Refining the welfare of immunocompromised mice receiving carbon tetrachloride (CCl_4) to induce liver fibrosis

VICKY WALCZAK-GILLIES[1,2], OLIVIA MATTHEWS[2], ALEX FYFE[2], ANNA HOY[2] and PHILLIP STARKEY LEWIS[2]

[1] Bioresearch & Veterinary Services, The University of Edinburgh
[2] In Vivo Pharmacology, Resolution Therapeutics, Centre for Regenerative Medicine, Edinburgh

Correspondence: vicky.walczak-gillies@ed.ac.uk and vicky.walczak-gillies@resolution-tx.com

Introduction

Carbon Tetrachloride (CCl_4) is a highly toxic substance. When administered consecutively at a low dose CCl_4 can model liver fibrosis in rodents and be used in the assessment of new anti-fibrotic therapies. RTx use immunocompromised mice which have a lower tolerance to CCl_4 than wild type mice.

We will discuss how we refined the care of the immunocompromised mice following each dose of CCl_4.

The mouse strains that were used are
- NSG – NOD.Cg-PrkdcSCIDIl2rgtm1Wjl/SzJ
- NOD-SCID – NOD.CB17-Prkdcscid/NcrCrl

Which were sourced from Charles River (UK).

The experiments were conducted in compliance with The Animals (Scientific Procedures) Act 1986 (ASPA). Mice were housed under standard conditions of 12:12 hour light-dark cycle, temperature 21 +/-2°C, with free access to chow (Irradiated RM3 and sterilised water) in sterilised individually ventilated cages (IVCs).

Method

Mice were administered CCl_4 using the intraperitoneal (IP) injection route twice weekly. All of the mice had their bodyweight (BW) monitored and were checked for any signs of ill health before each injection.

We reference the dosing criteria (Table 1) to determine when each mouse will receive a full dose, a half dose or if they will miss a dose. CCl_4 is diluted 1:4 with olive oil under sterile conditions. All humane endpoints are as described in the specific Project Licence that the work is being carried out under.

CONDITIONS TO TRIGGER A HALF-DOSE	
1	Last three weeks of the protocol
2	Recent anesthetic exposure
3	Losing 0.5g BW in two successive weighings
4	Losing 10% BW in a two-week period
5	If all mice in a cage lose 0.5g BW simultaneously
CONDITIONS TO MISS A DOSE	
1	An animal loses > 20% BW, allow 48 hours recovery and proceed

Table 1. Original dosing criteria.

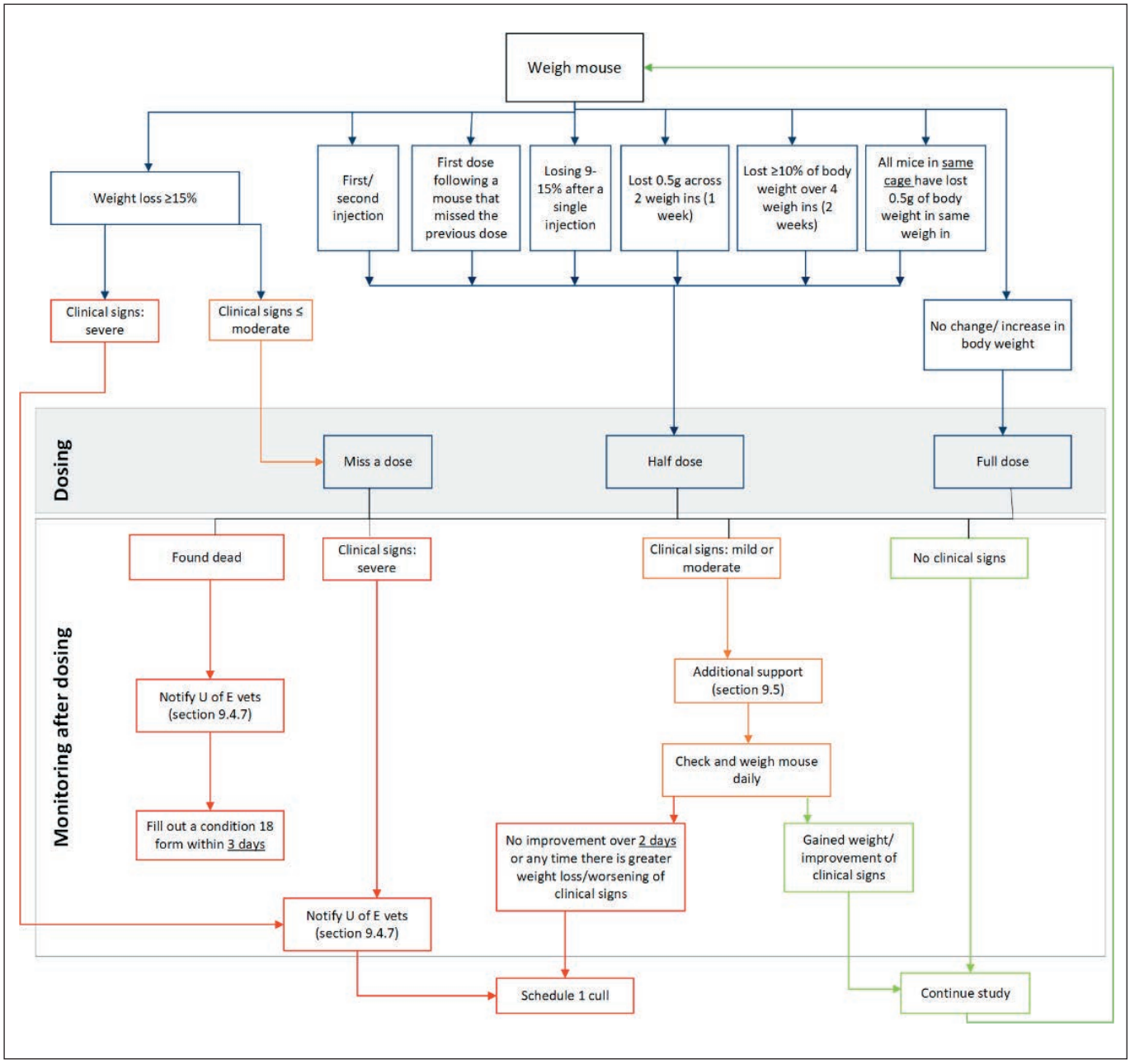

Figure 1. New dosing criteria action tree.

In addition, all mice under new dosing criteria:

- Must be > 23g bodyweight at the start of experiment.
- Have access to a gel pot post injection to increase hydration.
- Placed into a heating cabinet (26°C) for up to 5hrs post-injection.
- Are checked regularly throughout the day.
- Scored by body condition[1] and the NC3Rs' grimace scale.[2]

Results

Our method provides evidence that we can alter CCl_4 dosing and implement recovery techniques to enhance Animal Welfare, whilst maintaining sufficient levels of fibrosis in the liver.

Consequently, this has improved both Animal Welfare and scientific outputs, which in turn reduces the need to repeat studies and the overall number of animals required.

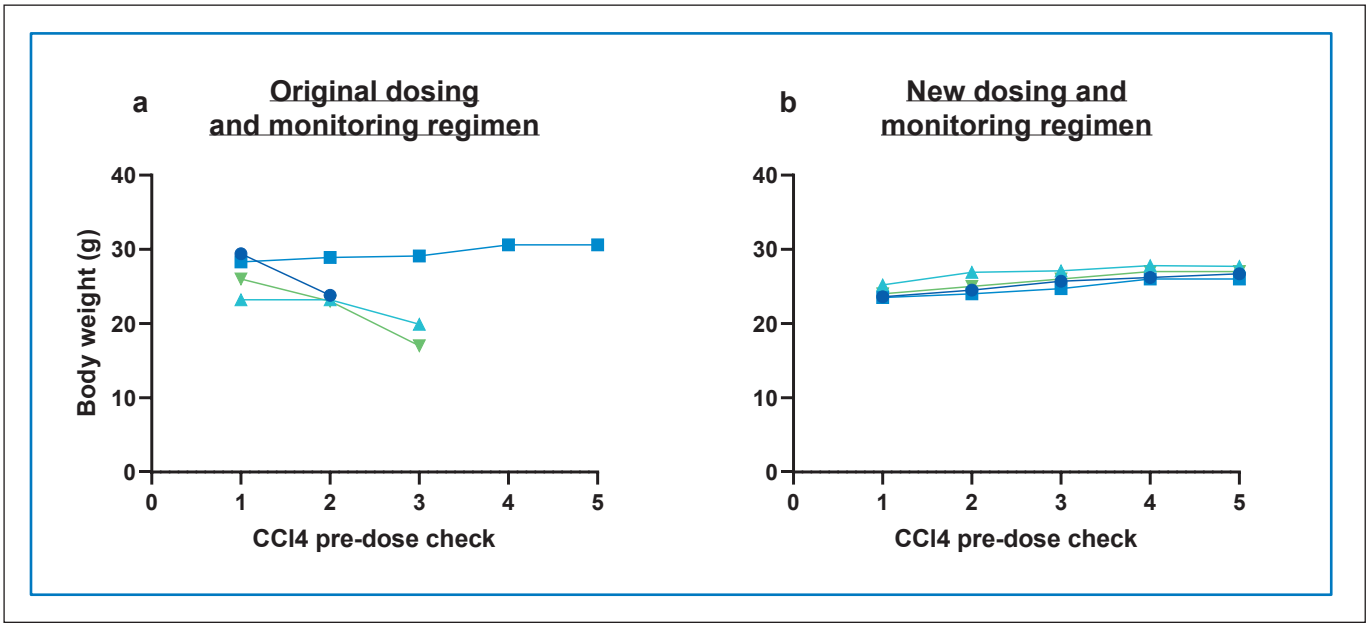

Table 2. Difference between the original and new dosing regimen.

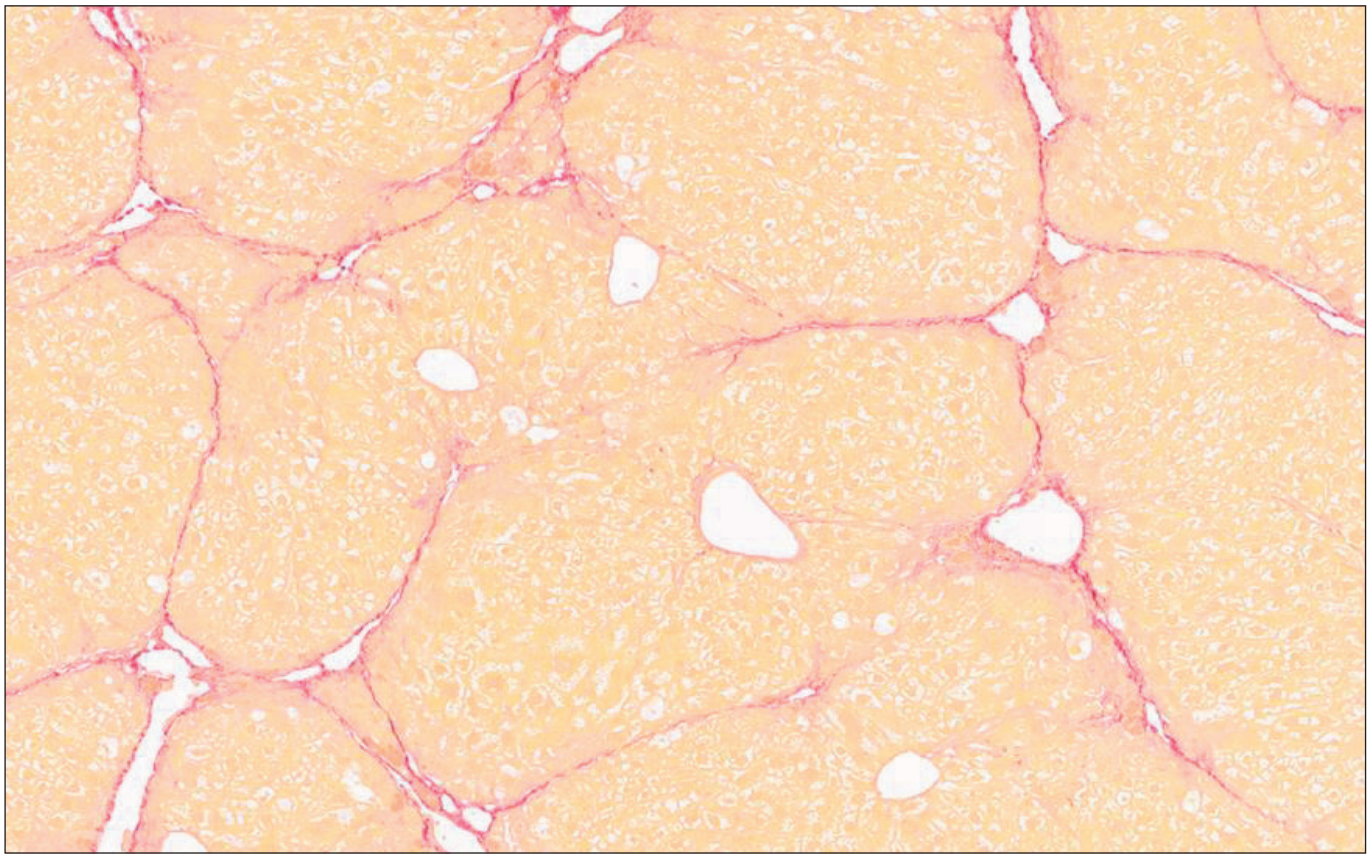

Figure 2. Representative image of PSR stained liver from a NOD-SCID mouse following a 12-week CCl_4 regimen.

Conclusion

We have successfully refined the care and welfare of the animals without compromising the science. This method can easily be adapted for any study.

The smallest actions can have the biggest impact.

References

1 **Burkholder, Tanya., *et al.*** Health Evaluation of Experimental Laboratory Mice. Current protocols in mouse biology vol. 2 (2012): 145-165. doi:10.1002/9780470942390.mo110217.

2 **Langford, Dale J., *et al.*** Coding of facial expressions of pain in the laboratory mouse. Nature methods vol. 7,6 (2010): 447-9. doi:10.1038/nmeth.1455.