Toxic Waste

Ending the use of non-human primates in toxicity testing

© Dr Hadwen Trust

An Antidote Europe Report

Andre Menache BSc(Hons) BVSc MRCVS
DIRECTOR, ANTIDOTE EUROPE

ANTIDOTE EUROPE

With thanks to the International Primate Protection League (IPPL)

About Antidote Europe

Antidote Europe is a non-profit NGO created by researchers from the CNRS (French National Centre for Scientific Research) whose goal is the promotion of sound science. Since its inception in 2004 it has actively pursued two main aims. One, the application of modern scientific methods to replace outdated animal experiments within the EU legislative framework on chemical risk assessment; and two, a public awareness campaign on avoidance of toxic chemicals.

About the Author

Andre Menache is a zoologist and veterinary surgeon. He has worked as scientific consultant and field representative for IPPL and other organisations, in Europe and internationally. He served as President of Doctors and Lawyers for Responsible Medicine for six years, and as a member of the National Council on Animal Experimentation for the Ministry of Health in Israel for eight years. Currently Director of Antidote Europe, Andre has been instrumental in the passing of legislation regulating animal research in Israel, and has made key amendments to the Helsinki Declaration on medical research.

Contents

The author gratefully acknowledges Louise Owen for support with this publication.

Published by Antidote Europe, 25 rue Jacques Callot, 66000 Perpignan, France
www.antidote-europe.org

Foreword

As a wildlife presenter for the BBC, I have been privileged to observe the behaviour of non human primates in their natural habitat. I have watched from really close up how they develop long-term bonds of affection and show emotions of happiness, fear and even jealousy. They most certainly have a sense of self and there is now documented evidence to show that non human primates grieve when members of their social group die.

I have never filmed inside a primate laboratory, nor would I want to. However I have seen undercover footage of monkey breeding farms and macaques undergoing invasive test procedures filmed inside laboratories – and I was shocked by those images. It is difficult to imagine the emotional and psychological trauma that these animals endure when they are separated from their family groups, transported in tiny cages to far away destinations, to be used as living test tubes.

We are told that toxicity tests are performed on non human primates to safeguard human health, because of their similarity to us. However, by the same token we have a duty and an obligation to afford them special protection. Given that modern science has the means to obtain the required safety data without the use of animals, we must act immediately and decisively to end those animal experiments.

Those of us who recognise our responsibility and stewardship towards primates should be their voice. The human mind is capable of great scientific innovation. Now is the time to combine that quality with the quality of compassion, to make this a better world for all – humans and non humans alike.

Dr Charlotte Uhlenbroek PhD
BBC Wildlife Presenter
September 2010

Toxic Waste Ending the use of non-human primates in toxicity testing has been endorsed by the following:

Aysha Akhtar MD MPH
Kathy Archibald BSc(Hons)
Jarrod Bailey PhD
Michael Balls CBE MA DPhil FSB
Marc Bekoff PhD
Alexander Belenky PhD
Janine Burns PhD
Theodora Capaldo EdD
Margaret Clotworthy PhD
Michael Coleman PhD DSc FHEA
Robert A Coleman MIBiol PhD DSc
Debra Durham PhD
Elad Faigin MD
Hope Ferdowsian MD MPH
Ray Greek MD
Jean Swingle Greek DVM DACVD
Nancy Harrison MD
Luc Hoffmann PhD
Robert C Jones PhD
Steve Kaufman MD
Anne Keogh MBBS MD FRACP
Andrew Knight BSc (Vet Biol) BVMS CertAW PhD MRCVS FOCAE
Marius Maxwell MD PhD
Shirley McGreal OBE PhD
Graham Moss RN BSc PhD
John J Pippin MD FACC
Claude Reiss PhD
Helene Sarraseca MSc
Niall Shanks PhD
Catrina Steedman BSc(Hons) AMSB
Erik Walum PhD
Clifford Warwick DipMedSci CBiol EurProBiol FRSPH FSB

1. Introduction

© IPPL

We live today in an era of the human genome and computational biology. What was unthinkable even ten years ago is now fast becoming reality. Great strides have been made, and continue to be made, in the sphere of molecular biology and personalised medicine. Why is it then that we continue to rely on 19th century science in the form of animal toxicity tests when it comes to human risk assessment of chemicals? This question is intended first and foremost for the regulatory authorities, whose primary responsibility is the protection of public health and the establishment of common guidelines with respect to toxicity testing.

Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin. (National Research Council [NRC], 2007, p. 1)

Indeed the gene sequencing of the human genome is no longer a vision but a reality. We now understand for example, why some individuals require significantly larger—or smaller—therapeutic doses of a drug or chemical than other individuals, based on their metabolic genetic profile. This has promoted the modern concept of personalised medicine, which although still developing, contrasts sharply with the traditional, but increasingly outdated concept of comparative medicine, which dates back to the 19th century. As a result, our regulatory system is in a state of transition somewhere between the two.

We are currently faced with a striking paradox, in which a group of scientists in one laboratory carefully records specific gene behaviour in human cells in response to a chemical, while another group of scientists in a different laboratory forcibly restrains a monkey fitted with a breathing mask and later kills the monkey, in order to study the fate of the same chemical—in humans.

The regulatory authorities, together with industry, are in a unique position to translate the NRC vision into action and to revolutionise our toxicity testing paradigm—from one based largely on animal data to one that is suited to the species in question—namely human beings. We need to examine whether it is political, rather than scientific, inertia that is the stumbling block to the progress of regulatory animal replacement. For example, Directive 2003/63/EC on the community code relating to medicinal products for human use makes the submission of animal data a legal requirement, whilst the submission of data obtained from human cell studies (e.g. pharmacogenomics) remains voluntary (US Food & Drug Administration [FDA] & European Medicines Agency [EMA], 2006).

We also need to question whether it is disingenuous for regulatory authorities to justify their continued requirement of animal tests based on the claim that there is insufficient human data. If there is no regulatory mechanism in place to enforce the collection of human data, there can be no informed comparison between animal and human data. We are now witness to a scientific culture that has neglected to systematically and diligently collate valuable human findings. Rather than pursuing animal studies, our policy makers need to invest resources into the expansion of existing human databases. The establishment of an integrated human toxicology program, together with data sharing should become a priority. Formaldehyde was discovered in 1859, yet it has taken the US health authorities 150 years to finally propose that this commonly used laboratory chemical be classified as a known human carcinogen (Department of Health and Human Services, 2009).

The development of sophisticated and modern non-animal methods of research and testing means that animal experiments have lost their credibility as the most scientifically tenable method available. This is already evident in the cosmetics industry, where a testing ban on the use of animals in the EU came into effect in March 2009, with a far wider-reaching marketing ban due to follow in 2013 (The European Parliament & The Council of the European Union, 2003). Significantly, many of the newly developed methods now used to test cosmetic products are likely to have applications in the pharmaceutical industry as well (Germain, 2009).

TABLE 1. **Primates used in scientific procedures in Great Britain in 2008 (Home Office, 2009).**

Species	Primary purpose of the procedure			
	Fundamental biological research	Applied studies – human medicine or dentistry	Protection of man, animals or environment	Total
Marmoset, tamarin	82	180	–	**262**
Macaque	122	2,630	340	**3,092**

There are also encouraging developments within the pharmaceutical industry itself. As an example, the rabbit pyrogenicity (fever) test is finally being replaced by a more accurate test method that utilises donated human white blood cells (Montag et al, 2007). The rabbit test was never formally validated to establish its reliability or relevance to humans and among the well-documented drawbacks, marked species and strain differences in sensitivity were noted (Hartung et al, 2001).

Forty eight percent of the world's primates are currently threatened with extinction (IUCN, 2009) and though not the only cause, the trade in primates for use by pharmaceutical companies and research institutions, has had its part to play in the decimation of wild populations.

Worldwide, it is estimated that between 100,000 and 200,000 primates are used in research and testing each year, mostly in Europe, Japan and North America (Hau & Schapiro, 2006). The most commonly used species are baboons *(Papio spp.)*, crab eating macaques *(Macaca fascicularis)*, rhesus macaques *(Macaca mulatta)* and vervet monkeys *(Chlorocebus aethiops)* (Carlsson et al., 2004).

On the grounds of predictivity to humans, replacing outdated animal tests is as much a public health imperative as it is an animal welfare issue.

2. Why are primates used in research and testing?


© IPPL

According to the European Commission's Scientific Committee on Health and Environmental Risks (SCHER), primates are needed chiefly in:

- the safety testing of pharmaceutical products
- research on infectious diseases
- studies of the human brain
- research on organ transplants

In the UK, almost all (95%) primate use is related to legislative requirements, primarily for toxicological purposes in pharmaceutical safety and efficacy evaluation studies.

The two main primate species used in the UK in 2008 were macaques and marmosets. Out of a total figure of 3,354 monkeys, macaques accounted for 3,092 or 92% (Home Office, 2009). Thus the macaque is currently the primate of choice to fulfil the need for a second, or "higher", mammalian species, after safety testing on rodents (usually rats) has been completed. The UK, France and Germany together accounted for almost all of the 10,000 primates used within the EU in 2008.

3. Current UK and EU legal requirements

The use of animals in regulatory toxicology is indicated in national as well as European legislation and guidelines, in the Chemical Testing Guidelines published by the OECD (2009) and Directive 2003/63/EC respectively. In its broadest sense, regulatory toxicology refers to the study of adverse effects of chemicals on living organisms. In the case of humans and animals, adverse effects may be categorised in several different ways. For example, chemicals may affect different target organs (e.g. skin, eye, liver, kidney). In addition, these effects may range in severity from mild irritation to irreversible tissue damage. A "time component" is also included in these studies to account for short-term (acute) and longer-term (chronic) toxic effects. Such animal tests are currently part of the regulatory requirements for pharmaceutical products intended for human use (e.g. Directive 2001/83/EC). Different regulatory requirements apply to industrial (i.e. non pharmaceutical) chemicals (e.g. REACH regulation 1907/2006).

The use of primates in research and testing remains a highly controversial issue. Concern has been expressed at many levels, including the European Parliament and EU member states. In the UK, the following recommendation was put forward by the Animal Procedures Committee in 2002, on their use in regulatory toxicology:

The use of primates in the safety assessment of pharmaceuticals can clearly only be justified under current UK legislation if the data obtained are both valid (relevant for humans) and necessary in order for a safety assessment to be made. Validity and necessity should be continuously monitored by retrospective comparison of test data with clinical experience, and the need for studies specifically on primates should be critically assessed before tests are carried out. The international pharmaceutical industry, in collaboration with regulatory authorities, has the major responsibility and the necessary access to data, to make these crucial assessments. (Animal Procedures Committee, 2002 p. 6)

As clearly stated in the Weatherall Report, *The use of non-human primates in research*, there is no mandatory requirement for the use of primates in pharmaceutical safety testing (Weatherall, 2006). Rather, these animals are selected out of caution of the risk that choosing another species may later prove unacceptable to the regulators, and thus result in costly delays in bringing a new medicine to market (Boyd & Smith, 2002).

The Weatherall report continues: *"Ultimately, the decision to use non-human primates for regulatory toxicology studies rests with the company developing the product."* (Weatherall, 2006, p. 93)

4. Toxicity studies

Historically, the first toxicity test performed was the acute toxicity study (Klaassen & Watkins, 1999). This test dates back to 1927, when it was originally used for the standardisation of potent and potentially toxic substances such as insulin, digitalis extracts, and diphtheria toxins (Hayes, 2008). In the absence of modern analytical chemistry, animals were used as crude living test tubes to calibrate the dose in question. These tests comprised single dose poisoning, in which 50 per cent of the test animals would die (Lethal Dose 50, or LD50). During acute poisoning, animals typically experience a rapid onset of toxicity and a short, but severe course of symptoms leading to death. Although the classical LD50 test has been replaced to a large extent by studies requiring fewer animals, acute toxicity is still employed, where death may be the endpoint of the experiment (OECD testing guidelines).

As far back as 1981, the highly respected toxicologists Gerhard Zbinden and Marilena Flury-Roversi compared lethal dose values from animal tests with those discovered in cases of accidental human poisoning, and concluded that the LD50 in animals "is of very little value" (Zbinden & Flury-Roversi, 1981). The significance of this statement becomes apparent in Table 2, which clearly illustrates the lack of concordance between oral lethal dose values in rodents compared with oral lethal doses in humans. A large study organized by the Commission of the European

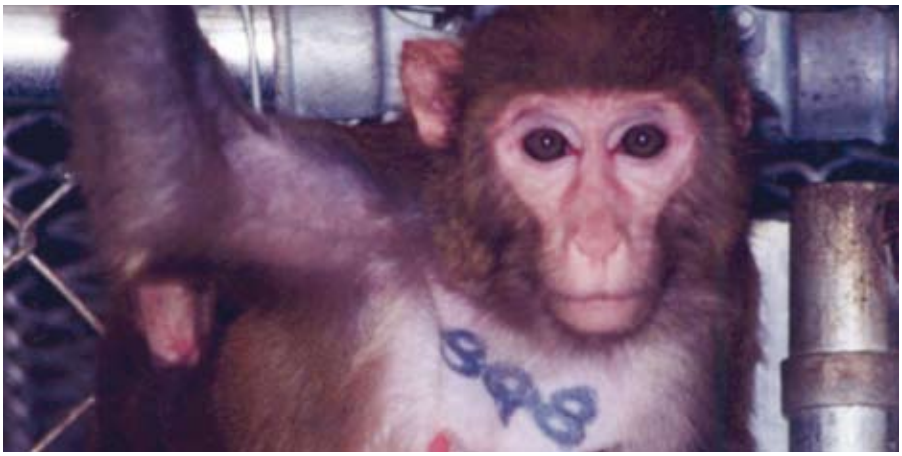
TABLE 2. A comparison of some oral LD50 doses for rats and mice, and mean oral lethal doses for humans reveals a substantial disconnect between lethality in humans versus rodents when exposed to different compounds (National Institutes of Health, 2001).

Chemical	Rat LD50 dose mg/kg	Mouse LD50 dose mg/kg	Average human dose mg/kg
Paracetamol	2404	338	271.4
Acetylsalicylic acid	200	232	385.7
Diazepam	352	45	71.4
Digoxin	28	18	0.1
Methanol	5619	7289	1569
Ethanol	7057	3448	4712.2
Malathion	290	190	742.8
Nicotine	50	3	0.7
Warfarin	2	3	107.1
Lindane	76	44	242.9
Chloroform	908	36	999.8
Altropine sulfate	585	456	1.7
Potassium chloride	2598	1499	285.5

4. Toxicity studies continued

Significantly, the UK National Poisons Information Service contains no LD50 data, but data relevant only to humans (Prof. A. Vale, personal communication, 11 September, 2009).

This is based on data obtained from accidental and deliberate human poisoning and overdose.



© IPPL

Communities involving 65 toxicological laboratories testing five substances in male rats produced same-species LD50s that varied by 20 to 40% (Hunter et al., 1979). Although modified, acute toxicity tests currently in use (as an alternative to the classical LD50) have also been criticised as not having been formally validated (Balls, 1991). These include the Fixed Dose Procedure, the Acute Toxic Class and the Up-and-Down Procedure, as well as the LD50 (dermal) and LC50 (inhalation) methods (OECD, 2009).

Acute toxicity studies for pharmaceutical development usually involve the administration of a single dose of test compound to two different mammalian species (typically a rodent species such as a rat, and a non rodent species, such as a dog or a monkey) often by two different routes. The animal is then observed for a period of up to 14 days for any clinical signs of toxicity, which may include changes in behaviour and body weight (NC3Rs, 2007). Pharmaceutical studies generally also require animals to undergo longer term tests such as the sub-acute toxicity program of 28 days, and the chronic test of 90 days. The longer term studies involve repeated exposure to the test compound.

TABLE 3. Toxicological procedures involving monkeys in 2008 in the UK. Taken from the Home Office report, *Statistics of Scientific Procedures on Living Animals 2008*.

Type of toxicological test or procedure	New world monkey	Old world monkey
Acute non-lethal clinical sign	–	34
Subacute limit-setting or dose ranging	30	324
Subacute toxicity	9	1,181
Subchronic and chronic	24	778
Toxicokinetics	–	493
Immunotoxicology	70	1
Other toxicology	–	705
Total	**133**	**3,516**

Note: the discrepancy between the number of macaques used (3,092) and the number of procedures in which these animals were used (3,516) is due to the fact that some animals underwent more than one procedure.

5. Conservation issues

Having examined the subject of toxicity testing from a regulatory perspective it is worth considering the animals themselves. Among macaques, the species most commonly used for toxicity testing was previously the rhesus macaque *(Macaca mulatta)*. In the 1960s and 1970s, researchers documented a drastic decline in wild rhesus macaques as they were being captured in huge numbers for export to Western laboratories (Malik, 1992; Southwick and Siddiqi, 2001). The Indian government subsequently banned primate exports in 1978, a ban upheld to this day. The resulting decline in available rhesus macaques has led to an increase in use of the crab-eating macaque *(Macaca fascicularis)*. This is now the species most regularly used in toxicology testing (Gad, 2007) and forms the overwhelming majority of primate imports to the UK (Department for Environment, Food and Rural Affairs [DEFRA], 2009, Response to Freedom of Information Request from IPPL) and to the USA (US Fish and Wildlife Service, Reponse to Freedom of Information Request from IPPL, 2010).

The IUCN Red List categorises this species as "least concern" because of its wide distribution and presumed large population. However, key data on numbers, distribution and population trends are lacking, and the threat that this increasing exploitation poses is now causing alarm among conservationists:

The greatest threat from the trade is in the Indochinese region, especially Cambodia where in 2003–2004 macaques began to be harvested from the wild, ostensibly for captive breeding for export to China and to the USA and elsewhere. The lucrative operations, however, may serve to "launder" wild-caught monkeys and appear to have resulted in their disappearance even from legally protected areas. (Eudey, 2008, p. 129).

The alleged "laundering" can be difficult to prove because there is no distinctive gut flora; nevertheless, there is evidence that these concerns are well-founded. In 1997, a US importer was fined for its role in shipping monkeys that were purportedly "born in captivity" when it was proved that the monkeys had been born before the exporter established its facility (McGreal, S., 2010).

6. Welfare concerns

There are disturbing ethical problems involved with the use of these intelligent primates in laboratories.
Firstly, transportation involves being packed singly in crates, before being shipped to countries half way around the globe. It is not uncommon for travel times to last up to 58 hours (Honess et al, 2004) and for some of the animals to die before, during, or after transportation to their destination.

Secondly, those that do survive this ordeal and reach the laboratory, or are actually bred in the country in which they will be used, then face an existence of incarceration in small steel cages, devoid of the environmental enrichment and rich social interaction for which they have evolved.

Macaques are extremely intelligent primates who naturally live in complex societies and form strong social bonds. Thus, it should be no great surprise that, in the isolated and unstimulating environment of the laboratory, macaques show signs of severe distress. Many macaques kept in standard laboratory cages exhibit stereotypical behaviour (Erwin & Deni, 1979). These can include more 'moderate' activities such as rocking, head-twisting and pacing back and forth, to more extreme behaviours, including self-biting, eye-poking, body-throwing and head-banging. A number of documented cases have led to wounds so severe that medical treatment was required (Pond & Rush, 1983; Reinhardt & Rossell, 2001).

In addition, monkeys undergoing tests – such as those required for the purposes of regulatory toxicology – are also continually under immense physiological and psychological stress due to the chemical testing regimen that is imposed upon them. Physical restraint often involves what is known as a primate chair, the use of which has been linked to a number of severe health problems including inguinal hernia and rectal prolapse as well as acute stress (Reinhardt et al, 1995). Violent dosing methods are also involved, such as nasogastric intubation or forced inhalation through a mask.

It is now well established that even routine laboratory procedures produce stress in macaques, which can lead to significant changes in physiological parameters correlated with stress (e.g., serum or plasma concentrations of corticosteroids, glucose, heart rate, blood pressure) (Balcombe et al., 2004).

In light of such confounding factors, the impact of such variables on results obtained from these studies should be questioned by the regulatory authorities in terms of their human applicability.

7. The chimpanzee as an animal model in toxicology

A discussion concerning the validity of monkeys as a model for human toxicity testing should be seen in the wider context of "higher" primates. In terms of evolutionary proximity to humans, the chimpanzee is our closest living relative, with whom we share about 98% of our DNA. The United States is the only country in the world to still use chimpanzees in biomedical research on a large scale, although the numbers are in decline with 1,600 animals used in 2000 (Strandberg, 2000) compared with 1,000 in 2009.

TABLE 4. Comparative in vitro metabolism of indinavir in primates (Chiba et al., 2000).

Species	Metabolite formation rate (mean figures in pmol/min/mg)
Rhesus macaque	74.5
Crab-eating macaque	84
Chimpanzee	26.7
Human	20.4

Note: Indinavir is an HIV protease inhibitor that shows marked species differences in metabolism in primates, including humans. Table 4 demonstrates that macaque monkeys are unique in their ability to metabolise this compound and therefore not predictive as models for humans (Chiba et al., 2000).

TABLE 5. Chromosome numbers.

Species	Chromosome number (per body cell)
Human	46
Chimpanzee	48
Macaque	42
Marmoset	46

Note: Table 5 illustrates chromosome numbers for various primate species. Chromosome numbers do not necessarily correlate with chromosome content as is illustrated by the fact that although humans and marmosets share the same chromosome number, they differ significantly in gross cellular anatomy from each other. Similarly, rhesus macaques and crab-eating macaques share the same number of chromosomes and yet possess species-specific polymorphisms, which in turn confer different levels of immunity against certain diseases (Flynn, 2009).

An ardent proponent of chimpanzee research once said that the chimpanzee is the "best possible model to predict the fate and effects of foreign chemicals in man" (Coulston, 1985, p. 182).

However, that view is not universally shared by all scientists:

It has been obvious for some time that there is generally no evolutionary basis behind the particular-metabolizing ability of a particular species. Indeed, among rodents and primates, zoologically closely related species exhibit markedly different patterns of metabolism. (Caldwell, 1992, p. 106).

Despite our close evolutionary proximity to chimpanzees, even small genetic differences have critical implications for using the chimpanzee as a predictive model for the study of human disease. For example, at least twenty genes implicated in human cancers are significantly different in chimpanzees. (Puente et al., 2006, Calarco et al., 2007).

In other words, when dealing with complex biological systems, small differences can have significant nonlinear effects so that two animals exhibiting a high degree of quantitative similarity can nevertheless show very different effects when identically stimulated (Shanks & Greek, 2009). Given that rapid genetic evolution has occurred in some parts of the human genome since its divergence from the last common ancestor with chimpanzees, it is not surprising that there is evidence to support the contention that chimpanzees are not good models for the study of important human diseases. While chimpanzees have been used extensively in the past by the pharmaceutical industry to test drugs, chemicals, and medical devices, their use as human surrogates yields a surprisingly poor track record. Chimpanzees are essentially immune to AIDS, hepatitis B and common malaria – three diseases that kill millions of people every year. A recent comprehensive literature analysis has revealed that chimpanzee studies have not been essential in the field of human cancer, or in the development of therapeutic monoclonal antibodies (Bailey, 2009).

Despite all of the scientific evidence to the contrary, proponents of the chimpanzee within the biomedical research community still consider this animal species to be the best animal model available. And yet this non-human primate has been spared by the regulators. This is partly based on their high cost of maintenance as well as their intelligence, physical strength and aggression, which requires skilled handling. It should also be noted that regulators rely on industry scientists to provide relevant safety data for their products. When it comes to selecting an animal species for drug testing, it is the drug manufacturer and not the regulator, who decides which will be the species of choice. The EU is an explicit example where the chimpanzee is not used in pharmaceutical drug development and testing. Industry and regulators have expediently "traded off" their premier animal model for smaller primates, such as macaques, baboons and marmosets. The fact remains, however, that the chimpanzee is a poor predictor of human response (Knight, 2008a), (Shanks & Greek, 2009).

SUMMARY

© IPPL & Tacugama Chimpanzee Sanctuary

1. The genetic similarity between chimpanzees and humans suggests (according to some researchers), that chimpanzees are the most appropriate animal model for biomedical research.
2. The available scientific literature indicates that the chimpanzee is in fact a poor predictor of human response for many important diseases.
3. The chimpanzee is not used for purposes of regulatory toxicology in the EU.
4. The chimpanzee is not used in the EU for the development and testing of pharmaceutical products.
5. If our closest relative is not a good predictor for humans then, *ipso facto,* no other more distantly related primate will be either.

8. The macaque as an animal model in toxicology

Based on evolutionary biology, humans and chimpanzees are estimated to have diverged five to seven million years ago. The divergence between humans and old world monkeys (such as macaques) goes back approximately 25 million years (Weatherall, 2006). From an evolutionary standpoint, macaques are clearly more distant cousins than chimpanzees. The macaque genome project has revealed that at least 200 genes evolved differently after humans and macaques branched off from each other (Gibbs et al., 2007). Other genes exist in both species, but are expressed differently. For example, humans and macaques both possess the gene for a tail. Macaques have tails, humans do not. Another example of a gene shared by both species is that associated with the disease phenylketonuria (PKU), which develops only in humans (Shanks & Greek, 2009).

The immune response represents another major difference between macaques and humans: the experimental monoclonal antibody TGN1412 caused a nearly fatal reaction in six healthy clinical trial participants in March 2006, despite having been shown safe in crab-eating macaques at a dose 500 times larger than that given to the human subjects (Department of Health, 2006). Earlier research had already shown that animal studies, including those conducted in primates, have limited predictive power for evoking an immune response in humans (Bugelski & Treacy, 2004). It has subsequently been demonstrated that in vitro procedures using donated human white blood cells could have predicted the events seen in the clinical trial and avoided this near tragedy (Stebbings et al., 2007).

Humans and macaques have a diverse genetic background as evidenced by numerous genetic polymorphisms (Kita et al., 2009). A genetic polymorphism is a difference in DNA sequence between individuals, groups or populations. An illustration of this is the human blood groups (A, B, O). Other genetic polymorphisms explain why some individuals require larger or smaller doses of a drug than the average. Macaques may vary genetically according to their geographic origin. Animals captured and bred in Vietnam, for instance, may respond differently in toxicological tests to those originating in the Philippines (Yasuhiro et al., 2009).

Historically, crab-eating macaques have been used in regulatory toxicology for the past 25 years or so. The principal reason for their selection by industry has been the increasing difficulty in obtaining the more traditional rhesus macaque, due to the previously mentioned primate export ban in India. Therefore, in order to ensure a constant supply, crab-eating macaques are captured from the wild in such countries as Cambodia and Laos (Eudey, 2008).

SUMMARY

1. The genetic similarity between macaques and humans is significantly less than that between chimpanzees and humans.
2. There are significant genetic differences between different species and subspecies of macaque, based on geographic origin.
3. There may also be a pharmacologically important genetic variation within a population of a particular species or subspecies of macaque.
4. There are serious welfare issues associated with the trapping, transport, incarceration and experimental protocols to which macaques are subjected.

9. Prediction in toxicology

Toxicology is both a science and an art. The science is the observational and data-gathering phase, whereas the art is the predictive phase of the discipline. When we fail to distinguish the science from the art, we confuse facts with predictions and argue that they have equal validity, which they do not (Gallo & Doull, 1993).

Those who claim that primate models are predictive must demonstrate that this claim is correct and the burden of proof lies with them. The fact that animal tests yield data does not necessarily imply that the data are relevant or reliable with respect to humans. Clearly, it is time for current risk assessment policy to undergo a paradigm shift with respect to human health in an era of modern, evidence based toxicology.

It could be argued that the field of regulatory toxicology has unwittingly fallen prey to confusing retrospective analysis with prediction. The classic study by Olson entitled "Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals" is often used as a poster to demonstrate the predictive power of animal tests (Olson et al., 2000). However, on closer inspection it becomes apparent that what the study evaluated was the concordance between adverse events based on clinical data with data generated in animal experiments (preclinical toxicology). According to Olson himself, "this study did not attempt to assess the predictability of preclinical experimental data to humans" (Olson et al., 2000). The Olson study is not without its critics. Taking into account the elements of sensitivity, specificity, positive and negative predictive value and concordance, Shanks and Greek comment that "If all the Olson Study measured was sensitivity, its conclusions are largely irrelevant to the great prediction debate" (Shanks & Greek, 2009).

It is apparent that the available scientific evidence (e.g. systematic reviews) does not support the thesis that the animal model is predictive for the human species (Horn et al., 2001, Knight, 2008b, Perel et al., 2006, Pound et al., 2004, Roberts et al., 2002).

> "Toxicology, like medicine, is both a science and an art. The science of toxicology is defined as the observational and data-gathering phase, whereas the art of toxicology is the predictive phase of the discipline. When we fail to distinguish the science from the art, we confuse facts with predictions and argue that they have equal validity, which they clearly do not."
>
> (Gallo & Doull, 1993).

The significant failure rate of pharmaceutical test compounds supports this view. Ninety per cent of test compounds that pass animal safety tests fail to make it through clinical trials in humans. Simply put, animal tests have a predictive power of 10% or one in ten (Shanks & Greek, 2009). Conversely, one could argue that some experimental compounds that might have had an acceptable risk profile in humans may be eliminated due to their toxicity in animals (Sankar, 2005).

In an era of evidence-based medicine, it is increasingly considered unethical to conduct more animal or human research while existing studies have not been systematically evaluated.

In a presentation given by Sir Iain Chalmers of the Scottish Wellcome Trust Clinical Research Facility, he summed it up as follows:

New research should not be designed or implemented without first assessing systematically what is known from existing research. The failure to conduct that assessment represents a lack of scientific self-discipline which results in an inexcusable waste of public resources. In applied fields like health care, failure to prepare scientifically defensible reviews of relevant animal and human data results not only in wasted resources but also in unnecessary suffering and premature death. All new research—whether basic or applied—should be designed in the light of scientifically defensible syntheses of existing research evidence, and reported setting the new research in the light of the totality of the available evidence, thus making clearer to readers what contribution—if any—new studies have made to knowledge. (2005)

The overwhelming majority of systematic reviews that have been conducted comparing treatment outcomes in animals and people show a huge discordance between the results obtained in people and in animals (Lindl et al., 2005).

The Medical Research Council (MRC) is a major funding source of fundamental research involving primates in the UK. Following the publication of the Weatherall Report, the MRC announced that it was:

...committed to undertaking a systematic review of the outcomes of such research over the past decade, which will aim to assess the overall efficiency and impact of research of this kind. The MRC believes firmly in commissioning research based only on its quality and potential benefits, and we are committed to conducting and evaluating research as openly as possible. (MRC, 2007)

Four years after the publication of the Weatherall Report, the MRC is still deliberating the feasibility of conducting such a systematic review (H. Finch, personal communication, MRC Head Office, 13 January, 2010).

10. Animal versus human data

"In terms of other types of modelling, e.g. physiologically-based pharmacokinetic (PBPK) models, real progress can only be made when there is a greater availability of human in vivo data (PK, efficacy, etc.) that are not currently in the public domain. These will be data that have been generated in clinical trials, many of which will have resulted in drugs not making it to the market for a variety of reasons. This is a potentially great resource of in vivo human data."

(Hewitt et al., 2009)

One of the major obstacles to the development, adoption and implementation of non-animal methods is the insistence by animal researchers and regulatory authorities that historical animal data be used in comparison with results obtained from modern human-based research methods. This is backward-looking, overcautious and lacking in innovation in the face of so many unsolved human conditions that are of intense interest to the public, such as neurodegenerative disease (e.g. Alzheimer's) and cancer. It is important to note that, according to a former head of the European Centre for the Validation of Alternative Methods (ECVAM), many currently accepted animal tests do not, and never could meet the criteria of scientifically validated test methods because they are not sufficiently reliable or relevant for a designated purpose (Balls & Combes, 2005).

There is a glaring regulatory double standard in research test evaluation in Europe and the USA. Whereas any non-animal method must comply with strict validation criteria before it can even be considered by regulatory bodies, currently accepted animal experiments have never been subjected to any formal validation procedure. In 2004, Home Office Minister Caroline Flint MP stated that "the Home Office has not commissioned or evaluated any formal research on the efficacy of animal experiments … and has no plans to do so" (Home Office, 2004). Across the Atlantic, Anita O'Connor (Office of Science, FDA) a few years earlier made a similar admission: "most of the animal tests we accept have never been validated. They evolved over the last twenty years, and the FDA is comfortable with them" (Greek & Greek, 2000, p. 57).

Much of this vital information has not been made generally available to the public at large. Moreover, raw animal test data generated by the drug companies—positive as well as negative results—would provide some much needed transparency into the drug regulatory process. Unfortunately, this type of information is generally confidential on the basis of commercial interest and intellectual property law. However, in its recommendations on the use of primates in research, the Weatherall Report stated: "Steps should be taken to make the results of toxicological studies involving non-human primates publicly available, in the same way as initiatives to register and publish the results of all human clinical trials." (Weatherall, 2006, p. 9).

Another avenue is to examine published scientific articles, some of which do provide useful information. One such article entitled "A European pharmaceutical company initiative challenging the regulatory requirement for acute toxicity studies in pharmaceutical drug development" describes an initiative to scrap acute toxicity testing since this category of test is considered redundant. The view that such tests have, in the eyes of their users, such low scientific and predictive credibility has apparently been held since the 1970s (Robinson et al., 2008).

The lack of progress in replacing animals in toxicity tests is not due to a shortage of scientific innovation. On the contrary, when industry scientists are instructed by their companies to provide solutions and to develop tests to replace animals, progress is made. Although industry has the necessary infrastructure and resources to develop tests that could replace living animals, the incentive to do so is often lacking. This is due in part to familiarity with submitting routine animal data, with which industry and regulators have become so accustomed.

Another issue is the cumbersome process required to develop a new test method, whose terms of reference will – ever increasingly – be based on human rather than animal data. On average, the currently accepted process of development, validation and regulatory adoption of a single non-animal test method will require about nine years to complete (Hartung, 2009). This period could be considerably reduced. For example, there could be existing human evidence of sufficient quantity and quality ("weight of evidence") to permit an evaluation of the performance of such a method for a particular purpose, without the need for additional laboratory work (Balls et al., 2006).

According to the Weatherall Report, pharmaceutical companies and regulators have chosen to use an "imperfect model" rather than no model at all. This is indeed a poor argument and oversimplification, given the wide range of advanced technologies available today. It would be more accurate to state that the choice today is between incomplete human data that is relevant to the species in question, versus complete animal data that is largely irrelevant to the species in question (i.e. humans).

Companies claim, in addition, that most new drug candidates fail toxicology testing for "off-target" effects, which cannot be predicted without a whole-animal system. However, since those "off-target" effects may well be species specific, it is quite possible that some, or even most, might not be relevant to humans. By the same token, potentially useful new drugs for humans may be lost because they were considered to have serious undesirable effects in animal models. For example, a survey of the US Environmental Protection Agency's (EPA) toxic chemicals database revealed that, for a majority of the chemicals of greatest public health concern, animal carcinogenicity data was inadequate to support classifications of probable human carcinogen or non-carcinogen (Knight et al., 2006).

© Kevin Robinson

The NRC report clearly stresses the role of human data:

"Human-exposure data may prove to be pivotal as toxicity testing shifts from the current apical end-point whole-animal testing to cell-based testing. Several types of information will be useful. The first is information collected by manufacturers, users, agencies, or others on exposures of employees in the workplace or on environmental exposures of the population at large. Such exposure information would be considered in the setting of dose ranges for in vitro toxicity testing and of doses for collecting data in targeted pharmacokinetic studies and in selecting concentrations to use in human PBPK models. Other valuable information will come from biomonitoring surveys of the population that measure environmental agents or their metabolites in blood, urine, or other tissues" (NRC, 2007, p. 79).*

The most convincing line of evidence for human risk is a well conducted epidemiologic study in which a positive association between exposure and disease has been observed (NRC, 1983).

*In terms of other types of modelling, e.g. physiologically-based pharmacokinetic (PBPK) models, real progress can only be made when there is a greater availability of human in vivo data (PK, efficacy, etc.) that are not currently in the public domain. These will be data that have been generated in clinical trials, many of which will have resulted in drugs not making it to the market for a variety of reasons. This is a potentially great resource of in vivo human data (Hewitt et al., 2009).

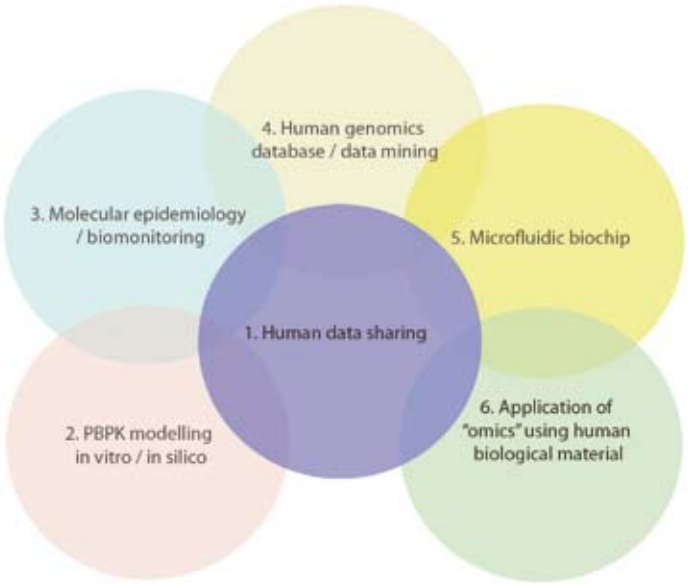
11. Replacement strategies

On one hand, testing on primates is ineffective and on the other, no single test is going to be predictive for all humans. What is required therefore is a tiered testing strategy, as has been proposed by the NRC in its document "Toxicity testing in the 21st century: a vision and a strategy", which incorporates human biology and high throughput screening.

1. Data sharing
Data sharing is a fundamentally important principle. Publicly available databases are providing an increasingly important resource for researchers and regulators alike (for example, the Comparative Toxicogenomics Database [ctd.mdibl.org] and the ChEMBLdb database [www.ebi.ac.uk/chembldb]). In the case of drug development, where the sharing of clinical data is crucial, measures can be put in place to protect commercially sensitive information as well as anonymising human biological material and clinical results to protect patient confidentiality (Thomas & Walport, 2007).

2. PBPK modelling in vitro/in silico
Physiologically-based pharmacokinetic modelling (PBPK) is a rapidly developing field that provides state-of-the-art modelling and simulation techniques to assist in candidate selection, accelerate drug development and improve clinical trial design. It could also be applied to regulatory toxicology for human risk assessment with respect to industrial chemicals (e.g REACH*).

3. Molecular epidemiology/ biomonitoring Although these concepts are not completely novel, such studies have been compromised by the lack of individual exposure assessment data that precisely quantify internal dose. However, with advances in analytical chemistry and molecular biology, direct biological monitoring of exposed populations is becoming increasingly possible. Biomarkers have been developed and validated in exposed populations that quantify individual exposure, susceptibility, and early markers of health effects and can be used to study relationships between exposures and environmentally induced diseases (Suk, 1996)



4. Human genomics database/data mining
As genomics research moves from an era of data acquisition to one of both acquisition and interpretation, new methods are being applied to organising and prioritising the data. The human genome has been extensively annotated with Gene Ontology for biological functions. The current challenge is to associate gene function with specific diseases, as illustrated by the Comparative Toxicogenomics Database initiative (Suk, 1996).

5. Microfluidic biochip
While a PBPK model mathematically simulates the absorption, distribution, metabolism and elimination (ADME) processes of living systems, such a model often requires parameters that are difficult to estimate, particularly those associated with kinetics of metabolism and mechanisms of action. A cell culture analogue system provides a physical replica of the PBPK model (Li, 2007).

6. Application of "omics" using human biological material
The voluntary submission of genomics data is already encouraged by regulatory bodies such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), as a means to ensure that regulatory authorities become familiar with the issues arising from the integration of pharmacogenomics in drug development (EC, EMA & FDA in joint public statement, 2006).

*REACH is the acronym for the European Union chemicals testing programme and stands for Registration, Evaluation, Authorisation and restriction of Chemicals (1907/2006/EC).

12. The way forward

The replacement of primates in regulatory toxicology is a responsibility that must be shared equally between industry scientists and regulators. As mentioned above, the lack of progress in replacing animals in toxicity tests is not due to a shortage of scientific innovation. The emphasis is therefore on teamwork in order to integrate 21st century toxicology into existing regulatory frameworks. The regulatory bodies at the forefront for the replacement of primate testing include the Committee for Medicinal Products for Human Use (CHMP) at the European Medicines Agency (EMA), the European Directorate for the Quality of Medicines (EDQM) and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). In terms of global harmonisation, the role of the ICH is absolutely pivotal, since it includes regulatory bodies from the US, Japan and the EU.

On the website of the ICH, the organisation's purpose is defined as "to increase international harmonisation of technical requirements to ensure that safe, effective, and high quality medicines are developed and registered in the most efficient and cost-effective manner. These activities have been undertaken to promote public health, prevent unnecessary duplication of clinical trials in humans, and minimize the use of animal testing without compromising safety and effectiveness (ICH, 2010). Their goal is "to promote international harmonisation by bringing together representatives from the three ICH regions (EU, Japan and USA) to discuss and establish common guidelines (ICH 2010)."

13. Conclusion

As stated earlier, there is no mandatory requirement for the use of primates in pharmaceutical safety testing.

This position, when accompanied by the science and ethics presented in this report, provides a logical basis from which to end the use of primates in pharmaceutical drug development and testing.

There are clear indications that primates, including the chimpanzee, have been a disappointment to the biomedical research community, in terms of failing to deliver much-needed answers for human health problems. Chimpanzees are not good models for human infectious diseases and equally poor as predictors of human response to pharmaceutical drugs and other chemicals. It is now only a question of time before the last remaining chimpanzees are finally removed from the laboratory and with them a scientific legacy that is out of step with modern toxicology. If the chimpanzee represents a failed animal model in our biomedical arsenal, the implication is that all non human primates should be replaced with modern scientific methods.

© Kevin Robinson

References

Animal Procedures Committee (2002). *The use of primates under the Animals (Scientific Procedures) Act (1986). Analysis of current trends with particular reference to regulatory toxicology.* Retrieved on 16 February, 2010 from apc.homeoffice.gov.uk/reference/primates.pdf

Bailey, J. (2009). An examination of chimpanzee use in human cancer research. *Alternatives to Laboratory Animals, 37(4),* 399-416

Bailey, J., Balcombe, J., Capaldo, T. (2007). Chimpanzee Research: An Examination of Its Contribution to Biomedical Knowledge and Efficacy in Combating Human Diseases. Retrieved on 16 February, 2010, from http://www.releasechimps.org/flawed-science/dangerous-and-unnecessary/the-case-to-end-chimpanzee-research/

Balcombe, J.P., Barnard, N.D., & Sandusky, C. (2004). Laboratory routines cause animal stress. *Contemporary Topics in Laboratory Animal Science. 43(6),* 42-51.

Balls, M. (1991). Why modification of the LD50 test will not be enough. *Laboratory Animals 25,* 198-206.

Balls, M. & Combes, R. (2005). The need for a formal invalidation process for animal and non-animal tests. *Alternatives to Laboratory Animals. 33(3),* 299-308.

Balls, M., Amcoff, P., Bremer, S., Casati, S., Coecke, S., Clothier, R. et al. (2006). The Principles of Weight of Evidence Validation of Test Methods and Testing Strategies. The Report and Recommendations of ECVAM Workshop 58a. *Alternatives to Laboratory Animals 34(6),* 603–620.

Boyd, K. M., Smith, J. A. (Eds.). (2002). *The Boyd Group papers on the use of non-human primates in research and testing.* Retrieved from http://www.boyd-group.demon.co.uk/Prefaceandsummary.pdf on16 Feb 2010.

Bugelski, P.J., & Treacy, G. (2004). Predictive power of preclinical studies in animals for the immunogenicity of recombinant therapeutic proteins in humans. *Current Opinion in Molecular Therapeutics, 6,* 10-16.

Calarco, J.A., Xing, Y., Caceres, M., Calarco, J.P., Xiao, X., Pan, Q., Lee, C., Preuss, T.M. & Blencowe, B.J. 2007. Global analysis of alternative splicing differences between humans and chimpanzees. *Genes & Development 21,* 2963–2975.

Caldwell, J. (1992). Problems and opportunities in toxicity testing arising from species differences in xenobiotic metabolism. *Toxicology Letters, 64-65,* 651-9.

Carlson, H-E., Schapiro, S. J., Farah I., & Hau, J. (2004). The use of primates in research: a global overview. *American Journal of Primatology, 63 (4),* 225-37.

Chalmers, I. (2005). From a presentation for the Scottish Wellcome Trust Clinical Research Facility, Edinburgh cited on the Sabre Research UK website. Retrieved on 16 February, 2010, from http://www.sabre.org.uk/#/background/4524921993

Chiba, M., Nishime, J. A., Neway, W., Lin, Y., and Lin, Y., and Lin, J. H. (2000). Comparative in vitro metabolism of indinavir in primates – a unique stereoselective hydroxylation in monkey. *Xenobiotica 30 (2)*, 117-129.

Coulston, F. (1985). Chimpanzees are the best possible model to test the fate and effects of foreign chemicals in man. *Regulatory Toxicology and Pharmacology, 5,* 182-189.

Department of Health. (2006). Expert scientific group on phase one clinical trials. Retrieved on 16 February, 2010, from http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_073165.pdf

Department of Health and Human Services. (2009). Federal Register, Vol. 74, No. 243. Retrieved on 16 February, 2010, from http://ntp.niehs.nih.gov/ntp/PressCtr/FRN/2009/74FR243RoC20091221.pdf

Erwin, J., & Deni, R. (1979). Strangers in a strange land: Abnormal behavior or abnormal environments? In J. Erwin, T. Maple, & G. Mitchell (Eds.), Captivity and Behavior (pp. 1-28). New York: Van Nostrand Reinhold.

EU (European Commission and EMA) and FDA Agree on Guiding Principles for Joint FDA EMA Voluntary Genomic Data Submission Briefing Meetings. (2006). Retrieved on 16 February, 2010, from http://www.fda.gov/InternationalPrograms/FDABeyondOurBordersForeignOffices/EuropeanUnion/EuropeanUnion/EuropeanCommission/ucm114343.htm

Eudey, A. A. (2008). The Crab-eating Macaque (Macaca fascicularis): Widespread and Rapidly Declining. *Primate Conservation, (23),* 129–132.

European Parliament and Council of the European Union. (2003). Directive 2003/15/EC amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. Retrieved on 16 February, 2010, from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:066:0026:0035:en:PDF

Expert Scientific Group on Phase One Clinical Trials (2006). Final Report. Retrieved on 16 February, 2010, from http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_073165.pdf

Flynn, S., Satkoski, J., Lerche, N., Kanthaswamy, S. & Smith, D. 2009. Genetic variation at the TNF-alpha promoter and malaria susceptibility in rhesus (Macaca mulatta) and long-tailed (Macaca fascicularis) macaques. Infect Genet Evol. 9(5):769-77.

Gad, S. C. (2007). *Animal Models in Toxicology.* Florida: Taylor & Francis Group.

Gallo, M.A., & Doull, J. (1993). History and scope of toxicology. In M. O. Amdur, J. D. Doull, & C. D. Klaasen (Eds.), *Casarett and Doull's Toxicology* (p. 3). New York: McGraw-Hill.

Germain, L. (27 September 2009). New EU funds for non animal testing. University World News. Retrieved on 16 February, 2010, from http://www.universityworldnews.com/article.php?story=20090925023713493

Gibbs, R., Rogers, J., Katze, M., Bumgarner, R., Weinstock., Mardis, E., et al. (2007). Evolutionary and biomedical insights from the rhesus macaque genome. Science, 316(5822): 222-34.

Greek, C. R., & Greek, J. S. (2000). *Sacred Cows and Golden Geese: The Human Cost of Experiments on Animals.* New York & London: The Continuum International Publishing Group Inc.

Hartung, T. (2009). A Toxicology for the 21st Century—Mapping the Road Ahead. *Toxicological Sciences 109(1),*18-23.

Hartung, T., Aaberge, I., Berthold, S., Carlin, G., Charton, E., Coecke, S. et al. (2001). Novel pyrogen tests based on the human fever reaction. The report and recommendations of ECVAM Workshop 43. *Alternatives to Laboratory Animals, 29(2),* 99-123.

Hau, J. & Schapiro, S. J. (2006). Non-human Primates in Biomedical Research. *Scandinavian Journal of Laboratory Animal Science, 33 (1),* 9-12.

Hayes, W. (2008). *Principles and Methods of Toxicology. (Fifth Edition).* New York: Informa Healthcare.

Hewitt, M., Ellison, C.M., Enoch, S.J., Madden, J.C. & Cronin, M.T. (2009). Integrating (Q)SAR models, expert systems and read-across approaches for the prediction of developmental toxicity. Reproductive Toxicology, (2009). DOI:10.1016/j.reprotox.2009.12.003.

Home Office. (2009). *Statistics of Scientific Procedures on Living Animals Great Britain 2008.* Retrieved on 16 February, 2010, from http://www.homeoffice.gov.uk/rds/pdfs09/spanimals08.pdf

Home Office response to Parliamentary Question by Michael Hancock MP. (2004). Retrieved on 16 February, 2010, from http://www.publications.parliament.uk/pa/cm200304/cmhansrd/vo040331/text/40331w06.htm

Honess, P.E., Johnson, P.J. & Wolfensohn, S.E. (2004). A study of behavioural responses of non-human primates to air transport and re-housing. *Laboratory Animals 38,*119-132.

Horn, J., de Haan, R.J., Vermeulen, M., & Limburg, M. (2001). Nimodipine in animal model experiments of focal cerebral ischemia: a systematic review. *Stroke 32,* 2433-38.

Hunter, W. J., Lingk, W., & Recht, P. (1979). Intercomparison study on the determination of single administration toxicity in rats. *Association of Official Analytical Chemists. 62(4),* 864-73.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. (2010). *Frequently Asked Questions (FAQs)* [online] Retrieved on 24 February 2010 from http://www.ich.org/cache/compo/276-254-1.html

IUCN Red List Version 2009. 2, Table 4A Retrieved on 24 February, 2010, from http://www.iucnredlist.org/documents/summarystatistics/2009RL_Stats_Table_4a.pdf

Kita, Y.F., Hosomichi, K., Kohara, S., Itoh, Y., Ogasawara K., Tsuchiya, H. et al. (2009). MHC class I A loci polymorphism and diversity in three Southeast Asian populations of cynomolgus macaque. *Immunogenetics 61(9), 635 -48.*

Klaassen, C.D., & Watkins, J.B. (1999). *Casarett and Doull's Toxicology: The basic science of poisons. (Fifth Edition).* New York: McGraw-Hill.

Knight, A. (2008). The beginning of the end for chimpanzee experiments? *Philos Ethics Humanit Med*; 3:16. Retrieved on 16 February 2010 from http://www.peh-med.com/content/3/1/16

Knight, A. (2008). Systematic reviews of animal experiments demonstrate poor contributions toward human healthcare. *Reviews of Recent Clinical Trials 3(2),* 89-96.

Knight, A., Bailey, J., & Balcombe, J. (2008). Animal carcinogenicity studies: 1. poor human predictivity. *Altern Lab Anim*; 34(1): 19-27.

Li, A.P. (2007). AltTox Forum. In Vitro Evaluation of Human Xenobiotic Toxicity: Scientific Concepts and the Novel Integrated Discrete Multiple Cell Co-culture (IdMOC) Technology. Retrieved on 16 February, 2010, from http://alttox.org/ttrc/toxicity-tests/repeated-dose/way-forward/li/

Lindl, T., Voelkel, M. & Kolar, R. (2005). Animal experiments in biomedical research. An evaluation of the clinical relevance of approved animal experimental projects. *Alternatives to Animal Experiments, 22(3),*143-51.

Malik, I. (1992). Consequences of Export and Trapping of Monkeys. *Primate Report, 34,* 5-12.

McGreal, S. (2010). Statistics on Primate Importation into the United States in 2009. *Laboratory Primate Newsletter, 49, (2),* 1-2.

Montag, T., Spreitzer, I., Loschner, B., Unkelbach, U., Flory, E., Sanzenbacher, R. et al. (2007). Safety testing of cell-based medicinal products: opportunities for the monocyte activation test for pyrogens. *Alternatives to Laboratory Animals, 24(2),*81-9.

MRC responds to the Weatherall report on research using primates. (2007). Retrieved on 16 February, 2010, from http://www.mrc.ac.uk/Newspublications/News/MRC003778

Mueller, W. F., Coulston, F., & Korte, F. (1985). The role of the chimpanzee in the evaluation of the risk of foreign chemicals to man. *Regulatory Toxicology and Pharmacology, Vol. 5,* 182-189.

National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). (2007). *Challenging the regulatory requirement for acute toxicity studies in the development of new medicines. A workshop report.* Retrieved on 16 February, 2010, from http://www.nc3rs.org.uk/downloaddoc.asp?id=559&page=22&skin=0

National Research Council. (1983). *Risk assessment in the Federal Government: managing the process.* Washington. DC: National Academies Press.

National Research Council. (2007). *Toxicity Testing in the 21st century: A Vision and a Strategy*. Washington, DC: National Academies Press.

OECD Chemicals Testing Guidelines. (2009). Retrieved on 16 February, 2010, from http://www.oecd.org/department/0,3355,en_2649_34377_1_1_1_1_1,00.html

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., et al., (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 32(1): 56-67.

Perel, P., Roberts, I., Sena, E., Wheble, P., Briscoe, C., Sandercock, P. et al. (2006). Comparison of treatment effects between animal experiments and clinical trials: systematic review. *British Medical Journal, 334*, 197.

Pond, C. L. & Rush, H. G. (1983). Self-aggression in Macaques: Five Case Studies. *Primates . 24 (1),* 127-134.

Pound, P., Ebrahim, S., Sandercock, P., Bracken, M.B. & Roberts, I. (2004). Where is the evidence that animal research benefits humans? *British Medical Journal 328(7438),* 514-7.

Puente, X.S., Velasco, G., Gutierrez-Fernandez, A., Bertranpetit, J., King, M.C. and Lopez-Otin, C. 2006. Comparative analysis of cancer genes in the human and chimpanzee genomes. *BMC Genomics 7,* 15.

Reinhardt, V., Liss, C. & Stevens, C. (1995). Restraint methods of laboratory non-human primates: a critical review. *Animal Welfare, 4,* 221-238.

Reinhardt, V. & Rossell, M. (2001). Self-biting in caged macaques: Cause, effect and treatment. *Journal of Applied Animal Welfare Science 4*, 285-294

Roberts, I., Kwan, I., Evans, P. & Haig, S. (2002). Does animal experimentation inform human healthcare? Observations from a systematic review of international animal experiments on fluid resuscitation. *British Medical Journal 324,* 474-476.

Robinson, S., Delongeas, JL., Donald, E., Dreher, D., Festag, M., Kervyn, S. et al. (2008). A European pharmaceutical company initiative challenging the regulatory requirement for acute toxicity studies in pharmaceutical drug development. *Regulatory Toxicology and Pharmacology 50,* 345–352.

Sankar, U. (2005). The delicate toxicity balance in drug discovery. *The Scientist, 19 (15),* 32.

Shanks, N. & Greek, R. (2009). *Animal Models in Light of Evolution*. Florida: BrownWalker Press.

Southwick, C. H., Siddiqi, M. F. (2001). Status, conservation and management of primates in India. *Wildlife Institute of India ENVIS bulletin, (1), 81-91.*

Stebbings, R., Findlay, L., Edwards, C., Eastwood, D., Bird, C., North, D., et al. (2007). "Cytokine Storm" in the Phase I Trial of Monoclonal Antibody TGN1412: Better Understanding the Causes to Improve PreClinical Testing of Immunotherapeutics. *The Journal of Immunology 179, 3325 -3331.*

Strandberg, J. (2000). Testimony on biomedical research and chimpanzees by John Strandberg DVM, PhD, before the House Committee on Commerce, Subcommittee on Health and Environment. (2000). Retrieved on 16 February, 2010, from http://www.hhs.gov/asl/testify/t000518a.html

Suk, W.A. (1996). Human biomonitoring: research goals and needs. *Environmental Health Perspectives. 104(3),* 479–483.

Thomas, R. & Walport, M. (2007). *Data Sharing Review.* Retrieved on 3 March, 2010, from http://www.justice.gov.uk/reviews/datasharing-intro.htm

US Food & Drug Administration (FDA) & European Medicines Agency (MEA). (2006). *Guiding principles Processing Joint FDA EMA Voluntary Genomic Data Submissions (VGDSs) within the framework of the Confidentiality Arrangement.* Retrieved on 16 February, 2010, from http://www.ema.europa.eu/pdfs/general/direct/pr/FDAEMA.pdf

Weatherall, D. (2006). The use of non human primates in research. (2006). A working group report chaired by Sir David Weatherall, FRS, FMedSci. Retrieved on 16 February, 2010, from http://www.acmedsci.ac.uk/images/project/nhpdownl.pdf

Yasuhiro, U., Matsushita, A., Osada, N., Uehara, S., Kohara, S., Nagata, R. et al. (2009). Genetic variants of CYP3A4 and CYP3A5 in cynomolgus and rhesus macaques. *American Society for Pharmacology and Experimental Therapeutics, (2009).* DOI:10.1124/dmd.109.029710.

Zbinden, G., & Flury-Roversi, M. (1981). Significance of the LD50-Test for the Toxicological Evaluation of Chemical Substances. *Archives of Toxicology 47,* 77-99.

Toxic Waste

Ending the use of non-human primates in toxicity testing

This report addresses the use of non-human primates in toxicity testing. There are clear indications that primates, including the chimpanzee, have been a disappointment to the biomedical research community, in terms of failing to deliver much needed answers for human health problems.

Chimpanzees are not good models for human infectious diseases and equally poor as predictors of human response to pharmaceutical drugs and other chemicals.

It is now only a question of time before the last remaining chimpanzees are finally removed from the laboratory and with them a scientific legacy that is out of step with modern toxicology.

If the chimpanzee represents a failed animal model in our biomedical arsenal, the implication is that all non-human primates should be replaced with modern scientific methods.

ANTIDOTE EUROPE

25 rue Jacques Callot, 66000 Perpignan, France
T +44 (0)20 8297 2129 **E** info@antidote-europe.org

www.antidote-europe.org