

# The Use of Rat Tickling to Improve Animal Welfare

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## Introduction

- Tickling is increasingly recognised as an effective means of improving laboratory rat welfare through mimicking natural play habits. After review of all the material that was available through the NC3Rs who promote this welfare initiative, it was felt that this initiative could have a positive impact on the stress levels of the rats used for inhalation studies at the Labcorp Huntingdon site, acclimatising them to the procedure more successfully while improving their general welfare.
- Initially two technicians completed the rat tickling certification that is available through the NC3Rs and an initial 13-week study was selected with agreement from study management to conduct the trial.
- The minimum recommended tickling time from research available is 15 seconds for 3 days. The certificated training recommended 15 seconds on/15 seconds off for a cycle of 2 minutes per rat, which was the approach that was adopted for the trial.
- The 3 days of tickling was to be conducted prior to any investigations or procedures conducted on the study, so it was started on the day after the animals arrived at the facility.

## Methodology

- Animals were removed from their home cage and tickled in a separate empty cage, where during the 15 seconds off they were free to move around the cage and conduct normal behaviour. The animal was flipped onto its back, pinned and then tickled, after which it was released.
- As expected, on the first day there were animals that were tense and slightly apprehensive of the procedure but as the session continued, the animals became more accommodating of the procedure and interaction from the technician. After a couple of tickling sessions, there were animals that were waiting for the interaction to continue. The male animals were much more susceptible to the interaction on day 1 than the female animals. As the tickling continued on days 2 and 3, more and more animals started to interact with the technicians' hands during the off time, rather than moving away to investigate the cage. There were animals that would wait upside down for the tickling to continue. It was identified that the female animals prefer the play to be a little rougher than the male animals, incorporating moving the animals side to side while tickling.



Image from NC3Rs rat tickling resource material

## Results

- A far greater impact was seen in these animals than expected. All staff that worked with these animals commented on how calm and amenable the animals were.
- From the success of the initial trial the tickling was rolled out to a rat carcinogenicity study with a 4-hour exposure. This was a bigger time commitment resource-wise due to the number of animals on this study, but it was felt the benefits outweighed this resource requirement. Additionally, more staff completed the certificated training as they wanted to be involved in this process and we found that it was really boosting staff morale tickling the rats.
- The data for red staining of the carcinogenicity study when compared to the last rat carcinogenicity study run is shown in Table 1. Here there was a difference in exposure lengths, the study where the animals were tickled was 4-hour exposure and the last rat carcinogenicity study run to compare to, a 1-hour exposure.

**Table 1. Comparison Between Tickled and Non-Tickled Rats in an Inhalation Study**

Trial Study – Rats Tickled			Comparative Study – Rats Not Tickled		
Day No.	No. of rats with red staining out of 90 animals	% of animals with red staining	Day No.	No. of rats with red staining out of 120 animals	% of animals with red staining
Day 1 of restraint training	3	3.3%	Day 1 of restraint training	54	45%
Day 2 of restraint training	1	1.1%	Day 2 of restraint training	62	51.6%
Day 3 of restraint training	0	0%	Day 3 of restraint training	104	86.6%
1	4	4.4%	1	81	67.5%
2	2	2.2%	2	91	75.8%
3	8	8.8%	3	61	50.8%
4	8	8.8%	4	66	55%
5	7	7.7%	5	43	35.8%
6	0	0%	6	31	25.8%
7	3	3.3%	7	14	11.6%

- The data from this review showed that the tickling was having a big impact on the percentage of animals seen with red staining and supported the theory that it was reducing the stress in the rats while on the exposure.
- We also found that maintaining tickling once a week with a 15-second tickle prior to performing each individual animal's Clinical Signs/Health check maintained the behaviour and reduced the stress level for the duration of the study.

## References

Link here the NC3Rs resource hub for rat tickling <https://nc3rs.org.uk/rat-tickling>

## Acknowledgements

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**Table 2. Comparison Between Tickled and Non-Tickled Rats in a 4-Hour vs. 1-Hour Carcinogenicity Study**

Trial Carcinogenicity Study – Rats Tickled			Comparative Carcinogenicity Study – Rats Not Tickled		
Day No.	No. of rats with red staining out of 550 animals	% of animals with red staining	Day No.	No. of rats with red staining out of 550 animals	% of animals with red staining
Day 1 of restraint training 30 Mins	3	0.05%			
Day 2 of restraint training 1 Hour	27	0.5%			
Day 3 of restraint training 2 Hours	117	21%	Day 1 of restraint training 20 Mins	0	0%
Day 4 of restraint training 3 Hours	110	20%	Day 2 of restraint training 40 Mins	4	0.07%
Day 5 of restraint training 4 Hours	103	18.7%	Day 3 of restraint training 1 Hour	74	13.4%
1	55	10%	1	108	19.6%
2	85	15.4%	2	85	15.4%
3	84	15.2%	3	108	19.6%
4	76	13.8%	4	121	22%
5	41	7.4%	5	194	35.2%
6	35	6.3%	6	165	30%
7	26	4.7%	7	132	24%

- From this data we could see again that the tickling had an impact on the stress levels of the animals and that the reduction in staining seen showed a reduced stress level particularly considering the different duration of exposures compared in this instance.
- Additionally, the number of animals lost during the restraint training period and the 2-week replacement period on both carcinogenicity studies was reviewed.

**Table 3. Number of Animals Lost During Restraint Training Period**

Trial Carcinogenicity Study – Rats Tickled		Comparative Carcinogenicity Study – Rats Not Tickled	
No. of tube deaths between Day -5 – Day 14	% of Animals	No. of tube deaths between Day -5 – Day 14	% of Animals
0	0%	13	2.3%

## Conclusion

- The improvement in the reduction of tube deaths was seen as a great refinement, particularly as the comparative study was a 60-minute exposure and the rats that were tickled were on a 4-hour exposure. It should be noted that the rat age and strain were consistent throughout this assessment to allow a comparison to be made.
- Due to the success of both trials, rat tickling has been rolled out to all rat inhalation studies at Huntingdon. After it was also highlighted that there was improvement in animal behaviour while having jugular blood samples taken, the other rodent toxicology facilities at Huntingdon and Eye are assessing the benefits to other dose routes.
- Although the project has introduced an additional procedure, this has refined the experience for the rats while on exposure, reducing animal stress and helping to reduce the number of restraint/tube deaths seen in rats. The project has greatly improved the welfare of the animals within the facility and helped to get the staff interacting with the animals in an enjoyable way for both themselves and the animals.