

Surplus animals/CO₂ euthanasia - questions you may have

Animals are living beings which have to be treated with respect and for which we are responsible – that is the consensus within our society. We, as animal welfare officers, consider unnecessary killing unacceptable. Here in the first part, we aim to explain why more animals need to be bred than used in animal studies and what we do to reduce their number. In the second part, we explain what happens to these animals.

Why do we need to breed animals for research?

To find new remedies to treat different human and animal diseases, animals with specific characteristics mimicking the investigated diseases are needed. This is achieved by using genetically modified animals, e.g. animals that develop diabetes. Today, thousands of animals with different genetic modifications exist for biomedical research.

Do the genetically modified animals suffer?

For the majority of animals, genetic modification does not have an impact on the animals' well-being or health. In fact, most of them cannot be distinguished from animals without genetic modifications. All laboratory animals are very closely monitored each day by specially trained and qualified animal caretakers and researchers and any change in well-being or health status is noticed.

However, for every new genetic modification, researchers have to demonstrate that animals are not suffering. As soon as an impaired well-being is noticed, the cantonal veterinary authorities must be notified. All breeding of constrained animal lines has to be justified and breeding of such animals needs to be approved by the veterinary authorities.

Why do we need to breed more animals than are used in animal studies?

The downside in the use of these genetically modified animals is that not all the offspring bear the desired characteristics and therefore not all can be used for a specific research. In some other cases, animals are not included in an experiment for other reasons, for example if only one sex is investigated. These animals are considered "surplus" animals.

Why is it in our interest to reduce the number of animals that are bred but not used?

Ethical considerations require us to reduce the number of animals used in research to an absolute minimum. Every researcher follows the fundamental principles of the <u>3Rs - Replace, Reduce, Refine</u>, which are implemented in the Swiss legislation, in research ethics and in our <u>Culture of Care</u>.

What can be done to reduce surplus animals to a minimum?

We aim at breeding as few animals as possible for an experiment by the following measures:

a. Breeding management

Case-specific schemes are developed according to current research projects. Therefore, state-of-theart knowledge on breeding strategies and continuous monitoring of strategies as well as a good communication between researchers and the animal facility management on breeding performance are inevitable to propagate only as many animals as needed. Online tools to select the best breeding strategy are used and continuously improved.

b. Targeted transgenic technologies to reduce breedings of complex genotypes

Emerging technologies of targeted genetic engineering (e.g. CRISPR/Cas9) provide the opportunity to more rapidly generate animals with a desired set of genetic alterations, rather than relying on multiple complex breeding schemes.

c. Cryopreservation

As in the case of human medically assisted reproduction, animal embryos or sperm can be frozen in liquid nitrogen and stored for many years in special facilities. Therefore, genetically modified animal lines that are currently not used in research can be cryopreserved. On this basis, they can easily be revived if living animals are needed again.

d. Connect researchers to share animals that are bred but not used

Specific online platforms have been developed to enable an exchange of animals or their organs among researchers. In Switzerland, the sharing platform "Animatch" (https://www.animatch.eu/) is offered by many research institutes or institute-specific sharing programs are available to optimize the use of each animal.

d. Rehoming

Animals can be rehomed, if they are healthy and the organs are not needed for scientific purposes. These animals can be placed with individuals or families and a new private home can be found. Some research institutions have developed specific programmes for this purpose and closely work together with animal welfare organisations. Animals which are genetically modified cannot be kept outside research institutes due to legal requirements on genetic engineering.

e. Use for training purposes

All persons that handle animals in research and conduct animal experiments need to follow a specific legally required basic education and continuous training in which they learn to use the latest methods that are respectful towards animal welfare. Some research projects require specific skills, e.g. surgical techniques, and researchers or veterinarians need to be well trained to perform medical interventions in an animal-friendly and scientifically robust manner. Like in human medicine, training is required by law and surplus animals can be used.

f. Feeding animals for zoological gardens

Zoos or falconries have a high demand for small rodents as feeding in their establishment. The use of surplus laboratory animals offers a good solution to meet this need and contributes to reducing the number of animals that are bred specifically for the purpose of feeding wild animals. Currently, only animals that are not genetically modified can be used for this purpose.

What happens with the surplus animals?

If animals cannot be used either for an experiment, training, rehomed or as feeding animals (see above), they are euthanized. This must be done with the minimal amount of anxiety, stress, suffering and/or pain for the animals.

Why is carbon dioxide (CO₂) used for euthanasia worldwide?

 CO_2 is widely used as it leads to unconsciousness (anaesthesia) quickly and ultimately to death. Mice and rats can be euthanized within their group and their home cage which significantly reduces the stress on them. There is no stress due to separation, new environment or immobilization for injection that could be caused by alternative methods. Furthermore, occupational safety and economical aspects are considered: CO_2 is safe to handle for the persons involved, easily available and technically easy to use.

What are the disadvantages of CO₂ for mice and rats? And why is it still used?

The major disadvantage of using CO_2 for euthanasia is that it irritates the airways which can induce anxiety, stress and pain for the animal. These effects can be reduced by using a controlled filling rate that is likely to have the lowest irritating effects and results in quick loss of consciousness. To date no practical and safe alternative method has been found. Sedation prior to the use of CO_2 causes stress to the animals as well and cannot be done on many animals at the same or within a short time. Alternative methods e.g. other gases are being investigated and hopefully will soon be successfully evaluated.