

## Recent examples of Alternative Methods developed by the Pharmaceutical Industry

### Computer technologies in early discovery process

- Companies use leading-edge computer technologies to predict more accurately how a potential medicine may act in the human body (for example, how it will be absorbed and distributed). This enables elimination of unsuitable compounds early in the discovery process and stops their progression into animal studies.
- Companies are pioneering in a new discipline called biosimulation, which involves computer models that simulate human beings as closely as possible. They participate in an EU Network of Excellence in Biosimulation (BioSim), which helps evaluate how biosimulation could contribute to future drug development and support the principle of The Three Rs.

### Imaging techniques

- New medicines for arthritis aim to relieve pain and improve mobility, and companies use a new system of imaging and analysing how an animal with arthritis walks so as to immediately detect any subtle changes in mobility following treatment. This is a major welfare improvement as the study is less stressful for the animal and can provide more accurate data using a smaller number of animals.
- Through the introduction of novel biological imaging technologies, companies have been able to refine the research process and reduce the number of animals needed in a variety of tests in biomedical research or for the screening and profiling of anti-parasitic compounds. For example, endoscopic investigation in live animals is now used to help evaluate the efficacy of treatments for intestinal parasites, rather than post-mortem studies, eliminating the need to kill the host animals.
- Safety assessment of novel compounds is a vital stage in drug development. Novel compounds can affect different organs in different ways and methods must be found to monitor these effects. MRI is an established non-invasive technique used for human imaging, but is now being used in drug safety assessment by imaging the rat at several time-points after dosing, allowing recovery between sessions. In this way, each subject becomes its own control: effects of compounds can be tracked over time, reducing the number of animals required for the study and increasing the scientific and statistical power. MRI lends itself well to serial imaging of the internal organs in animal species and humans. It provides an accurate and fast readout of compound effects and can contribute to the assessment of safety of such agents. As a result of these studies, MRI is now being used to monitor compound effects on adrenal size in humans.

### Use of in vitro liver cell to assess how compound is eliminated from the body

Companies use in vitro hepatocyte (liver cell) tests to assess how a compound is eliminated from the body, making it possible to discard unsuitable compounds without the need for animal studies. They have also developed an automated test for rat hepatocytes, the accuracy of which is proving less variable than the previous manual assay. These efficiencies mean that at this stage of research, it is possible to test more compounds and make compound selections without the need for animal studies.

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### Use of synthetic animal protein to partially replace animal tissues

Using synthetic animal protein in drug development has made it possible to replace some techniques which required the use of animal tissue, and the predictability has been improved of the animal studies that must still be done, leading to the use of fewer animals overall.

### Novel refined oral dosing method

The aim of this study was to implement a novel oral dosing administration that could potentially reduce the stress and morbidity associated with this standard gavage technique. The novel oral dosing method clearly offers a number of advantages over the oral gavage method in reducing restraint-induced stress and potential oesophageal injury.

### Measuring neurochemicals in the mammalian brain using in vivo microdialysis: increasing information whilst reducing animal usage

Brain chemicals (neurotransmitters) are critical for normal brain function and dysfunction is often associated with brain illnesses. Hence specific neurotransmitter systems can be targeted for the treatment of these diseases. In-vivo microdialysis provides great insight into brain function by allowing the sampling of neurotransmitters in the conscious brain. Previously, these techniques used one animal per experiment and generated information on a few neurotransmitters from one brain area. To significantly reduce animal usage, and enhance the information obtained, industry has developed methodologies which allow simultaneous measurement of neurochemicals from multiple brain structures in animals which are then re-used.. With the new technique, only 20 rodents would be necessary in studies which in the past required up to 100 individuals.

### New uses for existing drugs; the story of Solabegron; and the value of using human tissue

Irritable bowel syndrome (IBS) is a chronic disorder featuring recurrent abdominal pain and intermittent diarrhoea, often alternating with constipation. IBS is very common and is estimated to be present in 60% of patients that see a gastroenterology specialist. An existing molecule was identified as a possible solution to relieve pain and improve mobility. Safety of this drug was already proved in patients, but efficacy for reducing contractions in gut muscle had to be demonstrated. Human tissues were therefore used instead of in vivo animal studies.

### Reducing animal usage through enhanced experimental design

Biological experiments using animals are of great value in helping scientists to understand disease and evaluate new medicines. However, the statistician supporting the design and analysis of these studies has to balance the inherent inter-animal variability, which may compromise the scientists' confidence in the results, with the desire to minimise the number of animals used. Variability may be due to any number of factors, and is influenced by both the operator and the environment.

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The statistician's approach is to exploit this variation and strengthen the study design, allowing the scientist to control the variability by removing or reducing the influence of these factors. Complex experimental designs are sometimes mistakenly associated with increased animal numbers. In fact, exploring the full potential of alternative designs can help reduce sample sizes. Using three examples of different biological studies we highlight how use of these designs can reduce animal numbers:

- Factorial designs systematically investigate the influence of different factors in one experiment. In our example, using a factorial design to understand the underlying cause of a hypothermia side effect in transgenic mice used only 30% of the animals required for a non-factorial design.
- In cross-over designs, frequently used in clinical studies, every animal receives each of the treatments under investigation in sequence, resulting in the use of fewer animals. Using this design for a series of novel antipsychotic treatments in rats resulted in a 50% reduction in animal usage over a year.
- Nested designs allow investigation of factors other than animals that may increase variability. For example, when studying brain cell viability in an animal model of stroke, it was found that increasing the number of measurements taken for each animal allowed a reduction in the total number of animals used by 30%, without compromising the validity of the study.

### Replacing primates by transgenic mice for polio vaccine neurovirulence testing

For biological products such as vaccines, additional safety and quality measures must also be met. In some cases, regulatory authorities responsible for the safety of such vaccines, including oral polio vaccine, currently require that some of this testing be done using non-human primates. In accordance with WHO and European Pharmacopoeia specifications, as well as the US Code of Federal Regulations, batch testing of live polio viral vaccines still remains mandatory for public health reasons. This testing assesses if attenuated viruses remain safe and effective to be given to humans and is therefore an essential safety and quality measure. In addition, new primary batches ('seeds') of other live attenuated viral vaccines need to be tested before they are approved for use in people. Current legal and regulatory requirements mandate that this testing be conducted in animals, specifically in non-human primates. Recognising the sensitive character of testing using primates, pharmaceutical companies in a collaborative effort developed a transgenic mice which is today used or validated for use by all polio vaccine manufacturers.

### Removal of biological test types

During the last decade one company has succeeded in achieving regulatory approval for the total removal of all biological test types for product control in most countries. Only two test types remain to be removed to fully eliminate the use of laboratory animals for production control. In 2006 it once again applied to the US authorities for acceptance of removal of the biondentity test. This was declined, but the US authorities did accept a 75% reduction of the test frequency.

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### Use of a pressure chamber to reduce animal use in cartilage research

Using in-vivo models, the alterations of articular cartilage can be reliably quantified, but the cellular mechanisms controlling those changes are difficult to examine and remain largely obscure. In-vitro models offer a more direct way to study the cellular response to articular loading under controlled circumstances. An important advantage of in-vitro studies for biosynthetic experiments is that they are a closed system, that the experimental conditions can be controlled more precisely, and that the inter-individual variability can be reduced.

### Cryopreservation technology for reduction purposes

One company decreased the number of cattle and sheep used for strain maintenance of gastrointestinal nematodes through the introduction of cryopreservation technology. To ensure efficacy testing of new anthelmintics for cattle and sheep, a regular supply of nematodes at their different larval stages are required. Previously, this stock of nematodes and larvae had to be maintained in live animals. The company developed a cryopreservation protocol which allows certain nematode strains to be kept deep-frozen for several months. Strain maintenance in host animals is still required for other strains and to guarantee viability of preserved strains at regular intervals, but the cryopreservation protocol allows the reduction of host animals required for parasite passaging.

### Combination testing for batch release purposes

Similarly, the introduction of combination testing for cattle vaccine batch releases has reduced the number of animals required to test for potency and safety. By law, each new batch of vaccines must be tested for potency and safety. In the UK these tests were routinely carried out separately. By combining the protocols, however, a company has been able to address regulatory demands while at the same time reducing the number of animals by a factor of two. The revised protocol has been accepted by the regulatory authorities and should enter into effect during 2007.

### Using blood cells for pyrogenicity testing

Companies have played a leading role in the development of a novel pyrogenicity test using white blood cells instead of experimental animals. This test system is now undergoing final validation.

### Replacing cats for cat fleas breeding for efficacy testing

Another system has been implemented for use in the efficacy testing for new ectoparasiticides. A company that regularly needs large numbers of *Ctenocephalides felis* (cat fleas) for this process used to obtain them by breeding and feeding on host animals. An artificial system to breed fleas was developed whereby the entire life cycle of the flea can be performed in vitro. This innovation has allowed the complete transfer of flea breeding and maintenance from living animals to an in vitro system.

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### **Refinement: remote measure of temperature**

Refining test methods helps to minimize the distress experienced by animals. This can be achieved by means of technical innovations. One example of this is where rodents being used to test the efficacy of new antibiotics are implanted with a transmitter the size of a kidney bean. This enables body temperature and physical activity to be measured without the animal being removed from its accustomed environment. Evaluating body temperature and/or activity enables researchers to draw important conclusions about whether an infection is present (fever) and about its course. Another example is the use of high-resolution ultrasound devices which enable investigations to be performed without the need to penetrate the body.

### **Replacement: phototoxicity**

Industry was involved in the development of a laboratory test which detects whether the skin is made more sensitive to light by an active ingredient (a dangerous side effect of many substances). In this "3T3 NRU phototoxicity test", skin cells are exposed to ultraviolet light in a Petri dish. The sensitivity of this test was recognized by the OECD in 2003. Previously, animal studies were required to research this effect. These are now completely redundant.

### **Use of reconstituted human tissue culture models for identifying the irritant potential of chemicals used in the workplace**

Irritant dermatitis and ocular injury attributed to chemical exposure are ongoing issues with regard to occupational health and potentially can have serious impacts on quality of life as well as loss of productivity, particularly with regard to contact with severely irritating materials. An important step in control of irritants lies in the identification and characterisation of the chemical hazard which, for many years has relied on animal models. A company that conducts testing to determine the irritating properties of proprietary materials prior to their handling in large scale manufacturing operations has found that recent developments in the production of artificial human tissue models offer an opportunity for replacing, or significantly reducing the use of animals for irritant hazard identification. Furthermore, they are considered to offer a more physiologically relevant test system than other alternative methods proposed for this purpose, such as single cell monolayer culture. The Occupational Toxicology group within the company has adopted these models as part of a tiered approach to assess the corrosive and irritant potential of pharmaceutical process materials. Since the adoption of this strategy at the start of 2006, no animals have been used in the evaluation of dermal or eye irritation for any process material, resulting in a total cessation of animal use for this compared with testing which might have occurred using standard regulatory protocols.

### **Replacing the use of guinea-pigs with a cell-based assay to assess the duration of action of drugs for the treatment of asthma**

Inhaled beta-2 adrenoceptor agonists are widely used drugs to treat asthma and consist of those with a short duration of action used in rescue therapy, or those with longer duration of action, taken twice-a-day. A beta-2 agonist with a long duration of action which would only need to be administered once-a-day would be of great benefit to the patient.

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To measure the beta-2 agonists duration in vitro, one company is currently use an electrically stimulated guinea-pig isolated trachea prep. It has developed and characterised a cell-based washout assay to assess the duration of action of beta-2 agonists in Chinese Hamster Ovary cells recombinantly expressing the human beta- 2 adrenoceptor (CHO $\beta$ -2 cells). This makes it possible to replace the guinea-pig trachea assay for compound duration of action with this CHO $\beta$ -2 cell washout assay, thereby reducing animal usage by 15-20 animals per month as well as increasing throughput.

### **New technology for monitoring body temperature in mice provides new insights, improves welfare and maximises scientific value**

Implanted identification chips are widely used on companion animals, and such devices are frequently used to identify animals used in laboratory-based studies. Significant recent developments now make it possible to gain more information from individual animals and use smaller implants. It is possible to remotely monitor the temperature of individual mice in group-housing. Routine monitoring of temperature, especially in small mammals, is very important both in generating scientific information and to provide unequivocal measures of welfare and wellbeing. This significant development enables the collection of previously unobtainable but fundamental information from multiple mice within their natural social environment and thus reduces the number of animals required to produce meaningful results.

### **Evaluation of novel agents for stomach acid suppression using freely moving dogs**

The clinically available acid suppression therapies for the treatment of ARD fail to produce satisfactory relief from symptoms in many patients. This has necessitated the investigation for novel agents, with differing mechanisms of action, to reduce the symptoms of ARD. In the past, potential new medicines were tested using dogs restrained in slings for up to 8 hours to generate approximately 7 hours worth of data. With the need to investigate different mechanisms of action, the novel agents are now investigated using dogs that are free to move and wear custom made jackets for up to 24 hours. Within pockets attached to these jackets the battery-powered equipment monitors the acidity of the dogs' stomach and infuses a gastric acid stimulant used to simulate gastric acid secretion in patients. The equipment used with the dogs is similar to that used for clinical gastric acid studies and for patient controlled analgesic administration. Dogs are then administered the novel agents orally. The resultant data is then analysed using methods developed from clinical studies. These technology refinements allow the dogs to move independently and to be housed in their home runs for almost the entire duration of the experiment. In summary, these refinements: improve both animal welfare and quality of the data; allow the investigation of onset and duration of action to be performed in a single experiment of 24 hours duration, and allow the use of a modified experimental design which results in a reduction in the number of dogs used.

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### Welfare-enhanced cage design for primate toxicology studies

Traditionally the *Cynomolgus* Macaque (*Macaca fascicularis*) commonly used in regulatory toxicology studies was individually housed in caging of metal construction containing a single metal perch and grid floor. It is widely recognised that provision of social and environmental stimuli within a cage environment, that supports and encourages the animals in demonstrating natural behaviours, has a positive effect on health and well-being. Such benefits to the animal will enhance the scientific integrity of experimental work in which they are involved. In 2002 a purpose-built primate facility was opened at a company's laboratory to support the conduct of regulatory toxicology studies. This allowed the housing of six animals of less than 4kg bodyweight within each pen, giving a total room population of 48 animals. The modular construction allows for cage panels to be removed if necessary to open up a larger enclosed area. This housing system has allowed the animals direct interaction with their pen mates, the structural features of the cage and foraging substrate on the pen floor leading to noticeable improvements in demeanour and behaviour, and a reduction in adverse behaviour; notably a lack of stereotypic behaviours. The conduct of regulatory toxicology studies has been demonstrably enhanced through the use of group housing. This caging is recognised as having significantly advanced the standards of housing of non-human primates used for toxicology purposes.

### The reduction of primate use for the non-clinical prediction of abuse potential; Are rodents a suitable alternative?

Understanding the abuse potential of a new, centrally acting, drug is a key component for the non-clinical assessment of drug safety. The majority of abuse potential (AP) assessments have been performed using the non-human primate (NHP) self-administration model. In this experiment, monkeys are typically trained to press a lever for intra-venous delivery of a drug with known reinforcing properties (e.g. cocaine). A test drug is then made available for self-administration. Continued responding on the lever by the monkey for delivery of the test drug, allows prediction of the AP of the drug in man. In accordance with the 3Rs principal of reduction of animal use, and following recent guidance from the European Medicines Agency stating that, "where rodent or other non-primate models are available [for investigation of abuse potential] these deserve preference above primate models... provided the validity of the model used is sufficiently documented and... is technically feasible," a company sought to evaluate the validity of the rat self-administration model for assessment of AP. A review was undertaken to determine the concordance between findings from published rat, NHP and human AP assessments of drugs with differing mechanisms of action. AP assessments of 59 compounds demonstrated >90% concordance between data in rat and man, and NHP and man. However, published AP assessments for atomoxetine (a treatment for attention-deficit/hyperactivity disorder) and modafinil (a treatment for narcolepsy) in cocaine trained rats were unavailable. Thus, studies were performed to assess the reinforcing potential of these compounds. Atomoxetine, for which published data describe no self-administration in cocaine trained NHPs, was not self-administered by cocaine trained rats. Modafinil, for which published data describe self-administration in cocaine trained NHPs, was self-administered by rats. These data further support the rat self-administration model as an appropriate alternative to the use of NHPs for the non-clinical assessment of AP.

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### Reducing and refining the use of animals in cancer drug discovery: Measuring anti-tumour drug potency in normal, not tumour, tissue

The majority of effective anti-cancer drugs currently in use were selected based on their ability to inhibit the growth of tumours in rodents. However, some targets for anti-cancer drugs have specific and measurable effects in normal tissues and this may allow the effectiveness of an anti-cancer drug to be measured in animals without having to induce tumours. Companies use various techniques to investigate cancer drug targets in normal tissue rather than in tumour tissue. If the drug target does play a role that enables simple measurement, they use tests in healthy tissue to assess the effectiveness of new therapies in a normal animal. This benefits animal welfare in two ways: Refinement: because tumours do not have to be induced in an animal, and the duration of study is often shorter. Reduction: because it is possible to greatly reduce the number of animals used. For instance, in one project, the cancer drug target was found to affect blood pressure in normal animals. To look for activity of a number of potential drugs, blood pressure was monitored in a total of 12 normal rats. This replaced use of 800 mice with tumours that would previously have been required. In addition, the rats received only a single dose per test rather than the 10-14 daily doses to a mouse that would have been required to see tumour growth inhibition. This work also has additional scientific benefits in that the effects of a potential cancer drug on normal tissue are measured and understood which, in turn, gives information on likely clinical side effects. This knowledge can greatly assist patient safety during the clinical trials of new drugs.

### How radio-telemetry has reduced the numbers of animals used in the discovery of new medicines

Candidate medicines are routinely tested for effects on the heart, brain, blood circulation and breathing. Previously, companies measured many of these effects by inducing anaesthesia in dogs or rats and implanting catheters. At the end of the experiment, the animal would have to be killed without regaining consciousness. Now, all these biological signals can be transmitted from animals in their normal sleep/wake cycle using small, surgically-implanted radio-telemetry devices. The scientific benefits are: 1) it is possible to study the same animal on several occasions in their normal environment – instead of just once under anaesthesia. This way, several dose levels or repeated doses of the same medicine can be compared, or different medicines examined; making comparisons in the same animal rather than using different animals improves the statistical power of the study to give a clearer result. 2) The results are more relevant to humans as the animals are in their normal sleep/wake cycle and can be freely-moving during the experiments instead of being under anaesthesia. 3) The effects of the medicine can be studied for longer, e.g. up to 24 hours. Anaesthesia is normally only stable for a few hours. The 3Rs benefit is: A dramatic reduction in the number of animals used to generate data on a new medicine. For example, in 2005, one company reduced by 95% the number of dogs used to discover a new safe, long-acting, inhaled bronchodilator agent to treat asthma.

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### The contribution of imaging to the 3Rs

Some companies have for many years used advanced medical imaging techniques such as Magnetic Resonance Imaging in laboratory animals in their research. Use of these imaging techniques can make a significant contribution to the 3Rs: Reduction (Fewer animals to obtain same quality results) - the same animal can be scanned before and after treatment. This reduces data variability so in many cases fewer animals are needed. - Further reduction can be achieved using advanced statistical techniques. - Sometimes animal imaging can show a potential medicine is unlikely to work in man. Then the research project can be stopped immediately, which not only prevents exposure of any patient volunteers to a potentially ineffective drug, but also avoids any animal use in the essential safety testing that would have been necessary before a new medicine could be tested in humans (clinical trials). Refinement (less painful or distressing animal studies) - Advanced imaging techniques allow measurement of tiny changes in animals which, from a veterinary perspective, are quite normal and healthy. - Even where animals mimicking a human disease need to be used, it is often possible to reduce the disease to a much milder level. - Scanning helps decide which animals should be included in studies, and diagnoses any problems Replacement (eliminating animal studies) - While animals have to be used where data cannot be obtained from in vitro tests (and where ethics prevent seeking the data from humans in clinical trials), new translational "biomarker" science from scans in clinical trials can provide more data about the biological effects of drug candidates on patients, and over time biomarker data may eliminate the need for some animal studies.