

Jerald Silverman, DVM, Column Coordinator

Breeding without research

Sometimes an animal breeding protocol is like an overflowing bathtub: water continues to flow in and spill out, but the amount of water in the tub stays the same. Dr. Jonathan Spencer had a breeding colony of *Xenopus* frogs that was like the bathtub. Animals came and went, but all of the available tanks were always occupied. Kyle Sawyer, the animal facility supervisor, suspected that something was amiss because he never saw any requests to transfer frogs from Spencer's breeding protocol to his research protocol. After talking with the animal care technicians, Sawyer found that many frogs had been euthanized on Spencer's request. He asked the technicians what they thought the problem might be, and they quickly responded that there was no problem at all. Spencer continued to breed the frogs and

then euthanize them when space ran out. This had been going on for more than a year. So Sawyer delved deeper. He found that Spencer's IACUC protocols were approved for breeding nearly 1,000 frogs over a 3-year period and that he was approved to use half of them (females) for his research needs. Not knowing how to proceed, Sawyer went to the chairman of his IACUC.

Sawyer's basic concern was that frogs were being bred and euthanized but not used for research. He considered this a waste of life and wanted the IACUC to check Spencer's breeding and experimental records because the animal care technicians said that both males and females were being euthanized. The chairman understood Sawyer's concern and was sympathetic but noted that Spencer had an approved protocol for breeding and

that the euthanasia method was approved by the IACUC; in general, he felt that the IACUC should investigate concerns only when there was evidence of protocol non-compliance or animal abuse. In his opinion, neither had occurred. Nevertheless, he said he would bring the matter to the IACUC at its next meeting.

Before the full committee meeting was held, the IACUC determined that the observations of the animal care technicians were accurate. Spencer was breeding and euthanizing frogs and not using them for his approved research. When Spencer himself was questioned, he said that he had experienced some unforeseen delays but that he would start using the animals in the near future. What do you think this IACUC should do now in light of the facts presented?

RESPONSE

Spirit of the 3Rs

Gail Colbern, DVM, MS, DACT & Cheryl Aird, RVT, LATG, CPIA

Spencer wrote his protocol, had it approved and followed the procedures outlined in the protocol for breeding his frogs. The only problem is that he isn't doing the research that he outlined and justified in his IACUC protocol. Although Spencer's frogs are not covered by the provisions of the Animal Welfare Act, generally all IACUC protocols require "identification and appropriateness of the species and number of animals to be used"^{1,2}. The principle of the 3Rs applies specifically to "procedures that can cause more than slight or momentary pain or distress in animals, consistent with sound research design"³ and thus would not limit Spencer's breeding program. The description of the research project, as outlined in the original protocol, however, is clearly not being followed.

This situation, as Sawyer has pointed out, also does not follow the spirit of the 3Rs in reducing the total number of animals used. When Sawyer discovered the situation, he correctly requested the breeding and research records. The approved protocol allows Spencer to breed 1,000 frogs per year and to use half of them, or all of the females, in his research. If he has reached his breeding quota for the year, he must return to the IACUC for approval and justification for additional breeding to take place. As justification would require adequate description of the use of the first 1,000 frogs, the situation would be clearly defined for the IACUC. The IACUC could then determine whether 'unforeseen delays' constitute sufficient justification for Spencer to continue breeding these animals and, if so, for how much time or how many frogs.

Another consideration not specifically stated in the scenario is who is paying for this project. At a pharmaceutical company, Spencer may have to answer only to his supervisor and upper management for spending money to breed frogs that were not needed or used. This might reflect badly

in his performance evaluation. If, however, the frog breeding was being done with grant funding, then the institution, not the IACUC, is responsible for ensuring that funds are spent as outlined in the grant. If grant funds were being spent to breed and maintain frogs that were not used to complete the funded research, then the granting agency would expect the institution to report the inappropriate use of its funds, at the least. This could be considered grant fraud and, depending on the granting agency, may be pursued in other ways beyond the scope of this protocol review.

Sawyer was correct in questioning the ongoing breeding of frogs when no research was being done. This situation clearly violates the spirit of the principle of minimizing the waste of animal life. Sawyer was also protecting his institution by reporting a situation where funds may have been used inappropriately.

1. Animal Welfare Act, 9 CFR Chapter 1, Section 2.31 (1997).

2. Silverman, J., Suckow, M.A. & Murthy, S. *The IACUC Handbook* 2nd edn. 158 (CRC Press, Boca Raton, FL, 2007).
3. Office of Laboratory Animal Welfare. *Institutional Animal Care and Use Committee Guidebook* 2nd edn. 97 (US Department of Health and Human Services, Washington, DC, 2002, reprinted 2008).

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RESPONSE

Safeguard the 3Rs

Deepti Chadalavada, DVM

The IACUC has the responsibility to safeguard the 3Rs (reduction, refinement and replacement) recommended by the Public Health Service *Policy on Humane Care and Use of Laboratory Animals* and the *Guide for the Care and Use of Laboratory Animals*^{1,2}. The Public Health Service *Policy* and USDA Animal Welfare Regulations³ require research institutions and IACUCs to ensure that investigators have appropriately considered alternatives for animals in their research and are using the minimum number of animals by avoiding unintended breeding. It also suggests that to minimize the loss of animals, investigators should plan ahead and use appropriate statistical analysis in order to breed the number of animals necessary to obtain maximum information with minimal loss of life. It is the responsibility of the IACUC to ensure that such measures have been taken and that investigators are in full compliance with the regulations. It is also the responsibility of the IACUC to help assure high standards of animal welfare in the institution⁴. In this case, even though frogs are not a species covered by the USDA, the IACUC chairman cannot deny that this is indeed an IACUC issue of potential animal waste. Thus, the IACUC should be informed of the situation and the concern of the animal resources staff and should take appropriate action.

The IACUC should consider halting Spencer's frog breeding protocol until an appropriate resolution is developed to minimize the unnecessary waste of animals. The Dean or Chair of the Department should be notified about the current situation and

A word from OLAW and USDA

In response to the issues raised in this scenario, the Office of Laboratory Animal Welfare (OLAW) and the United States Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care (USDA, APHIS, AC) offer the following clarification and guidance:

The Public Health Service *Policy on Humane Care and Use of Laboratory Animals* is applicable to live vertebrate animals used in research, research training and biological testing and clearly applies to amphibians bred and used for research¹. The *Policy* does not explicitly require an institutional mechanism to track animal usage by investigators in IACUC-approved activities, but it does require proposals to specify and to include a rationale for the number of animals to be used and requires that number to be limited to the minimum necessary to obtain valid results. Accordingly, institutions need to appropriately monitor and document numbers of animals acquired (through breeding or other means) and used in approved activities. Monitoring should not exclude the disposition of animals that are inadvertently or necessarily produced in excess of the number needed or that do not meet criteria (e.g., sex) established for the specific study proposal².

The mandate in US Government Principle III to use the minimum number of animals necessary to obtain valid results is synonymous with a requirement to reduce animal numbers, which is one of the 3Rs^{3,4}. IACUCs, acting as agents of institutions, are expected to implement and routinely evaluate this aspect of the institutional animal care and use program to ensure compliance with the PHS *Policy*. When deviations from the approved number of animals occur, the IACUC should review the circumstances, take appropriate action to correct any noncompliance and report to OLAW and the funding agency as applicable.

The Animal Welfare Act⁵ defines an animal as "any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm-blooded animal, which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet. This term excludes birds, rats of the genus *Rattus*, and mice of the genus *Mus*, bred for use in research; horses not used for research purposes; and other farm animals, such as, but not limited to, livestock or poultry used or intended for use as food or fiber, or livestock or poultry used or intended for use for improving animal nutrition, breeding, management, or production efficiency, or for improving the quality of food or fiber. With respect to a dog, the term means all dogs, including those used for hunting, security, or breeding purposes."

Although the *Xenopus* frogs discussed in this scenario are not covered under the Animal Welfare Regulations⁵, the policies and procedures implemented by the IACUC must continue to ensure that proposals utilizing all covered species are in compliance with the Animal Welfare Regulations.

1. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals — Frequently Asked Questions*. Animal Use and Management, Question No. F.2. (US Department of Health and Human Services, Washington, DC, 2006, revised 2009).
3. Interagency Research Animal Committee, Office of Science and Technology Policy. U.S. Government principles for the utilization and care of vertebrate animals used in testing, research, and training. *Federal Register* **50**, 864–902 (1985).
4. Russell, W.M.S. & Burch, R.L. *Principles of Humane Experimental Techniques* (Methuen and Co., London, 1959).
5. Code of Federal Regulations, Title 9, Chapter 1, Subchapter A - Animal Welfare: Part 1 Definitions. (§1.1).

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the IACUC action. Future work on the animals (frogs) under this protocol should be resumed only after obtaining permission

of the IACUC by demonstrating to the committee that all of Spencer's "unforeseen" problems are resolved. Any future protocols

from Spencer should be approved only after the investigator has proven his competency and demonstrated to the IACUC that he has resolved all problems of animal waste by appropriate implementation of the 3Rs. Spencer should assure the IACUC that he is capable of minimizing the number of frogs in his breeding colony by providing good records of the numbers of animals bred, offspring produced and animals used. It is highly advisable that the IACUC monitor Spencer's work closely in the future to assure compliance with the regulations and the general intent of the 3Rs (to minimize the number of animals used in research, teaching and breeding protocols).

In summary, the role of IACUC in this situation is to oversee the breeding on a regular basis, to track the numbers of animals bred and used in the research, to evaluate the approved protocols during their semiannual inspections and to take necessary steps to correct any deficiencies.

1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC, 1996).
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).
3. Silverman, J., Suckow, M.A. & Murthy, S. *The IACUC Handbook* 2nd edn. 158 (CRC Press, Boca Raton, FL, 2007).
4. Office of Laboratory Animal Welfare. *Institutional Animal Care and Use Committee Guidebook* 2nd edn. 97 (US Department of Health and Human Services, Washington, DC, 2002).

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RESPONSE

Letter versus intent

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Spencer may be in compliance with the protocol approved by the IACUC, but we feel that this situation seriously deviates from one of the principles of the 3Rs (reduction). There is no direct use of the 3Rs, as presented by Russell and Burch¹, in the guidelines and regulations from our main regulatory bodies (USDA and OLAW), but the use of this principle is inferred. For instance, the *Guide for the Care and Use of Laboratory Animals*² endorses the *US Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*³, which lists “use of appropriate species, quality, and number of animals.” The 3R principles define reduction as the “minimum number of animals that will serve a useful purpose, yield statistically sound data and produce scientific benefit”¹. OLAW has indicated that federal mandates in US Government Principles III and IV are synonymous with the principles of the 3Rs and that the 3Rs should be incorporated into IACUC review and other aspects of the institution's program⁴. Spencer's breeding activities as currently conducted unnecessarily increase the number of animals needed to eventually carry out the studies that are outlined. In our opinion, this practice is irresponsible

and unacceptable. We believe that it is not in line with the objectives and mission of the IACUC.

In light of the facts presented, we feel that the IACUC should ask Spencer to cease breeding until he is prepared to transfer the animals to research studies. If he cannot cease breeding completely in order to maintain the colony, then the minimum level of breeding should be maintained and alternative uses for the unneeded animals should be sought, such as transfer to a different lab or even another institution. The IACUC should consider setting a policy for the management of animals that are ‘in house’ during a hiatus from activity, including breeding colonies.

1. Russell, W.M.S. & Burch, R.L. *Principles of Humane Experimental Techniques* (Methuen and Co., London, 1959).
2. National Research Council. Committee to Revise the *Guide for the Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC, 1996).
3. Public Health Service. *US Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training* (US Department of Health and Human Services, Washington, DC, 2002).
4. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals – Frequently Asked Questions*. Protocol Review Question No. 7. (US Department of Health and Human Services, Washington, DC, 2006; revised 2009).

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