

1 Sex Inclusive Research Framework (SIRF): an evaluation tool to
2 assess whether an in vivo research proposal follows the sex -
3 inclusive research philosophy.

4 Natasha A. Karp^{1*}, Manuel Berdoy², Lilian Hunt³, Maggy Jennings⁴, Angela Kerton⁵, Matt Leach⁶, Jordi
5 Lopez-Tremoleda⁷, Jon Gledhill⁶, Esther J. Pearl⁸, Nathalie Percie du Sert⁸, Benjamin Phillips¹, Penny S
6 Reynolds⁹, Kathy Ryder¹⁰, S Clare Stanford¹¹, Sara Wells¹², Lucy Whitfield¹³.

7 1: Data Sciences & Quantitative Biology, Discovery Sciences, R&D, AstraZeneca, Cambridge, United Kingdom

8 2: University of Oxford, Oxford, UK

9 3: Wellcome Trust, London, UK

10 4: RSPCA, Animals in Science Department, Horsham, UK

11 5: The Learning Curve (Development) Ltd, Ware, UK

12 6: Comparative Biology Centre, Newcastle University, Newcastle, UK

13 7: Queen Mary University of London, London, UK

14 8: The NC3Rs, London, United Kingdom

15 9: University of Florida, Florida, USA

16 10: Department of Health, Stormont Estate, Belfast, UK

17 11: University College London, London, UK

18 12: The Mary Lyon Centre at MRC Harwell, Harwell Science and Innovation Campus, UK

19 13: OWL Vets Ltd, Mildenhall, UK

20

21 *Corresponding author

22 Natasha.Karp@astrazeneca.com

23 Short title: Evaluating in vivo research with the Sex Inclusive Research Framework

24 Funding information:

25 Funding source:

26 Funding statement: "The funders had no role in study design, data collection and analysis, decision to
27 publish, or preparation of the manuscript."

28 Competing interests:

29 BP and NAK have shareholdings in AstraZeneca. EJP and NPdS are NC3Rs staff; role includes promoting the
30 ARRIVE guidelines and the Experimental Design Assistant (EDA).

31

32 Abstract

33 A new, interactive framework supports the evaluation of in vivo and ex vivo research proposals from
34 a sex inclusive research perspective delivering a traffic light classification, indicating whether a
35 proposal is appropriate, risky, or insufficient with regards to sex inclusion. This tool is designed for use
36 by researchers, (animal) ethical review boards, and funders to generate a rigorous and reproducible
37 assessment of sex inclusion at the proposal level, thus helping address the embedded sex bias in
38 preclinical research.

39 Why is there a need for a decision framework to assess the sex 40 inclusion position of research proposals?

41 Within preclinical research, there is an endemic and persistent sex bias whereby research is
42 predominately conducted with a single sex, typically male animals or male cell lines (1-3). This can
43 result in our fundamental biological knowledge being biased (4). To redress this imbalance and
44 improve the translation of scientific findings between humans and other animals, numerous funding
45 bodies have released inclusion mandates (5, 6) that require the automatic inclusion of both sexes
46 unless strong justification is provided.

47 To date, these policies do not require scientists to study differences between males and females *per*
48 *se*, but instead aim to improve the generalisability of studies by taking sex into consideration in the
49 design and statistical analysis. This can be achieved by estimating from both sexes an average
50 intervention effect and by visualising and analysing data in such a way that if there is a large sex
51 difference in the intervention effect this will be detected. For funders, regulators or ethical review
52 bodies to apply these policies in a systematic and consistent manner, there is a need for resources to
53 help assess whether a research proposal is compliant with sex inclusive mandates. Not only do
54 scientists struggle to include both sexes (1-3), but when data from both sexes are collected there is

55 often unequal representation (7) and inappropriate visualisation and analysis of the data (8, 9). It is
56 therefore important to not only encourage balanced inclusion but also appropriate analysis.

57 Research has shown that scientists are generally supportive of sex as an important biological variable
58 but there are barriers to implementing sex inclusive designs (10, 11). Frequently, the cited barriers are
59 culturally embedded misconceptions. These include the mistaken perspectives that females are
60 inherently more variable (12, 13), that sex differences will introduce variability in the data decreasing
61 statistical sensitivity (14), or that studying both sexes will increase the number of animals needed (10,
62 11) and hence the cost (15). Some researchers have identified logistic/welfare concerns (for example
63 the need for single housing to reduce male mouse aggression)(15). Fear of change is also a factor,
64 that has been identified as preventing a change to the status quo (10).

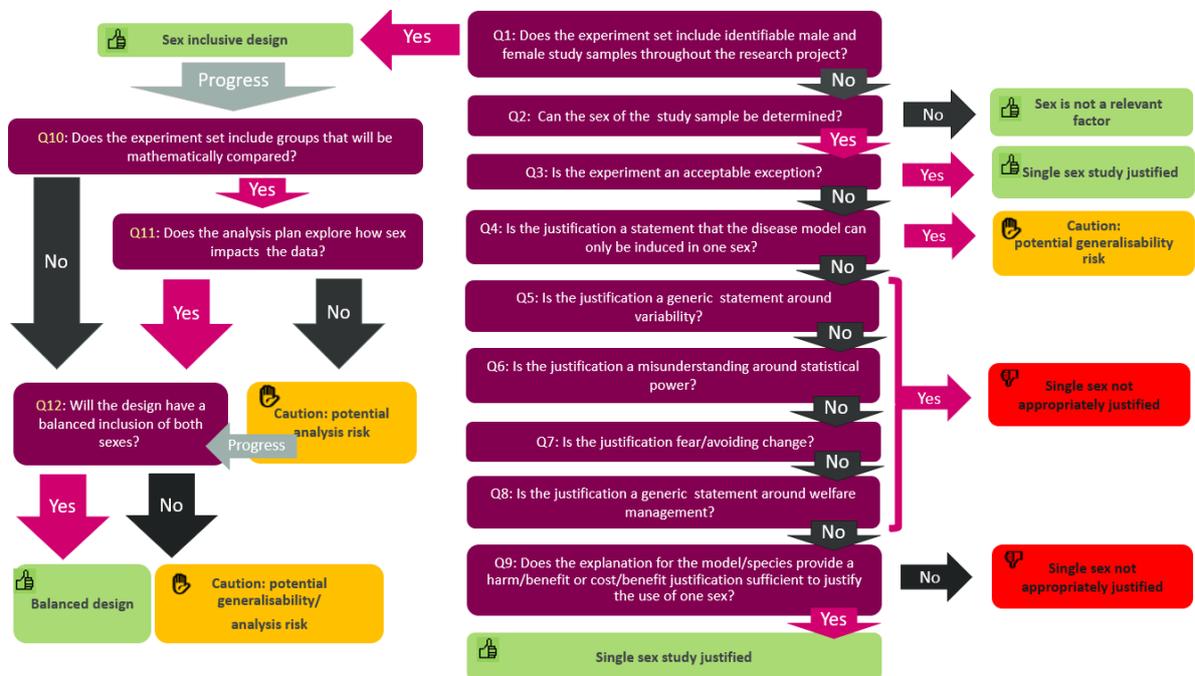
65 Here we present a framework to rapidly assess an in vivo or ex vivo research proposal to determine
66 whether the proposal is sex inclusive, balanced and whether analysis plans have included sex. When
67 a proposal includes only a single sex, the framework evaluates whether the justification is a
68 scientifically appropriate and a reflective assessment that is not based on common misconceptions.
69 The framework fulfils multiple objectives: (1) to provide transparency in the assessment process for
70 both researchers and those evaluating the proposals, thus aligning expectations, (2) to deliver
71 reproducible and unbiased evaluation of the proposal, and (3) to help address common
72 misconceptions, thus encouraging considered justifications that will enable a better understanding of
73 when sex inclusive research is possible.

74 The Sex Inclusive Research Framework

75 An original decision tree concept was initiated and developed by a working group of community
76 leaders, including representatives from industry, academia, ethical review committees and funding
77 review communities (**Figure 1**). In addition to the decision tree, detailed supporting information for
78 each question consisting of assessment advice and why the question was included is provided. This

79 framework has been tested in a grant review cycle by a reviewing panel and by several UK-based ethics
 80 review boards.

81 The framework can be executed via a pdf document or alternatively via an interactive web interface.
 82 The interface returns a report which can be submitted alongside the research proposal to the
 83 assessment body. To support the use of the framework (Supplementary 1 – SIRD document version),
 84 the website contains supporting information including a recorded seminar on the framework, FAQs
 85 (Supplementary 2 - SIRD FAQs), and some example classifications from a published dataset of single
 86 sex justifications (Supplementary 2 – Evaluation justification dataset).



87

88 **Figure 1: The Sex Inclusive Research Framework decision tree.**

89 Underpinning the Sex Inclusive Research Framework is a decision tree consisting of twelve questions which,
 90 when applied to a research proposal, results in the assignment of one or more traffic light outcome classification
 91 indicating whether a proposal is appropriate, carries some risk or insufficient with regards to sex inclusion.

92

93 Limitations of the framework

94 The present framework was developed to support the evaluation of *in vivo* or *ex vivo* research
95 proposals. If a proposal contains multiple sets of experiments, then the framework needs to be
96 independently applied to each. Adaption or the development of a separate framework is being
97 considered for *in vitro* research projects and will need development with a different set of
98 stakeholders.

99 The framework provides a structured set of questions to evaluate a proposal. However, many of the
100 questions require a subjective evaluation, which could lead to variations in the judgement reached.
101 The provision of supporting information for each question should mitigate that risk. Furthermore,
102 decisions for a question may shift in time as science/culture evolve. For example, different
103 communities might have a different ability to identify the sex of a sample when genetic testing might
104 be required, due to access to appropriate technology and the associated costs. This could move the
105 classification from one where the single sex is not appropriately justified to appropriately justified
106 when considering a cost/benefit evaluation.

107 Implementation of the framework

108 Effective implementation of sex inclusive research policies requires regulatory bodies to provide
109 training and guidance for applicants and evaluators (16). This framework aligns with this need by
110 providing a knowledge-base and practical support for the implementation of sex inclusive research
111 policies with a system openly accessible to staff, applicants and evaluators.

112 The framework may be used in several ways. Researchers could use the framework before submitting
113 a proposal or application to evaluate their position in a manner consistent with how it will be
114 evaluated by a funding panel or ethical review body. Research funding bodies or ethical review body
115 assessors could independently evaluate research proposals, either with the PDF format or through the

116 web tool. Alternatively, the assessors could request that applicants submit a report as evidence of the
117 applicant's assessment of their justification and review the classification provided.

118 The assessing bodies will need to explore the applicability of the framework to their specific area. For
119 example, the framework includes a question on whether the design has equal representation. If the
120 information is not collected during the application process, even in a situation where both sexes are
121 included; warnings might accumulate around the analysis or design. There are several options for the
122 assessing body: proceed with this potential risk, request additional information, adapt the decision
123 tree for their application process, or adapt the application process. An assessment of national funding
124 agencies sex, gender and diversity analysis policies, concluded that funders should provide applicants
125 and evaluators similar forms and instructions for consistency across the research process (16). This
126 framework could help provide a consistent evaluation for efficient engagement by the research
127 community.

128 Conclusions

129 Through this initiative, we aim to support the research community in using both sexes in the design
130 and analysis of preclinical experiments by providing a framework to differentiate genuine barriers
131 preventing the use of both sexes from culturally embedded misconceptions. Cultural change is
132 necessary to make sex inclusive research the standard for scientific rigor, excellence, and combating
133 sex bias in biomedical research.

134 References

- 135 1. Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav*
136 *Rev.* 2011;35(3):565-72.
- 137 2. Mazure CM, Jones DP. Twenty years and still counting: including women as participants and
138 studying sex and gender in biomedical research. *BMC women's health.* 2015;15(1):94.
- 139 3. Taylor KE, Vallejo-Giraldo C, Schaible NS, Zakeri R, Miller VM. Reporting of sex as a variable
140 in cardiovascular studies using cultured cells. *Biology of sex differences.* 2011;2(1):11.
- 141 4. Mogil JS. Qualitative sex differences in pain processing: emerging evidence of a biased
142 literature. *Nature Reviews Neuroscience.* 2020;21(7):353-65.

143 5. MRC. Sex in experimental design - Guidance on new requirements
144 [https://www.ukri.org/councils/mrc/guidance-for-applicants/policies-and-guidance-for-](https://www.ukri.org/councils/mrc/guidance-for-applicants/policies-and-guidance-for-researchers/sex-in-experimental-design/)
145 [researchers/sex-in-experimental-design/](https://www.ukri.org/councils/mrc/guidance-for-applicants/policies-and-guidance-for-researchers/sex-in-experimental-design/): UK Research and Innovation; 2022 [
146 6. NIH. NIH Policy on Sex as a Biological Variable [https://orwh.od.nih.gov/sex-gender/nih-](https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable2016)
147 [policy-sex-biological-variable2016](https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable2016) [
148 7. Stanford SC, Alexander S, Cirino G, George CH, Insel PA, Kendall D, et al. Considering and
149 reporting sex as an experimental variable II: An update on progress in the British Journal of
150 Pharmacology. *British Journal of Pharmacology*. 2023.
151 8. Weitowich NC, Beery A, Woodruff T. A 10-year follow-up study of sex inclusion in the
152 biological sciences. *Elife*. 2020;9.
153 9. Garcia-Sifuentes Y, Maney DL. Reporting and misreporting of sex differences in the biological
154 sciences. *Elife*. 2021;10.
155 10. Karp NA, Reavey N. Sex bias in preclinical research and an exploration of how to change the
156 status quo. *Br J Pharmacol*. 2019;176(21):4107-18.
157 11. Group MW. Sex in experimental design: summary report
158 <https://www.ukri.org/publications/sex-in-experimental-design-summary-report/>: UK Research and
159 Innovation; 2022 [updated 10th March 2023].
160 12. Becker JB, Prendergast BJ, Liang JW. Female rats are not more variable than male rats: a
161 meta-analysis of neuroscience studies. *Biology of sex differences*. 2016;7(1):1-7.
162 13. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and
163 biomedical research. *Neuroscience & Biobehavioral Reviews*. 2014;40:1-5.
164 14. Phillips B, Haschler TN, Karp NA. Statistical simulations show that scientists need not
165 increase overall sample size by default when including both sexes in in vivo studies. *Plos Biology*.
166 2023;21(6):e3002129.
167 15. UKRI. Inclusion of both sexes in research design - Call for input
168 [https://engagementhub.ukri.org/mrc-regulatorysupportcentre/inclusion-sex-in-research-](https://engagementhub.ukri.org/mrc-regulatorysupportcentre/inclusion-sex-in-research-design/2023)
169 [design/2023](https://engagementhub.ukri.org/mrc-regulatorysupportcentre/inclusion-sex-in-research-design/2023) [
170 16. Hunt L, Nielsen MW, Schiebinger L. A framework for sex, gender, and diversity analysis in
171 research. *Science*. 2022;377(6614):1492-5.

172

173 Author contribution

174 **Natasha A. Karp** Conceptualization, Project Administration, Methodology, Supervision, Validation,
175 Writing – Original Draft Preparation, Visualisation

176 **Manuel Berdoy** Methodology Writing – Review & Editing

177 **Lilian Hunt** Methodology Writing – Review & Editing

178 **Maggy Jennings** Methodology, Writing – Review & Editing

179 **Angela Kerton** Methodology, Writing – Review & Editing

180 **Matt Leach** Funding Acquisition, Methodology, Software, Writing – Review & Editing

- 181 **Jon Gledhill** Software, Writing – Review & Editing
- 182 **Jordi Lopez-Tremoleda** Methodology, Writing – Review & Editing
- 183 **Esther J. Pearl** Methodology, Validation, Writing – Review & Editing
- 184 **Nathalie Percie du Sert** Methodology, Validation, Writing – Review & Editing
- 185 **Benjamin Phillips** Methodology, Writing – Review & Editing
- 186 **Penelope S Reynolds** Writing – Review & Editing
- 187 **Kathy Ryder** Methodology, Writing – Review & Editing
- 188 **Clare Stanford** Methodology, Writing – Review & Editing
- 189 **Sara Wells** Methodology, Writing – Review & Editing
- 190 **Lucy Whitfield** Methodology, Writing – Review & Editing
- 191
- 192