

## Example classifications using the Sex Inclusive Research Framework

- The framework has been applied to a dataset of published justifications from a variety of manuscripts and the resulting classification determined (see Table below).
- This is a post publication analysis and the extract only included limited information which allowed a focus on whether the design was sex inclusive but limited the evaluation around the design and analysis elements.
- Dataset source: Woitowich, Nicole C., Annaliese Beery, and Teresa Woodruff. "A 10-year follow-up study of sex inclusion in the biological sciences" *Elife* 9 (2020): e56344.

| Category   | Published rationale                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Classification summary                                            | Classification using the structured framework                                            |
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| Behavior   | "Because adult males and juveniles of either sex rarely called, we report data from adult females only"                                                                                                                                                                                                                                                                                                                                                                             | Single sex study justifiable                                      | Q3 - acceptable exception (scarcity resource)                                            |
| Physiology | "As we have previously shown sex-specific downregulation of SIRT1 by MHF in male offspring (Nguyen et al. 2017), only male offspring were examined in the present study."                                                                                                                                                                                                                                                                                                           | Single sex study justifiable                                      | Q3 - acceptable exception (sex specific mechanism)                                       |
| Physiology | "No sex differences were detected on primary outcome variables; therefore, data from male and female subjects were pooled for analysis."                                                                                                                                                                                                                                                                                                                                            | Sex inclusive design                                              | From the information provided cannot assess the analysis or design                       |
| Physiology | "However, we observed in preliminary studies that female ob mice also have significant hyperoxaluria (~2.8-fold) compared with lean controls. We observed some difficulties when dealing with the female ob mice including finding bedding within their vaginal cavities mixed with smegma (a combination of shed skin cells, skin oils, and moisture) and in some cases blood, as well as an increased frequency of urine scalding (an acidic substance that can burn and irritate | Single sex study justified following harm/cost benefit evaluation | Q9 - cost/benefit justification provided (welfare, logistic issues in management - time) |

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|                       | the skin). Cleaning this out is quite time consuming, and we concentrated on male ob mice."                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                             |                                                                                                                                                           |
| Behavioral Physiology | Thirty-two male Long Evans rats were obtained at 51–75 g...PPA has been associated with neurodevelopmental disorders including Autism Spectrum Disorders, which tends to occur more commonly in males than females [15]. As such, male rats were the primary focus in the present study, albeit a limitation, as there is a lack of research on the effect of PPA on female rats.                                                                                                               | Single sex not appropriately justified                                                                                      | Q7 - fear of change. Acknowledges that the disease of interest does occur in both sexes and there is a lack of knowledge on the effect of PPA on females. |
| Physiology            | "To minimize extrinsic variability, we used adult wild-type mice (C57BL/6J, Jackson Laboratories) of the same sex (male) maintained on the same diet (normal) and exposed to the same light-dark cycles and limited exercise."                                                                                                                                                                                                                                                                  | Single sex not appropriately justified                                                                                      | Q5 - misconception on variability (baseline differences)                                                                                                  |
| Immunology            | "Experiments on Foxp3Sf mice and FoxpWT littermate controls were done in male mice because the Foxp3Sf allele is X-linked and the experiments could not be done in female mice. Th9 transfer experiments were all done in male mice because Foxp3Sf donor Th9 cells were obtained from male mice and could not be transferred to female recipients due to risk of rejection. All remaining experiments were done in both male and female mice and were age and sex-matched within experiments." | Some experiments: Single sex study justified following harm/cost benefit evaluation Other experiments: Sex inclusive design | Q9 - harm/benefit (risk of tissue rejection). For the inclusive experiments, the design and analysis could not be assessed from the information provided. |
| Immunology            | "We only used male mice in this study to avoid potential artifacts that may arise in gender mismatched adoptive transfer (AT) settings.                                                                                                                                                                                                                                                                                                                                                         | Single sex study justified following harm/cost benefit evaluation                                                           | Q9 - harm/benefit and cost/benefit justification provided                                                                                                 |
| Behavior              | "...we were unable to consistently distinguish between male and female prairie dogs and therefore pooled both sexes in analyses.                                                                                                                                                                                                                                                                                                                                                                | Sex is not a relevant factor                                                                                                | Q2 - sex of study sample cannot be determined                                                                                                             |

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| Behavior              | "While we tested too few females (n = 2) to perform statistical tests comparing males and females, we saw no evidence of sex-based differences in behavior."                                                                                                                                                                                                                                         | <b>Sex inclusive design with caution: potential generalisability/analysis risk</b> | Q1: Sex inclusive design with<br>Q12: generalisability risks due to imbalance inclusion.                                |
| Pharmacology          | "we did not have the power to examine for sex effects in the current design" "Gender (M:F) 31:16"                                                                                                                                                                                                                                                                                                    | <b>Sex inclusive design with caution: potential generalisability/analysis risk</b> | Q1: Sex inclusive design with<br>Q12: generalisability risks due to imbalance inclusion.                                |
| Endocrinology         | All data presented are from female mice....In addition, neuronal deletion of RANK has been shown to be involved in thermoregulation in female but not male mice, although it is unknown exactly which neurones are involved in this process. Therefore, we also used our female NPYcre/+;RANKlox/lox mice to investigate whether NPY neurones contribute to the control of thermoregulation by RANK. | <b>Single sex study justifiable</b>                                                | Q3 - acceptable exception (single sex mechanism)                                                                        |
| Neuroscience          | "In all experiments, male and female littermates were pooled together and analyzed as one group"                                                                                                                                                                                                                                                                                                     | <b>Sex inclusive design with Caution potential analysis risk</b>                   | Q1 - both sexes included but<br>Q11 - data pooled                                                                       |
| General Biology       | All animals analysed were P3 or younger, thus no sex determination was attempted. Analyses are thought to include animals of both sexes at approximately equal proportions."                                                                                                                                                                                                                         | <b>Sex is not a relevant factor</b>                                                | Q2 - sex of study sample cannot be determined (*could genetic testing have been used? Cost might have been prohibitive) |
| Endocrinology         | "Six of the eight participants were women and consequently the study was not designed to assess the effects of sex on the metabolic response to dietary intervention."                                                                                                                                                                                                                               | <b>Caution: potential generalisability/analysis risk</b>                           | Q12 - Inclusive design but unbalanced and no analysis for effect of sex possible                                        |
| Behavioral Physiology | "Only women were recruited to reduce unwanted variability in the design"                                                                                                                                                                                                                                                                                                                             | <b>Single sex not appropriately justified</b>                                      | Q5 misconception on variability (baseline differences)                                                                  |
| General Biology       | "studies in the fetal period used the male offspring while studies in the adult period used the female offspring, as ewe lambs are easier to group house when compared to growing rams"                                                                                                                                                                                                              | <b>Single sex study justified following harm/cost benefit evaluation</b>           | Q9 - harm/benefit and cost/benefit justification provided                                                               |
| Endocrinology         | Because collecting vaginal smears to control oestrous cycle in females would add an extra layer of stress to                                                                                                                                                                                                                                                                                         | <b>Single sex not appropriately justified</b>                                      | Q5 - misconception on variability (needing to control stage of female cycle)                                            |

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|                       | our experiments, we performed the experiments in male pups only.                                                                                                                                                                                                                    |                                                                   |                                                                                           |
| Physiology            | "We focused upon a single gender, as there are sex differences in glucose metabolism"                                                                                                                                                                                               | Single sex not appropriately justified                            | Q5 - misconception on variability                                                         |
| Endocrinology         | "Since historically, predominantly females are exposed to these estrogenic compounds through contraceptive use and estrogen replacement therapy, and lupus exhibits a strong female sex bias, for this study we investigated the effects of E2 and EE on female NZB/WF1 mice only." | Single sex study justified following harm/cost benefit evaluation | Q9 - disease specific explanation.                                                        |
| Endocrinology         | "Female subjects were not enrolled to avoid potential gender-dimorphic variations in bone microarchitecture. "                                                                                                                                                                      | Single sex not appropriately justified                            | Q5 misconception on variability (baseline differences)                                    |
| Pharmacology          | "Because female mice are known to have a higher expression of retinoic X receptors and higher sensitivity to FXR agonists than males (Kosters et al., 2013), we focused on females in this study."                                                                                  | Caution: potential generalisability/inference risk                | Q4 - model is induced in females                                                          |
| Neuroscience          | "Because we have detected no difference in phenotype between male and female animals in past or present study of this animal model, results from both sexes were combined."                                                                                                         | Sex inclusive design                                              | Q12 - Assuming equally inclusion and appropriate statistical testing to simplify analysis |
| Behavioral Physiology | "We chose to use male mice, as female mice tend to have twice the levels of circulating CORT as males, and these levels may shift in response to stage of the estrus cycle"                                                                                                         | Single sex not appropriately justified                            | Q5 - misconception on variability (baseline differences and female variability)           |
| Neuroscience          | "At P21, litters were weaned and only male pups were included in the experiments described below as gender-specific effects of VPA exposure have been reported"                                                                                                                     | Single sex study justifiable                                      | Q3 - single sex disease justification (*studying a phenotype which only occurs in males)  |
| Behavior              | "We could not compare the sexes as only four of the 25 wood mice were females. However, we have previously shown that the sex of wood mice has no effects on seed management "                                                                                                      | Caution: potential generalisability/inference risk                | Q12 - Sex inclusive design with generalisability risks due to imbalance inclusion.        |

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| Pharmacology  | "we used males exclusively in these experiments to minimize possible variability resulting from sex differences"                                                  | <b>Single sex not appropriately justified</b> | Q5 - misconception on variability (baseline differences) |
| Neuroscience  | "To avoid the influence of hormonal changes, only male rats were included in this study."                                                                         | <b>Single sex not appropriately justified</b> | Q5 - misconception on variability (female variability)   |
| Endocrinology | "We included only male mice to limit possible confounding off-target effects of tamoxifen in young female mice and other sex hormone turbulences of female mice." | <b>Single sex not appropriately justified</b> | Q5 - misconception on variability (female variability)   |
| Neuroscience  | "Using male mice ensured reduced gender-dependent variability in plaque deposition."                                                                              | <b>Single sex not appropriately justified</b> | Q5 - misconception on variability (baseline differences) |