Supplementary materials

Male F1 mice were weighed weekly (Supplementary Table 1), there was no significant differences in the mean weights in week 5,6 and week 8. In week 4 there was a significant difference between the Sire Exposed group and the Dam exposed group (P-value = 0.009). In week 7 there is a significant difference between Sire Exposed and Both Exposed group (P-value = 0.002). The Dam Exposed group had some stunted mice weighing around 11g at weaning, whose weights improved immediately after.

Supplementary Table 1: Weights (g) in F1 males

Group	**Parameter**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Control	Maximum	21.9	23.54	24.65	25.68	27.01
	Average	19.59	21.18	22.15	23.64	25.14
	Mininum	15.17	18.63	20.12	21.42	23.16
Sire Exposed	Maximum	23.37	23.47	24.15	26.77	26.77
	Average	21.50	22.05	23.38	24.21	24.93
	Mininum	19.74	20.83	22.44	22.07	22.07
Dam Exposed	Maximum	22.88	23.61	24.27	26.81	26.81
	Average	18.06	20.15	21.56	23.02	24.14
	Mininum	11.6	16.42	18.23	22.19	22.19
Both Exposed	Maximum	20.57	21.86	23.45	24.14	25.6
	Average	19.28	20.41	21.54	22.33	23.47
	Mininum	16.00	19.01	19.55	20.91	22.2

Female Mice were weighed weekly from age 4 weeks to week 8 and there were no significant differences among the groups as shown in Supplementary Table 2 below.

Supplementary Table 2: Weights (g) in F1 Female

Group	**Parameter**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Control	Maximium	18.17	18.1	18.27	20.22	20.03
	Mean	16.91	17.11	17.38	18.64	18.95
	Minimium	15.35	15.73	16.37	17.16	17.68
Sire Exposed	Maximium	18.04	18.53	19.4	20.68	21.26
	Mean	16.84	17.34	17.57	18.86	19.10
	Minimium	16.03	16.82	16.5	17.53	16.72
Dam Exposed	Maximium	19.11	18.13	18.54	20.22	21.31
	Mean	16.73	17.30	19.21	18.28	18.28
	Mininium	15.51	15.51	16.02	16.76	17.91
Both Exposed	Maximium	19.14	18.97	20	18.21	21.28
	Mean	17.18	17.50	17.54	17.82	19.76
	Mininium	14.53	16.37	16.37	17.23	18.35

The sex ratio of litters was observed in the four different groups in this study. It was then observed that lead exposure caused a variation in the sex ratio. With exposure resulting in the reduced ratio of female offspring when compared to male as shown Supplementary table 3 below.

Supplementary Table 3: Shows litter sex ratio among groups.

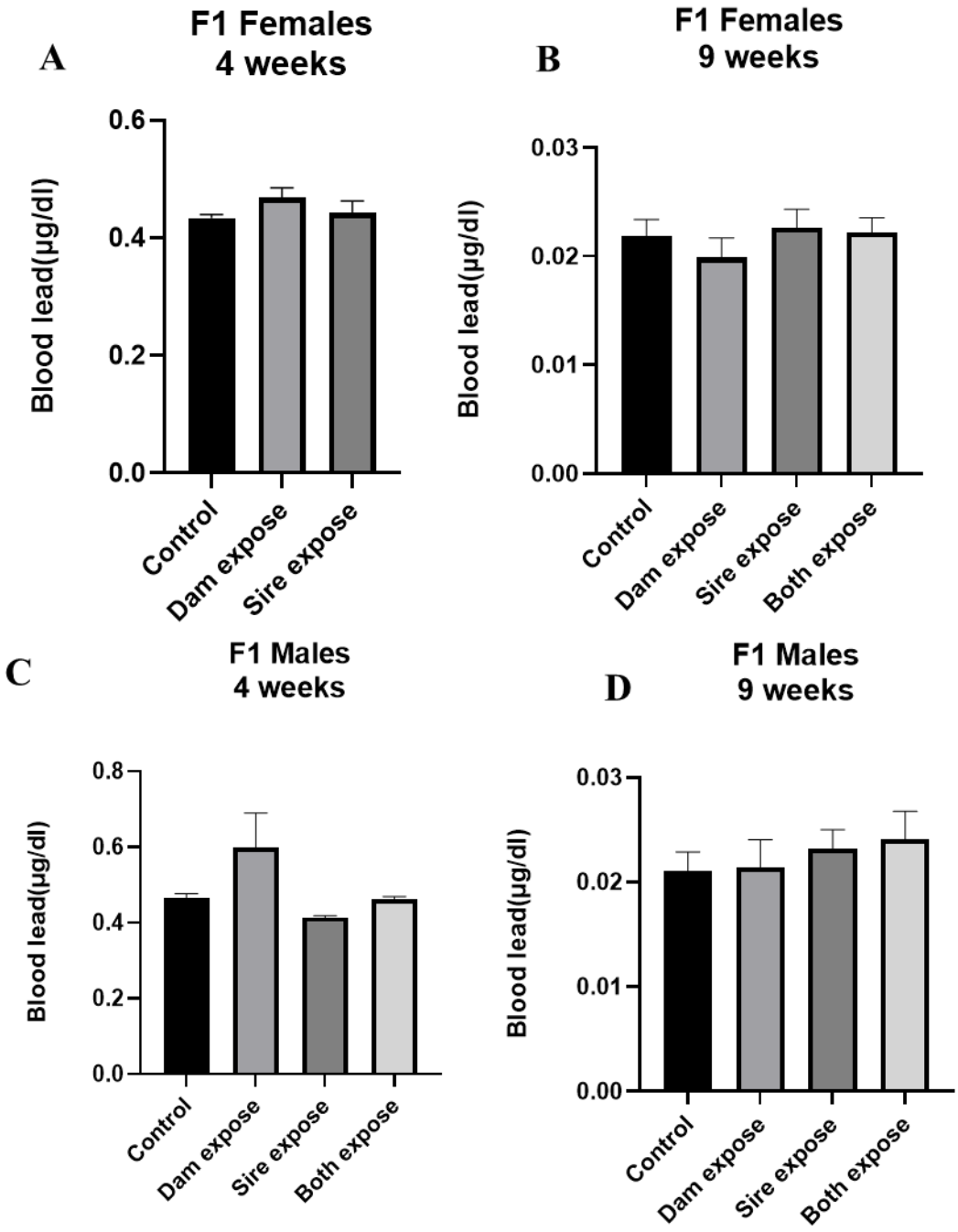
Group	Males	Females
Control	50%	50%
Dam expose	65%	35%
Sire expose	61%	39%
Both expose	63%	37%

Blood Lead Levels in F1 generation

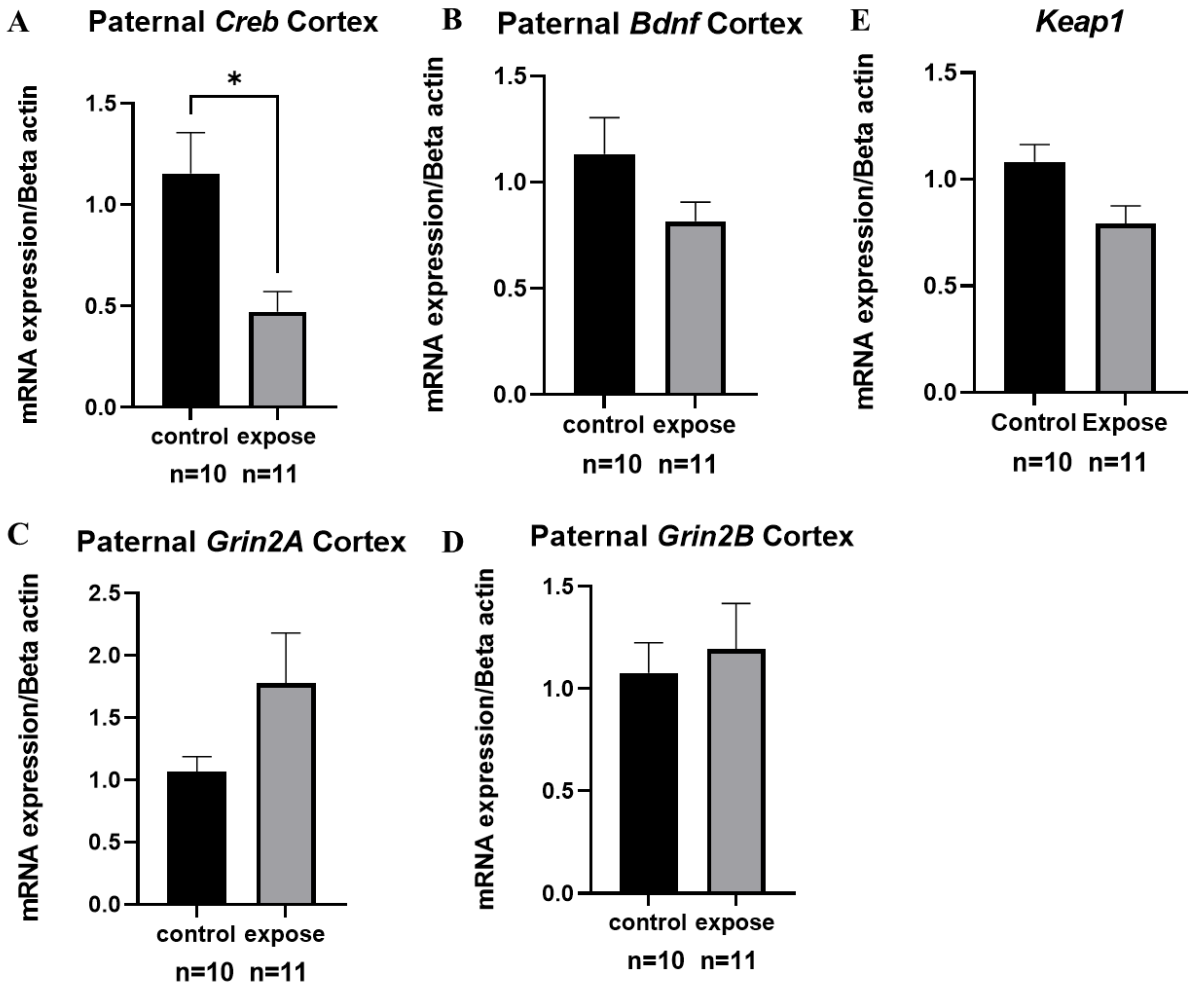
Blood Pb levels were measured in F1 generation to determine bioaccumulation and transfer from exposed mothers. There were no significant differences among the groups in both male and female groups. (Supplementary Figure 1 and 2).

Paternal prefrontal cortex genes were examined to determine if any changes could be observed. Creb was significantly downregulated in Pb exposed sires as shown in Supplementary Figure 2A. Grin2A (Supplementary Figure 2C) like that of their offspring was non-significantly upregulated. Grin2B (Supplementary Figure 2D) showed normal expression when compared to control. Nr2f was significantly upregulated, however keap1 (Supplementary Figure 2E) was non-significantly downregulated in the exposed sires.

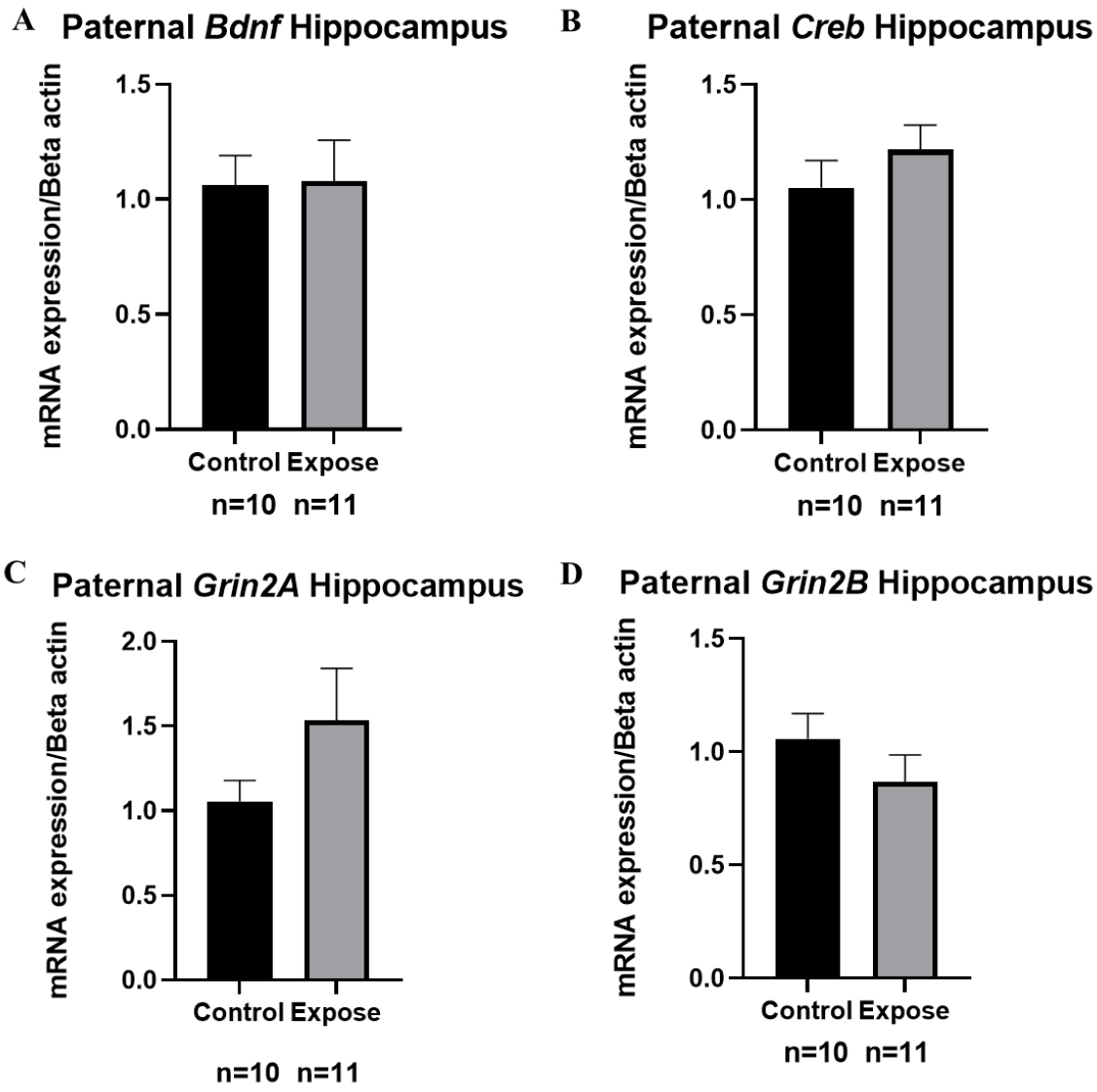
Paternal hippocampal mRNA expression levels of Bdnf, Creb, Grin2A and Grin2B. There was non-significant upregulation of Grin2A. Bdnf, Creb and Grin2B were normally regulated.



Supplementary Figure *1A* shows blood lead levels at 4 weeks and Figure *1B* shows blood lead levels at 9 weeks in F1 females. Supplementary Figure *1C* shows blood lead levels at 4 weeks and *1D* shows blood lead levels at 9 weeks.



Supplementary Figure 2 shows prefrontal cortex genes and testicular gene. Supplementary Figure 2A shows *Creb* expression levels, Supplementary Figure 2B shows *Bdnf*, Supplementary Figure 2C shows *Grin2A*, Supplementary Figure 2D shows *Grin2B* and Supplementary Figure 2E shows *Keap1* expression levels.



Supplementary Figure 3 shows hippocampal mRNA expression, Supplementary 3A shows *bdnf* expression, Supplementary 3B shows *Creb* expression, Supplementary Figure 3C shows *Grin2A* expression levels and Supplementary Figure 3D shows *Grin2B* expression in F0 sire males.

Supplementary Table 4: List of primers

Name	Sequence
Spink2	Forward: CATGAGACTCTCGACTCTTCCG Reverse: CGCACACAGGGTTGAGGTT
Pou5F1	Forward: CTTGCAGCTCAGCCTTAAGAAC Reverse: GCACCAGGGTCTCCGATTT
Crisp 2	Forward: TACTGTCCCAATCAAGACAACC Reverse: CACAAGGTGTTCCTTGTTGATATG
Bdnf	Forward: TTACCTGGATGCCGCAAACAT Reverse: TGACCCACTCGCTAATACTGTC
Creb	Forward: CCAAACTAGCAGTGGGCAGTATATT Reverse: GGTACCATTGTTAGCCAGCTGTATT
Grin 2A	Forward: CTCAGCATTGTCACCTTGGA Reverse: GCAGCACTTCTTCACATTCAT
Grin 2B	Forward: CTACTGCTGGCTGCTGGTGA Reverse: GACTGGAGAATGGAGACGGCTA
Beta-Actin	Forward: GTACTCTGTGTGGATCGGTGG Reverse: GCAGCTCAGTAACAGTCCG
Keap 1	Forward: ATGTTGACACGGAGGATTGG Reverse: TCATCCGCCACTCATTCCT
Nrf2	Forward: CCTCACCTCTGCTGCAAGTA Reverse: TCAAATCCATGTCCTGCTGGG
PPIA	Forward: CAGGTCCATCTACGGAGAGA Reverse: CATCCAGCCATTCAGTCTTG
GAPDH	Forward: GGTCCCAGCTTAGGTTCAT Reverse: CAATCTCCACTTTGCCACT

Supplementary Table 5: shows CT value of selected reference genes in first trail run.

RUN 1				
Group	Individual	GAPDH CT Value	Beta actin CT Value	PPIA CT Value
Control	C1	15.32697	15.68666	15.84749
Control	C2	16.69212	15.00539	15.95415
Control	C3	17.06668	15.8752	16.02423
Control	C4	17.89759	15.9946	17.34178
Control	C5	17.94913	15.59157	15.85979
Control	C6	17.44565	15.40437	16.72191
Control	C7	17.61228	15.90503	14.69256
Control	C8	16.35662	15.47632	17.31338
Expose	T1	16.20456	15.08564	15.68736
Expose	T2	16.93564	15.51711	14.50026
Expose	T3	16.86265	15.9786	16.68236
Expose	T4	15.16354	15.64809	17.6286
Expose	T5	17.59224	16.08731	18.82337
Expose	T6	17.53626	15.87617	17.97873
Expose	T7	18.14792	15.3315	17.17582
Expose	T8	17.31699	15.9314	17.1828

Supplementary Table 6: shows CT value of selected reference genes in second trail run.

RUN2				
Group	Individual	GAPDH CT value	Beta actin CT value	PPIA CT value
Control	C1	16.46793	15.65784	13.14844
Control	C2	15.54823	15.05299	15.0106
Control	C3	16.48954	15.10423	14.25053
Control	C4	17.40138	15.1017	13.91948
Control	C5	17.750	15.10565	14.92662
Control	C6	18.57142	15.17631	14.14266
Control	C7	17.91285	14.8488	14.13689
Control	C8	17.22471	14.86619	12.79246
Expose	T1	18.83699	15.80479	14.85937
Expose	T2	17.17774	15.13012	15.74629
Expose	T3	18.34385	15.27085	13.80686
Expose	T4	17.53303	15.31133	14.88873
Expose	T5	18.95068	15.98074	14.96893
Expose	T6	17.41922	15.04617	14.0075
Expose	T7	18.21537	15.40207	14.74077
Expose	T8	17.99582	14.99544	14.62141

Supplementary Table 7: shows CT value of selected reference genes in third trail run.

RUN3					
Group	Individual	Group	GAPDH CT value	Beta actin CT value	PPIA CT value
Control	C1	Control	15.20008	14.40775	15.84749
Control	C2	Control	16.01572	14.25703	15.95415
Control	C3	Control	15.88737	14.09925	16.02423
Control	C4	Control	16.66459	14.93578	17.34178
Control	C5	Control	16.55858	14.98195	15.85979
Control	C6	Control	15.69225	14.03901	16.72191
Control	C7	Control	15.90377	13.80109	16.0429
Control	C8	Control	16.62937	14.45649	14.69256
Expose	T1	Expose	17.91857	14.27263	15.68736
Expose	T2	Expose	19.10342	14.33329	14.50026
Expose	T3	Expose	17.95428	14.14912	16.68236
Expose	T4	Expose	19.72309	14.06207	17.6286
Expose	T5	Expose	16.70274	13.9841	18.82337
Expose	T6	Expose	17.97464	14.33997	17.97873
Expose	T7	Expose	16.42242	15.16294	17.17582
Expose	T8	Expose	17.14709	14.69135	17.1828

Supplementary Table 8: shows the Mean CT Value ± SEM from the three trial runs for selected reference genes.

RUN 1	GAPDH	Beta Actin	PPIA
Control	17.043 ± 0.316	15.617 ± 0.115	16.21941 ± 0.3110
Expose	16.969 ± 0.330	15.68198 ± 0.124	16.95741 ± 0.478
RUN 2	GAPDH	Beta Actin	PPIA
Control	17.170 ± 0.341	15.114 ± 0.0881	14.040 ± 0.272
Expose	18.059 ± 0.230	15.367 ± 0.125	14.704 ± 0.211
RUN 3	GAPDH	Beta Actin	PPIA
Control	16.068 ± 0.183	14.372 ± 0.148	16.060 ± 0.268
Expose	17.868 ± 0.399	14.374 ± 0.136	16.957 ± 0.478

The ARRIVE Essential 10: Compliance Questionnaire

Use this questionnaire to evaluate how well a manuscript complies with the ARRIVE Essential 10. It can be applied to any manuscript describing comparative experiments in living animals, by assessors such as journal staff, editors, or peer reviewers.

	Item	Question(s)	Answers
1	Study Design	Are all experimental and control groups clearly identified?	☒ Yes, for at least one experiment ☐ No
		Is the experimental unit (e.g. an animal, litter or cage of animals) clearly identified?	☒ Yes, for at least one experiment ☐ No
2	Sample Size	Is the exact number of experimental units in each group at the start of the study provided (e.g. in the format '*n*=')?	☒ Yes, for at least one experiment ☐ No
		Is the method by which the sample size was chosen explained?	☒ Yes, for at least one experiment ☐ No
3	Inclusion & Exclusion Criteria	Are the criteria used for including and excluding animals, experimental units, or data points provided?	☒ Yes, for at least one experiment ☐ No
		Are any exclusions of animals, experimental units, or data points reported, or is there a statement indicating that there were no exclusions?	☒ Yes, for at least one analysis ☐ No
4	Randomisation	Is the method by which experimental units were allocated to control and treatment groups described?	☒ Yes, for at least one experiment ☐ No
5	Blinding	Is it clear whether researchers were aware of, or blinded to, the group allocation at any stage of the experiment or data analysis?	☒ Yes, for at least one experiment ☐ No
6	Outcome Measures	For all experimental outcomes presented, are details provided of exactly what parameter was measured?	☒ Yes, for at least one experiment ☐ No
7	Statistical Methods	Is the statistical approach used to analyse each outcome detailed?	☒ Yes, for at least one analysis ☐ No
		Is there a description of any methods used to assess whether data met statistical assumptions?	☒ Yes, for at least one analysis ☐ No ☐ Not applicable
8	Experimental Animals	Are all species of animal used specified?	☒ Yes, for at least one experiment ☐ No
		Is the sex of the animals specified?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to species
		Is at least one of age, weight or developmental stage of the animals specified?	☒ Yes, for at least one experiment ☐ No
9	Experimental Procedures	Are both the timing and frequency with which procedures took place specified?	☒ Yes, for at least one experiment ☐ No
		Are details of acclimatisation periods to experimental locations provided?	☒ Yes, for at least one experiment ☐ No
10	Results	Are descriptive statistics for each experimental group provided, with a measure of variability (e.g. mean and SD, or median and range)?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to the type of data collected
		Is the effect size and confidence interval provided?	☐ Yes, for at least one experiment ☐ No ☒ Not applicable to the type of analysis used

ARRIVE

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Notes on questionnaire design

The ARRIVE guidelines are a useful resource for authors preparing manuscripts describing animal research, and also provide a framework to evaluate the transparency of those manuscripts. To assess reporting quality, numerous studies have in the past sought to operationalise reporting guidelines (including ARRIVE). Typically, this involves scoring a manuscript's degree of compliance with guideline items in a binary fashion (e.g. an item is either not reported or reported) [1-3], a graded fashion (e.g. not, partially, or completely reported) [4,5], or a combination of the two [6].

This questionnaire has been designed to be as concise and user-friendly as possible. The number of questions used to assess a manuscript's compliance has been kept to a minimum, and in most cases each question is designed to be answered in a binary fashion. Compliance with some Essential 10 sub-items is inherently impossible to judge in this way, instead requiring a subjective judgement on the level of detail provided. For this reason, not all sub-items are represented by a question in this questionnaire.

To facilitate binary answers, it has been necessary to identify the minimum information in a manuscript sufficient to comply with each question. The strengths of this approach include the relatively short length of the questionnaire (and the correspondingly low time burden of using it), and the avoidance of ambiguity that would arise from a graded answering system, in which an intermediate score (e.g. 'partially/insufficiently reported') could denote a number of distinct deficiencies in compliance with an item (e.g. either only part of the item was complied with, or only the reporting of some experiments in the manuscript complied with the item.)

Limitations of this approach centre on the necessity to identify the minimum information sufficient to comply with each question. In some cases, this has resulted in questions that require a guideline sub-item's criteria to have been fulfilled in the reporting of only one experiment in a manuscript. As a result, not all experiments in a manuscript may be described in a way that fulfils that criterion, despite the manuscript being considered to comply with the guidelines overall.

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