How should the IACUC handle unanticipated deaths?

Jerald Silverman, DVM

Some experimental procedures are more difficult to perform than others. Nevertheless, it is the responsibility of the IACUC to assure that all persons working with animals have been properly trained and can competently perform the procedures for which they are responsible. Proper training was especially important for the success of Dr. Ralph Osterman's cerebral trauma studies which required anesthetized rabbits to remain anesthetized for 12 hours after induced brain trauma. During that time Osterman studied the effect of various therapeutic modalities on brain microcirculation and brain temperature using noninvasive procedures. All animals were then euthanized without recovering from anesthesia.

Osterman's lab had sophisticated animal anesthesia simulation equipment which was used to train the technician-anesthetists who were responsible only for inducing, maintaining, and monitoring anesthesia. The approved anesthesia training had worked satisfactorily for the five years the protocol had been active and the veterinarians who periodically observed the studies had never requested any additional instruction.

In early June there were two experienced anesthesia technicians assigned to a typical 12 hour experiment, each working back-to-back six hour shifts. Two rabbits were anesthetized, one to receive the experimental treatment and one being a control. Unfortunately, the control rabbit died unexpectedly soon after the study began. When the study was repeated a week later with the same two technicians, once again the control rabbit died during the first six hours. This was quite unusual because this problem had never occurred previously with any of Osterman's studies. Osterman reported the two incidents to the IACUC as unanticipated adverse events. The institution's veterinarians had already performed necropsies but could not determine the cause of the rabbits' deaths, either grossly or through histopathology. The anesthesia machines were checked and were working properly. The anesthesia records did not indicate any unusual occurrences until just before death when there was an acute loss of cardiac activity. Osterman was upset but believed that the deaths were unfortunate coincidences and he wanted to move forward with his research. The IACUC discussed the problem but was unsure of what path to take. What do you think would be a proper action for the IACUC?

RESPONSE

Make the leap of faith, but finish looking first

Cheryl A Cheney

Osterman has been fortunate that throughout the five years of his study, the two control rabbits recently lost to cardiac arrest under anesthesia are the first. It is commendable that he promptly informed the IACUC of these untoward outcomes and involved the veterinarians in an investigation into why they occurred. Failing to discover any clear cause for the deaths following an assessment of the anesthesia equipment and necropsies on the bodies, the PI is prepared to chalk it up to bad luck and continue as before, and awaits the IACUC's okay to do so.

The PI and IACUC have equal interest in determining how to prevent future unanticipated mortality; it is unlikely Osterman would have suggested continuing the studies if he wasn't convinced these two deaths were beyond his control. Yet the IACUC first needs to consider whether all reasonable alternative explanations for the complications have been ruled out. A well-formed IACUC will reflect a diversity of expertise and opinion, and by brainstorming together they might identify other study elements to scrutinize. For example, hypothermia can dangerously increase the depth of anesthesia, so they could request confirmation that the thermal support system is also functioning properly.

In the absence of any actionable explanations for what went wrong, and in the interest of maintaining goodwill and an admirably transparent relationship, it is advisable for the IACUC to grant his request.

The Animal Welfare Act requires research facilities to review personnel qualifications with sufficient frequency to ensure individuals are qualified to perform their duties (§ 2.32, b)[1]. Although the two technicians present during the deaths have assisted Osterman on numerous prior study sessions without incident and appear to be maintaining appropriate anesthesia records, it would nevertheless be appropriate for the IACUC to ask for a veterinarian to be present during the next procedure these individuals will support, to assess whether refresher training is warranted and to advise on clinical interventions in the event that further issues arise.

If there is no evidence of noncompliance with the Animal Welfare Act, PHS Policy, or the Guide, there are no grounds to suspend the protocol, so the incidents will not need to be reported to the USDA. Since the deaths occurred under anesthesia, there would be no need to count these animals

under Category E in the annual USDA report, either (unless other protocol procedures the animals underwent warrant it).

We do not know if the study is PHS-funded, but even if it is, there would likewise be no need to report the incidents to OLAW, who acknowledge "that there may be levels of morbidity and mortality…that are not the result of violations of either the policy or the Guide", including "animal death or injuries related to manipulations that fall within parameters described in the IACUC-approved protocol"[2]. Given the inherent potential for some uncontrollable animal loss in the course of research, this wouldn't meet the criteria for reporting to AAALAC, either.

In summary, the PI has proven himself to be a conscientious animal user, and accepting his expert opinion and cautiously accommodating his keenness to proceed should satisfy the needs and obligations of all involved.

1. United States Department of Agriculture. *Animal Welfare Act and Animal Welfare Regulations* (2013).
2. National Institutes of Health, Office of Laboratory Animal Welfare. *Guidance on Prompt Reporting to OLAW Under the PHS Policy on Humane Care and Use of Laboratory Animals*. https://grants.nih.gov/grants/guide/notice-files/NOT-OD-05-034.html

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A Word from OLAW

In response to the issues posed in this scenario, the Office of Laboratory Animal Welfare (OLAW) provides the following clarifications:

This scenario describes the unanticipated deaths of control animals during a procedure that was conducted in the long-running study. The deaths were reported to the IACUC by the conscientious PI. The scenario asks, "How should the IACUC handle the situation?"

In addition to reviewing the report to the IACUC from the PI, the IACUC must further investigate the unexpected deaths to meet its oversight responsibilities under the PHS Policy, the *Guide* and the Animal Welfare Act and Regulations (AWAR)[1–3]. The investigation requires a thorough analysis by the IACUC, in cooperation with the research team, to discern any changes that may have caused the deaths. In this particular study, items to review include: 1) homeostasis of the animals (e.g., fluctuations in room temperature, fluid, or thermal support for the animal), 2) suitability of the animals for the study (e.g., animal conditioning, age, and weight), 3) fidelity to the IACUC-approved procedure (e.g., comparison of the protocol to the procedures and specific anesthesia actually used), and 4) condition of equipment (e.g., examination of maintenance records on all anesthesia and support equipment in use). Although the research team conducted their own investigation of both the equipment and animals, the IACUC may consider expanded consultation with veterinary pathologists, an independent diagnostic evaluation of the anesthesia machine by a certified technician, and a call to the rabbit supplier about any changes in health or genetics of the colony. To encourage continued engagement of the research team, a reasonable approach is to allow the research to continue with enhanced monitoring of the next procedure by the veterinarian and, if available, an anesthesia specialist.

If a cause is established, the IACUC may request amendments to the protocol to incorporate appropriate changes. Additional training may be necessary to improve responses to anesthetic complications and to engage rapid veterinary assistance. The IACUC may also find that the protocol needs to address an expected level of mortality.

If all procedures were performed according to the protocol and the deaths are due to individual rabbits' sensitivity to anesthesia, the incident is not reportable. If some aspect of the procedure was not done in accordance with the protocol or, for example, there was equipment failure, or inadequate thermal control or fluid support, then the IACUC must report to OLAW[4]. If the IACUC is unsure as to whether an incident is reportable, contacting OLAW by phone is the recommended approach.

1. Public Health Service. Policy on Humane Care and Use of Laboratory Animals (US Department of Health and Human Services, Washington, DC, 1986, revised 2015).
2. Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals 8th edn. 43–44 (National Academies Press, Washington, DC, 2011).
3. United States Department of Agriculture. Animal Welfare Act and Animal Welfare Regulations (2013).
4. National Institutes of Health. Guidance on Prompt Reporting to OLAW under the PHS Policy on Humane Care and Use of Laboratory Animals. Notice NOT-OD-05-034 (National Institutes of Health, Washington, DC, 24 February 2005).

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RESPONSE

Reporting is unnecessary, but preventing further unexpected deaths is key

Steven T Shipley & Jenny Estes

The question of action by the IACUC is twofold – regulatory concerns, and methods to correct the situation/prevent recurrence need consideration. The IACUC needs to first decide if this situation warrants a report to OLAW and/or USDA. For the question of OLAW reporting, PHS Policy IV.F.3 (ref. 1) states that a report is necessary for "serious or continuing non-compliance with PHS Policy," "serious deviation from the provisions of the *Guide*" or "any suspension of an activity by the IACUC". The AWRs §2.31 (d)(7) (ref. 2) state that reporting to APHIS (and the funding agency) is required if an activity is suspended by the IACUC/IO. Osterman's lab conducted all work compliant with an approved protocol and promptly reported the events to the IACUC. They actively cooperated with IACUC and the institution's veterinarians to evaluate the animals, records and anesthesia machines. Based on this scenario, reporting to either OLAW or APHIS is not warranted.

The IACUC now needs to decide how to best proceed by matching their action to the severity of the adverse events. The least intrusive option for the PI would be to simply agree with Osterman and accept that these incidents were bad luck based on the five-year track record of no previous unexpected adverse events. This would allow Osterman to proceed with his research

uninhibited, but there would be an inherent risk of a repeat adverse event since no corrective action would be taken. A much more aggressive option would be for the IACUC to either suspend the protocol or ask Osterman to voluntarily cease his research until he can submit a plan aimed at reducing the risk of anesthetic deaths. This is an overzealous response—Osterman and his team have been very cooperative and there might not be enough specific knowledge within the lab to create a comprehensive plan.

With a reputation for shutting down research after self-reported adverse events, an IACUC risks developing a culture of fear where PIs are not forthcoming with adverse event reporting. The appropriate middle ground in this situation is for the IACUC and veterinary group to work with the PI to develop modifications of animal procedures without suspending protocol activity. This should include having Osterman and his technician-anesthetists work under the auspices of a veterinarian (or veterinary anesthesiologist) for a period of time to refine animal use procedures along with thorough hands-on anesthesia training. This training should provide the technician-anesthetists (who were responsible only for inducing, maintaining, and monitoring anesthesia) with refined plans/algorithms necessary to respond to anesthetic complications before they become life-threatening. Veterinarian-provided training could incorporate more complicated scenarios than the animal anesthesia simulation equipment being used can provide, making problems leading to animal death less likely in the future. Working cooperatively with Osterman this way builds trust and improves relationships between scientists, the IACUC, and the veterinary group, while simultaneously improving animal care.

1. U.S. Department of Health and Human Services. Public Health Service Policy on Humane Care and Use of Laboratory Animals (2015).
2. United States Department of Agriculture. *Animal Welfare Act and Animal Welfare Regulations* (2013).

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RESPONSE

The time for active post-approval monitoring is now

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The unexpected death of any research animal is troubling, but especially when it appears that a trend might be forming. While in the case of Osterman's research, only two animals have died, it is incumbent upon the IACUC to determine the root cause of these unexpected deaths. The IACUC must, to the best of its ability, determine whether the root cause is due to inadequate training (a situation likely to incur other deaths) or a non-procedural issue that is unlikely to incur additional deaths (*e.g.*, an individual rabbit with undiagnosed cardiac disease). Osterman was correct in his timely reporting of the events before more unexpected deaths occurred. Rabbits are an "AWA-covered species." In the spirit of open communication, the IACUC should report the two deaths to the USDA while clearly stating that current evidence does not point to a non-compliance at this time[1]. Likewise, if the research is PHS-funded, OLAW should also be informed immediately[2]. The IACUC should report any findings of their subsequent investigations to these same agencies.

To aid in the IACUC investigation, the institution's veterinarians exercised due diligence in performing the necropsies on the two rabbits. The lack of gross anatomic and histopathological findings lends support that the deaths were not due to underlying disease conditions or anatomic anomalies. Likewise, the anesthesia machines appear to be in working order. This lack of findings, however, does not rule out a possible role that the anesthesia might have played in these deaths. For example, hypercapnia associated with physiologic dead space in the anesthesia circuit may not necessarily show up on necropsy. A root cause cannot be determined from the present investigation. Thus, I believe the body of evidence does not support an immediate IACUC suspension of Osterman's research, but, instead, the IACUC should institute active post-approval monitoring (PAM) of Osterman's rabbit protocol.

The Guide states that PAM "helps ensure the well-being of the animals and may provide opportunities to refine research procedures"[3]. Neither the AWAR or the PHS policy refer to PAM specifically by name. PAM can take several forms. One is the so-called "passive" form which relies on investigator self-reporting and the IACUC review of any adverse events reported on the annual and triennial protocol reviews required by the AWAR and PHS Policy, respectively[1,2]. In the case of Osterman's research, a more "active" PAM-approach is warranted because a potential procedural (and thus a training) issue with the implementation of the lab's rabbit-anesthesia protocol might be to blame. IACUC member(s) should observe the pre-anesthetic and anesthetic procedures from start-to-finish. At least one of the IACUC-appointed observers should be sufficiently knowledgeable in rabbit anesthesia. This could be one of the institution's veterinarians or an *ad-hoc* appointed observer, such as a board-certified veterinary anesthesiologist.

Should active PAM reveal deficiencies in the training and practice of anesthetic procedures as the root cause, the IACUC should suspend the protocol activities immediately[1]. To remove the suspension, Osterman should provide the IACUC proof of successful retraining of all staff involved in the rabbit anesthesia procedures. Additionally, it is within the IACUC's purview to stipulate that a continuation of active PAM is required as a condition of protocol reinstatement.

1. United States Department of Agriculture. *Animal Welfare Act and Animal Welfare Regulations* (2013).
2. U.S. Department of Health and Human Services. Public Health Service Policy on Humane Care and Use of Laboratory Animals (2015).
3. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).

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