



H. GOLLEDGE

An easy way out?

Scientists say they gas mice and rats with carbon dioxide because it is humane. It's also simple, cheap and keeps their hands clean. **Emma Marris** analyses the final seconds of the lab rodents' life.

"One uses CO₂ to knock the animal out, and it takes a bit longer than you would like — about a minute," explains Abigail Witherden, a kidney researcher at Imperial College London describing the death of a mouse. Then, to be sure: "you break its neck."

This is Witherden's preferred method of execution. "You feel more brutal if you've got a wriggling, living creature and you break its neck," she says, "rather than something that is probably already dead. It is worthwhile considering that some of these things are done for the benefit of the people, not the mouse."

Somewhere, right now, a lab mouse or rat is meeting its end, almost certainly by being gassed with carbon dioxide. The gas has become the preferred method because it is easy, cheap, and untroubling for the researchers. The rodent appears to drift off to a peaceful death. But some scientists say that CO₂ could cause pain and distress, and that it might be better to use a pricier anaesthetic, or a manual manoeuvre called cervical dislocation that breaks the animal's neck.

Tens or perhaps hundreds of millions of laboratory mice and rats are killed each year. The exact number is unknown, as there is no

reporting requirement in the United States, probably the heaviest user. In the United Kingdom, roughly 2.4 million rodents were used in experiments in 2004, according to the Home Office, which oversees lab animals¹.

Big sleep

Their use is on the rise. "While researchers' needs for animal models are declining, the use of rodents is increasing because of transgenic models — knockout mice and so forth," says George Goodno, spokesman for the Foundation for Biological Research, a Washington DC-based lobby group for animal research. Scientists can now buy transgenic rodents to

order, and their commercial production involves culling those that have the wrong genes, are unhealthy or have become surplus inventory.

In typical CO₂ gassing, one or more rodents are placed in a chamber and CO₂ is gradually added. As the CO₂ level rises, it acts as an anaesthetic and eventually knocks the rodent out. Then the lack of oxygen kills it. Adding CO₂ slowly is intended to avoid the pain caused by high concentrations, when the gas dissolves on the mucous membranes and forms carbonic acid. The hope is that the animals are out cold by this stage.

In the United States, rodents are sometimes put into chambers that have been pre-filled with CO₂, which kills them within 40 seconds, but might be more painful. The United Kingdom bans this method. None of these methods is foolproof. The Office of Lab Animal Welfare at the US National Institutes of Health (NIH) in Bethesda, Maryland, gets occasional reports of animals that wake up after being removed from the chamber. It is to avoid this that many labs, including Witherden's, break the gassed animals' necks.

There are rules about how to kill a mouse or rat, but many of the details are left up to the lab. The major guidance documents on killing



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methods for researchers in the United States, Europe and Japan are all being revised; the use of CO₂ is likely to be a point of dispute.

In the United States, researchers using federal funds must follow the 2000 report of the American Veterinary Medical Association Panel on Euthanasia². This report, though, is less of a how-to guide than a summary of the pros and cons of various methods. A few are deemed unacceptable; others, including gassing with CO₂ alone, are ruled as acceptable, because they consistently result in a humane death. Cervical dislocation is ruled as "conditionally acceptable", partly because it takes some skill. The report also considers the effects of various methods on the researchers who kill the animals — but also says that "there are occasions, however, when what is perceived as aesthetic and what is most humane are in conflict".

This February, a group of 34 scientists and those in the lab-animal business gathered at the University of Newcastle upon Tyne, UK, to discuss the possibility that CO₂ is not the most humane killing method. Huw Golledge, a neurophysiologist at Newcastle who helped to organize the conference, says that his work has convinced him that, at least with rats, loss of consciousness can be assured before the CO₂ reaches painful levels, if one adds the gas at less than 30% per minute. But before they pass out, are the rodents gasping for breath and panicking?

Breathless

"There is a strong suspicion that CO₂ levels that in no way cause them pain do cause them distress," says Golledge. Given the choice, rats will rapidly get out of a chamber containing CO₂ concentrations of about 15% or more³ — far below the concentration needed to knock them senseless.

What convinced Golledge was the work of Robert Banzett at Harvard University, who studies the distress caused by breathlessness, or dyspnoea, the sensation that one cannot breathe properly. "He has tried to make animal models for his human research," says Golledge, "so we got him over. My opinion on CO₂ until this meeting was that if you did it right, it was okay. Having heard what Bob Banzett said about dyspnoea, I think we need to clear that up."

Kathleen Conlee of the Humane Society, an animal-welfare organization based in Washington DC, doesn't need any more evidence. She thinks killing rodents with CO₂ alone is cruel, and prefers an anaesthetic gas — or, in cases where the anaesthetic would interfere with post-mortem measurements, the guillotine⁴. "We say, if there is a small number of animals, and the person is well trained, use decapitation. If you are doing a large number, use halothane or isoflurane, and then blast them with however much CO₂ you want."

"I feel strongly about it personally," Conlee adds. "The number of animals is huge; the

evidence is here; the alternatives are here. It is a no-brainer."

But moves away from CO₂ face two barriers: researchers' comfort levels and economics. "It is so widely used, and so convenient, and people have used it for so long, that it is going to be very difficult to change it," says David Morton, head of the centre for biomedical ethics at the University of Birmingham, UK, who helped to write a scientific report for the European Commission on the topic. The commission will use the report when revising its directive on lab-animal welfare⁵.



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The report argued for the use of anaesthesia before CO₂, saying killing with CO₂ alone "should be phased out as soon as possible". But in large operations, setting up facilities for an extra anaesthetic would involve extra costs and regulations. Still, Morton believes it is worth pushing for: "The ethical mandate for me is that if you can show there is a welfare problem, then you have to do something about it."

Other researchers, though, feel that the literature on CO₂ is too limited and contradictory to start moving away from it. "There are differences of opinion on the use of CO₂, and much of this stems from the inconsistency in the literature. There is very little consistency of



Hands-on: some researchers advocate cervical dislocation to ensure that gassed mice are dead.

methodology in these papers," says B. Taylor Bennett, associate vice-chancellor for research resources at the University of Chicago. "We need research done, but there are very few sources of funds for this type of research."

A good death?

Meanwhile, many researchers remain comfortable with using CO₂ alone. "If it is done properly, I don't have any problems at all using CO₂," says cell biologist Claire Pollock, who, when she began killing mice at the NIH's National Cancer Institute in Bethesda, allayed her fears by researching the science on the subject and throwing herself into her training courses. "I can't stress enough that any technique is only as good as the researcher," she adds. "I would much rather be put into a CO₂ chamber than be put into the hands of someone who doesn't know what they are doing for cervical dislocation."

Indeed, many researchers use their own intuition to decide what makes a humane death, perhaps because lab rodents can't tell us what they are feeling. And even in aversion studies, rodent behaviour can be hard to interpret. Golledge, for example, says he would prefer slowly rising CO₂ over a dive into a pre-filled chamber. Robert Banzett reportedly says he would make the opposite choice. Likewise, alternatives to CO₂, such as carbon monoxide, are discussed with analogy to humans. Inhaling carbon monoxide is a common method of suicide, largely because it is said to be painless. "And," points out Golledge, "you hear about people who have a malfunctioning gas boiler, and they just fall asleep [and die] in their armchairs." Another possibility is argon gas, to which lab rats seem less averse³. Argon is popular in the poultry industry and wins support even from animal-welfare campaigners. "It should be looked into," says Conlee. "Sometimes we hear about concerns with the anaesthetic gas [affecting] personnel, and that wouldn't be a concern with argon."

In any case, all of these lab techniques are undoubtedly more humane than most domestic methods of killing unwanted rodents, from snap traps that sometimes merely paralyze, to sticky traps that capture the mouse, requiring some kind of blunt instrument or drowning. "No matter what you do, it is going to be more humane than at home," says Witherden. ■

Emma Marris reports for *Nature* from Washington DC.

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