions have been separated from factual information. However, the researcher's background may be useful to the reader, certainly given that this is a delicate subject where so many interests and opinions come together. My own attitude towards animal experiments is generally a pragmatic but critical one. Animal experiments may be useful in order to proceed with biomedical science and medicine, but I tend to question the necessity of all these experiments. During the course of this research, I addressed many sides of the story in an open-minded but critical fashion, taking the views of both the proponents and opponents of animal experimentation into account. This has consequently increased my understanding of the need for a primate model in some cases. Nonetheless, my personal view is that the use of animals should be reduced to only the absolutely necessary experiments. I therefore hope that this study will contribute to the reduction and replacement of the use of primates in biomedical research.

I would like to thank all those who helped me during this study: first of all the respondents, without whom there would be nothing to say on the subject. It is regretful that some of these respondents have chosen to remain anonymous due to personal or commercial concerns. The British NC3R's allowed me to attend a workshop on the use of primates for biologicals, which was incredibly helpful. I would also like to extend my gratitude to Coenraad Hendriksen, Janne Kuil and Manon Vaal for their supervision, useful nuances and help with respondents, and Nina Cohen for her advice on how to conduct qualitative research. Finally, I would like to thank Jo Swabe for her correction of my English.

Frouke Pieters,
Utrecht, October 2006

Summary

More than one hundred thousand non-human primates are used for biomedical research annually worldwide. The United States, Europe and Japan are the main countries that use these primates. In the European Union, some ten thousand primates are used every year. These are mainly Old World species, which are primarily used for toxicological and safety purposes.

While the use of these animals leads to ethical objections, the scientific quality of primate studies is also the subject of discussion. This study investigates the extent to which the use of primates could be reduced or replaced through an analysis of the existing literature and interviews with stakeholders.

In fundamental studies on disease, different (animal) models are often used alongside each other; arguments regarding why primates are used are only seldom given. Primates mainly seem to be used for immunologic reasons, which exclude the use of any in vitro alternative. In preclinical research, it appears that primates are mainly used for conventional medicines, which is contradictory to expectations. Next to scientific arguments for the use of primates, non-scientific reasons also play a major role. Financial and regulatory issues appear to be the most important factors, which hamper the replacement or reduction of primate use. The necessity of using primates for routine safety tests has not been established.

The use of primates for both fundamental and preclinical studies is likely to increase in the near future, consequently increasing the need to replace or reduce the use of primates as much as possible. The most promising alternatives seem to be the use of other animal species, human tissue or volunteers and a tiered approach to reduce the number of primates. Although the use of other animal models is certainly not a 3R-alternative, this is generally regarded as some improvement and a first step towards complete replacement.

However, there are a number of factors that hamper the development and implementation of alternatives. These include problems with the selection of the relevant animal model, regulatory requirements, information, motivation and funding for alternatives, as well as personal and commercial interests. Furthermore, there are no criteria that a model has to fulfil, which makes it hard to judge the traditional model or an alternative method on its merits. This, together with safety issues and fear of liability, makes regulators slow to implement alternatives.

In view of this, the most relevant model for both fundamental and applied studies should be subject to discussion. For primate studies, advice on the relevance of the model could even be mandatory. Furthermore, the criteria that any model should fulfil must be decided and research into alternatives should be intensified. However, any stricter regulations on the use of primates should only be decided on in discussion with stakeholders, to prevent the outsourcing of primate studies to countries where animal welfare may be poorer.

Samenvatting

Wereldwijd worden jaarlijks meer dan honderdduizend niet-humane primaten gebruikt in biomedisch onderzoek, vooral in de Verenigde Staten, Europa en Japan. In de Europese Unie gaat het om zo'n tienduizend per jaar, voornamelijk Oude Wereld apen, en met name voor toxicologische en andere veiligheidstesten.

Het gebruik van apen in biomedisch onderzoek levert ethische problemen op, terwijl er ook discussie is over de wetenschappelijke kwaliteit van het primatenmodel. Daarom is door middel van literatuurstudie en interviews met betrokkenen geïnventariseerd in hoeverre het gebruik van primaten verminderd of vervangen zou kunnen worden.

In fundamenteel wetenschappelijk onderzoek worden regelmatig verschillende (dier)modellen naast elkaar gebruikt en zelden worden argumenten gegeven waarom het primatenmodel gekozen is. Het immuunsysteem lijkt de voornaamste reden te zijn, wat het gebruik van in vitro alternatieven uitsluit. In preklinisch onderzoek blijken, in tegenstelling tot verwachtingen, primaten voornamelijk voor conventionele geneesmiddelen gebruikt te worden. Naast wetenschappelijke argumenten lijken niet-wetenschappelijke een grote rol te spelen in het gebruik van primaten: financiële redenen en eisen van regelgevers belemmeren het gebruik van alternatieven. Voor routinematig veiligheidsonderzoek is de noodzaak van het gebruik van primaten niet aangetoond.

Waarschijnlijk neemt voor zowel fundamentele als preklinische doeleinden het gebruik van primaten toe in de nabije toekomst, wat het belang van vervangings- of verminderingsalternatieven onderstreept. De meest veelbelovende alternatieven zijn het gebruik van andere diersoorten, menselijke vrijwilligers of menselijk weefsel en een stapsgewijze aanpak om het gebruik van primaten te doen verminderen. Hoewel het gebruik van andere diersoorten geen 3V-alternatief is, wordt dit in het algemeen gezien als een verbetering en een eerste stap in volledige vervanging van primatenexperimenten.

Problemen die de ontwikkeling en toepassing van alternatieven belemmeren zijn: problemen met de selectie van diermodellen, eisen van regelgevers, informatie, motivatie en subsidie voor alternatieven, plus persoonlijke en commerciële belangen. De afwezigheid van criteria waaraan een (dier)model dient te voldoen maakt het moeilijk om traditionele en alternatieve methoden op hun waarde te beoordelen; samen met veiligheids- en aansprakelijkheidsoverwegingen zorgt dit ervoor dat regelgevers traag zijn met de implementatie van alternatieven.

Daarom moet er discussie plaatsvinden over het meest relevante (dier)model in zowel fundamenteel als toegepast onderzoek. Voor primatenstudies zou advies over de relevantie van het model zelfs verplicht kunnen zijn. Daarnaast dienen criteria voor deze modellen vastgesteld te worden en moet onderzoek naar alternatieven een extra impuls krijgen. Extra stringente regelgeving ten aanzien van het gebruik van primaten moet echter in samenspraak met belanghebbenden bepaald worden, om te voorkomen dat primatenonderzoek naar het buitenland geëxporteerd wordt, waar de omstandigheden wellicht minder goed zijn.

Chapter 1

Introduction

1.1 Motivation

In the Netherlands, hundreds of non-human primates are used for biomedical research every year. Figures on European level are tenfold higher, while globally tens of thousands of primates are involved [Carlsson et al., 2004; European Commission, 2005a; Hagelin, 2004b; United States Department of Agriculture, 2006; VWA, 2004b]. In comparison to other animal species, primates are only used in a very small percentage of experiments (generally about 0.1% in the European Union) [European Commission, 1999, 2003, 2005a]. However, the use of primates had lead to more intense ethical objections - from both the public and the scientific community - in comparison to the use of other species [Gagneux et al., 2005; Goodman and Check, 2002]. Furthermore, some scientists have questioned the scientific relevance and necessity of the use of primates in research [Nath et al., 2000], while others argue the opposite.

A distinction is often made between Great Apes and smaller primates. The use of chimpanzees has generated a great deal protest since, evolutionary speaking, these animals are our closest relatives and show evidence of a high degree of social and cognitive development. In 2001, Loek Hermans, the Dutch minister of Education, Culture and Science decided to put an end to experiments with chimpanzees in the Netherlands [Hermans, 2001; KNAW, 2001]. Other countries also placed restrictions on the use of chimpanzees: Sweden introduced a ban, New Zealand and the United Kingdom made it practically impossible to obtain permission for their use and Japan stopped using chimpanzees for invasive research [Anonymous, 2003b; Goodman and Check, 2002; Home Office, 2003].

The Dutch government, however, made a clear distinction with respect to other primates: minister Hermans explicitly stated that research on monkeys was still necessary, although in principle experiments on primates should be avoided, and reduced or replaced as much as possible [Hermans, 2001].

Despite the ban on the use of Great Apes, discussion on primate experimentation continued and intensified in the Netherlands. Three meetings with different stakeholders were organized in 2001-2002, in order to facilitate an open-minded discussion in which shared interests and solutions to primate experimentation could be found. Agreement was reached to solve the problems by investigating three scenarios: to improve the well-being of monkeys used, to improve the quality of ethical and legal restrictions on the use of primates and to improve the development and implementation of alternatives. A working party 'Primatenonderzoek in Nederland' ('Primate Research in the Netherlands') was established under the auspices of the Dutch 'Vereniging voor Proefdierkunde' ('Dutch Association for Laboratory Animal Science'). A sub-commission of this working party wished to promote this latter scenario and thus asked the Science Shop for Biology of Utrecht University to conduct a study into the possibility of replacing or reducing the use of primates in biomedical research. The results of this study and the contents of this report, however, do not necessarily reflect the opinion of the 'Vereniging voor Proefdierkunde'.

1.2 Definition of the problem

This study focuses on the scientific grounds for using primates in research and the possibilities for replacing them with alternative methods. The corresponding research question is:

- To what extent can the use of primates in biomedical research be replaced or reduced by other models and methods?

This central question will be answered through four sub-questions:

- What kind of research are primates used for and how many animals are involved?
- What are the reasons for using this model and the specific number of animals?
- To what extent would other (non-primate) animal models or animal-free methods be applicable?
- Do any factors hamper the replacement or reduction of primate use?

The aim of this research is to gain insight into the use of primates, the arguments involved and the possibilities for and limitations involved in reducing or replacing this animal model with alternative methods from a scientific and pragmatic point of view. Using the information obtained, sticking points in the reduction or replacement of primate experimentation with alternative methods are identified. Wherever appropriate, possible solutions to the obstacles found and general criteria for useful alternatives are formulated.

1.3 Scope and definitions

The scope of this inventory has been restricted in the following ways:

This study focuses entirely on the use of primates in biomedical research. Behavioural studies have thus been omitted from this survey.

In addition, the emphasis of this study is on the Dutch and European situation. More general questions with respect to the number of primates and types of research, as well as possible alternatives for biomedical research, have been investigated. However, the case studies focus on the Netherlands or Europe. Nevertheless, given that the scientific and pharmaceutical world is a global one, scientists or experts outside of Europe often had to be contacted to allow a more in-depth examination.

Alternatives

Not all 'alternatives' as proposed by Russell and Burch [1959] have been investigated. 'Replacement' and 'Reduction' have been studied, but 'Refinement' ('reducing the pain, suffering, distress or lasting harm experienced by the animal before, during or after the experiment') has not been taken into account in this study. However, the use of refinement alternatives may ultimately also lead to a reduction in the use of animals. While not investigated intensively, when such alternatives have been found, they will be mentioned.

Further to this, the option of using animal models other than primates has also been investigated. Although they are not replacement alternatives, according to Russell and Burch's definition, such models may provide a substitute to the use of primates. In this report, therefore, something is labelled as an 'alternative' when it is or could be used to ultimately answer the same research question as primates would have been used for. Eventually, the application of such an 'alternative' must lead to a reduction in, or replacement of the use of primates for this research question.

When alternative methods are used for research questions for which primates would not have been used in the first place, these methods will be discussed as interesting developments, but will not be regarded as direct 'alternatives' according to the definition used in this report.

According to the participants of a workshop on laboratory animal science in the Netherlands [Cohen and de Cock Buning, 2003], there are three groups of alternatives: at the level of the laboratory, at the transitional level and at the interdisciplinary level. The first would include the 3Rs as defined by Russell and Burch. At the intermediary level, more fundamental questions are involved: the quality of the scientific question and the models used, retrospective analysis and risk analysis. The interdisciplinary level includes more general attitudes as responsibility, risk acceptation and so forth.

The 'alternatives' discussed at the transitional and interdisciplinary level could certainly contribute to the reduction and replacement of animal experiments, though these are generally not regarded as alternatives according to the 3Rs point of view. In this report, these approaches will not be regarded as alternatives, but as problems with the current model or possible obstructions to the reduction or replacement of animal experiments. These will thus be discussed in the corresponding sections.

1.4 Approach

This study does not profess to be comprehensive; this is due to limitations in both time and scope. However, some general conclusions have been drawn by combining a general and extensive literature search with in-depth case studies, which have been carried out mainly by interviews with stakeholders.

The study consists of three stages:

Stage 1: Literature study

Firstly, a literature study has been conducted on the use of primates, the ethical and practical considerations involved and the possibility of alternatives. Primary scientific literature, reports from several organizations and factual information concerning the number of animals and regulations have been studied.

In the literature, however, there is a clear distinction between the views of researchers using primates, other scientists and animal welfare organizations with respect to the use of primates and possible alternatives. Moreover, very few arguments on the use of primates versus the possibility of alternatives are given in literature, which made it practically impossible to draw any firm conclusions from the literature study only.

Stage 2: Case studies

In order to acquire more in-depth information and arguments, case studies in fundamental biological studies, R&D (Research & Development) for human medicine and toxicological and other safety evaluations for medicine were selected for further research. For a subject to be selected as a case study, a significant number of primates had to be used for that topic in either the Netherlands or elsewhere in Europe. Furthermore, there had to be indications from the literature that alternatives might be applicable and the subject should not be regarded as an exception to the general category of research. The reasons for choosing each specific case study will be explained in greater detail in each of the corresponding chapters.

Given that the use of primates is a very sensitive topic, the accessibility of respondents was an important issue. This criterion also played a role when selecting the subjects for the case studies. Subjects were chosen for which in first instance preferably Dutch or European experts could be approached. Sometimes these contacts then referred to specialists from outside the EU.

Scientists were selected on the basis of their expertise and number of publications. Other respondents such as manufacturers, regulators and so forth have also been included where appropriate. In some cases, respondents were selected by an indirect procedure when previous respondents or supervisors to this study suggested contacting a specific person.

For all three case studies, experts with different points of view were contacted. In some cases, this was a combination of scientists using primates versus scientists using other methods; in other cases regulators or manufacturers were involved. As far as possible and applicable, the same topics have been discussed with each of these different respondents.

Stage 3: Report

This literature study and interviews with stakeholders resulted in this report. The stakeholders were informed that this report would be publicly available in advance. All findings from interviews have been used on an anonymous basis; a list of all respondents has been included at the end of this report in Appendix 1. All respondents on this list were previously informed of their inclusion and the parts of the interview that would be used in this report.

Within these limitations, this report attempts to provide an overview of the use of primates, the arguments involved from several viewpoints and the possibility of reducing or replacing them with alternatives methods, again from several perspectives.

1.5 Structure

This report combines the results from the literature study with the views of several stakeholders, although the three case studies are presented in different chapters.

Firstly, a general introduction on primate experiments is given, discussing figures and regulations as well as ethical issues. Furthermore, general examples of alternatives to animal testing are discussed briefly.

In chapters four to six, three cases for which most primates are used are discussed, starting with fundamental research, followed by preclinical research and finally safety testing. Each of these chapters is organized in the same way: they begin with an introduction on the use of primates for this category and findings from literature are presented, then the case study is discussed, after which the results from this case study are discussed in comparison with the general findings in this field.

Finally, in the last two chapters, the findings are combined and the use of primates, arguments, the possibility of using alternatives, current difficulties and future developments and solutions to replace or reduce the use of primates in biomedical research are discussed. In addition, some recommendations on these topics are presented.

Chapter 2

Use of primates

Although the use of animals in experiments is a subject that is surrounded by controversy and discussion, this is especially the case for the use of primates. In this chapter, these controversies will be discussed, as well as alternatives to the use of animals in general. In later chapters, alternatives specifically for primate studies will be discussed. However, some figures on the use of animals in biomedical research in general, and primates in particular, will first be provided.

2.1 Figures

Primates constitute a mere 0.1% of all animals used in experiments within the European Union. Rodents are the most frequently used: mice and rats account for 72% of all experimental animals in the EU: about 11 million animals in total each year [European Commission, 2005b]. The United States reports having used 1.1 million laboratory animals per year [United States Department of Agriculture]; in the Netherlands, a total of 633,155 animals were used in 2004 [VWA, 2004b].

These numbers, however, are hardly comparable, since regulations pertaining to the use of laboratory animals differ between countries. The figures from the European Union, for example, do not include the number of animals that are killed without previous intervention, as 'the killing by a humane method for the purpose of obtaining tissues, cells or body is not a procedure under the above definition, (...) and therefore need not be included in the table [Anonymous, 1997].

In the Netherlands, statistics on animal use are registered according to both the EU definition and national regulations. In the Netherlands, the humane killing of an animal without preceding procedures, but for one of the goals that fall under the definition of an animal experiment, is still regarded as an experiment, as opposed to EU statistics. In 2004, no primates were involved in such experiments. In addition, every experiment on an animal in the Netherlands is registered, while in the EU only the number of animals involved is actually tabulated. The re-use of an animal is thus not registered in EU statistics. Furthermore, toxicological research is not regarded as a main goal of an experiment in the Netherlands, but as a way of conducting an experiment [VWA, 2004a, 2004b, 2006]. For these reasons, two versions are available, which will both be presented here and in Appendix 4 and 5.

In the United States, animal experiments are viewed from a different perspective. Mice and rats (which in the EU make up 72% of all laboratory animals) are not registered at all: according to the Animal Welfare Act, 'the term "animal" means any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal, (...) but such term excludes birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research' [Anonymous]. The percentages for the use of one species compared to the total animal use cannot therefore be compared to the European statistics. The exact number, however, does include animals that have been humanely killed for tissue sampling.

2.1.1 Use of primates

Reliable figures on the use of primates do not exist for all countries, and no figures for countries as Switzerland, Norway and Eastern Europe are included in the European statistics. Furthermore, the exact use of primates for different goals can be retrieved from statistics. However, the analysis of national statistics is just one way to examine the use of animals: such information can also be retrieved by analyzing scientific publications. Nonetheless, this has its own disadvantages: not all experiments using primates will be published, nor is it always easy to retrieve these publications. In addition, even in the literature it can be hard to determine the exact number of primates used or whether the animals have been re-used.

Globally

Hans-Erik Carlsson investigated academic papers reporting the use of primates at a global level. Up to two-third of the animals in the articles he retrieved might have been re-used, but on the other hand, not all primate studies will actually be published. Indeed, in Carlsson's survey of papers published in 2001 [Carlsson et al., 2004], the number of primates used could only be deduced for 35% of all studies. In these studies, more than 41,000 primates were used. Based on these considerations, Carlsson estimated that globally every year 100,000 to 200,000 primates are used in biomedical research; approximately 27% of the research involves studying primates in their natural environments for conservation and ecological reasons, goals that are not included in the subject of this report.

A good example of the problems relating to retrieving data on primate use from scientific publications is a study by Joakim Hagelin [2004a]. He analyzed publications in which primates were used in 2001 and tabulated the number of primates involved. From EU countries, the percentage of retrieved animals from publications was about 37% of the total number used. According to this analysis of publications, most primates are used in North America, Japan and Europe. Japan, the third largest primate user according to Joakim Hagelin [2004b], accounts for two-thirds of scientific primate studies in Asia.

United States

According to the US statistics, 54,998 primates were used in 2004. This number has fluctuated for years around an average of about 53 thousand primates, but after a sharp decline in 2001, the number of primates is once again steadily increasing. The percentage of primates as compared to total animal use also seems to be slowly increasing over the years [Rowe and Lenz, 2000; United States Department of Agriculture, 2006]. See Figure 2.1 for details.

It is estimated that US based authors account for half of all publications using primates [Hau et al., 2000] and, according to Kathleen Conlee of The Humane Society of the United States (HSUS), the USA uses more primates (including Great Apes) per year in research than any other country in the world [Conlee et al., 2004].

Europe

According to EU-statistics, 10,362 primates were used in 2002 [European Commission, 2005a]. This number has changed little, although the use of animals has only been registered in EU-statistics for just a short period of time: in 1999 and 1996 respectively, 9097 and 10,681 primates were involved. In terms of publications, Europe accounts for a quarter of all papers in which primate use has been reported [Hau et al., 2000].

From the 15 reporting EU-countries in 2002, the UK, France and Germany are the foremost primate users, both in terms of percentages and absolute numbers. France is Europe's major user, with 3840 primates in 2002, 0.17% of their total animal use. The UK reached the same percentage and used 3173 primates, a number that seems to have increased throughout the past few years [Home Office, 2003, 2005]. Germany utilized 1844 primates, 0.09% of total animal use [European Commission, 2005a]. For more details, see Figure 2.2 .

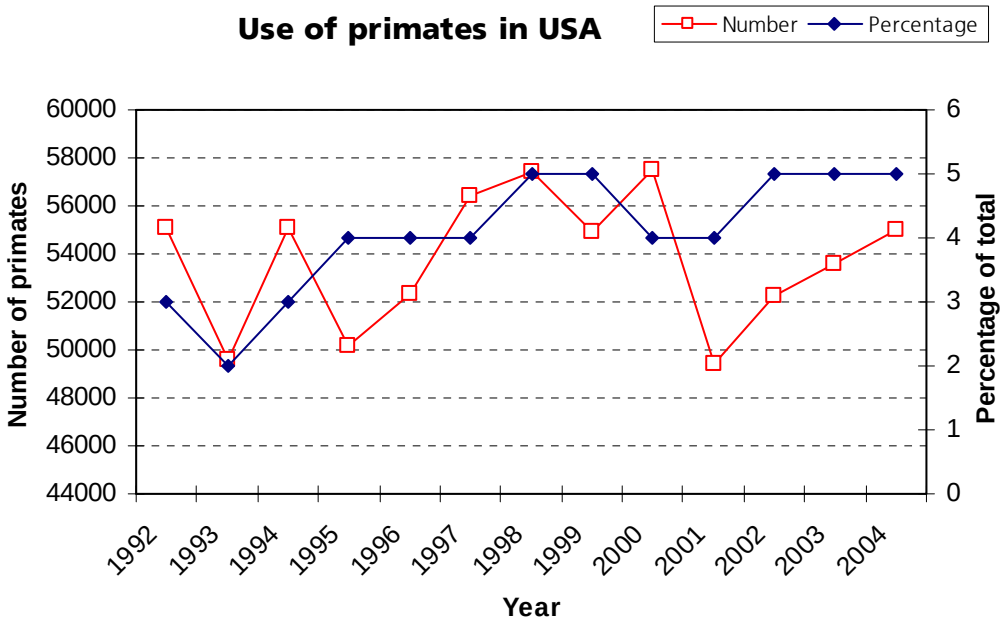

Figure 2.1 The use of primates in the United States of America [United States Department of Agriculture, 2006].

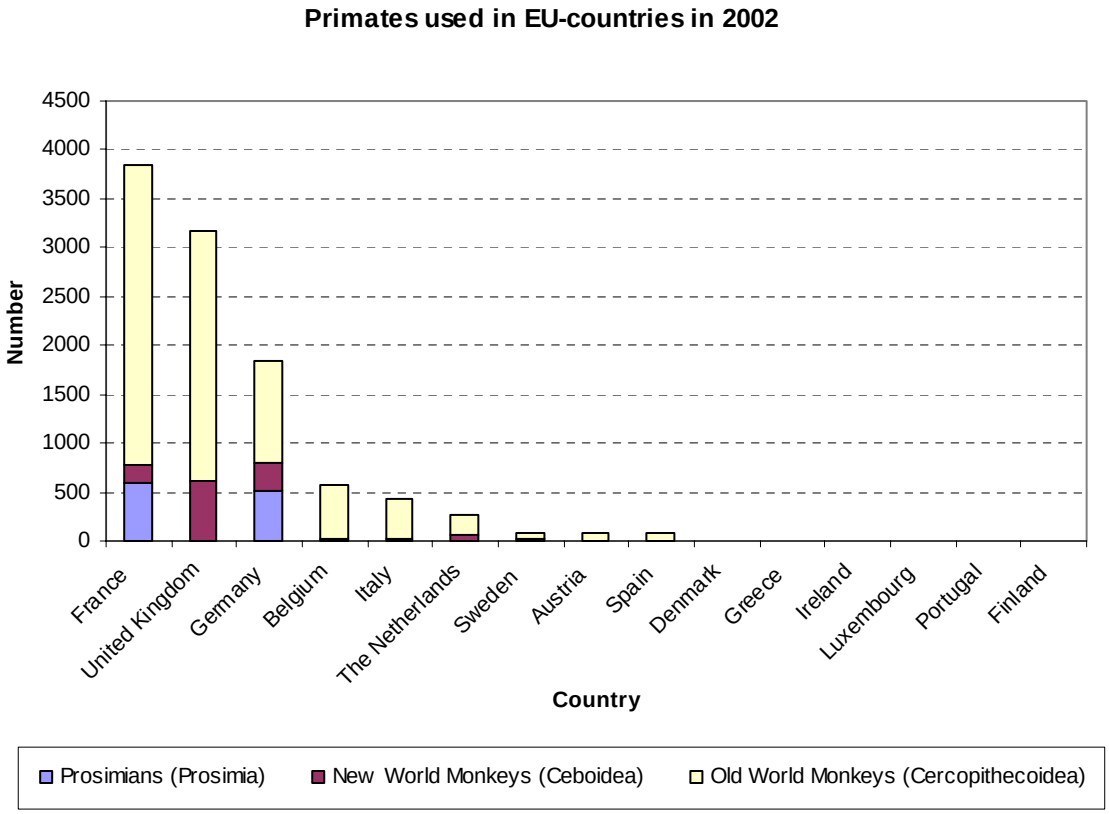

Figure 2.2 The use of primates in EU-countries [European Commission, 2005a].

The Netherlands

In the Netherlands, 600 to 700 experiments are performed on monkeys each year; a number that was reported to be declining since 2001, although the figures for 2004 have shown an increase [Schell and Tsang, 2005; Van der Eb, 2003; VWA, 2004a, 2004b, 2006]. A graphic representation of the use of primates can be found in Figure 2.3 .

According to the EU statistics (excluding re-use and humane killing of animals without preceding experiments), 289 primates were used in the Netherlands in 2004. According to national statistics, 701 experiments on primates were conducted, of which 403 primates were reported to have been re-used. The EU and national statistics differ markedly, mainly with respect to R&D (Research and Development), indicating that primates are often re-used for this goal [VWA, 2006].

Only 163 of 701 experiments were performed at universities. According to a KNAW-report and a letter from the Education Minister, the Biomedical Primate Research Centre (BPRC) in Rijswijk performed by far the most primate experiments in the Netherlands between 1995 and 2000, mainly on Old World primates. The Erasmus University in Rotterdam was second largest primate user in 1999, followed by the pharmaceutical company Organon and the Dutch National Institute for Public Health and the Environment (RIVM) [Hermans, 2002; KNAW, 2001]. With regard to the Dutch universities for which figures are publicly available, Rotterdam, Utrecht and Nijmegen used most primates in experiments in 2004 [VWA, 2006].

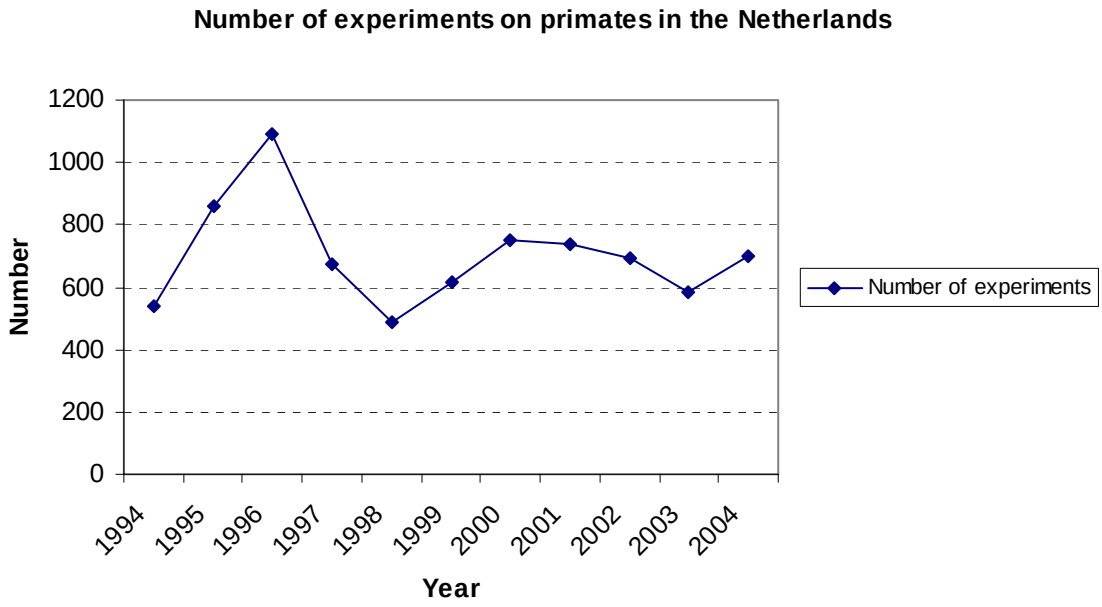

Figure 2.3 The use of primates in the Netherlands [Schell and Tsang, 2005]

2.1.2 Primate species

There are five major groups of primates: humans, apes, Old world monkeys, New World monkeys and prosimians. Together they form the biological order 'Primates', which can be divided is sub-orders and families. For more information on this taxonomy, see Textbox 2.1.

Textbox 2.1 Primate species

There are several possible taxonomical systems of primate species, and these have changed over time. The classification presented (a combined and simplified version of [Shoshani et al., 1996] and [Schwimmer, 1996]) might be outdated or even rejected, since the discussion on the exact taxonomic location of some primates is ongoing. In particular, the position of tarsiers is disputed, because this species has characteristics of both prosimians and monkeys. Historically they were categorized with the prosimians, but nowadays some think they actually belong with the monkeys and the taxonomic organization of monkeys had been changed.

Another discrepancy is the position of the chimpanzees. Traditionally, humans have been classified separately from the Great Apes. However, recent evidence shows that humans are not a separate lineage from the apes. For example, chimpanzees share more genetic material with humans — about 99% — than they do with gorillas [Hartman, 2005], indicating there actually is no division between Great Apes and humans and the classification has been revised, to show that humans are apes [The Boyd Group, 2002b].

Taking these ongoing discussions into account, the taxonomy of primates is roughly as follows:

Order Primates
- Suborder Strepsirrhini (prosimians: loris, lemur, galago)
- Suborder Haplorhini (tarsiers)
- Semi suborder Anthropoidea
  - Infraorder Platyrrhini (New World monkeys)
    - Subfamily Callitrichinae (marmosets, tamarins)
    - Subfamily Cebinae (squirrel, owl and spider monkeys)
  - Infra-order Catarrhini
    - Super-family Cercopithecoidea (Old World monkeys: macaques, baboons)
    - Super-family Hominoidea
      - Family Hylobatidae (Lesser Apes: gibbons)
      - Family Pongidae (Great Apes: orang-utans, gorillas, chimpanzees, bonobos)
      - Family Hominidae (humans)

On an evolutionary timescale, humans, chimpanzees and gorillas are closely related: current opinion is that the chimpanzee and human lines separated only around 4.5 million years ago. [The Boyd Group, 2002b] The prosimians, literally 'pre-monkeys', are evolutionary most distinct from humans.

Monkeys can be separated from apes by the fact that apes do not have a tail, and most monkeys do. The monkeys are divided into two major groups, one of which evolved in South and Central America (the New World monkeys) and the other in Asia and Africa (the Old World monkeys, including the macaques and baboons). Apes diverged from the Old World monkeys around 30 million years ago [The Boyd Group, 2002b].

All categories of non-human primates are used in biomedical experiments, although the exact species are not always registered. Reports state that around 65% of the primates used in research worldwide are Old World species. The cynomolgus monkey (*Macaca fascicularis*, crab-eating or long-tailed macaque) and rhesus monkey (*Macaca mulatta*) are most frequently used; the cynomolgus monkey tends to be the preferred and predominantly used model, while the rhesus monkey is utilized mainly because much background information on this species is available. 15% of primate species used worldwide are New World species, most often the marmoset (*Callithrix jacchus*). This species is considered especially suited to testing biotechnology products when at first there is little and expensive substrate available; due to its small size, only small amounts of a product are needed to test on this monkey. Around 10% of published research with primates worldwide involve the use of Great Apes, leaving the last 10% for prosimian use [Buse et al., 2003; Carlsson et al., 2004; FRAME, 2005b; Zuhlke and Weinbauer, 2003].

It is, however, unlikely that these publication figures truly reflect the actual use of individual animals: one respondent, for example, reported that in the United States 1300 out of the 55000 primates that are currently used per year are chimpanzees (2%). In the EU, no Great Apes are presently being used; Old World monkeys are most

frequently utilized. Indeed, Old World monkeys accounted for 78% of all primates used in 2002; New World monkeys and prosimians both account for 11%. Compared to the estimates of the use of primate species worldwide, it seems that the absence of the use of apes in the European Union has lead to a larger proportion of research being conducted on Old World monkeys [European Commission, 2005a].

Nonetheless, there are notable differences between individual countries in the EU, at least in 2002. Since this report focuses on the European and Dutch situation, the use of primates in specific countries has been analyzed. Different countries not only account for differences in numbers and species used, but also for the use of species for various purposes of experiments. It is noteworthy that prosimians were only used in France and Germany. Even more remarkable is that French researchers only used these primates for fundamental studies; they constituted virtually all primates used for this purpose. In contrast, Germany used them almost exclusively for toxicological studies. It should be noted that the use of prosimians in Germany diminished by more than 60% after 2002. Indeed, these primate species have only been used in relatively high numbers over a three year period [Anonymous, 2006]. These differences seem to be based on tradition or policy, but the exact grounds for these observations is certainly the subject of future research.

Other significant differences in the use of primates are the relatively large proportion of New World monkeys used in both the United Kingdom and the Netherlands, 19% and 23% respectively, and of Old World monkeys in Belgium and Italy: 96% of their total primate use [European Commission, 2005a].

2.2 Goals of primate studies

Laboratory animals are utilized for several purposes. Fundamental research offers insight into the processes that are involved in diseases. On the basis of such research, scientific institutes or commercial companies will then investigate the various possibilities for treating the illness. This might, for instance, lead to the development of a promising medicine, but, under the current regulations for the development of pharmaceuticals, such a novel therapy must also be tested on animals in order to ensure that it is both effective and safe for humans. Another series of animal tests is thus necessary.

Animals are also used for the production of vaccines and sera. Vaccines are essentially fabricated from dead or weakened micro-organisms. Animals might be used to produce the virus that eventually makes up a vaccine. Yet even if the vaccine produced in vitro, without the use of live animals, it must still be tested for safety before it may be used on humans.

Many animals are also employed for toxicological research for non-medical reasons. Numerous chemicals are used in our daily lives and food. These must be evaluated to exclude any unforeseen and potentially harmful effects. Animal tests often have to be performed and sometimes even primates are used for this purpose [Anonymous, 2005].

2.2.1 Categories in Europe

In general, the European Union has distinguished six categories of subjects for which animal experiments may be conducted [European Commission, 2005b]. A schematic overview of these categories is provided and further elucidated in Appendices 2 and 3. For the sake of clarity, the numbering of categories that is used by the EU will also be employed in this report. The six categories are as follows:

- Biological studies of a fundamental nature (2.2)
- Research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine (2.3, 2.4 and 2.5)

- Toxicological and other safety evaluations, including safety evaluation of products (2.6)
- Diagnosis of disease (2.7)
- Education and training (2.8)
- Other (2.9)

Appendix 6 presents a schematic overview of the number of animals used in the European Union for the different categories in 2002. The main goals of animal experiments were R&D and fundamental studies; with 4.8 million and 3.7 million animals respectively. Toxicological evaluations required the use of more than one million animals [European Commission, 2005a].

2.2.2 Subjects of primate studies

Europe

The statistics for primate use for the different categories are presented in Figure 2.4. The primary objective of primate use in Europe is for toxicological and other safety evaluations (2.6), specifically toxicity testing for human medicines. Not only are the highest number of primates by far used, but also the highest percentage when compared to all animal use: 0.6% of animals used for toxicity studies are primates, while this is generally 0.1%. Almost exclusively Old World monkeys are used for this purpose, both in absolute and relative terms. In the UK, the use of primates for safety evaluations has increased throughout the past few years, although it appears to be on the decrease for fundamental studies [Home Office, 2003, 2005].

The second aim of primate use in Europe is to conduct biological studies of a fundamental nature (2.2). Roughly the same numbers of prosimians, New World monkeys and Old World monkeys were used in the EU in 2002 for this purpose. In the UK, a remarkably high number of New World monkeys have been used for fundamental studies: more than twice the number of Old World monkeys used for the same purpose.

According to the statistics, 5535 primates were used for experiments relating to human and animal diseases in 2002. However, this figure cannot be correct. Erroneous reporting on the part of the United Kingdom meant that about 2400 more primates used for diseases were reported than for the separate categories. A figure close to 2100 is more likely to represent the actual use of primates for these purposes [European Commission, 2005a].

The Netherlands

According to EU statistics, 289 primates were used in 2004 in the Netherlands, mainly for fundamental biological studies; some of these primates were, however, used in R&D for human and veterinary medicine. According to Dutch national statistics, most procedures on primates were performed for R&D for humane and veterinary medicine and secondly for fundamental biological tudies. Appendices 4-5 illustrate the use of primates in 2004 in the Netherlands in comparison with the use of other animals.

Worldwide

On a worldwide scale, there are no known statistics relating to the experimental purposes that primates are used for. The aims of research are only recorded in publications using primates. Only successful scientific studies tend to be published, while most monkeys were shown to have been used in applied studies.

Hans-Erik Carlsson's investigation into the use of primates in literature worldwide found that most studies that use primates are conducted in the fields of microbiology and neuroscience studies [Carlsson et al., 2004]. In the United States, primates are reported to have been used mainly for HIV research, in addition to R&D for vaccines and medicines and in several fundamental studies. Chimpanzees have mainly been used for research into hepatitis [Archer, 2004; Conlee et al., 2004; Nuffield Council on Bioethics, 1996].

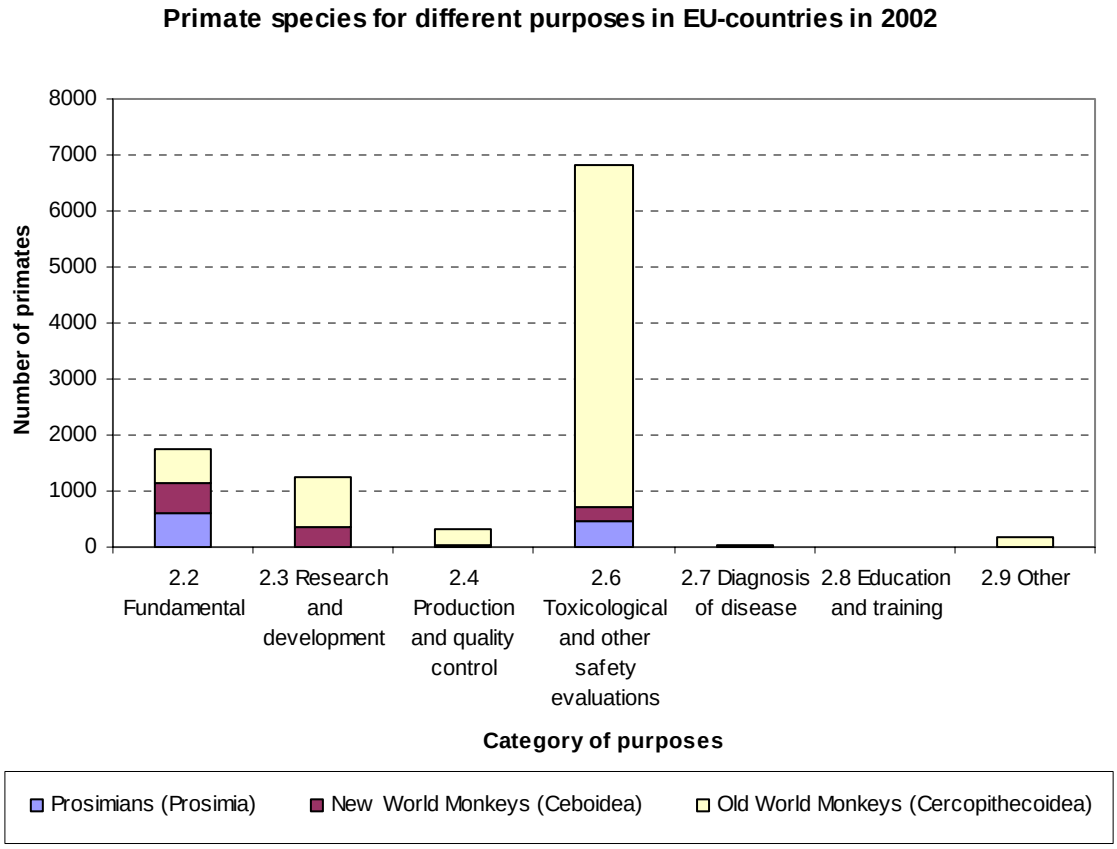

Figure 2.4 The use of primates for different purposes [European Commission, 2005a].

2.2.3 Discussion on differences in primate use

Although the differences in animal and primate use between the countries presented in this chapter are striking, it is not possible to draw any firm conclusions on the basis of these statistics. Within Europe, the number of animals used in biomedical research logically depends on the size of a country, the finances available and the presence of expertise for this type of research. For instance, France, UK and Germany, the three major primate users, are also the three largest countries in Europe. From this perspective, Germany, the largest country, even used relatively few primates; this certainly also applies to Italy. Belgium, however, used comparatively more primates.

It is even harder to give explanations for differentiation in primate use between continents, since differences between regulations and the registration of animals render any useful comparison difficult. It appears that there is generally less resistance to primate use in the United States. This could indeed facilitate the choice for this animal model.

More importantly, however, the scientific world is a global one. When scientists from several countries collaborate, the use of animals may occur and be registered in a different country to the one that initiated the study. For instance, a search on the use of primates in a German database on animal experiments [Anonymous] revealed that many fundamental neuroscience studies were performed at the German Primate Centre with the participation of Dutch researchers. In addition, another German study was found, which involved the participation of pharmaceutical company Solvay in the Netherlands.

This is even more applicable to pharmaceutical companies, since they often use Contract Research Organizations (CROs) as a part of their research. These CROs are commercial companies that perform various tests for

external parties, such as animal testing or clinical studies on humans. Any animals used by a CRO will be registered in the nation the CRO is located, not in the country of the company that has requested the studies.

The presence of CROs offering primates in a country is thus very likely to increase the number of primates reported in that country. There are no objective figures on the use of primates by CROs, but intensive protests against the use of primates have been held at Huntingdon Life Science in the UK and Covance in Germany. The presence of these companies may explain the high number of primates used in these countries as, according to a German animal welfare organization, about thousand primates, mainly Old World monkeys, are used by Covance in Germany every year. This company would account for more than half of the total primate use in Germany [Menschen für Tierrechte - Bundesverband der Tierversuchsgegner e.V., 2006]. On the other hand, this would mean that relatively few primates are used for non-contract research in Germany, when the size of the country is taken into account.

Nonetheless, this does not explain the high degree of primate use in France. A quick scan on the internet did not reveal any CROs in France that provide primates, although this certainly does not exclude their existence. One respondent confirmed that there are indications that a CRO is also located in France. Another explanation for the high level of primate use in the UK and France may be the presence of animal facilities at pharmaceutical companies, which use primates themselves. This is less easy to investigate given that most pharmaceutical companies are far from eager to disclose information on their use of animals, certainly when primates are involved. The use of primates by pharmaceutical companies will be discussed in greater detail in Chapter 5.

Re-use and goals

In the Netherlands, even more primates have been re-used than was estimated in literature. The re-use of animals also depends on the goals for which they are used. In general they cannot be re-used for safety evaluations, since these examinations involve the histopathological examination of tissue damage and the animals must be killed for this purpose. This kind of research seems to be the major goal of primate use worldwide, while such studies are generally not published in journals. The actual re-use of animals may therefore be lower than the two-thirds that was estimated on the basis of literature analysis. Given that almost no toxicology studies take place in the Netherlands, this may also partly explain the high percentage of re-used primates in the Netherlands [Carlsson et al., 2004; VWA, 2006].

2.3 Regulations on animal experimentation

2.3.1 General

In the Netherlands, the use of laboratory animals has been regulated since 1977 by the 'Wet op de dierproeven' (Animal Experimentation Act). This Act stipulates that animal experiments in the Netherlands are prohibited, unless a ethics committee has approved the research protocol in advance. This committee ensures that the animals will not suffer unnecessarily and that the benefit for humans will outweigh the cost to the animals.

Furthermore, the law stipulates that animal experiments will be prohibited if, according to experts, the goal of the experiment can be achieved by means other than animal experimentation, by using fewer animals or causing less suffering than in the experiment concerned [Ministry of VWS, 1977]. This means that an animal experiment will be deemed unlawful when a valid alternative is available.

A similar proscription has also been laid down in European regulations, both in the EU Council Decision [1998] as in the Council Directive concerning animals used for experimental purposes [1986].

The United States of America does not have any specific legislation with respect to laboratory animals, but parts of the 'Animal Welfare Act' [USDA, 2002] do deal with this subject. However, this Act does not apply to mice, rat or cold-blooded animals. The legislation stipulates that 'the principal investigator considers alternatives to any

procedure likely to produce pain to or distress in an experimental animal'. Nevertheless, according to this text, the use of animals is not forbidden if alternatives exist.

The present study has not investigated the existence of regulations on the use of animals in experimentation beyond Europe and the United States. Japan is likely to have laws of this kind, but what about China, India of African countries? Since these countries are rapidly developing and establishing laboratories, the use of animals is likely to increase and the level of animal welfare in such facilities might well be questionable.

2.3.2 Primates

The use of primates is limited by special regulations in many countries. Sometimes all primates receive special attention, in other cases the regulations only deal with Great Apes such as the chimpanzee.

The EU Council Directive [1986] concerning animals used for experimental purposes dictates that 'In a choice between experiments, those which involve animals with the lowest degree of neurophysiologic sensitivity (...) and which are most likely to provide satisfactory results shall be selected'. This regulation has implications for the use of higher animals such as primates, given that they are species that possess a high degree of neurophysiologic sensitivity. The question remains how the degree of 'neurophysiologic sensitivity' is determined and which species this concerns. Does this include the choice of mice over primates? What about the choice between dogs and primates?

The Netherlands did not implement this phrase in its own regulations, but some other countries have included a paragraph on the selection of species in their legislation. The British Animal (Scientific Procedures) Act 1986 (ASPA), for example, states that a project licence for use of non-human primates, cats, dogs and horses will not be granted unless 'no other species is suitable...or it is not practicable to obtain animals of any other species that are suitable for those purposes' [The Boyd Group, 2002a].

Restrictions, specifically on the use of chimpanzees, were also laid down in other countries: Sweden implemented a ban and both New Zealand and the UK made it practically impossible to obtain permission for their use; Japan also stopped using chimpanzees for invasive research [Anonymous, 2003b; Goodman and Check, 2002; Home Office, 2003]. In the Netherlands, experiments on apes have been prohibited since 2003 [Ministry of VWS, 2003]. In the US, there are no regulations or restrictions on the circumstances under which particular species, including monkeys and apes, may be used

2.4 Ethics

Animal experimentation is inextricably bound with a whole host of ethical issues. Given that the ethical acceptability of animal testing is not the focus of this report, these issues will not be extensively discussed here. Nonetheless, it is important to note that the use of primates meets with far greater resistance than the use of other species. In this section, the ethics of animal experiments and public attitudes towards this subject will therefore be dealt with briefly, paying special attention to the use of primates.

Often when the ethics of animal experiments are discussed, one assumption has already been made, namely that the use of animals in itself is not questioned at all. It is taken for granted that humans use animals for several purposes, thus accepting that humans are positioned 'above' animals in the species hierarchy. This premise will not be discussed further in the present report.

On the other hand, the intrinsic value of animals is also widely recognized. Dutch law explicitly states that the intrinsic value of animals forms the general guideline for the discussion of animal experiments. In the European Council Decision [1998] the following phrase also refers to the notion of intrinsic value: '(...) man has a moral obligation to respect all animals and to have due consideration for their capacity for suffering and memory. (...) Nevertheless in his quest for knowledge, health and safety man has a need to use animals where there is a reasonable

expectation that the result will be to extend knowledge or be to the overall benefit of man or animal, just as he uses them for food, clothing and as beasts of burden'.

2.4.1 Public opinion

A few studies on public opinion about animal experimentation have been conducted. Such investigations are hard to carry out in a subtle way, since most people tend to have a nuanced attitude on animal experiments, and answers may depend on the way in which the question is asked and the ends for which the animals are used.

In Table 2.1, the results of several studies on public attitudes towards the use of animals have been summarized. It may be concluded that the degree of approval is highest when a cure for life-threatening diseases is the research objective and lowest when the research goal is to assess the safety of household products etc. In the Netherlands, there are fewer moral objections to the use of primates for behavioural studies; the greatest objections are to primates being used as a biomedical research model and for the study of cognition [Cohen and de Cock Buning, 2003].

The correlation between public approval and the suffering of laboratory animals can be formulated as follows: when the expected suffering to the animals increases, public approval diminishes. When higher species such as monkeys are concerned, public approval lessens compared to the same type of tests on lower species such as rats and mice. According to the Dutch Intomart survey, the public makes no distinction between monkeys and dogs or cats. In general, the use of primates generates more resistance than the use of mice, but the difference between acceptance are not too great. There are differences between countries as well: in the UK, the use of primates is highly unpopular and strictly regulated, while in the United States, there is less moral indignation and the rules more permissive [Goodman and Check, 2002].

Table 2.1 Summary of studies on public attitudes towards the use of animals: Approval of animal expirements for several species and goals.

Study	**Level of suffering**	**Goal**	**Approval**			
			General	Mice	Dogs/cats	Primates
[MORI, 1999]	Not mentioned	Not mentioned	24%			
[Intomart GfK, 2004]			19%			
[MRC, 1999]			12%			
[MORI, 1999]		Speed up development of therapies to life-threatening diseases	45%			
[MORI, 1999]	No	Life-threatening disease		83%		75%
[Intomart GfK, 2004]		Chronic disease		74%	67%	67%
[MORI, 1999]	Some	Life-threatening disease		65%		52%
[Intomart GfK, 2004]	Intense	Chronic disease		34%	24%	24%

2.4.2 Practical / ethical problems with the use of primates

Other factors contributing to the ethical discussion on the use of primates in research related to the issues of the origin, transportation and housing of primates. These factors have been discussed more extensively in a document from the Scientific Committee on Animal Health and Animal Welfare [2002].

Origin

- Demand outstrips supply by breeding facilities [Nuffield Council on Bioethics, 1996], which results in primates being caught in the wild.
- This sometimes occurs in countries where monkeys are considered an agricultural pest and are hunted or killed for bush meat [Goodman and Check, 2002], though in other cases wild populations may be depleted because of capture: all primate species are to some extent endangered [IUCN, 2006].
- In the Netherlands, only animals obtained from breeding facilities may be used [Ministry of VWS, 1977]. However, even these facilities will often use wild-caught animals to establish their breeding programmes [Goodman and Check, 2002]. One respondent reported that they import primates from a breeding centre in China, which cannot guarantee that the animals have not been be caught in the wild.
- It has been recommended that only second generation offspring should be used for experimental purposes [Scientific Committee on Animal Health and Animal Welfare, 2002], though others state that this supply is insufficient to meet demand [EMBO, 2005].
- Capture causes considerable stress in animals. Moreover, they are kept at holding centres in the countries of origin, which have housing conditions and welfare standards that are possibly below the European standards [Ruhdel and Sauer].

Transportation

- 80% of all Old World monkeys used in the EU in 2002 derived from non-European Commission countries [European Commission, 2005a], most likely farms in China, Mauritius, Israel and the Philippines [Goodman and Check, 2002].
- Primates suffer stress during transportation. Most airlines now refuse to transport primates as a result of protests from animal-rights groups, so monkeys must endure a combined land and air transit of up to 58 hours. Incidents may cause suffering, distress and death [Bottrill, 2000a; Goodman and Check, 2002; GSK, 2001; Ruhdel and Sauer]
- It has been suggested that breeding facilities should be established in Europe, but this would lead to other objections [Balls, 1995]. Some animal welfare organizations fear that the very availability of primates would increase their use [Bottrill, 2000a; Goodman and Check, 2002].

Housing

- It is very hard to house primates in a naturalistic way under laboratory conditions.
- According to European guidelines, the current conditions under which rhesus macaques are housed are quite minimal. Each monkey may be housed individually, without enrichment materials, in a cage nearly twice its own height. There are plans to change these guidelines.
- During experiments, primates are generally housed individually.
- Poor housing conditions may affect the physiological reactions of the animal, thereby influencing the scientific quality of the study. It was reported that the immune system of individually housed primates was compromised.
- The conditions in the Chinese breeding centre from which the imported monkeys of one institution originate are said to be 'reasonably fulfilling our standards'.
- There are indications that some primates in experiments showed behavioural deformities such as auto-mutilation [BUAV, 2004; Cohen and de Cock Buning, 2003], but in general there is a perception that marmosets are less stressed by life in a laboratory environment than macaques [Animal Procedures Committee, 2002].

Re-use or retirement

- Prescott and Jennings [2004] argue that the complete life history of primates in experimentation should be assessed. Sometimes the animals may 'retire' as is organized for chimpanzees in the US, Australia and the Netherlands [Brent, 2004; Van der Hoeven, 2006].
- The British Home Office's view is that - particularly for Old World species - there are high costs involved in not re-using them due to problems with their sourcing, transport and acclimatization to a laboratory environment [Animal Procedures Committee, 2002].
- FRAME, however, has argued that the re-use of primates is not feasible due to the ability of primates to associate events with consequences [FRAME, 2005a].
- In the Netherlands, re-use of animals that have been subjected to the maximum level of distress [Ministry of VWS, 1977]

Relation

- One of the arguments against the use of higher animals is the nature of our relationship with pets and primates [Nuffield Council on Bioethics, 1996].
- The evolutionary close relationship between humans and primates is often given as reason for their use from a biological point of view. However, precisely the same argument can be used against the use of primates from an ethical perspective.

2.4.3 Higher development of primates

It is often argued that primates are highly developed animals, which possess cognitive functions that are even comparable to those of some humans. This would complicate their use as experimental animals for reasons of welfare and ethics. Research to verify such statements is still ongoing. It is therefore impossible to draw any firm conclusions in this report. An existing document published by the British Boyd Group [2002b], a forum 'for open exchange of views on issues of concern related to the use of animals in science', discusses much the evidence with respect to this issue.

Cognitive functions are hard to measure in animals that are not able to communicate with us directly, certainly when the experimental design must exclude the possibility of associative learning by the animal (instead of creative thinking) and unintentional influence of the results by the researcher. Therefore, artificial definitions that contribute to the level of cognitive functions are often used.

Intelligence

Most primate species are reported to be socially highly intelligent creatures [Van Hooff, 2003]. When comparing Great Apes with smaller monkeys, the latter seem to be less intelligent than the former, although exceptions have also been found. Moreover, other (non-primate) species may also have similar capacities: birds, in particular, have been shown to have complex cognitive capacities, including the ability to form concepts and communicate these symbolically; pigs and rats also display social behaviour and signs of intelligence [Nuffield Council on Bioethics, 1996; The Boyd Group, 2002b].

Theory of mind

Primatologists and other investigators of animal intelligence are still discussing Premack and Woodruff's [1978] question of whether primates have a 'theory of mind', using a variety of substitutes for this definition. It has been argued that in every case where nonhuman primate behaviour has been interpreted as a sign of theory of mind,

it may instead have occurred by chance or as a product of nonmentalistic processes [Heyes, 1998; The Boyd Group, 2002b].

- In humans, awareness of 'self' develops gradually in infancy and early childhood, with children only gaining full self awareness towards the end of their second year of life. The capacity for self recognition in a mirror appears to emerge at the same time. Great Apes alone have the ability to recognize themselves in mirrors, while other non-human primates do not [The Boyd Group, 2002b].
- On the other hand, studies of human development demonstrate that reflective thinking is not an all-or-none ability [The Boyd Group, 2002b].
- In a commentary on Heyes, Parker [1998], a scientist, argues that abilities are likely to be tied to neuroanatomy, and that the neuroanatomical study of the frontal lobes of humans and macaque monkeys has revealed that their basic architectonic plan is the same.
- On the other hand, it has been noted that it is unlikely that autobiographical memory will develop without language, and it seems likely that autobiographical, rather than episodic, memory is the key feature of the human conception of self.

Brain size

- The size of the brain compared to the rest of the body is also used as an indication for intelligence. The 'encephalisation quotients' (relative brain to body size) of humans is nearly three times greater than that of chimpanzees, more than three times that of squirrel and rhesus monkeys, and over four times that of marmosets - and it might be concluded that intelligence varies in similar fashion. On the other hand, large brains are not unique to primates. Dolphins have brains that are closer in size to those of humans than chimpanzees do, and elephants and whales have brains that are comparatively larger (in terms of relationship to body size) than marmosets.
- The neo (or frontal) cortex is the part of the primate brain that is associated with complex information processing. It is thought to be where 'conscious thinking' occurs and is associated with an ability to reflect on one's own thought processes. Neocortex size increases across the primates in similar fashion to overall brain size, with the human frontal cortex being proportionately larger than in other primates [The Boyd Group, 2002b].

Capacity to suffer

All of the characteristics discussed above may contribute to the capacity to suffer, or, more importantly, the capacity to realize one's own suffering.

- All animals are able to sense pain.
- One may argue that awareness of this pain may increase the distress experienced.
- At least some species of monkeys may have insight into their own thoughts. This could well be accompanied by a capacity to thinking about the future, although perhaps only in the relatively short-term [The Boyd Group, 2002b].
- One may question the effect of this ability on the welfare of primates in experiments. A primate with highly developed cognitive functions, which is able to anticipate forthcoming events, may put his own suffering into perspective, knowing that it will only be temporary.
- However, without periods of non-suffering, for instance, due to poor housing conditions, the same primate may realize his own distress, causing him to suffer even more.
- Yet what about a primate that is able to realize his own distress, but which does not have the capacities to foresee that the experiment will come to an end? One might reason that animals that do not possess fully

developed cognitive functions will suffer even more greatly under experimental conditions than more intelligent animals.

- At present, it is not possible to conclude which of these options is the case, or to what extent primates have an increased capacity to suffer compared to other animal species.

2.4.4 Discussion

The problem with defining the extent to which primates suffer more greatly under experimental conditions than other species is that we will never be able to know what other species feel, think and suffer. Moreover, there is also the problem of finding conclusive evidence of intelligence in other species.

Do primates then deserve to be accorded a special moral status? Some of the respondents in this study agreed, but this was on the grounds of housing issues in addition to their development. Compared to other non-rodent species used frequently in biomedical experiments, it is far more difficult to house and handle primates since these species have not been domesticated, in comparison to other non-rodents. Therefore, other species can adapt better to the unnatural laboratory conditions than primates, which increases the amount of distress of the latter. Furthermore, the Boyd Group also concluded that monkeys deserve a special moral status on grounds of their capacities for social and mental suffering.

In conclusion, since there are differences between primate species with respect to their intelligence and cognitive functions, it is hard to decide where to draw the line, also between primates and other animals. However, due to indications of higher development and, perhaps more importantly, difficulties with respect to sourcing and housing in laboratory conditions, there is reason to be extra prudent as far as the use of primates is concerned.

2.5 Problems and arguments

Aside from the ethical discussions on the use of animals in biomedical experiments, there is also scientific debate on the quality of animal models. Some have observed that the reasons for using (specific) animal models are not always quite scientific. A number of general points of criticism with regard to animal experiments and the use of primates will be discussed here.

2.5.1 Quality of animal models

People sometimes take extreme positions on the quality and relevance of animal experimentation. However, as the Animal Procedures Committee recognized in its review of the cost-benefit assessment of animal experiments, 'claims concerning the scientific validity of animal experiments cannot be argued in absolute terms. An absolute position that all animal experiments are scientifically invalid is untenable. However, so too is the opposite categorical position, that the validity of using animals in experiments is a forgone conclusion and should not be questioned.' Citing of cases where animal models have proved misleading does not in itself demonstrate that the approach is in principle misconceived. It only demonstrates the existence of invalid uses.

Moreover, what needs to be demonstrated is that the cases of failure are symptomatic of some general kind of flaw. To show this, it is not simply enough to appeal to the differences between species. What matters is whether these differences are relevant to the basis of the extrapolation that is being made [Animal Procedures Committee, 2003].

Fundamental biological similarities between animal species that are evolutionarily related make it at least sometimes possible to extrapolate results from one species to another, though it is recognized that this remains difficult. Some analogues of many human diseases exist, or can be induced in different species. In general, healthy

animals are used to study disease, or an artificial disease state is created, which is often not entirely representative of the human situation [Conlee et al., 2004; Ginis and Rao, 2003; Kuo et al., 1998].

Another problem is that information about individuals, re-use and housing conditions are seldom described in publications using primates. This renders it difficult to gauge the scientific validity of experiments, and hard for researchers to know that they are accurately reproducing each other's work. This is particularly disturbing in the case of reused animals, since the manipulations or experimental infections from earlier trials may well influence the outcome of later investigations [Carlsson et al., 2004; Conlee et al., 2004; Graham-Rowe, 2004].

2.5.2 Choice of model

If the objective of a study in animals is to discover the pathophysiological background of a disease in humans, then homology between animal and humans, at least for some of the relevant aspects of the disorder, is a prerequisite. Nonetheless, critical analysis of the literature revealed that, very often, these models are based on similarity of the phenotype rather than on homology [Van Zutphen, 2000].

Predicting the suitability of a species in advance of a study, however, can be difficult, because, for example, different species vary in how they metabolize test compounds. Differences that reduce the applicability of animal species to human physiology can be found in intestinal absorption, metabolism, distribution of a product in the circulation and tissues, and ultimately elimination or excretion from the body [Archer, 2004; The Boyd Group, 2002c].

Since there are no clear features to distinguish or decide the most appropriate animal model, different research groups may use different animal models to study the same problem. The Boyd Group [2002c] concluded that there can be sound scientific reasons for this, but that the choice of model might well be determined by tradition and convenience, rather than on strict assessment of validity. In practice, the real, perceived or anticipated requirements of regulators are an extremely important factor with regard to species selection for applied studies

The RSPCA suggests that the models used should be critically examined, which "might involve challenging the fundamental basis of a field of research that has traditionally been based on a particular animal model, but where an alternative approach might yield equally, or more, useful results" [Animal Procedures Committee, 2003].

On the other hand, there should already be background data on the 'normal' reaction of a model in order to be able to examine whether and to what extent this normal state is affected, and to judge the results of the test.

Time plays a role in this too. According to a review by the Animal Procedures Committee [2003], using a readily available animal model might be a legitimate factor in assessing the validity of the approach, if this model can bring results more quickly than developing and validating a different model or an alternative non-animal approach that might offer advantages. This would also depend on the nature and significance of the likely outcome, as well as the depth of consideration given to finding and developing an alternative. For example, speed would be of the essence if there were to be a need to rapidly develop new vaccines in the case of re-emergence of smallpox.

Some members of the Boyd Group believe that financial reasons, such as the costs to move to another species play a large part in determining species choice [The Boyd Group, 2002c].

Further to this, personal factors can also play a part. Research careers may be built on the use of particular models, and it can be difficult to change when there had been a great investment of time and energy into a particular approach [Animal Procedures Committee, 2003]. In addition, there is concern that the very availability of a particular animal model might determine the nature of the questions that are asked [Animal Procedures Committee, 2003; The Boyd Group, 2002c].

Practical factors are also the subject of discussion. It has been said that the committees, which assess the ethical and scientific quality of animal experiments, have a tendency to approve experiments prematurely [Cohen and de Cock Buning, 2003].

Chapter 3

Alternatives to animal use

3.1 Introduction

There are not only legal and ethical considerations for using alternatives to animal testing, but also practical and scientific grounds for doing so. Firstly, there are the costs involved in animal experimentation. Animals are generally more expensive than alternative methods, both in terms of their acquisition and housing and also the time-consuming care that they need. From a scientific point of view, alternative methods might yield better results, as unknown complex biologic mechanisms often play a role in the animal model, which may influence the research results.

Moreover, alternative refinement methods might yield better results as animals are subjected to less handling and thus less stress also, or can be followed over time. Eventually, this will lead to less variance and fewer animals being needed to obtain statistically significant results. As indicated in the introduction, this study devotes little attention to refinement techniques. The emphasis is instead on replacement. In addition, the use of other animal species as a substitute for primates will also be discussed. Unless stated otherwise, all information in this section is based on the "Handbook laboratory animal science" [Van Zutphen et al., 2003].

Primates

There is very limited information on alternatives to the use of primates in the existing literature. Many databases on alternatives exist, but only one German database was found with alternatives explicitly for primates [Anonymous]. In the literature, different animal models and animal-free methods have been described, but almost never as alternatives to the use of primates. In addition, most of these publications deal with the use of primates for fundamental research, while in fact most primates in the European Union are used for toxicological and other safety evaluations.

In a BSc thesis on the use of primates, two students from the Dutch Van Hall Instituut investigated the use of primates in the Netherlands, UK and Switzerland. Further to this, they conducted interviews with seven Dutch primate researchers and experts on their views regarding the possibility of replacing or reducing the use of primates in biomedical experiments, and the methods that are actually used [Schell and Tsang, 2005]. The results from this undergraduate investigation will be given here, but a more specific discussion on the possibility to use alternatives instead of primates will follow in the next chapters.

3.2 Alternatives

3.2.1 Animal-free methods

In vitro methods

In vitro methods are the most important group of alternatives to animal testing, which have been applied in

many kinds of scientific research. According to the interviews Schell and Tsang [2005] conducted with seven researchers and experts, in vitro methods are among the most frequently used alternatives, although not all in vitro techniques are explicitly used as alternatives to animal testing given that scientific research often makes use of these methods more generally. It is only an alternative when experiments on animals would otherwise have been conducted. Examples of these alternative in vitro methods are research on cell organelles, cells, tissues and organs. Mostly, these will be put 'in culture': kept in a medium that makes sure that all cells get the nutrients they require.

Specific cell types can be studied in vitro for the effects of a pharmaceutical compound or a pathogen on the cellular level. Mammalian cell lines can be used to predict specific aspects of in vivo activity and to quantitatively assess the relative sensitivity of various species (including human) to the product [ICH, 1997]. Genetically modified cells can also be used. By modification, a specific (e.g. human) receptor or an effect when this receptor is activated can be inserted. This enables researchers to study a specific receptor binding effect in vitro, which can be useful when evaluating new medicines.

In some cases, organs or tissues can be used instead of live animal models. Examples are the use of organs for educational purposes, but skin and eyes can be used for some toxicological irritation tests as well. With isolated liver cells from humans and animals, the rates and routes of metabolism of a drug molecule can be investigated to make predictions about initial doses in humans, but also about potential problems that may occur due to the formation of reactive entities. Such early testing in human-derived material can greatly speed up the drug development process, as well as reduce the number of animal studies necessary [Organon, 2005].

To a limited extent, it is also possible to create tissue in vitro by starting with cells that can both divide and differentiate, and adding the right chemicals to the medium. This can be done by starting with so-called stem cells. Research on how to grow organs in vitro is still ongoing: according to the European Centre for the Validation of Alternative Methods (ECVAM), companies are currently examining stem cell alternatives [Sinha, 2005].

The main advantage of in vitro models is the isolation of the system of choice. By this means, influences from other parts of the organism or systemic systems are excluded. In vitro models are often much more sensitive and exhibit less biological variance than the use of animal models. Moreover, the experimental conditions can be controlled and manipulated to a larger extent. Animal-free methods might show less variance, because complex biological mechanisms are not present or can be controlled.

The main disadvantage of in vitro testing is that cultured cells quickly lose many of their specialized properties when grown in culture because they are no longer in their 'natural' environment. Furthermore, not all cells can be put in culture or sustained for a prolonged period of time. This is difficult for specialized tissue and complete organs or small slides of the organ tissue. These have a relatively short lifespan in culture, since it is hard to provide nutrients to all cells in the organ. A constant supply of fresh organs is, therefore, required.

It is also difficult to extrapolate the results, since living organisms are more complex than separate cells or organs, and these may interact in a manner that cannot be studied in each system separately. For instance, the mechanism leading to toxicity may require the interaction of several different cell types; consequently much drug toxicity depends on long-term complex effects upon integrated functions that can only be assessed in a living animal [The Academy of Medical Sciences, 2005]. In vitro methods are, therefore, generally seen as viable for gaining more insight into fundamental questions, but cannot replace the use of primates in the final stages of the research.

Immunologic techniques

Immunologic techniques form the basis of a number of in vitro methods that are mostly used in diagnostics, the quality control of vaccines and fundamental immunologic research. Examples of these techniques are the enzyme-linked immunosorbent assay (ELISA), the hemagglutination test en de radio-immune-assay. In general, these methods

are very sensitive. However, sometimes they are not specific enough, which means that an animal test remains necessary.

Immunologic techniques have replaced the use of mice for the production of monoclonal antibodies: these can now be produced in in vitro systems, while previously so-called ascites mice were used for this purpose.

'Omics'

Relatively new methods, such as genomics, proteomics, metabonomics and transcriptomics, have generally been regarded as potentially useful methods with respect to species selection and the translation of animal findings to humans. The selection of the most appropriate animal model would prevent the unnecessary use of animals. Even within one animal species, the use of genomics may help in selecting individuals that are likely to respond in the desired way, thus reducing the use of animals. With metabonomics, small changes in a range of normal metabolites in intermediate biochemical pathways are detected, providing a 'fingerprint' of potential drug effects on, for instance, energy metabolism, liver or kidney function [Animal Procedures Committee, 2002]. This approach can facilitate the replacement by human studies as well.

In some cases, the use of genomics and proteomics was reported to be helpful in interpreting the results from animal experiments: for example, toxicity was found for a certain product in mice and rat. However, genomics revealed that the cause of this reaction was present in mice and rats, but not in humans; the product would thus be safe for humans. Genomics might therefore reduce the need to test for effects in other animal species.

Eventually such approaches may lead to a database of characteristic 'omic fingerprints that warn of likely hazard [The Academy of Medical Sciences, 2005]. One respondent, however, indicated that this might well lead to an increase in the use of primates rather than a decrease.

Physiochemical/biochemical techniques

Chemical techniques are used to characterize the composition of complex mixtures, for instance, by using High Pressure Liquid Chromatography (HPLC). This technique has led to the replacement of animal models in the regulatory quality control of hormone preparations. In addition, it can be used to investigate whether the concentration of certain (toxic) compounds is higher than allowed.

Mathematic models

Using the computer, compounds can be designed that contain structures to fit on the targeted receptors. This is called computer-aided drug design. This is followed by in vitro research and animal experiments, but the selection of active compounds can take place with much higher success rate, thus using less animals per compound in the end. Further to this, computer models may be used to predict and explain various biological reactions, such as the way in which a compound will be metabolized, distributed and excreted throughout the body.

3.2.2 Other animal models

Lower organisms

Micro-organisms such as yeast are exploited on a 'large scale' to produce antibodies or vaccines. Another example is the use of the horseshoe crab: a protein in their blood called Limulus Amebocyte Lysate (LAL) is used by pharmaceutical and medical device manufacturers to test their products for the presence of endotoxins, which are bacterial substances that can cause fevers and even be fatal to humans. Rabbits, which were previously used for this purpose, are now only used under special circumstances.

Embryos

In some cases embryos can be used instead of grown organisms. The advantage is that the nerves of embryos are not developed enough to be sensitive to pain. A mother animal is needed to collect eggs and to allow the embryos to grow to the size where they can be harvested and used for testing. Embryos can be used to test compounds for possible birth defects or cancer-inducing activity [Schell and Tsang, 2005]. The disadvantages of embryo use is that they are not fully developed organisms, which may mean that some reactions will differ from reactions in mature animals. Furthermore, they cannot be used over a longer period of time outside the mother animal, and not all procedures can be applied in the womb.

Other animal models

The possibility of using other animal models instead of primates will be discussed in this report, although this cannot be viewed as a replacement alternative in terms of Russell and Burch's [1959] 3R's definition. Several respondents contended that the use of primates encounters more problems than the use of other animal species, which meant that the replacement of primates with other species might be an option. Furthermore, when replacement with an alternate species is possible, this can be seen as an indication that the use of primates is not the only possible approach to answer the research question: other alternatives may thus also be applied.

However, one disadvantage of the use of non-traditional animal species in applied research is that they are not well-established and lack background data. In this regard, (transgenic) mice have been mentioned as possible alternative species. International guidelines also note the use of homologous proteins. Nonetheless, while useful information may also be gained from the use of homologous proteins, it should be noted that several factors may differ between the homologous form and the product intended for clinical use [ICH, 1997].

Another problem mentioned by a respondent is that results from the primate model can be translated to humans better, because the immune system of other animals such as mice is only partly representative of the human immune system. Furthermore, laboratory animals such as mice are often inbred-animals with a very specific genetic make-up. This increases the risk that results cannot be extrapolated to the more diverse human population.

3.2.3 Humans

Human material

Beside the use of animals as a model for humans, humans or human material itself could also be used. After all, humans are the best model for humans. Human material is already used. According Schell and Tsang´s [2005] interview data, human material is among the most frequently used alternatives.

In vitro models of the human liver could be used to study the metabolism of new pharmaceutical products. At present, this is mainly done using animal models, but there are many differences in the metabolic system of humans and animal models. Human blood cells are used as an alternative for the pyrogenicity test: human white blood cells react to the presence of pyrogenic compounds and this reaction can be determined in a test tube.

Yet, although long-term organotypic human tissue models are being developed, many existing in vitro systems based on human cells and tissues systems cannot be sustained for prolonged periods of time in culture. Research into model development therefore requires regular and sustainable sources of cells and tissues. However, hospital policies, a lack of human resources, and the views held by some medical professional bodies, mean that while there are often patients willing to make altruistic donations of tissues, including material such as placental tissue and cord blood, this material is not collected and made available for use. This is generally due to financial constraints, fears about liability for mishandling tissues, and the possibility that these procedures will detract from the primary purpose of patient care [FRAME, 2005a].

Another problem, according to a respondent, is that human material often derives from sick patients, and it may be hard to obtain healthy tissue. Furthermore, the human process of death generally takes a long time and leads

to extensive tissue damage.

Human volunteers / patients

In general, the use of human volunteers is presented in literature as the most promising alternative to the use of primates, although it is also restricted by ethical and practical dilemmas. According to the limited number of interviews conducted by Schell and Tsang [2005], human volunteers are amongst the most used alternatives for primates in primate research.

In particular, microdosing is reported. Using techniques, such as nuclear magnetic resonance spectroscopy, accelerator mass spectrometry (AMS), positron emission tomography, human pharmacokinetic data such as drug bioavailability, distribution to and elimination can be provided. Concentrations in the low picogram and femtogram range can be detected (a trillion of a gram up to one thousand of a trillion of a gram), which makes it possible to conduct human pharmacokinetic and metabolism studies using sub-toxic and sub-pharmacological doses of experimental drugs very early in the development process. Early markers of potential drug effects (biomarkers) can allow assessment of likely efficacy or toxicity, at point in time and at a drug dose far in advance of and below actual toxic thresholds [Animal Procedures Committee, 2002; Dr Hadwen Trust for Humane Research, 2005].

The method has already been endorsed by the FDA (Food and Drug Administration) (April 2005) and the European Agency for the Evaluation of Medicinal Products (January 2003) [Bailey, 2005]. A trial by the Consortium for Resourcing and Evaluating AMS Microdosing demonstrated around 70 per cent correspondence between microdoses and pharmacological doses. Early human microdose studies are reported to enhance the selection of optimal drug candidates and reduce the likelihood of later failure. By this means, animal tests for products that fail in humans later on can be avoided [Dr Hadwen Trust for Humane Research, 2005]. The Animal Procedures Committee recommends that the use of microdose studies should be encouraged as well. [Animal Procedures Committee, 2002].

A major concern with respect to this microdose approach is that the effects observed on administration of ultra-low doses may not correspond to those seen when therapeutically relevant doses are administered. Certainly, there are threshold limits below which no effect is detectable, but there is also the possibility that low doses may trigger events that are not seen at higher doses and vice versa. The situation is further complicated by the fact that most studies conducted in rodents as the first species are performed at doses which exceed the clinical range [FRAME, 2005a]. According to FRAME, early human studies are unlikely to replace animal tests entirely, not least because they are expensive to undertake and there is a shortage of human volunteers.

Another disadvantage is that the use of human volunteers will lead to a higher variance as compared to the use of laboratory animals. With the latter, the experimental conditions such as housing, food intake, genetic background and so forth are generally the same for all subjects, while for human volunteers there will be enormous differences [Evans, 1990]. Furthermore, research on human volunteers or patients takes much more time than research on animal models. [Huizinga, 2003; Laman, 2003].

Human embryos

The final alternative to be addressed here is the use of human embryos. In most Western countries, regulations severely restrict the possible use of embryos in science, and there are enormous ethical problems concerning their use. According to respondents, public opinion would favour the use of primates over the use of human embryos. However, there are indications that the use of embryos could be useful for research into certain serious diseases. Although not said in so many words, animals might be used for these goals at the moment, and apparently there are indications that embryos might be better models than the ones used now. In the Netherlands, the Embryo Act has been recently evaluated, which led (among others) to the following conclusions: "The Act enshrines the possibility, after the ban has been lifted, of creating embryos specifically for scientific research (...). The conclusion drawn is that statutory regulation may act as a barrier to scientific progress in the treatment of certain

significant health problems. It would be advisable to consider broadening these research areas" [Olsthoorn-Heim et al., 2006].

3.2.4 Reduction alternatives

Tiered approach

While attempts have been made to develop alternatives that can replace the animal experiment fully, in practice this has sometimes turned out to be impossible. In view of this, a tiered approach can be seen as useful: by determining and investigation each of the assumptions that are on the basis of an animal test, or by splitting the research question into several different ones, which can be studied using no or fewer animals. For instance, the corrosive ability of a compound can not only be tested in vivo, but also by using a tiered approach. If a compound turned out to be corrosive, it would not enter the market; identifying the compound's toxic characteristics at an early stage would thus be useful. Computer models may first be used, followed by chemical and in vitro methods; only if these prove negative, may an in vivo test be conducted. This approach has led to an approximately 50% reduction in the use of animals for toxicity tests, and less pain being inflicted on the animals used.

Telemetry

The use of telemetry may also reduce the number of animals used. It involves the implantation of a small transmitter that measures, for instance, blood pressure, body temperature etc. The signals from this transmitter are recorded by an external receiver, thus gathering data continuously and over a longer period of time. The animal would no longer need to be handled every time, thereby reducing the amount of stress and/or pain (and thereby probably variance between animals). Further to this, the continuous recording of data may reduce the number of animals necessary, since no animals would have to be killed during the experiment to investigate their functions.

Result sharing / collaboration

It is feared that pharmaceutical companies or scientists use animals, including primates, to conduct the same kind of tests independently of each other. Especially when products do not make it to the market, there is a risk that other companies might attempt to develop similar products. The sharing of research data is thus presented as a way in which unnecessary animal experiments can be prevented. Given that some information may be commercially sensitive, this could be done through a neutral third party [Animal Procedures Committee, 2002; FRAME, 2005a].

In general, respondents were positive about the idea of sharing results, certainly when these were negative and would otherwise not be published. However, the confidentiality of the data was deemed a problem, as well as a lack of money and the actual organization of a database of animal experiments: scientist and companies may not trust animal welfare groups with such information, and it is doubtful whether governmental organizations would assume the responsibility for organizing such database. The European industry, however, is said to be interested in establishing a database of studies and results.

Another potential reduction method could be the use of animals of just one gender. Both genders are usually used in testing procedures, but the use of one gender would suffice in, for example, investigative and exploratory studies, in certain pilot studies and before the onset of phase I clinical trials in male human subjects [Weekley et al., 2002]. Naturally, limiting the number of animals according to statistical analysis is another method of reduction.

3.3 Problems and arguments

Motivation

Problems and non-scientific arguments also go hand in hand with the use of alternatives. One of the key problems is that alternative methods have often been developed as a supportive assay rather than as a replacement alternative. Moreover, researchers lack the motivation to use or develop alternatives. A spokesperson for the Dutch Ministry acknowledges that a pro-alternative environment should be created in the educational programme for researchers and animal caretakers and in the management of pharmaceutical companies [Cohen and de Cock Buning, 2003]. Another respondent added that alternatives to the use of primates are likely to be found more quickly when primates are not as easily as available they are now. This was confirmed by the participants of a workshop on the use of primates [NC3Rs, 2006].

Experience

A lack of experience with alternative methods to animal experimental is viewed as a hindrance [Cohen and de Cock Buning, 2003], as well as the availability of information on alternatives. Many scientists seem to fail in their efforts to conduct an appropriate search for alternatives information [Janusch-Roi et al., 2000], and, at least in 2000, no thesaurus adequately covered the concept of the 3Rs, while this would be considered as a useful tool. A report to the US National Institutes of Health considered the legal requirement for a literature search on alternatives to be 'burdensome' [Bottrill, 2000b; Bottrill and Huggins, 2000; Stitzel and Todd, 2000]

In 2000, two databases providing ready-to-use information, consisting of evaluated information providing methodological descriptions of alternative methods at various levels and based upon a scientific review of each method were under development, by ECVAM in Italy and by ZEBET in Germany [Janusch-Roi et al., 2000]. However, when performing this inventory study on alternatives for primate use, no database was found to be helpful.

Funding

It is often argued that too little funding for research on alternatives is available. Though initiatives such as the British 3R's Centre and the Dutch Platform Alternatives to Animal Use exist, funding for research using animal models still largely exceeds the sums available for the development of alternatives. According to one respondent, the annual funding for the development of alternatives in the Netherlands amounts to about □ 900.00, which they deemed absolutely insufficient: a tenfold figure would be necessary to cover the costs of developing alternatives to animal experimentation. Furthermore, these alternatives (at least for the use of primates) are primarily refinement techniques. Methods to replace the use of animals are seldom developed.

In the US, there is governmental funding for human health studies only; alternatives can be part of a research project, but will always be regarded as 'extra work' and are thus not a priority. No funding for studies on alternatives as primary goal is available.

Validation and regulations

One complaint often made by scientists developing alternatives is that alternatives methods must be validated, while in most cases animal models has never been validated using the same stringent criteria [Newkirk, 2004]. The process of validation must shown that the alternative is just as good as the existing method, which is generally an animal model.

Theoretically, this might lead to a situation where the animal model and the alternative model represent fundamentally different endpoints. When the alternative would in reality be a better model than the animal model (which we will never be able to know in advance given that we do not experiment on humans), it would still be impossible to get it validated since it focuses on another aspect than the animal model does.

Another general complaint is that a validation procedure is a time-consuming, tedious and costly process. A spokesperson from the Ministry of Science in the Netherlands admitted that procedures to get alternatives validated, accepted and introduced are not particularly vigilant [Cohen and de Cock Buning, 2003]. A respondent in this study also asserted that regulations were hindrance, particularly given that many processes have now become international while different countries may feel differently about alternatives.

3.4 Introduction case studies

In the scientific literature, arguments are rarely given for why primates have been selected as the animal model for a specific research purpose. The same applies to why they have not used replacement alternatives. In view of this, the present study will investigate three case studies in greater detail.

These case studies cross the lines of categories of animal use as discussed in this chapter. In Chapter 4, the use of primates for both fundamental and applied research on human diseases is discussed. Chapter 5 examines the use of primates in preclinical studies for evaluating the efficacy or safety of pharmaceutical or biological medicines. Although many of these studies are to some extent obligatory, the details and the choice of model is mostly left up to the company developing the pharmaceutical or medical product in question.

Chapter 6 focuses on the use of primates for routine safety testing in the batch release of vaccines. These studies have provide detailed definitions of what studies have to be performed using which animal species. In chapter 7, the results from these case studies are combined, and conclusions and recommendations will be given.

Reliability

Although some bias may have arisen in the selection of case studies and thus specifications of the use of primates also, these studies were primarily chosen on the basis of the European statistics for primate use. In the details of the exact purposes of primate use, it is probable that the results have to some extent been coloured by these case studies.

It should first be noted that virtually all the information on this topic has been derived from interviews. It thus should be noted that there is a possibility that the interviewer, the author of this report, has influenced his respondents or vice versa. As noted in the introduction, the researcher's attitude towards the use of primates has been an open-minded but critical one. Attempts have been made to avoid influencing the respondents in a variety of ways. The interviews were semi-standardized: they all had more or less the same structure, a standard list of topics and detailed questions was used for all respondents in each case study. Since this investigation is all about arguments and attitudes, the interviews were topic-guided in order to give respondents space to express their views, the list of questions was only used when the initial answers to a topic did not cover the question completely. It is mainly in this decision to probe further - and the willingness of respondents to provide all answers - that bias could have arisen. However, this could also have happened during the course of the topic-guided interview, since this is inherent to the nature of this type of research.

In order to prevent bias by selecting respondents, several individuals from different backgrounds were included: for instance multiple regulators, or animal welfare organizations. However, this was not always possible since the timeframe in which this study was conducted was limited and many intended respondents were very busy. In itself this is not likely to have biased the investigation, though in some cases, respondents refused to cooperate with this research. It is very possible that this self-selection has created a predisposition. It is too easy to conclude that there was apparently something that should not have come out into the open, but it is likely that the reverse is true: companies and researchers who are willing to cooperate are likely to be the ones that have thought more carefully about the use of primates.

The major problem with this potential bias is that it has been hard to get to the experts on primate experiments or on alternative methods for these experiments. All researchers will be influenced to some extent by the animal models they generally use. An attempt to distinguish the extent to which this might have influenced the answer has been made. In this report, all relevant answers from all respondents will be provided, but the discussion of these influences have been separated from the factual answers. However, the possibilities for replacing or reducing primates may well be greater than can be concluded on the basis of these case studies, since it was difficult to find respondents with knowledge of primate experiments with respect to fundamental and preclinical research, but who were not involved in these studies themselves.

Chapter 4

Primate research for human diseases

In this chapter, fundamental and applied studies on disease will be discussed. In view of the scope of this project, fundamental studies are limited to biomedical subjects; behavioural experiments will thus not be addressed. Typically, the experiments discussed in this chapter aimed to acquire knowledge about a human disease, syndrome or disorder, or to find a treatment. In both cases, the focus is on the disease itself, rather than on the pharmaceutical product to treat it. The results of these experiments will probably be published in scientific journals. Applied studies, which are performed at pharmaceutical companies and not published in journals, will be discussed in Chapter 5.

In 2002, some 2100 primates were used in Europe for the study of disease. The main aim of the research was to study 'other human diseases', followed by human nervous and mental diseases. Fewer primates were used to study human cardiovascular diseases and human cancer [European Commission, 2005a]. See Appendix 6 for a graphical representation of these statistics.

4.1 Subjects of primate studies on disease

4.1.1 Use in Europe

Most primates in Europe are registered to have been used for research on 'other diseases'. According to the European Scientific Committee on Animal Health and Animal Welfare [2002], this probably involves studies on the mechanisms of HIV (Human Immunodeficiency Virus) infection, AIDS (Acquired Immune-Deficiency Syndrome), the development of HIV vaccines, and other infectious diseases as malaria, tuberculosis and so on. Other areas mentioned are research into shock, including septic shock, development of techniques, mainly for PET (Positron Emission Tomography) scans, investigations into reproductive function, dental research, immunological, anatomical and histological investigations, organ transplantation, investigations of coeliac disease, diagnostic procedures, ophthalmology and metabolic diseases. Finally, a number of primates are reported to have been used for studies on ageing, hepatic cirrhosis, and gene delivery.

In terms of publications however, fundamental neurobiology is the primary subject of primate experiments, according to a report from Krys Bottrill, who analyzed publications from EU-member states using primates between 1996-1999 [Bottrill, 2000a]. The second objective of primate experimentation is HIV research, followed by research into shock, neurodegenerative diseases, techniques and reproduction. Nonetheless, it can be questioned whether these data are still relevant and to what extent the number of publications is representative for the number of primates used. Therefore, publications between 2003 and 2005 from the Netherlands and the three countries in Europe that use most primates (France, UK and Germany) have been retrieved using a quick literature search on Embase. The keywords used were primate, rhesus, macaque, macaca, cynomolgus or monkey in either title, abstract or index terms, with restrictions to animal experiments and the country of the author address.

The results are presented below, together with Bottrill's findings. For each subject, the number of publications is also given in the title: the first figure is for the combined number of the UK, France and Germany, the second for the Netherlands.

Fundamental neuroscience 20+4 publications

Bottrill concluded that most publications in Europe related to fundamental neurobiological research. In Great Britain, according to statistics from the Home Office, in 2002, primates for fundamental research were used mainly for research on psychology, which would imply research on neurobiology [Home Office, 2003]. However, only 20 publications on this subject were actually retrieved from the three countries that use most primates in Europe. Four studies were found from the Netherlands, using only 1 or 2 primates per neuroscientific study.

HIV 18+4 publications

According to Bottrill's analysis, HIV was the next main subject primates are used for. In the Netherlands, 60 primates were used in 4 studies on HIV. 18 publications on HIV using primates as animal models were retrieved from the three other countries investigated.

Neurodegenerative diseases 31+2 publications

In the Netherlands. little work has been done on the fourth subject: neurodegerative diseases. Only two papers were found. France, Germany and the UK together produced 31 publications on Parkinson disease and Alzheimer disease in which primates were used experimentally. For this subject, larger numbers of animals are used than for fundamental neuroscientific research: several studies were found using 16 to 21 animals.

Immunology 17+6 publications

Immunology is number ten on the list. In the Netherlands most primates have been used with regard to transplantation, which is part of this subject: 91 primates in 3 experiments, all at one location. According to one respondent, no primates are used for transplantation studies at present. 15 publications on this subject came from France, Germany and the UK. MS or EAE (autoimmune encephalomyelitis) is also classified in this category, yielding three publications from the Netherlands (37 primates in total) and two more from Germany, France or the UK.

Infectious diseases 6+6 publications

With respect to infectious diseases, in Bottrill's report malaria was quite exclusively the domain of the Netherlands. Now, however, only one publication on malaria was found in the Netherlands, using 45 animals, and three from the other countries. Research on smallpox or monkey pox is not extensive in EU-countries that produce most publications on primate research. In the Netherlands and the United Kingdom, two publications about pox were published between 2003 and 2005, of which one used 35 primates. For respiratory syncytial pneumovirus, on which the Netherlands produced one publication with 14 primates, both UK and Germany published one as well. The Netherlands produced one publication on SARS using 14 primates. Germany, France and the UK published none. The Netherlands also published one primate study on measles (7 animals).

Other subjects

Research into shock was the third subject of primate studies in Bottrill's investigation, almost exclusively performed in the Netherlands. These experiments appear to have ceased, since no more studies on this subject could be retrieved. No publications on the development of techniques, reproduction and dentistry, fifth to seventh subject on Bottrill's list, were found in the Netherlands. The number of studies performed for these subjects in the EU remains unclear, due to the practical restrictions with regard to the keywords used. Toxicological studies and drugs R&D

(Research and development) are the next subjects on the list, but these are covered by other chapters and will not be discussed here. Finally, one publication on cancer was found in the Netherlands, for which 20 monkeys were used.

4.1.2 Alternatives

References questioning the value or proposing alternative models or methods for almost all purposes of primate use were found. These views will be discussed shortly, in the same order as above. The numbered references for the different subjects will be given in more detail at the end of the table.

Table 4.1 Use of primates and possible alternatives for several subjects

Subject, References	Use of primates, possible alternatives
Neuroscience References: 1,2,3,4,5,6,7,8,9, respondents	Primates are used for fundamental neuroscience studies because they are the only animals with well-developed frontal and temporal lobes. These portions of the brain are vital for advanced thinking, perception, attention, memory and planning. It is therefore said that research into higher cognitive functions can only be conducted in primates. Neuroscientists can also investigate the brain's basic functions. Studies on primates are needed to gain insight on cognitive functions and combine the macro- and microlevel of studies in humans and small animals respectively. There is much criticism on the relevance of this type of research on primates, as it is basically curiosity driven. Some say the experiments are useless, others point out the differences between primates and humans. The visual system of macaques and new world monkeys differ from the human one, as does the frontal cortex. Furthermore, when brains are similar in structure, this does not mean they are similar in function. With respect to the argument that it may yield evidence-based medicines, it is argued that the quest for drugs that modify human behaviour and brain function requires a subtlety not available through the study of primate brains. Another subject of disapproval is the use of primate chairs and water deprivation as experimental methods: this may not only cause stress and discomfort, but may consequently impair the results also. Potential alternatives to the use of primates could involve non-invasive studies with human volunteers, using MRI (Magnetic Resonance Imaging), TMS (Transcranial Magnetic Stimulation) and MEG (Transcranial magnetic stimulation) techniques. Some claim that the resolution of these techniques is insufficient, which is disputed by others. Mathematic models could help interpret the results, but they are not a research tool in themselves
HIV References: 6,10,11,12,13,14	Several different primate models have been used for HIV-research. There has been discussion on the relevance of some of these models. Only chimpanzees can be infected with HIV, for other primates the simian version is used: SIV (Simian Immunodeficiency Virus). However, the interaction of host with virus may differ in primates and humans. It has been stated that primates are necessary for the development of a vaccine, although human material could instead be used for fundamental studies. Others say that human cells or volunteers or other animal models as cats, cattle and transgenic mice could be used for vaccine studies as well, which is disputed by others. Mathematic models could also be used. Primates do not provide a good model for anti-HIV drugs; most knowledge could be gained by in vitro methods, while other sources state they are suitable for these studies as well.
Alzheimer's disease References: 1,14,15,16,17, respondents	Research on neurodegenerative diseases, of which Alzheimer's is one, has been mentioned as one of the subjects primates are necessary for (e.g. by KNAW and EUPREN). Primates are the only species that share the advanced frontal lobe brain functions that are most commonly involved in many neuro-degenerative diseases in humans. Furthermore, they are viewed as the only mammals that develop the plaques and neurofibrillary tangles that are symptomatic of Alzheimer's disease, though others state that their emergence in primate differs from

	humans. It has been stated that without primates, researchers would not be able to test new drugs to combat the condition. Others, however, say that progress in Alzheimer's research has been made using human material, not with animal experiments. A genetically modified mouse model has recently been created as alternative, and has proved invaluable in a long series of experiments to develop and test antibodies to treat the disease, which are now in clinical trials. Furthermore, (non-transgenic) rodents could be used in studies on the primary processes of Alzheimer's, and investments are being made to study parts of the disease on primate tissue.
Parkinson References: 14,15,16,17,18	Parkinson is another neurodegenerative disease for which the use of primates has been deemed invaluable. However, several different models are currently being used: rodents that have been treated with 6-hydroxydopamine and MPTP-treated primate species, including the macaque and marmoset. In all animal models, the brain cells of mostly young, healthy animals are destroyed by the toxic actions of the administered compounds. Although none of these animals model the cause, the cellular basis of the human disease or the progression of disease state, some researchers find the MPTP rhesus monkey the only valid animal model. Marmosets are considered less suitable because they lack neuromelanin in their brains, resulting in unstable disease states. Furthermore, they could not be trained like Old World monkeys. It has been reported that there have been developments to produce a transgenic mouse model for Parkinson. In addition, invertebrates and modern techniques as gene array technologies could be used for some of the more fundamental questions. Others state again that the major breakthroughs could have been achieved with various studies on humans.
Transplantation References: 14,19,20,21,22,23	Many primates are used in transplantation studies. There may sometimes be opportunities to reduce the number of animals per group. The use of other animal models or in vitro methods are said not to be representative of the human situation. Others state that results from primates are as hard to extrapolate to humans, or that pigs would be better models than primates, at least for xenotransplantation. Certainly for cell transplants, possible alternatives include in vitro methods or the use of human volunteers. A computer model that simulates the response of the immune system is being developed to predict immune activation in transplantation, but this is unlikely to become an alternative in the near future.
Arthritis References: 14,15,24, respondents	Rheumatoid arthritis is again one of the subjects where primates are deemed necessary. T-cell tolerance can play a role in the emergence of the disease, and this process differs between primates and other animal models. Primates are therefore thought to be necessary. Furthermore, not all factors that are involved in this mechanism are known, thus SCID (Severe Combined Immunodeficiency) mice cannot be used. Other approaches are, however, possible: transgenic animals can be used for fundamental questions, two rat models of arthritis and animal models with implanted human material are available. Human patients or their cells could also be studied, but the disease develops very slowly in humans so that would take a very long time. Why these could not be alternatives to the use of primates, except for questions where T-cell tolerance is involved, is not known. According to one respondent, the combination of rodent models with human studies excludes the necessity for primates for studies on arthritis. Refinement methods in the way this disease is induced could also indirectly reduce the number of primates, as well as decreasing suffering.
Multiple Sclerosis (MS) References: 8,14,15,25,26, respondents	Another disease, for which the use of primates is deemed necessary, is multiple sclerosis (MS). At present, no animal model is representative for this human auto-immune disease. One of the available models is Experimental Auto-immune Encephalomyelitis (EAE), in either rodents or primates. The immune and nervous system of rodents is reported to differ from humans. This is a reason to use primates, for it reflects the human situation better. Others, however, think that the rodent model is better, since primates usually develop EAE in hyperacute, destructive and often lethal forms, unlike human MS. It is reported that the disease is less aggressive in marmosets than in macaques. In rodents, models of chronic-relapsing EAE exist. The disadvantage of rodents, however, is that their size makes

	it difficult to study their brain by conventional imaging techniques, which is easier for primates (though harder for marmosets than for macaques). Alternatives to the use of primates include the use of primate tissue, the refinement of the experimental model and the improvement of MRI-techniques. Human volunteers are not an option since they cannot be experimented upon and this would take too much time. Other sources, however, claim that (non-invasive) studies on human patients have yielded more understanding of the disease than animal experiments
Malaria References: 6,14,15,26,27,28, respondents	It is said that primates are also needed for malaria research, because of narrow host range of the parasite and the similar immune system of primates and humans. Chimpanzees were found to not be a good model, but a few New World monkeys are reported to be the best animal model available, although it is not necessary to use them for all research questions. A problem with their use is the availability of tools to evaluate the immune response. Murine models show different pathology and immune defence mechanisms than described in humans. Human patients or volunteers could also be used as alternatives, but then only during the initial phase of infection (in volunteers that are deliberately infected and cured) or an existing disease state can be studied. Furthermore, products for the treatment or prevention of infection may have unexpected toxicity, which would need to be tested before administering the product to humans. Primates are thus said to provide the link between mice and humans.
Tuberculosis (tbc) References: 14,28,29,30	Tuberculosis is named as one of the diseases where primates are needed, because their immune system is similar to humans. Sources are contradictory on the best primate species: one states that the cynomolgus monkey is better than the rhesus monkey, while another asserts that the rhesus monkeys best resembles the human symptoms. These two species models show different susceptibilities. Primates could be used for several kinds of questions on tbc. Other animals, such as guinea pigs, rabbits and mice, are also used as tbc-models extensively, while cattle could be used as well. The problem with these animal models is the availability of immunologic agents, though this should not be a problem for the mouse.
Hepatitis References: 14,15,17,31,32,33,34	Hepatitis is again a subject for which the use of primates is said to be irreplaceable. There are different viruses that cause hepatitis, of which A and E were reported to infect New World and Old World species, while B, C and delta were limited to Old World species only. Hepatitis C could even only be studied in the chimpanzee, though they would respond to the virus differently than humans. Recently, a cell-line was found in which the interaction between host and virus can be studied. In the future, an artificial liver possibly could be used. Transgenic mice are a viable alternative as well, though it is unclear to what extent they can be used for immunization studies. Another animal model has also been found: in 1998 evidence was published that tree shrews (Tupaia sp) can be infected with hepatitis C virus, though the infection rate was not too high. Tree shrews are animals that are closely related to primates, but are evolutionary classified in a different order. Humans could also be used to study the infection, but since no symptoms are initially present, their use is excluded for initial infection.
Respiratory Syncitium Virus (RSV) References: 10	Research on RSV is being performed in primates, as well as humans, in vitro models and other animal models. The bovine disease shares many characteristics with the human version. Immunization studies in mice were not representative for humans, while results from primates were. New rodent models have been reported, though the virus would not proliferate in rodent lungs as in humans.
Reproduction References: 2,6,35,36,37,38	The reproductive system of macaques, most notably the cynomolgus monkey, is comparable to humans, both for men and women. Marmosets, however, differ markedly, but it is said that exactly these differences render them more useful than higher order primates for studying infertility in humans. The hormonal regulations between hypothalamus - pituitary gland - ovaries are said to be similar in humans and

primates. Other sources, however, report that the steroid-metabolism differs between primates and humans, which would impair their quality foor research on contraceptives

References

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3: [Lamme, 2003]	4: [Sauer, 2000]
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7: [Langley et al., 2000]	8: [Dr Hadwen Trust for Humane Research, 2005],
9: [Deutscher Tierschutzbund e.V., 2006]	10: [Osterhaus, 2003]
11: [BUAV, 2004]	12: [Lewis and Johnson, 1995]
13: [Smith, 2002]	14: [KNAW, 2001]
15: [Hau et al., 2000]	16: [Animal Procedures Committee, 2003]
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37: [Zuhlke and Weinbauer, 2003]	38: [Archer, 2004]

4.1.3 Introduction case study

Since it appears that alternatives are possible for many of the subjects for which primates are used, the question is why these alternatives are not applied. What are the arguments for continuing primate use and why can alternatives not be used?

In order to investigate this, one of the subjects for which many primates are used in the Netherlands and alternatives seem possible, has been chosen as a case study: HIV. Neurodegenerative diseases, for which even more primates are used, has not been studied intensively in the Netherlands. This subject was, therefore, deemed to be less suitable for a case study for practical reasons. Likewise, transplantation was also excluded as subject for a case study.

4.2 Case study: HIV

HIV research, the topic of this case study, is one of the major objectives of primate experimentation across the globe. The research focuses on several questions: fundamental studies try to clarify the mechanism of infection and pathogenesis of the virus, while applied studies search either for new antiviral medicines or vaccines against HIV. In this case study, vaccine research will be discussed; only limited attention will be paid to fundamental studies and research into new medicines.

The use of primates in the Netherlands for research into vaccines against HIV will first be described, followed by alternative models and methods and the extent to which these approaches or other methods could reduce or

replace the use of primates. Finally, arguments and problems concerning the use of primates or alternatives to study HIV will be discussed. Background information on HIV is presented text box 4.1

4.2.1 Vaccine studies on primates

Aside from the practices of safe sex, abstention and other behavioural changes, finding an effective vaccine against HIV-infection is one of the possible ways to prevent HIV infection. The antiretroviral medicines that are available are very effective, but cannot prevent or cure infection with HIV. This has thus lead to a worldwide search for possible vaccines. In the Netherlands, two research groups have used animals in their attempts to attain this goal.

Use of primates

One group uses primates in the search for optional approaches and optimal combinations for immunity against HIV. Vaccine candidates are tested in rodents first, and only if these animals develop a significant immune response to the product are primates used. The immune reaction of primates is then assessed, and if they show a significant response, the animals are infected to investigate whether they are protected against infection by the vaccine.

On an average base approximately 100-150 rhesus monkeys are used for about 5-7 studies per year. The primates generally display a good immune response to the vaccine, and thus are challenged with the virus.

At the other Dutch group, HIV-research is mainly conducted on human blood and cell lines. The research focuses on factors that influence the level of protection when infection has already taken place. It has been discovered that some HIV-patients show a slow progress in disease state because their cytotoxic T cells recognized certain proteins that are expressed early in infection. Primates were used to try to induce an immune response to these proteins. Two studies were performed using primates: in one, 4 monkeys were involved, in the other 12 primates were used in three groups of 4. The study was successful and is now proceeding to a clinical trial.

Relevance and quality of the model

Both respondents explained that primates were used because no other animals can be experimentally infected with HIV-like viruses. Only chimpanzees can be infected with the HIV-virus that infects humans, but after years of experimenting on chimpanzees, it was concluded that they did not sufficiently resemble the human situation [Bailey, 2005; KNAW, 2001]. They very seldom develop AIDS [Smith, 2002], and a first HIV-vaccine, which was based on research in chimpanzees, failed in humans [Archibald, 2005]. Nevertheless, some people still state that it is exactly these differences that are the reason that studying chimpanzees might yield interesting results [Bontrop, 2003; Schell and Tsang, 2005].

Other primate species are now used. These can be infected with either the naturally occurring simian immunodeficiency virus (SIV) or an artificial hybrid virus of SIV that expresses the HIV envelope proteins, called SHIV. These approaches have also received significant criticism.

There are several differences between SIV and HIV, which could make SIV a less suitable virus: it causes a weaker immune response and does not mutate as quickly as HIV. Moreover, several proteins from the virus differ from the ones in HIV [BUAV] and the host-virus-interaction of SIV with primates differs from the ones observed in HIV-humans [Letvin, 2005]. Nonetheless, infection of macaques with SIV results in a depletion of CD4+ T cells, which is comparable to the human reaction to HIV-infection [KNAW, 2001].

Another problem reported in the literature is that several species of macaques have been used alongside each other as models in HIV-research, which makes it hard, if not impossible, to interpret results [Smith, 2002].

Textbox 4.1 Background information on HIV

AIDS (acquired immunodeficiency syndrome) is caused by HIV (human immunodeficiency virus). HIV belongs to a class of viruses called retroviruses, and a subgroup known as lentiviruses. Other lentiviruses infect nonhuman species: the feline immunodeficiency virus (FIV) infects cats and the simian immunodeficiency virus (SIV) infects primates.
HIV progressively destroys the body's ability to fight infections. Most notably, immune cells called CD4 positive (CD4+) T cells are disabled and killed during the course of infection. These cells, sometimes called "T-helper cells," play a central role in the immune response. People diagnosed with AIDS may die of viruses or bacteria that usually do not make healthy people sick.
In Western countries, the median time from infection with HIV to the development of AIDS-related symptoms has been approximately 10 to 12 years, with a wide variation in disease progression.

AIDS was first reported in the United States in 1981 and has since become a major worldwide epidemic, growing most rapidly among minority populations. Worldwide, an estimated 38 million people were living with HIV/AIDS as of December 2003. Globally, approximately 5 million new HIV infections and 3 million AIDS-related deaths occurred in 2003 alone.
Among adults, HIV is spread most commonly during sexual intercourse with an infected partner.

Medicines, which interfere with the HIV lifecycle, preserve CD4+ T cells and immune function as well as delay clinical illness. Potent combinations of three or more anti-HIV drugs can reduce a person's "viral burden" (amount of virus in the circulating blood) to very low levels and in many cases delay the progression of HIV disease for prolonged periods. Antiretroviral regimens, however, have not been able to completely and permanently suppress the virus in HIV-infected people.
Efforts are taken to find vaccines against the virus. Other immune cells, CD8+ T cells, are critically important in the immune response to HIV. These cells attack and kill infected cells that produce the virus. Thus, vaccine efforts are directed towards eliciting or enhancing these killer T cells, as well as eliciting antibodies that will neutralize the infectivity of HIV.

Like all viruses, HIV can replicate only inside cells, commandeering the cell's machinery to reproduce. The virion, or viral particle, consists of an outer coat of the virus, known as the viral envelope, within which a core, or capsid, surrounds two single strains of HIV genetic material. HIV has nine genes: three of these, gag, pol, and env, contain information needed to make structural proteins for new virus particles, and six regulatory genes, tat, rev, nef, vif, vpr, and vpu, that contain information necessary to produce proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. Much of the research to develop a vaccine against HIV has focused on the envelope proteins.

One reason why it is hard to develop effective therapies against HIV, is the fact that some HIV invariably escapes the body's aggressive immune responses. This is due in large part to the high rate of mutations that occur during the process of HIV replication. As a consequence, many variants or strains of HIV develop in a person, some of which may escape destruction by antibodies or killer T cells.
In addition, much remains unknown about HIV, such as the pathogenesis: a detailed understanding of HIV and how it establishes infection and causes AIDS is crucial to identifying and developing effective drugs and vaccines to fight HIV and AIDS.
[NIAID, 2004]

The use of the SHIV-strain has also been the subject of critique, especially in the beginning when the virus was poorly characterized. According to one respondent, the construction of HIV in the SIV backbone was not as sophisticated then as it is now, and it might well be easier to obtain immunity with a bad construct. Later, questions were raised with respect to the extent to which results from SHIV-viruses are representative for the human reaction to HIV, since the disease symptoms of SHIV resemble the HIV-symptoms less well than SIV does. However, most scientists think that SHIV is a better model, because it contains an HIV envelope protein [Smith, 2002].

On the other hand, according to one respondent, all studies that have been conducted and showed good protection in the monkey still proved to be poor vaccines in the human population. Another respondent begged to

differ stating that vaccines, which fail in humans, had also failed in primates, or else the wrong vaccination strategy was used. References in the literature indicate that over the course of time more than 30 different candidate vaccines - mostly developed as part of chimpanzee or monkey experiments - have been tried in human volunteers in 70 early clinical trials, and five have gone into phase II trials. Nevertheless, none of these vaccines have shown genuine therapeutic results and progress has been disappointing [Bailey, 2005; Langley, 2002].

According to one respondent, there are a few dissimilarities between the monkey model and the human situation, which might explain the differences in the level of protection. First of all there are different strains of HIV, called 'clades'. Monkeys are challenged experimentally with just one virus population, while the human population is subjected to a heterogeneous mix of viruses. Furthermore, until recently, vaccines were based on clade B viruses, which mainly occur in Europe and North America. The focus of new vaccines is now based on the HIV clade C virus, which is most abundant in Africa and the Far East. One scientist working on HIV explained that using clade C was more difficult because there were no reagents available for it, which were present for clade B [IAVI Report, 2000]. Another scientist, however, contended that the experimental clade would not in fact be too important, since the biology of the different forms is similar. Ultimately, he argued, there are only nine possible proteins that could actually be used for immunizations. If a particular approach is found successful, the antigens used could easily be altered to other HIV-strains. He maintained that the reason why mostly European strains are used is that it is difficult to perform clinical tests in Africa for cultural and economical reasons. Another scientist agreed that - at least in 2000 - there was no evidence that a vaccine based on one clade would not work in another [IAVI Report, 2000].

A second difference noted by respondents was the route of virus transmission. In the early days monkeys were challenged with a high dose of virus, which is most of the time given intravenously. Humans are typically infected by (multiple) exposure to low doses via a mucosal route. It now appears that systemic immunization does not yield enough protection to prevent mucosal infection. Research on infected monkeys revealed that cells in the mucosa in the intestines become infected very early on in infection; a finding that was later confirmed in human patients. It is likely that in the first days of infection the virus strikes in such a way that the immune system never fully recovers. According to one respondent, the challenge is to prevent the virus from entering the body via these sites, which means that mucosal immunity is needed.

To investigate this, various SHIV-strains that are suitable for mucosal infection had to be constructed, and these had only been available for a few years. A multiple exposed low dose mucosal infection model to mimic the human transmission route was created, but the problem is that this is much more labour-intensive and one needs more monkeys for the experiment, as it takes a long time before one has enough animals infected in the test and control groups to draw conclusions.

Likewise, the other researcher agreed that the route of infection differs between humans and experimental models used, but added that as long as there is no vaccine, we will not know the importance of this difference. However, in the literature, differences in virus used and route of infection are also reported to account for the significant divergence in results from different laboratories [IAVI Report, 2000].

Despite the differences between primates and humans, both respondents stressed that this was still the only way to go: doing more fundamental research would not yield a vaccine earlier. One respondent even thought that it might be the other way around: perhaps we know too much. Vaccines have existed since the 18th century, at a time during which we knew almost nothing about viruses. Indeed, the creation of a vaccine has thus far been more a result of empirical science than knowledge. If we knew less about HIV, we would have proceeded to clinical trials earlier, which is the fastest way to develop a vaccine. Nonetheless, this is also the most dangerous route to vaccine development, which would probably have cost some lives. Given all that we know about the HIV virus, there is nobody who has dared to assume that responsibility.

The other scientist agrees that the solution must be found through empirical investigation. Furthermore, he argued that it would be ideal if a vaccine could be developed that gives protection to a high dose of HIV when administered intravenously. According to one respondent, results are gradually improving. In 2004, a clinical study with an HIV vaccine was published, which produced the best results thus far.

4.2.2 Replacement alternatives for vaccine studies

Many alternative approaches for HIV-research are reported in the existing literature. In this section, only the ones that apply to the use of primates for research specifically on HIV-vaccines will be discussed. According to two respondents, there are no such alternatives; in challenge studies one must be able to mount an immune response, which requires an animal. This immediately rules out the use of tissue, in vitro methods and physiochemical techniques. Moreover, primates are believed to be the only kind of animals that can be challenged with a virus, which is closely related to the HIV virus. In spite of this, institutes involved in AIDS research report having used other models before turning to primates. The question is why these have not been considered as replacement alternatives?

Animal-free methods

Reportedly in vitro studies on human cells and tissues have made the investigation of the immune-stimulating effects of potential vaccines possible [FRAME, 2005b]. Furthermore, in vitro studies are widely used to investigate new antiviral medicines; no animal models are needed for such efficacy studies [BUAV]. All respondents confirmed this, explaining that designing new antiviral medicine depends on general biomolecular science. Cells from patients could be used to test the compounds. One respondent reports that in vitro methods are used to check if antibodies from an immune response to the vaccine can bind to different HIV-strains. However, it was also stressed that a susceptible animal model is still required to investigate the protection level offered by a vaccine.

In vitro methods have also been used extensively for more fundamental research into HIV. One group is currently trying to develop an in vitro model for the mucosa to investigate how this route of infection works and possible intervention strategies. One cannot euthanize a monkey each time in order to investigate the order in which cells are infected, as is done with mice. Strictly speaking, this cannot be viewed as an alternative for the use of primates, since these animals would never have been used for this purpose. It can, however, improve the monkey model for vaccine studies, thereby reducing the number of animals needed in the future.

Mathematical models, which are also used in vaccine-research, cannot investigate the potency of a new vaccine. One respondent collaborates with a research group that uses theoretical models to help explain the results from their in vitro research. A scientist working on mathematical models explained that the models he has been working on mainly focus on fundamental questions relating to HIV, although the use of these models alongside experimental results can speed up the scientific progress and greatly contribute to identifying the most promising direction in which a solution may be found. Nevertheless, it does not provide an alternative to animal experiments, since the experimental data are needed to construct a model, which is used to analyze new data. These approaches are complementary.

Other animal models

The literature suggests that other lentiviruses with their natural animal hosts could be used instead of primates with SIV. Ungulates (hoofed mammals) and immunodeficiency viruses in cats and cattle may provide alternative animal models. The use of these animal species could reduce the number of primates used for HIV-research [Lewis and Johnson, 1995]. However, even animal welfare groups acknowledge that FIV (Feline immunodeficiency virus) and HIV are quite dissimilar [BUAV], as the respondents also observed. Humans have CD4 and CD8-cells, of which the first are infected by HIV. With respect to FIV, the disease process and the cat's immune system is very different from that of humans, which means that one would still need a control step in monkeys. In

addition, there are few monoclonal antibodies to characterize the CD4-cells in cats. In monkeys, one can use the antibodies that were developed for human use, given that these exhibit a certain degree of cross-reaction with monkeys. Cats are, therefore, not a good model for HIV, even when a FHIV construct could be produced as is done with SHIV.

Transgenic mice or SCID-mice have also been developed as an HIV-model [Lewis and Johnson, 1995]. According to FRAME [2005a], these have proved reliable models of viral infection and are in some ways more suitable than primates. These mice have been used in HIV-vaccine research by one of the respondents, but they did not turn out to be a good model either. The mice were given a human immune system, which is a difficult procedure. More importantly, there is a constant reaction between the human immune system and the mouse: a constant graft-versus-host disease. Consequently, the immune system is constantly activated and the animals do not survive for a long period. This influences the results, complicates their interpretation, and renders the experiment less reproducible. On the one hand, one is dealing with a human immune system, but it is impossible to ignore the mouse environment, which is influenced the human immune system. This last argument was also forwarded by another scientist.

Another reason that respondents provided preferring the use of primates to mice is the close relationship between primates and humans. The size of the animal was also mentioned: it is much harder to work with small mice than with large monkeys. One can only obtain only small amounts of blood from mice, which might not be sufficient for all the in vitro tests one has planned. Furthermore, it is much harder to inject them, which makes it more dangerous than injecting a monkey.

However, another respondent, who does not work with monkeys, thinks that mice could be used in the future. He believes that if a good mouse model was developed, a large proportion of the studies that are now done on primates could instead be conducted in mice. These animals are much more efficient to use, cheaper and yield fewer ethical problems. 99% of all immunological research is already done using mice, so this should not be the problem. The problem is that a model would have to be developed in which HIV could be studied.

Human volunteers

Studies on human volunteers, or clinical trials, can be alternatives to the use of primates. There are, however, several problems related to proceeding to clinical trials early in development. One respondent believes that, even for therapeutic vaccines given to HIV-patients, it unlikely that a medical ethics committee would permit this without preceding primate experiments. There are already good antiretroviral medicines available, which can keep the disease under control for those that are infected, and for uninfected people there is an unacceptable possibility that the vaccines will have a harmful rather than beneficial effect.

One could reason that this might be beneficial in third world countries, where most of the inhabitants have no access to expensive antiretroviral, but ethically this is a minefield, certainly when there is no proof of efficacy. There are known cases where the testing of products has led to irreversible neurological complaints. Primate tests provide the first indications of the possible harmful effects of a vaccine before it is given to humans. It is unlikely that medical ethics committees would approve of human trials without these animal data.

In 2000, Marc Girard, a scientist, asserted that he did not view primate models as a compulsory route to clinical trials, since there are problems in monkey models that make it hard to decide whether to move a candidate vaccine into human testing. Girard argued that there is currently no example of human and monkey data on the same vaccine from which a model could be built, which might guide these decisions. Consequently he believes that it is reasonable to test humans and monkeys in parallel [IAVI Report, 2000].

One respondent also noted that there is a scientific problem with respect to the use of humans for testing: humans cannot be used to perform effectivity studies in a controlled manner. Even in high risk-groups, one cannot

know when and how the infected individual came in contact with the virus or which clade of virus. It will, therefore, be impossible to determine the protection level.

According to Gill Langley, testing every candidate HIV vaccine in monkeys is almost as time-consuming as early clinical trials in human volunteers while human studies have to be carried out later on anyway. She suggests that more clinical trials should be performed and a newly developed in vitro method be used to give advance indication of efficacy in humans, before a full phase III clinical trial is conducted. Such an approach would be far more reliable and relevant than studies in a different species, which use a different virus [Langley, 2002].

4.2.3 Reduction alternatives

According to one respondent, the re-use of primates in HIV-vaccine studies is not possible, because an animal that has been used in an HIV immunogenicity study may have been sensitized by either the antigen or the vector used. It cannot, therefore, be re-used in a new HIV immunogenicity study, since one would never know whether a future reaction was due to the first or the second vaccine study, or maybe due to a unknown combination of the two. Animals can be re-used for other subjects, but primates that have been infected should be euthanized.

The number of primates used could, however, be reduced by having different studies share their control groups, which are immunized with a vaccine containing everything but the active ingredient. In practice, this would difficult to achieve since all experimental conditions, such as the vector, HIV clade and timeframe, must be compatible, which is seldom the case. What has been reduced, is the number of positive infection controls: these have been performed in the past, references to this historical evidence is used now.

A further reduction possibility relates to the size of the groups. Both research groups in this study undoubtedly calculated the number of monkeys needed in their experiment in advance. Yet still, it is notable that one used 12 and the other 20 monkeys per experiment. The use of fewer animals would not be advisable if this lead to statistically insignificant results, but differences in number such as these may warrant closer examination. One respondent agreed, arguing that proper experimental design and the statistical analysis of the required number of animals could reduce the use of primates in HIV studies.

Collaboration

Collaboration has also been mentioned in the literature as a reduction alternative. One scientist said that he would like to see a task force of experienced people in the field who agreed to standardize and validate their assays, share their reagents and perhaps exchange samples. This would provide access to more facilities than anyone one has individually. He also acknowledged that this would involve a significant amount of negotiation and discussion, since nations and governments may have their own scientific programmes. Moreover, this approach would not work if one has an idea and would like to conduct a quick test. In 2000, the trend was to produce new SHIV strains, which would have made it difficult to assess and compare results [IAVI Report, 2000]. In the Netherlands, the two groups do not have direct contact either. Their collaboration had come to an end because they had different interests.

Both respondents believe that the scientists who are working on an HIV vaccine are perfectly well aware of which vaccine candidates everyone is working on. However, they both admit that in theory experiments could be repeated without others being aware of them already having been done. One respondent argued that this does not happen in practice, especially as far as primates are concerned because these involve very labour intensive and expensive experiments. The other respondent, however, expressed the need for all different approaches to be registered at a central location. Yet he did not expect that this would improve matters given that we simply do not know the best way in which the research should be conducted. In addition, it would be hard to organize such a database due to widespread resistance against animal testing.

4.2.4 Other primate studies on HIV

Primates are not only used for the investigation of vaccines, but also for the production of antiviral medicines and fundamental research on HIV. According to several respondents (including primate researchers) and some of the literature [Bailey, 2005; BUAV, 2004], the use of primates is unnecessary for the development of antiviral medicines. Remarkably, even in 2001, the Royal Netherlands Academy of Arts and Sciences (KNAW) stated that the primate model was very suitable for testing anti-AIDS therapeutics [KNAW, 2001].

Fundamental studies on HIV have also been performed in the Netherlands, alongside the immunization experiments. According to a scientist who is not involved in primate experiments, the use of primates in fundamental research is very useful because you can perform experiments with them, infect them, and determine the state of the disease at the time the animal is euthanized. Primate work has yielded interesting and unexpected results, among which the confirmation of the hypothesis that the gut might be involved, which has now also been verified in humans. He regarded several groups, working with rhesus macaques and Sooty Mangabeys (*Cercocebus atys*), as important. These species are very interesting because they are natural hosts of SIV that do not get sick when infected; their immune system partly 'ignores' the virus. He believes that the reason for this reaction may provide a clue for a solution to the human disease.

Human material could be a very good alternative for fundamental primate experiments: in Paris, a group is currently investigating organs from human HIV patients who died in accidents. This is a unique approach that should be used more intensively. The use of data from patients has provided new information on the kinetics of HIV, and has led to new hypotheses. Multiple respondents thought there was insufficient investigation into HIV using human material or patients.

4.2.5 Future of primate studies for HIV

Naturally, all respondents hope that a vaccine for HIV will be found in the near future. One respondent thinks there is a chance that this will succeed: there will come a point when one knows enough to be able to simply change small characteristics to increase the level of protection of the current vaccines without having to conduct new animal tests. Another is more pessimistic about the chances, but still feels that the use of primates is making an essential contribution to the solution. On the other hand, no one knows whether a treatment will ever be found, so all research on primates may prove to be futile.

According to this scientist, everybody feels that an important clues would be missed if a decision to stop using primates was taken. Another scientist claimed that if primates could no longer be used, he would either stop his research or relocate it to another country, like America, India, and China, where conditions will probably be less favourable for the animals. A third respondent said that he would use lower animal species instead. However, this would mean that these could not be challenged and such a model would have to be created. This could take many years, and it would, he argued, take much longer before an effective HIV vaccine was developed.

4.2.6 Discussion

HIV is a major problem worldwide and current prevention methods have not halted the spread of the virus. In addition, a large part of the world's population has no access to antiretroviral drugs. A vaccine may, therefore, be the only way in which HIV infections worldwide could be prevented. As long as there is no vaccine, millions of people will continue to be infected every year.

In the search for an effective vaccine, some kind of organism is needed that can be infected with HIV or an HIV-like virus; this organism's immune system must resembles that of humans. Primates are currently the only model to test HIV vaccines, and their use is considered relevant for vaccine studies by all respondents, including those who do not work specifically with primates. Several objections to the use of primates are raised in the literature on this subject. These mainly concern differences between the primate/SIV model and the human infection with HIV. Primate

researchers willingly admitted that these differences are a flaw in the animal model, which renders extrapolations to the human situation problematic.

A more difficult question relates to the use of primates for fundamental studies on HIV. Respondents working on HIV-vaccines suggested that more fundamental research will not yield a vaccine earlier, but at least one of them was also involved in some fundamental research. One can argue that fundamental research is likely to pay off when it is designed to improve the animal model for the human situation or to gain insight in the mechanisms of infection that can be prevented with a vaccine. Yet why investigate the reasons why some species, such as chimpanzees or sooty mangabeys, do not develop AIDS-like symptoms? Is it realistic that this will yield a new approach that can treat patients or prevent infection in individuals who are susceptible to HIV? Is it likely that this protective aspect of their immune system can be mimicked in humans? Furthermore, the use of chimpanzees was deemed to not be relevant due to these considerations, amongst others. If so, then why would the use of mangabeys be relevant? Even more problematical is the fact that they are an endangered species, which also complicates their use in biomedical science from an ethical point of view.

Alternatives

(Transgenic) mice may be useful in the future, once a model has been designed that can be infected with HIV without too many side effects hampering extrapolation. Mice are already used in immunological studies. This means that differences between the mouse and human immune system are clearly not the impeding factor, nor is size. If the danger of injecting a small animal with HIV is an obstructing factor, this may easily be solved by sedating the mice.

Due to differences in the primate model and the human situation, humans are the best model for HIV. Greater efforts could be made to use data from patients or more initiatives, like the group in Paris, which collects data of deceased HIV-patients, could be taken. Although this concerns more fundamental research, which is not likely to yield a vaccine, it will probably contribute to our understanding of the virus and thus indirectly to a more effective way of finding a vaccine.

Naturally, humans cannot be deliberately infected with HIV in a controlled manner for ethical reasons. Only high-risk populations can be used, which are naturally exposed to a unknown combination of several HIV-strains. For this reason, clinical trials in humans are said to yield less useful data. However, such an approach, combined with in vitro methods to investigate the amount of immunity against several HIV clades before onset to phase 3 could yield more promising results than an additional series of trials in monkeys.

The costs are a factor, which is often mentioned as a reason why clinical trials cannot be done. However, primate experiments are also very expensive, and the costs of clinical trials are not always an issue. In Thailand, a phase 3 clinical trial with a vaccine is being conducted, despite the effectiveness of the product not having been confirmed in preceding trials [Belshe et al., 2004; Burton et al., 2004]. If this trial can pass ethics committees and sponsor reviews, one may question whether the costs and duration of these trials really are a hindrance.

Primate researchers think clinical trials on human volunteers will not be accepted by a medical ethics committee without proof of safety and efficacy in primates. Safety, however, can generally be assessed using other methods and other animal species than primates. Moreover, it has been demonstrated that efficacy in primates does not correlate well with human efficacy. Some endpoints will be needed in order to decide what products should proceed to human trials, of which primate data may be one. Nevertheless, there should be indications that these data resemble the human reaction better than previously, so the primate model can be improved.

Refinement of the primate model

The monkey model clearly needs refinement. One may question whether the route of infection and the dose of virus used in the challenge experiments should be revised in order to create a greater resemblance between the model and the human situation. Hopefully, this would also improve the correlation between primate and human

protection with a candidate vaccine. It therefore seems advisable to use the mucosal route of infection instead of the intravenous one.

As indicated by HIV-scientist Marc Girard [IAVI Report, 2000], too many primate models and approaches are being used simultaneously, without enough collaboration and communication. On the other hand, collaboration is a difficult issue. Nobody knows which approach will yield the best results and, one respondent noted,: one can only keep in contact with a limited number of research groups. Nonetheless, it is in the best interests of both science and laboratory animals for scientists to come together to share their experiences and discuss which approaches are likely to yield the best results. Respondents indicated that they met other scientists on informal occasions, but there have been no signals that this would lead them to join forces. Personal and national interests, beliefs and scientific competition may lead people to stick to the line of attack they believe in, as was already found with regard to animal models in general.

4.2.7 Conclusion

It may be concluded that primates are the only available animal model for research on HIV-vaccines at present. However, they generally are not needed for research on antiviral medicines and should, therefore, not be used. Fundamental studies on HIV can be conducted on primates, but the present study contends that such research should only be done when there is a reasonable chance that this will either improve the primate model of human infection, or yield new insights in possible treatments.

Although the primate model is the best one currently available, it still requires refinement since results from the monkey model do not sufficiently correspond with human data. Both the route of infection and the dose and clades of HIV used should be revised. Clinical trials should be initiated earlier, on the basis of the results of such an improved monkey model, in order to be able to distinguish promising candidates from failing ones in the best model available: the human.

4.3 Discussion on all studies using primates

4.3.1 Use of primates

The statistics presented in Appendix 6 and paragraph 4.1.1 are only indicative for the use of primates. Not all experiments are actually published. Furthermore, one single experiment may also lead to multiple papers being published. Although the search command contained a restriction on 'animal experiments', the retrieval of an occasional review or commentary article also cannot be excluded. In addition, studies that are assigned to one country may have actually been performed in an other. Bearing this in mind, it is likely that in Europe, primates are used most often for neurodegenerative diseases, both terms of absolute figures as the number of studies. HIV probably is the second most important research objective, and many fundamental neurobiological studies are also performed on primates, but these tend to use fewer animals per study.

Arguments

Fundamental differences between the immune system of humans and non-primate species are the main reason why primates are used for the study of disease. This is also why primates are used for research into transplantation, arthritis, multiple sclerosis as well as HIV. Sometimes primates are the only animal species that can naturally be infected with the chosen pathogen, as is the case for malaria and hepatitis. Other animal species may also be susceptible to other diseases, but primates are used since it is thought that they provide the best model. This is the case with respect to, for example, tuberculosis and respiratory syncytial virus (RSV). The exact reasons for

primate use in neurological studies are less clear. They are used because of their higher cognitive functions, the possibility to train them, and the anatomical similarity of their brain structure to that of humans.

The exact reason for using primates, or why alternatives cannot be used, is often unclear. There is discussion on their relevance and quality for almost all subjects, since even primates never completely model all aspects of the human situation. Sometimes, scientists seem to advocate the model they are specialized in. This was also one respondent's experience: the sponsors of primate studies request that research studies on primates concern human conditions, but in practice the researchers just want to use primates, and the human goal of a study is often only secondary.

Future developments

During the 1990-1997 period, the number of publications on research involving primates seems to have increased in Canada, Australia, Switzerland, South America and Israel [Hau et al., 2000]. More specifically, the use of primates is reported to have increased in neuroscience and genetic studies [Goodman and Check, 2002] and HIV research [Carlsson et al., 2004].

Fundamental studies are characterized by the freedom of research: it is the research group that decides which animal model should be used, perhaps with approval of the sponsor of the studies. Therefore, it is not likely that the use of primates for fundamental studies is going to change without the influence of policy-makers.

In the evaluation on the Dutch use of primates by the Royal Netherlands Academy of Arts and Sciences (KNAW), it was concluded that a reduction in the use of monkeys as experimental animals was not to be expected. A continued growth of biomedical research in general would require the use of suitable animal models [KNAW, 2001].

This increase in biomedical experiments is certainly anticipated for research on neurodegenerative diseases, since this is a growing problem within the aging population of the developed world. Since many primates are already used for such research, it is likely that primate use in this area will continue to increase. Furthermore, a respondent reported that it is likely that more funding will be made available for biomedical research, thereby increasing the use of primates as well.

4.3.2 Alternatives

A general conclusion on the possibility of replacing primates with alternative methods for studies of disease cannot be given, since all studies require different characteristics for the animal model and thus different alternatives also. There are, however, indications that some ways of reducing or replacing the use of primates is possible for many subjects. According to one respondent, HIV would in fact be one of the hardest goals to find alternatives for.

Immunological reasons are the main argument for using primates. Indeed, it is very difficult to replace them for that purpose, since a live organism is needed in order to study an immune reaction, and the immune system of other animals is quite different to that of humans. The use of human volunteers or human patients is, therefore, viewed as alternative for primates in these cases, together with transgenic animals. While humans cannot be used experimentally, but they can be used to study disease progression. The problem with this alternative is the fact that this may take more time and be more expensive. For neurological studies, human volunteers are a first possible alternative. This would require the use imaging techniques or tests on human post-mortem material. However, the resolution of these techniques and the quality of human material are the limiting factors. Moreover, it has even been argued that these human studies could generate even more questions, which in turn would be studied in primates.

Transgenic animals also offer an alternative for investigating certain aspects of the subject, mainly the more primary interactions. In the future, a transgenic animal could be developed, which may model the human immune system better than it does now. Such a model would be a major breakthrough, but it is also likely that it would also lead to an increase in the total number of animals used in biomedical research.

Other 'normal' animals could also be used in some cases. However, a major problem with the use of lower animals for studies where the immune reaction is involved is the availability of knowledge and reagents. For primates, reagents to study the human immune system can generally be used because of high cross-reactivity. However, these products are often unavailable for many other animals, except perhaps mice. For other animals, such as the dog, cat and pig, which are used relatively frequently, there is a lack of knowledge on their immune system. This would mean that research may well have to start from scratch. Ultimately, this may lead to a greater increase in the use of animals than if a model with more background information had been used.

4.3.3 Solutions

As indicated, other animal models apart from primates are available for research into several subjects. There often is no consensus on the best model for each situation. The persistence of the general idea that primates are the best, due to their close relationship with humans, may drive the use of these species in cases when there is no 'best' animal model present on scientific grounds. Therefore, there is a need for extensive studies on the quality of animal models for different subjects, which would best be performed by an independent investigator or a committee that represents all the different interests of scientists who favour their own models.

Such a committee could be established by extending Mr. Girard's proposal to increase collaboration and communication by the formation of expert groups on different subjects. This would not only reduce the use of primates, improve of the scientific quality of biomedical research, but also ameliorate the availability of reagents and expertise for alternative approaches.

However, it is unlikely that this will happen in the near future. It has already been noted that scientists have vested interests in using specific animal models, and research programmes and policies differ between groups and nations. There seems to be little interest in extensive collaboration or communication outside the established contacts and connections. It would be hard to draw a justified scientific conclusion on the basis of all these various opinions and hidden interests, though there might be a role for the sponsors of scientific research.

It would be a start to create a sense that primates should not be used unless absolutely necessary. Most respondents indicated that they shared this opinion, but the definition of what is 'necessary' can clearly not be established as long as there is no consensus on what model is most suitable under which circumstances. It would be helpful if scientists would better present their arguments with respect to why the model used was chosen and what alternatives they explored in their publications. In this regard, scientific journals could play a significant role by making the provision of such information a prerequisite for the publication of article.

Chapter 5

Preclinical studies

The use of primates for preclinical (or non-clinical) research by pharmaceutical companies encompasses several EU categories (see Appendix 3). Almost all primates of category 2.3 'R&D (Research and Development) and quality control of products and devices for human medicine and dentistry and for veterinary medicine' are likely to be used for preclinical research, although some of this research might have been conducted at academic institutions, which have been discussed in the previous chapter.

Some routine toxicological and other safety evaluations will also have been conducted in preclinical research. These are tabulated category 2.6: 'toxicological and other safety evaluation', although not all of the 6832 have been used for preclinical research. Since several categories are involved, the total use of primates for preclinical research by pharmaceutical companies can only be conjectured: this is estimated to be some 3500 primates per year in Europe.

5.1 Introduction

5.1.1 Process research and development

The development of a new therapeutic drug is a complex and lengthy process. It can take between 10 and15 years to bring a drug to market, which includes 2 to 4 years of pre-clinical development, 3 to 6 years of clinical development and additional time for dealing with the regulatory authorities, see Figure 5.1 .

The first stage of the process of drug development is the discovery of a possible active compound. In the past, many drugs were discovered accidentally (such as penicillin) or through the analysis of traditional medicines. Today, more systematic approaches are used. A computerized method is used to test thousands of potential targets with thousands of diverse chemical compounds in order to identify chemicals that have potential as drugs. Sometimes drugs are designed and synthesized based on the known structure of either a specific target or one of its natural ligands [The Wellcome Trust, 2006].

The initial screening may identify hundreds of potential drugs, but many of these will be eliminated in the first rounds of testing either because of toxicity or a lack of efficacy in cultured cells and animal models. Once the pre-clinical studies have been completed, the hundreds of promising compounds will have been whittled down to much fewer useful candidate drugs. Some of these may then be advanced to the clinical development stage.

Clinical development usually consists of phase I, phase II and phase III clinical trials. These are tests conducted on human volunteers and/or patients that provide more information on drug safety and activity. By the end of the clinical development phase, most of the investigational new drugs will have been eliminated on safety or efficacy grounds. Only very few compounds will actually be submitted to the regulatory authorities as a new drug application, which includes a request for permission to market them.

After approval, pharmaceutical companies have a short period of exclusivity before patents expire and other companies are able to market the same drugs as generics. This time is used to recoup the massive investment required to develop and launch a new drug [The Wellcome Trust, 2006].

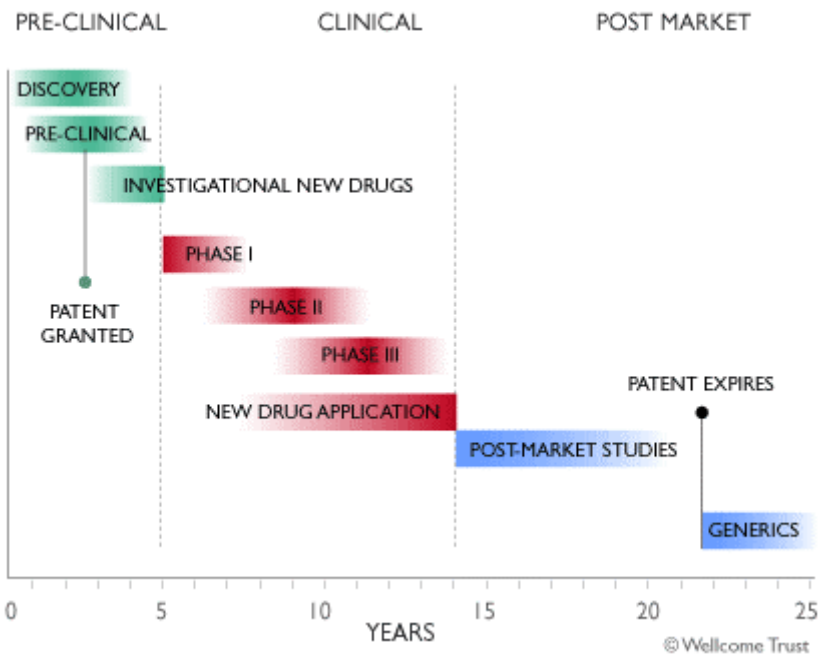

Figure 5.1 Process of drug development [The Wellcome Trust, 2006]

Contract Research Organizations

Companies do not always perform animal tests in house. Not all companies have the space or finances to house animal models, certainly not primates - which are expensive models to buy, house and take care of. Contract research organizations (CROs) may, therefore, be used. These are organizations that perform the requested tests on the requested animal model.

Some respondents believe that the use of CROs may increase the number of animals used in preclinical testing, and possibly influence the choice of animal model as well, since contract organizations have a commercial interest in 'selling' animal models. It can be beneficial for them to advise on the use of more animals or a more expensive model than is actually needed.

5.1.2 Type of products

In this report, two classes of pharmaceutical products are distinguished: conventional pharmaceuticals and biologicals. Conventional pharmaceuticals are small molecules, which can usually be produced by reproducible chemical and physical techniques.

Biotechnology-derived pharmaceuticals (biopharmaceuticals) are manufactured using biological materials and processes, such as the cultivation of cells, the extraction of materials from living organisms, or are produced from transgenic plants and animals using recombinant DNA technology [WHO, 2004]. This class of product is relatively new. The active substances include proteins and peptides, their derivatives and products of which they are components; examples are cytokines, plasminogen activators, recombinant plasma factors, growth factors, fusion proteins, enzymes, receptors, hormones, monoclonal antibodies and vaccines.

5.1.3 Type of tests

In pre-clinical development, several characteristics of a compound are studied, such as

- efficacy
- how it is absorbed and distributed, broken down and eliminated in the body: so-called ADME-studies or pharmacokinetics (PK),
- the adverse effects of the new investigational drug: toxicity studies.

The results of pre-clinical testing are also used to determine how to best formulate the drug for its intended clinical use, e.g. as a pill, aerosol or cream. Promising chemicals may be optimized in an attempt to alter their properties in subtle ways [The Wellcome Trust, 2006].

According to one respondent, efficacy is tested first, followed by PK studies. The last thing to be tested is the short term and 28 day's toxicity, together with specific studies that are required by regulators. This final set of tests will be done more or less at the same time. Finally, but mostly in parallel with the first clinical trials on humans, long-term toxicity and adverse effects on a foetus (reproductive toxicity) may be investigated.

The exact studies requested depend on the intended use of the product in humans: when it is designed to be used only once, no long-term toxicity is required. For instance, when no women of child-bearing age are in the proposed patient population, no reproductive studies need to be performed.

Not all tests are performed in animal models: early in the research phase tests in cell lines can provide evidence of mutations in the DNA, in which case development of the product is halted before animal experiments have even taken place.

5.2 Regulatory influence

5.2.1 Different regulations

Every country can have its own requirements allowing a new medicine onto the market. In practice, there are three important regulatory agencies for the approval of new drugs worldwide : the Food and Drug Administration (FDA) in the United States, the European Medicines Agency (EMEA) in London and the Japanese Pharmaceutical and Food Safety Bureau (PFSB) of the Ministry of Health, Labour and Welfare. They each have their own committees that evaluate the dossiers with results from preclinical and clinical tests, with respect to national or international regulations on safety data, which has to be submitted. Furthermore, additional experiments can be requested.

These regulatory agencies, especially the FDA, are reported to have considerable power [Animal Procedures Committee, 2002]. It is a common perception that these agencies are indeed driving the use of non-human primates in testing [The Boyd Group, 2002c].

In general, however, no explicit requirements on the use of primates exist. Pharmaceutical companies are required to test on animals, generally on at least two species: firstly, a rodent species (usually the mouse or rat), and a non-rodent as second species. The dog is usually the non-rodent species, but primates are used as a second non-rodent species of choice when the dog is unsuitable [Goodman and Check, 2002; The Boyd Group, 2002c]..

The harmonization of regulatory requirements was initiated as a result of differences in national requirements and the fact that pharmaceutical companies operate at a global level. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the

three regions to discuss scientific and technical aspects of product registration. Despite these ICH guidelines, there still are differences in requirements between regulatory authorities.

One such difference between the regulators is the approval to start clinical trials. In the US, this approval is called an IND and is issued by the regulatory authorities. In the Netherlands, human ethics committees approve clinical trials. They require proof in animal models that a compound is beneficial and safe before permission for a trial to start is granted. Later on, when applying for registration, the regulatory agencies in Europe require that the complete file is submitted, including both the animal experiments and clinical trials. These dossiers are thus often some ten thousand pages long.

5.2.2 Requirements for small molecules

For small molecules, primates are considered the second non-rodent species of choice, and are often used when the dog is deemed unsuitable. This is, for example, the case when the substance causes vomiting, or when the metabolite of the product is different in the dog than in humans. Next to this, two other tests for small molecules can be conducted in primates: if a compound has an effect on the central nervous system, a drug dependency study is required. There is, however, no consensus between pharmaceutical companies with respect to which of these models is most relevant: in general, the primate is seen as the gold standard. Efficacy studies are another subject, which may involve the use of primates in the preclinical phase of small molecules.

5.2.3 Requirements for biologicals

A critical review of experiences with the submission of applications for biopharmaceuticals has formed the basis for development of ICH guidelines with general principles for scientifically acceptable preclinical safety evaluation programmes for biologicals. This guideline is ICH S6 [1997] and it states the following:

"The biological activity together with species and/or tissue specificity of many biotechnology-derived pharmaceuticals often preclude standard toxicity testing designs in commonly used species (e.g., rats and dogs). Safety evaluation programmes should include the use of relevant species. A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope."

"Relevant animal species for testing of monoclonal antibodies are those that express the desired epitope and demonstrate a similar tissue cross-reactivity profile as for human tissues."

"Toxicity studies in non-relevant species may be misleading and are discouraged. When no relevant species exists, the use of relevant transgenic animals expressing the human receptor or the use of homologous proteins should be considered."

"Differences in pharmacokinetics among animal species may have a significant impact on the predictiveness of animal studies or on the assessment of dose response relationships in toxicity studies."

"Many biotechnology-derived pharmaceuticals intended for human are immunogenic in animals. The induction of antibody formation in animals is not predictive of a potential for antibody formation in humans."

While these sections of the guideline do not refer specifically to primates, the comments nevertheless imply that these might be the only appropriate species in certain cases. A human protein may not be pharmacologically or biologically active in the other animal species [Anonymous, 1997; Griffiths, 1999].

5.2.4 European market authorization

When the required tests have been performed, there are several procedures to get a new medicine approved: the Centralized procedure, the Mutual recognition procedure or the new Decentralized procedure.

In the case of the Centralized procedure, a dossier must be submitted to the European Agency for the Evaluation of Medicinal Products (EMEA) in London. The risk/benefit ratio of human medicinal products is determined by the Committee for Medicinal Products for Human Use (CHMP). Medicinal products, which have been approved via this procedure, are issued a marketing authorization that is valid throughout the EU. The use of this procedure is compulsory for medicinal products derived from a biotechnological process. For other innovative products, such as products with a new active substance, a company can choose whether to follow this procedure or the Mutual recognition one.

The Mutual recognition procedure (MRP) is a European authorization procedure, which is based on the recognition by EU Member States of a marketing authorization issued in another Member State. The assessment reports from the country that issued the first marketing authorization for a particular product (the reference Member State) are made available to the other Member States. These are expected to recognize this marketing authorization, unless there are serious objections regarding issues that constitute a potential risk to public health. In such a situation, arbitration by the CHMP can be requested. Once the procedure has been finalized, a marketing authorization is issued on a national basis in each of the countries concerned [Medicines Evaluation Board, 2006].

The Decentralized procedure is new since regulations only came into force in September 2005. It is comparable to the MRP, but concerned Member States are involved at an earlier stage of the evaluation than under the MRP [EGA, 2004].

Choice

One respondent indicated that each of these procedures has its own advantages. With both the MRP and the Decentralized procedure, a pharmaceutical company can choose a Member State of the European Union to act as Reference Member State, which will evaluate the dossier, and other countries (Concerned Member States) to recognize the conclusions of the chosen Reference Member State. By this means, one can choose not to have one's product registered in all countries. This can be an advantage when one anticipates difficulties with some countries, for instance if one is aware of resistance to the product or if one expects there will not be a market for the product in these countries. It is essentially a strategic choice.

With the centralized procedures, all 25 Member States of the European Union take part in the process that is facilitated by the EMEA in London. Decision is taken on basis of majority of votes. However, when certain member states have a strong opinion on a product, they may start influencing their CHMP colleagues, giving presentations to express their views. In such instances, there will always be member states that will follow this opposing member state.

Nonetheless, the advantage is that there is only one authority to deal with. Moreover, the process is much simpler, with only one consolidated list of questions from all member states, if necessary a hearing (but only one) and at the end of the procedure one approval leading to one European registration. It is easier and quicker. With the MRP, every Member State may ask their own questions, which makes it a more onerous procedure. New medicines are increasingly being submitted via the centralized procedure at the EMEA in London.

For the animal experiments it does not matter which procedure is followed. The guidelines are the same, so the experiments that must be conducted are independent of the procedure.

EPAR

A European Public Assessment Report (EPAR) is available for medicines that have been registered via the EMEA,. The EPAR is a concise document, which highlights the various reports produced during the centralized evaluation procedure, resulting from the review of the documentation submitted by the applicant, together with the scientific discussion at CPMP meetings. It is made available by the EMEA for information to the public, after deletion of commercially confidential information [European Medicines Agency, 1995-2005].

5.3 Use of primates

5.3.1 Primates for small molecules

No statistics were found on the use of primates with respect to small molecules, the conventional pharmaceuticals. Respondents, however, believe that primates are used only in a small percentage of cases for this class of products. The major use of primates is instead for biologicals.

5.3.2 Primates for biologicals

Literature references confirm that the emergence of biological medicine has increased the use of primates [Buse et al., 2003; Goodman and Check, 2002]. In fact, one speaker at the NC3Rs workshop on the use of primates for biologicals [NC3Rs, 2006] stated that the number of new drugs on the market has declined throughout the past few years, while the number of biologicals is actually increasing. These product thus make up a larger proportion of the new drugs, which are produced each year. Further to this, while the number of primates used in the UK was stable for many years, it has risen throughout the past four years. This is interpreted as another indication that primates are now often used in research and development for biologicals.

Numbers

In a presentation at the biologicals workshop, it was stated that the development of a typical antibody would involve the use of some 400 primates throughout a seven year period. The monkeys used in the first 11/2 years would be for PK studies and these are probably be non-naïve animals that are being re-used [NC3Rs, 2006].

Another speaker reported that, according to registration dossiers, some 2400 primates, mostly cynomolgus and rhesus monkeys, have been used for 29 new recombinant proteins. For monoclonal antibodies, around 1300 primates were used for 23 products. Of these 23 products, only 14 were tested on primates; again mostly cynomolgus and rhesus monkeys. The other products were intended for diagnostic use only, and thus had to be tested less intensively. If primates were used, on average between 80 and 90 monkeys were involved per product.

The numbers, however, differed enormously: between 3 and 70 primates were used for one single study. In repeated toxicity studies, there are cases where only 16 primates were used, but also ones where 60 animals were used in an experiment. Sometimes an excessive number of primates is used for one product: for example 150 cynomolgus monkeys, 80 rhesus macaques and 18 baboons. Other examples are the use of 40 cynomolgus monkeys and 50 rhesus monkeys, or 14 wild-caught cynomolgus monkeys for a product for single administration. The reasons for the use of so many animals is almost never given in the report.

According to a respondent, the reverse also occurs: often very few primates will have been used, which yields statistically insignificant results. In these instances, the primate experiments were considered to be useless and could better not have been performed at all.

5.3.3 EMEA figures

In order to get some idea of the use of primates in preclinical tests, all medicines registered by the EMEA for which assessment reports were available on the website by March 2006 have been analyzed for the presence of the words 'primate', 'monkey', 'baboon' or 'chimpanzee' in the text of the scientific discussion. By this means, medicines were retrieved for which tests on those animals were performed. However, the number of primates used is not always specified. More importantly, these reports only cover the product that made it to the market via this procedure, while many investigational drugs fail at some point during the development process, or other registration procedures are used. In clinical trials, about half of all human medicines fail, and then almost all animal experiments have already been performed.

In total, the assessment reports relating to 282 medicines were examined. Of these, 197 products involved the use of primates (70%), while for 85 products no primates were used (30%). In order to investigate the type of pharmaceuticals for which primates have been used, a random sample of 70 products was taken, of which 70% (49 products) had been tested on primates and 21 products for which no primates were used. On the basis of this sample, an analysis was made of whether the active compound was a small molecule (chemically synthesized), a protein (either recombinant, purified or chemically synthesized) or a monoclonal antibody. The results are presented below in Table 5.1.

Table 5.1 Use of primates vs other species for different classes of medicinal products (Mab = monoclonal antibody)

Product tested on	Total	Random sample	Active compounds in random sample		
			Small molecule	Protein	MAb
Primates	197	49	33	11	5
Non-primates	85	21	7	14	0
Total nr of products	282	70	40	25	5
Percentage tested on primates	*70%*	*70%*	*83%*	*44%*	*100%*

It appears that most products, which have been registered by the EMEA, have been tested on primates. The majority of the products tested on primates consist of a small molecule as an active compound. Proteins are the second class of compounds for which primates have been used, and monoclonal antibodies a third. The products that were not tested on primates were mainly recombinant proteins. Small molecules are tested on other non-rodent species as well, while no monoclonal antibodies were included that were tested on non-primates.

5.3.4 Introduction case studies

In the literature and EPARs, no arguments were given with respect to why primates were used instead of other alternatives. Therefore, a few products were selected that have recently (between 2002 and 2005) been registered via the centralized procedure at EMEA in London. These have been investigated as a case study and will be discussed below. All the information derives from the EPARs and interviews with the pharmaceutical companies involved.

5.4 Case study 1

5.4.1 Type of product

The subject of the first case study is a recombinant human protein with anticoagulant activity. Initially, this product was found to have anti-thrombotic qualities. However, it was finally registered for a completely different indication in humans and is only intended for short term use.

5.4.2 Use of primates

Experiments

Since the product was initially developed for its anticoagulant activities, several animal experiments have been performed to those ends. Firstly, anti-coagulant activity was studied in vitro using the blood of several animal models and humans. This was followed by some animal experiments, among which a study in rhesus monkeys and baboons

After these tests, the anti-thrombotic activity of the compound was set aside and another possible use of the product was identified. For this objective, primates were used once again. Efficacy was tested in baboon models for the disease state targeted (eleven baboons were used), and toxicity tests were carried out using rhesus and cynomolgus monkeys: repeated dose toxicity was investigated in rhesus monkeys, while single dose and long-term toxicity were performed in cynomolgus monkeys. Pharmacokinetics was tested in cynomolgus and rhesus monkeys (probably the same animals as used for toxicity testing), and immunogenicity studies were performed in rhesus monkeys.

The respondent indicated that in total less than 10 monkeys were used by the company itself, with only the minimum of two animals per dose group for toxicity testing. Eleven baboons were used for efficacy testing. In addition, all studies using cynomolgus monkeys were not conducted by the original company, but by a partner pharmaceutical company in Japan. The results of these studies were added to the registration file given that companies are obliged to report whatever they know about a product, including tests that were performed by second parties.

Reasons primates were used

The preclinical toxicology tests were decided on after consultation with the FDA. The company considered which tests would be scientifically balanced and had already conducted some animal experiments, such as dynamics and kinetics. The FDA was then contacted to discuss the toxicity tests that would be required in order to get an IND.

It was known that this molecule has very little biological activity in species other than primates, because it is a human protein. In vitro tests confirmed this: in humans only a very small amount was needed to prevent the blood from clotting, but for dogs and mice, much more was needed to see the same anticoagulant activity. One should not perform tests in any animal species it is known that the substance is not active in that species. Testing in other species than primates was thus considered irrelevant and the FDA agreed that primates were the only species that should be used, without the required second animal species.

According to respondent, the bare minimum of tests were performed. However, to prevent human volunteers being put at risk, some animal testing (on primates) was deemed necessary from an ethical point of view. In general, very small numbers of primates were used because the product was only intended to be used in the short term, which meant that no long term toxicity testing had to take place.

The first primate studies were a proof of concept to demonstrate anti-thrombotic activity in primates, which would ensure that it would work in humans. When the anti-thrombotic activity was left aside, there was no validated animal model for the disease targeted, which could predict the outcome of the study in humans. The company wanted some indication that the product would work for this syndrome. However, because the product is a human protein and the pathway it is involved in has major species differences, searching for a non-primate model was not considered to be helpful. One group from the US had developed a baboon model for this disease and tested it with the plasma-derived version of the same protein. A collaboration began to test the compound in a efficacy model, while the partners were afforded the opportunity to test their animal model.

Some experiments had to be repeated due to Japanese requirements. The Japanese partner had to repeat experiments that had already been performed. They used a different primate species for this since rhesus monkeys are more difficult to obtain in Japan than cynomolgus monkeys.

Discussion on relevance and quality

The baboon efficacy model was not validated. However, this was also not possible either given that no drugs for the syndrome were available before this one came onto the market. However, other products for this syndrome had been tested and proved helpful in this baboon model, but not in humans. The relevance of the model was, therefore, not known in advance.

The product did work in the baboon model, but after clinical trials the dose used turned out to be much higher than the human dose, and lethal effects of the syndrome occurred much earlier than in the human situation. The animal model did not appear to imitate the human situation exactly. The respondent explained that it is an artificial model, and the earlier lethal effects could explain the higher doses, though species specificity may be another reason. Since the product is a human protein, it may not work as well in monkeys as in humans.

According to the respondent, the number of animals involved in this efficacy study (eleven) was definitely too low to be able to produce a statistical relevant interpretation of the results. However, since all animals would die without treatment, there are limitations on the number of animals available. Even though no statistics could be applied, the tests gave some indications that the product may work, even though one cannot necessarily be certain that the same results will be seen in the human situation.

The respondent considered the tests that were conducted by the Japanese irrelevant. Japanese regulations were perceived by several respondents as being less scientific relevant, more like a long list of checkboxes. They were required to repeat experiments and perform long-term toxicity studies (2-week and 1-month) while the product would be used in clinics for only a short period of time. Furthermore, they used high doses of the compound, which caused severe bleeding and death in the monkeys, despite the warnings of the developing company that severe bleeding would occur.

The Japanese long-term studies must also have been influenced by a antibody response in the monkeys. In spite of the similarity of primates to humans, the monkeys involved in the study had a major immune response, which makes it hard to interpret the data. Antibodies are only formed after day 6 or 7, so the short-term data are acceptable.

The respondent contends that the monkey has been a good model in the preclinical research for this product. The studies on anti-thrombotic activity in rhesus monkeys predicted the results in humans, and the use of primates allowed the company to establish the highest dose in humans. Adverse effects were found at doses that were two times lower than in the monkey, which suggests that there are still species differences. However, given that the monkeys were healthy and the patients were very ill, one would expect to observe these effects at lower doses.

Nevertheless, the respondent believes that with the current state of knowledge, the animal models were not very helpful. However, it was stressed that this is just a personal opinion; in the literature, most people concur that if no signs are found in animals, that it is unethical to test the product on humans. Scientists criticized the preclinical testing for this product, arguing that insufficient animal testing took place and that the product was given to humans prematurely. However, it should be noted that these scientists were working on the same disease, using animal models other than the baboon. They may not be the most objective people to comment on this subject, since they have a vested interest. According to the respondent, the pharmaceutical industry was more understanding.

5.4.3 Alternatives

Respondents note that primates are closest to humans. At the same time, they are the most difficult to use, not only due to their availability (and the waiting time associated), but also because of the costs involved. Nevertheless, alternatives were considered not feasible for the following reasons:

Animal-free methods

A living organism would be needed because the disease in question is a syndrome and not just one simple biochemical reaction. In vitro methods were used in the species selection, but not as alternative to the use of primates. Indeed, in vitro tests with blood from monkeys showed the same reaction as human blood.

Other animal models

The respondent found it hard to judge whether other animal models would also have been possible. There has not been any model for the syndrome in question that has been more or less predictive for the results in humans. The monkey model was also not entirely predictive, but primates are closest species to humans to try as a model.

Toxicity testing could have been done in other animals, but doses much higher than in primates or humans would have to have been used. From the in vitro tests, it was found that 10-20 times more compound would be needed for dogs than for humans. Moreover, it was argued that one also requires some feeling of the dose range to aim for in clinical trials. Primates give a better sense of the effects in the human situation.

Other 'alternatives' mentioned in ICH S6 on biologicals are transgenic mice and the use of homologues proteins. According to the respondent, these methods were not possible. If one knocks out the same gene in several mice with the same background, one gets different biological properties. The results from these animals would therefore be hard to reproduce and to translate to the human situation. The use of mouse analogues of the human protein would also be hard to interpret. For the syndrome involved, mice differ enormously from humans, much more than monkeys do.

Humans

Microdose studies in human volunteers were used, but not as an alternative to the use of primates. The phase1 study was started with microdoses to prevent volunteers from mounting an immune response to the product. This was done because primates displayed an intense immune reaction. If monkeys do not mount an immune response, then humans probably would neither. However, the monkeys did have an immune reaction, thus phase 1 studies were initiated very carefully. In addition, the respondent notes that finding the right dose in humans is the best way to go.

Reduction alternatives

Since the company only uses a small number of primates, no reduction was deemed possible.

5.4.4 Discussion of case study 1

In view of the fact that the tests were conducted on primates, the arguments for why these species were selected and the problems relating to their relevance and quality, the issue of whether the use of primates may be considered necessary, and the extent to which alternatives could have been used, may be discussed.

Toxicity

The choice of a primate model for toxicity studies seems logical, since the product is a human protein and only primate blood showed a comparable response to the product as human blood. On these grounds, the primate seems to be the most relevant animal model, but this does not necessarily exclude the possibility of using other methods.

Animal-free alternatives could not have been used as an alternative to the final toxicity testing in animals as required by the FDA. Other animal species, however, though not an alternative in the strict sense of the word, did show evidence of a pharmacological reaction to the protein, and thus could not be rejected as irrelevant models. The FDA agreed that primates were the only relevant animal model. This, however, does not mean that the use of

primates was necessary. For dogs, the standard non-rodent species, doses 10-20 times higher than the predicted dose in humans would have to have been used, and extrapolation to the human dose level would have been more difficult. On the other hand, the respondent also indicates that determining the dose can best be done in humans. In fact, this is what happened: starting with a very low dose and slowly increasing it. Only after the clinical trials could it be verified that the dose level found in primates was similar to the dose found in humans.

One can even wonder what would have happened if the dose level in primates, for whatever reason, was found to have been five times higher. Would the drug then have been administered as a starting dose to humans at exactly that level? Certainly not. Results from animal tests are required in order to gain some indication of the dose level, but administration will always be given first in lower doses.

Would it then not have been possible to do the toxicity testing in other species, for instance, dogs? Since in vitro tests provided an indication of the extent to which the activity of the compound differs between dogs and humans, could this factor not have been used as a safety level when extrapolating the results from dogs to humans? For instance, when a dose level of 100 mg/kg was found in dogs, but it was known that dogs needed 10-20 times more compound to show a reaction, one could estimate the human dose of 5-10 mg/kg. For safety reasons, first administrations would start at doses well below this estimate, e.g. on 0.1 (g/kg. Even when differences in reaction in vitro would turn out to show exponential differences in vivo (thus 10-20 times more effect in vitro would mean 100-400 times more in vivo), this would still be on the safe side. Why would such an approach not be possible?

In fact, a same approach was applied to the first clinical trials. A NOAEL (No Adverse Effects Level) of 5 mg/kg/hr was calculated on the basis of primate studies. The first studies in humans started with 0.5 (g/kg/hr, ten thousand times lower than the level calculated from primate studies. It is remarkable that these data are still requested, when they apparently are not subsequently used.

Efficacy

It is recognized that some kind of efficacy studies will have to be performed, both to give the pharmaceutical company some indication that the product is worth developing, as well as to provide some proof of concept to the regulatory agencies. Since this product is a recombinant human protein, one may reason that the working mechanism in humans could also be provided by just scientific literature. However, the protein is involved in a syndrome together with several other molecules, and it is never known for sure what will happen when the level of one of these proteins will be increased by the administration of a drug. It thus seems rational that this would be tested in some organism in advance. This rules out all animal-free alternatives.

It has been indicated that several animal models existed, but none of them could have been validated for the disease state for which the product in this case study was intended. At the same time, the disease is more a syndrome than a simple disorder. The pathway of the targeted syndrome was reported to differ enormously between species, and the species specificity of this human protein would limit the use of less closely related species.

The choice of using a primate seems to be primarily based on this species specificity; the general idea of primates as being 'the best model' is a secondary consideration. However, could it have been possible that the very presence of a primate model for this disease influenced the choice to use it? If one validated animal model of the disease had existed, being a non-primate, this probably would have been used; that is, at least, if the human protein showed some activity in that animal. It must therefore be conclude that the primate was considered to be 'the best model', even though it was no more validated than the other models available.

One topic, which has not been discussed thus far, is the extent to which human volunteers or patients might have been used as alternatives to the use of primates. This is an ethically difficult topic. New products for cancer are usually administered to human patients quite early on in the development process, certainly when the patients cannot be successfully treated in any other way. The syndrome targeted by the product in this case study is also often fatal; 30% of patients with severe disease die within a month. One may question whether in the case of such a serious

disease is also worth trying the product on some seriously ill patients early in the development process. On the other hand, while there is a 30% chance of dying, there is also a 70% chance of survival without the risk of being harmed by a novel medicine. The general principle for clinical studies is that it should not cause the volunteers or patients anything more harm than they are already suffering as a result of their medical condition. It is a very difficult question whether an increase of survival rate, which is already 70%, is worth the chance of experiencing unknown, and potentially fatal, side-effects. Furthermore, since even the efficacy of the product would not be known in advance, it must be concluded that no testing on human patients would be possible for this product for ethical reasons.

5.5 Case study 2

Case study 2 consists of two products, both produced by the same pharmaceutical company. Since both products were discussed with the same respondent, they have been presented together in this case study and will be named product X and Y.

5.5.1 Type of product

Product X is a recombinant human protein, which is intended to alleviate some side-effects of cancer therapy. According to the respondent, the product is very old, which means that the work done on this compound may not reflect the kind of research that is done today. The initial tests would have been conducted 8-10 years ago, but there were probably some obstacles in the development process that caused it to lay on the shelf for a few years.

Product Y is a small molecule, which is intended for treatment of certain diseases of the thyroid gland over a longer period of time. The EPAR for this product does not contain very much information on the preclinical tests that were carried out on it.

5.5.2 Use of primates

Tests performed

Non-human primate models based on lethal irradiation were used as an efficacy model and 6 monkeys were used for pharmacokinetic studies for product X. Several toxicity studies were conducted on primates, using several dosages, durations and routes of administration. These tests consisted of a 3-day toxicity test, one repeated dose toxicity test of three days with five days in between, one 28-day toxicity test and two preliminary studies. In total, at least 88, but probably some 100 primates were used for toxicity studies. In the 28-day toxicity group, where the product was administered intravenously, antibodies against the human protein were detected.

The efficacy of product Y was studied using rat models, while pharmacokinetics and repeat-dose toxicity was assessed using both dogs and monkeys as non-rodent species. The number of animals involved is not specified in this EPAR, nor are the dose groups or routes of administration given. There are indications, however, that a 3 month study and at least one of another duration have been carried out, with at least one recovery group, several dose groups and possibly different administration routes. With three animals in three dose groups (the normal minimum) this would mean that at least 22 primates will have been used for toxicity. It is likely that more primates will have been used. In addition, some non-naïve primates will have been used for pharmacokinetics, which are normally re-used after a wash-out period of about one month.

Reasons primates were used

The respondent could not give explicit reasons why primates were used as opposed to other models. He explained that in general, when both dogs and primates are used, there must have been a reason to think that the toxicity profile would be different in the dog as compared to the monkey.

According to the respondent, the process for choosing an animal model is different for biologics than for small molecules. For small molecules, the primary driver in the choice of the non-rodent species is the metabolic profile of the compound. If humans produce a major metabolite that the dog does not, but the monkey does, then the monkey will be chosen. The metabolic profile is assessed early in development using in vitro methods.

There are two factors that influence the preclinical species choice for biologics: the biological activity and the immunogenicity of the compound. The biological activity is assessed by either looking at the homology of the protein between species (when the compound is a therapeutic protein), or by assessing binding activity and functional activity in vitro when the compound is an monoclonal antibody. Immunogenicity, however, cannot be studied in vitro and it is hard to predict whether this is going to occur.

Apart from scientific considerations, the respondent suggests that the regulatory authorities may be the driving forces behind the use of primates. It is important to make a good case to regulatory authorities in order to convince them that the right tests on the right animal models have been performed.

Discussion on relevance and quality

According to the respondent, the studies mentioned above for product X were no longer typical either. It is not unusual to test the extent to which the product is effective by using more extreme situations than the human conditions the product is developed for. However, today there must a very strong justification to use an extreme endpoint as lethal irradiation; this would probably no longer be used.

In addition, the number of animals for toxicity testing would be reconsidered. It would be typical to use three dose levels and the minimum number of animals required by the regulatory guidelines. In general, it was reported that more time would now be spent on species selection with regard to this compound.

The respondent initially thought product Y had only tested in dogs. General reasons to use both species were given: when there were indications that the either the metabolites or toxicity of the product would differ between species and man. The only indication from the EPAR is a reported 'low systemic exposure' of the dog, 'possibly due to frequent vomiting'. The primate did not seem to have had any other additional value: pharmacokinetics even was reported to be similar in all species used (mice, rats, dogs and primates) and humans.

The possible immune reaction is another factor when deciding on an animal model. According to the respondent, this decision is driven by a lack of data: the primate immune response is well-understood, while there is poor understanding of the canine immune system. The immune response in the primate can be much better measured than in the dog. Furthermore, there is a belief that the most immunogenic part of an monoclonal human antibody is more immunogenic in dogs than in primates. At the same time, the respondent explained that when an immune response occurred, one could attempt to solve it by increasing the dose, thus overwhelming the immune reaction.

It was asked whether the dog would be chosen as a non-rodent species in the event of a protein or monoclonal antibody having biological activity in the dog, unless there were possible problems with an immune reaction. The respondent replied that this would be a difficult decision. If a company decided to use dogs instead of primates, they will probably have to argue the case before the regulatory authorities. They would therefore have to consider any potential delay this might cause and whether this was worthwhile. The respondent acknowledged that a delay in the development process is always even more expensive than using a primate.

It was thought that the company would use the dog and plead their case with the FDA, if they believed that it was the right model. According to respondent, it would be harder to make a justifiable argument for smaller companies, since these tend to have less extensive resources and possess less data.

Sometimes the FDA may require a company to use both species when they have information on the product, e.g. when other pharmaceutical companies are working in the same area as well. This has been the case for one

group of drugs that a number of companies were working on; some choosing to use only monkeys and others only dogs. When that data made it to FDA, they realized that these species showed different toxicity profiles and the companies were asked to use both species.

In general, it was stressed that tests may be carried out differently today than they were for these products. According to the respondent, the industry has changed in general. There is a much greater awareness and/or concern for the ethical issues surrounding primate use, which has led to the greater scrutiny of the justification for use of primates instead of other laboratory animal species. In some cases, this may have been due to the changes in attitudes of individual scientists, a general maturing of the science of laboratory animal care and welfare, and perhaps a general shift in public opinion too.

5.5.3 Alternatives

Most approaches that were mentioned as 'alternatives' were used in the preclinical development process, but not as alternative to the use of primates. These approaches may lead to a reduction in the use of these animals, for instance when a compound is dropped as consequence of the results of these methods before primate tests are performed. This can occur mainly after tests on in vitro models with human or animal cells and other animal models

In conclusion, the respondent deemed it impossible to replace primate tests, even with all the available alternative methods. It is a gold standard, since all these methods can only tell you something about what could happen, but not what will happen in vivo, in a living animal or person.

Animal-free methods

Respondents argued that the use of human tissue has two major limitations: often the quality of the tissue is not too good, and one can only examine the metabolic characteristics of one tissue. Only part of the reaction can be investigated, no whole body response e.g. toxicity of metabolites (that come from the liver) on the kidneys. This is mainly a problem for chemicals, less for biologics, since there is less reason to investigate the whole body response for biologicals.

Other animal models

In general, according to respondent, other living animal models could be used as an alternative, but again mainly as an alternative that helps to reduce the use of primates, rather than replace them. Certainly for biologics, it would be hard to use other animal models because of either a lack of biological activity or issues of immunogenicity.

The common perception of transgenic mice was that the primate model is more accurate than transgenic mice, since the latter may have a completely different reaction to the compound. Moreover, primates are deemed better models because of immunogenicity problems in transgenic mice.

Several problems with respect to homologues were discussed. Data on homologue proteins are difficult to obtain for the dog. Furthermore, the use of surrogate antibodies in the rats or mice provides information about the biology of the target, but this does not mean that the same will happen when the clinical compound is administered to people. Gene function differs between species, which means that the same target might induce a different response in vivo. Moreover, the structure of the clinical candidate may be sufficiently different from the structure of a surrogate molecule, that when using a surrogate molecule one may fail to predict something that could be very catastrophic in people.

Humans

According to the respondent, the use of human volunteers or microdose studies cannot contribute to changes in primate use given that these methods are employed after animal model studies have taken place.

However, if unexpected toxicity is found in the first human studies, in vitro techniques, such as genomics, will then be used to investigate these further. Ten years ago, more primate tests would probably have been conducted instead.

Reduction

As discussed in 5.5.2, the decision on what model to use and the number of animals involved, would be assessed in a different way in the future. There are apparently sometimes possibilities for the reduction in the number of primates used.

5.5.4 Discussion of case study 2

Unfortunately, the respondent in this second case study was unable to provide details on the reasons why tests on primates were conducted for these products. The reasons that were given, which are more general of nature, are informative but not very factual. This makes it hard to discuss the necessity of the use of primates, or the possibility of replacing them with other methods, for this specific case study.

Product X

Some 100 primates were used in the preclinical process for the recombinant protein, product X. The respondent indicated that both the number of animals, the extreme endpoint of lethal irradiation used, would be decided on differently now and more time would be spent on species selection. Biological activity and immunogenicity would have influenced the species selection. However, there are indications that the product was not too species specific: in the EPAR it was reported that rats also displayed a response to the biological action of the compound. It may be questioned whether dogs would not have either.

Immunogenicity, the other factor in the choice of animal model, can also be discussed. Antibodies to the compound were observed in both the primates and the rats and rabbits used in the preclinical testing of this product. The primates used did therefore mount an immune response, which was said to have increased the exposure of the animals to the product.

It thus seems that both species specificity and immunogenicity were not crucial reasons for choosing a primate model instead of other non-rodent species. The product was shown to be active in the rodent, and primates did mount an immune response just as it was feared dogs would. The only problem was related to the understanding of the dog immune system.

It is noteworthy that the respondent said that they would have used dogs if a compound was active in the dog and have argued the case with the FDA, if they thought the dog was the relevant species: it seems that this could very well be the case for this product. This begs the question of why was the use of other animal species not discussed with the FDA. The respondent even indicated that the FDA does not give advice, but only indicates whether the tests performed were good enough to provide an IND afterwards. Various other respondents stated to the contrary that a scientific advice on preclinical tests is possible in the US, and this even occurred with respect to the previous case study.

In general, the responded argued that this product was quite old and the tests, which had been carried did not reflect the way in which they would be conducted today. The product was left lying on the shelf for a couple of years before it was rediscovered and developed for the current indication. However, is it not possible that this delay was caused precisely because of the use of primates? Could it be possible that an approach with several tests, a comparatively large number of animals per group and primates as species could have been chosen to speed up the development process after this delay, and to make sure that the data were accepted by the regulatory agencies? For that question to be answered, one would need to know more about when the patent was granted and how long the delay was. At the moment, this question remains unanswered.

Product Y

It is even more difficult to discuss the use of primates, their necessity and the possibility of replacing them with alternatives with respect to product Y, a chemical, since even less information on this product was available from the respondent or the EPAR. The general reason for using both dogs and primates is that there must have been indications that the either the metabolites or toxicity of the product would differ between species and man. No indications for either of these were found in the EPAR.

The most striking feature, however, that primates were used while this product is a chemical, and that dogs were used as well. The only indication the EPAR gives is that a 'low systemic exposure' of the dog was reported, possibly due to frequent vomiting. This is a reason, which is often given for using another species. It is notable that this was not reported during the initial pharmacokinetics studies, which means that the dog was used in the toxicity studies as well. Moreover, the initial selection of all standard species for pharmacokinetic studies is also noteworthy. Why were both mice and rats as rodents and dogs and primates as non-rodents selected? Was it to be sure that the relevant species was chosen? Or was it to save time? Again unanswered questions.

5.6 Alternatives

Several alternatives for animal tests were discussed in Chapter 3. The respondents'' views on their application and limitations, specifically with respect to preclinical testing, will be discussed here.

5.6.1 Animal-free methods

Respondents indicate that the use of tissue or cells as alternatives to the use of primates is very limited. Cultured cells are used, but not as an alternative for primates since extrapolation from cultured cells is very difficult, while primates are used when a high level of prediction is needed. Genetically modified cells, for instance with a human receptor, are used comparatively often and sometimes even as an alternative to the use of primates. However, the problem is that not all biological reactions can be studied and the cells may have changed because of their unnatural environment in culture; primary tissue can also not survive long enough to study long-term effects.

It was reported that computerized techniques are now being used more frequently. According to one respondent, these can provide an alternative for pharmacokinetics and dynamics studies, although another contended these results would always need to be checked in animals.

According to the respondents, new techniques such as genomics will not offer alternatives to primate use, at least not in the foreseeable future. The prediction level of these approaches is very uncertain, as is the possibility for testing for chronic effects.

It thus can be concluded that in vitro techniques do not offer a direct alternative to the use of primates. These techniques can be used earlier in product development to identify whether a compound might make it to the clinic, thus eliminating primate experiments for failing products. This may indirectly reduce the use of primates, though it can be questioned whether this is the actual effect of such a technique. The question remains whether it would not lead to a higher throughput of candidates, more compounds being investigated over a shorter period of time and thus a same (or higher) number of animal experiments. Moreover, it is unclear whether a method that reduces the use of primates by eliminating poor quality products will ultimately reduce the number of animal tests.

5.6.2 Other animal models

In general, respondents agreed that animals (including primates) are still needed to evaluate the biological effects of compounds. However, it can be questioned whether other animal models can be used instead of primates, even though these are not a 3R alternative.

Most respondents from the pharmaceutical industry do not regard transgenic animals, which were given as a possible alternative for the testing of biological in ICH S6, to be good alternatives to the use of primates. They are used, but their usefulness as an experimental model has not yet been verified. One problem is that not every transgenic animal is the same as the wild-type, while laboratory mice normally are almost genetic twins. After the insertion of a gene in the transgenic mice, one must therefore carry out multiple generations of backcrossing with the original strain, in order to get a model that resembles the standard strain. This takes a lot of time (over a year) and even then one still does not know whether the gene inserted into the mouse will be expressed in the same way as in humans, or whether the reaction to the compound will be identical. The respondents thus contended that - for new pharmaceuticals - transgenic animals could only replace the use of primates for routine procedures, since every time a new compound with another mechanism will be involved.

The problem associated with surrogate molecules, another alternative mentioned in ICH S6, is that they are not the clinical candidate and it is not known whether this will have the same results; this was a view generally shared by the respondents. According to one of them, the surrogate molecules' success in predicting the clinical outcomes should be investigated. They are, however, used in the initial development phase for efficacy studies; one respondent gave an example of the use a homologue in the mouse for a very species specific human protein. This is frequently done with respect to monoclonal antibodies in order to increase the knowledge of a certain compound. Nevertheless, a primate study would still have to be performed as a final examination, and it was argued that one can question whether the use of primates is still necessary when a lot has already been learned from other studies.

Embryos could also be used to assess reproductive toxicity, but, according to a respondent, these are not predictive enough.

Based on the case studies, it can be concluded that one obstacle to the replacement of primates in biomedical research is the fact that the 'most relevant' species must be chosen. Yet what are criteria? How 'perfect' does an animal model have to be? When is an animal model that is responsive to the agent not the most relevant species? It may be precisely these questions that drive the use of primates, which are considered 'the best model' given that they are closest to humans in evolutionary terms. This is, however, not always a scientific argument, but one of intuition.

For all three products investigated in these case studies, it was concluded that there was a lack of compelling arguments to justify the use of primates for at least some of the tests performed on primates. In most cases, it appears that it was possible for other species to be used as well. Another interesting finding is that none of the assessment reports, which were studied for this inventory, appeared to report the use or consideration of non-traditional species as a non-rodent species. However, background data on animal models is needed in order to know which reactions are normal; this data may be lacking for non-standard species.

At present, companies differ in their view on the quality of transgenic animals and surrogate molecules as experimental models. This may change in the future, when more experience of them has been gained. Nonetheless, the amount of time involved in backcrossing will continue to be a problem in the development of these species.

5.6.3 Humans

Human tissue is used sometimes to test the cross reactivity of monoclonal antibodies. However, this does not replace the use of primates since, according to a respondent, the technique is only used during development.

Most respondents were pessimistic about the possibility of using microdose studies in humans. It was noted that this would take a very long time, up to two years. Moreover, the toxicity of even low doses can never be ruled out, putting the volunteers at risk. More importantly, toxicity could never be studied in humans; animal experiments would thus remain necessary. At the biologicals workshop, a case was mentioned of an antibody, which had no cross-reactivity in cynomolgus monkeys, and there was no rodent equivalent of the target to use as a homologue. No toxicity studies were therefore possible. After discussion with the FDA, it was agreed that tests using a very low dose of only 0.001 mg/kg could start in humans. However, the company was still reported to have carried out a toxicity study in cynomolgus monkeys, since they believed the FDA required it. In reality, the FDA deemed it pointless.

According to one respondent, the use of human volunteers for pharmacokinetics would also be very limited. In preclinical toxicity testing, the exposure of the animal model to the compound must be known. PK-studies would thus have to be carried out using the animal model. Other respondents indicated that microdose studies would only be a viable alternative when no relevant animal model was available.

Human volunteers can, therefore, only be used for microdose toxicity studies with assessment of biomarkers and pharmacokinetics. This will not lead to an enormous reduction in the use of primates. Another problem with the use of human volunteers, specifically for monoclonal antibodies, is that nothing can be done to reverse toxic effects when these happen to occur. Moreover, as one respondent pointed out, there is a possibility of a progressive immune reaction, even when starting in very low doses.

5.6.4 Reduction alternatives

For reduction alternatives, respondents indicated that the tiered approach is always used as part of the testing process. This is, however, not an alternative to the use of animals. One respondent said this was not often used for telemetry, while another respondent indicated this method was going to be used in the future.

Most pharmaceutical companies are open to the idea of sharing research data. Some indicate that this is already done with the FDA as the middle man. Others say that failed research studies are shared on incidental basis on the scientists' own initiative. Likewise, respondents differed in their opinions about the reduction in primate use that could be achieved using this approach. In general, it was argued that some reduction could be achieved. Nonetheless, confidentiality would be an important issue. One pharmaceutical company indicated they were not going to submit their research data.

In general, respondents indicated to make result sharing work, the benefits for pharmaceutical companies would have to be obvious. Money would be less of an issue to overcome the problems with confidentiality than time, but improved safety for human volunteers might be the best argument for sharing results.

Most respondents did not comment much on the use of reduction alternatives. One respondent noted that the number of primates is not always statistically balanced: mostly, a minimum of three primates is used with two extra to be sure. However, from case studies 1 and 2, it seems that in some instances many more primates are actually used. A justification for the number used is seldom given. In this regard, there is a possibility for reducing the number of primates used, as well as for preventing the unnecessary use of monkeys.

Furthermore, respondents indicate that the questions primates are used for could be asked in a different way, avoiding the use of primates altogether: e.g. when a compound binds to heart tissue, this can be studied using in vitro methods before using primates. A tiered approach could thus be used more often; however, this would also take more time.

5.7 Discussion

5.7.1 Use of primates in preclinical research

The first striking observation about the use of primates in preclinical research is that 70% of medicinal products registered via the centralized procure have been tested on primates. This number is far higher than the one, which is generally given by respondents who estimated the proportion of products tested on primates to be about 25% maximum. The explanation for this high number cannot be found in differences between registration procedures, since according to a respondent, these do not influence the use of animals.

Biologicals

One factor that does influence this figure is the fact that all biologicals must be registered via this centralized procedure, while other products (small molecules) can also be registered by the other routes. This consequently results in an overrepresentation of biologicals in the list of products on EMEA's website, and thus in the samples taken for this investigation as well. This overrepresentation, however, is more likely to have lowered the proportion of products for which primates were used than to have increased it: 16 out of 30 biological products in the sample had been tested on primates, which is less than the average 70%.

The finding that only 50% of biologicals was tested on primates contradicts the references in the literature and the respondents' answers, both of which generally stated that the development of biological was one of the major subjects where primates were used. Furthermore, according to them, these products are tested mainly on marmosets, while these species were seldom found in the reports. Chimpanzees were, however, found in two cases both monoclonal antibodies.

A possible explanation for the comparatively low use of monkeys for biologicals is the fact that of the 4 biologicals in the sample that were not tested on primates, at least 7 were insulin-variants and two were vaccines. It is generally acknowledged that insulin-variants can be tested in other non-rodents beside the primate (for instance the dog), since these proteins are not species specific. The inclusion of so many insulin-products may have been coincidence. For monoclonal antibodies, however, it was reported that the majority of these products were not tested on primates, since these were only for diagnostic use. Nevertheless, in the sample taken for this analysis, only MAbs were retrieved that were tested on primates. As only 5 of these products were included, which also might have happened by chance; another explanation is not likely.

Small molecules

The key observation is, therefore, that most products for which primates have been used are small molecules, not biologicals. As regards this class of compounds, nothing is known about the number of primates involved per product or per test, nor on the relevance of their use. The reason for using primates has also not been elucidated by the case studies, since two of the products involved were instead biologicals. The small molecule, which was studied in more detail, only had a very short EPAR on the preclinical tests that had been carried out. Moreover, the respondent could not comment on details. This should subject to further investigation in the future.

Some indications can be given on the basis of the non-representative findings from the sample examined: at least 6 out of 33 conventional products that had been tested on primates were antiviral agents for the treatment of HIV. Primates may have been used as an efficacy model, which is striking given that in the previous chapter the conclusion was drawn that primates are not necessary for the development of anti-HIV-drugs. Why these animal models were used is an other question that has to be answered.

Another goal could be drug abuse studies. Indeed, at least four chemical medicines in the sample that had been tested on primates were agents for the central nervous system. Yet this does not explain the high percentage in

total. The reason why so many conventional products have been tested on primates should be elucidated by a future study.

Limitations

It should, however, be noted that the results from this sample have several limitations. Firstly, all medicines listed on the EMEA website have been examined with respect to the use of primates and drugs from all time periods were retrieved. If one takes into account that fact that animal experiments may have been conducted some 10 years ago even for recent registrations, one may question whether these total results of animal testing are still relevant.

Although this may go both ways, since the proportion of biologicals increased from 36% of the total number of primate tests in 2003 [Motola et al., 2005] to 43% in the first quarter of 2006. Many more of them are in the pipeline, and if respondents are correct in their supposition that mostly primates are used for their development, then the use of primates may even increase in the future.

Another point of discussion is the difficulty in labelling compounds. In many cases, it was not stated whether the compound was a small molecule. Indeed, in a few cases where the production process was not entirely clear, it could be the case that a compound was given the wrong label. It is, however, unlikely that this has influenced the results significantly.

Another possible bias in the results can be found in relation to the products where it was not apparent what animal had been used at all, or whether primate studies had been newly performed or only referred to. This may have been the case for less than 10% of products, however it is unlikely that the actual use of primates would differ with that percentage from the figures presented. A variation of 5% does seem possible.

5.7.2 Relevance and quality

There was also some criticism on the quality of the primate model, mainly from animal welfare organizations, which presented examples of extrapolations from primates to humans that have failed in the past [BUAV, 2004; FRAME, 2005a]. Their publications are based on literature: several studies have tried to correlate side-effects in humans with toxicity found in animal testing.

Prediction

Olsen et al. [2000] found that for existing drugs, the side-effects in humans were predicted by total animal testing in about 70% of cases; non-rodents accounting for 63%. There were few correlations across species. A critique on this study is given by Bailey [2005], who argues that in retrospect it is simple to find some animal model from all species used in toxicology replicating the human condition. Prediction, knowing in advance which species will be suggestive of the human condition would be another matter. He contends that dogs and monkeys (apart or together) would be no more predictive for human toxicity than tossing a coin. Several studies are cited in his review to underline this opinion.

Foster [2005] concluded that although companies 'have an inbuilt prejudice that the primate will more closely mimic effects that might occur in man in the clinic, insofar as the liver is concerned, there are many instances where the dog has been more representative of human exposure and metabolism and there is little evidence to show that the nonhuman primate is consistently better than dog in predicting human liver toxicity.' Indeed, a respondent reported that about 70% of all primate experiments show predictions for the human situation; a number that would be valid for all animal experiments [Olson et al., 2000].

Relevance

In the experience of one respondent, at least 50% of all primate experiments in the preclinical process were irrelevant (inadequate question), wrongly performed (e.g. with incorrect doses) or unnecessary. However, only one

study on the relevance of choosing primates to test new medicinal products is known, which is a confidential report based on biotechnology products that made it onto the market [Van der Valk, 2005]. In general, the conclusion of this investigation was that 80% of primate studies for recombinant proteins were considered relevant. Dogs could have been used instead of primates for 6 out of 29 products.

When both dogs and primates were used, but the arguments cannot be retrieved from the dossier, it is hard to decide whether the use of primates was relevant. According to one respondent, the company could have decided to use primates regardless, but it is possible that dogs also mounted an immune response to the product, and therefore the test was repeated in primates. One cannot conclude that either the primate or the dog study was irrelevant.

Another important conclusion of the present report is that the argumentation was often insufficient, and regulatory authorities made no comments on the eventual unnecessary use of primates in their assessment reports.

Numbers

Several respondents indicated independently of each other that the use of primates often involves numbers of animals that are statistically not robust, since the number of animals available is often used instead of scientifically balanced numbers, or a standard number of animals is used without any statistical rationale.

5.7.3 Reasons for the use of primates

As discussed above, the arguments for using primates are often inadequate. This conclusion was reached on the basis of the EPARs and the case studies carried out for this report. The arguments most often used will be discussed in the following section.

Scientific

Scientific reasons are most often used to justify the use of primates. For example, epitope-expression and tissue cross-reactivity is regarded as being very species specific, which means that primates are considered to be the only relevant model. This has not been demonstrated with respect to the two proteins discussed in the case studies, but these arguments may well be valid for other products. However, for small molecules, the metabolite of the product would a valid reason for using primates, or the argument that primates would be more predictive than other animal models for agents that work on the central nervous system. However, the industry has not achieved consensus on this topic, thus it cannot be said that this is a relevant or irrelevant argument.

Another scientific argument for using primates is the risk of an immune reaction. According to one respondent, primates are often used when there is a chance of an immune reaction, because the immune response in primates may be better characterized than in dogs, and the target or sequence homology data may not be attainable for dogs. The extent to which this argument is valid is unclear, but there are indications that even a strong immune response does not have to be a problem for studies that only take 28 days. The immune response is not always most profound in the highest group, and with an increase of dosing, it may be overruled.

It is hard to predict whether an immune response will be found in primates, and this does not predict whether an immune response in humans will occur: this is also reported in ICH S6. Nonetheless, primates are often used to interpret the likelihood of a human immune reaction. One respondent contends that it is likely that humans will have an immune reaction to the product if primates also mount an intense immune response. Furthermore, if primates do not response with an immune reaction, humans are not likely to do so either. However, since most products are in between in the grey area, it is hard to interpret how to translate these results to the human situation.

The smaller size of primates was also mentioned in literature. Since biotechnology products are quite expensive, a smaller animal would be cheaper to use. According to Smith [2001], 30% of marmoset-users found that the smaller size of the marmoset compared to the dog, was a reason to use the primate model. Some respondents

also agreed with this, but in reality it was found that marmosets are not commonly used. The same argument is made for other monkeys, such as the cynomolgus monkey, given that they are also smaller than dogs.

Tradition

Scientific tradition and primates' close relationship to humans were also reported as arguments in the literature. In Smith's [2001] study, 30% of marmoset-users believed that the marmoset was an appropriate model since it is closer to humans than dogs, and thus would predict the safety of compounds in humans better. In case studies, these arguments were not used in so many words, but one respondent confirmed that the existence of large amounts of background data was a reason for using cynomolgus monkeys. Furthermore, the finding that not too much effort seems to have been paid to species selection in the second case study confirms these grounds. The primate was simply thought to be the 'best model'. This is an intuitive argument, which is based on tradition and evidence of close relationship, but it is not necessarily true. Nor does it justify the choice of primates as a species.

Financial

Financial or commercial grounds for using primates were not given directly in case studies, although these arguments clearly played an indirect role. The use of primates itself has been deemed 'expensive and burdensome'. Indeed, such financial reasons are good reasons to argue against their use. However, if no primates were used, more clinical trials would have to be conducted. Or, as one respondent put it, "at the moment 1 out of 10 compounds make it to the market. When primates could not be used, maybe 20 or 30 substances must be developed before one makes it to the market. This will make the end product immensely expensive; so either we go bankrupt, or medicinal products grow unaffordable."

Time was also used as an argument for primate use, mainly by respondents: every day of delay in the development process can cost $ 1million, which greatly exceeds the costs of using primates. Although the industry does not admit it in so many words, time does indeed seem to be an issue, at least for some companies. As other arguments were not always convincing, it was thought that time was one of the major drivers in the decision to use primates for products in case study 2. Whether this is representative for the whole field should be investigated further.

Time is also a reason not to develop alternatives. Respondents argued that every primate experiment differs from the other in preclinical development, and it would be no point in developing an alternative for each of them. This is simply too expensive and time-consuming to be of interest to the pharmaceutical industry.

CROs have other financial reasons for using primates, but no respondents from the pharmaceutical companies said they had experience with CROs stimulating the use of primates out of commercial interest. The possibility was, however, recognized by one respondent, but on different grounds: CROs are not as close to the regulatory bodies as pharmaceutical companies, which means that they tend to take the safer route. Companies may be more aware of the extent to which they can argue with the FDA than the CROs, which just follow the rules. Indeed, the client relies on them to guide the product through the regulatory requirements.

In addition, some references from literature indicated that (non-European) CROs may drive the use of primates: Japan's largest CRO has entered a collaboration to develop specifically non-human primate models of human diseases for medical research and drug discovery [Anonymous, 2004], and an American CRO is specialized in primate models [Alpha Genesis Inc., 2006]. In both these examples it is not the right animal model that is sought for a experiment, but an experiment for an animal model. This not only lacks scientific arguments, but is also likely to increase the use of primates.

CROs may also play a role in the re-use of animals. According to one of the respondents, pharmaceutical companies may be reluctant to re-use animals that have first been used by another company, since there is a feeling

of distrust about what has been done with the animals. Therefore, companies request naïve animals, to be sure to get results that have not been influenced by unknown preceding experiments. This means that most animals at CROs will in fact only be used once and killed afterwards. It has been argued that if companies have their own animal facility, this may reduce the number of animals used, since they will be re-used more often. However, others think that the very availability of primates may increase their use as well. Perhaps both claims are correct, and the difference between using CROs or having one's own facilities is not too great.

Regulatory influence

There are some examples of questionable requirements that stimulate the use of primates. ICH S6, on the preclinical testing of biologicals, contains the following ambiguous sentences: "An animal species which does not express the desired epitope may still be of some relevance for assessing toxicity if comparable unintentional tissue cross-reactivity to humans is demonstrated. (...) Where it is not possible to use transgenic animal models or homologous proteins, it may still be prudent to assess some aspects of potential toxicity in a limited toxicity evaluation in a single species"

This implies that when no relevant species is available, tests may be done in some non-relevant species. This is contradictory to the text, which states that testing in non-relevant species is discouraged. One speaker at the NC3Rs workshop [NC3Rs, 2006] stressed that testing in a species not expressing the desired receptor, but showing some non-specific binding is useless. "Molecules do not act if they do not bind. However if you would find binding in a species that does not express an epitope for the compound you are studying, the FDA may require you to use that species in testing. This is a nonsense requirement, as the binding may be non-specific. These tests have very limited value, and so do these requirements."

Or, as another attendant testified, when the cell-bank gives no target effect and multiple species show off-target effects, the use of monkeys is probably required. It might be reasoned that this species will have the highest chance of showing biological activity.

Another section of ICH S6 states: "Some information on absorption, disposition and clearance in relevant animal models should be available prior to clinical studies in order to predict margins of safety based upon exposure and dose." Data on pharmacokinetics are thus required before the start of clinical trials, while elsewhere in the guidelines it is stated that the predictiveness of this information is not always apparent. This seems contradictory.

For instance, several studies on primates for monoclonal antibodies were found that reported the half-life of the antibody in the animal. However, PK data for MAb vary significantly in animal species and are of limited predictive value for humans, given that the half-life of a typical MAb in humans is known to be 28 days.

Another example: when an immune response is suspected in humans, the first clinical trials will be started at a very low dose. When primates have had an immune response, some response may occur in humans. As one of the respondents indicated, primates generally have some kind of immune reaction. Dose finding will, therefore, mainly be done in humans. It then seems irrelevant to request PK data from animal species, certainly when it is known that these differ from humans.

Further to this, there are some examples of regulations, the use of which was questioned by a speaker at the biologicals workshop [NC3Rs, 2006]: for instance the necessity of 28 days toxicity and 3 months toxicity studies for oncology products before being allowed to keep phase 1 patients on treatment. In industry the perspective is that 4-6wks data should be enough. In addition, according to a respondent, there is a trend that long-term toxicity studies are being more frequently requested by the FDA. Furthermore, the value of reproductive studies on primates has also been questioned: these animals are used when no cross-reactivity with rats and rabbits is found. However, have results ever been found that were not predicted?

Western pharmaceutical companies consider the Japanese regulatory agency to be the most stringent. Various respondents stated that they may require tests that we, in Europe and America, think are unnecessary. In

Japan, all products must be tested for long-term toxicity, even when the product is only intended for short term use. Moreover, if a product is not supposed to be used during pregnancy because of known risks for that class of compounds, the Japanese regulators will also require data to support this view, which means that reproductive tests will also have to be performed conducted unnecessarily. In addition, two studies have to be done for biologicals that are not considered useful in other regions. One is the assessment of tissue distribution and the other is single dose acute toxicity studies, both of which are required in primates.

These Japanese regulations also increase the use of animals in the rest of the world, since pharmaceutical companies also want to market their products in Japan. Respondents indicate that all tests will, therefore, be carried out in line with these Japanese guidelines.

The Japanese authorities, however, also require histopathology data. This means that human volunteers are not viable alternatives to the use of animals, since these data cannot be collected using humans. For example, when registering the pneumococcal vaccine Prevnar, which has been successfully used in over 30 million American children, the human epidemiological data were of no interest to Japanese regulatory authorities, who required new NHP data instead [Bailey, 2005].

Finally, the regulatory authorities also pose a problem with respect to the replacement of primates with alternative methods. One respondent observed that regulatory authorities tend to regard alternatives as extra methods, rather than as replacement methods for the use of primates; certainly as long as they have not been validated. In vitro assays are regarded as tests for a certain aspect of the complete test and they are therefore reluctant to replace the animal test with such an incomplete assay. According to respondents, the only chance of replacing or reducing the use of primates is if the FDA would become more open to alternate points of view.

However, both the literature and respondents reported that a problem with these findings on regulatory requirements is not these requirements themselves, but the perception of those who are the major drivers in the use of primates. Most of the findings here are based on interviews and are, therefore, also perceptions. So where does the problem lie: in the requirements or the perceptions?

Companies do what they think regulators want to. The use of non-human primates is regarded as the best way to secure regulatory approval [The Boyd Group, 2002c], and studies are designed to satisfy the most demanding regulator worldwide. There is a concern that the use of primates may have become more a matter of convention than a deliberate choice [Animal Procedures Committee, 2002]. Several respondents reported that there is a prevailing notion that it is impossible to get some medicines through the FDA without primate data.

Time

The time associated with gaining regulatory approval is even more important. Commercial pressures may make companies unwilling to take the risk of using an animal other than a primate, since the use of a primate is less likely to cost the industry time and money (and possibly other animals) in the long run [Animal Procedures Committee, 2002]. This was confirmed by a respondent who contended that monkey experiments are carried out quite early in the process of development as a 'proof of principle' to show the pharmacological activity of the substance, or primate experiments are conducted while phase I clinical trials have already started; all to save time.

Consequently, pharmaceutical companies are believed to maximize the number of animal experiments, because they would rather conduct animal experiments that turn out to be unnecessary than take the chance that new experiments are requested afterwards. Everything is calculated on the basis of the time remaining before the patent on the active compound expires, since this is when the company can start to recoup their investments. Even where it is believed that another animal model, or presumably a non-animal alternative, would be more appropriate, it would take more time (and money) to discuss this with the regulatory authorities than to perform the standard tests in what is perceived as 'the best model'.

5.7.4 Future developments

Use of primates

It has been acknowledged that the spin-off of developments in molecular genetics and biotechnology will have great impact on the future use of laboratory animals [Van Zutphen, 2000]. It has been said that the pharmaceutical industry's view is that its future lies in developing entirely new classes of neuroactive drugs to combat the increasingly important neurological diseases of old age [Animals Procedure Committee, 2002]. In fundamental research, it has already been expected that the use of primates might increase for this field, which apparently is to be expected in preclinical studies as well.

Due to an increase in the development of biologicals, the use of primates for preclinical tests could also increase over the years. Personalized medicine may lead to the development of more diverse and very species-specific products, thus increasing the use of primates, although it may also exclude testing on primates because of the specialization on personal circumstances, although the possible immune response would continue to pose a problem. The Human Genome Project may have a similar influence: more products may be developed, which may need to be tested on monkeys or even Great Apes, but it is also possible that greater understanding of the genome may yield better understanding of the mechanisms of actions of biotechnology-derived pharmaceuticals, and that less testing on animals would, therefore, occur. However, it was noted that growing consumer concerns about the safety of chemicals, coupled with an increasingly litigious society, may also create pressure to test substances more widely in animals before moving to humans, including the use of non-human primates [The Boyd Group, 2002c]

Alternatives

New techniques, such as genomics, proteomics and metabonomics, might be used to check for the most relevant animal model, which again might increase the use of primates, if it turns out that they are more relevant for products where the dog is currently chosen as the non-rodent species.

All respondents from the pharmaceutical world were highly pessimistic about the possibility of replacing the use of primates in the future. Most thought that, as long as animal experiments are deemed necessary, the use of primates will be part of this; rough estimates were that maybe in 20 to 30 years it might be possible to develop medicines without animal testing.

Regulations

Furthermore, one respondent reported that an increase of experience in biopharmaceuticals has lead to regulations becoming stricter for the preclinical data, which are required for these products. One may very well question the consequences for the primate data after the recent incident with TGN1412, a monoclonal antibody, which produced serious adverse effects in humans during a recent London trial. This product had been tested by 28-days repeated dose toxicity studies in cynomolgus monkeys and some PK studies in rhesus monkeys [MHRA, 2006]. These animal models clearly did not predict the severe human reaction to the product, although other factors in the preparation or dosing of the compound may also have played a role in the human reaction.

However, if the conclusion is finally drawn that these problems were due to differences between humans and primates, two options seem possible: either it is recognized that animal models, including primates, are not always representative for the human situation and alternative methods (using human material or microdose studies) are applied, or that apes should be used for these products instead of monkeys. Since regulations only tend to become stricter instead of more relaxed, it may be likely that the second option will be the case. Yet then again it will only be a matter of time before a product is tested in chimpanzees and still turns out to fail dramatically in humans.

At the same time, many respondents noticed that because the public discussion on the use of primates was increasing, regulators are now becoming more aware of unnecessary or irrelevant primate experiments and inclined

to say something about it. Furthermore, the use of primates is becoming increasingly expensive as a result of housing regulations and need for security due to threats and attacks.

Contract Research Organizations

An increase in regulations and costs is also likely to increase the interest in using Contract Research Organizations (CROs), notably the ones that are located outside of Europe. Some companies are already moving their tests abroad, mainly outsourcing them to countries to outside Europe. China is eager for biotech companies to use CROs or for them to build their own facilities in China. On its website, a Chinese CRO advertises "high quality pre-clinical research services with very competitive cost and virtually no waiting period for our clients" and "the high cost and long waiting period for studies using non-human primates has been a significant barrier to many drug development programmes" [Starvax Inc., 2005]. Even an American CRO is now moving to China, building his own centre there because of the costs [Levine, 2004].

According to one respondent, who agreed that the scientific quality of Asian CROs would currently meet international standards, more companies will move their testing sites once the differences in time and money associated with these are great enough. In general, it is acknowledged that this will have a negative effect on the use of animals and their welfare. Or, as one respondent put it, for a monkey in research it does not matter where he is killed, but how he is treated during life.

5.7.5 Solutions

It will be difficult to solve the scientific problems quickly. Although the argument of the immune reaction has be proved to not always be relevant, the lack of knowledge of the immune system of other species and an absence of reagents for them leads to primates being selected as the preferred species. This can be solved by an increase of research into immunology, not only for humans but trying to translate this to in vitro models as well. The development of reagents for other animal models could reduce the use of primates, but would be accompanied by an increase in the use of other animals.

Other solutions are based on influencing regulatory requirements. According to both the respondents and literature, scientists and companies should come together to discuss safety issues, how much one can base on technology and the predictive power of the milder toxic symptoms [The Boyd Group, 2002c]. Pharmaceutical companies should, therefore, join forces: combined forces and experience could counteract the power of regulators, who request primate use while this is not necessary.

However, it is not only regulations that pose a problem, but also the perception of regulations. Pharmaceutical companies themselves decide what tests they will conduct on which animals .Regulatory authorities offer the possibility of asking for 'scientific advice', but according to one respondent, in Europe this is almost always only done for the clinical trials, not for the initial animal experiments. The case studies revealed that large differences between companies exist: where one (US-based) company reported that they will always ask for advice with regard to their last animal experiments before obtaining an IND, the other did not even think that this was possible.

One respondent therefore proposed a solution to improve communication between regulators and pharmaceutical companies, while preventing unnecessary primate use. For primate experiments, it would be made compulsory to ask for scientific advice. However, this respondent also pointed out that regulatory authorities would not be too keen on this because scientific advice in name is not binding, but in practice this advice makes the regulatory authorities morally responsible. Imagine if something happens after registration, then the consumer will turn to the authority to ask why no experiments had been conducted on monkeys.

The pharmaceutical industry's reaction to this proposal varied, some being enthusiastic and recognizing that scientific advice might well be helpful to prevent studies from being replicated or to improve regulatory guidelines, which could help to reduce the number of primates used.

However, if a committee was to be asked for scientific advice, it should have knowledge of practically all compounds that had been worked on worldwide; it would not be helpful if it was just a national or continental authority. Furthermore, the respondents indicated that a negative scientific advice from European authorities would be ignored by the FDA or maybe even aggravate them, or drive companies outside Europe. In addition, the right people should sit on such a committee. If it consisted mainly of people who favour primate experiments or fear any unforeseen effects, it might even have an adverse effect. How this should be prevented is an issue that remains to be solved. Finally, the administrative burden to companies should not be increased too much. It might be an option to combine this scientific advice with the ethical approval for which companies also have to apply; by this means, there should not have to be an extra delay in the process.

Chapter 6

Routine safety evaluations

6.1 Introduction

Even after registration, tests have to be carried out for certain immunological medicines (vaccines in particular) and for stable blood products to ensure that every production cycle has the same characteristics as originally tested. This independent check of every batch produced is compulsory by law, because biological medicines are complex and variable and the original material and/or the product may be infected with viruses. There may be serious consequences if a defective batch was distributed for use [RIVM, 2006].

Many of these consistency tests can be done by modern biochemical techniques, but sometimes animal tests are requested, most notably for the potency and safety testing of vaccines. Since these tests have to be performed on a routine basis on every batch of the product, the use of animals can be extensive. For vaccine batch release testing, this has been estimated at 10% of the total use of animals in biomedical research [Hendriksen and Gupta, 2000].

Regulations

European legislation for the routine safety evaluation of medicinal products has been laid down in the 'European Pharmacopoeia' (Ph. Eur.), which provides detailed descriptions of the tests that have to be carried out. The United States have their own requirements, which have been set down by the US Food and Drug Administration (FDA) in the 'Code of Federal Register' (CFR).

The use of primates is sometimes demanded by these regulations. According to information from an antivivisection organization, the overwhelming majority of primates used in scientific procedures and for medical purposes is for the development and testing of vaccines [BUAV]. Information from the EU is even more specific, pointing out that primates are used on a large scale in Europe for the production and quality control of polio vaccine, specifically the attenuated oral vaccine [Anonymous, 1997]. The regulatory safety testing of polio vaccine will, therefore, be examined in greater detail in a case study. Background information on polio can be found in Textbox 6.1.

6.2 Case study: OPV

6.2.1 OPV background

IPV vs. OPV

There are currently two types of polio vaccines. The inactivated polio vaccine (IPV), which comprises an inactivated wild-type virus, is an injectable vaccine developed by Jonas Salk. In contrast, Albert Sabin's vaccine is an oral poliomyelitis vaccine that consists of attenuated viral strains: OPV.

Both types of vaccines have their advantages and disadvantages. The two main differences between the vaccines are the working mechanism and the mode of application. IPV provides antibodies that circulate in the blood.

This means that polioviruses can still (though in lesser extent) colonize the intestines, and be excreted with faeces. IPV thus provides individual protection of vaccinated subjects.

OPV is made using live, attenuated strains of the virus. This helps to produce antibodies in the intestine that prevent the infection itself, stopping the spread of the virus. The attenuated virus in the vaccine multiplies in the digestive tract and leaves the body through the stools. People who live around the vaccinated children will also be infected by vaccine virus and will thus be vaccinated indirectly. The drawback is that this attenuated virus may revert to a pathogenic form, causing new cases of polio.

Another difference between IPV and OPV is the mode of application. As OPV is given orally, it is easy to administer and practical for use during immunization campaigns in developing countries: it can be given by virtually anyone. IPV not only requires a needle and syringe, but also specialized training in order to assure safe administration.

Furthermore, there are also costs associated with both vaccines. OPV is cheaper to produce and administer, but the combination of IPV in other injectable vaccines does not result in any major additional costs. This, however, is only effective when these vaccines are already given on a routine basis (All preceding information from [Sanofi Pasteur SA, 2005]).

Virtually all developing countries use OPV to protect children against polio. However, in order to eradicate poliomyelitis, not only wild type viruses but viruses derived from OPV must be eradicated as well. In 2002, 178 out of 212 countries used only OPV; together the vast majority of global annual births (122,7 out of 131,8 million). Continued routine vaccination with OPV could in fact compromise the complete eradication of poliomyelitis. In this context, the Inactivated Polio Vaccine (IPV) has an important role when eradication of the wild type virus has been pronounced. This is to prevent circulation of oral vaccine-derived viruses and wild viruses that could reappear. With the global progress towards polio eradication, many developed countries have switched from routine OPV immunization to IPV [Sanofi Pasteur SA, 2005].

Textbox 6.1 Polio and polio vaccination

Polio (fully 'poliomyelitis') is caused by poliovirus, of which humans are the only natural hosts. Three serotypes of poliovirus are known: 1, 2 and 3. The poliovirus is transmitted only from one person to another, principally by contact with feces-contaminated water or food. It enters the body, reaches the lymph nodes and enters the bloodstream, from where it can reach the spinal cord and effect motor neurons, causing paralysis. The virus is excreted in the stools for three to six weeks, by which means others can get infected.

In some 70% of cases, the virus does not manage to penetrate the intestinal barrier since the body produces protective antibodies, and infection is thus not apparent. In approximately one quarter of cases, the infection results in non-specific symptoms and clears up in less than one week. The destruction of motor neurons occurs in less than 1% of cases.

It has been estimated that on average only one of every 200 infections results in paralysis. This means that poliomyelitis virus may circulate silently for many months or years from patient to patient before a case of paralysis reveals the existence of the virus. No therapy is known, and vaccination is the only available method of prophylaxis.

Thanks to massive vaccination, eradication of polio can be achieved. In 1988, polio was endemic in 125 countries and more than 350,000 cases of poliomyelitis were reported. The last case caused by a type 2 wild virus was recorded in October 1999 and this type may already be eradicated. In 2003, only 6 endemic countries were left, with only 784 cases being reported. Despite the WHO's (World Health Organization) striving for complete eradication, cases of polio were reported from 11 countries in 2005 that were thought to be polio-free in the preceding year.

[Sanofi Pasteur SA, 2005]

Manufacturers

GlaxoSmithKline (GSK), located in Belgium, is the world's largest supplier of polio vaccines to developing countries with a production of 1 billion vaccine doses per year [GSK, 2001]. Two other suppliers are also located in Europe and several in Asia.

6.2.2 Production

Process

The production sequence of any vaccine is roughly as follows: first a (attenuated) pathogen strain is selected and long-term stored. This is the prime source of virus from which a vaccine is produced; for polio this is stored centrally. Taking a little bit from this master seed, the virus is multiplied and another homogenous series of vaccine virus is produced, which is called the working seed. Small amounts of virus are taken from this working seed lot in order to produce the actual vaccine against polio. This yields a 'batch' or 'harvest' of micro-organisms [Osburn et al., 1996].

Since bits are taken from the master and working seed lot, these need to be renewed over time. With regard to OPV, one manufacturer reported that a new working seed lot is needed about every five years and a new master seed lot needs to be produced more or less every 20 years.

Substrate

Polio viruses are grown in primate or human cells in the test tube. Three types of cell substrate may be used: primary monkey kidney cells, human diploid cells (MRC-5 or WI38) or a continuous cell line derived from African Green Monkey kidney cells: Vero cells [Wood and Macadam, 1997].

Around the world, various companies use different approaches. It is possible that the majority of companies use monkey kidney cells, which are obtained by killing monkeys and removing their kidneys. With regard to the OPV-manufacturers listed by the WHO (World Health Organisation), it was found that all non-European manufacturers used these primary cells [Haffkine Biopharmaceutical Corporation Ltd; Panacea Biotech, 2006; PT Bio Farma (Persero), 2002]. GSK reported to have stopped using primary cells in 2002 [GSK, 2001], while another European manufacturer has been using Vero cells since 1982 [Langley, 2002].

Respondent argued that the scientific advantage of using immortalized Vero cells or human cells is that they are well-characterized, well-controlled and yield a stable substrate for stable production. Once a cell-bank has been established, these cells can be used for every next production cycle and need not be re-tested for safety of the cells, as is the case with primary monkey tissues [Langley, 2002]. Ultimately, they are even cheaper.

The disadvantage is that the virus that is yielded from long-lived cells is not as good as the one obtained from primary monkey cells. Consequently, the product is more dilute and the cost of the vaccine increases, which also means that more monkeys will be used in the neurovirulence tests (NVT) on each vaccine batch [Langley, 2002]. A respondent confirmed that the yield is somewhat lower when cultured cells are used. It was said that companies compensate for this by increasing the volume of vaccine produced.

Safety

The production of polio is very delicate. During production, certain factors are essential to prevent the virus from mutating back to the neurovirulent form. Temperature and the duration of incubation are the most crucial parameters; cultures must be incubated at a constant temperature in the range of 33-35 °C and the virus must be harvested no later than 96 hour after infection [Wood and Macadam, 1997].

According to a respondent, the choice of a seed is another factor that influences the safety. When either the master seed or the working seed reverts to a more neurovirulent form, then so will all the vaccine that has been produced from it. As a consequence, the first four batches of a new seed lot are tested to confirm that they have not

reverted to a neurovirulent form. However, if a well-standardized production system is used, it is not expected that any working seed lot will mutate back [EDQM, 2004].

6.2.3 Monkey neurovirulence test

When OPV was licensed in 1962, the neurovirulence of the preparation was tested in a different way than it is today. Moreover, several test methods were used throughout the world. The use of monkeys dates back to the days of Sabin. When developing his vaccine, he used several passages of inoculations in the central nervous system of monkeys. In 1980, a collaborative study to evaluate different ways to assess residual neurovirulence of oral polio vaccine was set up under WHO auspices. This eventually led to a test prescribed by the WHO, which has since been adopted by all OPV-using countries in the world.

Alongside these WHO requirements, the test has been described in detail in several other regulations, including the European Pharmacopeia (Phar. Eur.) [EDQM, 2004, 2.6.19]. Until the mid 1990s, the US Code of Federal Regulations (CFR) also contained detailed prescriptions on vaccine production, similar to what is done in Europe today. However, they now serve as a guideline, and detailed prescriptions are contained in the license that is issued by the FDA to the manufacturer. The CFR on OPV was withdrawn when the US switched from using OPV to IPV in 2001.

The WHO and Phar. Eur. specifications of the monkey neurovirulence test (monkey NVT) do not differ much from each other, so they will here be discussed concurrently.

Procedure

In the monkey NVT, the preparation is injected in the anterior horns of the lumbar part of the spinal cord of a group of Old World (Macaca or Cercopithecus) monkeys. At the same time, a different group with the same number of monkeys is injected with an international standard reference preparation of the same virus strain. Depending on the neurovirulence, the virus climbs further up the spinal cord. The animals are observed for 17 to 22 days for signs of poliomyelitis and are then killed. A specified number of slides is made at specified levels of the spinal cord up to the brain.

These slides are microscopically examined for lesions, during which assessment scores for the histological signs of damage are given. For instance, a '2' is assigned when there is 'some neuronal lesion' and a 3 when there is 'more neuronal damage' and significant inflammation. According to respondents, this is a relatively subjective process: when is a lesion small and when is one 'more extensive'? The slides are therefore sent to the national control laboratory (NCL) for independent reading after the manufacturer has examined them. In practice, it appears that various experts generate quite similar results.

The results from the monkeys in each group are then combined, which yields an average mean histological lesion score. According to the statistical criteria, the comparison of this result with the score for the reference preparation leads to an acceptance or rejection of the vaccine.

Probability curves demonstrate that there is a 99% chance of rejecting a monovalent bulk with just a two-fold increase in virulence compared to the reference, whereas there is a 1% chance of rejecting a batch that is not significantly different from the reference [Wood and Macadam, 1997].

According to all respondents, the use of a concurrent reference is an absolutely necessity, given the very principle of a biological assay. Monkeys may differ in their reaction due to biological differences between the individuals. Without a positive control of the reference test, one does not know whether one's test was sensitive enough or how to interpret the results.

Numbers

According to the respondents, the number of monkeys involved in the NVT was determined after a detailed statistical analysis in the 1970's during the development of the current WHO requirements. At least 11 positive

monkeys are needed for type 1 and 2, and 18 for type 3 strain of poliovirus, given that this is the strain with the highest variability in results. Several additional monkeys are inoculated for all three strains because some animals may turn out to be invalid. Positive monkeys are those that exhibit specific neuronal lesions of poliovirus in the central nervous system [EDQM, 2004, 2]. When no needle track is visible on the slides, the inoculum was injected improperly. WHO requirements state that 12 or 20 monkeys usually have to be inoculated in order to get positive results. Respondents indicate that 12-15 animals are used for type 1 or 2, and some 24 monkeys for type 3.

Thus about 13 primates are needed for the neurovirulence test of type 1, plus 13 primates for the concurrent reference test. For type 2, the equivalent number (26) of primates are needed for every batch. Type 3 necessitates the use of 48 primates per batch.

The extent of the worldwide use of primates for this purpose is not known, although it has been estimated that more monkeys are used for this test than for any other single biomedical purpose [Hendriksen, 2002]. Using different calculations (see Appendix 7), it has been determined that probably between 4000 and 5000 primates are used every year for this test worldwide.

Alternatives

Alternatives to the neurovirulence test are given in all regulations. An in vitro test for Sabin poliovirus type 3 has been validated, which is highly predictive of in vivo neurovirulence. This 'Mutant Analysis by PCR and Restriction Enzyme Cleavage' (MAPREC) assay shows specific mutations that make a strain of poliovirus more neurovirulent and is indicative for inconsistency in the production process. Another alternative is the use of transgenic mice. This method will be discussed in the first section, MAPREC will be the topic of section 6.2.9.

6.2.4 Reduction opportunities for the monkey neurovirulence test

One may question why the numbers of primates used for the neurovirulence test cannot be reduced. What about the number of monkeys in each test? According to respondents, there is not much reduction possible there. The primates are not inbred animals and react differently, probably because of differences in their genetic make-up. The results are said to be extremely variable. For type 3 polio vaccine, the strain with the highest variability, it is not extraordinary when the standard deviation of the group results exceed the mean. The number of monkeys needed has been based on statistics in which the results of thousands of monkeys have been analyzed. Using fewer animals would produce statistically questionable results and render the test useless.

The use of cell bank systems instead of primary monkey cells and other modern improvements in production process might have decreased variance. With less variance, fewer monkeys would be necessary in order to obtain reliable results. According to one respondent, European companies have indeed improved their technique greatly, with test rates better than they have been. However, for other places, the precisely the opposite has occurred. Another respondent noted that the need for testing well-controllable cell-line-derived versus monkey-derived vaccine has never been discussed. However, one regulator did not regard the use of changing regulations as a requisite for obtaining a certain statistic instead of absolute numbers; he deemed the requirement of numbers to be one of the advantages of the current regulations. According to this respondent, European manufacturers are very good in critically reviewing the data in order to assess how many monkeys are needed. In other countries, they just have a routine of using 15 or 16 animals in order to get the results accepted and not having to complete the whole test.

What can be done in order to prevent the whole test from being repeated, according to this respondent, is a top-up of animals. Sometimes one finds 10 or 9 positive animals. This is said to be a valid test, technically. Instead of repeating the whole test with 13 new animals, what is done is a top-up of 3 or 4 extra animals in order to get the required results.

Valid results

According to all respondents, the key factor in obtaining valid results is the injector's competence. The injection is a delicate operation: it requires fine skills to get the preparation into the correct motor neurons without doing damage. The number of animals that turn out to be invalid therefore depends on the experience of the technician performing the injection. Only a few operators are qualified: at one important manufacturer, only two people have been trained to perform the injection. The number of primates needed can differ between companies. Number of 85 to 110 animals have been reported by different manufacturers to be needed for the three strains together [Langley, 2002]. However, the extra training of technicians would require the use monkeys, which has been deemed unacceptable.

Reference testing

The testing of reference vaccine in parallel - in the same number of monkeys on the same days - can also be called into question. In several countries, the test was (sometimes) conducted without concurrent reference, as indicated by this passage from the revoked US CFR regulations §630.16(b) (5): Alternative procedures in case of monkey shortage: 'In the event of a shortage of test monkeys and upon approval of the Director, Centre of Biologics Evaluation and Research, a monovalent virus pool may be tested without concurrent testing of the corresponding type Reference Attenuated Poliovirus. In such a case, the magnitude of the neuropathology of the monovalent virus pool shall be compared with the magnitude of the neuropathology exhibited in all previous tests of the corresponding Reference Attenuated Poliovirus' [WHO, 2002a]. However, none of the respondents were in favour of such approach.

What one manufacturer does is to the accumulate data for reference tests. The results from a tested batch are not only checked against the reference, but also against the accumulated data of preceding references. Furthermore, two sets of monovalent vaccine are tested concurrently against one reference. According to the respondent, it is not possible to extend this approach and test more batches at once, because of logistic problems. Firstly, there are human limitations: only a certain number of monkeys can be injected on any one day, and regulations oblige that all groups of monkeys are injected within two days. Secondly, there is not enough space to store three batches before testing them, nor to house all the primates that would be necessary for the test procedure.

In conclusion, all respondents confirmed that the discussion should not focus on an eventual reduction of the number of monkeys, but on the necessity of using monkeys at all.

6.2.5 Problems and arguments monkey neurovirulence test

Relevance

The first issue with respect to the use of monkeys for neurovirulence testing is whether it models the way humans become paralyzed as a result of polio. After all, humans become infected after oral intake, which causes paralysis only in 2% of cases, while monkeys are infected via direct intraspinal inoculation. Respondents and the literature concurred that this is an artificial route, which may have nothing to do with the way in which humans are infected naturally [Wood and Macadam, 1997]. Furthermore, the exact reason why polio sometimes causes paralysis is still unknown. The distribution of lesions in the central nervous system can be virtually the same in paralytic cases and non-paralytic cases, but the severity of injuries depends on the neuropathogenicity of the infecting virus [Mueller et al., 2005]. This is an indication that the lesions observed in monkeys are correlated with human paralysis.

The main reason why this test is done is to ensure that the vaccine still has the same characteristics as the Sabin's strains did. The assumption is that virulence in monkeys is an exaggerated test of virulence in humans infected by the oral route. The demonstrable safety of Sabin's strains in humans supports this assumption, as well as the increased neurovirulence of monkeys with respect to revertant strains [Wood and Macadam, 1997].

Even when the monkey NVT has served well in avoiding cases of polio after infection, this still does not prove that it would be necessary to carry out the test. Indeed, no batch of types 1 or 2 vaccine has ever consistently failed

the monkey test [Langley, 2002]. A respondent explained that failed batches have been looked for when evaluating the factors that influence neurovirulence. Four batches of OPV type 1 that failed the test were found, but on re-evaluating these original batches, they passed. Since the test is deliberately designed in such a way that 1% will be tested false-positive, four false-positive samples might be a reasonable number to expect out of the few hundred batches that have been produced throughout history.

Over a 12-year period, 81 batches of type 1 and 34 type 2 batches would have passed the test without any problems. The only serotype that ever failed the test was type 3. About two dozen failing batches around the world were found that indeed failed again when the test was reproduced. In literature, 4 failing batches out of 89 were mentioned for type three, but manufacturers have switched to a seed that is less neurovirulent in monkeys. [Wood and Macadam, 1997]. Respondents have had the same experience: a routinely produced vaccine never fails the monkey NVT, though laboratories from outside Europe are reported to have failed more often than European manufacturers.

Re-testing

According to a respondent, the test was performed twice in the US: both by the manufacturer and the NCL. However, since the US stopped using OPV in 2001, this is no longer an issue. There are indications that the same was happening in the UK for OPV that had been produced and tested outside Britain, although others claimed that these animal facilities have now closed down. The reason OPV was tested twice in the US was that Dr. Sabin required it; he said that he would not be comfortable when it was tested solely by the pharmaceutical company. For the UK, similar anxieties have undoubtedly also played a role.

Reasons

Hence, the question is why this test is carried out at all when a vaccine (almost) never fails the test. Several respondents argued that one needs some reassurance that the quality of the product is fine, and if one did not carry out the tests, one simply would not know. The reason the test is still performed is, therefore, for reassurance and historical reasons. A vaccine is just a red liquid and before one gives it to newborn babies, one will need some kind of reassurance that it is a similar liquid to the one that was produced before, and similar to the one that was tested in clinical trials and originally licensed by Sabin.

Then again, testing for neurovirulence is also no guarantee for safety. This has also been stressed by some publications and many respondents: vaccine, which passed the NVT, has resulted in vaccine-associated cases of polio. In addition, one of the respondents gave an example of a vaccine that was proposed by Hilary Koprowsky in the 1960s that had a much superior ability and lower neurovirulence in monkeys compared to Sabin strain. Everybody expected that it would be safer in humans. However, when it was tested in clinical trials, there were several hundred cases of paralysis. While it was perfect in both laboratory tests and monkeys, it had devastating effects in humans. Another respondent gave an example of a case in the past, when some batches of OPV made it onto the market when they should not have been released. More cases of polio were subsequently reported. This would indicate a correlation between the occurrence of polio and the results of the monkey test. However, this correlation simply cannot be validated. At present, exactly what causes neurovirulence remains unknown.

Another reason why the NVT is conducted is the risk associated to both companies and regulators. Or, as one respondent put it: if one stopped testing, and if for any reason some people developed polio after immunization, who would want to take the responsibility? No company has ever abandoned these tests, because there is a very rare number of adverse reactions to the vaccine. About one person per every million doses suffers paralysis. However, if the company ceases testing and the next time there is a case of a reverse reaction, there is a risk that they could be sued. No company will, therefore, even contemplate dropping the test, simply for such legal considerations.

Companies, however, argue that they conduct these tests since they are required to do so by regulations. They cannot drop any test at their will without first obtaining permission from regulatory authorities. Nonetheless, these regulators are concerned about the consequences of the distribution of a neurovirulent vaccine: not only for the people involved, but for the whole vaccination programme. When people become concerned about the quality of the vaccine, this has led to populations choosing not to take the vaccine. This concern makes them reluctant to change regulations with respect to the NVT: all countries know that this is a key test, and changes may lead to difficulties with acceptance of the test results in some poorer countries where the regulators are not as sophisticated as in Europe.

For these reasons, it is argued that some comparative test is needed and the monkey test was all there was for a long time. Many respondents stress that it was informative and served a useful purpose when there were no alternatives, but now we do. Many respondents therefore question whether there still is a need for in vivo neurovirulence testing at all.

6.2.6 Alternative: transgenic mouse

The transgenic mouse model was developed in 1990 [Ren et al.]. The mouse model has been engineered to express the human poliovirus receptor, which normally is only present on human and primate cells [Wood and Macadam, 1997]. These transgenic mice, however, can be infected with each of the poliovirus serotypes by various routes and develop clinical signs and morphological lesions in the central nervous system similar to those observed in primates.

According to one respondent and a reference, the mice need to be inoculated intraspinally in two dilutions. The majority of mice then develop flaccid paralysis, unlike monkeys, and a clinical dose response occurs. The dilutions are chosen in such a way that, in general, in both groups between 10 and 60% of the animals shows signs of paralysis. Sometimes this is one limb, sometimes 2, but maybe 3 or 4.These symptoms can be scored by observing the symptoms of paralysis after two weeks. The number of animals with symptoms is counted and the severity of paralysis is scored, and again these scores are compared with the scores of a reference preparation. Using a modified histological scoring system is also possible. [Wood and Macadam, 1997].

Respondents seemed to contradict each other with regard to the number of mice needed in this test: one contended that 20 animals per group are sufficient, while WHO requirements impose the use of 32 mice per group [WHO, 2002b]. The background of this contradiction remains unsolved; it would seem logical when less mice were used per group than is the case for monkeys, since these transgenic mice are inbred animals and thus should show less variance.

Regulations

The WHO initiated a validation study on the use of transgenic mice as a replacement alternative for the monkey NVT, initially restricted to poliovirus type 3, which historically is the most difficult strain to control [Wood and Macadam, 1997]. The transgenic mouse model was accepted in 1999 as an alternative for this strain [Langley, 2002]. Moreover, in 2003 a study was published which reported that this alternative had been validated for all three strains [Dragunsky et al., 2003].

The European Pharmacopoeia describes it as 'a suitable alternative to the monkey neurovirulence test for neurovirulence testing of types 1, 2 or 3 vaccines (...)' [EDQM, 2004]. In WHO requirements, it is only allowed for type 3 poliovirus and being evaluated for type 1 and 2 [WHO, 2002b]. Recent WHO documents [2005], however, indicate that the mouse NVT has been approved as an alternative to the monkey test for all three OPV types.

Why the mouse test has not been accepted for types 1 and 2 yet, remains unclear. The WHO suggests that some revisions may take place next year, since data are currently being accumulated to make absolutely sure that the test will yield the same results. Extra accumulation of data is necessary because validation studies have not been done

under all the different circumstances and only on the routine basis. One respondent compared this to registering a new vaccine: one can do a phase three study, but it is only after phase four that one is really confident that it actually works. One respondent reported that currently the validation can be completely done for type 2 and is well underway for type 1, although a manufacturer said that they have already applied for registration for type 1, and type 2 will be the last one to become implemented. Results are said to be satisfactory for all three strains.

'To avoid confusion' it was decided that the test in simians will remain the gold standard for evaluating the neurovirulence of OPV, and should be used to validate new virus seed lots or changes in the manufacturing process [Dragunsky et al., 2003; WHO, 2005]. According to respondents, this is because of all the historical experience accumulated over the years. In some cases, one wants to be absolutely sure that the test being conducted is the best way to characterize the vaccine: for new seed lots or if there are any questions on the interpretation of test results.

The mouse test may only be used 'once a laboratory qualifies as being competent to perform the test and the experience gained is to the satisfaction of the competent authority' [EDQM, 2004]. A standard implementation process must be completed before laboratories are qualified. According to respondents, this is done because the injection technique is very delicate, even more difficult than in the somewhat larger monkey spinal cord [Dragunsky et al., 2003]. This might be called a disadvantage to the model, though in practice everybody who tried has succeeded in this part of the implementation process.

Progress

In general, progress to implement the transgenic mouse test has been very slow. One manufacturer, which had been involved in the validation study for type three, is still not allowed to use this test as an alternative for the monkey test: they are waiting for national authorization. According to a respondent, the review of a registration file to be allowed to use the alternative can take up to a year. This is regarded as a very long period of time, although since it is recognized that regulators want to be sure, and that it takes time to answer all legal questions. It makes sense, but at the same time the respondent thinks that it could have been done much sooner.

It is not known why it takes so long. One respondent contended that the manufacturer involved would have started using mice instead of monkeys from 1st January, but this clearly is not the case. In addition, it was mentioned that two other manufacturers would switch to the mouse test in 2006, while another respondent indicated that these manufacturers would not even have completed the implementation process. Only one manufacturer would have, as well as 3 out of 4 National Control Laboratories.

6.2.7 Problems and arguments transgenic mice alternative

Advantages

According to all respondents, the mouse test has only advantages, at least for the Western world. The main advantage reported is the fact that mice are not monkeys: although it is recognized that some people may think differently about this, in general primates are considered to be 'more valuable' than mice.

The financial consideration is also important because monkeys are much more expensive and harder to obtain. This, however, applies only to the Western world, since in some other countries wild monkeys are abundantly available and even considered vermin. Furthermore, the availability of transgenic mice is not unlimited either, certainly not in non-European countries. According to a respondent, these manufacturers will probably continue to test on monkeys.

The mouse test is also quicker than the monkey NVTl, which is another advantage noted by both the respondents and the literature: the mouse test takes 2 weeks as opposed to the 1.5-2 months needed for the monkey test [Dragunsky et al., 2003].Moreover, it does not require a highly qualified pathologist.

Most important, perhaps, is the fact that it is as safe as the monkey test. According to one respondent, the mouse test would not be used if it was even slightly less safe than the monkey NVT. Thus, as one respondent concluded: on any count the transgenic mice are better than monkeys.

Disadvantages

There are, however, disadvantages with respect to transgenic mice. The implementation procedure was viewed as a hassle, certainly given that the test has not been validated for all three serotypes and monkeys will remain necessary. Furthermore, ethical concerns are also not eliminated, especially since transgenic mice are required and the number of animals needed is higher than for monkeys [WHO, 2005].

There may also be problems relating to controlling the results. For the monkey NVT, the slides that were made by the manufacturer, were checked by the NCL. Respondents also say the opposite with respect to whether this is possible for the transgenic mouse test: one argues that this is not possible given that symptoms of paralysis are scored. Another respondents and literature references state that histological slides of the mouse spinal cord could be made, but that this yields no additional information and is not easy; it requires greater effort and makes the procedure more expensive and lengthy [Dragunsky et al., 2003; Wood and Macadam, 1997].

Two other options are also possible: either the NCL comes to the manufacturer to do an independent scoring, or they repeat the complete test themselves. One respondent suggested that both methods are currently being used: in a random 10% of cases, the NCL repeats the test, next to an independent scoring of the symptoms. The reason why this is done is not quite clear, though it has been said that gaining experience by the NCL could be a motive. Another respondent thinks that there is no strong scientific justification for carrying out the test twice.

Problems

What then are the reasons why this model is still not used? For type 1 and type 2, this remains unclear. One of the problems could be the availability of good and bad vaccines, which are practically unavailable for types 1 and 2 since they never fail the monkey test. In addition, according to one respondent, shifting to the mouse test would also necessitate the use of the new molecular methods MAPREC. Another respondent explained that it always takes a couple of years to go through a period of validation with manufacturers and their national authorities. In this period, both methods will have to be used next to each to give statistical evidence that the new test is just as good.

6.2.8 Alternative: MAPREC

In 1991, a paper was published that correlated the neurovirulence of type 3 OPV in the monkey test with mutations in the viral genome [Chumakov et al.]. This has been the basis for a new alternative, completely based on molecular biology: Mutant Analysis by Polymerase chain reaction and Restriction Enzyme Cleavage (MAPREC). This assay detects and quantifies mutations in the genetic material of the virus, which are correlated with higher neurovirulence in monkeys.

In the test, this segment of DNA is amplified and then treated by a restriction enzyme, which will only cut the strain of DNA when the mutation is present. After electrophoresis one thus observes complete DNA molecules and maybe a shorter segment that has been digested by the enzyme, which is the proportion of neurovirulent virions.

Regulations

MAPREC has been accepted by the World Health Organization since 1999 as a method of ensuring consistency of polio vaccine production, but only for type 3 [WHO, 2002b]. In 1999 the WHO Expert Committee also reported that MAPREC has been established for type 3 vaccine and is being widely used by both manufacturers and national control authorities [Langley, 2002]. It is, however, not deemed to be replacement for the monkey test: any

type 3 vaccine batch that fails MAPREC should not be tested further in monkeys, but if the vaccine is negative this must still be confirmed in a monkey NVT.

If MAPREC is also to be accepted for types 1 and 2, this test will replace the current in vitro test, which is the rct40 test: Replicating Capacity Temperature 40 °C. This test measures the sensitivity of poliovirus to grow at high temperatures. Only the more virulent virus is able to grow at 40 degrees, which is another way of testing how it may revert to wild-type status.

Progress

The WHO has been evaluating the use of MAPREC for types 1 and 2 vaccines for several years. In 2001 it was already suggested that MAPREC for types 1 and 2 might be approved that year [Langley, 2002]. Respondents currently state that MAPREC is in the process of being introduced for type 2, and hopefully for type 1 as well. For type 1, there are problems with the availability and quality of reference materials, which is the first stage of the process. One then has to use the test on an ongoing basis, and a third party has to review these data.

Studies for type 2 have been done for several years; the next step is to accumulate data to confirm that the results are the same for different laboratories over the world. Since this strain is produced least often, this may take some time. It has been said that this strain is ready to go, though there seems to be some disagreement between regulatory bodies as to when there will be enough data on type 2 to allow this method to be used. They prefer to change the regulations for type 1 and type 2 at the same time. Therefore, the implementation of type 2 will depend on the outcome of the studies for type 1. However, if it looks like there will be a problem with type 1, respondents believe that moves could be made to introduce type 2 as soon as possible.

6.2.9 Problems and arguments MAPREC

Molecular basis

Two different mutations in the virus must be analyzed for type 1. The designed test is supposed to work on both mutations at the same time, but according to one respondent, many laboratories are unable to get the test to work for both mutations at once. This is most probably due to a problem with the primers, which have some sort of interaction. It may be necessary to make new more up-to-date primers or conduct the two tests independently of each other. Another respondent stresses that the primers are not the problem, but the experience of laboratories that are working with them.

Only one site is involved for type 3. However, several sites are involved for type 1 and 2 and it is much less certain that these are actually involved with neurovirulence given that these strains hardly ever revert to neurovirulence. Since multiple mutations can be involved with increased neurovirulence for strain 1 and 2, regulators have considered whether MAPREC is suitable for testing for all possible revertant viruses. One can get a percentage of mutants using different primers, but no one is certain that this actual measures the relevant conditions of neurovirulence. It is feared that a vaccine, which contains other mutations that can cause neurovirulence, might slip through. According to a scientist involved in the development of the test, however, mutations that are located in a specific region in the virus have a perfect correlation with high neurovirulence, also for type 1 and 2.

An approach such as micro array, which is able to measure multiple mutations at the same time, has been forwarded as a promising solution. According to one scientist, a micro array method is available, but is far less sensitive. In MAPREC, the specific mutations looked for are amplified by PCR. This is less well possible with micro array, since this method seeks to identify multiple mutations.

Another point of contention between respondents is the correlation of MAPREC with the monkey NVT. One respondent claimed that there is not a very good correlation, which was strongly disputed by another, who argued that the correlation was perfect. However, for types 1 and 2, no single batch of neurovirulent vaccine could be found, so in fact there was nothing to compare. Therefore, the vaccine was intentionally spoiled to make it fail the monkey

neurovirulence test. Then it was found that a strain 1 vaccine will only fail the monkey neurovirulence test when it consists of more than 30 to 50% of revertant virus. MAPREC could detect a percentage of about 1-2%. It is, therefore, tenfold more sensitive.

It has thus been argued that knowing the molecular basis or the direct correlation with the monkey test is not relevant, since at least strain 1 of the vaccine is very hard to spoil. Moreover, MAPREC is actually a better method than the monkey NVT. This was also confirmed in the literature [Rezapkin et al., 1998].

In vitro testing

If the mutation measured by MAPREC correlates with the NVT-results for type 3, and the degree of correlation for type 1 and 2 is irrelevant, then why is MAPREC not regarded as a replacement alternative for the monkey test?

One respondent reported that he also thought initially that MAPREC was the ultimate solution. However, he suggests that nobody is apparently ready to replace in vivo tests entirely with in vitro methods, even if there is consistency. He thinks this is a matter of experience; the millions of doses that have been tested using monkey NVT and in the future the same might be true for the transgenic mice. According to this respondent, neither producers nor regulators are ready to rule out all animal testing.

The scientist involved in the development of MAPREC had a similar experience. Regulations stipulate that a vaccine should be tested before release by at least one in vivo and one in vitro test. When he turned to the WHO for validation, they argued that since both an in vitro and an in vivo alternative had been developed, these could replace the existing in vivo and in vitro tests. MAPREC could, therefore, offer a substitute for an in vitro method, not replacing the use of animals. That was the best they could agree on. He contended that as long as there is a requirement for a vaccine to be tested in animals, the case for alternatives cannot be won.

Delay

Another problem encountered by the aforementioned scientist is the regulatory authorities' level of understanding. When he tried to push MAPREC through the WHO, there were mostly people from the older generations. He contends that making safety assessment by looking at the X-ray film with black spots was beyond their experience, especially for classical virologists who do not fully appreciate the value of molecular tests. He claimed that they might have had a hard time understanding what a restriction enzyme is, and how it may be related to neurovirulence. It remains alien to them, and thus they may be not as comfortable in replacing an animal method.

One respondent thought that administrative considerations are the reason why MAPREC is still not accepted as a fully fledged alternative. The process of international approval is very lengthy and cumbersome and involves many people from different countries, collaborative studies, meetings, etc. It has already taken ten years to get MAPREC through the WHO for just one type of OPV.

6.2.10 Discussion NVT and alternatives

If the eradication of polio is to be attempted, then OPV is the best vaccine to use. In comparison with IPV it is much easier to administer, cheaper and because OPV is excreted along with faeces, some non-vaccinated people will mount immunity against polio infection too. For these reasons, it currently is the best vaccine to administer to the developing countries.

Use of primates

Moreover, for any vaccine, some kind of test is necessary to show consistency with the product licensed. However, one may question whether the monkey neurovirulence test is the best test to apply, especially since alternatives are now available. One respondent thinks that it now makes little sense scientifically and we should have

stopped at some point when after several decades it turned out that no failed vaccine lots had been discovered, at least for type 1 and type 2. Another contends that we could now rely purely on consistency in production, thanks to modern techniques that yield more consistent cell-line-derived vaccine.

Furthermore, since the mice alternative has been validated, it can be questioned whether the use of monkeys goes against the European Council Directive [1986], given that animals with a 'lower degree of neurophysiologic sensitivity' can be chosen, although this may be subject to discussion.

Reduction

It must be possible to reduce the use of monkeys. Using cell-banks instead of primary monkey kidney cells reduced the number of monkeys needed for their kidney cells and provides a more stable production, with less variation in test results. There are indications that European manufacturers could use fewer animals per group in order to get statistically significant results. Notably the number of primates in the reference group should be reassessed, since the use of historical evidence next to the experimental outcome could reduce the number required. There is no statistical need to use the same number of animals in the test group as for the reference preparation.

Another option for reducing the number of monkeys is the exchange of trained personnel. The surplus of monkeys that is needed for the neurovirulence test is mainly determined by the skills of the injector. It could, therefore, be an option to only let the best few injectors administer all the injections. The travel costs and payment should weigh up against the savings of monkeys, since these animals are reported to cost a few thousand Euro each. Another possibility is the establishment of a European reference centre, which could perform all tests on any appropriate model. In fact, this was one of the recommendations made during a scientific workshop organized by WHO [2005]. By these means, the use of monkeys could be reduced by an estimated 10%.

Finally, the number of neurovirulence tests depends on the size of batches that are produced. Every batch needs to be tested, thus producing larger batches means more doses for the same number of NVTs. A reduction in the use of animals could thus be achieved if production were centralized at a few large manufacturers worldwide. However, in practice this is unlikely to happen since smaller companies already exist, and many countries may prefer to produced the vaccine under their own supervision.

Replacement

Replacement alternatives are also available (if the mouse test is regarded as a replacement for the use of monkeys). The WHO itself says that 'efforts towards characterizing the vaccine virus as much as possible by alternative tests, especially involving the use of non-primate models, should continue as a high priority. Only if such tests do not provide enough assurance of safety should the monkey neurovirulence test be performed' [WHO, 2005]. Therefore, a thorough discussion on the reasons that alternatives are not used more often, is justified.

Despite successful validation and advantages compared to the monkey test for all three strains, no manufacturer has yet been allowed to use the transgenic mouse test on routine basis. Furthermore, only one manufacturer appears to have actually applied for this permission, though this has not been confirmed by other sources. The implementation process, rather than the check by control laboratories, seems to explain why the test is still not being used.

The limitation of the mouse test is that it will not be used in non-EU countries (maybe with exception of Japan). In these other countries, monkeys are more readily available and cheaper than mice. A further limitation is the use of transgenic animals would entail larger numbers of animals being used as compared to the monkey test. This makes the alternative ethically complex. According to one reference, the modification of the transgenic mouse test to function in the test tube is thought possible, given that primary cell cultures from the mice are also susceptible to polio virus [Langley, 2002]. No more information from literature or respondents has been found on this subject, but this approach seems promising.

Then there is also MAPREC. Despite validation for type 3, it is not yet a replacement alternative but it might indirectly lead to some reduction in the use of monkeys, although it is unlikely that any vaccine, at least from European manufacturers, is ever going to fail MAPREC, thereby diminishing its use as a reduction alternative.

For type 1 and 2, there has been discussion on the correlation to the monkey test. For type 1, MAPREC has been shown to be much more sensitive than the monkey test, so it should be considered as a better approach. Scientists at the US Centre for Biologics Evaluation and Research recommended that it should be introduced as a complete replacement for the monkey tests, once consistency of production has been established within a company [Langley, 2002]. In sum, it is more sensitive, probably much quicker, cheaper (although apparatus and primers are costly, this should be less than the costs of monkeys and trained personnel) and does not involve the suffering of any animals.

One general problem in the application of alternatives is knowledge and skills. Laboratories cannot simply switch from using monkeys to mice, or to MAPREC: some will have methodological difficulties [Dragunsky et al., 2003]. This, however, cannot be a reason why the test has still not been applied after several years.

A problem with the implementation of the transgenic mouse test seems to be that it is indirectly linked with MAPREC. Moreover, since MAPREC has suffered delays for strains 1 and 2, this is probably why the mouse test has also been delayed. This interlinking of the two methods, however, seems totally irrelevant. At present, they are each alternatives to different tests: the mouse test for the monkey NVT, and MAPREC for the rct40 test.

Problems and arguments

The first problem is the delay at the authorities. The authorities have been accumulating data for both the mouse test and MAPREC for several years since the method has been validated as an alternative. The absence of scientific criteria, which an alternative has to meet, is the main cause of this delay. Without these guidelines, data can be gathered endlessly. It now boils down to the question whether the individual regulators feel comfortable enough to change the regulations. Moreover, such a decision is even harder to take when no guiding principles are available. This is illustrated by the statement that one respondent from a regulatory body made: "[despite all good results,] I would still be reluctant to do it very quickly".

The basis of this hesitancy is probably a perception that there should be no risks associated with taking the decision. It seems that regulators want to be more than 100% sure than any risk has been excluded. Extra delay is not a problem for the regulators, since this leads to the accumulation of extra data, and the more data they have, the more comfortable they are that will be an accurate test under all circumstances. As one respondent put it: "it is a vaccine that is going to be given to healthy children, thus you want it to be as safe as possible. I would not be the first to say that the animal test can be dropped".

At the same time, a manufacturer indicated that consistency of production is the main factor, and that any test to confirm that consistency, whether MAPREC or a monkey NVT, would be enough for him, at least for type 3.

Another factor also plays a role for the WHO: the risk of delaying the eradication process. This may also be a reason for why the implementation of alternatives has been delayed. It is feared that governments from developing countries may not understand why a key test, which is seen to ensure safety, would be replaced by another method. Replacement by an animal-free methods would certainly generate questions on the quality. However, it should be possible to replace the monkey test with alternatives without causing worries in developing countries. It has already been done for the mouse test for type 3, so why would it not be possible for animal-free methods to be introduced? It should be possible, even if this may require some education.

A further problem for both regulators and manufacturers is liability. According to respondents, this is another reason why data is being accumulated. In the United States, malpractice lawyers have been involved in claiming millions of dollars per case of adverse events after polio vaccination in settlements in medical malpractice court. One respondent believes that this has forced the companies to be very conservative in their approach.

A manufacturer reacted ambiguously to the question of whether they feared liability. On one hand, they would be comfortable with any consistency test, but, on the other, it was acknowledged that if one drops any test and anything happens, one will have a problem. However, liability is not their primary fear, but they say they are concerned about their mission to produce safe and effective products.

Another problem is that regulators and manufacturers seem to point the accusing finger at each other with respect to who does not want to change the regulations. The manufacturer says that if regulators relaxed their requirements, then they would be comfortable with it and go along. One regulator, however, says that he cannot see the manufacturers doing it in the next fifty years.

Solutions

First of all, the criteria that an alternative has to meet in order to replace an animal test should be decided in advance and the basis for these criteria must be explained. It then might be an option to distinguish between European and non-European manufacturers. The WHO has not said it in so many words, but the European manufacturers seem to do a better job than the non-European ones and would trust the former to use MAPREC exclusively as a consistency test. This, however, is not the case for non-European manufacturers, and it may be questioned whether they have the skills to use it correctly, since some European laboratories already have difficulties in getting it to work. Nonetheless, all manufacturers must meet the same regulations, which makes it hard to change them.

Furthermore, there are differences in the availability of monkeys and transgenic mice. A possible solution would therefore be to abandon the one-size-fits-all principle of current regulations and use manufacturer-specific requirements as is done in the US. By this means, the quality of the product will be ensured, while recognizing that differences in consistency between manufacturers may justify other approaches. Furthermore, the use of primates for OPV worldwide is certainly not going to change if Europe does not make the first move.

One respondent asserts that the validation and implementation process is so slow, because there is nobody driving the principle of change through regulations; the regulators themselves have no interest in doing so. Someone needs to push things now and then.

Such a leader should be someone not only with a vested personal or professional interest, but also access to regulators and manufacturers. They should be open to discussion; animal welfare groups are generally regarded as being biased. A scientist might, therefore, be the best option, but the scientist who developed the alternative tests has lost interest now that the US has changed to using IPV instead. The best option may to find someone from an OPV-using country, or a scientist with good knowledge of the problems and access to regulators. Public opinion might also play a role in this and help speed up the process.

Future

In time, the problem might also solve itself. Polio may either be eradicated, or new technology will become available and a new generation of decision-makers may emerge, who are more comfortable with molecular methods. Many respondents think that polio will be eradicated before new alternatives will be developed or existing ones will be accepted as replacement for the monkey test. However, eradication was initially predicted for 2005, but daunting scientific, logistical, and financial challenges for achieving eradication still remain.

In 2004 the WHO made two assumptions with respect to the need for OPV; the worst case scenario being that eradication was not possible by the end of 2005, in which case 8.9 billion doses would be needed until 2010. For routine and stock, 825 million doses would still be needed annually after eradication [WHO, 2002c]. However, it is now clear that even this 'worst case scenario' has not been realized. It appears more likely that from now on some 9-10 billion doses will be needed, if not more.

Furthermore, the risk of bioterrorism is no longer viewed as a remote hypothesis. It is highly probable that many countries will not take the risk of allowing generations of children to grow up with no immunity against such a pathogenic agent [Global Polio Eradication Initiative, 2005].

It therefore seems that even if OPV can be stopped, other polio vaccines will still be required, for instance s-IPV: a vaccine made from killed Sabin strains. Large amounts of OPV would then still have to be produced in order to produce Sabin strains, which is accompanied by the risk that it may mutate back to a neurovirulent form. According to some respondents, it would not make much sense to test every batch for neurovirulence, since the product would be inactivated. Nonetheless, another respondent, who is much more closely involved in assessing future requirements, says that there should be some testing of the bulks to ensure that they are indeed still Sabin strains and have not been transformed into "wild-type" strains under harsh production conditions.

Some kind of polio vaccine will, therefore, continue to be produced for many years; this will probably be s-IPV, which has been tested for neurovirulence. The need to focus on replacement of the monkey test will thus continue for the next decades. The best hope is that this may become easier due to the accumulation of data, a change in the legal climate, education or a shift in the people responsible or the development of an other alternative that does meet all the requirements (when these are formulated). The development of an in vitro test from transgenic mouse cells could also offer a promising solution.

According to a respondent, the best practice for the future would be to develop new vaccines with molecular tests, which should be created along with the vaccine development. Once it has been licensed, it is very hard to change anything.

6.3 Other routine safety tests on primates

A monkey neurovirulence test is also carried out for other live vaccines that could be neurotropic. This does not occur on a routine basis for every batch, but in general only when major changes take place such as a new seed lot. Recently, a scientific workshop on neurovirulence tests for live vaccines was held [WHO, 2005], from which the following findings are derived.

Neurovirulence test for other vaccines

There are problems with respect to when and on what model a neurovirulence test should be conducted. The criteria for classifying a virus as neurotropic are unclear; there are cases where the specified neurovirulence test was not able to distinguish between acceptable and unacceptable preparations, as was the case for a mumps vaccine. A neonate rat model, however, has shown promising results for this vaccine.

For three other vaccines, measles, rubella and varicella, it has been concluded that it is not apparent that the neurovirulence test provides relevant data with respect to vaccine safety, and the need for neurovirulence testing of new seed preparations is thus difficult to justify. Nevertheless, new vaccine strains must be tested on neurovirulence using the monkey model. The reason given is that no alternative model is available and there are some cases of neurovirulence associated with these viruses.

With respect to another vaccine for yellow fever, it was concluded that despite the fact that the reference preparation is not a good control, it has not been demonstrated that vaccines that pass the test are more acceptable than those that fail. The relevance of the test has thus not been demonstrated and since there are no objective criteria to pass or fail, seed lots should still be assessed by the monkey NVT.

For the Rota virus vaccine, it was concluded that there was no scientific reason to perform a neurovirulence test, while for influenza 'it is questionable whether [demonstrating the absence of neurovirulence] justifies the use of

a monkey neurovirulence test and other models should be considered'. The monkey NVT was only considered 'highly relevant' for polio.

Discussion

In summary, the meeting concluded that 'the validity of the model applied should be crucial, but in many cases the monkey NVT is likely to be applied despite absence of good data qualifying its use'.

There were questions on the quality or relevance of this test for five products, yet it was still recommended that it should be carried out. In one case, this was motivated by the absence of alternatives; in other cases it is unclear why this conclusion was drawn. In addition, it remains unclear why doubts for the need of a monkey neurovirulence test on influenza vaccine were expressed, while this 'need to look for other models' was less present in other cases (besides mumps).

It is, therefore, remarkable that nowhere in the document was the (chance of) neurovirulence being assessed using animal-free methods discussed. When alternative models were mentioned, they always implied animal models. The reason for this also remains a mystery. This is reminiscent of OPV: the animal-free alternative does not seem to have been accepted, because no animals have been involved.

6.4 Conclusion

Based on the findings from the case study in the present report and results from a scientific meeting on neurovirulence testing, the use of primates and the possibility to replace them for vaccine safety tests in general can be discussed. Although the reasons for using monkeys for other products than OPV have not been investigated in greater detail, a few findings are still apparent.

6.4.1 Use of primates

The neurovirulence test requires thousands of monkeys worldwide each year, many of which are used in Europe. The main reason that primates are used for the neurovirulence test is historical. There is also a scientific reason: no other animal species is known that can be infected with polio. However, the relevance of the monkey neurovirulence test has been questioned both for OPV as well as for other vaccines. Sometimes the absence of alternatives is given as an explanation for the continued use of the monkey test. However, given that these alternatives are available for OPV and monkeys are still used, it may be questioned whether the existence of alternatives would change the regulations for these other vaccines. History and legal safeguarding have provided the grounds for the arguments with respect to why these alternatives have not been accepted or used for OPV; the same may very well be the case for other NVTs.

6.4.2 Alternatives

There are possibilities for alternatives, both to reduce this number and to replace them with other methods. The reduction of the use of monkeys seems possible by exchanging the best personnel, centralizing production or by reducing the number of animals according to the variance of the test. However, respondents agreed that discussion should take place on replacement, rather than on reduction.

No alternatives are available for most of the NVT's, besides a baby rat model for mumps vaccine. There are, however, two alternatives for the consistency testing of OPV: a transgenic mouse model and the molecular method known as MAPREC. Despite successful validation studies and several advantages to the monkey test, neither of these methods have been accepted as a replacement alternative for the monkey test. This delay has been caused by a seemingly endless accumulation of data by the regulatory authorities in order to be more than sure that the test

is as good as the monkey test, in order not to put the eradication process in danger or because of liability issues, which each of them says the other fears. Nevertheless, the main problem for the complete replacement of animal tests seems to be a feeling that an animal method is better. All other arguments can be reasoned back to this one topic.

6.4.3 Solutions

The best solution would be to centralize the production of OPV at one or two large European manufacturers. However, since the developing world is one of the major users and companies in developing countries already exist, this is not a realistic option. Three things could, therefore, be done:

Firstly, clear guidelines on the criteria for testing of vaccines need to be established and the relevance of current test should be discussed comprehensively. By this means it will be clear to everybody whether an alternative has a chance, and what needs to be done in order to be allowed to implement it. These issues can now drag on for years.

Secondly, regulators should abandon the general requirements for the safety testing of OPV and start with manufacturer-specific requirements, allowing the most consistent products to be tested without the use of animals. This might encourage the others to also improve the quality of their products.

Thirdly, someone is needed to drive these changes.

Chapter 7

Conclusion

The central question of this inventory study is the extent to which the use of primates in biomedical research can be replaced or reduced by other models and methods. This question has been subdivided in four topics: the use of primates, reasons for their use, possible alternatives and problems concerning alternatives. In this chapter, each of these themes will be the focus of a separate section. In addition to this, some possible solutions will be presented.

7.1 Use of primates

7.1.1 Numbers

More than 100 thousand individual primates, mainly Old World primates, are used for biomedical research every year worldwide. The United States, Europe and Japan are the main countries that use primates. Primates also appear to be re-used quite often. The ethical question is whether the increase of suffering to the individual animal weighs up against the use of fewer new animals. Naturally, there should be a limit to the amount of distress caused to one animal, but since there are problems relating to the sourcing, transport and housing of primates (mainly Old World species), the additional costs of not re-using them might also be high [Animal Procedures Committee, 2002]. This problem could best be solved by providing the animals with the best housing conditions possible, also during or in-between experiments, using as few animals as possible and re-using them as long as the amount of distress is limited.

7.1.2 Goals of primate use

In Europe, major goal of primate experimentation is 'safety evaluations', followed by 'fundamental research'. There is no reason to expect that the findings based on European statistics differ greatly from other parts of the world. In Europe, a comparatively large number of primates are used for the safety testing of oral polio vaccine (OPV), which is no longer done in the US. However, it appears that safety tests for new medicinal products are conducted more frequently in the US than in Europe: for most products that were examined in detail for a case study, the relevant spokesperson was found in the United States. The amount of fundamental research carried out using primates in the US is not known, but there is no reason to think this would be less than in Europe. Indeed, research on HIV is one of the major aims of primate use in America.

The production and safety testing of OPV probably is the major goal of primate use in Asia, since some local manufacturers are likely to produce smaller batches than their European colleagues, thus using comparatively more monkeys. In addition, primary monkey kidney cells are used for the production of the vaccine, which also requires the use of monkeys. Safety testing of new medicines and fundamental research may also be conducted in Japan on a

large scale. Moreover, Contract Research Organizations (CROs) in developing countries as China are also an increasingly important factor with respect to primate use.

There are no figures on the use of primates for the rest of the world, including Eastern Europe, Australia, Canada, South America, Africa and the Middle-East. Primates are probably used in these countries, at least for production and testing of OPV and some fundamental research. It is, however, not likely that primate research is substantial, since few publications deriving from these continents were found in the literature. CROs in these countries, however, play an important role in primate use.

7.1.3 Future developments

The use of primates for both fundamental and preclinical studies is more likely to increase in the near future than diminish. However, an increasing resistance in public opinion has lead companies to review their use of animals more carefully, which may lead to a further decrease in the number of animals used and the distress per experiment in the future. On the other hand, such resistance may lead companies to outsource their animal studies to countries where animal welfare may be even poorer.

7.2 Reasons

7.2.1 Fundamental studies

In fundamental research, experiments may be conducted on primates despite the fact that other models could also be used (see Table 4.1). Arguments on the grounds for choosing primates as experimental models are seldom given in scientific literature, but the present study concludes that differences in immune systems between humans and non-primate species are the main reason for using primates in fundamental biomedical studies. As far as fundamental neurology studies are concerned, primates are used for their higher cognitive functions, the possibility of training them and the anatomical similarity of their brain structure to humans. Anatomical or physiological similarities also provide the basis for some other primate studies.

In addition, primates were considered to be the best model by the researchers who use them. It is unclear to what extent this is based on scientific evidence, or whether tradition, convenience, or experience with the primate model may play a role; this should be investigated in greater detail with respect to the various subjects for which primates are used.

7.2.2 Applied studies

Safety evaluations are the main goal of primate studies: including the testing of new medicines and the consistency testing of the oral polio vaccine. The reason for using primates for new medicines is almost never explained in the registration files either. While it has been acknowledged that the main reason to select primates as a non-rodent should be scientific, other arguments also seem to play a significant role. The two main ones are time (which equals money) and regulatory requirements.

Scientific arguments for using primates for biopharmaceuticals are the exclusive epitope-expression and tissue cross-reactivity in primates (this argument is most frequently used) and the risk of an immune reaction. However, in the case studies, examples of primate use were discovered in spite of the fact that the product was also active in other models. Furthermore, primates often mounted an immune response against the product. Although this response might be better characterized in primates than in other species, it does not necessarily exclude the use of alternate models. In the literature, the smaller size of monkeys compared to dogs was given as an argument for testing biopharmaceuticals on primates, but in case studies this did not seem to play a major role.

More importantly, contrary to most references and respondents, the present study found that primates in preclinical safety testing seemed to be used mainly for conventional products. The reasons for their use remain unclear, though possible explanations may include the exclusion of dogs as experimental model, the notion that primates are better models than rodents for drug abuse-studies or other non-scientific arguments. This topic should be investigated in greater detail using a different set of case studies.

Regulatory influence

The argument of regulatory requirements is frequently used to explain the use of primates. However, in practice, it seems that it is the perception of the actual requirements that plays a role, since regulators report that there are studies that are being conducted, which are not considered relevant. There seems to be little discussion with regulators on the actual requirements, even in cases where these requirements are considered irrelevant by the pharmaceutical industry (examples in paragraph 5.7.3). With respect to routine tests, companies and regulators even point the finger at each other regarding the issue of why the requirements have not been changed.

Time and the costs associated with discussions on regulations is an important factor for the pharmaceutical industry, although both were not directly mentioned by respondents. Tradition also plays a role, since primates are often considered to be 'the best model'; a notion that is primarily founded on tradition and intuition, rather than concrete evidence. The selection of primates as a pre-clinical model seems to be conceived as a way to avoid discussion on the quality of the animal model, thus safeguarding the quick registration of a new medicinal product, and more time to recoup investments. The costs of using the expensive primate model is often weighed up against preventing a delay in the registration and marketing process.

Some financial and traditional aspects may also play a role in routine evaluation. When primates are not used in product development, there is a risk that legal settlements will have to be paid to people who have suffered adverse reactions. Both regulators and companies want a compound to be absolutely safe: in some cases, regulators request studies on primates just to be sure. In general, companies and regulators are not inclined to take risks by using other methods than the historical ones, which are typically deemed to be the best: primates. This argument was also apparent with respect to the routine safety evaluation of OPV. Despite the fact that alternatives have been available for years, their use has not been permitted because regulators want to be absolutely sure that the compound is safe.

Number of animals

Reasons for the number of animals involved in primate studies are seldom given in reports or by respondents either. The reasons for differences between research groups and companies thus remain unclear. There are some examples of seemingly unnecessarily high or statistically insignificant low numbers of animals, which may be worth further investigation.

7.3 Alternatives

In general, regulators and respondents accept that primate experiments should be avoided, reduced and replaced as much as possible. In the following section, the overall possibility of replacing or reducing primate experiments will be discussed.

7.3.1 Animal-free methods

According to respondents, tests that can be conducted without the need animals are the hope for the future, but many were sceptical about the timeframe: this may take many decades or never be achieved at all.

In vitro methods

In vitro methods regarded as the most important group of alternatives to animal testing and these methods are already used extensively. Primates, however, are primarily used mostly because the nature of the studies require an animal model. In vitro models are not living organisms and generally cannot be used to replace or reduce the use of primates. There are indications that some in vitro methods could replace the use of primates, but the main obstacle with respect to achieving this are the regulatory requirements.

In vitro methods can sometimes be used for subquestions, which help to gain more fundamental insight in subjects that are studied on primates. In this regard, they can be viewed as an indirect reduction alternative. Animals are often used for fundamental studies while the exact mechanism of disease is unknown. Although a complete biological system might be needed to study all effects, investigating parts of the disease process by in vitro methods may yield insights that improve the quality of animal experiment and indirectly reduce the use of animals. Respondents from the pharmaceutical industry report that in vitro methods are already used to large extent, but not as a direct alternative.

One of the possible in vitro alternatives is the use of primate tissue. Whilst primates will probably have been killed to obtain the material, using primary primate tissue may extend knowledge and improve the primate model in the long run. Moreover, it may be possible to carry out multiple tests on tissue from one monkey, thus reducing the overall number of animals. Moreover, tissue from older animals that were to be euthanized anyway could also be used, which means no extra animals would be needed.

Immunologic/physiochemical/biochemical techniques

New techniques, such as genomics and micro-arrays, may help to guide species selection, prevent the unnecessary use of animals and help interpret results. Within one species, the use of genomics may help to select individuals that are likely to respond in the desired way, thus reducing the use of animals. However, this is no alternative to the use of animals, and may even increase the use of primates when using these methods reveals that they are the only appropriate species.

It was reported that these methods were generally used in the quality control of vaccines, but even for this category they were not found to be alternatives to primate use, although in some cases they may well serve as such. While MAPREC could be used as an alternative to the monkey test, it has not yet been accepted for mainly non-scientific reasons.

Mathematical models

Mathematical models can be used to predict various reactions. However, this cannot be seen as a viable alternative since these reactions will generally still have to be checked in an (animal)model. Mathematical models may also help in the interpretation of experiments, but it is questionable whether this will actually reduce the number of experiments. Moreover, for the foreseeable future, it will not be possible to rely on results from mathematical models alone, except maybe with respect to some routine examinations. No examples, however, were found, certainly not for experiments on primates. Mathematical models were also not found as an alternative in pharmacokinetics or pharmacodynamics studies either.

7.3.2 Other animal models

Lower organisms

Lower organisms, in the sense of invertebrates, are not regarded as alternatives to primate use, since primates are specifically used due to their high development and close relationship to humans, which automatically excludes lower organisms as alternatives.

Other animal models

Other animal models, although not alternatives in the sense of the 3 Rs, are the most promising methods for reducing or replacing the use of primates. Many subjects are studied in fundamental studies using other animal models alongside primates, which indicates that at least for some parts of the subject it is possible to use other species. In all case studies, there was some possibility of using other animal models than the primate for preclinical research. Many options are available for this: using transgenic mice or homologue proteins for biologicals, rodents for the effects on the central nervous system, dogs or non-standard species such as the pig in cases where the dog is not suitable. However, for both transgenic animals and dogs, this will generate other ethical problems, which means that it is questionable whether it is indeed an improvement. Respondents agreed that most primate workers automatically think about other animal models when the use of primates was excluded, but it should be considered whether they offer a real alternative.

In addition, it has been argued that a lack of background data is hampering the use of non-standard species, while the creation of transgenic animals with enough background data costs a significant amount of time and money. These will, therefore, only be useful for routine procedures, which excludes preclinical development and the testing of new medicines. With regard to homologue proteins, it is uncertain whether the clinical candidate with give the same reaction. Another problem is that these homologues may be difficult to obtain for species other than the mouse and the primate due to a lack of knowledge.

Embryos

Embryos could be used to test for reproductive toxicity, but they would be not predictive enough and therefore cannot be viewed as a viable alternative to the complete biological system of primates. In general, they were not even considered as experimental models.

7.3.3 Humans

Human material

The use of human tissue could be another alternative for the use of primates, at least for fundamental studies and some preclinical studies in a tiered approach. Although it has been reported that human liver material can be used to study the metabolism of new pharmaceutical products, it seems that this approach has not been widely used. Human tissue is sometimes used by the pharmaceutical companies to test cross reactivity of monoclonal antibodies, but three major limitations for the use of human material are reported: often the quality of the tissue is not good due to long intervals before the tissue can be harvested and prepared. Furthermore, one only examines the metabolic characteristics of one kind of tissue, instead of a whole body response. The third problem is that no system is in place to coordinate supply and demand of human tissue.

Human volunteers

Human volunteers are another promising alternative to the use of primates. Non-invasive techniques could be used on human volunteers for several neurology experiments. The resolution of these techniques could, however, pose a problem, and animal experiments would still be necessary to link these experiments to more fundamental results. Humans could also be used for some studies on disease; either by infecting and curing them in the case of infectious diseases, or for studies using patients. There are indications that the investigation of human patients has lead to major breakthroughs for a variety of diseases. Researchers using animals presented several ethical objections to the use of human volunteers, although sometimes these may be overcome.

Humans could be used in microdose studies for preclinical and toxicological experiments. However, there are four major limitations. Firstly, ethical problems with experiments on humans make it impossible to perform studies with efficacious doses on patients that are not terminally ill, and no tissue can be assessed for histopathologic

changes. Biomarkers of toxicity or small biopsies for toxicity evaluation could resolve parts of the lack of tissue evaluation, but these methods were not mentioned by any of the respondents from applied studies. In fundamental studies, biopsies have been taken from human patients, but not as an alternative to primate use.

Time is another problem, since studies on volunteers take far longer than animal experiments. A third factor is the costs associated, which are much higher for the use of humans than for the use of animals. The only preclinical studies that humans could be used for, therefore, are pharmacokinetics. However, the fourth limitation is the requirement that the exposure of the species used for safety evaluations must be known, thus animals will have to be used for PK studies anyway. Volunteers will thus only be used as replacement alternative when no relevant animal model is available.

7.3.4 Reduction alternatives

In general, several options for the reduction of the number of primates were found in this study. The number of animals per group and the number of experiments could be reassessed, plus the use of primates sometimes could be restricted to one sex: for instance when no gender difference is likely, or when no gender difference was found in preceding tests. Furthermore, collaboration and communication of groups working on the same subject could reduce the number of primates used for fundamental studies, preclinical studies and routine safety evaluations. However, there are several problems with regard to such collaboration, which will be discussed in the following section.

It is reported that the pharmaceutical industry uses the tiered approach on a routine basis, although not as a direct alternative to primate use. Only when a compound is dropped as a result of preceding tests will primate use be avoided. However, there are indications that this approach could involve more direct alternative methods to reduce primate use, and it can be questioned whether the final check in an animal model cannot be replaced as well.

7.4 Problems concerning alternatives

As some of the case studies in this report indicate, given that it seems possible to replace or reduce the use of primates, the question remains why the implementation of alternatives is hampered. Several problems and arguments were given In the literature (see chapters 2 and 3), some of which have been confirmed in case studies. Other arguments were also found.

7.4.1 Selection of relevant model

The literature suggests that one of the key problems is that there is no formal way of deciding the relevant (animal) model for a certain research goal, and different groups may use different ones. A lack of consensus on the quality of the different methods appears to be impeding the replacement of primate studies with other (animal) models. Even within the primate model, this has led to differences between groups using the primate model, leading to results that are consequently hard to extrapolate. Scientists seem to keep to their own model in which they can continue their course of research, and discussion is complicated by the fact that there is 'freedom of research' and the notion that studying a difference is also valuable.

In preclinical research, the 'most relevant' species is selected from two standard animals: the dog or the monkey. Non-standard species are not used because of a lack of background data. If there are any indications of problems with efficacy, immunogenicity, metabolism or acceptance of the compound in the dog, then the primate will automatically be chosen. Moreover, since predicting the suitability of the relevant species in advance is difficult, in some cases the primate may be chosen automatically, because of the notion that primates are always 'the best model'. However, different companies seem to have divergent strategies for selecting the most relevant species. The exact process of species selection by pharmaceutical companies should thus be investigated in a future study.

Given that the use of other animal species seems to be the best way of reducing primate use in the near future, this may increase the use of the standard non-rodent species: the dog. Although the extent to which this may be ethically viewed as an improvement is questionable, in general the respondents in this inventory thought that it would. It was stressed that the use of dogs should be reduced and replaced as much as possible too, but because of practical problems with acquisition, transportation and housing, the reduction in primate use was seen as a high priority. Furthermore, the replacement of primate studies with another animal model could be seen as a first step toward complete replacement, as at least it indicates that primates are not absolutely necessary, clearing the way for 3R alternatives.

7.4.2 Regulatory requirements

This report concludes that the perception of regulatory requirements is one of major reasons why pharmaceutical companies use primates, while regulators themselves have a tendency to favour the use of primates as well. Further to this, there are indications that the replacement of primate studies for regulatory purposes has been impeded because regulators want to rely on animal data. As regards medicinal products, it is required that they are tested on at least a rodent and a non-rodent species. In order to omit one of these tests, one has to show that it is impossible to conduct them, and animal data can be requested even when it is scientifically irrelevant. This hampers the application of a tiered approach, though technically it might be possible to test for several effects using in vitro methods.

This reliance on animal models seems to be even more of a problem for routine safety testing, since available and validated alternatives for the consistency testing of OPV are not accepted. As for CROs, there are no direct indications that they affect the use of primates versus alternatives, although it has been suggested that they are more inclined to take the safe route (using primates) and are less willing to discuss requirements with regulators than the pharmaceutical companies themselves.

7.4.3 Practical problems with replacement alternatives

The literature reports that there is not only a lack of information on alternatives, but also the motivation and budget to develop them. This study found that gathering information on alternatives is indeed a problem. Moreover, it has been noted that many scientist were also unaware of possible alternatives. If information is hardly available, and no clear arguments on the quality of the different models can be found, how can researchers be expected to use another approach than the one to which they are accustomed?

Respondents confirmed that the lack of a pro-alternative environment is one of the main problems, which poses an obstacle to the discovery and use of alternatives. It might be beneficial to conduct more research on alternative methods, since in the past this has yielded alternatives for some cases where primates were previously considered as the only relevant model: hepatitis C (tupaias), oral polio vaccine (transgenic mice and a genetic method) and biopharmaceuticals (transgenic mice and mice with homologues).

However, developing alternatives is often not beneficial. Most primates are used for non-routine tests. Developing an alternative not only takes time and money, but also animals to get it validated. When this would be done for every single test or compound, it would require more animals than when a readily available model was used and only limited funding is available.

Another problem with respect to the development of alternatives is that primates mostly are used when a complete organism is needed, and the immune system is often involved. A lack of knowledge of the immune system of primates (including humans) and non-primate animals largely prevents the use of other animal species instead of primates. Moreover, reagents are also often unavailable for other species. While mice are used for many fundamental immunology studies, respondents argued that their immune system did not sufficiently resemble the human one.

Using transgenic mice could be an alternative when techniques have been developed to provide these mice with a human immune system.

Transgenic mice can be used as alternative for tests that are conducted on a routine basis, but the validation process takes a couple of years. In addition, it has been noted that even after validation, there are no clear guidelines as to when an alternative can actually be approved.

7.4.4 Practical problems with reduction methods

Collaboration could reduce the number of primates used. However, it will be very hard to get scientists to discuss the relevance of their model and approach with an open mind. For pharmaceutical companies, commercial interests pose the major stumbling block for result sharing. Furthermore, it should be questioned who could organize the database without being suspected of some vested interest or prejudice.

The role of animal ethics committees has also been condemned since they would allow too many experiments, especially at CROs. However, these committees are restrained by their own background and expertise, and it may be practically impossible to judge whether the use of primates is necessary for subjects on which one has no expertise. Furthermore, several respondents stressed that (at least in the Netherlands) ethical committees assess the ethical justification of the test, not the scientific background of the research or the model used: this is to be indicated by the researcher who requests the experiment. Indeed, some primate experiments could very well pass these committees while other models could in fact be used.

7.5 Recommendations

7.5.1 Solutions

Discussion on different models

The use of different animal models for the same research subject by different research groups and the selection of different models as preclinical model for a same class of compounds by pharmaceutical companies should be discussed by scientists using these models. This could be take place at congresses on specific diseases, where researchers using different models are present, or at other meetings.

Since various factors are likely to hamper informal collaboration or sharing research data, another possibility is the establishment of expertise centres or committees. Knowledge on different research strategies could be brought together in these committees. For instance, an expert centre on HIV should involve scientists using all relevant animal models, mathematical approaches, in vitro tests and human volunteers or material. It is more difficult to decide who should participate with respect to preclinical research, but members should include regulators from several continents and other parties to prevent the unnecessary use of primates. These participants should not create any resistance among the pharmaceutical companies, nor should they have any commercial interests in the proposals of experiments. Respondents emphasize that such committees should be aware of all previous primate studies, making it an international organization.

Expert committees could perhaps be grouped with respect to disease subjects, which means that the committees for fundamental and applied research could be shared. Another possibility is the extensive evaluation of the quality of animal models for different subjects, which can best be performed by an independent investigator. The RSPCA offered a similar idea, which "might involve challenging the fundamental basis of a field of research that has traditionally been based on a particular animal model, but where an alternative approach might yield equally, or more, useful results" [Animal Procedures Committee, 2003].

Mandatory advice

It may be argued that researchers themselves will not ask any centre for advice on the quality of their models because of their personal interest in using the animals they are specialized in. Therefore, it may be beneficial to require the approval of such centre by law. In fact, two respondents already proposed mandatory scientific advice for the use of primates independently of each other. This would be comparable to the way in which experiments on humans are approved in the Netherlands: for experiments on certain groups of human volunteers e.g. unborn children and mentally disabled, approval of a special centralized committee is needed, while 'normal' experiments on humans are approved by local medical ethics committees.

Several respondents were positive about this idea, but only if such centres possessed greater expertise on the subject than the current committees, and they should replace current procedures, thus not delaying the process.

Criteria

However, in order to allow expert committees to function, there should be consensus on the criteria of a relevant model, or the background of requesting tests. At the moment, this is neither clear for animal models, nor alternatives. In these international expert committees of regulators and scientists, the regulatory requirements for the use of primates and other animals could also be discussed and the criteria that a model has to meet could be formulated. In future, this may facilitate the development and implementation of alternative methods, as well as the eradication of irrelevant tests.

Likewise, the arguments on the use of a certain model and why alternative methods are not used should be recorded in scientific publications and European Public Assessment Reports. More openness from all parties could be an improvement in general, both by creating understanding for the use of animals and forcing people to think about possible alternatives.

7.5.2 Conditions

More research is needed in order to solve the problem of the lack of knowledge of the immune system and to increase the understanding of how results can be translated from one species to another. More reagents for other animal models might create a possibility for using those instead of primates. However, in order to increase research on these topics, more funding and a more prestigious atmosphere with respect to research on alternatives will be needed. Scientists must be familiarized with alternatives earlier in their education. This would certainly require a rigorous shift in politics.

Many of the solutions presented in the present report depend on some kind of funding, while several respondents indicated that funding is also one of the limiting factors with respect to the reduction or replacement of animal use. It thus is essential for funding for the development of alternatives to be made available on a routine basis.

Finally, requirements on the use of primates or alternatives should be formulated in discussion with primate users to prevent them from moving their tests to countries with fewer regulations or cheaper test facilities, which would likely impair the welfare of primates used in research while not enhancing scientific quality either. The most important regulatory authorities should also be involved to prevent them from ignoring the outcome of a discussion and to continue to request tests that are regarded as irrelevant. Perhaps these authorities could even exchange their experiences and best practices, although one respondent thought that the involvement of different regulators would only result in endless discussions.

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Appendices

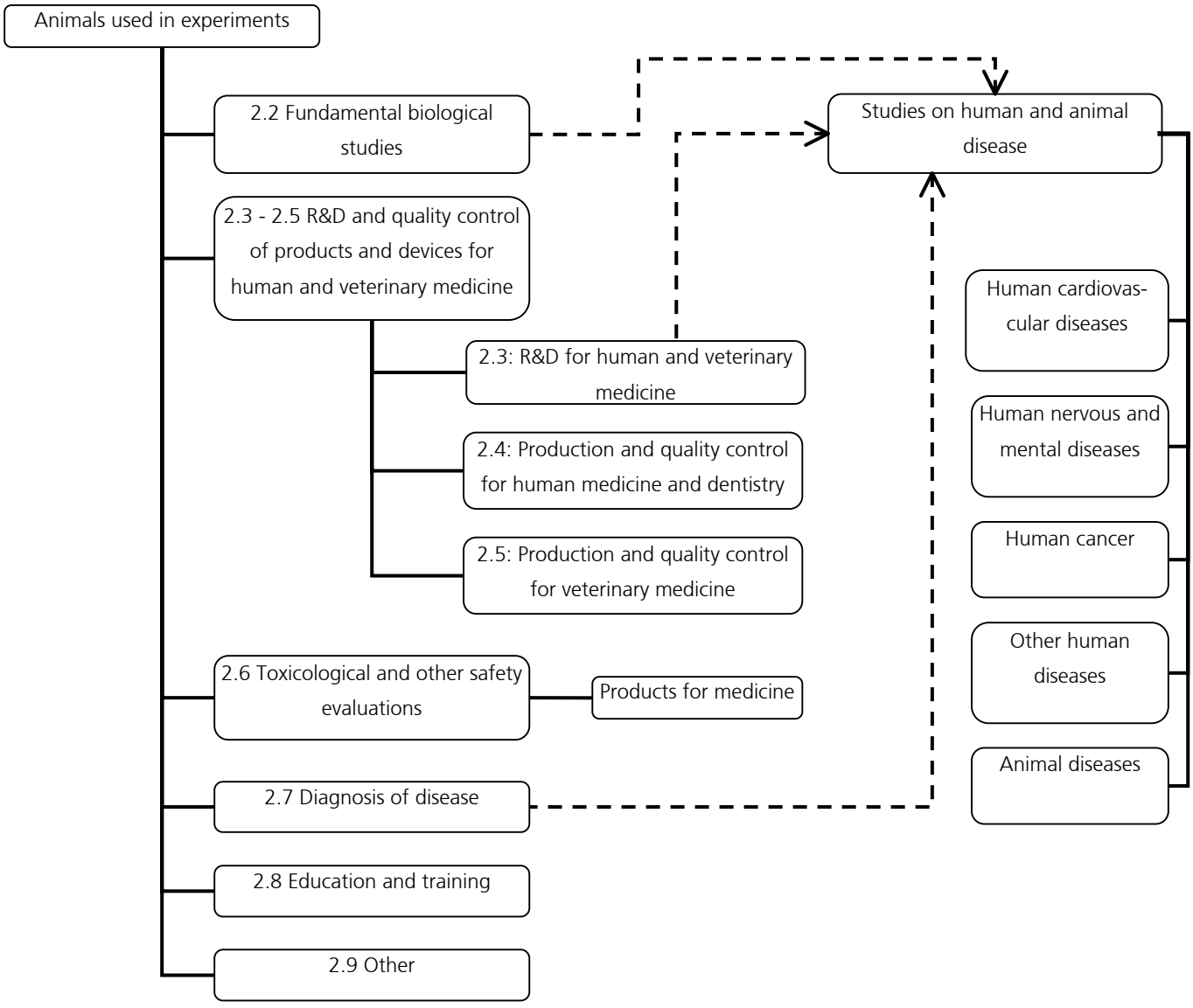
Appendix 1

List of respondents

Dr. Aileen Aherne, Academy of Medical Sciences, United Kingdom
Anonymous, anonymous pharmaceutical company
Anonymous, Eli Lilly, United States of America
Anonymous, Erasmus Medical Centre, the Netherlands
Anonymous, GlaxoSmithKline Biologicals, Belgium
Anonymous, GlaxoSmithKline Biologicals, Belgium
Dr. Jeffrey Bajramovic, Biomedical Primate Research Centre, the Netherlands
Prof. dr. Rob de Boer, Utrecht University, the Netherlands
Dr. Willy Bogers, Biomedical Primate Research Centre, the Netherlands
Dr. Kathryn Chapman, NC3Rs, United Kingdom
Constantin Chumakov, PhD, DSci, Center for Biologics Evaluation and Research, United States of America
Dr. Tineke Coenen – de Roo, NV. Organon, the Netherlands
Kathleen Conlee, BS, Humane Society of the United States, United States
Roland Dobbelaer, Dr. Sc., Federal Public Service Health, Food Chain Security and Environment, Belgium
Prof. dr. Peter Heidt, Biomedical Primate Research Centre, the Netherlands
Dr. Jan-Willem van der Laan, Centre for Biological Medicines and Medical Technology, the Netherlands
Dr. Scott Lambert, World Health Organization, Switzerland
Dr. Renato de Leeuw, NV Organon, the Netherlands
Dr. Helen Munn, Academy of Medical Sciences, United Kingdom
Dr. Mark Prescott, NC3Rs, United Kingdom
Dr. Vicky Robinson, NC3Rs, United Kingdom
Prof. dr. Joost Ruitenberg, Royal Netherlands Academy of Arts and Sciences, the Netherlands
Dr. Huub Schellekens, Utrecht University / Medicines Evaluation Board, the Netherlands
Drs. Henk Smid, ZonMW, the Netherlands
Drs. Tessa van der Valk, Utrecht University, the Netherlands
Dr. David Wood, World Health Organization, Switzerland

Appendix 2

Overview of EU categories on animal use



Appendix 3

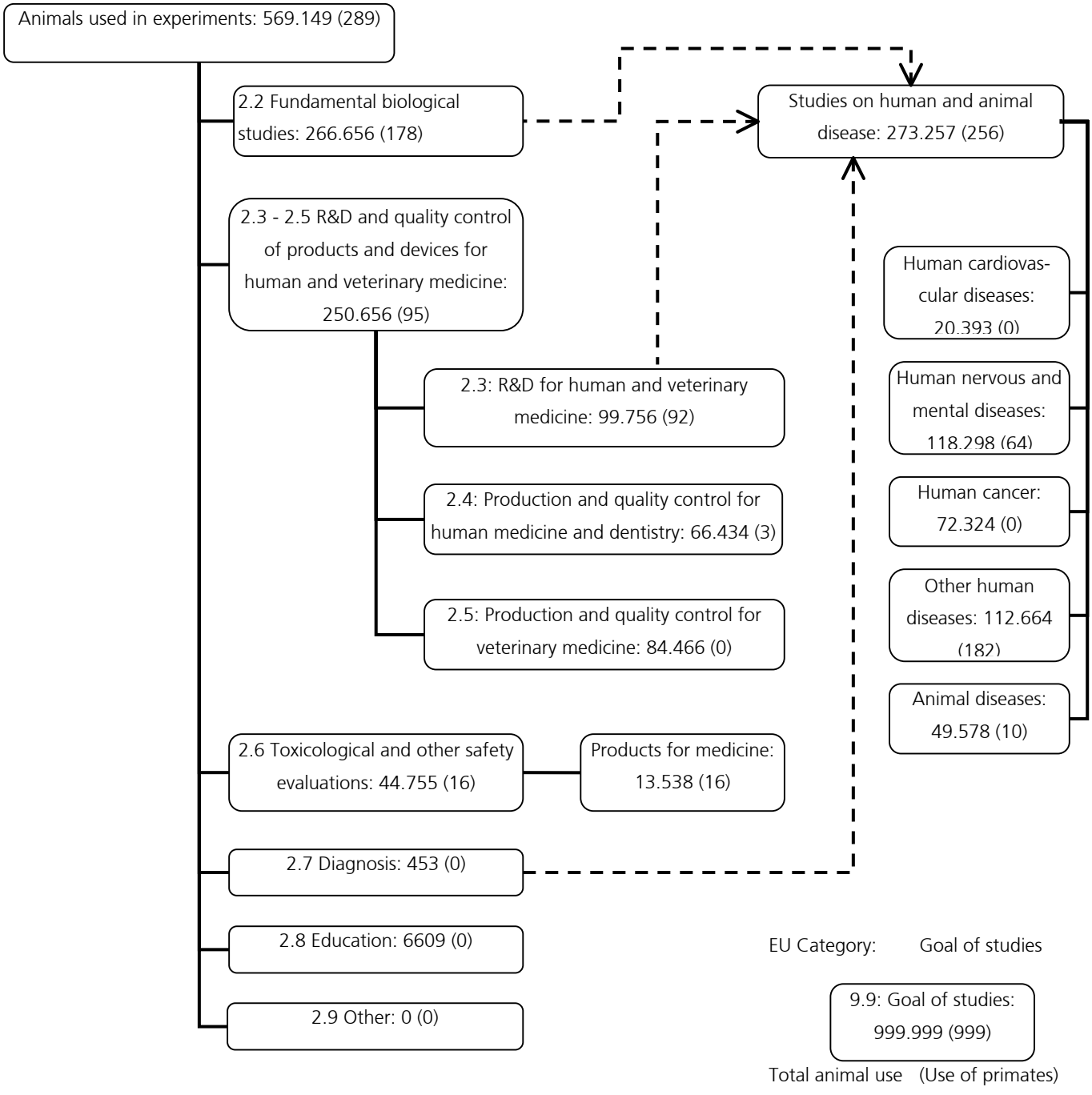
Explanation on EU categories of animal use

- Biological studies of a fundamental nature (2.2): this category deals with studies that are designed to add to the stock of knowledge about the normal and abnormal structures, functioning and behaviour of living beings; including fundamental studies in toxicology.
- Research, development and quality control of products and devices for human medicine and dentistry and for veterinary medicine (2.3, 2.4 and 2.5): category 2.3 tabulates animals used for applied research, which is aimed at the identification, characterisation and development of medicinal products and any other substances that could potentially be used for curative, palliative, prophylactic or prosthetic purposes in humans and animals. Routine testing is not included in this table. 2.4 comprises of animals used for the routine production of biological material used routinely in human medicine and dentistry, as well as animals used in quality control of the final product and its constituents and any controls carried out during the manufacturing process for registration purposes. Animals used in routine safety evaluation processes carried out for regulatory purposes are not included. Animals used for the same procedures but for veterinary medicine instead of human medicine, are tabulated in category 2.5.
- Toxicological and other safety evaluations, including safety evaluation of products (2.6): includes studies carried out on any product or substance to determine its potential to cause any dangerous or undesirable effects in humans or animals as a result of its intended or abnormal use, manufacture or as a potential or actual contaminant in the environment. This category is subdivided to classify animal use by the types of substances that have been studied.
- Diagnosis of disease (2.7): contains animals used in procedures for the diagnosis of human or animal disease, including diagnosis of suspected poisoning.
- Education and training (2.8) comprises of animals used in education and training at all levels.
- Other (2.9) includes animals used for the production and maintenance of infectious agents, vectors and neoplasms and animals used for the production of biological material, insofar as these are not used for the purposes defined in any other category.

Parts of categories 2.2, 2.3 and 2.7 together form a category in which research on diseases are tabulated. A further breakdown in the following diseases is made: human cardiovascular diseases, human nervous and mental diseases, human cancer (excluding carcinogenic risks), other human diseases and studies specific to animal diseases [1997].

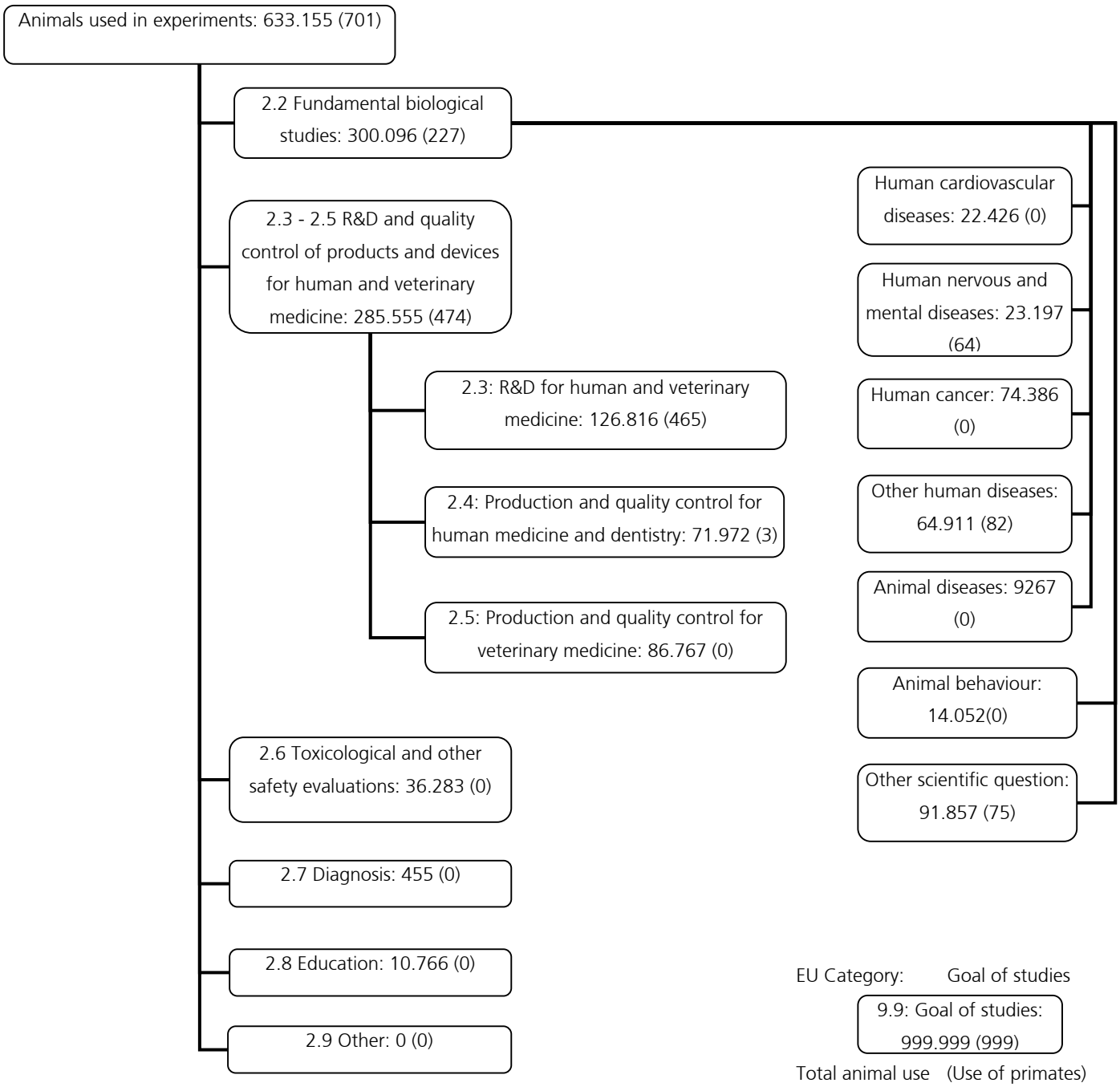
Appendix 4

Use of primates in the Netherlands in 2004 (EU statistics)



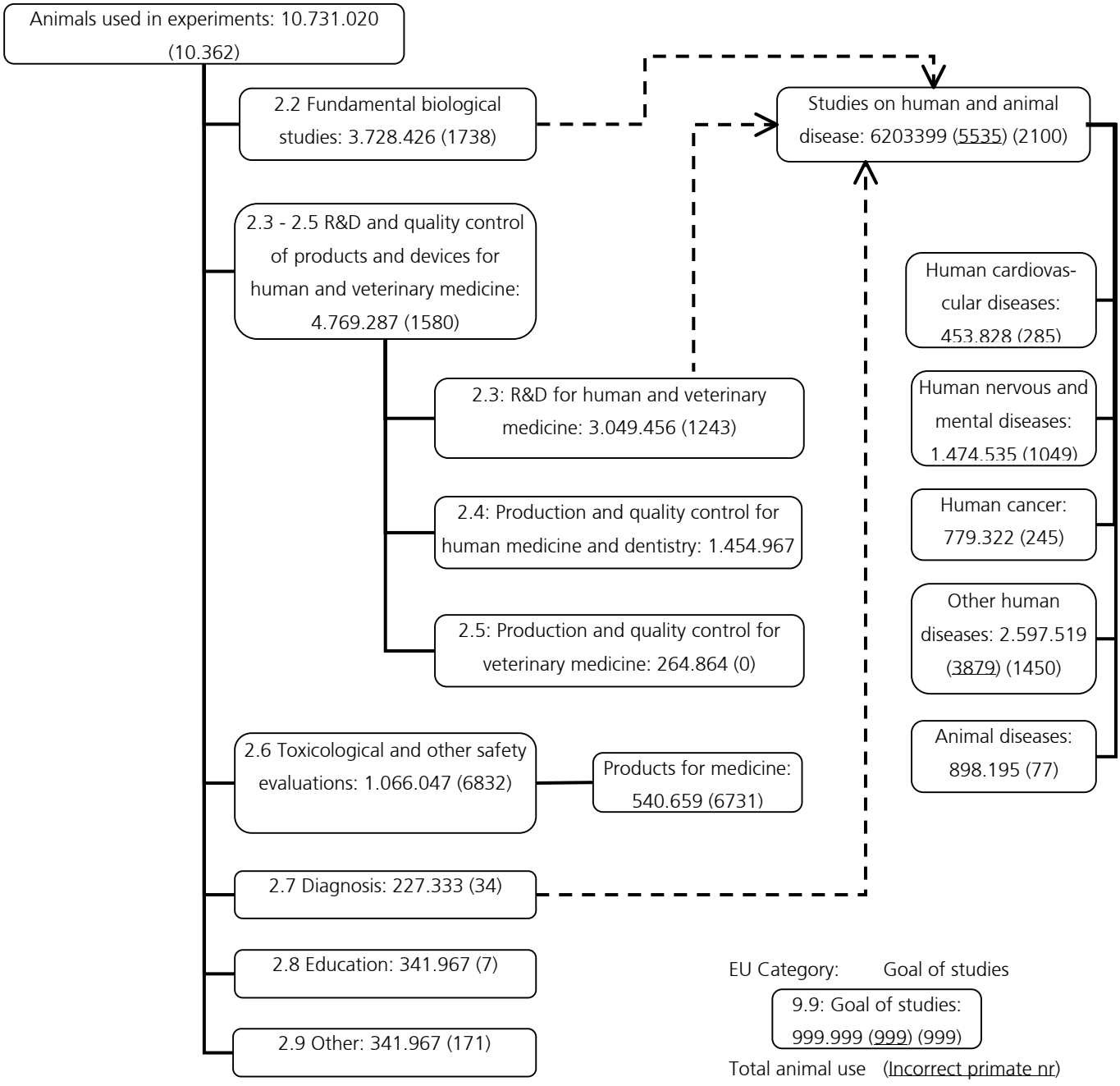
Appendix 5

Use of primates in the Netherlands in 2004 (NL statistics)



Appendix 6

Use of primates in the EU in 2002



Appendix 7

Calculations on use of primates for OPV

Primates are used for the production and testing of oral polio vaccine (OPV), which consists of three strains in different amounts. Therefore, the frequency of neurovirulence testing differs between the strains. There are several ways to calculate the total use of primates for neurovirulence testing:

- According to one respondent, for the United States' 300 million population, the NVT was carried out every 2-3 years for type 2, while type 1 and type 3 had to be tested 3-4 times a year. Thus a complete session of testing trivalent OPV would require one test for type 2 plus about nine tests for types 1 and 3. This would mean the use of 692 primates in 21/2 years, plus 100 primates every five years for the testing of a new working lot.
- When the US ceased using OPV in 2001, the use of primates decreased by more than 8000 animals in one year. Many of these animals may be used for the (repeated) NVT; this would mean that many more had been used for that goal than calculated above.
- With a world population of 6 billion, where most countries use OPV for immunisation, this would come down to an estimated thirty thousand primates in five years for worldwide neurovirulence testing: some six thousand primates a year.
- According to a WHO document, the size of bulk produced varies amongst manufacturers, and thus the number of doses per neurovirulence tests. Somewhere between 16 and 75 million doses would be tested per NVT [2003a], but it is unclear whether a complete set of NVT's for the three types are meant, or just one test for one strain.
- Another WHO document states that in 2004 3 billion doses of OPV were required [WHO, 2002c], which would mean some seventy neurovirulence tests. If a complete set of tests was meant, this would involve about 7000 primates, if separate NVT's were meant, about 3200 primates would be used each year.
- The Belgian manufacturer has been reported that 300-400 monkeys per year are used for neurovirulence testing, while it produces 0.6-1 billion doses per year. On this basis, some less than 2000 primates would be used per year [Langley, 2002].
- However, this is a large manufacturer, and smaller ones would produce fewer batches with the same number of NVT. In addition, there are numerous countries that produce their own OPV and do not depend on WHO supplies.

The results from these calculations differ between 7000 primates per year or even more, to an optimistic 2000 primates per year for the neurovirulence test. It is likely that around 4000-5000 primates are used every year on a global scale for the neurovirulence test