Europeans for Medical Progress

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APC Primates Report 2003: Response from Europeans for Medical Progress and Antidote-Europe

Introduction

Europeans for Medical Progress (EMP) and Antidote-Europe are two scientific organisations concerned with human health protection, and not with animal protection. We welcome the opportunity to respond to the APC Primates Report, and state at the outset our informed, professional opinion that animal use in regulatory toxicology and as models of human diseases are so poorly predictive of the human situation that, on balance, they result in more human harm than benefit. This is true not just for species such as mice and rats but also for our closest evolutionary relatives, despite the *prima facie* genetic and biochemical similarities of many non-human primate (NHP) species to humans.

In this document we respond to the recommendations and comments of the APC in the Primates Report and in the rapporteur's report of the Home Office Primates Stakeholders Forum. To illustrate our points and substantiate our claims, we show many examples of the human harm that reliance upon data from NHP use in regulatory toxicology and other fields of research has caused. We also discuss the merits of alternative forms of research and testing that are more relevant to human medicine, and which should be adopted as a matter of urgency in preference to primate models which, quite simply, have failed to deliver despite decades of opportunity.

We do not deny that, historically, there may have been examples of medical progress that have relied upon NHP experiments. These successes, however, must be assessed alongside the failures and the human harm that has resulted from them. If the failures outnumber the successes, as we believe they do by a significant margin, then it is time to replace the model with the technologically superior, more predictive methods of research that are now available.

Specific Responses to the APC Primates Report / Rapporteur's Report

Recommendation 2. *"We believe that the development and implementation of non-animal alternatives to replace the use of non-human primates must be accepted within industry and the international regulatory arena as a high priority goal, which requires immediate and dedicated attention. A coherent appropriately resourced strategy must be developed to achieve this goal."*

We applaud the committee on this recommendation, their insistence that industry 'can do better,' their opinion that non-animal alternatives might constitute 'better science' and their conclusion that there are major advantages to be gained from the replacement of NHPs.

However, we disagree with the verdict that there are 'no quick wins in sight,' meaning that we must concentrate on reduction and refinement in preference to replacement, and that gains in the former '2 Rs' are more likely in the short term than gains in the latter. These 'gains' might be true if the focus is restricted to animal numbers and/or suffering, but any gain with regard to human health is questionable at best. If the committee accepts that 'better science' is a tangible advantage of the acceptance, development and implementation of non-animal alternatives, then it is surely counter-productive to restrict replacement to the 'back burner' in favour of reduction and refinement. We believe (and show here as far as space allows) that animal models are demonstrably poor, and therefore their refinement and reduction cannot translate to any benefit for human medicine.

Replacement is the only scientifically valid way forward and a multitude of technologies has already been developed. When used together, these are clearly more predictive of the human situation than the animal (and often NHP) models that are current standard practice. For example, recent advances with microarray technology allied to computer learning methods have shown huge promise in toxicity testing. One group developing this is SimuGen (Cambridge, UK), who have stated a view shared by many, "The proof is overwhelming that it will work…..the long

term plan is to replace animal testing…..the technology is already there, but drugs regulators are very strict as to what is appropriate and safe."

Recommendation 3. *"We believe it is essential to instigate a detailed examination of regulatory policies on species selection in toxicity testing..."*

"Could the Home Office also comment on whether other regulatory authorities could be persuaded to disclose their policy – reportedly confidential – on the choice of second species?"

We agree that an examination of this type, in conjunction with full disclosure of the policies of regulatory authorities, would be of real scientific value. We, alongside other international groups representing thousands of similarly-minded scientists and doctors, have argued long and hard that it is not scientifically possible to reliably extrapolate findings from one species to another. This conviction was echoed by the Toxicology Working Group of the House of Lords select committee on Animals in Scientific Procedures[1], who said that 'the formulaic use of two species in safety testing is not a scientifically justifiable practice, but rather an acknowledgement of the problem of species differences in extrapolating the results of animal tests to predict effects in humans' and 'the reliability and relevance of all existing animal tests should be reviewed as a matter of urgency.' In addition, the Nuffield Council on Bioethics recently concluded that 'there is a need for continuing review of the scientific case for using animals in research and testing. It is axiomatic that any such use should be accompanied by active and critical reflection on the validity and relevance of the models and research studies[2].'

"The forum understood that the thinking behind this APC recommendation was that pharmaceutical companies often used primates for reasons other than scientific necessity. However it was not clear what the evidence for that view was."

The evidence for this view is that the scientific necessity of NHP use has never been demonstrated by those that advocate it. In fact, in addition to myriad examples illustrating the folly of NHP use, there is anecdotal evidence to embarrass those advocates. For example, Dr Robert Ruffalo, President of R&D at Wyeth, cited at an international conference in March 2005 an example involving the pneumococcal vaccine Prevnar. Successfully used in over 30 million American children since the year 2000, the human epidemiological data were of no interest to Japanese regulatory authorities, who required new NHP data instead. Indeed, the fact that human trials often take place concurrently with or even prior to animal toxicology is evidence that animal tests are performed for regulatory and legal, rather than scientific, reasons. Further evidence is provided by the Association of the British Pharmaceutical Industry in a paper entitled ''The selection of marmoset monkeys in pharmaceutical toxicology[3]' which documents considerations of cost, practicality and convenience as reasons for their use.

"There may be a transient, but significant, increase in animal numbers whilst information to inform discussion of suitability was gathered on the basis of comparative studies."

After decades of animal-based toxicology, there is more than enough data with which to perform comparative studies. In fact, a number of such studies have been completed, which reveal levels of discordance between results from animals and humans of between 67% and 96%[4,5] Last year a review of the evidence that animals are reliable predictors of toxic effects in humans found the evidence to be 'fragmentary,' pointing to 'significant over- and under-prediction of adverse effects from animal studies that varies with the particular organ or system.' Furthermore, it showed the false positive rate to be astonishingly high: even in NHPs it exceeded the success rate in two-thirds of the forms of toxicity studied[6].

Recommendation 4. *"The Home Office should insist that a full range of in vitro toxicokinetic / metabolism screening be done before…the selection of a second (non rodent) species for drug safety evaluation."*

We fully support the committee's decision that a full range of *in vitro* ADMET (how a drug is Absorbed, Distributed, Metabolised and Eliminated, and its Toxicological properties) studies be made obligatory during the course of drug development. However, we believe that the range, diversity, relevance and performance of batteries of powerful *in vitro*, *in silico*, human *ex vivo*, microdosing and other studies negate any data that can be provided from animal models; it follows that using data that is directly relevant to humans as the basis for the choice of a poorly predictive *animal* model is of questionable scientific merit. Therefore, scientists should be required to justify their use of a particular animal species against human-specific methods, rather than simply against another animal species.

In fact, among scientists in academia and in the pharmaceutical industry itself, the inability of animal-based pre-clinical models (including NHPs) to assess human ADMET properties has been acknowledged for decades:

- Dr Brimblecombe of Smith Kline and French commented in 1981, '…the present tests are well known to us but that does not make them good. There may be better tests around, but we have no incentive whatsoever to look for them at the moment. In fact, quite the reverse [because of the fear that regulatory agencies will require the new tests in addition to the current ones[7].'
- Professor Caldwell of St. Mary's Hospital Medical School, London observed, '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[8].'
- William Bains, chief scientific officer of Amedis Pharmaceuticals (Cambridge,UK) estimates that 50% of all drugs in development fail to progress to the market because of problems associated with ADMET and 50% of all drugs that do make it to market have problems associated with ADMET[9].
- 'One of the major challenges facing the drug discovery community is the limitation and poor predictability of animal-based strategies.....many drugs have failed in later stages of development because the animal data were poor predictors of efficacy in the human subject… One of the overriding interests of the pharmaceutical and biotechnologies industry is to create alternative development strategies that are less reliant on poor animal predictor models of human disease… [10].'

"It was recognized and acknowledged that tremendous progress has been made in identifying and progressing appropriate preliminary studies and that industry deserves credit."

We agree that credit must be given to those who have conceived and developed many of the studies and tests that provide more pertinent and reliable data to aid in the prediction of human drug response. In this way, drugs are being and have been developed precluding any reliance on animal pre-clinical data, at least until regulatory guidelines demand it: examples include anti-HIV protease-inhibitor and nucleoside-analogue drugs[11-16], some of which are known to have ADMET properties that vary wildly between animal species and that can be particularly dissimilar in NHPs[17]; Novartis' leukaemia drug Glivec that was designed and developed *in vitro*[18-21], and several drugs in the development pipeline from the company Pharmagene, one of which, for cystic fibrosis (PGN0052) is in late-phase clinical trials[22].

Recommendation 6. *"The availability of animal tissue for comparative in vitro studies should be improved and we urge the pharmaceutical industry, the Home Office and ERPs to promote in-house tissue sharing and further promote tissue banks."*

There is no need for us to repeat our well-founded views on the use of animal tissue here. We believe the committee's encouragement of the use of *human* tissues, which are readily available through numerous channels such as the UK Human Tissue Bank[23], members of the British Association for Tissue Banking[24], and various commercial suppliers is far more apposite.

Recommendation 7. *"The use of highly sensitive analytical methods to provide human pharmacokinetic data should be further developed and the resources provided to move*

technologies from the research phase to the stage where they can be routinely used. Early ultra-low dose studies in human volunteers should be encouraged."

We concur with this recommendation, but strongly dispute the objections raised by the stakeholder forum. Even the Food and Drug Administration (FDA) has accepted that 'the tools of the last century are being used to develop the drugs of the 21st,[25]
Concerns were raised about limitations of these techniques; about increased animal use in later stages of development if more drugs pass the screening process; about the expense, infancy and limited availability of microdosing; about predictability and human safety, and we must address these here.
All scientific techniques used in drug development have limitations; the critical question to ask is which combination of them provide the most relevant, predictive and reliable data with which to proceed to clinical trials. Currently the drug development process is heavily animal-based, relying on methods that correlate poorly with the human response, which is largely responsible for the fact that over 90% of drug candidates fail during human clinical trials. As discussed earlier, a comprehensive battery of human-specific tests presents us with the shortest 'leap of faith' possible when proceeding to the clinical phase of development. To continue using animal (often NHP) models that have never been validated and with all their limitations, while insisting upon extreme validation criteria for alternative methods is unscientific.
The claim by the forum that more animals will be used in pre-clinical development if prior human-based screening and discovery methods are adopted is not a valid reason to resist them, even if this were true. The truth is that more pertinent human-based screening and discovery methods will help to deliver the 'fail early, fail cheap' holy grail of the pharmaceutical industry, leading to the progression of less compounds, but better compounds, to clinical trials where the real expense lies. If drug companies extend this human-centric approach to the development phase, as they can and should, they may well find such compounds have a lower chance of adding to the adverse drug reaction (ADR) statistics that are now responsible for harming and killing a number of people beaten only by cancer, heart disease and stroke. With regard to the 'predictability' and 'human safety' concerns of the forum, moving away from animal-based methods is absolutely critical to address these.
Events have shown the forum's concerns about microdosing to be unfounded. Even if expense were a genuine issue, it is surely a drug company's foremost concern that any drug they develop is launched with the best human efficacy and safety data available? It is perhaps timely here to reiterate the burden of expense in the pharmaceutical industry; R&D costs are typically one quarter of the advertising and marketing budget, and only 15% of this is for pre-clinical studies.
So if expense is not a real issue, is the relative infancy of the technique itself? Notably, microdosing was recently rigorously tested with 5 drugs known for their difficult pharmacokinetic properties, in a trial designed for the technique to be unlikely to succeed. This 'CREAM' (Consortium for Resourcing and Evaluating AMS Microdosing) trial was pronounced a real success[26], and the technique has also been given a seal of approval by the FDA (April 2005) and the European Agency for the Evaluation of Medicinal Products (EMEA, January 2003[27]).

Recommendation 8. *"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."*

Validity and relevance for humans are surely pre-requisites for *any* techniques used in the safety assessment of drugs. It is incredible, therefore, that so little analysis of these aspects has been carried out for what is an intrinsic part of drug development. Earlier in this document we briefly summarised some of the studies done to date. In addition, the Olson study cited by the APC[28] found that if a side effect occurred in humans, there was an animal that it also occurred in about 70% of the time. Of course in retrospect it is easy to find an animal from all available species used in toxicology that replicates the human condition. *Prediction*, knowing in *advance* which

species will be suggestive of the human condition is another matter, and this is where the animal model fails. The Greaves study[6] cited earlier revealed an alarmingly high false positive rate. It also does not stand up to statistical scrutiny: much of the evidence supporting the predictive nature of animal toxicity was derived from an earlier study[29] with incorrectly calculated false positive and false negative rates[30]. Correctly manipulated statistics actually demonstrate that dogs and monkeys, even when the results are taken in tandem, are no more predictive of human toxicity than a coin toss; they give no weight of evidence to prior odds that a particular compound is toxic to humans, and they actually show that toxicity results for animals (including NHPs) and humans are independent of one another.
It is therefore already abundantly clear, despite the relative paucity of studies that have examined the plethora of toxicological data available, that animal studies in pre-clinical drug development are neither valid for nor relevant to humans. Often, data from NHPs, our closest genetic relatives, correlates with human data less than that from other more distant species. Even Ralph Heywood, former director of Huntingdon Research Centre, stated 'the best guess for the correlation of adverse reactions in man and animal toxicity data is somewhere between 5 and 25%'

Recommendation 10. *"The predictive value of data from primate studies should be investigated by comparing the results of pre-clinical and clinical studies on drugs that have progressed to clinical use."*

"Such a comparison could provide an indicator of study validity with respect to materials that subsequently make it into humans."

This information would be essential in helping to determine the true predictive value of NHPs as a pre-clinical model, but would of course only provide limited information: many 'False Positive' compounds would be missed because they did not progress to clinical trials. We do, however, encourage this endeavour to confirm beyond doubt whether the use of NHPs (and indeed other animals) in pre-clinical toxicology is a scientifically justifiable undertaking. More evidence of this type would hasten the move towards better drug development methodologies, translating to safer and more effective drugs for society.
The concerns of certain members of the forum are again insubstantial:

"Simply acquiring the data would not provide sufficient information to critically appraise decisions on whether or not to use primates."

Acquiring this data is essential, and is better than doing nothing to scientifically appraise primate use at all. Put simply, NHP data is either predictive (as it should be) or not. If this data shows a lack of correlation, then the data is not predictive, and NHP use is not justifiable.

"*An informed choice of second species would have been made before the data was generated, and it would be difficult to determine what the findings might have been if another species had been used."*

This is precisely the point; one cannot determine what the findings might have been for another species because it is impossible to extrapolate such data between them; this includes humans.

"*As clinical studies are not designed to produce or evaluate toxic effects in humans, correlation of findings related to toxicity would not be expected."*

This is an extraordinary claim: clinical studies may not be *designed* to produce toxic effects, but one of their accepted and important roles is most certainly to evaluate those that do occur. Many ADRs are detected during clinical trials, and these can be retrospectively compared with those arising in pre-clinical NHP models. Correlation is then possible.
If, as claimed above, clinical trials are not designed to evaluate toxic effects then it must be assumed that this is one of the roles of pre-clinical animal models. We refer once again to the lack of predictivity of animal toxicology, to the numbers of reported deaths in clinical trials despite extensive animal data[31], and to the increasing rates of morbidity and mortality from ADRs.

"As the findings in the second species can prevent materials reaching clinical trials, and prior to this many compounds will already have been screened out based on other species' toxicity/in-vitro/in-silico, such a study would also not be informative about the decisions not to proceed to humans taken on the basis of the findings in the second species."

Findings in the *first* species do not always signal the end of the drug assessment process, and so important comparative information between animal species could be gathered. This information could further be used to assess the correlation and predictive nature of the animal models with regard to humans, for example when compounds proceed to clinical trial despite some adverse findings in the animal models, or in cases where compounds 'pass' the animal-based tests but fail in clinical trials.

"Companies could be encouraged to expand on these studies."

We agree.

Recommendation 13. *"The design and sequence of pre-clinical safety studies needs to be reviewed. We ask the Home Office to consider whether measures need to be taken to prevent overlap of rodent and non-rodent studies, actively discouraging any simultaneous testing in rodents and primates in order to shorten the time course of drug development."*

We think a review of the practices involved in pre-clinical studies is long overdue, and absolutely essential in light of the current level and rate of growth of ADRs. However, we believe that the focus should not be on a simple alteration of a protocol that tacitly accepts the worth of animal (including NHP) models: the increasing amount of negative evidence with regard to the scientific validity of these, coupled with technological advances that have presented many better alternatives, demand that nothing short of a complete overhaul will suffice.

"However, tests on rodent species can fail to identify toxic effects (which will more accurately predict the effect on man) found in primates."

Tests on rodent species cannot predict toxic effects found in NHPs; they can fail to correlate with them, and often do. It follows that there is also a poor correlation between rodents and humans, and of course between NHPs and humans. Such poor correlation is found between all species, and even between different strains of the same species, and is responsible for the complete lack of predictivity between species. Statistics do not support the statement made above by the forum that toxic effects found in NHPs more accurately predict the effect on man; we therefore refer back to our statements above concerning a complete overhaul of pre-clinical studies, the support for this from the House of Lords select committee on Animals in Scientific Procedures and the Nuffield Council on Bioethics[1,2], and other statistics and quotations above that constitute much of the evidence base for this.

Annex A. *"Species selection is important for both ethical and scientific reasons; and it must be made on a sound scientific basis."*

"Since pharmaceuticals are being developed for use in the human, the most appropriate species will be the one that most closely resembles the human."

"…the Primates sub-committee considers for the reasons set out in chapter 3 of the report that the choice is currently insufficiently justified. We consider that this problem is significant…"

The only species that is scientifically justifiable for selection is *Homo sapiens*, for all the reasons outlined above. The argument for animal-based pre-clinical testing has always been the necessity of testing in a 'whole system.' When the available whole systems are quite clearly, in every respect, the 'wrong systems,' this argument fails to hold. This can be illustrated simply by looking at a small number of examples of how species differences between humans and NHPs manifest themselves in drug development:

- Hormone Replacement Therapy – prescribed to millions of women, and thought to protect against heart disease and stroke on the basis of NHP experiments, HRT is now known to

increase the risk of these diseases, as well as breast cancer in humans. The Chairman of the German Commission on the Safety of Medicines described HRT as 'the new Thalidomide.' It has caused up to 20,000 cases of breast cancer over the past decade in Britain alone[32].

- AIDSVAX – after successful results of this HIV vaccine in chimpanzees, it failed to protect any of the 8000 human volunteers in clinical trials[33].
- Teratology (risk of birth defects) – in the wake of Thalidomide, animals, often NHPs, have been used to assess the risk of foetal damage during pregnancy as a result of exposure to drugs and other chemicals. Results from NHPs correlate with known human teratogens only 50% of the time, less even than results from more evolutionarily distant species such as rats, hamsters and ferrets[34,35]. Aspirin, for example, is teratogenic in monkeys though not in humans.
- Asthma – Isoprenaline doses, derived from animal data, were too high for humans and killed thousands; mainly children. An attempt to replicate this in NHPs was unsuccessful[36].
- Opren (for arthritis) killed 61 people and harmed over 3500, despite showing no problems in NHPs[37].
- Flosint (another arthritis drug) caused hundreds of severe ADRs and several deaths, despite being well tolerated in NHPs[38].
- Carbenoxalone (for gastric ulcers) caused heart failure, which could not be replicated in NHPs[37].
- Amrinone (for heart failure) caused severe haemorrhaging in 20% of long-term patients, despite showing no warning signs in NHPs[39].

Summary

The Biological Basis of Human/NHP Differences

The differences between NHPs and human beings are clear to see, not just in the list of points made here, but more simplistically just by looking at individuals from all primate species. Depending on the methods of calculation humans are 97-99% genetically identical to chimpanzees, our closest evolutionary relative. Yet the physiological and biochemical differences that manifest due to this relatively small degree of genetic variation are immense[40-43]. On a superficial level we don't look alike or behave similarly; on a deeper level our biochemical differences mean that we suffer from different diseases, respond in different ways to infectious agents, have different metabolisms, and find different substances toxic. In short, an awful lot of subtle biochemical differences combine to make us very different indeed.

We are now learning why this is so, and it carries through to NHP use in biomedical research: NHPs are just too different from human beings to serve as 'surrogate' humans in this way. They cannot be predictive of the human situation; one cannot rely on data from NHP experiments when extrapolated to humans. NHP research can be 'tweaked' in order to retrospectively correlate with and 'confirm' human data, but this should not be taken as evidence of its worth. When *extrapolated* to humans, NHP research has caused untold amounts of harm, a fraction of which is detailed here; directly by causing human suffering and death, and indirectly by delaying medical progress and diverting research funds from more appropriate methodology.

Those small genetic differences between us explain why this is the case. Some of these differences lie within the structural genes, i.e. those genes that make proteins and enzymes that 'make up' the body and allow it to function. Tiny differences can completely change a gene's function and ability to do its job. Most of the differences, however, are now known to lie within the regulatory regions of our DNA; particular genes and sections of DNA that are involved in turning other genes 'on' and 'off' and modifying how their products do their jobs and interact with one another, in response to a variety of signals and stimuli. These can act highly promiscuously, and exert 'avalanche' effects upon hundreds of other genes. A small difference, therefore, can have far-reaching and extreme effects. For example, striking differences have been found in the levels of gene expression between humans and chimps in the brain and liver[44].

The Way Forward

Many scientific techniques exist that perform significantly better than NHP-oriented research, and are directly responsible for the great strides we are now making towards treating and curing the most widespread and debilitating human diseases. Batteries of human-based tests provide reliable and relevant information on which to base further research and to speed the translation of research to the bedside.

These technologies include microarrays and other DNA technologies; proteomics and metabolomics; mathematical and computer modelling; epidemiology; human clinical research; myriad *in vitro* molecular biological techniques; microfluidics devices harbouring many types of human cells in an almost 'natural' environment and interacting with one another; and many more. Studies of brain function and neurological disorders account for much NHP research, yet the most dramatic differences between us and other primates are in the brain. Human brains can now be studied non-invasively using a huge array of imaging techniques such as positron emission tomography (PET), magnetoencephalography (MEG), magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI) and many others. The Academy of Life Sciences at Aston University, for example, shows the progress to be gained from multidisciplinary human clinical research.

Perhaps the most exciting technology with regard to drug development is 'microdosing,' in which nanogram doses of new drugs are traced through the human body. Employed as 'Phase 0' clinical trials, microdosing provides extensive information about a drug's pharmacokinetic properties in a human environment and has already been endorsed by the FDA (April 2005) and the European Agency for the Evaluation of Medicinal Products (January 2003)[27]. The APC recommended that the use of microdosing in place of NHPs should be encouraged. Several companies exist whose mission is to develop and/or test drugs in an exclusively human context, such as Pharmagene, who observe; 'no animal species is sufficiently similar to man to act as a wholly reliable surrogate - indeed there is extensive evidence that the use of animal (non-human) tissue can result in the generation of potentially misleading information' and Biopta, whose rationale is 'proof of concept in man.'

Time for Objectivity

The limitations of nonhuman primate research and the risk of excessive faith in its results have not yet been widely appreciated by many. For a variety of political reasons, few scientists openly question whether primate models are a reliable research method. One of the main obstacles to open discussion and debate is that researchers, threatened by those who oppose their modus operandi, believe that the best strategy is to defend *all* animal research regardless of its actual value[45,46] and to discourage and actively suppress criticism from within their ranks[47-50].

We believe it is time for objectivity, transparency and honesty in the assessment of NHP models and their contribution to medical science. Only by ensuring this can we be confident that we are utilising scientific technology to the full, performing the best translational research possible, and making real progress towards the relief of human suffering and disease.

References

1. House of Lords Select Committee on Animals in Scientific Procedures, HL150-I, July 16th 2002
2. The Nuffield Council on Bioethics. The ethics of research involving animals. 2005. http://www.nuffieldbioethics.org
3. Smith D, Trennery P, Farningham D, Klapwijk J. (2001). The selection of marmoset monkeys .Callithrix jacchus. in pharmaceutical toxicology. Laboratory Animals. 35, 117-130.
4. Lumley and Walker. Animal Toxicity Studies: Their Relevance for Man Quay 1990 p73.
5. Spriet-Pourra C and Auriche M. (Eds) 1994. SCRIP reports PJB, New York and *Animal Toxicity Studies: Their Relevance for Man* p57-67. Quay 1990.
6. Greaves P, Williams A and Eve M. First dose of potential new medicines to humans: how animals help. 2004. Nature Reviews Drug Discovery **3**;226-236.
7. Dr R. Brimblecombe, Vice-President of Research and Development, Smith, Kline & French Laboratories, in *Risk-Benefit Analysis in Drug Research,* ed. Cavalla, p 153, 1981.

8. Caldwell, J. 1992: "Species Differences in Metabolism and their Toxicological Significance", *Toxicology Letters* 64 5, pp. 106.
9. Hodgson, J. ADMET—turning chemicals into drugs. Nature Biotechnology 19, 722-726 (01 Aug 2001).
10. Dr M. G. Palfreyman, Dr V. Charles and J. Blander, The importance of using human-based models in gene and drug discovery. DDW (Drug Discovery World), Fall 2002, p.33-40.
11. Seelmeier, S *et al* (1988). Human immunodeficiency virus has an aspartic-type protease that can be inhibited by pepstatin A. Proc. Natl. Acad. Sci. 85:6612-6616.
12. Vacca, JP *et al* (1994). L-735,524: an orally bioavailable human immunodeficiency virus type 1 protease inhibitor. Proc. Natl. Acad. Sci. 91:4096-4011.
13. DeVita VT Jr., Hellman S, Rosenberg SA. AIDS Etiology, Diagnosis, Treatment, and Prevention, 3rd Edition. Philadelphia, JB Lippincott, 1992.
14. Mitsuya H, Weinhold KJ, Furman PA, et al.(3'-Azido-3'deoxythymidine (BS A509U). Proceedings of the National Academy of Science USA 1985;82:7096-7100.
15. Soudeyns H, Yao X-J, Gao Q, et al. Anti-human immunodeficiency virus type 1) activity and in vitro toxicity of 2'-deoxy-3'-thiacytidine (BCH 189), a novel heterocyclic nucleoside analog. Antimicrobial Agents and Chemotherapeutics 1991;35:1386-1390.
16. Roberts NA, Martin JA, Kinchington D, et al. Rational design of peptide-based HIV proteinase inhibitors. Science 1990:248;358-361.
17. Chiba, M *et al* (2000). Comparative in vitro metabolism of indinavir in primates - a unique stereoselective hydroxylation in monkeys. Xenobiotica 30:117-129.
18. Lydon NB, Adams B, Poschet JF, et al. An E. coli expression system for the rapid purification and characterization of a v-abl tyrosine protein kinase. Oncogene Research. 1990;5:161-173.
19. Geissler JF, Roesel JL, Meyer T, et al. Benzopyranones and benzothiopyranones: a class of tyrosine protein kinase inhibitors with selectivity for the v-abl kinase. Cancer Research. 1992;52:4492-4498.
20. Druker BJ, Lydon NB. Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukaemia. J Clin Invest. 2000;105:3-7.
21. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nature Med. 1996;2:561-566.
22. http://www.pharmagene.com/ (Active programmes).
23. http://www.ukhtb.org
24. http://www.batb.org.uk/
25. http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html
26. http://www.xceleron.co.uk/metadot/index.pl?id=2224&isa=Newsitem&op=show
27. European Agency for the Evaluation of Medicinal Products (EMEA). 2003. Position paper on non-clinical safety studies to support clinical trials with a single microdose. CPMP/SWP/2599/02.
28. Olson H *et al*. 2000. Concordance of the toxicity of pharmaceuticals in humans and animals. Regul Toxicol Pharmacol 32(1);56-67.

29. Schein PS, Davis RD, Carter S, Newman J, Schein DR, Rall DP The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. Clin Pharmacol Ther. 1970 Jan-Feb;11(1):3-40.
30. Personal communication from a professional statistician.
31. Marshall E. Science 2000; 288:951.
32. Gray S. Breast cancer and hormone-replacement therapy: the Million Women Study. Lancet. 2003 Oct 18;362(9392):1332.
33. Expert Rev Vaccines. 2003 Dec;2(6):731. AIDSVAX fails to prove efficacious in large-scale trial.
34. Schardein JL 1993. Chemically Induced Birth Defects, 2nd edn rev. Marcel Dekker, New York.
35. Bailey J, Knight A and Balcombe J 2005. The future of teratology research is *in vitro*. Biogenic Amines Vol. 19, No. 2, pp. 97–145.
36. Carson S *et al*., 1971. Pharmacologist 18:272
37 Eason CT *et al*., 1990. Regulatory toxicology and pharmacology 11:288-307
38. Mann RD 1984. Modern drug use, an enquiry on historical principles. MTP press
39. Eason CT *et al*., Human toxicology 1987. 6:436
40. Watanabe, H. *et al*. DNA sequence and comparative analysis of chimpanzee chromosome 22. *Nature* **429**, 382-388 (May 27, 2004).
41. Hacia JG. 2001. Genome of the apes. Trends in Genetics 17(11); 637-645.
42. Gagneux P and Varki A. 2001. Genetic differences between humans and great apes. Molecular Phylogenetics and Evolution 18(1); 2-13.
43. Enard W, Khaitovich P, Klose J *et al*. 2002. Intra- and interspecific variation in primate gene expression patterns. Science 296(5566); 340-343.
44. Ruvolo M. Comparative primate genomics: the year of the chimpanzee. Curr Opin Genet Dev. 2004 Dec;14(6):650-6.
45. Gluck JP, Kubacki SR. Animals in biomedical research: The undermining effect of the rhetoric of the besieged. *Ethics and Behavior* 1991;1:157-173.
46. Weibers DO, Leaning J, White RD. Animal protection and medical science. *Lancet* 1994;343:902-904.
47. Kaufman SR. Animal protection and medical science [letter]. *Lancet* 1994;343: 1574.
48. Kaufman SR. UR researchers refused to debate. [Rochester] *Democrat and Chronicle* July 19, 1991.
49. Dunayer J. Censored: Faculty who oppose vivisection. *Z Magazine* 1993;April:57-60.
50. Arluke A. The ethical socialization of animal researchers. *Lab Animal* June 1994, pp 30-35.