Session V-2: Systematic reviews of animal experiments

Session V-2: Oral presentations

V-2-543

Systematic reviews of animal studies: a necessary step to take

M. Ritskes-Hoitinga, C. Hooijmans, R. de Vries, A. Tillema, J. van Luijk, G. J. van der Wilt and M. Leenaars

Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

M.Ritskes@cdl.umcn.nl

Within evidence-based medicine, Systematic Reviews (SR) are routinely performed for clinical studies. The phases when performing a SR are: Phrasing the specific research question/objective of the study; doing a systematic search for original papers in at least 2 databases; selecting relevant papers; doing a quality assessment; extracting data and performing meta-analyses; synthesizing the data, interpreting results and writing the paper. Animal studies are used as a preparation and risk assessment for clinical studies, however, SRs of animal studies are not yet routinely performed, even though there are very good reasons for doing so. SRs of animal studies will lead to (1) better quality science (Kilkenny et al., 2009; Sena et al., 2010), (2) improved patient safety (Pound et al., 2004), and (3) the prevention of unnecessary duplication of animal studies and 3R implementation (Hooijans et al., 2010a).

We have therefore developed practical guidelines for research and teaching and training. The Gold Standard Publication Checklist has been developed as a checklist for optimal planning, design, executing and reporting of animal studies in order to make sure that all necessary elements are covered (Hooijmans et al., 2010a). To do a more effective literature search for animal publications, our group has developed two validated search filters for the databases Pubmed (Hooijmans et al., 2010b) and Embase (de Vries et al., submitted). A search guide, describing which steps to take, is currently under development (Leenaars et al., in preparation). Several SRs on the choice of animal models and translational validity are currently being executed. Over the last 2 years we have already incorporated education and training on SRs of animal studies together with a workshop on a more effective literature search in our FELASA category C courses for researchers.

References

Hooijmans, C. R., Leenaars, M. and Ritskes-Hoitinga, M. (2010a). *ATLA 38*, 167-182.

Hooijmans, C. R., Tillema, A., Leenaars, M. and Ritskes-Hoitinga, M. (2010b). *Lab. Anim. 44*, 170-175.

Kilkenny, C., Parsons, N., Kadyszewski, E. et al. (2009). *PLoS One 4*, e7824.

Pound, P., Ebrahim, S. and Sandercock, P. (2004). *BMJ 328*, 514-517.

Sena, E. S., van der Worp, H. B., Bath, P. M. et al. (2010). *PLoS Biol. 8*, e1000344.

V-2-355

Study quality and publication bias in experimental studies of neurological diseases

E. S. Sena, H. M. Vesterinen, K. J. Egan and M. R. Macleod

University of Edinburgh, Edinburgh, UK

emily.sena@ed.ac.uk

The poor conduct and reporting of animal experiments, due to compromised internal and external validity and the presence of publication bias, have been implicated in the discrepancies between the results of animal and human studies. Systematic review and meta-analysis have proven to be useful tools in quantitatively estimating the impact of study quality on the outcome of animal studies.

Assessment of publication bias in 499 focal ischaemia publications using 1300 animals identified that 1 in 6 experiments remain unpublished, which leads to an overstatement of efficacy of at least 30%. Furthermore, only 3% of studies report performing a sample size calculation, and about a third of studies report random allocation to group and blinded assessment of outcome – both associated with overstatements in reported efficacy. These findings are not unique to experimental stroke. In publications reporting the use of transgenic mouse models of Alzheimer's disease only 16% report random allocation to group, 22% report blinded assessment of outcome and no publications performed a sample size calculation. In publications of experimental autoimmune encephalitis (a model of multiple sclerosis) efficacy was substantially overstated in those reporting measures to avoid bias (random allocation to group: 20.6% [95% CI 11.4-29.7] versus 41.6% [36.7-46.5] and blinded assessment of outcome: 29.8% [19.8-39.8] versus 41.0% [36.2-45.8]).

Quantitatively estimating the impact of potential sources of bias has allowed us to develop good laboratory practice guidelines but also to highlight the impact of not disseminating data and the value of reviewing evidence before embarking on clinical trials.

V-2-163

The ARRIVE guidelines to improve the retrospective analysis of animal studies

N. Percie du Sert

National Centre for the 3Rs, London, UK

nathalie.percie du sert@nc3rs.org.uk

Systematic reviews and meta-analyses are commonly used in clinical trials. There is also increasing awareness about their utility for analysing preclinical data, for example to assess the evidence supporting the effect of a treatment or to evaluate and improve the predictability of animal models. Two examples of systematic review and meta-analysis of animal data will be presented: first investigating the effect of anti-emetic drugs in a ferret model to identify opportunities to refine the model of chemotherapy-induced emesis, and second comparing the self-administration of opioids in rats and non-human primates to provide science-based evidence for the choice of species in models of abuse potential.

One of the hurdles to carrying out meta-analyses of animal studies is the poor quality of animal data published and it is therefore essential to improve the reporting of animal studies. To address this, the NC3Rs, in consultation with scientists, statisticians, journal editors and research funders, developed the ARRIVE guidelines (Animal Research: Reporting *In vivo* experiments). The guidelines consist of a check list of 20 items describing the minimum information that all scientific publications reporting *in vivo* research should include, in order to maximise the availability and information gained from animals and allow in-depth critique by scientific peers. Their goal is to guide authors and reviewers during the publication process to ensure completeness and transparency. They also enable an objective assessment of the quality of studies included in a systematic review.

The ARRIVE guidelines were recently endorsed by many high quality journals and major bioscience funding bodies in the UK; international dissemination will ensure a wider impact and improve the quality and comprehensiveness of scientific reporting.

Session V-2: Poster presentations

V-2-077

Harnessing opportunities in non-animal asthma research for a 21st century science

G. L. Buckland

Humane Society International, London, UK

gbuckland@hsi.org

The asthma field has relied heavily on animal use to model this human disease. Yet despite decades of intensive funding and animal experimentation, the incidence of asthma continues to increase, and only two new classes of asthma drug have progressed from the laboratory to the clinic in the last 50 years. Some fundamental research questions are still the mainstay in laboratories, and there is growing recognition of the need to more fully incorporate the "Three Rs" principle of Replacement, Reduction and Refinement in this area of research. At the same time, progress in research techniques with the potential to reduce, or in some cases replace, the use of animals is reaching a level where commitment and integration are necessary. Asthma research could benefit from a "21st century" targeted strategy incorporating multidisciplinary research from computational modeling to three-dimensional *in vitro* systems. There is growing consensus that progress in this field rests on the linking of disciplines to make research directly translatable from the bench to the clinic. With this in mind, the current research status of asthma will be critically examined, with a focus on the animal models currently employed, together with a look to the future, and to methodologies which have already shown their value and could be incorporated into a robust, and potentially more human-relevant research strategy.

V-2-166

Meta-analysis of the application of weight of evidence (WoE) and read-across for the assessment of repeat-dose systemic toxicity

S. Freeman[1], J. Rowland[2], M. Mishra[3], C. Goebel[4], F. Schellauf[5] and J. Scheel[6]

[1]Farino Consulting, Surrey, UK; [2]GSK Consumer Healthcare, Surrey, UK; [3]GSK Knowledge Center, Gurgaon, India; [4]P&G, Darmstadt, Germany; [5]COLIPA, Brussels, Belgium; [6]Henkel AG & Co. KGaA, Duesseldorf, Germany

julia.scheel@henkel.com

Animal welfare considerations, as laid down in the 2013 deadline for a ban on animal testing according to the 7th Amendment of the European Cosmetics Directive, demand the use of non-animal alternatives to evaluate repeat-dose systemic toxicity. A meta-analysis of open literature and databases was initiated by the COLIPA (European Cosmetics Association) Safety Assessment Task Force to identify non-animal approaches that are currently available and already applied in risk or safety assessment in various sectors, the focus being on examination of the practical application of WoE and read-across in the assessment of hazard and risk for toxicological effects usually assessed by repeat-dose toxicity studies. The analysis included subacute, subchronic and chronic toxicity, toxicity to reproduction and carcinogenicity. The search included PubMed/Medline, Toxline, OECD dossiers, HERA risk assessments, CIR reports, SCCNFP/SCCP/SCCS opinions and RIFM group assessment reports. Overall trends and examples from this meta-analysis are presented, which is considered a complementary exercise to other research projects in this area like the SEURAT-1 cluster co-funded by the European Commission and COLIPA under the 7th Framework Programme. The aim of this set of related projects is to establish testing and assessment strategies in order to finally replace animal testing in this area.

V-2-244

Fewer animals – more quality data with process improvement and engagement in Refinement, Reduction and Replacement (3Rs) culture

H. Parenteau, H. Maurice, M. Perez-Marcogliese and M. Roumi

AstraZeneca R&D, Montreal, Canada

heli.parenteau@astrazeneca.com

In vivo studies are necessary to answer questions about poorly known targets and to develop safe and efficacious medicines for unmet needs in chronic pain. At our institution, we have implemented a working model that combines efficient processes, refined scientific methodologies, guiding principles and an engaged culture to attain best 3Rs practices. A stringent discovery phase screening cascade reduces the number of compounds from several thousands to tens of most advantageous ones prior to *in vivo* testing. Annual 3Rs improvements are incorporated in site/department goals and cascaded as individual objectives for scientists and animal care staff. Our animal use protocols undergo critical 3Rs evaluation by the animal care committee (ACC). Sectional representatives were included in the ACC to facilitate protocol review and ensure the close integration between science and ethics. Statistical expertise is provided to researchers to strengthen the science whilst optimising study designs to minimize animal numbers. A new animal management system linked to the protocol system ensures rodents can be efficiently used to obtain maximal data and to track animal use for protocols. Finally, different microsampling methods and study designs are used routinely to reduce animals for pharmacokinetics (up to 70%).

V-2-247

The ECVAM search guide – Good search practice on animal alternatives

A. J. Roi[1], B. Grune[2], D. Butzke[2], A. Luch[2], G. Pellegrini[1] and J. Kreysa[1]

[1]European Commission, Joint Research Centre, Ispra, Italy; [2]ZEBET – Federal Institute for Risk Assessment, Berlin, Germany

annett.roi@jrc.ec.europa.eu

The "ECVAM Search Guide" is aimed at untrained database users and will be most relevant, where comprehensive searches are required, as part of authorisation processes for animal experiments and where regulatory requirements mandate the application of the 3Rs.

A major challenge to locate relevant high quality information about a proposed field of scientific investigation is the exponential increase of scientific publications in the recent past. Over the last years the electronic resources, originally developed to offer a potential solution to this problem, have shown a similar proliferation. The question arises: how best to search for information specifically on the 3Rs (replacement, reduction, refinement of animal use) in the World Wide Web that is heterogenic, constantly changing and growing?

The ECVAM Search Guide provides a systematic step-by-step search procedure and user guidance to facilitate the location of the desired information on 3Rs alternatives in addition to an inventory of relevant resources providing an answer to the question: What can I find where?

The project has been initiated and sponsored by ECVAM and represents the outcome of a close collaboration with the National German Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) and an international project advisory team composed of scientists and representatives of ethical and regulatory authorities in support. Its publication as a handbook and on the Internet by ECVAM is expected for this year.

V-2-267

Reduction of animal use in toxicity studies in the pharmaceutical industry: fact or fiction?

E. Johansson, B. Granath, A. Annas, E. Jalkesten and A. Blomqvist

AstraZeneca R&D, Södertälje, Sweden

Elin.Johansson@astrazeneca.com

A systematic animal welfare culture, based on the principles of the 3Rs, has been implemented over recent years within Safety Assessment at AstraZeneca. In order to review the actual outcome of different projects/activities aimed at reduction, refinement or replacement (and combinations of these), all 3R improvements implemented since 2006 have been registered within the unit. The most prominent finding was the large reduction in the number of animals used, which is the focus of the data presented. "Reduction" projects/activities (40) were listed and categorized, and numbers of animals spared in toxicity studies, including regulatory studies, were estimated. Study directors, animal technicians, molecular toxicologists, genetic toxicologists, safety pharmacologists, reproductive toxicologists, pathologists, clinical pathologists and veterinarians all contributed to these changes resulting in diminished animal use. The implemented reduction activities were a result of improvements in study design (18 activities), method development (12) and collaboration between departments (10). All these activities together resulted in reductions in use of rats (51%), mice (34%) dogs (19%) and rabbits (8%). Importantly, concomitant refinement improvements were made in some cases as well. These data clearly indicate that reduction of use of animals in toxicity studies, including regulatory studies, is achievable by systematic use of 3R principles in study designs and that building a strong 3Rs culture in everyday work at all levels of the organization is essential to achieve this. The data show that systematic implementation of 3R principles in the Safety Assessment paradigm enhances ethical use of animals in research, without compromising the scientific quality of the study.

V-2-284

Reduction in the number of species required for design verification studies

D. Broughton and A. Welling

Ethicon EndoSurgery, Cincinnati, USA

Dbrought@its.jnj.com

Design verification studies for Ethicon Endo-Surgery, Inc.'s HARMONIC® energy devices are performed to evaluate vessel sealing performance. The HARMONIC® instruments must be able to ligate and transect vessels within a specific diameter range (<5 mm). Historically, verification studies utilized multiple animals and two species (porcine & caprine): one species for large diameter vessels (3-5 mm) and another for small diameter vessels (<2 mm). Each animal provided a maximum of four data points and two blood pressure challenges. Internal studies indicated similarities between vascular physiological functions, biochemical properties, histological healing parameters and the absence of differences in evaluative measures for vessel sealing between the two species. Therefore, it was concluded that each species could be used interchangeably in evaluation of efficacy in blood vessel cutting and coagulation. The goal of this study was to validate the swine model for verification studies.

An exploratory procedure was performed to identify vessels in the small (<2 mm) and large (3-5 mm) diameter range. Surgery was performed in 4 pigs. Four pigs implied 32 vessels <5 mm (8 vessels per pig) were targeted for vessel sealing.

Eight vessels were identified, 4 small and 4 large. One blood pressure challenge was utilized to test the durability of the seals. Vessel sealing performance was evaluated and compared favorably to historical norms. In conclusion, one species, porcine, can be utilized in place of two species for verification studies.

V-2-495

Is the baboon model appropriate for endometriosis studies?

J.-P. Dehoux, S. Defrère and J. Donnez

Université catholique de Louvain, Brussels, Belgium

dehoux@chex.ucl.ac.be

Endometriosis, characterized by the growth of endometrial tissue outside the uterine cavity, is a common gynecological disorder affecting 10% of reproductive-aged women. The baboon is commonly used as a model for the study of human reproductivity. Previous studies have shown successful endometriosis induction after injection of autologous menstrual effluent into the pelvic cavity of baboons, resulting in the formation of endometriotic lesions with gross morphological characteristics similar to those seen in women. The aim of our study was to determine (i) the prevalence of spontaneous endometriosis and (ii) the incidence of induced endometriosis after transcervical resection of the endocervix in baboons. Between February 2009 and July 2010, a total of 41 baboons underwent diagnostic laparoscopy. In a first step, 30 subsequently underwent transcervical resection of the endocervix. In a second step, 20 of them underwent uterine horn resection. Two out of 41 baboons were diagnosed with spontaneous endometriosis (4.8%). Twelve months after the surgical procedure to induce endometriosis, 8/29 animals (one died) presented with endometriotic lesions diagnosed by laparoscopy and confirmed by histology. The cumulative incidence of induced endometriosis in our model was thus 27.6%. In two baboons, endometriosis disappeared over time, resulting in a final rate of 20.7% (6/29). In conclusion, our data lead us to doubt that the baboon is a relevant model for endometriosis, since our observations suggest that baboons develop extensive and effective mechanisms, lost by women in the course of evolution, to cleanse and renew their peritoneum.

V-2-513

Open-access journals and the increased availability of animal alternatives information

M. W. Wood[1], C. Howard[2] and L. A. Hart[1]

[1]University of California, Davis, USA; [2]Johns Hopkins Center for Alternatives to Animal Testing, Baltimore, MD, USA

mwwood@ucdavis.edu

Online publication of full-text journal articles has greatly simplified and increased access to scientific literature, including literature concerning animal alternatives and animal welfare. However, the open-access publishers and journals are those responsible for making access truly equally accessible. Without requiring paid subscriptions, open-access journals may be read by anyone with access to the internet. This poster will describe a few options for authors interested in locating an open-access publisher, as well as identify those open-access journals most likely to be of relevance to Congress attendees.

PubMed Central, directly related to the NIH (National Institutes of Health) Public Access Policy, is a free digital archive of biomedical and life sciences journal literature. BioMed Central and PLoS (Public Library of Science) publish peer-reviewed scientific and medical research literature freely and available as public resources. These are just a few examples of the options available to the scientist, for both research and publishing.

V-2-515

People making information matter

D. Jensen

U.S. Department of Agriculture, Beltsville, USA

danna.jensen@ars.usda.gov

Within the United States, there are requirements to address alternatives in the context of the 3Rs. This has been a regulatory requirement since the 1985 amendments to the Animal Welfare Act. To this day most people within the regulated community still have difficulty in "addressing alternatives." Over time the research community has come to understand that they must search the literature in order to address unnecessary duplication and to determine if alternatives are available. With the advent of the internet, Google, online databases, and generalized information access at your fingertips, much of the regulated research community believed that they could adequately address alternatives by plugging in a few keywords to get all they need. The error in this thinking is that they are only working with computer language and not with a true understanding of information access. When it comes to addressing alternatives, it is vital that people (those trained in information access-information providers) be involved. Utilizing the knowledge of "Information Providers" is a must in gaining the most relevant information. Only with the knowledge of people can the information results "truly matter" to the research community. Examples of computer vs. information provider will be provided.

V-2-562

How the 3Rs can benefit from systematic reviews

J. van Luijk, M. Leenaars and M. Ritskes-Hoitinga

Radboud University Nijmegen Medical Centre, Central Animal Laboratory and 3R Research Centre (3RRC), The Netherlands

J.vanluijk@cdl.umcn.nl

Searching for available 3R methods is not an easy task. Survey results indicate that existing 3R possibilities are regularly not found nor implemented (Leenaars et al., 2009; Van Luijk et al., submitted). Relevant 3R information is scattered over various sources such as: databases, persons and text books. Conducting systematic reviews (SR) will not be the ultimate answer for finding all existing 3R possibilities; however it does have great potential for maximizing 3R implementation (Hooijmans et al. 2010).

When planning and designing a new animal experiment, a systematic search for relevant literature can answer questions on the animal model or the novelty of the experiment. This systematic search is an important step in doing SRs. Therefore SRs can contribute to a more evidence-based use of animal models and will prevent unnecessary duplication of animal experiments. SRs will also contribute to more transparency in the animal experiment designing process and the choices made. Assessing the legitimacy of chosen methods is not an easy task according to Animal Welfare Officers and members of Animal Ethics Committees (AEC) (Van Luijk et al., submitted). Systematic Review steps such as systematic search for literature and quality assessment of primary studies will provide important insights into the decision process and provide a better basis for the ethical evaluation.

During the presentation I will elaborate further on how SRs contribute significantly to the quality of animal experiments, and on how SRs can be of benefit to society, policy makers and animal welfare.

References

Hooijmans, C. R., de Vries, R., Leenaars, M. et al. (2010). *Br. J. Pharmacol. 162*, 1259-1260.

Leenaars, M., Savenije, B., Nagtegaal, A. et al. (2009). *ATLA 37*, 297-303.