SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

FORMAT BY SYRCLE (WWW.SYRCLE.NL)
VERSION 2.0 (DECEMBER 2014)

Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	Discomfort due to toe and ear clipping in rodents	
2.	Authors (names, affiliations, contributions)	KE Wever – SYRCLE, Radboudumc, The Netherlands – study design, study selection, data extraction, data analysis, RoB assessment, manuscript writing, manuscript approval FJ Geessink – SYRCLE, Radboudumc, The Netherlands – study design, search, study selection, data extraction, data analysis, RoB assessment, manuscript approval M Brouwer, SYRCLE, Radboudumc, The Netherlands – study selection, data extraction, RoB assessment, manuscript approval A Tillema – librarian, Radboudumc, The Netherlands – search design, manuscript approval M Ritskes-Hoitinga – SYRCLE, Radboudumc, The Netherlands – study design, manuscript approval	
3.	Other contributors (names, affiliations, contributions)	R de Vries – SYRCLE, Radboudumc, The Netherlands – project supervision	
4.	Contact person + e-mail address	KE Wever, kim.wever@radboudumc.nl	
5.	Funding sources/sponsors	The Netherlands Organisation for Health Research and Development, commissioned by The Netherlands Ministry of Economic Affairs	
6.	Conflicts of interest	The authors report no conflicts of interest	
7.	Date and location of protocol registration	21 October 2015	
8.	Registration number (if applicable)	NA	
9.	Stage of review at time of registration	Systematic searches and pre-screening completed	
	B. Objectives		
	Background		
10.	What is already known about this disease/ model/ intervention? Why is it important to do this review?	Rodents, especially mice and rats, are the most frequently used laboratory animals in biomedical research. They are usually identical in appearance and housed in groups. Individual identification of the animals is often necessary during breeding, daily care or experimental procedures, and several possible identification methods are in use. Selection of the best method of individual identification depends on several factors, including species, age, skin pigmentation, study duration, and technical expertise available. The ideal identification method should be effective and practical, but it should also be minimally invasive in terms of pain and/or distress to the animal, since this can interfere with animal welfare and distort the experimental results. It is therefore important to assess the effect of identification methods on animal welfare. Toe clipping is an individual identification method mostly used in mice, which can be applied in newborn and very young animals. The toe may be	

		clipped at the very distal part of the second phalanx (Figure 1) to remove the entire nailbed, or a larger portion of the toe may be removed. The removed tissue can be used for genotyping. Ear clipping or punching (notching) is used to identify individual adult rodents (mostly mice and rats). Using a special puncher, holes or notches are made in the ear according to a chart/system, in order to ensure a valid identification. The punched or clipped tissue can be used for genotyping. The ethical justification to perform these methods is a matter of debate, since both methods are likely to cause pain and/or distress. Both methods require restraint of the animals, and may permanently affect the wellbeing of the animal. For instance, toe clipping might impair the mouse's ability to grip, groom and feed, as well as to alter the animal's gait. However, the evidence for the discomfort caused by toe and ear clipping has not been systematically reviewed. We will therefore conduct a systematic review of the evidence on discomfort due to ear and toe clipping, in order to better inform animal researchers, welfare officers, policy makers and other stakeholders when making decisions on the choice of identification method for rodents.  Figure 1 Schematic picture of the site of distal phalanx removal – adapted from Dahlborn et al. 2013	
	Research question		
11.	Specify the disease/health problem of interest	Discomfort due to toe or ear clipping	
12.	Specify the population/species studied	Rodents	
13.	Specify the intervention/exposure	Toe or ear clipping	
14.	Specify the control population	No intervention, or restraint only	
15.	Specify the outcome measures	Outcomes related to discomfort, suffering, pain or distress in the animal undergoing the intervention	
16.	State your research question (based on items 11-15)	What is the effect of toe or ear clipping on the level of discomfort, suffering, pain or stress in animals undergoing this intervention? Sub-questions: what is the quality of the evidence on this topic? Which factors influence the effect of toe or ear clipping on discomfort? How (e.g. at what age, with which technique) can discomfort due to toe and ear clipping be minimized? How are toe and ear clipping related to each other in term of the discomfort caused?	
	C. Methods		
	Search and study identification		

17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	X MEDLINE via PubMed X Web of Science ☐SCOPUS X EMBASE ☐Other, namely: ☐Specific journal(s), namely:	
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: see below NB: our current search strategy is designed to also identify studies on tail clipping, which will be labelled for future use.	
19.	Identify other sources for study identification	X Reference lists of included studies ☐Books X Reference lists of relevant reviews ☐Conference proceedings, namely: X Contacting authors/ organisations, namely: • Federation of European Laboratory Animal Science Associations (FELASA) Working Group on animal identification • Personal communications with authors of included studies • Google searching ☐Other, namely:	
20.	Define search strategy for these other sources	-check each reference list from the included studies for possible relevant studies which were not found by our search in the databases -identify relevant reviews (FELASA) and check the reference list for possible relevant studies which were not found by our search in the databases -email the authors of included studies to ask for relevant unpublished data (grey literature) Any literature obtained from these sources will be evaluated for inclusion in full by two independent reviewers.	
	Study selection		
21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Phase 1: pre-screening on title and abstract to remove references with no relation at all to the review topic Phase 2: screening on title and abstract Phase 3: final inclusion or exclusion based on full-text	
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Phase 1: one reviewer (FG) assesses all references for relevance to the review topic. Excluded references are checked by KW. Phase 2: each reference is assessed by two independent reviewers (KW, FG and MB) using EROS. Disagreements are resolved through discussion. Phase 3: each reference is assessed full-text by two independent reviewers (KW, FG and MB) using EROS. Disagreements are resolved through discussion.	
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria: studies with a control group (no intervention or restraint only) versus an intervention group, or observational studies Exclusion criteria: case reports	
24.	Type of animals/population (*e.g.* age,	Inclusion criteria: rodents, any age or sex	

	gender, disease model)	Exclusion criteria: non-rodents	
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: toe or ear clipping. This includes distal phalanx removal, toe removal, ear notching, ear punching and ear tagging Exclusion criteria: no toe or ear clipping applied	
26.	Outcome measures	Inclusion criteria: outcomes related to discomfort, this includes, pain, stress, disease and mortality Exclusion criteria: outcome measures not related to discomfort or distress	
27.	Language restrictions	Inclusion criteria: all languages Exclusion criteria: none	
28.	Publication date restrictions	Inclusion criteria: all years of publication Exclusion criteria: none	
29.	Other	Inclusion criteria: animals undergoing only toe or ear clipping Exclusion criteria: animals undergoing additional co-interventions, except for restraint.	
30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: 1. Article without original data (e.g. review, editorial) 2. Not an in vivo animal study 3. Not an ear or toe clipping in rodents 4. No relevant outcome measures 5. Case study 6. Unsuitable co-intervention 7. Article not retrievable Selection phase: 1. Article without original data (e.g. review, editorial) 2. Not an in vivo animal study 3. Not on ear or toe clipping in rodents 4. No relevant outcome measures 5. Case study 6. Unsuitable co-intervention 7. Article not retrievable NB: our current search strategy is designed to also identify studies on tail clipping, which will be labelled for future use.	
	Study characteristics to be extracted (for assessment of external validity, reporting quality)		
31.	Study ID (*e.g.* authors, year)	Author, title, year of publication	
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	Number of experimental groups, number of control groups	
33.	Animal model characteristics (*e.g.* species, sex, disease induction)	Species, strain, sex, age, weight, housing conditions	
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	Identification method, site of clipping, frequency of intervention, animal age at intervention	
35.	Outcome measures	Time of outcome assessment, outcome measures determined (list)	
36.	Other (*e.g.* drop-outs)	Number, reason	
	Assessment risk of bias (internal validity) or study quality		
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	RoB performed for controlled studies only. At least two reviewers will assess the risk of bias and study quality of all selected studies. Discrepancies will be dealt with through (written/non-written) discussion.	

38.	Define criteria to assess (a) the internal validity of included studies (*e.g.* selection, performance, detection and attrition bias) and/or (b) other study quality measures (*e.g.* reporting quality, power)	☐By use of SYRCLE's Risk of Bias tool[4] X By use of SYRCLE's Risk of Bias tool, adapted as follows: additional scoring of reporting of any randomisation, reporting of any blinding, reporting of a power calculation ☐By use of CAMARADES' study quality checklist, e.g [22] ☐By use of CAMARADES' study quality checklist, adapted as follows: ☐Other criteria, namely:	
	Collection of outcome data		
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	For both toe and ear clipping, preliminary screening shows a wide range of outcome measures in use, for example pain-related behaviour, mortality, weight/growth, stress hormones, vocalizations, heart rate, blood pressure, grimace scale, grip tests, histology etc. The indirectness of the outcome measures to accurately assess discomfort in rodents may differ and is a matter of debate. We therefore aim to first provide a complete overview of outcomes measured. We aim to perform meta-analysis for any discomfort-related outcome measure reported by 3 or more articles, separately for toe and ear clipping. We aim to assess at minimum the following outcome measures: • Mortality • Pain related behaviour • Stress hormone levels • Weight/Growth • Heart rate • Blood pressure • Mobility (for toe clipping)	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	Preferred method of extraction: numerical data from text or tables. If data are only presented graphically, graphs will be measures using digital image software. In case of missing data, we will contact authors in an attempt to retrieve additional information. In case of no response within three weeks including a reminder, the study will be excluded from analysis.	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	At least two reviewers will independently extract data. Discrepancies will be dealt with through (written/non-written) discussion.	
	Data analysis/synthesis		
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	A descriptive summary of all included articles and their outcome measures. If possible, meta-analysis will be performed for any discomfort-related outcome measure reported by 3 or more studies (separately for toe and ear clipping).	
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	A meta-analysis will be performed if ≥3 studies report on a specific outcome measure. For subgroup analysis a minimum of 3 studies per subgroup is required.	
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	To be determined	
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	We expect heterogeneity between the included studies, due to the explorative nature of animal studies and the expected low standardisation. We will therefore perform a	

		random effects analysis, since this model is more suitable to handle data with expected high heterogeneity.	
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	(residual) I^2 and adjusted R^2	
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	• Age of the animal at time of intervention • Frequency of intervention • Strain • Site of clipping	
48.	Any sensitivity analyses you propose to perform	To be determined	
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	If applicable, we will perform a Holm-Bonferroni correction for testing multiple subgroups. If one or more subgroup analyses cannot be performed due to insufficient data, the p-value will be adjusted accordingly. Also correction for multiple use of control group will be performed by dividing the number of animals in the control group by the number of comparisons performed with this control group	
50.	The method for assessment of publication bias	Produce funnel plots and visual analysis of these plots for outcome measures containing 20+ studies. We are aware that funnel plots of SMD are susceptible to distortion and will omit the assessment of publication bias if this is suspected for our dataset. In addition, we aim to perform Egger's test for small study effects for outcome measures containing 20+ studies	
Final approval by (names, affiliations): On behalf of my co-authors, Kim Wever			Date: 27-10-2015