5th RSPCA international meeting

Cumulative Severity

Leiden 2023

Summary Report

In October 2023, the RSPCA co-organised the latest in our series of international events promoting the sharing of knowledge and approaches to avoiding and reducing suffering, and improving the welfare of animals used in research and testing. The in-person meeting, which attracted almost 160 participants from 13 countries, had a focus theme of 'cumulative severity'. It was delivered in association with the University of Leiden, Leiden University Medical Center (LUMC), the Netherlands National Committee for the Protection of Animals used for Scientific Purposes (NCaD), the Dutch Platform of Animal Welfare Bodies (IvD-Platform) and the Dutch Association for Laboratory Animal Science (DALAS).

After a welcome and introduction from Nelleke Verhave from Leiden University Medical Center, the first session opened with an update on the '**Focus on Severe Suffering' initiative**, and an overview of the '**Roadmap** to Reducing Suffering' by Barney Reed from the RSPCA. The Roadmap is a practical exercise that establishments can use to focus on procedures that have the potential to cause 'severe' suffering, identify contributing factors and find ways of avoiding or refining these. The exercise is most effectively applied by a group of individuals who hold a variety of roles and expertise; the RSPCA website provides all the information and guidance necessary for you to try the Roadmap at your own institution. This was followed by a presentation by Ludo Hellebrekers from the Central Authority for Scientific Procedures on Animals (CCD). The CCD, which holds the authority for granting project licences in the Netherlands, performed a review of severe suffering over the years with the aim to identify trends, establish approaches for implementing refinements and to evaluate cumulative severity. Key messages from this exercise included that collecting good quality data from **retrospective assessments** is a useful tool for supporting the harm-benefit analysis and can also lead to greater awareness with respect to cumulative effects, and the positive impact of applying clear and effective humane endpoints in reducing severe suffering.

The remaining sessions of the first day were devoted to **case studies in basic and applied research**. Hanneke Willemen, from the University Medical Center Utrecht, introduced her research on **chronic pain** and described the difficulties in identifying pain in rodents, as prey animals, who may have evolved to 'hide' signs that they are experiencing suffering. Hanneke described a recently published study on chronic osteoarthritis in mice which applied multiple pain assessment methods such as the von Frey test, and a dynamic weight-bearing device, which also helped to identify humane endpoints as well as providing scientific data. Current work is also ongoing in the field of chronic pain to study molecular pain mechanisms using *in vitro* 'pain-on-a-dish' which aims to replace or reduce the number of mice experiencing the cumulative effects of chronic pain.

In the next presentation, Marloes Hentzen from MSD Animal Health explained how severity classifications can be difficult when working across 13 species. Marloes described a protocol of **infectious bursal disease** and explained how multiple refinements can be applied to refine the protocol and identify humane endpoints. For example, extra observations are performed in case unpredictable effects occur. Also, a detailed scoring system is used which covers a variety of adverse effects and uses a weighted calculation of points to identify clear humane endpoints and reduce cumulative suffering. A post-study welfare evaluation is also performed to identify any opportunities for improvements. Next, Stéphanie De Vleeschauwer from KU Leuven described her ongoing work in creating the **OBSERVE guidelines** which aims to provide oncology researchers with clear guidance on specific *in vivo* cancer model refinements. The guidelines, which are being developed by an expert steering committee, will describe clinical signs associated with tumour categories, and describe refinements during tumour development. This resource aims to provide robust tumour-specific guidance, promoting standardised animal welfare practices across studies and organisations.

Following this, Stéphane Marinesco from Lyon Neuroscience Research Center presented a scoresheet which was developed to monitor animal welfare following **traumatic brain injury** (TBI) in rats and used as a strategic approach to reduce suffering. The scoresheet monitors specific parameters for TBI including a grimace scale, mouth injury and hydration levels. It uses a points system on a variety of parameters to identify humane endpoints. The use of the scoresheet also helped to establish a scientific outcome that rat models of TBI using this approach appear to recover after 5-7 days. The next speaker was Marjolein Kikkert from Leiden University Medical Center who explained the importance of health monitoring in mouse models of **MERS Coronavirus**. Lethal models are widely used in infectious disease research and so procedural refinements and humane endpoints are essential to reduce suffering. Health monitoring sheets which include parameters such as weight, consciousness, behaviour and respiration were used to determine humane endpoints, and as a result, instances of animals being ‘found dead’ were rare.

Finally, the **keynote presentation** was from Bernice Bovenkerk, an associate professor of social science from Wageningen University who described how **cognitive complexity** plays a role in suffering. She outlined and critiqued the assumption that animals with more cognitive complexity ‘suffer more’ than those with less cognitive complexity, but explained that this is multifaceted. For example, more cognitively complex animals can in some cases cope better with pain, if the pain is short and the animals understand the pain will be over quickly. On the other hand, in the case of cumulative suffering, an animal with more cognitive complexity may cope less well, because they anticipate the pain will continue. Overall, it is deemed problematic to assume that less cognitively complex animals experience less suffering than more complex animals.

Key messages and action points from day 1

- Obtaining good quality information on severity is essential for focusing efforts. There is still room for improvements regarding how Retrospective Assessments and actual reporting of severity are carried out, and how this information is considered and utilised at both institutional and national level.
- There must be robust ethical discussion in any case where animal use is proposed - especially so where 'severe' suffering may be involved.
- When considering the assessment of 'harms' (such as by conducting a Harm-Benefit Analysis) it is important to think about *all* aspects that contribute to and affect an animal's overall quality of life. Everyone involved must continue to think carefully and challenge assumptions on what causes suffering, how it is experienced by the animal, and what steps can be taken to reduce or avoid it.
- Animals may become habituated or sensitised to procedures making it difficult to assess cumulative severity. Welfare assessment protocols should be regularly reviewed to ensure information related to this is captured.
- Clear, specific and precise humane endpoints should always be identified, agreed, and implemented. There are continuous opportunities for improving humane endpoints throughout a study, and for sharing this information - both within and between establishments.
- There is evidence to suggest that increased cognitive complexity *may* cause an animal to experience greater suffering in some circumstances, however a precautionary approach should be applied to all animals.

The second day commenced with speakers describing how they have **applied the Roadmap in practice** to avoid and reduce severe suffering. Carla Bol, an attending veterinarian from Charles River Laboratories, presented an example of how to use the Roadmap as a strategic approach to reduce the severity of suffering in animals used for **safety testing** in an industry setting. She provided an example of a study that tested propylene glycol in rabbits to determine tolerability and the appropriate dosage range. The Roadmap was used to assess procedural effects, and they redefined the humane endpoint as the point at which toxicity is evident without causing severe suffering. In the next presentation, Jeanette Lorteije and Manon van Hulzen from Radboud University Medical Center presented a case study from academia which described a research study involving **limb amputation and replantation** in pigs. Jeanette and Manon used the Roadmap to identify areas of reducing cumulative severity in this model such as group housing pigs prior to surgery, and providing safe social contact post-surgery. They also reviewed specific aspects of the procedure including anaesthesia, surgery, and postsurgical recovery to identify refinements such as providing appropriate levels of analgesia and frequent post-surgical monitoring by a familiar handler.

The second session of the day, focusing on **cumulative severity**, opened with a talk from Wim De Leeuw of the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) who described the importance of accurately determining **prospective assessment** of severity, including cumulative severity. Wim explained that predicting severity is essential in order to identify factors which can be mitigated to reduce suffering. The Roadmap can be used to strategically assess each experience of the animal, and implement actions with a positive impact.

Following this, participants separated into smaller groups for a **workshop on cumulative severity**. Groups were provided with an example of a severe procedure which involved either mice or zebrafish. The Roadmap worksheets 1 and 2 were provided so that participants could discuss how to implement refinements to the experimental procedure, as well as over the lifetime experiences of the animals. In the following discussion session, all groups were able to apply refinements to the procedures using the Roadmap technique. Some key points identified by participants included: having a range of expertise and disciplines present within each group greatly facilitated the identification and critical evaluation of the suggested methods; and protocol-specific welfare assessment score sheets are essential for identifying humane intervention points.

The third session, titled '**Making it work - roles and responsibilities**', featured four presentations from speakers who hold different responsibilities related to the use of animals in scientific research. Tineke Coenen, animal facility manager at Leiden University Medical Center, opened with an introduction to how **animal facility management and animal caretakers** can contribute to animal welfare. Tineke described how routine husbandry practices can be refined in order to reduce cumulative suffering. The presentation highlighted how adopting refined breeding practices, including avoiding continuous breeding and providing raised platforms for dams, can help minimise cumulative suffering in mice and prevent incidents of animals being found dead. The next speaker, Stéphane Marinesco, led a discussion on the **experiences of a scientist** working with Animal Welfare Bodies (AWBs). Stéphane described ways in which the newly formed AWBs in France have already contributed to enhancing animal welfare, such as reducing single housing. Participants also discussed the challenges they encountered. During the discussion, the idea of merging the grant proposal and ethical review processes was considered as a potential way to streamline the procedures and enable more effective discussion of refinements and humane endpoints.

In the next presentation, Jukka Puoliväli from Charles River Laboratories shared his **experience as the Chair of an Animal Welfare Body**. He discussed the positive impact of the AWB on animal welfare improvements, highlighting a specific example involving high levels of aggression identified in male mice sourced from an external supplier. To address this issue, they conducted a small study comparing the ordering of male mice at 4 weeks and 7 weeks of age. The results demonstrated that ordering males at 4 weeks significantly reduced the need for single housing (and also resulted in a cost saving of 38 euros per mouse). The final presentation in this session was delivered by Penny Hawkins of the RSPCA. She discussed strategies for **optimising engagement between scientists and Animal Welfare Bodies.** A working group established by the RSPCA has developed a valuable resource that outlines effective ways for researchers and AWBs to engage constructively. This includes recommendations for AWBs to clarify their project review processes, timing, and information requirements. It also emphasises the importance of scientists familiarising themselves with AWBs to facilitate successful communication of their research.

In the final session, Penny Hawkins led a discussion on **assessing the impact** of initiatives to reduce severe suffering. Severe suffering has decreased steadily in the UK and EU over the last few years. This has been achieved by improved experimental design such as the application of earlier humane endpoints, and cultural factors such as better communications

within teams and AWB involvement. Participants emphasised the significance of improved communication and fostering a Culture of Care in minimising suffering. Additionally, they pointed out that in order to assess initiatives to reduce severe suffering, there must also be good data collection and transparent reporting of positive and negative outcomes.

Key messages and action points from day 2

- The net impact, both positive and negative, of all events determines the welfare of the animal over their lifetime. The 'marginal gains' approach can be used throughout an animal's lifetime to apply many small improvements which can collectively make a larger and significant positive difference to animal welfare.
- Suffering is not limited to experimental procedures; animals can also experience negative mental states, such as distress and boredom, due to housing and husbandry that does not meet their needs. The role of animal technicians and animal facility management are vital to optimise the welfare of animals.
- Improving animal welfare is not only beneficial to animals and scientific research, but can also be cost-effective.
- The effect of interventions on animals may change over time, so animal welfare assessment must be reviewed and improved throughout a study.
- In order to gain the greatest benefits from the Roadmap to reduce severe suffering, input is required from a wide range of roles including scientists from different areas of research, veterinarians, animal technicians, Animal Welfare Body members (including lay or non-affiliated persons) and regulators.
- Animal Welfare Bodies should evaluate their relationships with researchers to ensure that they are working most effectively.
- The Culture of Care in a research institution is key to ensuring good communication and a positive attitude towards reducing severity within an establishment.

Further information

Visit the RSPCA 'Focus on Severe Suffering' website for the latest information and resources on this topic.

The RSPCA would like to thank all of the speakers at the meeting, our sponsors, and Leiden University for co-organising this event and providing the venue.

This summary report has been produced by the RSPCA Animals in Science Department.

October 2023