

THE THREE RS IN THE PHARMACEUTICAL INDUSTRY:
PERSPECTIVES OF SCIENTISTS AND REGULATORS

by

NICOLE PATRICIA FENWICK

B.Sc., The University of Guelph, 1995

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Animal Science)

THE UNIVERSITY OF BRITISH COLUMBIA

August 2005

© Nicole Patricia Fenwick, 2005

Abstract

Interviews with six drug regulatory reviewers and 11 pharmaceutical research scientists were used to explore their perspectives on obstacles and opportunities for greater implementation of the Three Rs (Replacement, Reduction, Refinement) in drug research and development. Participants generally supported the current level of animal use in the pharmaceutical industry and viewed *in vitro* methods as complementing, but not replacing, the use of animals. Obstacles to greater use of the Three Rs cited by participants included the lack of well-researched and validated non-animal alternatives; the need to use certain numbers of animals to achieve statistical validity; some regulatory requirements; reluctance by industry and regulators to depart from established patterns of animal use; the priority of commercial objectives ahead of the Three Rs; and concerns that less animal testing could jeopardize human safety. Opportunities identified for the Three Rs included the development of better animal models including genetically modified (GM) animals; pursuit of more basic knowledge, notably drug action on gene expression; re-use of animals in successive studies; greater use of pilot studies to avoid full-scale studies of unpromising drugs; using sufficient numbers of animals per test to avoid repeating inconclusive studies; regular review of animal data and regulatory requirements; and following the regulatory option of combining segments of reproductive toxicology studies into one study. In some areas greater implementation of the Three Rs seemed well aligned with industry priorities; examples included phenotypic characterization of GM animals and validation of alternative methods. In other areas, wider use of the Three Rs may require building consensus on areas of disagreement including the usefulness of death as an endpoint, the suitability of re-using animals, and whether GM animals and the use of pilot studies contribute to Reduction. The Three Rs, with their emphasis on decreasing use of animals, may also be incompatible with the goal of protecting human safety and the commercial objectives of the pharmaceutical industry.

Table of Contents

Abstract	ii
Table of Contents	iii
List of Tables	iv
Acknowledgements	v
Co-authorship Statement	vi
Chapter 1 Introduction	1
Animal use in the pharmaceutical industry	1
The Three Rs and alternative methods in the pharmaceutical industry—previous research.....	6
Thesis research.....	15
Chapter 2 Methods	18
Research methods.....	18
Limitations of research methods	26
Chapter 3 Results	29
Views on animal use in pharmaceutical research and development	29
Views on the use of <i>in vitro</i> alternative methods.....	33
Obstacles to Replacement and Reduction	37
Suggested opportunities for greater implementation of the Three Rs.....	43
Chapter 4 Discussion & Conclusions	48
Discussion.....	48
Summary of conclusions and suggestions for further research	54
References	57
Appendix 1: Behavioural Research Ethics Board Approval Certificate	63
Appendix 2: Letter of Initial Contact	64
Appendix 3: Consent Form	66
Appendix 4: Interview Schedule	68
Appendix 5: Notes for Preamble to Interviews	69

List of Tables

Table 1: Area of expertise, degree(s) and academic research experience of 11 pharmaceutical research scientist participants	20
Table 2: Years of experience in drug research and development of pharmaceutical research scientist and regulator reviewer participants	21

Acknowledgements

My thanks must first go to the study participants for taking the time to speak with me about the sensitive topic of animal research. I am in debt to them for sharing so openly their experiences and personal views.

I would also like to thank my supervisory committee, Susan Cox, Dave Fraser, Gilly Griffin, Marc Levine, and Dan Weary. I am extremely grateful for everyone's willingness to come together across disciplines and assist me with this somewhat atypical project. I would like to especially thank my supervisor Dave Fraser for his encouragement and guidance throughout the research process, and his careful editing of all my writing, even though on sabbatical. Thanks also go to my fellow students, Cathy Schuppli and Carol Morgan, who have shared their experiences with qualitative research and provided me with very helpful insight during all phases of my research.

I have immensely enjoyed my time in the Animal Welfare Program and I have learned about all aspects and areas of animal welfare, well beyond my own thesis. For this I thank all the program members for the many great presentations, discussions and ideas. I also gratefully acknowledge the support of the Animal Welfare Program in sponsoring this research.

Finally, I would like to thank my family for all their support, patience, and understanding from when I first left my job and returned to school until the final days of writing up. Thank-you Jason, Alice, and now Joe.

Co-authorship Statement

Nicole Fenwick, graduate student, was responsible for the study's design, data collection and analysis, and drafted the thesis manuscript. David Fraser, graduate supervisor, acted in the typical role of a graduate supervisor and contributed to the interpretation and presentation of the findings.

Chapter 1 Introduction

Animal use in the pharmaceutical industry

The pharmaceutical industry uses animals in order to discover and evaluate the pharmacological and toxicological effects of new human medicines. According to the Declaration of Helsinki animal experimentation, where appropriate, should precede biomedical research in human subjects (World Medical Association 2004). Some use of animals by the pharmaceutical industry is required by regulatory agencies that review applications to sell new drugs and to test drugs in human subjects. National guidelines regarding testing requirements are issued in the United States by the Food and Drug Administration (FDA) and in Canada by Health Canada. There are also international guidelines produced by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, or ICH. This organisation is composed of the regulatory agencies of the United States, Europe, and Japan as well as experts from within the pharmaceutical industries from each of these regions (ICH 2004). Canada is involved in the ICH process as an official observer. Hence, Canada does not participate in final decisions regarding ICH guidelines, but Health Canada applies ICH guidelines when reviewing applications for drug approval.

Drug development and approval typically involves both animal and human studies. The animal-based studies required in support of proposed new drugs are often collectively referred to as “non-clinical safety studies” or “preclinical studies”, whereas studies undertaken in humans are called “clinical studies”. The goals of preclinical studies are to characterize any toxic effects and to determine dose-response relationships of a new drug compound. Information from preclinical studies is used to estimate a safe starting dose for human clinical trials and to identify parameters for clinical monitoring (ICH 1997). In clinical trials the goals

are to demonstrate the safety of the drug in healthy humans (Phase I trials), and then the safety and efficacy in patients (Phases II and III trials) (Gad & Chengelis 1995). Regulatory agencies usually require that preclinical data submitted for review must be from tests that were conducted to Good Laboratory Practice (GLP) standards. These standards for the conduct and reporting of studies are in place to ensure the quality and integrity of the data. Regulatory agencies evaluate whether GLP standards have been met by auditing documentation and inspecting laboratories.

Preclinical studies include tests that evaluate non-target pharmacological effects, pharmacokinetic characteristics, and genetic, acute, chronic, and reproductive toxicological effects of the drug (ICH 1997). All these studies are conducted in healthy animals. In non-target pharmacology or “safety pharmacology” studies, animals are used to examine whether a new drug can affect physiologic or pharmacologic responses other than the intended response (Meyer & Svendsen 2003). Pharmacokinetic studies determine how a drug is absorbed, distributed, metabolised and excreted. In genetic toxicity or “genotoxicity” testing, the goal is to identify whether the drug induces mutations or causes chromosomal damage and this is evaluated using both *in vitro* and *in vivo* studies (Meyer & Svendsen 2003). Acute toxicity studies evaluate undesirable biological effects (also known as adverse effects) that occur in a short period of time (up to 14 days) after administration of a single dose of the drug (Meyer & Svendsen 2003). If appropriate for the type of drug, chronic toxicity is evaluated through repeat-dose studies, where animals are exposed to the drug for periods of 28 days, 90 days or one year, and carcinogenicity studies, which expose animals to the drug for the average lifespan of the animal (e.g. 24 months for rats).

Regulatory guidelines require that acute and chronic toxicity studies be conducted in two mammalian species with one of these being a non-rodent species (ICH 1997). Rats and mice are the most used rodent species and dogs are the most used non-rodent species, with occasional use

of miniature pigs and non-human primates. For carcinogenicity and reproductive studies typically only rodent species are required.

Numbers of animals used by the pharmaceutical industry

Unfortunately there are no clear statistics that show the number of animals used annually by the pharmaceutical industry in North America. In Canada data on the number of animals used for experiments, and the purpose of their use, are collected annually by the Canadian Council on Animal Care (CCAC), a national peer-review body which oversees laboratory animal use. However, these numbers are reported only by institutions that are participants in the CCAC program and not all private sector animal-using companies in Canada take part. In Canada in 2002 the total number of animals reported used was 2,103,135 and the three most used taxonomic groups were mice (accounting for 36.1 percent of the total), fish (28.9 percent) and rats (15.8 percent) (CCAC 2005).

In the United States in 2002 the number of animals reported used was 1,137,718 (United States Department of Agriculture 2003a). However, numbers of mice, rats and birds used are not reported because they are excluded from the definition of animal in the relevant legislation, the Animal Welfare Act (United States Department of Agriculture 2003b). Because rodents are frequently used as laboratory animals, various attempts have been made to estimate total United States animal use, including mice and rats. One estimate of total United States animal use for 1995 was 13.9 million (Orlans 1998), calculated by assuming that only 10 percent of animals used in experiments are officially counted. Rowan et al. (1995, as reported in Gauthier 2004) estimated that 14 to 21 million animals were used in biomedical research in the United States in 1992 but no rationale for the estimate was described. Gauthier (2004) estimated that 28.8 million animals were used in experiments in the United States in 1992. For this estimate, Gauthier first calculated the United States-to-Canada ratios of numbers of dogs, cats, rabbits and

hamsters used. Next, the average of these ratios was multiplied by the total number of animals used in Canada in 1992 to estimate animal use in the United States.

The number of animals used directly by the pharmaceutical industry is not possible to determine for either Canada or the United States. In Canada in 2002 a total of 281,196 animals were used for “studies for regulatory testing of products for the protection of humans, animals or the environment” (Purpose of Animal Use 3), and 175,085 animals were reported used for “studies for the development of products or appliances for human or veterinary medicine” (Purpose of Animal Use 4) (CCAC 2005). Combined, these categories account for approximately 22 percent of the total animal use in 2002 and include animal use in the pharmaceutical industry. In 2003 the United Kingdom, which keeps more detailed statistics related to animal use in the pharmaceutical industry, reported that “pharmaceutical safety/efficacy evaluation purposes” accounted for 63 percent of all toxicology procedures on animals (Home Office 2004). The United Kingdom also reported that pharmaceutical research and development accounted for 400,000 procedures (or 17 percent of procedures) for “fundamental and applied studies other than toxicology, regulatory or safety purposes” (Home Office 2004). It is not clear how estimates from Canada or the United Kingdom may relate to animal use in the United States however, given the greater extent of the pharmaceutical industry in the United States compared to Canada it is likely that the pharmaceutical industry accounts for at least 22 percent of total animal use in that country.

Animal welfare concerns relating to use of laboratory animals by the pharmaceutical industry

Concern for laboratory animal welfare has been expressed in both scholarly and animal advocacy literature. The term ‘animal welfare’ has been variously defined; however, I will adopt a commonly accepted tripartite model used by many animal welfare scientists (e.g. Duncan & Fraser 1997) which sees animal welfare as involving 1) an animal’s basic health and

functioning, 2) an animal's affective states, and 3) an animal's ability to perform important types of natural behaviour. For laboratory animals, concern for the three areas of welfare may be reflected by considering, for example, whether the animal is injured (basic health), whether the animal is experiencing pain and distress (affective state), and whether the animal is being housed in a way that allows exercise and other behaviour appropriate for the species (natural behaviour). These animal welfare concerns do not, of course, exhaust the range of ethical concerns associated with laboratory animal use. For example, it is generally accepted that animals should not be used or killed unnecessarily (e.g. CCAC 1989).

There are also ethical and animal welfare concerns that apply especially to laboratory animals used in commercially-directed research and testing, such as in the pharmaceutical industry. Four such concerns have been discussed in the literature. First, limited access to commercial data has resulted in a lack of objective assessments of how well animals are functioning as predictive models for humans (e.g. Olson et al. 2000; Greaves et al. 2004; Preziosi 2004; Rawlins 2004). Second, in much biomedical research, causing harm to the animal is incidental rather than integral to the experimental procedure, whereas in commercial toxicity testing the goal of the experiment is to produce a toxic effect in the animal, often (presumably) with associated pain and/or distress (Smith & Boyd 1991; Balls 1994; Rollin 2003). Third, there is a legal obligation to use animals in testing and a legal obligation to ensure that products are safe. This requirement to adhere to regulatory guidelines considerably restricts how test procedures can be modified by animal ethics committees in response to concerns raised during ethical review (Purchase 1999). Fourth, the confidentiality of experimental data produced in a commercial setting may result in duplication of experiments with animals (Smith & Boyd 1991).

In biomedical research the concern for animal welfare is often acknowledged by reference to Russell and Burch's principles of the "Three Rs" (1959). The Three Rs are: Replacement (replacing whole animal models with non-whole animal models), Reduction (reducing the number of animals required to a minimum), and Refinement (minimizing harms to animals in both husbandry and experimental procedures). Application of the Three Rs involves consideration of the use of "alternative methods" a term that refers collectively to scientific methods that use fewer animals; minimize pain and distress; use cells, tissues or animals of lower sentience; or do not involve animals at all (Smyth, 1978, as reported in Balls et al. 1995). Use of the Three Rs is widely accepted in animal research communities and is typically used by ethics committees to guide their review of animal care and use protocols. The European Union's Directive 86/609 and legislation in the United Kingdom include reference to the Three Rs (Stephens et al. 2001). The United States Food and Drug Administration (1992) includes the Three Rs in a policy statement on animal use in regulatory testing, and in Canada the CCAC similarly includes reference to the Three Rs as fundamental principles for animal-based research and regulatory testing (CCAC 1989). The Three Rs are also used as guiding principles in pharmaceutical company policies related to animal care and use (e.g. Glaxo Smith Kline 2001; Pfizer 2002; Eli Lilly 2004).

The Three Rs and alternative methods in the pharmaceutical industry—previous research

A limited amount of research has examined pharmaceutical industry use of laboratory animals and use of the Three Rs and alternative methods. In the following sections I review some of the approaches and the findings of research that has attempted to examine the contributions of non-whole-animal and animal experiments, how successfully the Three Rs have been used in animal-based research, and how the Three Rs fit with regulatory guidelines.

Research analysing the contributions of non-whole-animal and animal experiments

One approach to assessing animal use has been through critical reviews of the contributions of non-whole-animal and animal experiments. Many of these reviews are specific to a certain field of science and not to the pharmaceutical industry. They are also generally written with the aim of improving the science in the field, rather than reducing or replacing animal use. For example, Brandon et al. (2003) reviewed the “pros and cons” of *in vitro* methods in human hepatic drug research. They found that techniques using isolated human perfused livers were very representative of *in vivo* situations but are limited by practical considerations, whereas various cell culture and subcellular fraction models are useful only for limited purposes. Overall these authors concluded that *in vitro* models are presently unable to replace *in vivo* models but do offer Reduction opportunities.

In another example from the biomedical literature, Pound and colleagues (2004) analysed seven published review articles that examined how animal studies had informed human clinical medicine. They reported that the review articles all identified methodological problems in animal studies that led to incorrect interpretations of results. These observations led Pound et al. to conclude that more frequent, thorough reviews of animal contributions to clinical medicine are required in order to increase experimental validity and benefit, to decrease unnecessary duplication, and to promote adherence to the principle of Reduction. Similarly, Greaves et al. (2004) focused on how animal use increases the safety of new human medicines by analyzing published reviews that examined the predictive power of preclinical data. These authors concluded that 90 percent of the toxicities that can be detected in animals are detected in studies lasting one month or less, and that information obtained from dog studies is generally more predictive of adverse effects in humans than data from rodents or monkeys. They also concluded that there is currently no evidence to show that genomics technologies and analysis of

the structure-activity relationships of drug compounds (sometimes proposed as alternative methods) have contributed information to drug safety assessments. Greaves et al. (2004) noted however, that their analysis was limited by lack of access to proprietary data and that evaluation of preclinical data would be facilitated by publication in peer-reviewed literature.

Another approach to assessing contributions of animal-based methods has been citation analysis. In one example Dagg (2000), in an analysis of animal-using cancer research papers, calculated the ratio of citations received to the number of animals used in the paper, based on the assumption that a higher number of citations indicates that a paper was more useful to other scientists. Dagg (2000) concluded that animal ethics committees are not being sufficiently vigilant to curtail unnecessary experiments in the field of cancer research. However, this broad conclusion was based on the assumption that there was an ethical problem with experiments that used animals but received few citations.

The contributions of animal experiments have also been assessed through retrospective data analysis. In a study that used proprietary data provided by pharmaceutical companies, Olson et al. (2000) correlated the toxicities observed in human clinical trials with toxicity data from the corresponding preclinical animal studies for 150 selected drugs. They found that 71 percent of the toxicities observed in human clinical trials were identified by the preclinical animal studies. However, the authors acknowledged that their study was limited by the lack of data from drugs that were toxic to animals and may have been toxic to humans, but were never tried in humans. In a similar study also using unpublished proprietary data, Broadhead et al. (2000) assessed the contributions of toxicity studies conducted in dogs. This analysis concluded that use of the dog in studies of less than three months duration did provide additional information on potential toxicities that were not identified in rodent studies. They also concluded that in studies of longer duration, the use of only one species rather than two may

provide sufficient information and that more investigation is required on this point. Proprietary information was also used by Smith et al. (2005) in a workshop to develop a new study design for non-regulatory drug toxicity studies in the dog. These authors concluded that the proposed design would use fewer dogs and collect information on a greater number of clinical parameters than dog toxicity study designs currently in use. These papers show that the approach of retrospective data analysis is useful in understanding the contribution of animals; however, it requires access to preclinical data, which are often proprietary.

Research analysing use of the Three Rs and alternative methods

Use of the Three Rs has been examined by looking at each 'R' individually and by assessing them collectively. In a critical review of Replacement, Balls (1994) noted that the range of methods used to replace animals includes avoiding unnecessary duplication; using physical and chemical experimental techniques; using mathematical and computer models; using organisms of lower sentience such as invertebrates, plants and microorganisms; using early developmental stage vertebrates; using *in vitro* methods, such as tissue slices and cell cultures; and human studies. This review also described the difficulty of validating methods to replace animals, and the role of non-governmental organizations in promoting and facilitating validation. In his conclusion, Balls (1994) proposed that more effort should be put towards maximizing the usefulness of non-animal methods in toxicity testing. He suggested that individuals conducting regulatory tests need to be more "realistic" about the scientific limitations of animal tests and more willing to consider alternatives; that more creativity be applied to the development of alternatives; and that regulators consider data from alternatives in place of traditional methods.

Festing's (1994) review of Reduction noted that there are few surveys of research that examine the quality of experimental design and statistical analysis. Of these, most have concluded that poor quality of experimental design has often led to a waste of resources and in

some cases to wrong conclusions. Festing (1994) also used a case study approach to analyse the experimental design and statistical analysis of three toxicology research papers, and to explain how better experimental design could have allowed all three studies to use fewer animals while also improving the science. Stephens et al. (2002) reviewed possibilities for Reduction in regulatory testing and concluded that improvements to experimental design, use of genetically defined animals to minimize statistical variance, and the use of non-animal methods to screen test substances before proceeding to *in vivo* tests would contribute to Reduction. Stephens et al. (2002) also concluded that an obstacle to Reduction is the default practice of using greater numbers of (less expensive) smaller animals.

Refinement was examined by Flecknell (1994) in a review of laboratory animal pain assessment and alleviation. The author concluded that reliance on anthropomorphic criteria for pain assessment is not sufficient and that objective methods for detecting pain and distress still need to be developed. Pain assessment in laboratory animals and the administration of analgesics (Refinement) was also examined in an ethnographic study of animal researchers (Phillips 1993). This author found that although researchers could agree that animals feel pain, the researchers rarely noticed occurrences of pain and suffering in their own laboratories. Stephens et al. (2002) reviewed possibilities for Refinement in regulatory testing and concluded that opportunities include improved pain and distress relief, and use of experimental endpoints that occur before adverse reactions are clinically observed.

Carlsson et al. (2004) surveyed animal-using scientific papers that were published in major journals in 1970 and 2000 to try to assess whether implementation of the Three Rs has increased over time. This survey tabulated the number of animals used in each paper and factors related to animal health and husbandry such as microbiological status, bedding material and cage size. The frequency of reporting this information was then compared between papers

published in 1970 and 2000. The authors concluded that an increase in reporting of these details represented an improvement in application of Refinement, and the decrease in animal numbers represented an increase in the application of Reduction. However, this approach to assessing the implementation of the Three Rs seems limited by the unclear association between the factors reported in published papers and the actual application of the Three Rs by scientists.

Application of the Three Rs has also been examined by authors who drew on their own experience to discuss the successes and the challenges they faced when trying to implement the Three Rs. In one such paper a regulator identified lack of knowledge, together with resistance to the Three Rs concept, as obstacles to the acceptance and implementation by scientists (Zurlo 2000). Another regulator noted that uncertainty regarding the interpretation of data from alternative methods was a barrier to regulatory and industry acceptance (O'Connor 1997). In an article that reviewed the achievements of the Three Rs in the development and testing of biologicals, Hendriksen (2000) identified “frustrations” related to applying the Three Rs, such as slow regulatory acceptance of alternative methods, and scientists who do not consistently support the Three Rs in their work. Similarly, Clark (1994) and Richmond (2002) noted several barriers to the acceptance of alternative methods in industry including the difficulty of validating alternative methods, lack of scientific knowledge, lack of desire to change regulatory guidelines to include alternatives, fear of litigation, and the conservative attitudes of regulators. While these commentary papers highlight the observations and insight of experienced individuals, the conclusions may be difficult to generalize from because they were written from a personal perspective and may be regarded as anecdotal.

In another approach to analyzing application of the Three Rs, organizations dedicated to implementation of the Three Rs have co-ordinated workshops that brought together experts to make recommendations on how the Three Rs could best be implemented. One such workshop,

coordinated by the European Centre for the Validation of Alternative Methods (ECVAM), was titled “Reducing the Use of Laboratory Animals in Biomedical Research: Problems and Possible Solutions”. This workshop concluded with some general recommendations for reducing animal use by improvements to research strategy, experimental design and statistical analysis, interpretation of results, legislation, and education of scientists (Festing et al. 1998). Another workshop coordinated by ECVAM was titled “Pharmacokinetics in Early Drug Research”. Workshop participants, who were from both the alternatives community and the pharmaceutical industry, reviewed the current *in vivo* and *in vitro* methodology for providing early pharmacokinetic data in drug research. The workshop report noted that participants supported the view that current *in vivo* pharmacokinetic studies used more animals than the minimum that would be required if *in vitro* data were used more consistently in pharmacokinetic studies (Leahy et al. 1997). The report also recommended ways to use animal studies more effectively such as by increasing development of toxicological databases, and it identified specific research needs such as research to optimize techniques for preservation of metabolic functions in *in vitro* preparations (Leahy et al. 1997).

The Three Rs and pharmaceutical regulatory guidelines

Application of the Three Rs in pharmaceutical regulatory guidelines has been examined from various perspectives. Some authors have assessed regulatory use of animals across industries. At a conference that brought together regulators and scientists from both the chemical and pharmaceutical industries, Stitzel et al. (2002) concluded that there was scope to merge chemical acute oral exposure tests and pharmaceutical safety pharmacology tests into a single acute toxicity study. Purchase (1999) examined ethical issues associated with animal use in regulatory guidelines for toxicity testing. This review noted that even if regulatory toxicity studies undergo review by an animal ethics committee, the testing cannot be altered in response

to comments arising from the review. However, since the regulatory guidelines themselves have not undergone independent ethical review Purchase (1999) concluded that independent ethical review of such regulatory guidelines could result in improvements to animal welfare.

Some research has more specifically assessed the compatibility of the Three Rs with pharmaceutical regulatory requirements. Three studies reviewed safety pharmacology studies, which are studies that typically use dogs to investigate any undesirable pharmacodynamic effects of a potential drug (ICH 2001). Luft and Bode (2002) reviewed ways to integrate safety pharmacology measurements into toxicity studies. They concluded that in some cases if a few additional animals were added to a toxicity study then safety pharmacology could be assessed in parallel with toxicity, and a separate study would not be required. A similar conclusion was reached by Greaves et al. (2004) who, in a review of the contributions of preclinical data to drug safety evaluation, also identified that some of the same data were collected in toxicology and safety pharmacology studies, and that these could be combined into one study. In their review of how *in vitro* methods could be applied in regulatory testing of safety pharmacology Wakefield and colleagues (2002) noted that when *in vivo* studies are conducted early in the development process those studies will likely need to be later repeated using GLP standards. They concluded that *in vitro* approaches could contribute significantly to the early evaluation of safety pharmacology and assist in decreasing the number of *in vivo* studies.

The compatibility of the Three Rs with pharmaceutical regulatory requirements has also been reviewed more broadly. Tweats (2000) and Schechtman (2002) reviewed aspects of internationally harmonized regulatory guidelines that are consistent with the Three Rs and noted that harmonization contributed considerably to Reduction of animals used in pharmaceutical regulatory testing. Schechtman (2002), further noted that the Three Rs approach is implemented in several guidelines; these include a guideline for carcinogenicity testing that identifies

situations where the test is not necessary, a guideline for genotoxicity that outlines provisions to use *in vitro* tests, and a chronic toxicity guideline that allows studies of shorter duration. Snodin (2002) reviewed the use of *in vitro* methods and opportunities for Three Rs in pharmaceutical toxicology from the perspective of European Union regulatory authorities. He noted several opportunities to apply the Three Rs in pharmaceutical toxicology testing, including the validation of alternative methods using internationally accepted validation criteria, use of transgenic animals, and development of the fields of toxicogenomics and toxicoproteomics. He also noted that the lack of biological complexity of non-animal models made them unlikely to serve as Replacements, and concluded that short term prospects are “brighter” for Reduction and Refinement.

Summary of previous research

In summary, a wide range of approaches including review articles, personal commentary, and workshops have been used to analyze the use of the Three Rs and alternatives. Most of the research which analyzed the contributions of non-whole-animal and animal experiments found value in both types of studies. However, most concluded that continual review of animal studies would increase their value and may contribute to Reduction, but that in the pharmaceutical industry the scope for analysis of the contributions of animal data is severely limited by lack of access to proprietary information. Research that analyzed use of the Three Rs and alternatives has generated many general suggestions on how to replace animals and to achieve Reduction and Refinement, as well as more specific suggestions for Reduction such as through alternate experimental designs.

Similarly, workshops and collaborative studies with participants from both the alternatives community and the pharmaceutical industry resulted in additional suggestions on how the Three Rs could be specifically pursued in the pharmaceutical industry. Research that

examined the Three Rs and regulatory guidelines has identified some areas where progress in the Three Rs has already been made. Specific opportunities to implement the Three Rs in some aspects of safety pharmacology testing were also identified, as well as other general ways forward. Several authors, based on personal experiences, highlighted possible obstacles to greater use of the Three Rs and one author proposed that ethical review of regulatory guidelines which require animal use may contribute to improved animal welfare.

Overall, there is limited research into the Three Rs in the pharmaceutical industry and very few studies assessing the usefulness of the way animals are currently used in pharmaceutical research and regulatory testing. Of the existing research several studies focussed on Reduction of the number of dogs used. In general, Reduction appears to be more positively viewed, while Replacement appears to be viewed as less likely.

Thesis research

As noted above, a variety of ways to apply the Three Rs and alternative methods in the pharmaceutical industry have been suggested, but empirical research has been limited. Therefore I proposed to further investigate where animal use in the pharmaceutical industry could be replaced, reduced or refined.

A number of possible research approaches could have been followed. The usefulness of literature surveys and citation analysis approaches, such as those by Carlsson et al. (2004) and Dagg (2000), are restricted by the limited publication of pharmaceutical industry data and the unclear relevance of these analyses to assessing use of the Three Rs. Two studies noted above (Broadhead et al. 2000; Olson et al. 2000) examined the usefulness of animal toxicology drug studies through retrospective data analysis. This type of research is logistically complex as it requires multiple authors and access to proprietary data provided by the participating

pharmaceutical companies. Similar studies to examine the predictive value of other animal tests, although desirable, would be very difficult to conduct.

Workshops on implementation of the Three Rs that included pharmaceutical industry scientists appeared to be successful in generating specific recommendations. In order to draw on the knowledge and experience available in the industry, one approach to my thesis research could have been to conduct a survey of scientists and regulators regarding the Three Rs. However, this approach was rejected for two reasons. First, I could not find previous empirical studies which asked these populations about their perspectives; therefore not enough was known to be confident that meaningful survey questions could be generated. Second, not enough information was available to guide the selection of an appropriate population to sample.

Given these limitations, I decided to adopt a qualitative approach using personal interviews with industry scientists and regulatory reviewers. Qualitative research is used to clarify values, ideas, and interpretations; to generate hypotheses; to describe complex problems in detail; and to reveal how interdependent individuals, groups, and institutional components interact (Sofaer 1999). Denzin and Lincoln (2000 p3) explain that “[q]ualitative research involves the studied use and collection of a variety of empirical materials ... that describe routine and problematic moments and meanings in individuals’ lives”. Some examples of empirical materials include case studies, personal experience, interviews and artifacts. In the health care research literature, qualitative research has been described as the “systematic collection, organisation, and interpretation of textual material derived from talk or observation” (Malterud 2001 p483). Qualitative research methods are not used for hypothesis testing or for generating statistically representative results. Instead, they allow the researcher to proceed inductively and to be open to topics or ideas raised by research participants.

Qualitative research has been used in disciplines such as environmental research (e.g. Satterfield 2001), health research (e.g. Cox & McKellin 1999; Lippman 1999) and policy evaluation (e.g. Hurley 1999). It has also been used to understand issues arising from animal use. Examples include studies of animal shelter workers (Arluke 2003; Reeve et al. 2004), exploration of the attitudes of veterinary students (Herzog et al. 1989), studies of animal care and use committees (Schuppli & Fraser in press), and studies of issues in wildlife rehabilitation (Dubois & Fraser 2003). Qualitative approaches have also been used to explore topics related to laboratory animal use such as views on use of animals in research (Arluke 1994; Michael & Birke 1994; Paul 1995), use of animals in teaching (Arluke & Hafferty 1996), and researchers' perception of animal pain (Phillips 1993). They have been used to explore other issues related to the pharmaceutical industry, such as the different strategies followed in preclinical toxicological studies (Parkinson et al. 1996), whether harmonization of international regulatory guidelines improves or harms the safety of new medicines (Abraham & Reed 2003), as well as changes to industry research culture (Varma 2000).

I have used qualitative research methods to attempt to improve our understanding of where laboratory animal use may best be replaced, reduced and refined in the pharmaceutical industry. I conducted in-depth interviews with both pharmaceutical research scientists and regulatory reviewers in order to 1) explore the value that participants place on animal and *in vitro* data in drug research and development, 2) have participants identify both opportunities and obstacles to the goal of decreasing animal use in drug research and development, and 3) identify areas of agreement and disagreement in the perspectives and opinions of study participants, and to compare these with the Three Rs literature.

Chapter 2 Methods

Research methods

For this exploratory study I used a “focused ethnographic” research design which involved semi-structured interviews with pharmaceutical research scientists and drug regulatory reviewers to ask directly about their opinions and perspectives regarding animal use.

Ethnography is an applied branch of qualitative research that uses participant observation and interviews to identify and explore an issue, and to answer questions which cannot be addressed with other approaches (LeCompte & Schensul 1999). A traditional ethnographic study, typified by many anthropological studies, involves a broad research focus within a cultural group (Morse & Richards 2002). “Focused ethnography” is characterised by a topic that is identified before the start of the study, participants that may not know each other but share certain features, and use of only one ethnographic data collection method (Morse & Richards 2002). The approach in this study is focused ethnography because 1) the research collected perspectives specifically regarding animal use in drug research and development, 2) the participants’ shared feature was experience with the pharmaceutical industry, and 3) the data collection strategy was confined to interviews.

Sampling

The sample was not randomly selected and I was not seeking to be statistically representative of the views of participants. Instead, this study used criterion-based or “purposive” sampling.

With this sampling method potential participants are chosen because they have particular characteristics which relate to the study’s central questions (Schensul et al. 1999; Ritchie et al. 2003a). As mentioned, participants were pharmaceutical research scientists (hereafter called “scientists”) or government drug regulatory reviewers (hereafter “regulators”). I selected

participants who (1) used or reviewed animal data when making decisions about human drugs, (2) were based in North America, and (3) were not formally affiliated with advocacy organizations promoting either animal welfare or the use of alternative methods. These criteria provided some boundaries for participant recruitment.

Prior to participant recruitment this study received ethical review and approval from the University of British Columbia Behavioural Research Ethics Board (Appendix 1). Some participants were found through personal contacts or referrals by colleagues. Others were found by referral from the initial participants in a process known as chain referral or snowball sampling (Morse & Richards 2002). In addition, regulators from one agency were found through the agency's public relations department. The first direct contact with all participants was made with a letter which described the study, provided the name of the person who referred them, and described how their confidentiality would be protected (Appendix 2). A consent form was also mailed with the letter of initial contact (Appendix 3). Upon receiving the letter some participants contacted me by email or telephone, while with others I followed up the letter with a telephone call to determine their willingness to participate. Participation was voluntary with no remuneration. Twenty letters of initial contact were sent out to individuals and 17 interviews were completed, six with regulators and 11 with scientists. Of the three individuals who received contact letters but were not interviewed, none refused directly. Instead, two individuals (a scientist and a regulator) did not respond to my follow-up telephone message or email, and a third (a scientist) agreed to be interviewed and then later refused because s/he was too busy.

Participants

The scientists included toxicologists, pharmacologists, and clinical researchers, and thus had varying types of involvement in the pharmaceutical industry. The regulators were from both the United States Food and Drug Administration and Health Canada and were responsible for

conducting primary reviews of preclinical data submitted in support of drug approval applications. Participants' areas of expertise included human medicine, immunology, molecular biology, pathology, pharmacology, physiology, toxicology and veterinary medicine, or some combination of these (for example veterinary pathology). Two participants had experience in both industry and regulatory roles, seven had academic research experience apart from graduate school, and two of these had remained primarily academic researchers. More detail about the areas of expertise, degree(s) and academic experience of the scientist participants is provided in Table 1, however this information is not linked together for the regulators, as it could compromise anonymity if combined with other background information. The length of professional experience in drug research and development averaged approximately 24 years, with a range of 8 to > 40 years (refer to Table 2 for more detail). Therapeutic areas in which participants had experience included analgesics, antibiotics, anti-inflammatories, anti-virals, biologics, immune-modulators, ophthalmics, psychiatrics, and therapies for cancer, cardiovascular, central nervous system, and gastro-intestinal diseases. The sample included four females and 13 males.

Table 1: Area of expertise, degree(s) and academic research experience of 11 pharmaceutical research scientist participants

Participant	Area of expertise	Degree(s)	Academic research experience apart from graduate school
1	Pharmacology and toxicology	PhD	no
2	Veterinary pathology	DVM, PhD	yes
3	Human medicine	MD, PhD	no
4	Toxicology	PhD	yes
5	Molecular biology	PhD	no
6	Physiology	PhD	yes
7	Veterinary medicine	DVM, MSc	yes
8	Human medicine	MD	yes
9	Immunology	PhD	no
10	Pharmacology	PhD	no
11	Pharmacology and physiology	PhD	yes

Table 2: Years of experience in drug research and development of pharmaceutical research scientist and regulator reviewer participants

Years of experience	Number of scientist participants	Number of regulator participants
< 10	1	-
10 - 20	6	2
21 - 30	4	1
> 30	-	3
Total number of participants	11	6

Interview process and questions

Interviews were conducted from June 2003 to May 2004. I conducted one semi-structured interview with each participant either in person (seven) or by telephone (10). Approximately 11 to 13 open-ended, prepared questions were asked (Appendix 4). Unplanned questions were also used to allow me to respond to unanticipated comments made by the participants (Morse & Richards 2002), hence not every participant was asked exactly the same questions. The interviews lasted between 25 and 105 minutes. Interviews were tape recorded, and I also took handwritten notes. Participants were identified by pseudonyms.

Prior to telephone interviews participants were asked to return the signed consent form by mail or by fax. At the start of in-person interviews participants were asked to sign the consent form and give it directly to me. At the start of all interviews I provided background information about myself, reiterated information about the study that was contained in the letter of initial contact and asked participants if they had any questions for me prior to the interview (Appendix 5). I then asked for permission to begin tape recording. I began with questions asking participants to describe their background in the pharmaceutical industry and their experiences using animal models. Next they were asked if they could describe: any animal tests which are “less important”; any occasion where animal data were “misleading”; their perspective on “use of animal death as a toxicological endpoint”; and their opinion on the “role of *in vitro* data in drug research and development”. Participants were then asked about

“opportunities for decreasing animal use”, “obstacles to decreasing animal use”, impact of the regulatory environment on animal use, and the role of transgenic animals. The terms “Three Rs”, “Replacement”, “Reduction” and “Refinement” were not used in order to keep the questions as open-ended as possible.

These questions were ‘piloted’ during the first two interviews of the study and subsequently revised to improve clarity. In addition, early in the study I added questions to the interview schedule in response to interesting comments made by participants. For example, when one participant unexpectedly spoke of the usefulness of animal death as an endpoint in toxicology studies, I added the question, “What is your perspective on the usefulness of death as an endpoint?” to the interview schedule.

Data analysis

The interviews were transcribed verbatim and analysis of the transcripts occurred concurrently with data collection. In order to describe and provide insight into the range of views, analysis focussed on searching for commonalities and differences among participants’ responses.

Analysis also focussed on identifying how responses related to the Three Rs in order to provide a framework for discussion. I also generated lists of the opportunities and obstacles for the Three Rs from the responses of participants to those questions.

I used the strategy of coding to assist me in developing my understanding of the interview transcripts. Coding is an umbrella term for the process of organising qualitative data to assist in retrieval and interpretation (Coffey & Atkinson 1996). It also has the purpose of assisting the researcher in generalizing from the data (Morse & Richards 2002) and “interacting with the data” (Coffey & Atkinson 1996 p30). I used several coding strategies throughout analysis. I began the process of coding simply by reading through each transcript and making marginal notes next to the interview text. I then began to organize the data (quotations from

participants) systematically by generating coding categories from my marginal notes and from comments made by participants in the transcripts. This is an inductive approach known as “open coding” and is commonly used in the qualitative research method of Grounded Theory (Corbin & Strauss 1990).

I also used a more descriptive approach to coding which was useful for increasing my familiarity with details of the data (Morse & Richards 2002). With this approach I generated a list of specific, narrowly-focused queries regarding the data and reviewed the transcripts looking for information which related to my specific queries. For example, one query was, ‘Is this participant for or against use of death as an endpoint?’ and it allowed me to catalogue participant viewpoints in a concise way.

Because my questions were fairly uniform from interview to interview, I was also able to organize the data according to the interview questions. This involved the use of preliminary codes (which were related to the interview questions) and also inductive codes (which arose from participant responses) when needed. For example, one preliminary question-related code was ‘*In vitro*’ and I used it to group all the participants’ comments relating to the use of *in vitro* data. This code was directly related to an interview question about the role of *in vitro* data in drug research and development, but I also assigned this code to other comments about *in vitro* data. An example of a more inductive code was ‘Business Needs’ where I grouped all participants’ explanations of how commercial pressures influenced animal use.

Throughout coding and analysis I reviewed the transcripts by reading paper copies and by using the qualitative analysis software program QSR N6 (QSR International Pty Ltd, 2002). I initially used QSR N6 while trying to do open coding. I read through the transcripts on-screen and sorted segments of text into various categories. I also used the software when I began coding based on my interview questions. The software provided a simple way to search the

transcripts, and made it easier to double check exactly what participants had said and in what context.

Interpretation and writing

The methodological literature uses various words and phrases to describe the process of moving beyond description to analysis. Conceptualization and “doing abstraction” are terms used by Morse and Richards (2002 p134). LeCompte and Schensul (1999 p147) refer to the analysis process in ethnography as “making sense of the data”. Ritchie et al. (2003b) explain that the outcome of analysis is the identification of key themes, concepts and categories. To facilitate interpretation I wrote memos to record ideas and insights about the data. I did this using both the QSR N6 software memo feature and by writing in a notebook kept for the purpose. Memo-writing occurred throughout the research process.

I also followed a variation of a more structured technique called ‘thematic charting’ to assist me in moving from coding to interpretation. Thematic charting is a process in which the researcher summarises the key points of each piece of data and organizes it into a chart according to proposed themes and sub-topics (Ritchie et al. 2003b). Doing this provided me with an overview of the data and helped me to compare the perspectives which existed among the participants. By organizing the data this way it was easier for me to see the range of perspectives among participants and items which did not fit into a code.

Another technique I used to assist with interpretation was Chenail’s Qualitative Matrix (Cole 1994). This matrix instructs a researcher to identify patterns in the data by looking for ‘Central Tendencies’ which describe how data come together into common themes, and ‘Ranges’ which are the differences within a theme. The matrix also prompts researchers to deliberately look for ‘Expected Results’ which conform to the literature and researcher

assumptions, as well as ‘Unexpected Results’ which differ from the literature and researcher assumptions (Cole 1994).

The usefulness of looking for unexpected results was illustrated by some unanticipated perceptions about genetically modified (GM) animals in drug research and development. A few participants commented that the increased use of GM animals would contribute to Reduction of numbers of animals used for drug regulatory tests. This was counter to my own view, and to many conclusions in the literature that GM animals, because of the large numbers required to create them, were leading to increased use of animals overall. After puzzling over this perception I realised that those participants were responding to questions about animal use solely within the context of the numbers of animals used in experiments, not taking creation of the GM animals into account. I may not have realised the context of their comments if I had not paid attention to this unexpected result.

Quotes from participants have been used to illustrate the research findings and to allow the reader to more readily evaluate the conclusions I have drawn. The quotes have been presented mostly verbatim, although they have been ‘cleaned’ by removing interjections (“um”, “you know”) and by adding punctuation. In the selection of supporting quotations I tried to ensure that each participant was represented and that the context of the quotes was maintained. This required carefully reviewing transcripts to re-check my interpretation of what participants said. To maintain anonymity I have taken care not to use identifiable quotes from regulators whose identity might readily be recognized. I have also not identified gender or linked different quotes from the same participant, although doing so may have potentially been more interesting for the reader.

Limitations of research methods

Two points regarding the presentation of research results are commonly stressed in the qualitative methodology literature. One is the importance of providing clear, detailed, and explicit explanations of the research procedures (as above). The other is a formal recognition of the limitations of those procedures, and the effect they may have on research findings. The general purpose of these is to allow readers to understand how conclusions were derived from the data (Malterud 2001). Research findings may be limited by the research paradigm, researcher bias, and research procedures.

Research paradigm

Researchers inevitably have certain assumptions regarding the nature of knowledge and how it can be acquired (epistemology) (Snape & Spencer 2003), and certain biases regarding the research topic. To assist in clarifying epistemology, it is customary for qualitative researchers to locate themselves in an interpretive framework or paradigm. A research paradigm is “a way of looking at the world; interpreting what is seen; and deciding which of the things seen by researchers are real, valid, and important to document” (LeCompte & Schensul 1999 p41). Therefore research paradigms have a clear role in guiding the research process.

I would locate myself partially within the post-modern paradigm since I acknowledge that I cannot be a neutral, objective data collector and that through the process of interviewing I am shaping the research (Legard et al. 2003). I also incorporate an element of the critical theory paradigm (LeCompte & Schensul 1999) because I do have an agenda for change which is motivating the research. I locate myself outside a positivistic research paradigm, which rests on assumptions of the objectivity of the researcher and the ability to conduct value-free enquiry (Snape & Spencer 2003).

Researcher bias

Qualitative researchers also need to acknowledge their bias toward the research topic. Denzin and Lincoln (2000 p19) explain that “All research is interpretive; it is guided by a set of beliefs and feelings about the world and how it should be understood and studied”. This is evident in this study because my concern for animal welfare motivated the research and I view the objective of decreasing animal use positively. These views, which may have biased my interview questions, were not necessarily shared by participants.

My experiences working in the pharmaceutical industry may bring another type of bias. While the experience was helpful to me when developing my interview questions, locating participants, and building rapport, it may also slant my analysis because I likely share a number of assumptions with my participants. For example, the assumption that scientific inquiry is important, and the assumption that animal experimentation cannot currently be completely replaced in drug research and development. My implicit acceptance of these points may make me less critical than a complete ‘outsider’ who approaches the topic from a different background. In addition, my understanding of the complexities and regulations of the drug approval process may make me more sympathetic to the difficulties that my participants perceive they face.

Research procedures

The use of interviews to collect data placed some limitations on this study. Responses represent the views and opinions of the participants at the time of the interview and cannot be viewed as either definitively true or false (Denzin & Lincoln 2000). With only one interview per participant, the study design did not allow for follow up or for participants to change or clarify earlier comments. This study may also be limited by the sampling method. First, my use of chain referral to recruit participants may have limited the diversity in the sample (Ritchie et al.

2003a). Second, because I could include only those who agreed to participate, the research findings may not be generalizable to the entire population of pharmaceutical scientists and regulators. Third, some individuals seemed unwilling or uninterested in participating but may have done so out of loyalty to the individual who referred them; their lack of interest may have made their responses less thoughtful. Fourth, the sampling method did not result in reaching saturation (i.e. the point at which the researcher can anticipate what will be said); and therefore many views and opinions that exist among pharmaceutical scientists and regulators may not be represented in this study.

This study was also limited by my inexperience as a researcher, and because I was learning about qualitative methodology as I was going. My underdeveloped skills as an interviewer were revealed by sometimes awkwardly phrased questions, unnecessarily rigid adherence to the interview schedule, and missed opportunities to follow up on interesting points. In all these areas I improved as the study progressed; however these uneven skills may have influenced the overall quality of the interviews.

Chapter 3 Results¹

This study reports on the views and perspectives of scientists and regulators in four areas. First, views on animal use in pharmaceutical research and development are presented, followed by views on the use of *in vitro* alternative methods. Next, the obstacles to Replacement and Reduction that were identified by participants are listed. Finally the opportunities for greater implementation of the Three Rs as suggested by participants are listed. This study did not find two distinct clusters of responses – one from scientists, one from regulators. Instead, most views were shared by one or more members of each group, and a wide range of perspectives existed among all participants.

Views on animal use in pharmaceutical research and development

When asked to describe their views on the use of animals in drug research and development, most participants stressed the importance of animal data in determining the safety of new medicines before human trials, and generally felt that drug studies in animals were predictive for humans. A greater variety of views were expressed on four topics.

1. Appropriateness of animal use

Several scientists and regulators felt strongly that animals were currently used appropriately and at a minimum level. One scientist affirmed that there was “no superfluous use” of animals. A regulator could not think of any information obtained from animals that was extraneous to the approval of new drugs. Another scientist noted that in comparison to basic research, regulatory testing for drugs uses far fewer animals, and two others felt that on occasion not enough animals

¹ A version of this chapter has been submitted for publication to *Animal Welfare* (co-authors N. P. Fenwick and D. Fraser)

are used. Only one participant (a scientist) expressed the view that animal use was currently “excessive”.

2. Difficulties of using animal data

Many participants commented on the difficulties associated with evaluating animal data and selecting animal models. One scientist summarised this with the phrase, “All models are wrong, but some models are useful”. Another simply said, “Models can’t tell you everything”.

Participants also referred to the complications of judging the relevance to humans of a toxic effect in animals. In particular, participants observed that one species of animal may be similar enough to humans to model a pharmacological effect, but may not model a toxic effect, and thus another species may be needed to study toxicity. They also mentioned the level of skill required to both create and properly use some animal models, noting that conflicting results can be generated by different laboratories or by using animals provided by different vendors. A few participants felt that these difficulties could be further compounded when animal data are interpreted solely by non-clinician scientists (i.e. those without medical or veterinary training) because these scientists may not have a “whole animal perspective” and may wrongly attribute clinical observations to a drug effect.

3. Animal death as an endpoint in drug studies

When I asked participants about their perspective on using animal death as an experimental endpoint (13 were asked), three different viewpoints emerged. Most participants (five scientists and four regulators) did not think that animal death is necessary as an endpoint, and some were adamant that death provides no useful information. One scientist explained:

By toxicity, I don’t mean that every single animal has to be sick, or that you’ve got animals dying, or major lesions or whatever ... If you had most of the animals, or all of the animals, in the high dose group who lost more than 10 percent body weight while on

study — that would be considered a toxic response, so you don't have to have the mortality.

However, two scientists viewed animal death as a legitimate endpoint for some drug discovery research, and as an unfortunate but unavoidable consequence of some toxicology studies. This view was explained by one of the scientists with reference to sepsis research:

Now, there are occasions when they do need death as an endpoint. Sometimes there's such a narrow therapeutic margin that they need to find that ... Sepsis by definition is almost always a fatal disease, and a lot of times what they are looking at is: can we increase survival time? The other thing about sepsis is it is a very complicated disease, and there's not simple models for it. And we do have sepsis models [where] they say they'll euthanise the animal before it dies, but the truth of the matter is you can't generally get to it fast enough. You'll look at the animal and it looks fine, and an hour later it'll be dead.

Last, two regulators felt that knowing the dose which causes death in animals is necessary to fully understand a drug. One explained:

The object of a toxicology study is to produce toxicity in the animal, and we want to see the spectrum of toxicity. I call it the tox profile; that includes death. Naturally you don't want to take your high dose and kill all your animals, because that's basically a waste, but you want to let some of them die ... maybe 20 or 30 percent of your high dose.

4. Use of genetically modified (GM) animals

I was initially interested in what participants thought about the use of transgenic² animals because of the many welfare problems associated with them (Buehr et al. 2003), and because interest in transgenics is growing in the pharmaceutical industry (e.g. Burki 1995; Harris 2001; Lindsay 2003). However, when I asked about transgenic animals many responses covered GM animals in general, so both transgenic and other GM animals (such as knock-outs) are discussed.

Many participants saw use of GM animals as an important research tool in the discovery of disease therapies, hence as “qualitatively useful”. However, there was much less support for GM animals in regulatory testing. A reason cited was that “a lot of those [GM] animals are not characterised very well” and are typically not thoroughly phenotyped. In toxicology studies, this was believed to make it difficult to distinguish between drug effects and naturally occurring pathologies. One regulator commented that s/he would not make a regulatory decision based on a transgenic GM animal model because they are not yet reliable.

Many participants also referred specifically to the attempt to validate the p53 knockout mouse model which was investigated as an alternative to the mouse 2-year carcinogenicity regulatory assay, and was subjected to an international validation trial coordinated by the International Life Sciences Institute (ILSI). This GM animal model has a deletion of one allele of the p53 tumour suppressor gene and such mice develop tumours more quickly than other mice (Robinson & MacDonald 2001). Two regulators in this study were dissatisfied that this and other GM models were embraced too quickly. One regulator expressed concern that the p53 model was adopted for regulatory purposes before the final (negative) results of the validation trial were known:

² The term “transgenic” refers to organisms whose normal genome has been altered by introduction of a gene by a manipulative technique. The term “genetically modified” (GM) refers more broadly to organisms whose genome has been modified by any type of gene modification. Therefore, transgenic animals are a type of GM animal.

I think we jumped on a band wagon before the model was validated ... ILSI did that lovely project and showed that indeed the positives aren't always positives and the negatives aren't always negatives, so what do you do with the data?

Participants expressed opposing views regarding whether use of GM animals will reduce or increase animal use by the drug industry. Some participants believed that GM models will promote Reduction because they have less genetic variation and hence fewer animals would be needed to achieve statistically valid results. Also, they speculated that if animals are genetically modified to be closer to the human genome, then results would be more relevant to humans, and perhaps less repetition of studies would be needed. However, other participants believed that use of GM animals will not promote Reduction. For example, they remarked on the large number of animals required to create transgenic lines with one scientist noting, "It's gone against the general trend in Reduction in the numbers of animals used". These participants also believed that the high degree of variability currently seen in GM animals does not allow smaller numbers to be used in a study.

Views on the use of *in vitro* alternative methods

I asked participants to describe their views on the role of *in vitro* data in drug research and development. Responses indicated that participants interpreted the term to include both non-whole-animal and non-animal methods. Responses related to how and when to use *in vitro* methods, limitations of *in vitro* methods, and doubts regarding Replacement of animal models by *in vitro* models.

How and when to use in vitro methods

Almost all participants saw *in vitro* data as restricted to the role of supporting whole animal data, but most also agreed that *in vitro* work made very valuable contributions to drug research

and development, and was as important as the whole animal work. A common perspective was to see *in vitro* assays as a first step in the drug research process, to “pick the most promising ones [drugs] to do preclinical animal studies”. Similarly, a scientist explained that in toxicology, *in vitro* methods could be used to “knock out the compounds that are potentially toxic ... [so] you don’t have to carry them forward into the animal”. Another scientist explained that “we do all of our early work using these models” and thus “conserve our animal resources for those compounds [and] those programs where we feel they have the greatest probability of success”.

Other scientists, however, believed that potential drugs should still be tested in animals at an early stage in order to assess whether time and resources should be further invested. Several scientists also noted that there was not always a sequential progression of experiments from *in vitro* to animal studies:

I think a lot of times we sort of think of the whole research process as being linear ... you synthesize a drug, you go to laboratory tests, you go to animal tests, you go to people, but in fact there is a lot of back and forth.

Limitations of in vitro methods

Some participants felt that although *in vitro* tests are very valuable to drug research and development, too much is currently expected of these methods.

First, participants commented that a limitation of many *in vitro* methods is their dependence on *in vivo* databases. They explained that this decreases the usefulness of the *in vitro* test for new classes of drugs which have not yet been tested in animals. As an example one regulator cited Structure Activity Relationships, which attempt to predict a new chemical’s biological activity by comparing it to similar chemical structures; this however requires previous knowledge of how compounds with similar structures affect animal models.

Second, the difficulties of validation, and the resulting lack of available validated *in vitro* tests, were raised as limitations. One scientist described the difficulty of validating alternatives against *in vivo* studies, noting that:

With an *in vitro* model you have to validate that model, and ‘validate’ being a legal term ... means that you have to be able to reproducibly, reliably show an endpoint, and that endpoint has to be relevant. Our *in vivo* models have never been validated. We never validated the rat as a predictive species for human toxicity. We never did that, but the rat’s the standard, so everything we do now is validated against it. That’s a problem.

Third, many participants described *in vitro* methods as being limited by their lack of biological complexity. As one scientist explained, an *in vitro* result is “taken away from all its normal checks and balances”; therefore, questions that could be asked of an *in vitro* test were necessarily much simpler than those that could be asked of an animal model. Another scientist described this in terms of the strengths and weaknesses of *in vitro* methods:

You can screen a thousand or 10 thousand or a million compounds and get that yes-or-no answer. That’s the strength. The weakness is that every time we try to ask more questions than that, we are bitterly disappointed by the outcomes. So for example, you’d say, ‘I’ve got the cell culture; I’ve got the drug target; I wonder if I can use this to predict cardiovascular safety?’ The answer is no!

Doubts regarding Replacement of animal models by in vitro models

Participants appeared doubtful that alternative methods could completely replace the use of animals. Several specifically stated that they do not believe that animal models can be replaced entirely. For example, a scientist said, “I think *in vitro* tests are for sure going to be valuable to all concerned parties, but ultimately they are never going to fully supplant the use of animals”.

Another expressed frustration with the concept that batteries of *in vitro* experiments could be validated as a Replacement for trying a drug in an animal:

With an *in vitro* model you don't validate it as a Replacement for an animal study ...

This concept in the eighties was: if we did batteries of *in vitro* assays we wouldn't have to do animal studies. That doesn't work. It doesn't work, and in retrospect, I'm surprised now that we really thought it would work, because it doesn't make sense.

Some participants were also not convinced about the usefulness of 'Relative Replacement' methods (those which involve the use of cells and tissues from animals). One regulator said:

I think it's [Relative Replacement methods] over-relied-on at the moment because I don't think we really know what it means in a lot of instances — Caco-2 cells [permeability assay], some of the p450 hepatocyte cultures. Certainly we've shown that the Purkinje Fibre assay is not an effective predictor for the human experience. I think there's lots of examples where perhaps we're jumping in feet first, rather than making sure that it proves effective for extrapolation.

Another regulator noted that *in vitro* methods for predicting long-term toxicological effects are not available:

Suppose you are looking to see whether or not you get necrosis of the liver. Sure, you can take an isolated liver out and add a high concentration of a compound to it, and see in a period of time whether necrosis occurs. [But] It'll be a different type of area that becomes necrotic ... because you don't have blood circulation through the liver ... If you are dealing with compounds intended to be given chronically, you don't have a chronic *in vitro* assay.

Obstacles to Replacement and Reduction

Both spontaneously and in response to specific questions, all 17 participants identified one or more obstacles to decreasing animal use in the pharmaceutical industry. I grouped these into seven issues.

1. Lack of alternative methods

Most participants, both regulators and scientists, identified a lack of appropriate alternative methods as a main obstacle. Participants also commented that some alternative methods may be unavailable because industry has difficulty gaining access to human biological material for *in vitro* experiments, and because of the economic costs associated with implementing new alternative technologies. Some also felt that progress in alternatives was not being made. One scientist expressed surprise that there are not more alternatives available “considering all the years we have been working on them”.

2. The need to establish statistical validity

Many participants identified the requirement for statistical validity as a reason why fewer animals could not be used in regulatory studies. One regulator explained that, “You do really have to show that there is a ... statistical difference between different groups. It’s hard to think of doing less than say 10 animals per group to look at all the different things one looks at in a toxicology study”. A scientist felt that s/he was not the appropriate individual to answer questions about Reduction, commenting “You’re asking a question that really should be addressed to the statistician”. Another scientist commented that application of statistical methods to reducing animal numbers in experiments was hampered by a shortage of statisticians.

3. Regulatory requirements

When I asked about obstacles to decreasing animal use, six scientists (but no regulators) identified “regulatory requirements” although some acknowledged that drug companies also play a role in creating and maintaining regulations, for example through participation in international regulatory harmonization processes. Some participants identified the repetition of studies to conform to Good Laboratory Practices (GLP) standards (a regulatory requirement) as a source of unnecessary use of animals, in particular for non-human primates. However, only two examples of specific problematic regulations were provided. One scientist criticized the regulatory requirement to have two routes of administration for an acute toxicology study for drugs that will be dosed only orally in human clinical trials (Food and Drug Administration 1996). This scientist viewed the requirement as “a bit of a waste” of animals, and expressed frustration that drug companies seem content to just go along with the requirement:

There are some regulatory agencies in the world that still want to see two routes of administration in the acute studies. So what it comes down to is: many companies, they play the game. They go along and they say, ‘this is what the regulatory agencies are going to want; we’re just going to go ahead and do the study. We know it doesn’t make sense but we’re just going to do it’.

Another scientist cited the requirement for two rodent species for carcinogenicity studies, noting, “it is a big and on-going debate as to whether you gain anything additional by running a second species”.

Some scientists also described uncertainty over the expectations of regulators. One scientist mentioned that although a regulatory agency might not specifically require a particular animal study, a particular reviewer may still expect to see it. Similarly, different perceptions were expressed regarding whether non-GLP data were ever acceptable to regulators. Some

participants (both scientists and regulators) felt that regulators would accept non-GLP studies on a case-by-case basis if they were done with adequate documentation, but one regulator maintained that GLP animal studies are always necessary to protect regulatory agencies from fraudulent data.

4. Human safety concerns

Many participants identified the need to protect human safety as an obstacle to replacing or reducing animal use. Regulatory agencies in particular were perceived to be responsible for protecting public safety and ensuring the safety of medicines. A scientist explained:

I think regulatory agencies take a longer period to adjust to changing science ... They have to be convinced because they are on the hot seat of having to protect human safety, and so ... the weight of the scientific evidence has to grow very strong in order for them to change their mind. I see that as one of the major obstacles.

Similarly, when speaking of why *in vitro* alternative methods are not readily adopted by regulatory agencies, one regulator explained that “we really need to be assured that this method is adequate, and that is the issue for us and why we probably do not move as quickly as we’d like, because we need that extra assurance”. The same regulator also claimed that public opinion polls have confirmed the preference of society to have regulatory agencies remain conservative when it comes to evaluating new medicines—although the participant did not identify the relevant opinion poll.

Two scientists touched on the sense of personal responsibility they had felt when drugs they worked on ended up having toxic effects in humans. One described that it felt “really scary” to find out they had gone into clinical trials with a drug which had “the potential to cause harm” in spite of having tested it appropriately in animals. Another explained their reaction

when a drug was found to have serious side effects after it was approved for use in the general population:

There's a sense of ownership and a sense of pride when the drug gets out to market and is successfully used to treat human diseases and conditions. And then when you find out that the drug you developed — that you thought was non-toxic and efficacious — ends up killing people, it's kind of a devastating blow.

5. Resistance to change

A number of participants used the phrase “resistance to change” when discussing obstacles to decreasing animal use. Regulatory agencies were perceived by scientists to be reluctant to depart from established procedures. One scientist complained that regulators adopted a “box checking” mentality while reviewing new drug submissions, just checking if certain tests were completed instead of reviewing whether the tests were scientifically relevant. This scientist believed that not all tests were necessary all the time, and that drugs should be evaluated more on a case-by-case basis.

Both scientists and regulators perceived that drug companies are resistant to change. Referring to LD₅₀ tests, one scientist explained that companies “got stuck on a lot of these tests where they just do things because historically that's the way it had been done”. A regulator felt that the industry's resistance to change was due to the comfort level of the status quo: “That is how they've always done it and they've been successful ... That's what they are comfortable with, so that's what they are going to use”.

This was echoed by a scientist who commented:

Most of the drug companies do have kind of a set pattern that they follow for the development of a drug, and so this gets back to the original question of ‘are they doing studies they don't have to do?’ Well yeah, because they do pattern how they develop a

drug, and they just find it easier to say, ‘Ok we’re going to go ahead and we’re actually going to run this drug through mice and run it through rats and put it through dogs, [and] we’ll put it through monkeys.’ And then they just go ahead and do that in sequence without really giving a lot of thought about does it make sense.

Another scientist noted a division in thinking in the industry regarding the adequacy of current methods for drug evaluation in animals:

I think that there are two camps ... There’s your group of people who believe that what we’re doing now [in animal studies] is sufficient and we don’t need to ask any more questions. And there’s a group who believe that what we’re doing now is Neanderthal and we’re not even now asking the right questions, let alone coming to the right conclusions. So ... it’s really polarized.

A few participants felt that resistance to change would be overcome only in response to external pressures. For example, two participants (one regulator and one scientist) felt that only political pressure would force the wider adoption of alternatives to animals. A few scientists also remarked on the effectiveness of animal care committees to “push” scientists to consider alternatives.

6. Commercial goals

Several scientists pointed to the commercial nature of pharmaceutical research and development as an obstacle to Replacement and Reduction. A scientist expressed frustration that attempts to use as few animals as possible may be compromised by timelines and difficulties in scheduling studies:

Too many times what I see is this overlapping of studies where ... you don’t have all the information that you need to actually go in and properly set up the next level studies.

And that continues all the way through the development process, and the excuse that’s

always given is: ‘We’ve got corporate timelines ... We’ve got to meet what our shareholders want. They want us to get this drug to market’ ...

Similarly, another scientist spoke of how responding to changing business pressures can sometimes cause scientists to rush into animal experiments:

Often there’s a situation where things happen very quickly. Priorities change and someone’s identified a candidate drug from somewhere, and we want to test it — we want to test it fast. We don’t have experience with the model ... [but] we’ll throw the compound in there to just start, and we may end up with nothing really, because we don’t really understand the model very well and the result you get is virtually uninterpretable.

One scientist observed that protecting a company from litigation may prompt some companies to do the maximum amount of animal safety testing regardless of whether there is a scientific basis: “At least in the US, where people will sue you at the drop of a hat, I mean that’s a huge obstacle because we’re not willing to take any risks”.

Some scientists questioned whether it is the responsibility of drug companies to develop and validate alternatives and commented that it may be particularly difficult for small companies. As one observed, the development, validation and implementation of alternatives is very expensive and the cost is not really part of a drug company’s core business:

It’s expensive to look for an alternative way ... It’s not just being creative and coming up with a method. Really we have to validate it, so if you’re going to replace something you need to make sure your Replacement is at least as good as, if not better than, what you’re replacing ... It’s very resource intensive ... And why would you? You might as well run a 30-day rat study instead.

Scientists disagreed over whether attempts to save money within the company would encourage less animal use. One scientist did not think that costs would deter animal use because “drug companies have lots of money”. In contrast, another scientist identified economics as a potential influence to decrease animal use:

People are no longer going to be paying the price that they are right now for our drugs, and ... animal testing is very, very expensive. Anything that we can do *in vitro*, in a computer model, is way cheaper. And again, when we're screening ... tens of thousands of drugs ... we can't be running them all through animal tests ... [and] the sooner we decide [a drug] isn't going to work, the more money we've saved.

7. Development of patent extension products

Finally, two scientists observed that the drive to extend existing product patents (rather than create novel medicines) also results in use of animals, but for possibly lesser benefit. One explained:

There will be more attempts to come up with slightly improved versions of existing drugs, and yet those slightly improved versions still have to go through the entire development process. So in that sense, maybe it's not so much that there are going to be more animals used, but they're going to be used less profoundly.

Suggested opportunities for greater implementation of the Three Rs

When asked to identify opportunities for decreasing animal use in the pharmaceutical industry, two participants (one regulator and one scientist) responded that they did not feel that too many animals were being used, and thus could not think of ways to reduce animal use. Responses from the other 15 participants identified ten possibilities.

1. Ask more basic questions

Several scientists felt that better use of animal models would be achieved if toxicology studies sought more basic information such as changes in gene expression caused by a drug (“predictive toxicogenomics”) in addition to phenotypic information such as tissue damage. One scientist explained:

I think that the animal models that we use are a lot more predictive than we give them credit for because we are not asking the right questions ... For example, you can see the effects of a carcinogen on the liver in seeing the induction of DNA damage-repair genes without ever seeing tumours.

2. Develop and select better animal models

Several participants felt that by developing or selecting better animal models, less repetition of experiments would be required and hence animal use would be reduced. One scientist noted that in regulatory experiments “the way you minimize the use of animals is to select the appropriate species to begin with”. Similarly, a regulator felt that drug companies “should be doing a better job of looking at the physiology and the anatomy and basic physiological parameters that these animals have” in order to improve their selection of species.

3. Use pilot studies

Some scientists identified the strategy of doing pilot studies that use smaller numbers of animals to select which drugs should progress to large GLP toxicology studies. One explained:

I really do believe that this approach of doing pilot and definitive [GLP] studies actually does in the long term reduce animal numbers. It may seem initially like it’s increasing because of the repetition, but because of the volume of drugs that we’re screening ... it allows us to not have to run definitive [GLP] studies on a lot of our compounds.

4. Re-use animals as appropriate

Participants suggested Reduction strategies which involved some re-use of individual animals. Some scientists proposed altering animal study designs so that they are similar to human clinical drug trials. One scientist suggested developing models where “an animal is used for a series of investigations” to examine different drugs in sequence. To avoid killing some of a study’s animals at each data collection timepoint another scientist suggested doing “longitudinal studies in the same animal” so that fewer animals would be needed to achieve the desired sample size.

5. Use sufficient numbers of animals to avoid repeating inconclusive experiments

Several participants also explained that it is equally important to use sufficient numbers of animals in a study to make the experiment statistically meaningful and therefore acceptable from a regulatory point of view. This was felt to contribute to Reduction in the long term by avoiding repetition of inconclusive studies. These participants felt that, in general, industry scientists currently erred on the side of not using enough animals and that this resulted in repetition of experiments.

6. Reproductive toxicity guideline

One regulator noted that the regulatory guideline for reproductive toxicity studies (ICH 1994) provides an opportunity to reduce animal numbers, but that this is rarely used by drug companies:

In rat reproduction studies you can use rats for your Segment One studies, where you look at the fertility and ease of ... animals copulating and stuff like that, or for the Segment Two study, you can do that separately, where you look at organogenesis, or Segment Three which is ... pre-birth and post-birth. They can be combined into one, so you can do dosing throughout ... [and] rather than using 60 animals for reproductive toxicology studies you get to use 20 and combine all three of the segments.

7. Regular review of animal data and regulatory requirements

Regular review of existing animal data was proposed by both scientists and regulators as an opportunity for decreasing animal use and a practice which should be routinely followed by both drug companies and regulatory agencies. Many participants mentioned that drug companies should develop correlations between *in vitro* and *in vivo* data for their own compounds.

Through actively looking for correlations one scientist felt that some use of animals to study the pharmacology of drugs with well validated receptor-targets could be replaced with *in vitro* methods:

You may say ‘Well, I don’t really need to use an animal model anymore. I have enough information based on clinical findings and I know that my compound exhibits the right *in vitro* profile and the right pharmacokinetic profile, that there really isn’t much point in taking it into an animal [disease] model. I have enough information from other sources’.

A few participants expressed the view that regulatory agencies should review their requirements more frequently to see if information is still needed or used in assessing new drug safety. For example, one regulator pointed to the need to review “what is being done currently and figuring out ... how large the studies and how long the [future] studies should be based on past experience”. This regulator suggested taking a “cut-off” number of animals and reviewing how much additional information is provided by use of animal exceeding this cut-off number.

8. Require companies to publish certain data

One scientist suggested that a legal requirement for companies to publish some of their data could lead to less duplication of work.

9. Use historical control groups

Using historical control groups was suggested by a scientist as a way to reduce animal use; however, one regulator specifically expressed distrust of studies using historical controls.

10. Refinement

Although I did not ask directly about Refinement, several participants commented on it.

Refinement was acknowledged by many participants to be a reasonable and achievable goal and “really our biggest opportunity”. Suggestions for Refinement included developing less invasive ways of evaluating animals – for example “put the animals in a scanner instead of sacrificing their brain”; using animals that are more suited to confinement – for example “you can keep a pig happier in confinement than you can a monkey”; and using pain control methods to “try and reduce pain and suffering of the animal in some of these tests”.

Interestingly, several participants saw the use of GM animals as a Refinement. They believed that since some GM animal studies can be of shorter duration than non-GM studies, Refinement would be due to fewer in-study deaths because “you don’t lose as many on study when the study’s shorter”. Using pilot toxicological studies was similarly described as a Refinement. One scientist explained that with pilot studies the overall number of animals which suffer serious toxic side effects is minimized since those drugs do not progress to larger GLP studies which use more animals.

Chapter 4 Discussion & Conclusions

Discussion

This study did not find two different clusters of responses between scientists and regulators. A few points were raised only by the scientist participants, such as the role of commercial goals and regulatory agencies' resistance to change as obstacles to reform. Overall, however, there was no evidence of large differences between regulators and scientists that would need to be resolved for progress in the Three Rs to be made.

Most previous research relevant to this thesis has been reported in the Three Rs literature – that is, studies whose goal was to achieve or advocate greater use of the Three Rs in biomedical science. Therefore, it is helpful to compare the results of this study with findings and conclusions from the Three Rs literature.

Areas of agreement between industry, regulators and Three Rs advocates

Many of the opportunities identified in this study have also been discussed in the Three Rs literature (e.g. Tweats 2000; Combes et al. 2002; Richmond 2002; Stephens et al. 2002). In particular, the views of participants agreed with those of Three Rs advocates in four main areas.

First, some participants acknowledged the need for better models because animal data are not without problems. Animals may not reflect human responses; an animal may model pharmacokinetics but not toxicology; and individual animals vary in their responses. Therefore the industry, like animal advocates, sees a need for improved animal and non-animal methods to replace less effective animal models.

Second, some participants identified improvements to current use of animals as opportunities to reduce animal use. These included routine review of animal data, asking basic questions at the level of gene expression, use of historical control groups, and more careful

species selection. These approaches are also advocated by the Three Rs community (e.g. Balls et al. 2000). These strategies may be particularly well aligned with industry as they may also contribute to improved scientific quality of drug studies.

Third, some participants identified the difficulty of validation and the lack of validated alternatives as key problems for expanded use of alternative methods. Validation of alternative methods is necessary if pharmaceutical companies and regulatory agencies are to adopt them. Therefore, validation is a priority for Three Rs advocates (Balls et al. 1995) and is supported by the pharmaceutical industry and regulatory agencies in North America. For example, validation activities for regulatory purposes in the United States are co-ordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), a multi-agency organization that includes the FDA (ICCVAM 2005). Although alternatives used for regulatory purposes must undergo a stringent validation process (Snodin 2002), validation for ‘in-house’ use – a less onerous form of validation – could allow companies to replace animals in some drug development experiments (Clark 1994).

Fourth, some participants commented on the need to better understand the phenotypes of GM animals. These comments generally related to trying to improve the scientific value of GM animal tests, for example by allowing investigators to distinguish between the effect of the genetic modification and actual effects produced by the drug being studied. Improved phenotypic characterization of GM animals has also been identified by animal advocates as a way to improve animal welfare. Given that some types of genetic modifications can cause welfare problems (Buehr et al. 2003), phenotypic characterization of GM animals could also help to reveal any special needs or problems, including the animal’s handling and housing needs (Jegstrup et al. 2003). Thus, characterizing GM animals would promote Refinement in using these animals as well as possibly allowing Reduction. In these four areas in particular, there is a

high level of agreement not only between scientists and regulators, but also between these groups and advocates of the Three Rs. These areas could form the basis of work for better implementation of the Three Rs which would have wide support by all interested groups.

In addition to these broad opportunities, some participants also made two specific suggestions for Reduction: removing the acute toxicity testing requirement for two routes of administration if the drug only has one, and using the option in the ICH regulatory guideline for reproductive toxicity which permits combining three sections into one study.

Areas for consensus building

In this study five contentious points were also raised. First, a few participants saw the re-use of animals as an opportunity for Reduction, and suggested structuring animal studies in a manner similar to human clinical trials. Traditionally, re-use of animals has not been promoted by animal welfare advocates because of the potential to increase harms to individual animals such as pain or stress from handling. Re-use has also been hampered by the small size of mice and rats as these animals can provide only limited blood samples and need to be sacrificed to examine tissues. However, re-use is more commonly done with larger animals such as dogs (Broadhead et al. 2000); and with the advent of newer and less invasive methods of analysis, such as telemetry and imaging technologies (Stephens et al. 2002), re-use of smaller animals may become more feasible. Nonetheless, effects on individual animal welfare must be considered before this type of approach can be advocated; hence re-use may need to be judged on a case-by-case basis by weighing the different options to achieve the least overall harm (Russell & Burch 1959).

Second, some participants suggested that use of GM animals could contribute to Reduction, but other participants asserted that GM animals increase the numbers of animals used overall. Similarly, in the Three Rs literature both perspectives have been expressed (e.g.

Buehr et al. 2003). Current methods of GM production involve a large increase in animal use but a good model, once developed, could reduce numbers needed in studies. Also, newer methods of GM production may prove less hit-and-miss, and so might produce GM models more efficiently (Schuppli et al. 2004).

Third, some participants saw the apparent repetition of studies to GLP level as a source of increased animal use, while other participants viewed this practice as a way to both reduce overall numbers by decreasing the number of large GLP studies which are conducted, and to refine experiments by subjecting fewer animals to severe toxic effects. Some participants also felt that animal studies did not always need to be conducted to GLP standards, but at least one regulator felt that GLP was the only acceptable standard. There is a need to create consensus on these points. Pilot studies may well save animals and minimize overall harms if they are followed by a judicious switch to GLP at a certain point to avoid or minimize repetition. Similarly, non-GLP studies may be acceptable for regulatory agencies under specific circumstances and clarification of these circumstances would be useful.

Fourth, two regulators expressed the opinion that determining the lethal dose is important to understand the full toxicological profile of a new drug, and two scientists viewed death as a legitimate endpoint in some drug discovery research, and as an unavoidable consequence of some toxicological studies. Death is generally not a legislated regulatory requirement (Richmond 2002; Stokes 2002) except for some biologics, and these views run counter to common calls for humane endpoints. If the belief in the usefulness of death as an endpoint is widespread among regulators and scientists, it could detract from efforts to refine animal use in drug research and development. Hence, consensus is needed on where, if ever, death is useful.

Fifth, complete Replacement of animals with non-animal testing methods was not accepted by participants in this study as a reasonable or achievable goal whereas Relative Replacement (i.e. use of methods which involve the use of cells and tissues from animals) was more supported. Participants generally viewed data obtained from *in vitro* methods as always requiring follow-up confirmation by tests in whole animals. This apparent reluctance to pursue Replacement on the part of industry scientists and regulators could hinder progress in the Three Rs, particularly if alternatives are equated only with non-animal methods (Zurlo 2000). Thus there is a need to create consensus on what are realistic goals for Replacement so that the pharmaceutical industry, regulatory agencies and advocates of the Three Rs can pursue the same objectives. Probably much can be achieved in relation to Relative Replacement, even if study participants are correct in the strongly held view that complete elimination of animal studies is not feasible.

Other obstacles to the Three Rs

Concern for human safety was identified as an obstacle to reducing and replacing animal use in regulatory tests. Human studies, although proposed as a Replacement by advocates of the Three Rs (e.g. Balls 2002) were never mentioned by study participants as an opportunity. Many participants felt that testing which moved away from using animals could put human safety at risk. This concern draws on past tragedies with unsafe medications used by the general public, but not tested, or incompletely tested on animals (e.g. Gad & Chengelis 1995; Schechtman 2002). Studies showing how animal studies have contributed to the prediction of human safety risks (e.g. Broadhead et al. 2000; Olson et al. 2000) may further support this view. For scientists or regulators who perceive that any move away from animal tests may increase the risk to humans, protecting human safety presumably justifies the costs to animals; hence the Three Rs, with their emphasis on decreasing use of animals, may be seen as incompatible with

the goal of protecting human safety. These concerns may perhaps in future be alleviated by advances in medical and scientific understanding that lead to improved validation of non-animal alternatives.

Another obstacle is that many scientists and regulators did not perceive their companies or agencies to have a mandate to pursue the Three Rs. The objective of a drug company is to achieve commercial success with new medicines, and any substantial diversion of resources for Three Rs purposes without a clear commercial benefit may not be acceptable. Patent extension products, which from a Three Rs perspective may seem to duplicate animal studies, benefit the company as they extend the financial returns on a drug. Because pharmaceutical companies must also follow the guidelines of regulatory agencies, some participants felt that industry could not take the lead in implementing the Three Rs. The mandate of regulatory agencies is to protect human safety; hence they too may feel that diversion of resources to pursue the Three Rs is inappropriate. Moreover, since some participants identified problems over clarity between regulators and industry, industry may be unclear as to how regulators will evaluate data from alternative methods and therefore reluctant to use them for regulatory purposes (O'Connor 1997). Neither the industry nor the regulators presumably want to be perceived as using animals in a needless or excessive way, yet neither group perceives itself to have a mandate to take the initiative. Here again, progress will require more consensus on who has a mandate to pursue the Three Rs and how the work should be funded. In this case greater acceptance of the Three Rs may only be achieved through government legislation or adoption of the Three Rs in corporate vision statements, both of which are likely to occur only in response to changes in the values or perceived values of North American society.

Summary of conclusions and suggestions for further research

The goal of this exploratory study was to improve our understanding of where laboratory animal use may be replaced, reduced and refined in the pharmaceutical industry. The research was guided by three objectives: first, to explore the value that participants place on animal and *in vitro* data in drug research and development; second, to have participants identify both opportunities and obstacles to the goal of decreasing animal use in drug research and development; and third, to identify areas of agreement and disagreement in the views of study participants, and to compare these with the Three Rs' literature.

Most of the regulatory reviewers and pharmaceutical research scientists in this study could identify opportunities for greater implementation of Reduction and Refinement within the North American pharmaceutical industry. Specific examples included the development of better animal models including genetically modified (GM) animals; pursuit of more basic knowledge, notably drug action on gene expression, re-use of animals in successive studies; greater use of pilot studies to avoid full-scale studies for unpromising drugs; using enough animals per test to avoid repeating inconclusive studies; regular review of animal data in regulatory requirements; and following the regulatory option of combining segments of reproductive toxicology studies into one study. Some of the opportunities identified by participants may result in scientific improvements as well as improvements to animal welfare, such as the phenotyping of GM animals, routine review of animal data, and more careful selection of appropriate species. Some opportunities identified for Reduction and Refinement are more contentious; these include re-using animals in multiple studies to achieve Reduction, and the use of pilot studies to reduce animal use overall. In such cases, there is a need for consensus-building and perhaps the development of guidelines specifically for the pharmaceutical industry to assist in implementation of the Three Rs.

This study found substantial obstacles to the implementation of Replacement. Although most participants viewed *in vitro* data as valuable, they did not see the complete Replacement of animals in drug development and testing as either feasible or desirable. In particular, participants were concerned that pursuit of the Three Rs might jeopardize human safety, and did not seem to perceive that their organizations have a mandate to pursue the Three Rs. In this case, greater implementation of Replacement may require the development of realistic goals for Replacement in the pharmaceutical industry, a clearer sense of who has a mandate to pursue the Three Rs, and consensus-building on the relationship between the Three Rs and human safety.

Further research into the perspectives of pharmaceutical research scientists and regulatory reviewers may be helpful in increasing understanding of contentious issues and building consensus. Some topics identified as contentious in this study include acceptance of the use of animal death as an endpoint, the role of GM animals in regulatory testing of drugs, and the feasibility of the goal of Replacement. It may also be interesting to research scientists and regulators knowledge of the available alternatives.

This study found minimal differences between the perspectives of regulators and scientists; however, due to the small, non-random sample used it is not possible to know if this similarity of views would also be found in the larger population. To investigate this further it may be interesting to conduct a survey with statistically representative samples of regulators and scientists. In this sample the individual participants had quite diverse backgrounds and were focussed on different areas of drug research and development and that may be part of why saturation was not reached. Further interview-based studies with regulators or pharmaceutical scientists in a particular discipline (e.g. toxicology) may expand and reach saturation on their views.

The cost of developing and implementing alternatives was raised by participants as an obstacle. Therefore it would be interesting to interview individuals involved in the financial and business management side of the pharmaceutical industry to find out their perspectives regarding the allocation of resources and how those decisions are made with regard to Three Rs and animal welfare. Finally, in this study participants identified Refinement as a more attainable and reasonable goal than Replacement. Further research into the types of Refinements which would be acceptable in the pharmaceutical industry would be useful, for example pain relief and environmental enrichment.

References

- Abraham J and Reed T** 2003 Reshaping the carcinogenic risk assessment of medicines: international harmonisation for drug safety, industry/regulator efficiency or both? *Social Science & Medicine* 57: 195-204
- Arluke A** 1994 "We build a better beagle": fantastic creatures in lab animal ads. *Qualitative Sociology* 17: 143-158
- Arluke A and Hafferty F** 1996 From apprehension to fascination with "dog lab": the use of absolutions by medical students. *Journal of Contemporary Ethnography* 25: 201-225
- Arluke A** 2003 The no-kill controversy: manifest and latent sources of tension. In: Salem D J and Rowan A N (eds) *The State of Animals II: 2003* pp 67-83. Humane Society Press: Washington DC, USA
- Balls M** 1994 Replacement of animal procedures: alternatives in research, education and testing. *Laboratory Animals* 28: 193-211
- Balls M, Goldberg A M, Fentem J H, Broadhead C L, Burch R L, Festing M F W, Frazier J M, Hendriksen C F M, Jennings M, van der Kamp M D O, Morton D, Rowan A N, Russell C, Spielmann H, Stephens M L, Stokes W S, Straughan D, Yager J D, Zurlo J and van Zutphen B F M** 1995 The Three Rs: the way forward. The report and recommendations of ECVAM Workshop 11. *Alternatives to Laboratory Animals* 23: 838-866
- Balls M, van Zeller A-M and Halder M E** 2000 Background to the Three Rs declaration of Bologna, as adopted by the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, on 31 August 1999. In: Balls M, van Zeller A-M and Halder M E (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation. Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences* pp 17-22. Elsevier Sciences B V: Amsterdam, The Netherlands
- Balls M** 2002 Future improvements: replacement in vitro methods. *Institute for Laboratory Animal Research Journal* 43: S69-S73
- Brandon E F, Raap C D, Meijerman I, Beijnen J H and Schellens J H** 2003 An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. *Toxicology and Applied Pharmacology* 189: 233-246
- Broadhead C L, Betton G, Combes R, Damment S, Everett D, Garner C, Godsafe Z, Healing G, Heywood R, Jennings M, Lumley C, Oliver G, Smith D, Straughan D, Topham J, Wallis R, Wilson S and Buckley P** 2000 Prospects for reducing and refining the use of dogs in the regulatory toxicity testing of pharmaceuticals. *Human & Experimental Toxicology* 19: 440-447
- Buehr M, Hjorth J P, Hansen A K and Sandøe P** 2003 Genetically modified laboratory animals—what welfare problems do they face? *Journal of Applied Animal Welfare Science* 6: 319-338
- Burki K** 1995 Prospectives of transgenic animals for the pharmaceutical industry. In: van Zutphen L F M and van der Meer M (eds) *Welfare Aspects of Transgenic Animals, Proceedings of EC-Workshop October 30, 1995* pp 48-57. Springer: Berlin, Germany
- Carlsson H-E, Hagelin J and Hau J** 2004 Implementation of the 'Three Rs' in biomedical research. *The Veterinary Record* 154: 467-470
- CCAC** 1989 Ethics of Animal Investigation.
http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/POLICIES/ETHICS.HTM
Accessed on: 22 May, 2005

- CCAC 2005 Facts & Figures CCAC Animal Use Survey-2002.
http://www.ccac.ca/en/Publications/Facts_Figures/aus2002.htm Accessed on: 15 April, 2005
- Clark D G** 1994 Barriers to the acceptance of in vitro alternatives. *Toxicology In Vitro* 8: 907-909
- Coffey A and Atkinson P** 1996 Concepts and coding. In: Coffey A and Atkinson P (eds) *Making Sense of Qualitative Data: Complementary Research Strategies* pp 26-53. SAGE Publications: Thousand Oaks, USA
- Cole P M** 1994 Finding a path through the research maze.
<http://www.nova.edu/ssss/QR/BackIssues/QR2-1/cole.html> Accessed on: 21 August, 2004
- Combes R, Schechtman L M, Stokes W S and Blakey D** 2002 The International Symposium on Regulatory Testing and Animal Welfare: Recommendations on best scientific practices for subchronic/chronic toxicity and carcinogenicity testing. *Institute for Laboratory Animal Research Journal* 43: S112-S117
- Corbin J and Strauss A** 1990 Grounded theory research: procedures, canons and evaluative criteria. *Qualitative Sociology* 13: 3-21
- Cox S M and McKellin W** 1999 'There's this thing in our family': predictive testing and the construction of risk for Huntington Disease. *Sociology of Health & Illness* 21: 622-646
- Dagg A I** 2000 Animal experimentation in cancer research: a citation analysis. *Journal of Applied Animal Welfare Science* 3: 239-251
- Denzin N K and Lincoln Y S** 2000 The discipline and practice of qualitative research. In: Denzin N K and Lincoln Y S (eds) *Handbook of Qualitative Research* pp 1-28. SAGE Publications: Thousand Oaks, USA
- Dubois S and Fraser D** 2003 Conversations with stakeholders, part I: goals, impediments, and relationships in wildlife rehabilitation. *Journal of Wildlife Rehabilitation* 26: 18-26
- Duncan I J H and Fraser D** 1997 Understanding animal welfare. In: Appleby M C and Hughes B O (eds) *Animal Welfare* pp 19-31. CAB International: Wallingford, UK
- Eli Lilly** 2004 Animal Care and Use Policy. <http://www.lilly.com/about/policies> Accessed on: 17 March, 2004
- Festing M F W** 1994 Reduction of animal use: experimental design and quality of experiments. *Laboratory Animals* 28: 212-221
- Festing M F W, Baumanns V, Combes R D, Halder M, Hendriksen C F M, Howard B R, Lovell D P, Moore G J, Overend P and Wilson M S** 1998 Reducing the use of laboratory animals in biomedical research: problems and possible solutions. The report and recommendations of ECVAM Workshop 29. *Alternatives to Laboratory Animals* 26: 283-301
- Flecknell P A** 1994 Refinement of animal use—assessment and alleviation of pain and distress. *Laboratory Animals* 28: 222-231
- Food and Drug Administration** 1992 Animal Use in Testing FDA-Regulated Products.
<http://vm.cfsan.fda.gov/~dms/cos-205.html> Accessed on: 26 January, 2004
- Food and Drug Administration** 1996 Guidance for Industry: Single Dose Acute Toxicity Testing for Pharmaceuticals. <http://www.fda.gov/cder/guidance/pt1.pdf> Accessed on: 18 February, 2004
- Gad S C and Chengelis C P** 1995 Human health products: drugs and medical devices. In: Chengelis C P, Holson J F and Gad S C (eds) *Regulatory Toxicology* pp 9-49. Raven Press Ltd: New York, USA

- Gauthier C** 2004 Overview and analysis of animal use in North America. In: Balls M, Firmani D and Rowan A N (eds) *The Three Rs at the Beginning of the 21st Century: Proceedings of the Fourth World Congress on Alternatives and Animal Use in the Life Sciences, 11-15 August 2002 New Orleans, Louisiana, USA* pp 275-285. Alternatives to Laboratory Animals: Nottingham, UK
- Glaxo Smith Kline** 2001 Care and Ethical Use of Animals. <http://science.gsk.com/about/animal-care.htm> Accessed on: 12 August, 2004
- Greaves P, Williams A and Eve M** 2004 First dose of potential new medicines to humans: how animals help. *Nature Reviews Drug Discovery* 3: 226-236
- Harris S** 2001 Transgenic knock-outs as part of high-throughput, evidence-based target selection and validation strategies. *Drug Discovery Today* 6: 628-636
- Hendriksen C F M** 2000 Replacement, reduction and refinement and biologicals: about facts, fiction and frustrations. In: Balls M, van Zeller A-M and Halder M E (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation. Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences* pp 51-62. Elsevier Science B V: Amsterdam, The Netherlands
- Herzog H A, Vore T L and New J C** 1989 Conversations with veterinary students: attitudes, ethics and animals. *Anthrozoos* 2: 181-188
- Home Office** 2004 Statistics of Scientific Procedures on Living Animals in Great Britain 2003. http://www.official-documents.co.uk/document/cm62/6291/6291-old.pdf#xml=pdf_hl?STEMMER=en&RGB=ff00ff&WORDS=statistics+scientific+procedures+&DB=off-docsa&URL=http://www.official-documents.co.uk/document/cm62/6291/6291-old.pdf Accessed on: 15 April, 2005
- Hurley R E** 1999 Qualitative research and the profound grasp of the obvious. *Health Services Research* 34: 1119-1136
- ICCVAM** 2005 About ICCVAM. <http://iccvam.niehs.nih.gov/about/overview.htm> Accessed on: 27 July, 2005
- ICH** 1994 Guideline for Industry. S5A Detection of Toxicity to Reproduction for Medicinal Products. <http://www.fda.gov/cder/guidance/s5a.pdf> Accessed on: 20 November, 2004
- ICH** 1997 Guidance for Industry: M3 Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals. <http://www.fda.gov/cder/guidance/1855fml.pdf> Accessed on: 19 February, 2004
- ICH** 2001 Guidance for Industry: S7A Safety Pharmacology Studies for Human Pharmaceuticals. <http://www.fda.gov/cder/guidance/4461fml.pdf> Accessed on: 18 February, 2004
- ICH** 2004 Official Website for ICH. <http://www.ich.org> Accessed on: 08 November, 2004
- Jegstrup I, Thon R, Hansen A K and Ritskes Hoitinga M** 2003 Characterization of transgenic mice—a comparison of protocols for welfare evaluation and phenotypic characterization of mice with a suggestion on a future certificate of instruction. *Laboratory Animals* 37: 1-9
- Leahy D E, Duncan R, Ahr H J, Bayliss M K, de Boer A B G, Darvas F, Fentem J H, Fry J R, Hopkins R, Houston J B, Karlsson J, Kedderis G L, Pratten M K, Prieto P, Smith D A and Straughan D W** 1997 Pharmacokinetics in early drug research: report and recommendations of ECVAM Workshop 22. *Alternatives to Laboratory Animals* 25: 17-31
- LeCompte M D and Schensul J J** 1999 *Designing & Conducting Ethnographic Research*. Altamira Press: Walnut Creek, USA

- Legard R, Keegan J and Ward K** 2003 In-depth interviews. In: Ritchie J and Lewis J (eds) *Qualitative Research Practice: A Guide for Social Science Students and Researchers* pp 139-169. SAGE Publications: London, UK
- Lindsay M A** 2003 Target discovery. *Nature Reviews Drug Discovery* 2: 831-838
- Lippman A** 1999 Embodied knowledge and making sense of prenatal diagnosis. *Journal of Genetic Counselling* 8: 255-274
- Luft J and Bode G** 2002 Integration of safety pharmacology endpoints into toxicology studies. *Fundamental & Clinical Pharmacology* 16: 91-103
- Malterud K** 2001 Qualitative research: standards, challenges, and guidelines. *Lancet* 358: 483-488
- Meyer O and Svendsen O** 2003 Animal models in pharmacology and toxicology. In: Hau J and van Hoosier G L (eds) *Handbook of Laboratory Animal Science, Second Edition* pp 11-37. CRC Press: Washington DC, USA
- Michael M and Birke L** 1994 Accounting for animal experiments: identity and disreputable "others". *Science, Technology & Human Values* 19: 189-204
- Morse J M and Richards L** 2002 *Readme First for a User's Guide to Qualitative Methods*. SAGE Publications: London, UK
- O'Connor A M** 1997 Barriers to regulatory acceptance. In: van Zutphen L F M and Balls M (eds) *Animal Alternatives, Welfare and Ethics*. Elsevier Science B V: Amsterdam, The Netherlands
- Olson H, Graham B, Robinson D E, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B and Heller A** 2000 Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regulatory Toxicology & Pharmacology* 32: 56-67
- Orlans F B** 1998 Number and species of laboratory animals used worldwide. In: Bekoff M (ed), *Encyclopedia of Animal Rights and Animal Welfare* pp 215-217. Greenwood Press: Connecticut, USA
- Parkinson C, Thomas K E and Lumley C E** 1996 The timing of preclinical toxicological studies: pharmaceutical company approaches to toxicity testing in support of initial clinical investigations. *Regulatory Toxicology & Pharmacology* 23: 162-172
- Paul E S** 1995 Us and them: scientists' and animal rights campaigners' views of the animal experimentation debate. *Society and Animals* 3: 1-12
- Pfizer** 2002 Laboratory Animal Care and Use.
http://www.pfizer.com/are/about_public/mn_about_laboratory_use.html Accessed on: 12 August, 2004
- Phillips M T** 1993 Savages, drunks and lab animals: the researcher's perception of pain. *Society and Animals* 1: 61-83
- Pound P, Ebrahim S, Sandercock P, Bracken M B and Roberts I** 2004 Where is the evidence that animal research benefits humans? *British Medical Journal* 328: 514-517
- Preziosi P** 2004 Science, pharmacoeconomics and ethics in drug R&D: a sustainable future scenario? *Nature Reviews Drug Discovery* 3: 521-526
- Purchase I** 1999 Ethical review of regulatory toxicology guidelines involving experiments on animals: the example of endocrine disrupters. *Toxicological Sciences* 52: 141-147
- Rawlins M D** 2004 Cutting the cost of drug development? *Nature Reviews Drug Discovery* 3: 360-364
- Reeve C L, Spitzmuller C, Rogelberg S G, Walker A, Schultz L and Clark O** 2004 Employee reactions and adjustment to euthanasia-related work: identifying turning-

- point-events through retrospective narratives. *Journal of Applied Animal Welfare Science* 7: 1-25
- Richmond J** 2002 Refinement, reduction and replacement of animal use for regulatory testing: future improvements and implementation within the regulatory framework. *Institute for Laboratory Animal Research Journal* 43: S63-S68
- Ritchie J, Lewis J and Elam G** 2003a Designing and selecting samples. In: Ritchie J and Lewis J (eds) *Qualitative Research Practice: A Guide for Social Science Students and Researchers* pp 77-108. SAGE Publications: London, UK
- Ritchie J, Spencer L and O'Connor W** 2003b Carrying out qualitative analysis. In: Ritchie J and Lewis J (eds) *Qualitative Research Practice: A Guide for Social Science Students and Researchers* pp 219-262. SAGE Publications: London, UK
- Robinson D E and MacDonald J S** 2001 Background and framework for ILSI's collaborative evaluation program on alternative models for carcinogenicity assessment. *Toxicologic Pathology* 29: 13-19
- Rollin B E** 2003 Toxicology and new social ethics for animals. *Toxicologic Pathology* 31: 128-131
- Russell W M S and Burch R L** 1959 *The Principles of Humane Experimental Technique*. Methuen & Co Ltd: London, UK
- Satterfield T** 2001 In search of value literacy: suggestions for the elicitation of environmental values. *Environmental Values* 10: 331-359
- Schechtman L M** 2002 The safety assessment process—setting the scene: an FDA perspective. *Institute for Laboratory Animal Research Journal* 43: S5-S10
- Schensul S L, Schensul J J and LeCompte M D** 1999 *Essential Ethnographic Methods: Observations, Interviews, and Questionnaires*. Altamira Press: Walnut Creek, USA
- Schuppli C A, Fraser D and McDonald M** 2004 Expanding the Three Rs to meet new challenges in humane animal experimentation. *Alternatives to Laboratory Animals* 32: 525-532
- Schuppli C A and Fraser D** in press The interpretation and application of the Three Rs by animal ethics committee members. *Alternatives to Laboratory Animals*
- Smith D, Combes R, Depelchin O, Jacobsen S D, Hack R, Luft J, Lammens L, von Landenberg F, Phillips B, Pfister R, Rabemampianina Y, Sparrow S, Stark C and Stephen-Gueldner M** 2005 Optimising the design of preliminary toxicity studies for pharmaceutical safety testing in the dog. *Regulatory Toxicology & Pharmacology* 41: 95-101
- Smith J A and Boyd K M** 1991 Ethical considerations in the use of animals in toxicology and toxicity testing. In: Smith J A and Boyd K M (eds) *Lives in the Balance: The Ethics of Using Animals in Biomedical Research. Report of the Working Party of the Institute of Medical Ethics*. Oxford University Press: Oxford, UK
- Snape D and Spencer L** 2003 The foundation of qualitative research. In: Ritchie J and Lewis J (eds) *Qualitative Research Practice: A Guide for Social Science Students and Researchers* pp 1-23. SAGE Publications: London
- Snodin D J** 2002 An EU perspective on the use of in vitro methods in regulatory pharmaceutical toxicology. *Toxicology Letters* 127: 161-168
- Sofaer S** 1999 Qualitative methods: what are they and why use them? *Health Services Research* 34: 1101-1118
- Stephens M L, Goldberg A M and Rowan A N** 2001 The first forty years of the alternatives approach: refining, reducing, and replacing the use of laboratory animals. In: Salem D J

- and Rowan A N (eds) *The State of the Animals: 2001* pp 121-135. Humane Society Press: Washington DC, USA
- Stephens M L, Conlee K, Alvino G and Rowan A N** 2002 Possibilities for refinement and reduction: future improvements within regulatory testing. *Institute for Laboratory Animal Research Journal* 43: S74-S79
- Stitzel K A, Spielmann H and Griffin G** 2002 The International Symposium on Regulatory Testing and Animal Welfare: Recommendations on best scientific practices for acute systemic toxicity testing. *Institute for Laboratory Animal Research Journal* 43: S108-S111
- Stokes W S** 2002 Humane endpoints for laboratory animals used in regulatory testing. *Institute for Laboratory Animal Research Journal* 43: S31-S38
- Tweats D** 2000 A review of reduction and refinement of regulatory toxicity studies for pharmaceuticals. In: Balls M, van Zeller A-M and Halder M E (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation. Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences* pp 783-791. Elsevier Science B V: Amsterdam, The Netherlands
- United States Department of Agriculture** 2003a Fiscal Year 2002 Animal Welfare Act Annual Report of Enforcement.
<http://www.aphis.usda.gov/ac/awreports/awreport2002.pdf> Accessed on: 15 April, 2005
- United States Department of Agriculture** 2003b Code of Federal Regulations, Title 9, Chapter 1, Subchapter A-Animal Welfare.
http://a257.g.akamaitech.net/7/257/2422/14mar20010800/edocket.access.gpo.gov/cfr_2003/pdf/9cfr1.1.pdf Accessed on: 15 April, 2005
- Varma R** 2000 Changing research cultures in U.S. industry. *Science, Technology & Human Values* 25: 395-416
- Wakefield I D, Pollard C, Redfern W S, Hammond T G and Valentin J-P** 2002 The application of in vitro methods to safety pharmacology. *Fundamental & Clinical Pharmacology* 16: 209-218
- World Medical Association** 2004 Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. <http://www.wma.net/e/policy/b3.htm> Accessed on: 13 September, 2004
- Zurlo J** 2000 Obstacles to acceptance and implementation of the Three Rs by scientists. In: Balls M, van Zeller A-M and Halder M E (eds) *Progress in the Reduction, Refinement and Replacement of Animal Experimentation. Proceedings of the 3rd World Congress on Alternatives and Animal Use in the Life Sciences* pp 1383-1387. Elsevier Science B V: Amsterdam, The Netherlands

Appendix 1: Behavioural Research Ethics Board Approval Certificate

Appendix 2: Letter of Initial Contact

Dear [potential participant],

We would be very grateful if you would be willing to participate in a University of British Columbia study of how animal data informs drug development in the pharmaceutical industry.

The research is being carried out for a M.Sc. thesis. The objectives of the study are to understand which information is most important to progress a drug from animal tests to human clinical trials, to identify areas of agreement and disagreement in assessment of the value of animal data and to identify promising areas for the replacement, reduction and refinement of animal use in drug research.

This research will be conducted by a graduate student who has several years work experience in the pharmaceutical industry. The objectives and design of the study have had substantial input from a member of the UBC Faculty of Pharmaceutical Sciences and a member of the Canadian Council of Animal Care, both of whom are supervisory committee members for the student co-investigator.

You have been asked to contribute due to your experience with the drug development industry and evaluation of animal data. In addition, you were personally recommended by [name of referral known to the potential participant] as a possible participant for this study. We would like to learn about your experiences, perspectives and concerns, if any, regarding the use of animal data in the pharmaceutical industry and therefore would like to schedule an interview with you at a time and location of your choice. During this interview you will have the opportunity to give your own opinion, in confidence, on issues relating to animal use in the pharmaceutical industry. The interview will be semi-structured, which means that you will be asked questions on specific themes but you will be free to answer in any direction you would like.

The interview will take between 1 and 1.5 hours and will be audio-taped. The identity of participants who participate in this study will be kept strictly confidential. All documents will be identified only by code number and kept in a locked filing cabinet. Participants will not be identified by name in any reports of the completed study. Computer files will be protected by password so that only the principal and co-investigators can access them.

If you have any questions or desire further information with respect to this study you may contact Principal Investigator or the Co-Investigator at the numbers or e-mails listed below.

Principal Investigator:	Dr. David Fraser Professor, Animal Welfare Program Faculty of Agricultural Sciences and W. Maurice Young Centre for Applied Ethics University of British Columbia Tel: (604) 822-2040 E-mail: fraserd@interchange.ubc.ca
Co-Investigator:	Nicole Fenwick, M.Sc. Student Animal Welfare Program, Faculty of Agricultural Sciences University of British Columbia Tel: (604) 822-5715 E-mail: nfenwick@interchange.ubc.ca

If you would be willing to participate, please feel free to contact the Co-Investigator by telephone or e-mail. Alternatively, the Co-Investigator will telephone you during the next 2 weeks. We would greatly appreciate your assistance in what we believe will be a unique study that capitalizes on the knowledge and experience of individuals involved in drug development research.

Yours sincerely,

David Fraser

Nicole Fenwick

Appendix 3: Consent Form

Study Title: Exploring the Scope for Implementation of the 3 Rs in Pharmaceutical Research

Principal Investigator:

Dr. David Fraser
Professor, Animal Welfare Program
Faculty of Agricultural Sciences and W. Maurice Young Centre for Applied Ethics
University of British Columbia
Tel: (604) 822-2040
E-mail: fraserd@interchange.ubc.ca

Co-Investigator:

Nicole Fenwick, M.Sc. Student
Animal Welfare Program, Faculty of Agricultural Sciences
University of British Columbia
Tel: (604) 822-5715
E-mail: nfenwick@interchange.ubc.ca

Purpose:

The purpose of this study is to understand how data from animal testing informs drug research and how this may be applied to the replacement, reduction and refinement of laboratory animal use in the pharmaceutical industry. You have been asked to contribute due to your experience with the evaluation of animal data within the drug development industry.

Study Procedures:

Our goal is to learn about your experiences, perspectives and concerns (if any) regarding the use of animal data in the pharmaceutical industry. If you agree to take part in this study you will be asked to participate in an interview conducted in person by the student co-investigator at a time and location convenient to you. It will be semi-structured which means you will be asked questions on specific themes but will be free to answer in any direction you like. The interview will take between 1 and 1.5 hours. The student co-investigator will take hand-written notes and, with your permission, audio-tape the interview.

Confidentiality:

The identity of participants in this research study will be kept strictly confidential. All documents will be identified only by code number and kept in a locked filing cabinet. Participants will not be identified by name in any reports of the completed study. The interview will be audio-taped and written transcripts will be generated. Computer files will be protected by password so that only the principal and co-investigator can have access to them.

Contact for information about the study:

If you have any questions or desire further information with respect to this study, you may contact either the Principal Investigator or the Co-Investigator at the numbers listed above.

Contact for information about the rights of research subjects:

If you have any concerns about your treatment or rights as a research subject, you may contact the Research Subject Information Line in the UBC Office of Research Services at 604-822-8598.

Consent:

Your participation in this study is entirely voluntary and you may refuse to participate or withdraw from the study at any time.

Your signature below indicates that you have received a copy of this consent form for your own records.

Your signature indicates that you consent to participate in this study.

Subject Signature Date

Signature of a Witness Date

Appendix 4: Interview Schedule

- 1) I would like to get an understanding of your background so could you tell me a bit about your career?
 - a. *What is your academic background?*
 - b. *What therapeutic areas have you worked in?*
 - c. *Have you worked in big pharma and small pharma?*
- 2) Can you describe your involvement in drug development?
- 3) Can you describe how the information from animal tests assists you assessing new compounds?
 - a. *In assessment of the usefulness, safety*
 - b. *What information is likely to stop a drug going into clinical trials?*
- 4) Can you describe any circumstances where animal tests are done but maybe aren't as necessary? Examples?
 - a. *any animal tests which are less important in advancing a drug?*
 - b. *information from animal experiments which wouldn't stop a drug going into clinical trials?*
- 5) How do you interpret conflicting animal data?
 - a. *Can you describe any examples of where animal data misinformed you?*
- 6) What is your perspective on the use of animal death as a toxicological endpoint?
 - a. *Is it useful? How?*
- 7) What is your opinion on the role of *in vitro* data in drug R&D?
 - a. *Can in vitro data be extrapolated to humans?*
 - b. *Can you describe any examples of when you felt you were misinformed by in vitro data?*
 - c. *What types of in-vitro technologies would you like to see developed or improved?*
- 8) What are the opportunities for decreasing animal use in drug development?
- 9) What do you think the obstacles are for decreasing animal use?
 - a. *Do you think the pharmaceutical industry will increase its use of research animals? Why?*
- 10) How is the regulatory environment impacting animal use?
- 11) What do you think the role of transgenic animals in drug development will be?
- 12) Now knowing my topic and interests, is there anything else you think I should have asked you? Anything else to add in general?

Appendix 5: Notes for Preamble to Interviews

Introduction for Interviews

- undergraduate degree in biochemistry
- worked for over six years in the pharmaceutical industry
- primarily as an analytical chemist but also drug formulation and stability
- want to talk about how laboratory animals are used in the pharmaceutical industry
- I am interviewing you because I feel that people within the industry are in the best position to give opinions and information regarding animal use and advice on how use of animals may be reduced or replaced
- it is very important for me to get candid opinions and answers to my questions
- reiterate that your identity will remain completely confidential
- will it be ok to tape record?
- Any questions for me?
- Sign confidentiality sheet