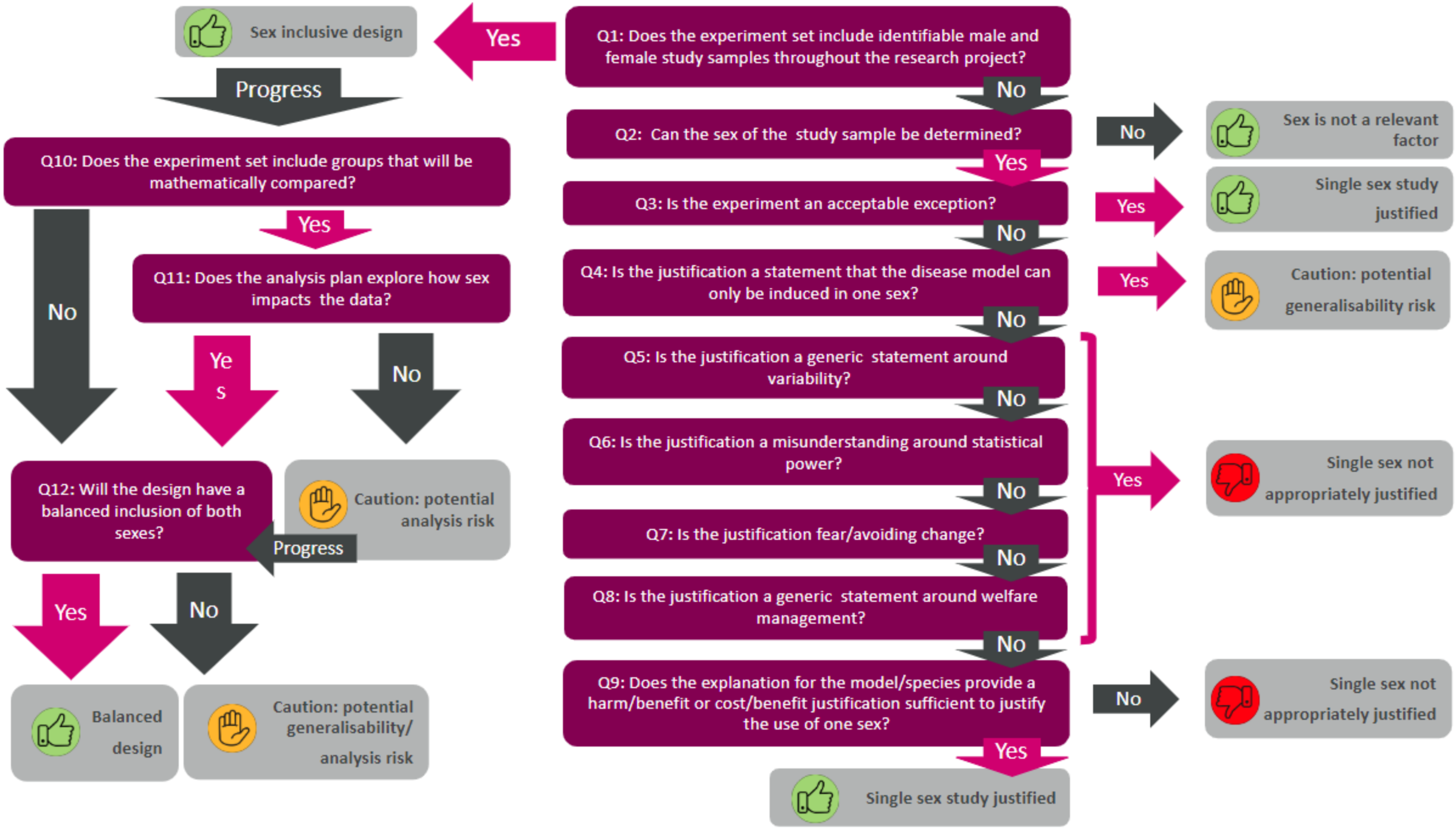

Q1: Does the experiment set include identifiable male and female study samples throughout the research project?
Yes
Sex inclusive design
Progress
No
Q2: Can the sex of the study sample be determined?
No
Sex is not a relevant factor
Yes
Q3: Is the experiment an acceptable exception?
Yes
Single sex study justified
No
Q4: Is the justification a statement that the disease model can only be induced in one sex?
Yes
Caution: potential generalisability risk
No
Q5: Is the justification a generic statement around variability?
No
Q6: Is the justification a misunderstanding around statistical power?
No
Q7: Is the justification fear/avoiding change?
No
Q8: Is the justification a generic statement around welfare management?
No
Yes
Single sex not appropriately justified
Q9: Does the explanation for the model/species provide a harm/benefit or cost/benefit justification sufficient to justify the use of one sex?
No
Single sex not appropriately justified
Yes
Single sex study justified
Q10: Does the experiment set include groups that will be mathematically compared?
Yes
Q11: Does the analysis plan explore how sex impacts the data?
No
Yes
No
Q12: Will the design have a balanced inclusion of both sexes?
Caution: potential analysis risk
Progress
Yes
No
Balanced design
Caution: potential generalisability/ analysis risk

Examples “Caution is required”

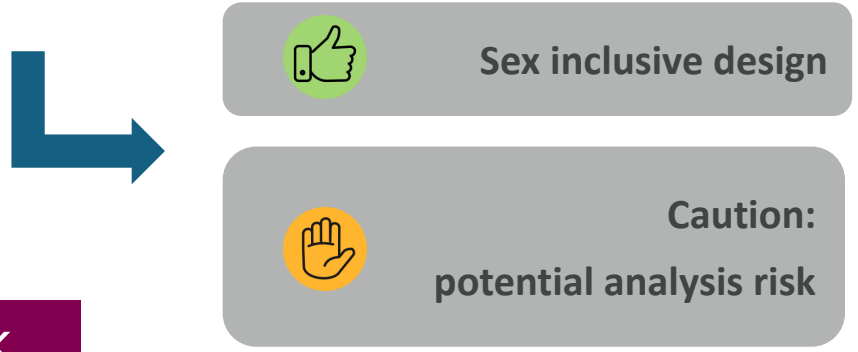
Arises?

- Unbalanced inclusive designs
  - **Generalisability/analysis risks**
- Inclusive designs that do not consider sex in the analysis
  - **Analysis risks**
- Studies for disease which effects both sexes but the model can only be induced in one
  - **Generalisability risk**

Example scenario

'In all experiments, male and female littermates will be pooled together and analyzed as one group“ (Woitowich, 2020)

- Q1 – inclusive? Yes
- Q10 – Groups compared? Yes
- Q11 – analysis considers sex? No



Decision to proceed depends on reflection on the risk

Examples "Single sex not appropriately justified"

Arises?

Misconceptions

- "Females are more variable"
- "Including both sexes will increase the variation in my data"
- "Including both sexes will double the sample size needed"

Fear/Avoiding change

- "My previous data is all in one sex"
- "Sex hasn't been shown to date to matter"

Example scenario

"We plan 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." (Woitowich, 2020)

Q1 – inclusive? No

Q2 – Can the sex be determined? Yes

Q3 – acceptable exception? No

Q4 – disease model induction issue? No

Q5 – generic statement around variability Yes

Single sex not appropriately justified

Examples “proposal is appropriate outcomes”

Exception?

“Female mice implanted with patient derived ovarian cancer tumours” (Woitowich, 2020)

Q1 – inclusive? No

Q2 - Can the sex be determined? Yes

Q3 – acceptable exception? Yes

Single sex study justified

Harm &/or Cost evaluation versus benefit

“Th9 transfer experiments will be done in male mice because Foxp3Sf donor Th9 cells are obtained from male mice and could not be transferred to female recipients due to risk of rejection.” (Woitowich, 2020)

Q1 – inclusive? No

Q2 – can the sex be determined? Yes

Q3 – acceptable exception? No

Q4 – disease model induction issue? No

Q5:8 – misconceptions/fear of change? No

Q9: Cost &/or harm versus benefit? Yes

Single sex study justified

Case study

A researcher intends to run a study on renal function, attempting to evaluate the impact of compound X on blood urea nitrogen (BUN) levels.

The researcher typically works with only males, and conducts a power calculation which indicates that group sizes of 10 control and 10 compound X mice are appropriate to detect the effect size of interest. The researcher has no prior information about the impact of the compound in female mice, but would like to run a sex-inclusive study. **They conduct the following power calculations:**

Historic standard deviation: 3.5
Historic control mean: 9.5
Target effect size: 50% change
Power with *n* = 10: 0.82

As the researcher intends to use a 2-way factorial ANOVA to analyse the data (factors - treatment and sex), they take the intended sample size (10 per group), and evenly split the study between female and male mice (5M & 5F control, 5M & 5F compound X).

Since the factorial analysis handles the sex-related variation in the data, under most circumstances power to detect the treatment effect is retained. Under the circumstances power for the treatment effect is lost (e.g., the treatment effect is opposite between the sexes), power to detect a statistical interaction between treatment and sex increases, and important biological information is gained. As the researcher gains more information about the impact of compound X on renal function, they may conduct further precise power calculations (e.g., if the variances between the sexes substantially differ).