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SCHER

The need for non-human primates in biomedical research, production and testing of products and devices

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCHER adopted this opinion at its 27th plenary on 13 January 2009

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Scientific Committee members

Herman Autrup, Peter Calow, Wolfgang Dekant, Helmut Greim, Wojciech Hanke, Colin Janssen, Bo Jansson, Hannu Komulainen, Ole Ladefoged, Jan Linders, Inge Mangelsdorf, Marco Nuti, Anne Steenhout, Jose Tarazona, Emanuela Testai, Marco Vighi, Matti Viluksela

Contact:
European Commission
Health & Consumer Protection DG
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc8-Secretariat@ec.europa.eu

ACKNOWLEDGMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion:

Prof. W. Dekant (*Chairman and Rapporteur*), University of Würzburg, DE

Dr E. Testai, Istituto Superiore Sanità, IT

External Experts:
Dr. C. Bernardi, Preclinical Compliance Unit, Nerviano Medical Sciences, IT

Prof C. Cavada, University of Madrid, ES

Dr D. Jones, EMEA Safety Working Party, UK

Prof. D. Morton, University of Birmingham, UK

Dr E. Procyk, Stem Cell & Brain Research Institute, FR

Prof. M. Spangberg, Swedish Institute for Infectious Diseases Control, SW

Dr C. Stark, Systemic and Reproduction Toxicology Unit, Bayer DE

Dr J.W. van der Laan, EMEA Safety Working Party, UK

Dr K. Wickstrom, Medical Products Agency, SW

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1. BACKGROUND

Directive 86/609/EEC[1] on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes provides for controls of the use of laboratory animals, and sets minimum standards for housing and care as well as for the training of personnel handling animals and supervising experiments.

Since 1986, important progress has been made in science and new techniques are now available requiring specific attention, which the current Directive does not provide. Nor is the use of animals with a higher degree of neurophysiological sensitivity, such as non-human primates, specifically regulated. Therefore, DG ENV is currently revising the Directive.

The revision addresses issues such as compulsory authorisation of all experiments, inspections, severity classification, harm-benefit analysis and compulsory ethical review. In addition, specific problems relating to the use, care and acquisition of non-human primates are addressed.

In 2002, the Scientific Steering Committee (SSC) highlighted the continuing need to use non-human primates in biomedical research.

Since then, a number of publications have been made on the need to replace the use of non-human primates in biomedical research due to ethical and scientific reasons. In July 2007, Animal Defenders International, National Anti-Vivisection Society and Lord Dowding Fund for Humane Research published a response to the SSC statement.[2,3] The most recent publications include December 2006 a report "The use of non-human primates in research" by Sir David Weatherall and a subsequent response to it by NC3Rs.[4,5]

Animal protection Non-Governmental Organisations (NGO)s campaign on the phasing out of all experiments on non-human primates. The arguments in support of phasing-out range from ethical to scientific. The essential element of the discussion is on the availability of alternative methods to replace the use of non-human primates. See e.g. British Union for the Abolition of Vivisection report "Ending Primate Experiments - Meeting the challenge" by Dr Katy Taylor and David Powell.

On 25 September 2007, the European Parliament adopted a declaration (0040/2007) urging the Commission to end to the use of great apes and wild-caught monkeys in scientific experiments; and to establish a timetable for replacing all primates in scientific experiments with alternatives.

The Commission stated in its response that with the current scientific knowledge a timetable with a fixed deadline to phase out the use of non-human primates in biomedical research was not possible[6]. However, the science is evolving rapidly in this field and novel technologies, such as genomics and computer modelling, are gradually emerging.

In order to participate in this debate in a balanced manner, independent scientific information is needed on the latest status of the possibilities to replace the use of non-human primates. DG ENV would therefore like to request the Scientific Committee on Health and Environmental Risks to issue an opinion in this context.

To support the co-decisions procedure and the related discussions at the European

[1] OJLSSS, 18.12.1986

[2] The Scientific Steering Committee: "The need for non-human primates in biomedical research", statement adopted 4-5 April 2002: http://europa.eu.mťcoinm/food/fs/sc/ssc/out253_en.pdf

[3] http://www.ad-mtemational.org/admmydownloads/ssc_response_english.pdf

[4] http://www.acmedsci.ac.uk/images/projectiihpdownl.pdf

[5] http://www.nc3rs.org.uk/downloaddoc.asp?id=563

Parliament and the Council, the scientific opinion would need to be available by the end of 2008.

Use of non-human primates in scientific procedures

Around 12 million animals are used each year in scientific procedures in the EU, and of these, around 10,000 are non-human primates.

Due to their genetic proximity to humans and highly developed neurophysiology, the use of non-human primates in scientific procedures raises specific ethical questions and practical problems in terms of meeting their behavioural, environmental and social needs in a laboratory environment. The capture of non-human primates from the wild is stressful for the animals and increases the risk of injuries and suffering during capture and transport. Furthermore, the use of non-human primates for scientific purposes is of the highest concern to some citizens.

Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes was adopted to improve the controls on the use of experimental animals and to harmonise practices in the area of animal experimentation in the EU. Article 7 of the Directive provides that *"an experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available."* It is therefore in the spirit of the Directive to encourage methods, which will ultimately replace the use of animals in experiments.

Further on Article 7 states that *"When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected. Experiments on animals taken from the wild may not be carried out unless experiments on other animals would not suffice for the aims of the experiment. "*

Currently, non-human primates are only used in circumstances where no alternative methods are available and no other species may suffice for the purposes of the research. NHPs account for less than 0.1 % of the total number of animals used in the EU (European Commission, 2005). The legislation on marketing authorisation of pharmaceuticals (EU 2001/83 as amended) requires the use of a relevant non-rodent species and as modern biotechnology-derived pharmaceuticals exert highly human-specific pharmacodynamic properties there is the need to study the safety in a species as closely related as possible to humans. As a consequence of these developments the majority (67%) of the non-human primates are used in scientific procedures for the testing of pharmaceutical products and devices for their safety and efficacy. The rest are used for biological studies of a fundamental nature as well as for the research and development of products and devices for human medicine, dentistry and veterinary medicine. Their use is claimed to be essential according to the state of the art and according to the scientific community (e.g. FP7 programmes sponsor scientific research using NHPs in several research programs such as on immune based diseases (e.g. multiple sclerosis), neurodegenerative disorders (Parkinson's, Alzheimer's, etc), infectious diseases (HIV, Malaria, TB, Hepatitis, SARS, etc.) and other serious diseases (Weatherall, 2006). But others disagree for a variety of reasons including ethical, alternative approaches and scientific quoting misleading data (BUAV statement, November 14 2008, Dr Hadwen Trust 2008).

Some alternative techniques are available and are successfully used to reduce the need to resort to non-human primates. In the EU, the proportion of NHPs in the total number of laboratory animals used in research is only one third (<0.1 % compared to approximately 0.3 %) of the ratio in the US. However, it is understood that, even with the current scientific knowledge, there are not enough validated alternative methods

available to replace the use of non-human primates in all areas of biomedical research at present.

2. TERMS OF REFERENCE

In view of the above, the Commission asks the Scientific Committee on Health and Environmental Risks to issue a scientific opinion on:

- The areas of research (fundamental, translational and applied) and testing of products and devices in which non-human primates are used today;
- The currently available possibilities to replace their use either with methods not entailing the use of animals or by resorting to other species of animals including genetically altered animals by type of research or testing;
- The scientific outlook as to their replacement in short, medium and long term by type of research and areas of testing with a view to establishing a specific phasing-out time-table;
- The opportunities for the reduction and refinement of their use in areas where no replacement can be foreseen in medium or long term as per the principles of the "3Rs"[6];
- Research areas that investments should be made to advance replacement, reduction and refinement of the use of non-human primates in scientific procedures.
- Possible implications in biomedical research (e.g. immune based diseases, neurodegenerative disorders, infectious diseases and serious diseases) should the use of non-human primates be banned in the EU

The mandate for SCHER specifically excludes ethical, economic, cultural and social aspects of NHP use as this is dealt with by other groups within the EU Commission and the EU Parliament.

3. OPINION

3.1. The areas of research (fundamental, translational and applied) and testing of products and devices in which non-human primates are used today

3.1.1. Overview on the use of NHPs in research and testing

Toxicity testing of pharmaceuticals in NHPs, under certain circumstances, represents an important part of the safety assessment of new low and high molecular weight pharmaceutical compounds and the use of NHPs in neurosciences and infectious disease research has generation important new insights into brain function and prevention of infectious diseases in humans. More than 100,000 non-human primates (NHPs) are used annually (Hau and Schapiro, 2006; Pieters, 2007) for biomedical research worldwide, with the USA, Japan and Europe as the main users. In 2005, the use of NHPs in the EU was 10,451 animals, representing 0.09% of the total (primate and non-primate) number of animals used (EC, 2007). Both the percentage and the absolute numbers have not substantially changed since 1999 (European Commission, 1999, 2003, 2005).

Nearly 100% of New World (NW) primates (e.g. marmosets) used for experimentation are captive bred and have been for sometime, in some cases are at F 4/5 generation. However, for Old World (OW) primates, the figure is around 95% and most of these are F1s (i.e. the offspring between wild-caught captive F0; F2 are offspring of F1 x F1 - long

[6] The "3Rs" Principle (Replace, Reduce and Refine the use of animals in experiments) were first established in a book "The Principles of Humane Experimental Technique" by W.M.S. Russell and R.L. Burch, l959

term captive but both F1 and F2 can be considered to be 'purpose bred')[7]. The majority of these animals are not bred in the EU, but in China, Indonesia, Vietnam, Cambodia, the Philippines, and Mauritius. It is still common practice in some breeding establishments to replace breeding stock with wild-caught animals to avoid in-breeding, and one way to reduce this dependence on wild caught animals is to use the F1 generation for future breeding stock and not for research. Alternatively, to avoid inbreeding, breeding establishments could first consider an exchange of F0 males in a similar way to zoos. Regardless, it is likely to take a considerable time before sufficient F2 generation animals are available to meet research needs (EFSA 2005; FELASA, 2006).

In regard to purpose breeding, there are several other aspects to be considered: The majority of the OW primates used for scientific procedures are still F1 generation animals, so that increasing the number of F1 animals available for breeding purposes could increase the number of wild-caught breeders needed to produce sufficient F1 animals both for scientific purposes and for breeding purposes for F2 animals for Europe during the transition time. In addition, experience of some of the breeders is that breeding from F1 and F2 generations has resulted in some unexpected difficulties not only because of in-breeding, which can be overcome, but also because of a decreased birth rate, poor mothering, a higher incidence of reduced birth weight, and diabetes in the off-spring (ILAR conference 2008). However, against this is the scientific value of using purpose-bred animals to produce accurate, reliable and reproducible data (EFSA 2005). If using non-purpose bred animal results to generate data these may be more variable or less robust than when using purpose bred animals (see EFSA 2005, Appendix A, ETS 123 Council of Europe, 2007).

According to the information provided during a survey made by SCHER, wild-caught animals are rarely used in academia, but exceptions include research in aged animals and research in pregnant animals (breeding rate higher in wild-caught animals). Of the 72 academic institutions responding to the survey, only 4 used wild-caught animals. In addition, at only 6 sites the majority of the animals used were F1s. In the pharmaceutical industry (survey in 18 companies and 4 contract research organisations using NHPs), only two companies used wild-caught baboons or wild-caught Cynomolgus. For Cynomolgus, 18 of the 22 institutions used F1s and 9 institutions used 80 to 100% F2s. Of the 6 institutions using Rhesus, 3 used F1s. As outlined below, the majority of OW primates are used for applied research and safety testing, so that it has to be assumed that the majority are F1s. This further supports the assessment of EFSA that phasing out use of the F1 generation in the EU in research will take 'considerable time' (no time was specified), others suggested that it would be at least 15 years (FELASA, 2006).

The European Commission regularly publishes the statistical data on the number of animals used for experimental and other scientific purposes in the EU (Report for the Council and the European Parliament in accordance with Article 26 of Directive 86/609/EEC). The 'Fifth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union' (EU, 2007) covers data collected in 25 Member States and gives an overview on the year 2005 with the exception of France who reported data from 2004. According to the Report, Great Apes were not used in experiments in the EU in 2005. Just for comparison to the 10,451 NHPs used in Europe, 54,998 NHPs were used in the USA in 2004. This number has fluctuated for years around 53,000, but after a sharp decline in 2001, the number of primates used in the US is again steadily increasing.

However, comparing NHP use in 2002 and 2005 in the EU, the number of prosimians decreased by 38% while NW primates increased by 31% (Table 1). Member States reported that these changes were attributed to increases in the number of studies

7 There is no definition of which generation (F1 or F2) is to be considered as 'purpose bred' in either Directive 86/609/EEC or in the EFSA report 2005, and much confusion has arisen because of this. Both F1 and F2 are not born or raised in the wild, but F1s have wild caught parents.

performed for pharmaceuticals and in toxicological safety testing, which are the main uses of NHPs (EC, 2007).

Table 1 - Changes in NHP use between 2002 and 2005 in the EU

Species	Animal n° in EU 25 2005	Animal n° in EU 15 2002	Change since 2002	% change since 2002	Animal n° in EU 15 2005	Change since 2002 in EU 15	% change since 2002 in EU 15
Prosimians (Prosimia)	677	1095	-418	-38.2	677	-418	-38.2
NW Primates (Ceboidea)	1564	1192	372	31.2	1564	372	31.2
OW Primates (Cercopithecoidea)	8208	8075	133	1.6	8151	76	0.9

The higher use of NHPs in some member states (see Table 2) is likely due to the presence of Contract Research Organisations (CROs) performing NHP testing. The number of animals used by a CRO is registered in the member state where the CRO is located, rather than in the country of the company requesting the studies.

Table 2 - NHP used in different EU countries

Species	**AT**	**BE**	**CZ**	**DE**	**EL**	**ES**	**FR**	**IT**	**NL**	**SE**	**UK**	**Total**
Prosimians (Prosimia)	0	0	0	99	0	0	578	0	0	0	0	677
NW Primate (Ceboidea)	0	0	0	408	0	1	433	17	50	12	643	1564
OW Primate (Cercopithecoidea)	56	449	51	1579	1	81	2778	395	277	63	2472	8202
Total	56	449	51	**2086**	1	82	**3789**	412	327	75	**3115**	10443

It is noteworthy that prosimians were only used in France, mainly for fundamental biology studies, and in Germany, where they were used almost exclusively for safety testing

Origin

It should be noted that in 2005, prosimians were, for the first time, all of EU origin. A similar trend is observed with the NW primates where an increasing number was either of EU or European Convention (ETS 123) origin (accounting for about 95%). Also, OW primates coming from the EU increased, but are still below 25%. It has also to be noted that OW primates are often re-used, mainly in R&D (Research and Development) and testing. According to EU statistics, 111 prosimians, 410 NW primates and 1,740 OW primates were re-used. As an example, in the Netherlands in 2004, 289 NHPs were used in 701 experiments; 403 of these Experiments involved the re-use of NHPs (Pieters, 2007).

Species used

All categories of NHP are used in biomedical experiments. The cynomolgus (*Macaca fascicularis*) and rhesus monkey (*Macaca mulatta*) are most frequently used. The cynomolgus tends to be the most widely used species, but rhesus is also used mainly because of available background data. NW primates, mainly marmosets (*Callithrix jacchus*), are sometimes said to be suitable, due to their small size, for testing products that are generally only available in limited amounts. However, thus far this is not confirmed in the dossiers provided to the regulatory authorities.

Reasons for use

The European Union distinguishes six categories of research areas were animal experiments may be conducted. These are present in the forms to be used by Member

States (MS) to communicate data on use of experimental animals.

The six categories defined for use of animals in experimental purposes in Europe are the following:

1. Biological studies of a fundamental nature (2.2)
2. Research, development and quality control of products (2.3) and devices for human medicine (2.3), dentistry (2.4) and for veterinary medicine (2.5)
3. Toxicological and other safety evaluations, including safety evaluation of products (2.6)
4. Diagnosis of disease (2.7)
5. Education and training (2.8)
6. Other (2.9)

The distribution of NHP testing in relation to the different purposes, according to the six categories defined by the EU is reported in Table 3. No NHPs were used specifically for veterinary medicinal products (2.5). However, it should be realised that many veterinary medicinal products are the same as those developed for humans. The largest use is for 'Toxicological and other safety evaluations, including safety evaluation of products' (67% - mainly OW primates). When these data are analysed to identify the type of products tested in the safety evaluation included within category 2.6, 82% are products/substances/devices for human medicine and dentistry, being the rest used within the more unspecific category: other toxicological/safety evaluation (EC, 2007).

Table 3 - NHP used for different purposes (see above for description of categories)

Species	**2.2**	**2.3**	**2.4**	**2.6**	**2.7**	**2.8**	**2.9**	**Total**
Prosimians	384	0	0	97	0	0	196	677
NW primates	357	327	43	650	16	5	166	1564
OW primates	715	654	373	6257	0	37	174	8210
Total	**1456**	**981**	**416**	**7004**	**16**	**42**	**536**	**10451**
% of the total NHPs used	13.9	9.38	7.9	67	0.15	0.41	5.1	
% with respect to total animal used	0.036	0.026	0.029	0.68	0.008	0.21	0.054	0.09

Within the category 'Toxicological and other safety evaluations, including safety evaluation of products' (2.6), the number of NHPs used for safety testing for regulatory submissions is 6,992, representing almost 100% of the animals used in the category. In safety testing, 51% of the NHPs are used in sub-chronic and chronic toxicity studies and 34% for studies after a single administration to identify non-lethal clinical signs. A smaller percentage was used in 'Developmental and reproductive toxicity' (5.8%) and for other tests (7.9%). No studies were conducted where lethality was the primary endpoint. These percentages are more or less the same for all MS using NHP.

In fundamental biology research, the use of NHPs is detailed in Table 4.

Table 4 - NHPs used for different purposes in fundamental biology research

Species	Human cardiovascular diseases	Human nervous and mental disorders	Human cancer	Other human diseases	Studies specific to animal diseases	**Total**
Prosimians	0	383	0	0	0	383

NW primates	58	204	2	810	0	1074
OW primates	53	167	179	2882	9	3290
Total	**111**	**754**	**181**	**3692**	**9**	**4747**
% of total NHP use	2.33	15.9	3.81	77.8	0.19	
% total animal use	0.026	0.05	0.020	2.28	0.006	0.068

For the UK, statistics are available for 2007 (HO Stats, 2007). The number of procedures using NHP was 3,964, down by 240 (6%) from 2006, mainly due to a decrease in use of both OW and NW primates. Less than one percent of toxicological procedures performed used NHPs in 2007. Many primates were re-used since some of the procedures used on them had only a mild effect (such as taking blood samples).

It is important to note that major investments to improve housing conditions for NHPs have been made over recent years with 'enriched' and social group housing.

Based on response to a SCHER survey to pharmaceutical companies, experiments with substantial severity represent only a very small percentage (< 0.1 %) of NHP use.

3.1.2. Selection of non-rodent species for toxicological studies and rationale for using NHPs

Safety testing of chemicals is performed by a combination of many different approaches including animal experimentation. Whereas for industrial chemicals, toxicity testing in non-rodents is not required, inclusion of a non-rodent species is required in the safety assessment of pharmaceuticals. However, most of the safety testing for pharmaceuticals is also performed in rodents. Furthermore, non-animal methods play an important role in candidate drug selection and selection for further testing in animals, as well as for the selection of the animal species (Sietsema and Schwen, 2007). It needs to be noted that only a very small percentage of pharmaceuticals initially selected for further development are finally introduced into the marketplace since they fail on the bases of lack of efficacy or unwanted toxic effects predicted by the safety testing. While safety testing of new pharmaceuticals and other medical products represents one of the major uses of NHPs, only few candidate pharmaceuticals are actually tested in NHPs. Normally, there is no routine requirement for the use of NHPs as a second species.

Animal safety testing of pharmaceuticals is intended to safeguard human subjects used in the clinical trial studies through risk assessment based on the results of animal experiments. The Declaration of Helsinki[8] is a set of ethical principles developed by the World Medical Association (WMA) for the medical community regarding human experimentation. It states that the wellbeing of the human subject should take precedence over the interests of science and society.

International regulatory authorities including the European regulatory authorities therefore require that the safety of a new medicinal product is supported by a variety of non-clinical data prior to the start of clinical studies. The scope of testing is regulated in the EU by Council Directive 2001/83/EEC and its amendments.

[8] Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

As a consequence, the European Medicines Agency (EMEA) Committee for Medicinal Products for Human Use (CHMP) prepares scientific guidelines, in a global harmonisation process in the framework of the International Committee for Harmonization (ICH), to help applicants prepare marketing-authorisation applications for medicinal products for human use. The safety guidelines are written to ensure that duplication of studies is not required for various regions in the world. These guidelines also indicate that the non-clinical studies are performed in "relevant species", and that pivotal studies for risk assessment of pharmaceuticals, such as the repeated dose toxicity testing, have to be performed in two species, one of which must be a non-rodent.

In Europe, medical products tested in NHPs over recent years include all classes of pharmaceuticals and the main reason is the fact that no other species showed the same primary pharmacodynamic response. NHPs are also selected when they represented a well-established model for pharmaceuticals of that class or are the most relevant species for detecting known side effects. In addition, NHPs are used in testing because of recommendations from regulatory agencies including the US FDA, the EMEA, the Japanese authorities, and the WHO. For vaccines, some European Pharmacopoeia monographs, the US Code of Federal regulations (US CFR) and WHO monographs require that bulk and/or seed lots of live viral vaccines are tested for safety (i.e. neurovirulence) or potency on defined numbers of NHPs.

The species for toxicity testing selected based on its similarities to humans with regard to pharmacology and pharmacokinetics, including biotransformation and in certain cases also where anatomical similarities are essential. The use of a non-rodent species for the characterisation of new medicinal products aims at limiting the uncertainty in the extrapolation process from animal toxicity data to the human situation. Such uncertainties are species variation, scaling from small, short-lived animals to large, long-lived species, and use of a homogeneous animal population (NCB, 2005). Dogs are most frequently used as the non-rodent species, and NHPs are only used when testing is considered essential for safety assessment.

The CHMP has defined criteria on the demonstration of relevance of an animal species to predict human safety (EMEA/CHMP/SWP/28367/07).

The scientific requirements specific to the substance include:

- Presence of the required pharmacodynamic binding site and response
- Similarity to human toxicity or pharmacokinetic profile based on *in vitro* data or prior experience with related compound(s) of the same class
- Similarity to human in aspects of anatomy or physiology of specific organ systems
- Indication for the need of an additional species to investigate a toxic effect or the effects of a significant metabolite in humans which is not produced in the original non-rodent species

The ABPI and Home Office (2002)[9] gave additional specific recommendations on the selection and justification of the relevance of an animal species for safety testing:

- Use of a well characterised species may be quicker and require fewer animals
- Unknown and contradictory neurophysiological sensitivity (meant to reflect differences in suffering, harm etc) of the species (e.g. dog vs. pig)
- Public perception (e.g. dogs and other pets)
- Limited availability of new pharmaceutical in early stages requesting small size animal to allow fast development of new pharmaceutical for serious medical condition

[9] ABPI and Home Office (2002) Non-rodent selection in pharmaceutical toxicology: A 'Points to Consider' document, developed by the ABPI in conjunction with the Home Office.

According to all these recommendations, NHPs should only be used when it is scientifically demonstrated that none of the other non-rodent species commonly used in safety testing is appropriate for the purpose of the study.

To illustrate, safety testing in the NHP may be preferred over that in other mammalian species in the following cases*:*

- Due to the similar menstrual cycle and the anatomy and physiology of the mammary gland of NHP females and human females, NHPs (cynomolgus monkeys) are the more pertinent species in term of predictivity of relevant reproductive effects (Buse et al., 2003; Cline, 2007; Luetjens et al., 2005) and are therefore often chosen as non-rodent species for classes of compounds, which are expected to provoke effects on the female genital organs.
- Regarding the ocular system, the retina of NHP and man show some unique features (e.g. both NHPs and humans have a macula lutea/fovea) not found in other mammals (Stone and Johnston, 1981) and therefore NHPs represent a more relevant model of specific ocular effects for discovery and development of new pharmaceuticals as compared with other species.
- NHPs are less susceptible to vomiting than dogs. Thus, pharmaceuticals with an emetic effect in the dog may be tested in the monkey (Weber, 2005). Vomiting does not only limit exposure of the pharmaceutical administered, but is also a major hurdle to accurately characterise early effects on behaviour and on the cardiovascular system.
- The blood coagulation system of NHP is more similar to humans than that of any other species (Abildgaard et al., 1971; Lewis, 1996) and thus, NHP are often the most suited model for humans to assess potential toxicity of coagulation factors and anti-coagulation agents.
- NHPs are the most appropriate animals to characterise safety of many biotechnology-derived pharmaceuticals, especially monoclonal antibodies, since the most relevant species for testing is selected based on species-specific aspects of the immune system. Monoclonal antibodies are highly specific to their targets and accurate prediction of 'on-target' effects requires testing in a species which shows cross-reactivity, thus frequently requiring testing in NHPs as only species cross-reacting with humanised monoclonal antibodies (APBI-NC3Rs, 2006; Weatherhall, 2006); (Chapman et al., 2007).
- Recent regulatory guidance for assessing human drug abuse of new central nervous system (CNS) pharmaceuticals may further increase the need for testing in NHPs, since all CNS-active pharmaceuticals with properties indicating stimulant, depressant, hallucinogenic, or mood-elevating effects require an evaluation of abuse liability (EMEA/CHMP/ SWP/94227/2004). Whilst the rat is in principle acceptable for self-administration studies in the EU, NHPs are preferred in Japan.
- Historically, non-primate species have been used for reproductive toxicity studies, generally mice, rats and rabbits. However, rodents and rabbits are not necessarily the most accurate predictor of teratogenicity or reproductive toxicity in humans due to differences in placental anatomy and number of foetuses. In addition, they are not suitable models for all aspects of human reproductive toxicity, specifically for the investigation of agents suspected or known to interfere with the menstrual cycle. In such cases, NHPs may be more predictive for human toxicity. The male cynomolgus is also a good model of male fertility in specific cases (Ehmcke et al., 2006; Millar et al., 2000). Rodents can also not be used to assess the safety of novel hormonal intrauterine devices or cognitive dysfunction associated with the menopause (Schlatt et al., 2008; Wistuba and Schlatt, 2002). The need for NHPs in specific aspects of reproductive toxicity testing is exemplified with lenalidomide, a compound recently approved to treat multiple myeloma. Lenalidomide is structurally related to thalidomide, a known human teratogen that caused severe

birth defects during the late 1950s and early 1960s when given to pregnant women suffering from morning sickness. In rodent reproductive toxicity studies, lenalidomide did not induce teratogenic effects. In rabbits, although reproductive toxicity was evident, no limb abnormalities were observed, while in a group of animals treated with high doses of thalidomide, there was a significant incidence of multiple limb abnormalities (Revlimid, EPAR). Studies with thalidomide in monkeys have shown high similarity in teratogenicity to that documented in humans, both in terms of doses and types of malformation (Hendrickx et al., 1983). Since the Cynomolgus monkey appeared highly relevant for humans, both with regards to pharmacological and toxicological effects of lenalidomide, reproductive toxicity testing in NHPs was requested by the CHMP. These studies showed that lenalidomide produced malformations (short limbs, bent digits, wrist and/or tail, supernumerary or absent digits) in the offspring of primates that received the drug during pregnancy. Consequently, lenalidomide is expected to be teratogenic also in humans and specific precautionary measures need to be taken if lenalidomide is to be given to women of child-bearing potential.

The development of therapeutical monoclonal antibodies has resulted in an increased need for primates in reproductive toxicology since the immune system in macaques is much more similar to humans compared with rodents. In addition, the ontogeny of the immune system differs between rodents and primates, including humans, in that the immune system in rodents is less mature at time of birth. Rodent safety testing may therefore miss potential effects in a critical phase of development *in uterus.*

The need for specific safety and efficacy testing of drugs used in paediatrics in "young" animals may further increase the use of NHPs since the age-dependent development in NHPs is very similar to humans and NHPs may therefore be preferred over rodents as a test species.

Selection of the non-rodent species for safety testing is considering species specificities and also recognizes that toxicity testing in NHPs is not always predictive of all aspects of human toxicity. For example, regarding liver toxicity, the dog may be more representative of human metabolism than NHPs and in general, hepatobiliary toxicity in humans has been poorly predicted from animal studies (Peters, 2005). Although the NHP is the most representative species with regards to several of the aspects of the human immune system, there are important differences in, for example, parts of the T-cell intracellular signalling pathways. This was illustrated in the TGN1412 case which resulted in severe side effects in humans, but induced only a weak signal in NHPs (Waibler et al., 2008). Existing animal models also have limited capability for prediction of certain types of drug allergy in humans (Bala et al., 2005).

Therefore, for a safety assessment of a medical product it is required that all relevant information gathered from a variety of animal and non-animal models including computer-based prediction of biotransformation for a final conclusion based on a weight of evidence approach (Boobis et al., 2008; Doull et al., 2007).

Examples for major new treatment options for debilitating diseases where NHPs have been used in the safety assessment as the best available model for humans due to close similarities in physiology and anatomy are the development of a humanised recombinant antibody to treat severe asthma (EMEA, Xolair), an antibody directly injected into the eye to inhibit vascular endothelial growth factor thus preventing neovascular age-related macula degeneration often resulting in blindness (EMEA, Lucentis) and the approval of prostaglandin analogues in eye drops to decrease intraocular pressure thereby preventing loss of vision in patients with glaucoma (Stjernschantz, 2001).

3.1.3. Uses of NHPs in research regarding treatment and prevention of infectious diseases

The development of safe and effective intervention strategies against emerging and currently circulating human pathogens, like vaccination and treatment with antibiotics,

antivirals and other medicines, are urgently needed. The three major global health threats are HIV, malaria and tuberculosis (TB), but new pathogens, such as the SARS-corona-virus and avian/pandemic influenza viruses, are emerging. In the developing countries, AIDS, malaria and TB are major sources of morbidity and mortality and have a severe impact on the economic burden for affected families (Russel, 2004).

Several vaccines currently used to protect humans against fatal infectious diseases have been developed through studies in NHPs. Before new candidate vaccines can be evaluated for efficacy in humans, their efficacy has to be assessed in animals. For several infections, NHPs are the only animal species susceptible to the infectious agent and proof-of-concept of candidate vaccines can therefore only be studied in these species. As an example, the final confirmation of the efficacy of smallpox vaccines must be performed in NHPs, using exposure to monkeypox (EMEA/CPMP/1100/02). Other infectious agents for which NHPs have been a valuable resource for vaccine research include influenza virus, paramyxoviruses, flaviviruses, arenaviruses, hepatitis E virus, papillomavirus, Mycobacteria, Bacillus anthracis, Helicobacter pylori, Yersinia pestis, and Plasmodium species (Gardner and Luciw, 2008). Furthermore, to understand the mechanisms of protective immune responses induced by candidate vaccines, it is critical to use an animal model in which the immune system closely mimics that of humans. The choice of the animal model for testing new vaccines and drugs will largely depend on the nature of the pathogen. Many human pathogens have co-evolved with their primate hosts for many millions of years, and a process of mutual adaptation of pathogen and host has taken place. For this reason, many studies on pathogenesis and subsequent intervention studies are most effectively carried out in NHPs.

Vaccination studies conducted in rodents are not easily translatable into clinical trial protocols, due to qualitative differences between rodent and human immune systems. Therefore, the validity and the quality of the induced immune response to the vaccine need to be assessed in an animal model that is genetically very close to humans. Safety assessment of the candidate vaccine, which is required before entering into clinical trials, needs also to be performed in NHPs.

The use of NHPs may be necessary for the rapid identification of newly emerging infectious diseases with pandemic potential. For example, studies on NHPs led to a rapid development of the appropriate intervention strategies, which effectively prevented a pandemic spread of the SARS-coronavirus (Osterhaus et al., 2004).

HIV

The spread of HIV/AIDS can probably not be stopped without the use of an easily accessible vaccine. However, the immediate goal is not to develop a 100% effective vaccine, but a vaccine that at least partially protects against HIV infection and also protects against development of AIDS in patients already infected. A 50% effective vaccine given to just 30% of the population, could reduce the number of new HIV infections in the developing world by more than half in 15 years (IAVI, 2006).

Preclinical studies in NHPs play a key role in AIDS vaccine development (Morgan et al., 2008). Vaccine efficacy data are generated from immunised NHPs challenged with either simian immunodeficiency virus (SIV) or chimeric simian/human immunodeficiency (SHIV) virus (Letvin, 2005). Macaques infected by SIV develop clinical signs very similar to those in humans infected by HIV. In addition, the development of the disease in macaques is predictable from the viral load in the blood at early stages of infection (Sato and Johnson, 2007). Mucosal immunity is known to play a critical role in the susceptibility of humans to HIV infection. In this context, NHPs, which are also susceptible to infection via the mucosal route and thus mimic the natural infection in humans, are a unique model that currently cannot be replaced by *in vitro* systems (Yuki et al., 2007).

In addition to their traditional utilisation to judge vaccine safety and immunogenicity, NHP models are also employed to probe fundamental mechanisms of primate immune system regulation, to investigate pathogenic mechanisms of AIDS, and to optimise immunisation strategies involving novel vaccine vectors (Staprans and Feinberg, 2004).

Animal models can only be validated after successful trials in humans and the determination of correlates of protection. The HIV-vaccines tested to date in phase III trials in humans have failed to achieve the desired protective threshold. Therefore, we cannot at present judge the full validity of the currently used NHP models for vaccine research. However, NHP models yielded data on immune responses to vaccines congruent with clinical data (Makitalo et al., 2004; Sandstrom et al., 2008). This finding suggests that primate models are valuable as adjunctive testing systems to prioritise future therapeutic and vaccine strategies (Haigwood, 2004). In fact, there is now a growing consensus in the field that candidate vaccines should be studied even more thoroughly in NHPs before moving into large and expensive clinical trials (Morgan et al., 2008).

Tuberculosis

According to WHO (WHO, 2008), approximately one third of the world population was infected with Mycobacterium tuberculosis in 2006, and more than 1.7 million people die from tuberculosis (TB) every year. The current TB vaccine (BCG) was developed at the beginning of the 20th century, and is still the most widely used vaccine worldwide. However, its efficacy is varying. In the last 15 years, new strategies to improve or replace BCG have led to several candidate vaccines being evaluated in human clinical trials. These vaccines are based on the "prime-boost" principle, and have been extensively tested in animals, including NHPs, before clinical trials (Ly and McMurray, 2008).

As for many infectious diseases, there is no ideal experimental animal model for TB, and information has to be gathered from studies in various animal species. NHPs develop pulmonary granulomas in response to Mycobacterium tuberculosis and show an immune response similar to humans. There are differences between macaque species in response to vaccination and protection against infection. Comparative studies in these closely related species are likely to provide insight into mechanisms involved in protection against TB (Langermans et al., 2001).

The mouse and guinea-pig provide important and often complementary answers to TB vaccine questions. Before proceeding to human studies, however, it is necessary to perform confirmatory studies on efficacy in NHPs (Kaufmann, 2000). Only the most promising vaccine candidates are considered for NHP experiments.

Malaria

In 2005, the WHO/UNICEF reported that approximately 350 to 500 million people were infected with malaria (WHO/UNICEF, 2005). More than one million die from the infection each year; many of them are children under 5 years of age. The challenges to develop a successful vaccine are great, as there are 4 species of malaria that infect people. In addition, during the course of malaria infection, the human host is confronted by four distinct life cycle stages of the parasite. Each of these life stages presents new antigens (targets) to the immune system. Human genetic differences can also affect the level of immunity in response to a vaccine. Therefore, a vaccine against *Plasmodium falciparum*, the most serious malaria parasite, must account for the genetic diversity of both the parasite and the human host, and provide effective immunity against all different life cycle stages of the parasite.

The owl monkey (*Aotus*) and the squirrel monkey *(Saimiri)* are the only species (besides the chimpanzee) that are susceptible to the human malaria parasite and they are used (in very limited numbers) to test the potential efficacy of human malaria vaccines (Gysin et al., 1996; Herrera et al., 2002). The rhesus macaque has also been used to study the immunogenicity of candidate vaccines, without studying protection against infection (Stewart et al., 2006). A candidate vaccine developed with the use of NHPs is now in Phase III studies. Although many challenges have yet to be overcome, the development of an effective malaria vaccine is likely (Dolan and Stewart, 2007).

Other infectious diseases

As an example of other infectious diseases, hepatitis C virus infects about 170 million people worldwide (WHO, 1999). The search for a vaccine against this disease is complicated by the fact that the only species besides humans that is susceptible to the hepatitis C virus is the chimpanzee. In earlier phases of hepatitis C vaccine development, *in vitro* techniques and other animal species are often used, and chimpanzees are only used for testing the efficacy of very promising candidate vaccines. Recently, a vaccine capable of eliciting virus-specific immune responses in baboons and genetically altered mice was developed. When testing this vaccine in chimpanzees, a significant immune response to the hepatitis C virus was elicited, and the virus became essentially undetectable in the infected animals for at least a year (Contie, 2007).

In Europe, studies with chimpanzees are not performed, and research groups that are studying this virus must utilise laboratories in USA and other parts of the world to perform the necessary experiments.

3.1.4. Use of NHP in Neuroscience

Research in neuroscience collects knowledge on how the brain works in healthy human subjects and after disease and injury in humans and in experimental animals, including NHPs. Basic research is required in order to better understand how the brain works normally and in pathological conditions (Editorial, 2008). The main reasons to use NHPs in neuroscience are the close similarities between NHP and human brains in terms of overall anatomy, cellular structure and chemical communication, functional and cognitive abilities, neural circuitry, and in brain injury and diseases. This knowledge is useful, not only to understand effects and consequences of brain and spinal cord damage in humans and to devise therapies, but also help to construct new experimental models *in silico* and *in vitro*, and to develop new computational technologies. There is a continuous iteration between basic, translational, and applied medical research in neuroscience (Fitzsimmons et al., 2007; Moritz et al., 2008).

Neural injuries and diseases encompass disorders like epilepsy, cerebrovascular disease, depression, addiction, Alzheimer's disease and other dementias, Parkinson's disease, and multiple sclerosis. These diseases have an important impact on society in terms of number of affected humans and their relatives affected (WHO NeuroAtlas, EBC). In 2004, 127 million, or one in three, European citizens were living with a brain disorder at a total cost of €386 billion (EBC, 2008). Brain research received 8% of the life science budget in the European Commission's Fifth Framework Programme of research (FP5, 1998-2002), and 10% of the FP6 budget (2007-2010), a proportion that is likely to grow (EBC, 2008).

The unique role of NHPs in neuroscience research

Although major advances have been made in past 50 years, our knowledge on human brain function is still limited and the use of NHPs remains crucial for a significant advancement of neurosciences (Weatherhall, 2006).

Much of our current understanding of nerve cell function is based on studies in animals such as the cat, rat and even invertebrates such as squid where brain structure and circuitry is much less complex as compared with humans. However, the organisation of nerve cells in a complex system such as the human brain is more likely be understood by studying a similarly complex primate brain. In fact, only because of recent studies in NHPs, the existence of primate-specific developmental features was discovered (Bystron et al., 2006; Dehay and Kennedy, 2007; Garcia-Cabezas et al., 2008; Letinic et al., 2002; Meyer et al., 2000; Sanchez-Gonzalez et al., 2005; Smart et al., 2002). Moreover, most current human neuroscience research is based on evidence first discovered in NHPs, e.g., the neural bases of working memory (Goldman-Rakic, 1995), dopamine-based learning (Schultz, 2002), motor function (Georgopoulos, 2000), and mirror systems (Fabbri-Destro and Rizzolatti, 2008; Rizzolatti and Fabbri-Destro, 2008).

Experiments using invasive neurophysiological recordings in NHPs raise some ethical concerns; however, our understanding of the functional organisation of the brain areas

involved in vision, sensation, hearing, motor control and cognition in primates and humans has significantly benefited from of such work. An example is the research on primate visual pathways that led to a Nobel Prize in the early 1980s which, as well as elucidating visual centres and the mechanisms in NHPs, went on to discover similar pathways in humans (Rees et al., 2000; Tootell and Taylor, 1995). NHPs currently provide the only model to systematically study the relationships between the activity of a single nerve cell and higher cognitive functions. It is relevant to note, however, that in some instances, such as during brain surgery, neurophysiological recordings can also be made on humans (Alonso-Frech et al., 2006).

Some non-invasive research techniques, such as transcranial magnetic stimulation (TMS) are used both in humans and NHPs, and provide highly relevant information on how the brain works, thus saving invasive studies (e.g. (Ellison et al., 2007). However, TMS can only be used to study human brain areas near the surface of the skull. If deeper areas need to be studied, it will require invasive methods (causing permanent and reversible lesions) in animals, including NHPs.

Motor control is another area where the use of NHPs has been instrumental for basic understanding of the production and control of arm movements (grasping and reaching) leading to brain-computer interface technologies which are of major relevance to help alleviate the consequences of brain lesions and spinal cord injuries (Moritz et al., 2008).

Only small aspects of the complex interactions in the brain can be studied using *in vitro* techniques. More complex interactions can be studied using brain slices, but this requires the sacrifice of animals. Cell cultures can be used to study synaptic mechanisms and cellular events occurring in single cells. For example, to study the role of neuromodulatory molecules (like dopamine) on a neural network requires the use of intact tissue because the location of receptors on the different cells at different stages of a local network is one of the important keys to understand how the neuromodulation will act on information processing by the network. Such mechanisms have been studied on rodents, in particular rat, brain slices. However, the characteristics of e.g. cortical arrangement of neural networks and distribution of the different dopaminergic receptors within the cortical layers differ greatly from rodents to primates. Although there are evolutionary trends of variation in receptor localisations between primate species, there are drastic changes between rodents and primates. Therefore, conclusions based on *in vitro* data are limited.

NHP models and treatment of diseases

Pain

The use of animals in pain studies for the basic mechanistic understanding and for the development of therapies is one of the most controversial areas of research, but it has been estimated that chronic pain of moderate to severe intensity occurs in 19% of adult Europeans, seriously affecting the quality of their social and working lives (Breivik et al., 2006). A workshop was recently held to review and discuss the potential and challenges of using ethically conducted studies in human patients and volunteers to replace animals in certain areas in pain research and in the development of new therapies (Langley et al., 2008), and it appeared possible in some areas.

It should be noted that pain research is mainly performed in rodents and very rarely with NHPs. Research on neuropathic (chronic) pain is still a major issue since neither animals nor healthy humans are good models.

Neurochemistry

The study of the neurotransmitters (chemicals transmitting information between neurons), their receptors, transporters and enzyme systems in the human brain is key to understanding normal and pathological brain function, as well as to reveal possible targets for treatment. Basic mechanisms on neurotransmission and neuromodulation can be studied *in vitro* and in non-primate species (such as rodents and rabbits) (Carlsson, 1993; Vandecasteele et al., 2008). However, due to the large evolutionary distance

between humans and rodents, the study of neurotransmitters and related molecules at the level of the entire brain requires research on NHPs. In fact, data on rodents may be misleading as their brain physiology and biochemistry is different from those of NHPs and humans (Bjorklund and Dunnett, 2007; Garrick and Murphy, 1980; Howell and Wilcox, 2002).

Neurological diseases

Brain disorders such as depression, schizophrenia, attention deficit hyperactivity disorder, autism, drug addiction and obsessive compulsive disorders, all involve malfunctioning of the highly developed primate frontal lobes and their interactions with other parts of the brain. This is also true of conditions such as traumatic head injury, Huntington's disease, stroke and some types of dementia, which also involve interactions between multiple systems in the brain (Gil and Rego, 2008; Van Hoesen et al., 2000). Such conditions cannot, or only partially, be reproduced in non-primate species. Even if NHP are not ideal models for the above conditions, their complex brains make them more suitable to further advance knowledge than non-primate species (Yang et al., 2008).

NHPs have been used to investigate neurological diseases occurring in human neonates. For example, rhesus can be a surrogate model for asphyxia insults at birth and accurately reflects the mechanisms and neuropathology seen in human newborns suffering from this condition. More recently, a premature baboon model developed for evaluation of bronchopulmonary dysplasia has been applied to the investigation of cerebral development and injury, revealing a high similarity in neuropathology to the premature human infant (Inder et al., 2005).

Stroke research does not use large numbers of NHPs, but the importance of their availability as a research tool is significant. An example of NHP use in stroke research was the development of the drug NXY-059 (Marshall et al., 2003) that had significant effects and reduce functional disability following ischemic stroke. The NXY-059 studies in NHPs were regarded as crucial in designing the SAINT trials in humans. While the SAINT I clinical trials showed some promise, the SAINT II trials revealed no significant effects. The review procedures from animal trials to clinical studies have been published and suggested a need, not to change the model, but the criteria for publication, evaluation and use of animal testing (Macleod et al., 2008).

Important efforts are being made to develop better models of psychiatric disease by screening large populations of animals to detect the presence of a similar disease in NHPs. In the study of depression, simple tests for anti-depressant-like activity of pharmacological substances are often based on known classes of therapeutically successful existing anti-depressant agents, but investigators have also endeavoured to reproduce factors in the laboratory that are believed to initiate depression in humans. Studies on large macaque colonies show that some individuals, in particular those of low social rank, express behavioural signs comparable with some human depression syndromes. Neuroimaging has shown activity deficits in NHPs, comparable with those in humans, thereby supporting their use for further neurobiological characterisation and modelling (O'Neil and Moore, 2003).

A model of Parkinson’s disease (PD) in NHPs has been highly valuable in studying its pathophysiology (Beal, 2001; Boulet et al., 2008; Emborg, 2004; Hamani and Lozano, 2003; Mounayar et al., 2007). The application of deep-brain stimulation (DBS) in humans with Parkinson’s disease derives from experiments in a NHP model showing that destruction or high-frequency stimulation of certain areas in the brain reversed Parkinsonian symptoms. Over 40,000 patients have now been treated with DBS worldwide, and there are 160 DBS centres in Western Europe. In addition, DBS is showing promise in other brain conditions such as drug resistant cases of depression, obsessive compulsive disorders, and Tourette’s syndrome. However, there are still significant cases of unpredicted adverse effects, and numerous patients who are not suitable for DBS show there is still a need for further research such as advancements in

DBS, local delivery of neural active substances, the application of neurotrophins and stem cells, gene therapy, and molecular neurosurgery (Hamani and Lozano, 2003).

Stem cell technology, like induced pluripotent stem cells, opens the possibility to use somatic cells of an individual to repair his own tissue, thereby removing some of the ethical issues concerning embryos and the problem of rejection. These technologies are being currently developed for the repair of brain tissue in Parkinsons, Huntingtons, in stroke and in spinal cord and brain injuries, but will likely require safety and efficacy testing in NHPs (Brundin et al., 2008; Chen and Palmer, 2008; Roh et al., 2008).

Research on sensory and motor systems has led to the new field of neuroprosthetics to restore the severe loss of sensory abilities or movement capabilities in paralysed patients (Weatherhall, 2006). The development of brain machine interfaces (BMI) has much benefited from neurophysiological experiments in NHPs showing that it is possible to use natural brain cortical neural activity to drive computers, robots, and artificial limbs, to restore volitional control of movement to paralysed limbs, and/or to compensate for perceptual deficits (Fitzsimmons et al., 2007; Moritz et al., 2008).

New fields in neurosciences

New fields and techniques to assess brain structure and function, such as Magnetic Resonance Imaging (MRI, now routinely used for diagnosis in humans), and functional MRI (fMRI), are rapidly developing and increasingly employed in human clinical and experimental studies. Even though they are powerful means to study brain function, these techniques also have important limitations as they only measure blood flow, which is an indirect measure of neuronal activity (Logothetis, 2008; Vanzetta, 2006; Vanzetta and Grinvald, 2008). Other valuable non-invasive techniques, that provide useful information on how the brain works, include multichannel electroencephalography (EEG) and magneto-encephalography (MEG), both of which have a better temporal resolution than fMRI, but still have poor spatial resolution. Thus, fundamental research, at the level of single neuron activity (that may be invasive), is still needed to obtain information about how the brain works to improve understanding of pathological changes. Additional non-invasive novel technologies, like magnetic resonance spectroscopy (MRS), provide information on the chemical composition of the brain. They represent potentially powerful tools to gather data on brain biochemistry; however, at present the information they can provide is much cruder than that provided by more precise methods, like positron emission tomography (PET), ligand binding, micro-dialysis and immunochemical techniques.

Improvement of molecular and cell biology has reached a point where genetically modified NHP models of brain diseases are becoming available (Yang et al., 2008). Such genetically modified models will, potentially, be of even higher predictability power for human outcomes than rodent genetically modified models.

3.1.5. Use of NHPs in Xenotransplantation

The shortage of organ donors for transplantation is a major societal problem and the waiting lists continue to grow due to limited organ supplies. Only a minority of patients who may benefit from a transplant will be able to receive one and 10-20% of patients on the waiting list for organ transplants will die before a donor organ becomes available (McManus et al., 1991; Leichtman et al., 2008). Furthermore, as the transplants themselves will also need replacing, this will exacerbate the situation. Novel sources of organs may help to reduce this shortage. In addition to treatment of the terminal failure of organs such as kidney, lung, liver and heart, transplantation is also being seen as a therapy for other diseases such as cystic fibrosis and for patients affected by diabetes and Parkinson disease.

The pig represents the most likely candidate as a source animal and NHPs represent the only useful proof-of-concept species. However, there are serious immunological incompatibilities between pigs and primates based on a specific immune response (anti-αGal), and only OW primates (such as baboons and cynomolgus), great apes and

humans have anti-αGal antibodies. While rodents have been generated that exhibit an anti-αGal immune response, the response is weak, severely limiting the credibility of results in experimental models of xenotransplantation (Gock et al., 2000). In addition, there are several concerns over infections transmitted between source animals and humans, physiological functioning, and long term side effects due to the degree of immunosuppression required.

However, some xenotransplants have demonstrated a 2-3 year recipient survival in life-supporting NHP models. This indicates that long-term survival of a xenotransplant is achievable, even when clinically acceptable immunosuppressive regimens are used (Zhong et al., 2003).

3.1.6. Conclusions

SCHER concludes that, from a scientific point of view, the use of NHPs, at the present time, is essential for scientific progress in a number of important areas of disease biology research and in safety testing:

- **Development** of pharmaceuticals, in particular safety testing, to assess potential toxicity in animals to identify unacceptable adverse reactions in humans. For specific pharmaceuticals including antibodies, NHPs may represent the most relevant animal model for specific aspects of toxicity testing because of their close similarity to humans.
- Understanding the pathophysiology of **infectious diseases** such as HIV/AIDS, where the NHP is the only susceptible species and therefore the only useful animal model to study the disease, and to develop safe and effective vaccines and therapies.
- Learning how **complex brains** of primates, humans included, are structured and function. Again, NHPs are the best model due to their close similarity to humans with regard to brain complexity and function. In addition, NHPs are the best model for some human brain conditions and have been critical in developing and testing novel and current treatments.
- Developing and testing **xenotransplantation** methodologies.

3.2. The currently available possibilities to replace NHP use either with methods not entailing the use of animals or by resorting to other species of animals including genetically altered animals

SCHER recognises that there are promising developments that have replaced NHP use. A number of alternative methods (either *in vitro* or using other animal species) have been developed and implemented over the last decade (e.g. the TgPVR21 transgenic mouse model for neurovirulence and potency testing of poliomyelitis vaccines) (EDQM).

The position of the SCHER relating to the use of animal testing in the context of the assessment of hazards and health risk assessment of chemicals, the **"Three Rs"** concept of **R**eplacement, **R**efinement and **R**eduction of animal use for experimental purposes has been stated before. The continuation of the high level of human health and environmental protection is identical to the position of the former CSTEE. This position is outlined in detail in opinions by the CSTEE in 2004 and by SCHER in 2005 (CSTEE, 2004; SCHER, 2005). This position can also be extended to areas of basic research where information generated will have a major influence on understanding basic physiological functions and mechanisms of pathophysiology, when a benefit to prevent or treat humans diseases can be expected in the longer term.

In the opinion of SCHER, animals should only be used in medical research when it is unavoidable and when appropriate and validated alternative methods are not available. Replacing animals in medicine research is a long and difficult process and application of *in vitro* or *in silico* methods are often not yet feasible due to highly complex systems and limited knowledge of basic biology and pathophysiology. In addition, experimental

models not using animals are often developed in medical research as complementary methods as they may only address questions at sub-cellular or single cell level, or, at best, at the level of interactions between a very limited number of cell types. When whole body integrated systems need to be examined, animal models have to be used in order to better understand the interactions between different cells in an intact organ, and between different organs. The importance of combining all approaches at the cellular, organ and whole body level are vital to a full understanding of the scientific issues.

SCHER also recognises that when animals are used as models of human conditions or as surrogates for humans, there are limitations to the accuracy with which the animal model reflects the pathophysiology, pharmacology or toxicological susceptibility of humans. In the cases examined in this opinion, the use of NHP is considered essential because other species provide demonstrably unsatisfactory models in crucial respects.

It should not be forgotten that humans are also used in experiments whether healthy human subjects, patients participating in clinical studies, and tissues from bio-banks. Furthermore, it is important that there is a constant feedback and iteration between human and animal research, as well as *in vitro* studies, to improve our knowledge and to make animal and human experiments more meaningful.

Safety testing of pharmaceuticals

In safety testing, regulatory requirements and scientific considerations may almost mandate the use of NHPs if NHPs represent the non-rodent species resembling humans most closely regarding pharmacodynamics and pharmacokinetics. It needs to be noted that testing of new pharmaceuticals in NHPs represents only a very small part of the total safety and efficacy testing. Results obtained in NHPs are introduced into the risk assessment process, which integrates all information from safety testing based on a weight of evidence approach. The total replacement of animals, including NHPs in testing for safety, is not possible based on present knowledge. Arguments against phasing out NHPs in safety testing of pharmaceuticals are therefore identical to those regarding using rodents for toxicity testing, i.e. incomplete knowledge of integrated body systems and pathophysiology, poor representation of pharmacokinetics by *in vitro* systems, and the absence of NOAEL or benchmark doses vital for human risk assessment (SCHER, 2005).

Regarding safety testing of the highly specific monoclonal antibodies and the other biotechnology derived products, NHPs are often the only relevant model for humans. In certain cases, genetically modified rodents, carrying the human pharmacological target, may replace NHPs. This requires, however, that downstream signalling is relevant for humans and that the alternatives are sufficiently well characterised. At present, genetically modified rodents as well as testing of the homologous protein in rodent species are usually considered as supportive data and not as replacements for the use of NHPs by regulators (Anonymous, 2008).

Micro-dosing is sometimes postulated to be able to replace some animal testing. Microdose studies in humans are considered to be clinical trials in accordance with the EU Clinical Trials Directive and, therefore, have to be supported by animal toxicity studies Therefore, micro-dosing cannot replace animal testing, and administration of chemicals or pharmaceuticals to humans in low doses to study pharmacokinetics and toxicokinetics (biokinetics) (Amberg et al., 1999; Monster et al., 1976) has been used for a long time in research. Recent developments in analytical chemistry such as LC/MS-MS or accelerator mass spectrometry have only refined microdose studies due to more simple sample workup and higher sensitivity. Micro-dosing in early human studies only investigates pharmacokinetics and is performed after administration of very low single doses (max. of 100-fold below the pharmacologically active dose in animals). As a prerequisite for performing microdose experiments in humans, single dose toxicity data in an appropriate animal model are needed to ensure that the microdose given to humans can be considered a safe dose. Thus, toxic effects are not expected in humans and a toxicity profile cannot be established. Toxicity in animals is the relevant endpoint in all safety testing and this can thus not be studied with micro-dosing. However, compounds with an

unfavourable human pharmacokinetic profile are not further developed and in that sense, the use of animals in toxicity testing may be reduced due to earlier termination of an unpromising compound. On the other hand, if a compound shows a favourable human pharmacokinetic profile in micro-dosing, all standard animal safety tests are needed for further clinical development, so that micro-dosing in humans can also result in an increase in the number of animals used for a specific compound (single dose toxicity study plus standard tests) (EMEA, 1994).

The US National Academy of Sciences has recently issued a report on "Toxicity testing in the 21st century". The report discusses a "vision" to reduce the need for animal testing based on a combination of *in vitro* testing, "omics"-technologies applied to *in vitro* systems, and physiologically-based pharmacokinetic modelling within the next decades. Animal testing should only be used when unclear results are obtained or specific concerns are present. However, it needs to be noted that the mandate of the NAS committee was restricted to environmental chemicals where daily human doses are much lower then those used in therapy with pharmaceuticals. Therefore, the conclusions of this report cannot be applied to pharmaceutical safety testing at the present time.

Infectious diseases

In infectious disease research and vaccine development, there are no ideal small-animal models for studying HIV infection. A model for investigating immunogenicity is the Trimera model, where a human immune system is introduced into wild-type mice. However, conclusions are limited since the viability of the human cell transplant is short and so the Trimera model is mainly used for the investigation of short-term immunity and rapid screening of candidate HIV vaccines (Ayash-Rashkovsky et al., 2005).

Generating a genetically modified mouse permissive to infection with HIV is difficult, since all species-specific factors needed for complete HIV replication in mice have not been identified. Recently, a mouse model (HIV/MuLV) has been established based on the infection by HIV-1 enveloped by a mouse retrovirus envelope. Since the mouse immune system is intact, studies of HIV candidate vaccines and adjuvants can be made over longer time periods compared with other models (Boberg et al., 2008). Similar to the Trimera model, HIV/MuLV-challenge system is primarily useful for screening candidate vaccines, but further testing of such vaccines requires studies in NHPs.

It has been claimed that the failure to achieve protection against infection in clinical studies of HIV vaccines invalidates the use of NHPs in preclinical vaccine studies (Gordon and Langley, 2008). However, over the years, new knowledge about the virus and how the immune system interacts with it has been gradually collected both in humans and in NHPs. As a result, new and better animal models have continuously been developed. In parallel, based in part on observations in these studies, *in vitro* techniques have given us a better understanding about fundamental reactions at a cellular level in the immune system. To date, *in vitro* systems only show that a given formulation can induce an immune response in human cells and cannot demonstrate that this immune response protects the host against viral infection. Therefore, NHPs cannot be replaced in this area of research at present and *in vitro* methods can only be regarded as complementary techniques.

Many alternative methods, without the use of NHPs, have also been used in search for a malaria vaccine. After publication of the genome of *Plasmodium falciparum*, the most important human malaria parasite (Gardner et al., 2002), genomics-related technologies, recombinant DNA and cell engineering have increased the knowledge about genes and pathways involved in human malarial infections. However, no *in vitro* system today mimics the complex biology of the human malaria parasites and the interaction with the immune system of the host. Infection of mice with sporozoites of *Plasmodium berghei* or *Plasmodium yoelii* to evaluate liver-stage protection by candidate malaria vaccines has not translated readily to effective malaria vaccines in humans. Thus, mice can be used to dissect basic parameters required for immunity, but may not represent preclinical vaccine models (Schmidt et al., 2008). While NHPs also have their limits in the studies of

protective immunity, they will likely be needed to develop human malaria vaccines in the future.

A cell culture system with a transfected human hepatoma cell line has been developed (Bartenschlager and Lohmann, 2001) that allows research on hepatitis C anti-viral treatment without the use of NHPs. However, for development of vaccines, it is still necessary to test the efficacy of candidate vaccines in chimpanzees.

Neurosciences

Magnetic resonance imaging (MRI) is now routinely used for diagnostics in humans. Functional MRI (fMRI) is also widely used in most fields of neuroscience. It is often considered as an alternative to replace research involving NHP. MRI measures changes in vascular parameters and relies on a link between neural activity and vascular variations (blood flow, oxygenation levels). It is thus only an indirect measure of neural activity. Moreover, fMRI is limited because our knowledge on neurovascular coupling and its underlying mechanisms is incomplete (Weatherhall, 2006). Recently, these issues became highly relevant in the context of how to correctly interpret the signals used for functional brain imaging (Vanzetta, 2006). In addition, the temporal and spatial precision of fMRI is low, measuring variations in blood oxygenation levels in the order of seconds, far from the millisecond range at which neural cells process information. Also, fMRI and other neuroimaging techniques (EEG, MEG) give large-scale functional views by being able to record activity or activations from the entire brain. Because of these characteristics fMRI studies cannot replace studies collected with invasive microelectrode techniques; these are much more precise anatomically and temporally. In summary, fMRI and microelectrode studies in NHP are complementary. In fact, the use of fMRI and of other haemodynamic-based functional brain imaging with NHP is of fundamental interest to bridge the vast knowledge acquired in NHP research with established techniques and data acquired in humans, as well as to understand the neural bases of what is measured using fMRI and to validate the technique itself (Logothetis, 2008; Vanzetta and Grinvald, 2008).

The very promising diffusion imaging techniques (DWI, DTI) use magnetic resonance imaging for the non-invasive detection and tracing of neural fibres. However, while the potential of DTI to study connectivity in normal and diseased human brains is highly significant, this technique still needs to be validated and evaluated by histological studies. Current shortcomings are lack of anatomical precision, the inability to evaluate the direction of fibres, and the fact that algorithms interpreting signals acquired with diffusion imaging are based on untested hypotheses (Tuch et al., 2005).

Computer modelling is rapidly improving and is expected to reach significant importance in the domain of robotics and the development of machines based on neural knowledge. Many laboratories integrate computer modelling in their research; it is important to acknowledge, however, that most available models still have poor prediction rates due to limitations in our present understanding of basic brain physiology.

Theoretical approaches to simulate brain anatomy and function depend on empirical data for verification. Neurophysiological and neuroanatomical investigations are the main sources for the development of biologically plausible computational models and neural network models require detailed knowledge on the characteristics of individual neurons, their connectivity and pharmacology. These are acquired with invasive neurophysiological methods. Moreover, knowledge of how the brain works in healthy individuals is important to better understand the pathophysiology of disease.

The Blue Brain project is the first comprehensive attempt to reverse-engineer the mammalian brain, in order to understand brain function and dysfunction through detailed simulations. The first phase was reached in 2007 with a complete modelling of a unique rat cortical column of 10,000 neurons that required the full computational power of a supercomputer. A realistic model of a primate brain will have to contain up to 100 billion neurons. While this approach is necessary for the advancement of neuroscience, there is no foreseeable time when an artificial model of a primate brain will be feasible.

Xenotransplantation

In vitro models may be useful in the initial investigations into the presence or absence of antibodies and receptors and rodent models of transplantation and xenotransplantation are usually simpler than primates, especially with regard to the measures required to control the immune response. However, they cannot replace long term studies of function in animals including NHPs.

3.3. The scientific outlook for NHP replacement in the short, medium and long term with a view to establishing a specific phasing-out timetable

The scientific progress in the highly complex and interacting areas of basic and applied research, which use NHPs, is difficult to predict. Therefore, a specific timetable cannot be defined. Based on the presently available science, the total replacement of NHPs in many areas of use, either by other animal species or by non-animal methods, is unlikely to be achieved in the foreseeable future.

Safety testing

Due to our still limited understanding of pathophysiology and mechanisms of toxicity, an adequately justified need for the use NHPs as the best surrogate models for humans in specific parts of the safety testing for pharmaceuticals and monoclonal antibodies is very likely to remain for the foreseeable future. Use of alternative non-rodent species may only reduce the number of NHPs for testing, but increase the use of other species.

Infectious diseases

Regarding HIV research, it is possible that a genetically modified mouse strain with a human-like immunity, in which complete HIV replication can take place, may be available in the future. Still, one of the biggest hurdles is to know how the immune response in a mouse model will actually translate into the protection of humans, especially since correlates for effective protection against HIV infection in humans are not known. It is therefore necessary to continue the HIV vaccine development in NHPs in order to learn as much as possible about the immune response. Human vaccine clinical trials will deliver immunological and efficacy data that may reveal the relevance of the immunity and protection studied in the NHP models. Such information can then be utilised to develop better models with genetically modified mice and/or *in vitro* studies. However, a mouse model for HIV vaccine research cannot totally replace the use of NHPs. From a regulatory point of view, studies on the efficacy and safety of the candidate vaccine in a relevant surrogate species, such as NHPs, will remain necessary before starting clinical trials in humans.

Neurosciences

Computer modelling and a wider use of modern imaging techniques such as MRI and PET may complement experimentation in NHP (see below). Because whole brain imaging technologies and invasive neurophysiological methods give qualitatively different information on brain function, those methods remain complementary and not alternatives. The future advancements in the development of non-invasive technologies will need to be regularly assessed.

Xenotransplantation

The development of artificial organs and tissue engineering may replace or reduce the need for NHPs, but, currently, artificial organs are mostly extra-corporeal devices and are not an alternative to organ transplantation. Moreover, complex functions of organs such as the liver cannot yet be replicated artificially.

3.4. The opportunities for the reduction and refinement of NHP use in areas where no replacement can be foreseen in medium or long term

3.4.1. Reduction

1. Reductions in routine toxicity testing in NHPs may be achieved by a more careful evaluation of results of biotransformation studies in other mammalian species, including other non-rodents. An improved screening for biotransformation may help to identify the animal species most similar to humans and may thus reduce the use of NHPs in cases when the dog is not a suitable non-rodent species. However, this may only shift testing to other mammals such as the minipig. Improved information exchange between industry may also help to reduce NHP uses as discussed for a reduction in dog use for safety assessment (Smith et al., 2002).
2. Overly restricting the re-use of NHPs may result in an increased number of NHPs being used for experimental purposes, and care has to be taken to differentiate re-use from continued use.
3. The use of NHPs in reproductive toxicity studies (now 15-20 animals per group, in 4 groups including control) with therapeutic monoclonal antibodies may be reduced. In the standard test design, the number of animals per dose group is selected to enable detection of the incidence of malformations. This standard study design involves giving antibody to this number of pregnant females between days 20 and 50 of pregnancy. Placental transfer in this period is very low to absent and, therefore, the risk of skeletal malformations induced by monoclonal antibodies is very low. Conventional studies on embryo-foetal development (so-called segment 2 studies) to look for consequences of *in vivo* transfer over the human placental barrier could be combined with peri-postnatal studies. The period at risk is the latter part of pregnancy where deviations can be induced in more functional parameters (kidney function, immune function).
4. A reduction of NHP use in safety testing of pharmaceuticals is also possible by harmonisation requirements for safety testing (for example regarding group sizes) by different regulatory agencies. A high variety in the number of NHPs used for safety testing, depending on the character of the product and its potential use in humans, is observed for biotechnology-derived pharmaceuticals registered in Europe between the late 1980s and 2003. A very low number is used for testing of diagnostic antibodies (used only once), whereas a much higher number of NHPs (around 300) may be used for testing a therapeutic monoclonal antibody. A reduction may be possible by improving and harmonizing the study designs needed for safety testing.
5. The application and implementation of the "Three Rs"-concept in the use of NHPs could be improved by facilitating greater collaboration between establishments, and by enhancing the exchange of information leading to a reduction in primate use as experiments will not have to be repeated. For example, a European network of laboratories using NHPs as well as laboratories working to replace animal experiments should be established to allow access to a pool of information and resources and the sharing of expertise, tissues and ideas for implementing the "Three Rs". Accessible databases, like those developed for neuroanatomical and pathology data could be very useful in this context.
6. More transparent information for choice of species and justification including numbers of animals used for specific testing should be available in public assessment reports.
7. Genetic and genomic research and the increased possibilities to create genetically modified humanised rodent models may lead to a possible reduction and partial replacement of NHPs models used for the development of vaccines and treatments against infectious diseases. However, a time frame for the successful completion of these studies cannot be given.

8. It may be possible to reduce the need for NHPs in xenotransplantation by using stem cell research and tissue engineering. However, these areas are still in an early research stage and far away from clinical applications.

3.4.2. Refinement

Refinement encompasses not only causing the minimum level of suffering consistent with obtaining the scientific objective, but also promoting the welfare of animals whenever possible.

1. Council of Europe revised Appendix A of ETS 123 of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes on which Commission Recommendation 2007/526/EC is based, sets down new standards for husbandry in NHP studies that have been recommended for adoption in the EU (Revised Directive 86/609/EEC). Refinements in the housing and care of animals that meet their mental needs will result in better animal welfare and better models. It is important that the standards laid out in Appendix A are adopted as soon as possible, and even exceeded as new scientific information on the mental needs and psychological wellbeing of NHPs is discovered. Poor housing and care standards will cause avoidable suffering and are likely to produce animals less suitable for studies, particularly in the neurosciences. Pharmacological and other data, e.g. cardiovascular such as heart rate and blood pressure, may also be affected by adverse welfare states such as pain, distress and fear.
2. Improved recognition of suffering in NHPs (e.g. behavioural signs) is needed, as well as understanding its impact on the animals, and requires further research. Recognition is key to taking any further action such as alleviating such suffering (through the use of anaesthesia and analgesia after painful procedures), and avoiding causing such suffering. This may include improved experimental design (see below), acclimatisation of animals, habituation to procedures such as training, with use of positive training techniques involving rewards, and ensuring the competence of all those involved in the NHP care and experimental procedures.
3. Experimental design strategy should be optimised, e.g. staging experimental challenges so that mild stimuli precede more severe ones, humane endpoints and withdrawal from study when a validated endpoint has been identified, early endpoints when object of the experiment has been achieved, or before if there is any significant pain and distress. Such strategies may also include not being able to achieve the scientific objectives as they will be frustrated by the degree of animal suffering. Moreover, identifying key lines of research at an early stage, the use of pilot studies, use of non-invasive technologies e.g. bioluminescence imaging, PET/MRI (micro and macro), long term and tissue friendly implants, telemetry may all result in refining research by causing less suffering.
4. The use of MRI and fMRI should help to refine the use of animals in neurophysiological and neuropsychological studies. Technologies like MRI are a way by which some intracerebral procedures or follow-up of brain interventions can replace euthanasia for target validations and invasive processes. MRI may also be applied to refine toxicological studies by avoiding invasive procedures.
5. In studies on vaccine development, early and humane endpoints should be included in the study design (e.g. viral load and CD4 cell counts in HIV vaccine studies, early disease symptoms monitored by using imaging techniques in Tb vaccine studies, etc).

3.4.3. Recommendations

Based on the available scientific evidence, at the present time, SCHER sees no valid scientific reasons to support a discontinuation of the use of primates in basic and applied research, or in the development and testing of new drugs. However, this position should be regularly reviewed in the light of validated alternatives that are constantly being developed.

To comply with all "Three Rs" alternatives, SCHER recommends investment and activities in the following areas of NHP use:

1. All uses of NHPs should be carefully justified before any work begins and any such justifications should be carefully monitored by the Member States and subjected to a retrospective evaluation at the end of each project.
2. Better research strategies (including the development of non-invasive methods which can be used in human volunteer studies, and the development of new *in vitro* and *in silico* technologies) should be supported and encouraged. Although the replacement of NHP use by new technologies should be encouraged, it should always be subjected to a careful scientific evaluation.
3. The anticipated benefits of NHP studies and scientific progress in developing alternative methods should be regularly assessed to ensure that validated alternatives are adopted as soon as they are reasonably and practical available. Regular meetings (e.g. workshops, conferences) should be organised by those involved in NHP research and supported by the Commission, to stimulate scientific discussion and exchange of information between researchers within the various fields of NHP use and scientists working actively with, and advocating for, alternative methods.
4. The development of accessible and comprehensive databases and collaborative users networks should be promoted covering aspects such as data sharing, tissue sharing, exchange of knowledge and information e.g. on animal models, alternative to animal models, in house data and experience, in order to further the "Three Rs" in European NHP research. Such networks should also include laboratories engaged in developing replacements for NHP use with non-animal methods.
5. Networking of facilities breeding and maintaining NHPs for experimental purposes should be supported by those involved in NHP research and by the Commission, in order to advance knowledge and competence in the areas of animal housing, care and breeding. The objectives should be the improvement of animal welfare, the standardisation of procedures and methods, and the availability of NHPs. Implementation of improved standards of husbandry and care, as laid out in Commission recommendation 2007/526/EC (Council of Europe Appendix A) should be achieved at the earliest opportunity to ensure good welfare and to support good science. This should include higher biosafety level facilities.
6. The predicted degree of severity for NHP work should be limited to moderate. However, derogation for predicted severe severity should be approved and justified by the competent and independent authority of the MS where the work is taking place.
7. In the specific context of NHP use, the use of other non-primate species, such as minipigs or genetically modified rodents, to replace NHPs should be further investigated and encouraged.
8. The use of wild-caught NHPs for experiments should be discouraged for both scientific and animal welfare reasons.
9. In view of the concerns raised by NHP users, breeders and suppliers regarding the transition towards using only F2 generation or higher in research, an evaluation of the animal welfare, scientific and economical aspects of such a use is recommended to take place on a regular basis, starting 5 years after the implementation of the revised Directive 86/609/EEC.

3.4.4. Other recommendations

10. All work done supported from sources in Europe with partners outside the EU should meet European standards of care and welfare in order to promote good science. Compliance should be assured by the research partners.
11. Sectors using NHPs and developing alternatives should organise global networks to exchange information on the "Three Rs", including giving clear and consistent guidance on the criteria for the use of NHPs, supported by the Commission.
12. Further negotiations with Non-European countries (such as the USA and Japan) for international harmonisation should be carried out on the requirements for safety testing of pharmaceuticals and vaccines (e.g. animal numbers, study design, endpoints) involving NHPs.

3.5. Promote research into areas that advance replacement, reduction and refinement in the use of NHPs in scientific procedures

- Further research in the use of genetically altered animal models or other suitable mammalian species in testing of vaccines and pharmaceuticals. However, the ethical aspects of such use should also be considered.
- Improved understanding of pathophysiological mechanisms (e.g. cell-cell interactions in the development of a toxic response) of toxicity to improve *in vitro* test systems to actually resemble the complex interactions likely to be involved in the pathophysiological responses to toxicants to serve as basis to develop better *in vitro* models.
- Improved understanding of the comparative physiology of the immune system in NHPs, humans and other non-rodents to develop improved models with higher predictivity.
- Research into the recognition of suffering in NHPs, and its classification, its avoidance, and its alleviation should be carried out as quickly as possible. This would help in addressing the predicted adverse effects and severity bands, any retrospective assessment of animal suffering, and limiting any pain and distress. A better understanding of social needs and housing requirements of NHPs and how to meet them best in experimental environment is also needed.
- Research to develop new accessible technologies should be supported in order to refine experimental procedures on NHPs. Such technologies might be non-invasive procedures such as imaging, biocompatible implants such as telemetry and data loggers. It is conceivable that their refinement will also greatly benefit non-invasive research of human brain structures. Furthermore, access for researchers using NHPs to facilities with newer technologies should be encouraged.
- Further research into use of stem cells to restore organ function may replace the need for NHPs in xenotransplantation,
- More research into an improved understanding of the neural basis of brain neurodynamic responses for the physiological assessment of the welfare of primates and scientific validity is required.
- More research is required into the stress-reducing potential of social housing, environmental enrichment, positive reinforcement training and other measures of behavioral management in relation to experimental procedures with NHPs.
- Creative work to stimulate or inactivate deep brain regions (Roth et al., 2007) may provide in the future novel means for non-invasive brain studies, including safe human brain research that could reduce, and, in specific instances, replace NHP experiments. Such work should be funded and encouraged.

4. LIST OF ABBREVIATIONS

ABPI	Association of the British Pharmaceutical Industry
BUAV	British Union for the Abolition of Vivisection
CHMP	Committee for Medicinal Products for Human Use
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
EBC	European Brain Council
EFSA	European Food Safety Authority
EMEA	European Medicines Agency
FDA	US Food &Drug Administration
FELASA	Federation of European Laboratory Animal Science Associations
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization of Technical requirements for Pharmaceuticals for Human use.
NHP	Non-Human Primates
NW	New World
OW	Old World
PET	Positron emission tomography
SARS	Severe Acute Respiratory Syndrome
SCHER	Scientific Committee on Health and Environmental Risks
TB	Tuberculosis

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6. COMMENTS FROM STAKEHOLDERS

Comments received from the Public Consultation on the working mandate and the call for information.

The SCHER website was opened for comments on 15 May 2008 and the deadline for submission was 6 June 2008. In total, 628 scientific articles and 84 comments were received from non governmental organisations, industry, academia, public authorities and individuals.

In evaluating the responses from the consultation, submitted material has only been considered for revision of the opinion if:

1. it is directly referring to the content of the report and relating to the issues that the report addresses,

2. it contains specific comments and suggestions on the scientific basis of the opinion,

3. it refers to peer-reviewed literature published in English, the working language of SCHER and the working group,

4. it has the potential to add to the mandate and the opinion of SCHER.

Each submission which meets these criteria has been carefully considered by the Working Group. The scientific rationale of the opinion has been revised to take into account relevant comments and the literature has been updated with relevant publications.

Comments received from the Public Hearing

SCHER has undergone a public hearing with stakeholder representatives with scientific expertise in the field to address particular topics of the draft opinion. The public hearing was held on 6 November 2008 and stakeholders who contributed to the consultation on the working mandate were invited to participate. Fifths stakeholder representatives including NGOs, industry, research institutes, university and (Governmental) scientific committees participated in the meeting.

Contributions related to the scope of the public hearing, given at the hearing or in writing by the 12th of November 2008, were considered by SCHER. All the relevant contributions were taken into account in the revision of the final version of the opinion.

7. GLOSSARY

Biokinetics	Absorption, distribution, biotransformation and excretion of chemicals from the body of mammals
In silico	Biological experiments performed entirely in a computer or via computer simulation.
In vitro	Technique performing a biological experiment in a controlled environment outside of the living organism
In vivo	Biological experiment that takes place in or on the living tissue of a whole, living organism
Infectious diseases	Diseases caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi; the diseases can be spread, directly or indirectly, from one person to another
Microdosing	Administration of chemicals in small dose to humans to study biokinetics
Monoclonal antibody	Antibody produced by one type of immune cell and. binding only to specific antigens
New World Primates	Small to mid-sized primates native to Central and South America
Old World Primates	Medium to large in size primates native to Africa and Asia
Primate	Mammal of the order Primates, which includes anthropoids and prosimians, characterized by refined development of the hands and feet, a shortened snout, and a large brain. It includes humans
Reduction	Use of fewer animals
Refinement	Methods which cause least harm to the animals
Replacement	Alternative methods that replace animal testing
Three Rs	Acronym for **R**eplacement, **R**eduction and **R**efinement
Transgenic mouse	Transgenic mice contain extra genetic material coming from any other animal and integrated into the genome in every cell.
Xenotransplantation	Transplantation of living cells, tissues or organs from one species to another such as from pigs to humans