University of Wollongong Animal Ethics Committee

Position Statement on Intraperitoneal Injections in Rodents

Background

Intraperitoneal (IP) injection is used widely in research involving laboratory rats and mice. Advantages include rapid absorption of substances, suitability for administering relatively large volumes, and technical simplicity when compared to the intravenous route.

At the same time, however, there is considerable evidence to show that IP injection is an unreliable route for administration of substances in rodents.

Articles in the peer-review literature have reported that instead of being deposited in the peritoneal cavity, substances are often deposited (either fully or partially) into unintended sites such as the caecum, abdominal fat or subcutaneous tissues. This is often referred to as misinjection. The following table provides a summary of some reported misinjection rates.

Misinjection rate	Species	Reference (full details attached)
14%	Mice	Steward et al (1968)
40% to 100%	Mice	Walwoort (1991)
10% to 20%	N/S	Claassen (1994)
17%	Mice	Gaines Das and North (2007)
6%	Rats	Coria-Avila et al (2007)
17%	Rats	Zatroch et al (2017)
7% to 17%	Mice	Ballard (2009)
13% to 59%	Rats	Ballard (2009)
3% to 10%	Mice	Inoue et al (2009) + 3 cited refs
5% to 16%	Mice	Wokke (2017)

Due to concerns over the reliability of the IP route, UOW conducted a pilot study in an attempt to identify factors that might reduce the misinjection rate. Unfortunately the large number of variables did not allow firm conclusions to be drawn on how the technique could be refined, although it was found that misinjection occurred in all cohorts with an overall rate of 24% (see attached summary).

While the problem of misinjection is not always mentioned in animal research guidelines, it is occasionally highlighted. Training resources linked to the UK's National Centre for the 3Rs website, for example, state "…this is an inherently unreliable technique, since inadvertent injection of some material into the gut, abdominal fat and subcutaneous tissues is a relatively frequent occurrence."[1]

Probably the most common site for misinjection is the caecum. While it is sometimes recommended that injections should be made into the right hand side of the abdomen to avoid this, studies have shown that the position of the caecum is variable in both rats and mice: it may be located on the left hand side in perhaps 30% of animals, and it can also extend across both sides.

The unreliability of the IP route is of particular concern in situations where even a single misinjection can have a major impact on outcomes. This can apply, for example, when animals are undergoing sensitisation or immunisation, or in procedures involving administration of cells, micro-organisms or imaging agents such as luciferin. Misinjection of substances such as analgesics, anaesthetics or antibiotics also raises animal welfare concerns.

[1] http://www.procedureswithcare.org.uk/intraperitoneal-injection-in-the-rat/ and http://www.procedureswithcare.org.uk/intraperitoneal-injection-in-the-mouse/

Although it is sometimes suggested that withdrawing the plunger of the syringe to test for aspiration of gut contents can be used to determine whether the needle has entered the bowel, simple evaluation of this technique using post mortem specimens shows that it does not work because the consistency of the gut contents prevents it being drawn into the needle.

Aside from the problem of misinjection, the IP route raises a number of other concerns, especially:

- Adverse effects on animal welfare. The technique is likely to be painful if the needle injures an abdominal organ or if the substance being injected is irritant to the surfaces of abdominal viscera. Anecdotal experience has also suggested that rodents show increasing aversion to IP injection when subjected to it repeatedly.
- Potential complications from IP injection, although probably uncommon, may include sepsis from bowel puncture, unobserved haemorrhage, visceral adhesions, and sequestration of injected substances in granulomatous inflammation (especially if oil-based).
- Pharmacological relevance must also be considered. The IP route is seldom used in human medicine so its use in animals may reduce the utility of animal models. Furthermore, substances absorbed from the peritoneal cavity will enter the portal circulation and may therefore be altered by the liver before reaching the target organ.

Conclusion

Based on these lines of evidence, the AEC believes that IP injection should only be used when a strong case can be made that other routes of administration are unsuitable.

Applications to use IP injection should therefore show that careful consideration has been given to:

- An explanation of why other routes are unsuitable. Wherever possible, this should be supported by peer-reviewed literature.
- Quantitative validation to show that the substance being administered by the IP route is achieving the required level systemically.
- Statistical methods that take into account a possible misinjection rate of at least 15%.
- Training procedures that directly evaluate the misinjection rate of the operators (e.g. IP injection of dye followed by post mortem examination with a consistent high success rate).
- Post mortem examination at the conclusion of the study to check for evidence of complications.
- Engaging staff who hold current competency documentation to administer substances by a more reliable route if no members of the research team have the relevant training.

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References

1. Publications cited on IP injection in rodents.

Misinjection rate	Species	Reference
14%	Mice	Steward et al (1968) Errors in the technique of intraperitoneal injection in mice. Appl Microbiol 16(9) pp 1418-9
40% to 100%[a]	Mice	Walwoort HC (1991) Assessment of distress through pathological examination. In: Animals in Biomedical Research: Replacement, Reduction and Refinement: Present Possibilities and Future Prospects (Hendriksen CFM, Koeter HBWM, eds). Amsterdam: Elsevier, 265–71. [Cited by Gaines Das and North – see below]
10% to 20%	Not stated	Claassen V (1994) Neglected factors in pharmacology and neuroscience research. In: Techniques in the Behavioral and Neural Sciences. Vol. 12. (Huston JP, ed). Amsterdam: Elsevier. [Cited by Gaines Das and North – see below]
17%	Mice	Gaines Das, R and North, D (2007) Implications of experimental technique for analysis and interpretation of data from animal experiments: outliers and increased variability resulting from failure of intraperitoneal injection procedures. Lab Animals 41: 312–320
6%[b]	Rats	Coria-Avila et al (2007) Cecum location in rats and the implications for intraperitoneal injections. Lab Anim (NY) 36(7):25-30
17%	Rats	Zatroch et al (2017) Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats. BMC Vet Res 13:60 pp1-7. DOI 10.1186/s12917-017-0982-y
7% to 17%[c]	Mice	Ballard (2009) Intraperitoneal route of administration - how accurate is this technique? An Tech and Welf. 8(1) pp.17-18
13% to 59%[c]	Rats	Ibid.
3% to 10%	Mice	Inoue et al (2009) Comparison of subcutaneous and intraperitoneal injection of D-luciferin for in vivo bioluminescence imaging. Eur J Nucl Med Mol Imaging (2009) 36:771–779. DOI 10.1007/s00259-008-1022-8. Also 3 cited references.
5% to 16%	Mice	Wokke, E (2017) Refinement: Evaluating stress and accuracy of different intraperitoneal injection techniques in mice. Thesis.

[a] n = 25

[b] Misinjection was only assessed by visual examination for injury to abdominal organs.

[c] First figure is injection on right hand side, second figure is injection on left hand side. All injections conducted after death.

2. Summary of study into reducing the IP misinjection rate.

The study was led by the UOW Animal Welfare Officer Dr Sarah Toole under Animal Ethics approval no. AE1432r17A20. C57BL/6 mice that were surplus from another institution were injected by the IP route with a blue dye then humanely killed and a necropsy conducted immediately to observe the location of the dye.

The misinjection rate was recorded for 6 operators of varying experience and using a variety of needles and techniques. Although there were some cohorts in which no misinjections were recorded, none of these were consistent across all operators or techniques. The misinjection rate varied between different operators, ranging from 4% to 53%. Misinjection rates for different needle lengths ranged from 9% to 32%. Unfortunately, it became apparent that due to the many possible variables (needle, operator, syringe type, left or right side, angle and depth of injection, position of animal), statistical analysis was not possible.

Prior to this, a study on rat cadavers injected IP with blue dye immediately after death found a misinjection rate of approximately 50%. It is possible, however, that this high misinjection rate is at least partly the result of post mortem changes such as loss of muscle tone.