The adverse affect of adverse effects

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Abstract

Within most animal research facilities there will be occasions when a project Standard Condition 18 report needs to be submitted if the severity limits specified in the licence or other controls stated have been or are likely to be breached. For example, breaches may occur if a new transgenic line exhibits an unknown phenotype that was not accounted for in the Project Licence (PPL) or if death has unexpectedly occurred as a direct result of a scientific procedure.

Adverse effects are often experienced in research studies but at the time of publishing the results, they are rarely mentioned. The ARRIVE Guidelines 2.0,[1] recommend detailing scientific implications of a study, including adverse effects but this is not part of the Essential 10 areas that should be covered in a publication. By sharing the unexpected adverse events more widely in the research community there is the opportunity to refine techniques, thereby reducing the number of animals used and the potential pain, distress and lasting harm they may experience.

This paper will address how research facilities could encourage the research community to share the unexpected adverse events they have encountered and how they overcame them to successfully meet the scientific aims and objectives of their research.

Keywords: adverse effects, ARRIVE guidelines, refinement, reduce

Introduction

When considering adverse effects it is important to understand when they go from being an expected adverse effect to unexpected. The Animals in Science Regulation Unit (ASRU),[2] recognises that during the course of research unexpected adverse effects may occur in the following situations:

- An unanticipated source of pain, suffering, distress or lasting harm.
- Not included in the harm benefit analysis that underpins the project licence.
- Animals undergoing procedures are found dead as a consequence of those procedures.
- Mortality is not an authorised adverse effect in the relevant protocol. However there are situations where death is an authorised adverse effect and an example of this is myocardial infarction studies where is typically a 20% mortality rate.

A Project Licence (PPL) Standard Condition 18 (SC18)[3] requires the PPL holder to notify the Secretary of State if constraints on severity or observance of other controls as described in the PPL have been breached or are likely to be. Situations resulting in a SC18 notification being completed range from mild behavioural abnormalities to death. The following questions need to be considered when deciding if a PPL SC18 notification is required:

- Is an animal experiencing adverse effects due to the regulated procedures being carried out?
- Where the adverse effects observed specified in the PPL or are they truly unexpected?
- Are the adverse effects exceeding the severity limit of the protocol or are they likely to?

An informed decision needs to be made based on the knowledge of what an animal is known to have

experienced or likely to experience and consider what actions will be taken by those responsible for the animal.

Protocols within PPLs have an overall severity limit ranging from non-recovery to severe. Examples of unexpected adverse effects and how these severities have been breached at KCL are:

- Mild – Mice treated with a cannabinoid-related drug appeared to be sluggish and hypoactive approximately 1-hour post-dosing. The severity for this protocol was not a concern but the adverse effects observed had not been listed within the protocol.
- Moderate – Mice were dosed with a carcinogen (4-NQO) in their drinking water for 16 weeks before reverting to standard water for a further 8 weeks. The mice experienced rapid weight loss, were cold to touch and in a moribund state. The severity of this protocol risked being breached with the adverse effects leaning towards severe severity. The adverse effects noted where not stated in the protocol.
- Severe – Rats administered a single intraperitoneal dose of DSP-4 toxin as part of a study looking at the induction of cancer pain. Death was not an authorised adverse effect and was not necessary had there been adequate understanding and control of the adverse effects.

Project licences detail the expected adverse effects that may occur during a specific protocol and these can be general in terms of the animals' overall appearance and condition, or they can be more specific and provide information on how likely the adverse effects are to happen. Protocols in Project Licences clearly state the expected adverse effects that may occur due to the procedure being carried out on animals but there are instances where unexpected adverse effect can occur. In some cases, it may be possible with refinements to overcome these events and for the study to reach completion with the objectives being achieved.

The objectives of the present study are outlined below:

- To demonstrate platforms currently available that could be used for sharing unexpected adverse effects.
- To gather information regarding why the research community do not typically report unexpected adverse effects.
- To find an effective way to disseminate information relating to unexpected adverse effects.
- To show the need for openness around unexpected adverse effects within journal publications.

By achieving these objectives, Animal Welfare might be positively impacted by preventing unnecessary pain, suffering and lasting harm.

Method

A key objective during this project was to find an effective way to disseminate information regarding unexpected adverse effects to research communities. To be able to do this it was important to reach out to the research community at King's College London to identify if they actively detailed unexpected events in their publications and what would cause them to not detail this information. In the first instance a short anonymous questionnaire via Microsoft Forms was created for PPL holders to complete.

1. Have you experienced any unexpected adverse effects during an experiment that involved regulated procedures on an animal?

2. Have you submitted a Standard Condition 18 report to ASRU due to unexpected adverse effects?

3. If you have experienced adverse effects, did you include them in any associated publications?

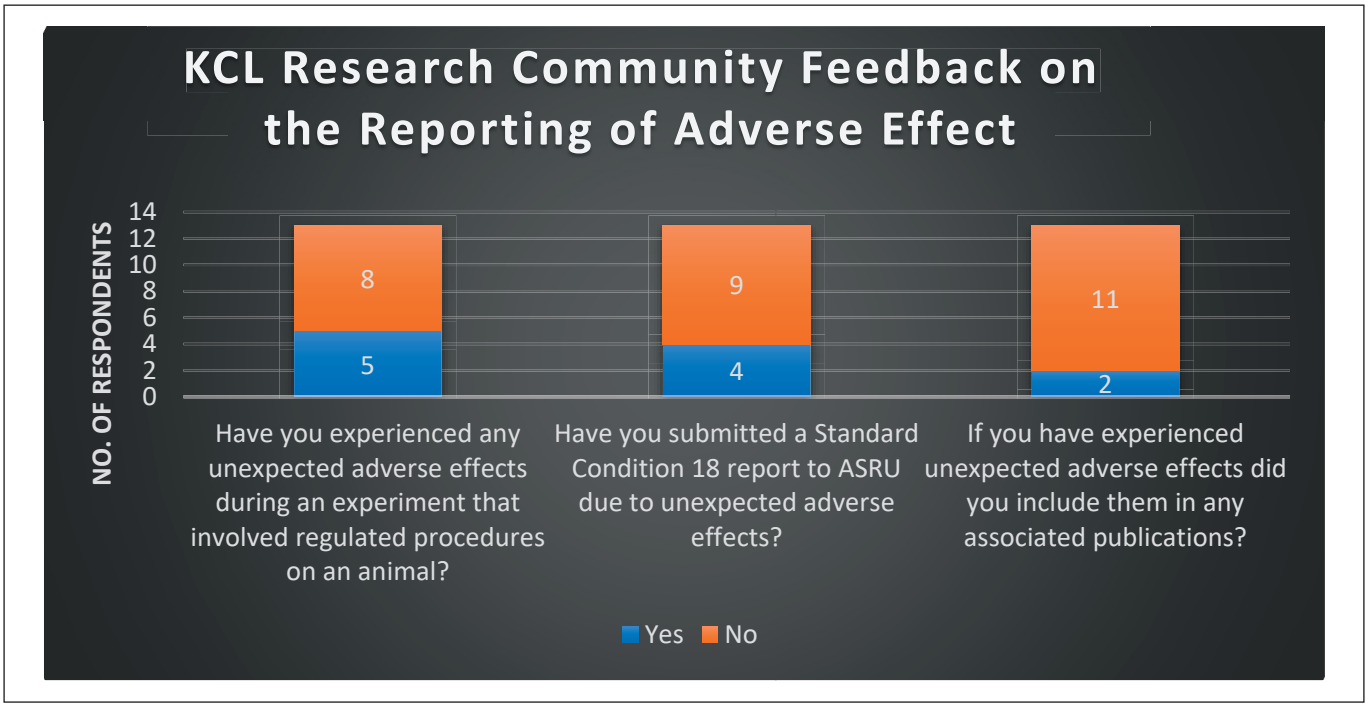
4. Would you publish unexpected effects in any future publications?

The questionnaire was sent to all active PPL holders at King's. It was important that the questionnaire was anonymous to encourage PPL holders to provide honest and provide accurate feedback relating to unexpected adverse effects. Questions 1-3 were single answer questions. Question 4 required a long answer, and all participants were required to answer Question 4 regardless of whether they had experienced unexpected adverse effects.

Results

The graph overleaf details the responses collected from the anonymous PPL questionnaire.

It was important to find out whether the research community would publish unexpected adverse effects moving forward. This question provided great insight into the reasons why they would or would not publish this these details. Limited word counts and publishers requiring manuscripts to be streamlined prevented researchers from publishing adverse effects. Other researchers would only publish the adverse effects if they felt they directly related to the animal model or experiment. Another interesting response explained they would not include adverse effects because it did not fit the aim of the paper and the information was already available. They did not mention if they had looked at refining the procedure to prevent the adverse effects from occurring or whether they had implemented any interventions.



Graph 1. KCL Research Community Feedback on the Reporting of Adverse Effect.

Would you publish unexpected effects in any future publications? Please answer even if you responded no to Q1-3.
Possibly yes.
Great question. Not sure - often there are very restrictive word limits etc, and manuscripts have to be very streamlined. I think if it was relevant to the main point of the paper then yes, otherwise probably no. But maybe we should!
Yes, if I felt they were directly related to the model or experiment in question and may be important for other researchers to know. I have previously reported special diet modifications used to help improve outcomes in disease models.
Yes, included in publication to inform future studies and wider community. There was no need to report as rats were carefully monitored and culled immediately. The issue was discussed with the NVS internally.
Yes, if I thought it would advance our scientific understanding.
Slightly depends on what the adverse effect was and how “preventable” it would be in the future. So, if it was just one animal and for example, something unfortunate happened during surgery that I thought was unlikely to happen to others, then I probably wouldn’t, although may mention it in a “protocols chapter”. However, if it affected several animals and could be prevented easily then I would definitely mention it.
The adverse events we encountered were linked to original set up of a new surgical technique, not due to the treatment we wanted to eventually test in our project. Any adverse event directly related to the test treatment would of course be reported in any publication of our data.
I feel like it might depend on whether the adverse event is at all relevant to the procedure. I think I would, in the Methodology section. Good luck with the research!
It depends on what the adverse effects were – whether related to the procedure or welfare of animals.
We have not written these experiments up for publication because of the unexpected effects, which led us to halt the trial of the drug.
No. this would not fit the aim of the paper. Also, the there was some literature that has previously reported and discussed the unexpected adverse effect.
Yes, if relevant to topic and publication.
Yes.

Figure 1. Examples of responses from questionnaire.

Discussion

Following recent findings relating to adverse effects at King's College London, it has become evident that adverse effects are rarely included in publications where animals are used in research. This study therefore aimed to explore the platforms currently available for sharing unexpected adverse effects, reasons why adverse effects may not be reported by researchers, effective means of disseminating information and how to create better openness around this subject.

This study found that there are various factors that prevent and/or inhibit the author from including details relating to animal care and monitoring and interpretation/scientific implications.

Researchers had mentioned the requirements of the publishers and how this can impact what is included in publications. General searches of publications that include the use of animals in research do not mention the expected or unexpected adverse effects. Could this lack of inclusion be due to manuscript requirements and the lack of adherence to the ARRIVE guidelines 2.0.?[2] It was important to assess what different publishers require on submission. Looking at three publishers it was evident there is inconsistency in this area. The British Journal of Pharmacology (BJP),[4] submission requirements are very thorough and detailed. BJP specifically reference the adherence to the ARRIVE Guidelines 2.0.[1] Authors must provide a scientific justification for the animal species and model used for each study. An ethical statement for experimentation must be provided that is recognised worldwide. Inclusion of animal welfare assessments and interventions carried out during the course of the study are a requirement. Nature Scientific,[5] requires the use of animals to be reported in accordance with the ARRIVE guidelines 2.0.[1] However Nature Medicine,[6] does not state that the animals used in research must be included but they do encourage the use of their protocol exchange system where researchers can deposit their tested protocols for others to use when replicating studies. Lastly eLife,[7] Sciences also require ethical approval during the submission stage but does not require details of animal welfare or interventions that may have occurred.

With the pandemic halting research for several months, KCL planned for research to restart on campus in July 2020 as restrictions began to ease. To enable this study plans were implemented. Study plans details the PPL and protocol steps being carried out and the known adverse effects that the animals may experience as well as the endpoints. Study plans are completed by the PPL holder or Personal Licence holder (PIL) and are submitted to the Deputy Director of Biological Services. This ensures the individual carrying out the study has the appropriate competencies and the plan of work is covered by the PPL. Following this the study plan is sent to the Named Animal Care and Welfare Officer (NACWO) of the relevant facility for final approval. Study plans are widely accessible by all animal care staff and the implementation of them has allowed for unexpected adverse effects to be more easily detected. When unexpected adverse effects have occurred these are discussed with the researchers, NACWO, Deputy Director and the Named Veterinary Surgeon (NVS) and a Home Office Inspector, if necessary, to assess why they have occurred and what can be done to move forward in a positive direction.

The ARRIVE Guidelines 2.0, are a great driving force in improving the reporting of animals used in research. The original guidelines have been reviewed since they were published in 2010 and the checklist has been updated with the classified items placed into two sets, the Essential 10 and the Recommended Set.[1] Despite the improvement in the guidelines there is division between the Essential 10 and the Recommended set. While some publishers do adhere to the guidelines, particularly the Essential 10 there is a lack of inclusion of topics included within the Recommended Set which includes the adverse effects, interventions, welfare assessments and additional monitoring.

In December 2021 ARRIVE published a series of actions for Universities, Journals and Funders. The Action Plans,[8] encourage activities that organisations can undertake to implement the guidelines into their policies and procedures. The Action Plan for Universities[8] includes three key actions for raising awareness and adherence to the guidelines. They include ensuring the institute has a public statement on its website endorsing the use of the guidelines, promoting the resources provided by the NC3Rs.[9] The NC3Rs' website is a great tool for new and updated resources that can be fed back to the research community and technicians. Lastly discussing SC18s,[3] at Animal Welfare & Ethical Review Bodies (AWERBs) and the issues that arose and how they were dealt with.

The Knowledge Hub is facilitated via the Animals in Science Committee (ASC).[10] The knowledge hub is a great tool for AWERB members to connect while promoting best practice for animal use in science and supporting a positive culture of care to all aspects of animal welfare. Due to the information that may be shared the knowledge hub operates on a private platform and access is invite only. Unfortunately, this is a tool that is not well known within the research community and currently only has 115 members within the UK.

At KCL change has started locally with the implementation of study plans which has provided greater understanding of the research for managers and technicians while also highlighting the expected adverse effects, it brings into focus the unexpected when they do occur. Ensuring SC18s are discussed during AWERB meetings to share knowledge with the committee. To be able to facilitate

change we plan to create an adverse effect repository to capture unexpected adverse effects and events occurring across the whole of Biological Services and enabling change within our Establishment. At a national level further adherence to the guidelines from publishers is needed and further inclusion of topics within the Recommended Set that clearly explain the animal experience, adverse effects and additional monitoring regimes. AWERB members should be encouraged to become members of the Knowledge Hub and share interventions, monitoring regimes and welfare assessments put in place to achieve the project aims and objectives. Lastly taking change to an international level we have the publishing of papers in worldwide journals and attending international conferences to not only learn but to share experiences.

In conclusion, this study has found that publishers requirements often influence whether adverse effects, welfare assessments and interventions are included in paper submissions. This is often caused by word limits and the need for the paper to be streamlined. Other factors include whether the researcher considers the inclusion of this information relevant to other researchers and whether the adverse effects experienced could be prevented.

Greater awareness of both the unexpected and expected adverse effects are needed to prevent other research groups from experiencing the same issues and animals potentially experiencing unnecessary pain, distress, and lasting harm. There are occasions where adverse effects can be overcome by implementing welfare assessments and interventions and it is this information that should be more widely available to the research community. By shining the light on this important topic, it is hoped that other institutions will begin to share adverse effects experienced internally and for publishers to see the positive impact sharing this information will have on the research community.

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