

Providing animal technicians with the latest news from the NC3Rs

# Tech3Rs

Welcome to the latest edition of Tech3Rs. In each issue, we share updates on recent advances in the 3Rs and highlight new resources, research and events.

This newsletter is for animal technicians working in research establishments to help identify opportunities to embed the 3Rs in practice and ensure high standards of animal welfare. If you have any ideas for future issues or are working on a 3Rs approach you would like us to feature, please get in touch – we would love to hear from you! You can email us at [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk).



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Don't miss the  
next issue!

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## Zebrafish skin swabbing

We have collaborated with experts to create an online resource on a refined method for collecting DNA from fish.

Zebrafish are common experimental models, and DNA is routinely collected from these animals to determine their genetic makeup (genotyping). The current standard procedure for this is fin clipping, which involves placing the fish under non-terminal anaesthesia and removing a portion of the caudal fin with a scalpel, but this can impact their health and welfare.

Instead of fin clipping, a swab (such as a cotton bud) can be used to collect mucus from the flank of the fish, which can then be processed to extract DNA. Zebrafish that are swabbed rather than fin clipped are less stressed after the procedure and placing the fish under anaesthesia is not necessary, further reducing the welfare impact. The technique has also been validated for stickleback and may be suitable for other small bony fishes.

Skin swabbing has been gaining interest as a refined method for DNA collection, and a growing number of researchers and technicians are asking whether swabbing is appropriate for their needs and how to perform the technique correctly. We have created a resource containing information on skin swabbing vs. fin clipping, and how to establish a skin swabbing protocol.

The resource includes frequently asked questions answered by experts in zebrafish welfare. Questions include how fin clipping and skin swabbing compare as methods for DNA collection, and how fish should be restrained during the swabbing procedure. If your facility is not ready to begin skin swabbing, the resource also contains information on other potential refinements to the zebrafish genotyping procedure.

**To learn more about zebrafish skin swabbing as a refinement, and for useful tips on trialling this technique in your facility, visit [www.nc3rs.org.uk/zebrafish-swabbing](http://www.nc3rs.org.uk/zebrafish-swabbing).**

**To read the original research on refining DNA collection for small laboratory fishes, please see: Tilley CA *et al.* (2020). Skin swabbing is a refined technique to collect DNA from model fish species. *Scientific Reports* 10: 18212. doi: [10.1038/s41598-020-75304-1](https://doi.org/10.1038/s41598-020-75304-1)**

**If you have trialled skin swabbing, or you currently use this technique, we would love to hear more about your experiences. Please email us at [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk).**

# 3Rs champions

We want to help you share your ideas for putting the 3Rs into practice. In this issue of Tech3Rs we feature two technicians who are championing the 3Rs at their establishments.

**Lucy Onions is a senior *in vivo* support technician and NACWO at the University of Leicester. She spoke to us about habituating mice prior to export.**

## What 3Rs idea have you developed?

The preferred method of transferring mouse strains between establishments is to transfer frozen embryos and sperm (see [www.nc3rs.org.uk/sharingarchiving](http://www.nc3rs.org.uk/sharingarchiving)). If this isn't possible the only option is to transport live animals.

Transport can be a stressful experience for mice, and this is especially the case for longer journeys. Over the past two years my colleagues and I have been refining the way we prepare our mice for export. We now spend a week preparing them before they are transported.

## How did you develop this project?

Historically mice would be put into a new, unused transport box with hydrogel. However, we now know how important smell is to mice and we also know that they are fearful of novelty. This combination means that a new transport box is an alien environment to a mouse and being placed into one is a stressful experience.

Mice are transported socially to reduce stress, and travel in the same compartment as familiar cage mates. However, sometimes the double-sided transport box will contain unfamiliar mice in the other compartment, which is a

potential source of stress. To address this, we now house the mice that will be transported together in a segregated double-decker cage for a week before transport, so they can become accustomed to the presence and smell of their travel companions.

We also use this period to habituate the mice to the food that will be available during transport, providing them with floor food, soaked diet and hydrogel. Exposing the mice to the different diets beforehand also allows us to spot individual preferences. Some mice completely ignore the hydrogel; in these cases, we make sure they have more than enough soaked diet for the journey.

During the seven-day process, we habituate the mice to being transferred and contained within the transport box. On day three we transfer the mice into the box that they will travel in, along with used nesting material and a variety of diet. We allow them to explore the new environment for thirty minutes, before returning them to their double-decker cage. We leave the used nesting material in the transport box so that when we repeat the process two days later the box already contains the scent of the mice.

When the mice go into the transport box for the first time they are unsettled – they scurry and scuddle. When we repeat the process, the mice are notably calmer. On the day of transit, we transfer the mice to the transport box an hour before travel,

with all their home-cage contents. This includes their cardboard enrichment items, such as tunnels or shelters, so the mice can take refuge or shred the cardboard during transport. Facilitating these species-specific behaviours is another way to improve the wellbeing of mice.

## How has this project impacted you?

If my mice are stressed, I am stressed, so transporting animals is a tense time. I can't relax until I know they have arrived safely. I will always worry, but knowing that they have the best environment that we can give them and we have done our best to prepare them for the journey provides some reassurance.

**Chago Bowers is an animal health technician at the University of Chicago. He spoke to us about switching from using sentinel mice to environment monitoring for health surveillance.**

## What 3Rs idea have you developed?

Previously, soiled-bedding sentinels were used to monitor the health of our mouse colonies, including the animals in our rodent quarantine program. Mice were ordered in specifically to be sentinels; they would be group-housed, exposed to soiled bedding from colony animals and culled for sampling at predetermined timepoints.

I have taken the technical lead on making the switch from using sentinels to instead adding collection media to exhaust air ducts. The collection media accumulates environmental samples in the form of dust. I then send these samples for laboratory analysis to detect pathogens. This has almost entirely replaced the need for sentinel mice, saving 1,676 mice annually.

## How did you develop this project?

We were asked to take part in a trial by our rack supplier and my manager approached me to coordinate this. We started with a few racks and it grew from there. A big part of my role was thinking about how this would work in a practical sense, and the



Pairs of mice inside a double-sided transport box prepared for transfer



Chago holds collection media placed inside a holder that fits in the exhaust plenum of an IVC rack

overall logistics of the new approach. I had to consider our new schedule for sampling and washing racks. I also had to think about the ergonomics of fitting and accessing the collection media; this turned out to not be too challenging with the help of some stools. It became evident early on that this method was superior in terms of sensitivity, cost and, after the initial set up, our time – this naturally led us to roll it out across the rest of our program.

#### How has this project impacted you?

I have been involved with some publications on this topic, but just being part of making the change happen has been rewarding. The major impact for me has been the reduction in number of mice that we are required to cull for health monitoring. It was always stressful on the days we had to collect the sentinel mice for tissue sampling. I have been working as a technician for over twenty years and that all adds up. Collecting exhaust air dust samples has been such a refreshing change and the switch has improved the quality of my work life.

**Chago encourages anyone concerned about the cost of switching to read the University of Chicago's study: Luchins KR *et al.* (2020). Cost comparison of rodent soiled bedding sentinel and exhaust air dust health-monitoring programs. JAALAS 59(5): 508-511. doi: 10.30802/AALAS-JAALAS-20-000003**

Visit [www.na3rsc.org/health-monitoring](http://www.na3rsc.org/health-monitoring) to learn more about environmental monitoring for rodent health surveillance, including the recording of an NA3RsC/ NC3Rs webinar on the topic.

**Would you like to be featured in our next issue, or find out more about the refinements featured above? Please email [tech3rs@nc3rs.org.uk](mailto:tech3rs@nc3rs.org.uk).**

# Spotlight on the new NC3Rs website

Our website has a fresh new design and we have made improvements to how you search for relevant information. You can now filter content by your job role and the species or model you are interested in. You can also narrow down your search to focus on the type of information you are looking for, such as guidance on husbandry, or e-learning modules on how to refine procedures. We hope these changes will help you find the most useful information as quickly as possible when you visit us online. Two of our newest resources are highlighted below.

**For more information on how our website has changed and to leave us feedback, visit [www.nc3rs.org.uk/newwebsite](http://www.nc3rs.org.uk/newwebsite).**



## Reducing aggression in group-housed male mice

Within a research environment, mice are exposed to husbandry conditions that can disrupt social stability and limit the ability of subordinate animals to escape from aggressors. This can lead to serious aggression-related welfare issues including stress, injury and even death. However, removing the aggressor or subordinate from the cage can cause further social disruption, and single housing of mice also presents a welfare concern.

We have created a new resource that provides recommendations for reducing aggression between group-housed male mice. These recommendations are based on peer-reviewed literature and the findings of an international crowdsourcing project led by the NC3Rs, which obtained data from 137,580 male mice at 44 UK facilities with the help of animal technicians.

**For more information on how you can reduce aggression without resorting to single housing, visit [www.nc3rs.org.uk/mouseaggression](http://www.nc3rs.org.uk/mouseaggression).**



Preclinical Research Facility, University of Leicester

## Playpens for rats

Our new web resource on rat playpens answers common questions and provides practical tips for setting up a rat playpen in your facility.

A playpen is an enriched space provided as an addition to a standard cage, allowing animals to exercise and engage with a wider range of environmental enrichment. Access to a larger, more structurally complex enclosure allows rats to perform behaviours important for their health and welfare, such as stretching upright, climbing and digging.

Playpen sessions have been shown to increase indicators of positive affective state (mood) in rats and reduce the impact of a negative experience. Other potential benefits of providing rats with playpens include reducing handling-associated stress, facilitating habituation to new objects and improving technicians' job satisfaction.

**Visit [www.nc3rs.org.uk/ratplaypens](http://www.nc3rs.org.uk/ratplaypens) to explore the resource, or read our news story on new research into the welfare benefits of playpens for rats at [www.nc3rs.org.uk/playpenstudy](http://www.nc3rs.org.uk/playpenstudy).**

# Highlights from the NC3Rs and beyond

## Non-aversive handling: the evidence base

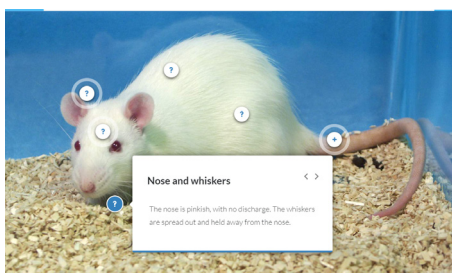
In 2010, a study led by Professor Jane Hurst showed that picking up mice by the tail results in aversion to the handler and high anxiety. The same study demonstrated two refined, non-aversive handling methods: picking mice up using a tunnel or cupped hands.

Since Professor Hurst's initial work, which won the 2010 NC3Rs International 3Rs Prize, the evidence base that non-aversive handling techniques improve mouse welfare has grown considerably, with over 20 studies now published from research groups around the world. At AALAS 2021, Dr Mark Prescott (NC3Rs) gave a presentation summarising the evidence base for improved animal welfare and scientific outcomes when non-aversive handling methods are used to pick up mice.

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**This thirty-minute presentation is now available to watch online:**  
[www.nc3rs.org.uk/MHevidence](http://www.nc3rs.org.uk/MHevidence).

**If your facility has yet to make the switch to non-aversive handling methods, visit the NC3Rs mouse handling resource for practical tips and testimonials from technicians who have implemented this refinement:**  
[www.nc3rs.org.uk/mousehandling](http://www.nc3rs.org.uk/mousehandling).



## New e-learning modules on pain in research animals

Two new e-learning modules on post-operative pain assessment and pain alleviation have been developed by Emeritus Professor Paul Flecknell of Research Animal Training with the support of the NC3Rs.

Module EU21.7 provides detailed practical information on choosing and using analgesics, including details on different types of analgesics, routes of administration, doses and potential adverse effects. The module also describes issues you might encounter when managing pain following surgery.

Module EU21.8 focuses on how to assess post-operative pain, with images and videos demonstrating rodents experiencing different levels of pain to help you spot the signs. The module describes some of the problems associated with pain recognition and management in animals and gives suggestions to help overcome these. There is also an opportunity for you to assess and score pain levels and receive feedback on your performance.

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**Visit [module.researchanimaltraining.com/nc3rs](http://module.researchanimaltraining.com/nc3rs) to access the courses and learn more about post-operative pain assessment and pain management.**



## Harmonising welfare assessment for non-human primates

It is essential to accurately assess the welfare of non-human primates used and bred for research, but there is little consensus on what should be measured. To harmonise views on which welfare indicators for macaques and marmosets are reliable, valid and feasible in a laboratory context, Drs Mark Prescott (NC3Rs), Matthew Leach (Newcastle University) and Melissa Truelove (Yerkes National Primate Research Center) organised a Delphi consultation of researchers, veterinarians and animal technicians at the 2019 NC3Rs Primate Welfare Meeting. The results are now published in *F1000Research*.

For macaques, self-harm behaviour, social enrichment, cage dimensions, body weight, a health monitoring programme, appetite, staff training and positive reinforcement training were considered key welfare indicators. By combining these results with those of an earlier expert consultation, the authors have developed a new welfare assessment protocol to support those working with macaques in assessing, monitoring and maximising their health and wellbeing. The GEN-MAC protocol is intended for a generalised assessment of wellbeing that can inform and augment existing tools tailored to specific projects, procedures and adverse effects.

The authors encourage all laboratories with macaques to trial the GEN-MAC protocol and to provide feedback so they can validate and enhance this new tool for the benefit of the non-human primate research community.

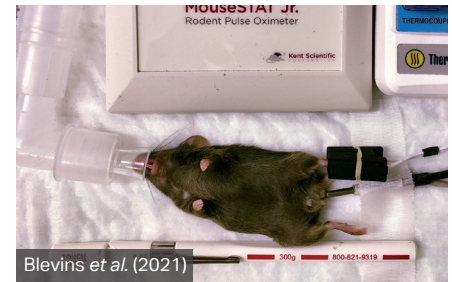
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**Prescott MJ et al. (2022). Harmonisation of welfare indicators for macaques and marmosets used or bred for research. *F1000Research* 11: 272. doi: [10.12688/f1000research.109380.1](https://doi.org/10.12688/f1000research.109380.1)**

**Download the GEN-MAC protocol as an Excel spreadsheet: doi: [10.25405/data.ncl.19106960](https://doi.org/10.25405/data.ncl.19106960)**

# 3Rs papers of interest

Each issue we feature recent 3Rs papers, providing summaries and links to the full articles for further reading. This issue we look at skin preparation before surgery and perioperative support for mice and rats.



## Evaluation of 3 alcohol-based agents for presurgical skin preparation in mice

- This study compared the performance of three commercially available antiseptic agents to that of alcohol and povidone-iodine, investigating reduction in bacterial population of mouse skin, as well as skin irritation or impaired healing, when these compounds were applied before surgery.
- The hair-clipped skin of euthanised C57BL/6J mice was treated with Duraprep™, Chloraprep™, Hibiclens®, or alcohol and povidone-iodine. Skin swabs were obtained before, immediately after, and 20 minutes after skin preparation. All antiseptic agents were equally effective at reducing bacterial populations immediately and after 20 minutes.
- Skin irritation and wound healing were assessed in Balb/c mice that underwent recovery surgery following skin preparation with one of the four agents. Wound healing was not affected by the choice of antiseptic agent and there was no evidence of skin irritation in any of the groups.
- These findings indicate that all three alcohol-based preparation agents are as effective as povidone-iodine and alcohol for the antiseptic preparation of mice. These alternatives are easier and faster to apply, which could encourage higher uptake of proper skin sterilisation before mouse surgery.

Huss MK *et al.* (2021). *JAALAS* 59(1): 67–73. doi: [10.30802/AALAS-JAALAS-19-000053](https://doi.org/10.30802/AALAS-JAALAS-19-000053)

## Using waterless alcohol-based antiseptic for skin preparation and active thermal support in laboratory rats

- This study aimed to confirm the efficacy of a commercially available waterless alcohol-based antiseptic (Avagard™) for skin disinfection prior to rat surgery, and explore whether prewarming can improve perioperative body temperatures for *SD-Bdnf<sup>em1+/-</sup>* rats to aid recovery from anaesthesia.
- Skin swabs were collected before and after antiseptic treatment and after surgery. Analysis of the swabs showed bacterial counts were effectively diminished following treatment with the antiseptic, which means that the waterless alcohol-based solution is effective at creating an aseptic environment on rat skin.
- Prewarming of the rats was achieved in three ways: warm-water blanket under the rat's cage to warm the cage, warmed cage plus intraperitoneal administration of warmed saline, or warmed saline only. The control group didn't receive any of the above prewarming interventions.
- None of the prewarming treatments significantly affected body temperature before or during surgery, or improved anaesthetic recovery times in rats. The researchers also observed that the use of a heated surface during surgery can thermally support rats, avoiding the need for additional interventions such as warm fluid administration.

Hankenson FC *et al.* (2021). *JAALAS* 60(3): 365–73. doi: [10.30802/AALAS-JAALAS-20-000128](https://doi.org/10.30802/AALAS-JAALAS-20-000128)

## Effects of oxygen supplementation on injectable and inhalant anaesthesia in C57BL/6 mice

- This study looked at how oxygen supplementation affected physiological parameters and depth of anaesthesia in C57BL/6 mice anaesthetised with low, medium or high doses of injectable anaesthetics or isoflurane.
- Mice anaesthetised via injection received either 100% oxygen supplementation or nothing. Mice that did not receive supplemental oxygen were significantly hypoxic, as shown by their low peripheral oxygen saturation levels.
- Oxygen supplementation significantly shortened the time during which deep anaesthesia was maintained following injection. At medium anaesthetic dose, oxygen supplementation significantly reduced respiratory rate (hypoxia can lead to increased respiratory rate).
- Mice anaesthetised with isoflurane breathed supplemental 100% oxygen, room air, or medical air with 21% oxygen. All mice that breathed room air were hypoxic during anaesthesia. The amount of isoflurane required to achieve anaesthesia was not affected by the type of air breathed.
- These results suggest that mice anaesthetised either via injection or with isoflurane are routinely hypoxic. Oxygen supplementation is recommended for all anaesthetised mice, particularly when high doses are given.

Blevins CE *et al.* (2021). *JAALAS* 60(3): 289–97. doi: [10.30802/AALAS-JAALAS-20-000143](https://doi.org/10.30802/AALAS-JAALAS-20-000143)

Visit [www.nc3rs.org.uk/rodentsurgerywebinar](https://www.nc3rs.org.uk/rodentsurgerywebinar) to watch a recording of an NC3Rs webinar on good surgical technique and other refinements to incorporate into rodent stereotactic surgery procedures. More information on anaesthesia can be found at [www.nc3rs.org.uk/anaesthesia](https://www.nc3rs.org.uk/anaesthesia), including links to freely available NC3Rs-funded e-learning modules on anaesthesia.

# Upcoming events



## IAT Congress 2022

**Tuesday 29 March – Friday 1 April**  
**Harrogate, UK**

The 2022 Congress of the Institute of Animal Technology (IAT) will feature workshops and presentations covering a range of topics, including improving the welfare of ageing mice, environmental enrichment for zebrafish, and the evolution of the rat playroom.

We will be running an in-person workshop on managing aggression in laboratory animal species at 11:00 – 12:30 on Wednesday 30 and Thursday 31 March. Join us for tips on reducing and dealing with aggression in mice, rats, rabbits, dogs, pigs and macaques. Delegates can now book onto workshops using the details emailed by the IAT Congress Committee ([website@iat.org.uk](mailto:website@iat.org.uk)).

Visit [eu.eventscloud.com/website/4554/](http://eu.eventscloud.com/website/4554/) to learn more and register before Monday 21 March.



## FELASA Congress 2022

**Monday 13 – Thursday 16 June**  
**Marseille, France**

The 2022 Congress of the Federation of European Laboratory Animal Science Associations (FELASA) will focus on the central theme of communication. Sessions, workshops and keynote speakers will focus on the topics of communication with and between animals; communication and the culture of care; and communication between colleagues, trainers and trainees.

If you do not wish to attend the event in-person, FELASA offers the option of virtual attendance through the online congress platform.

Find more information, including the preliminary programme and details on how to register, at [www.felasa2022.eu](http://www.felasa2022.eu).



## UFAW International Conference 2022

**Tuesday 28 – Wednesday 29 June**  
**Edinburgh, UK**

The Universities Federation for Animal Welfare (UFAW) invite in-person attendees to network and socialise face-to-face at their International Conference 2022. For those who do not wish to travel, the talks will also be streamed live online, though the workshops will not.

The conference will include topics such as the use and misuse of measures of welfare, new methods to assess or improve welfare, and how to improve the quality of animal welfare science.

Early registration closes on Thursday 31 March. More information can be found at [www.ufaw.org.uk/ufaw-events/advancing-animal-welfare-science-2022](http://www.ufaw.org.uk/ufaw-events/advancing-animal-welfare-science-2022).

## Apply now for the AAALAC International Fellowship Award

Applications are now open for IAT- and AALAS/CALAS-registered animal technicians to apply for the AAALAC International Fellowship Award. Every year the fellowship recognises two outstanding individuals who have made, or have the potential to make, significant contributions to the field of laboratory animal care and use.



Past winners of the award include John Waters and Stephen Woodley, who shared details of their inspiring careers in animal technology in previous issues of Tech3Rs (Issue 7 and Issue 11 respectively, both available to read at [www.nc3rs.org.uk/tech3rs](http://www.nc3rs.org.uk/tech3rs)).

The deadline to apply for IAT-registered technicians is **Wednesday 1 June**.

To find out more about the award, including key dates and requirements, visit [www.aaalac.org/awards/fellowship-award](http://www.aaalac.org/awards/fellowship-award).