

Maximising the success of rat bile duct cannulation studies: recommendations for best practice

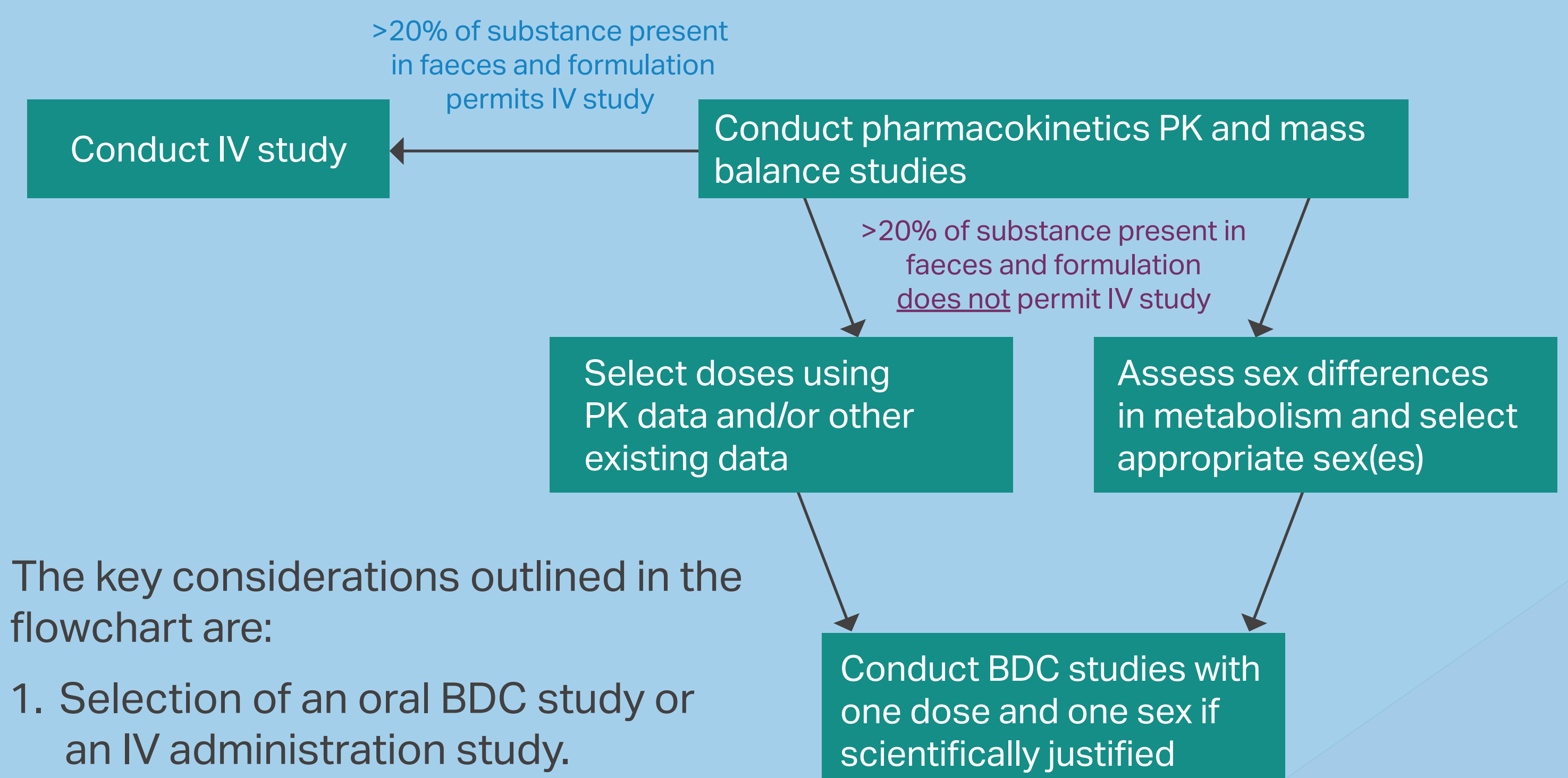
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Introduction

- Bile duct cannulation (BDC) studies are carried out to support assessment of absorption, distribution, metabolism and excretion profiles of agrochemicals and pharmaceuticals.
- They are most commonly conducted using the rat and often to OECD Test Guideline 417.
- They are triggered when >20% of the test substance is present in faeces following oral dosing, for substances that cannot be dosed intravenously (IV).
- Preparation of the animals for BDC studies is technically complex, requiring relatively invasive and skilled surgical techniques.
- The dosing and bile collection aspect of the studies can also be intricate, involving the single housing of animals within metabolism cages (Metabowls®) and implantation of cannulas that are at risk of twisting and/or blocking.
- Failure of the patency of a cannula while on study can result in insufficient data being generated to meet the objectives, and therefore repetition of the study.
- The NC3Rs recently convened a working group across three major contract research organisations that routinely carry out BDC studies.
- The group identified opportunities for best practice for BDC studies to maximise the amount of useful data generated whilst ensuring the highest possible standards of animal welfare.

Considerations prior to starting BDC studies

Before animals are prepared for a BDC study, some key factors should be considered to establish whether the BDC study is most appropriate and necessary to answer the scientific questions, and if so how the studies should be designed with regard to sex and strain of animals used and doses tested.



The key considerations outlined in the flowchart are:

1. Selection of an oral BDC study or an IV administration study.
2. Inclusion of one or both sexes on BDC studies.
3. Selection of doses and number of dose groups.

Recommendations for ensuring high success rates once BDC studies are triggered

1. General considerations

- **Monitoring of success rates**
 - There should be recording and close monitoring of success rates across all aspects of the study to aid in identifying key areas to focus on for improvement should there be sub-optimal success rates.
- **Impact of additional cannulations**
 - Additional cannulations tend to negatively impact success rates therefore full justification for the inclusion of additional cannulas should be provided.
- **Weight of animals selected for study**
 - Animals should be at least 200-250 g, preferably 225-275 g, at the time of surgery to ensure that the bile ducts are of a sufficient size for cannulation.
- **External influences**
 - Hydrogel/DietGel (nutrient fortified water gel) tubs and water softened pellets can be beneficial during the first few days after surgery, but it should not be necessary to offer oral rehydration solutions during the bile collection phase of study, particularly since the infusion of replacement bile salts into the duodenum provides supplementary fluids and by this stage animals should be sufficiently recovered from surgery to maintain normal hydration. If other supplements are given these should be standardised between animals in terms of the amount given and the timing of administration since they could otherwise affect the outcome of the study.

3. Post-operative considerations

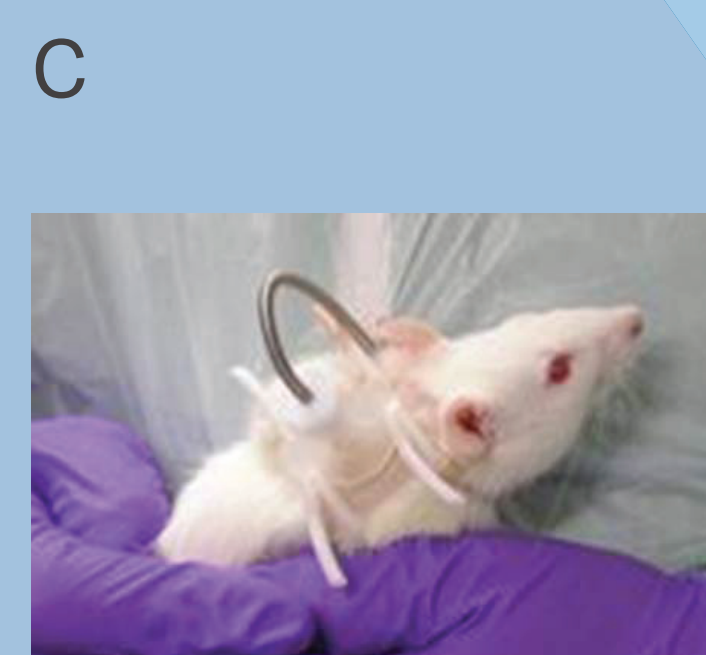
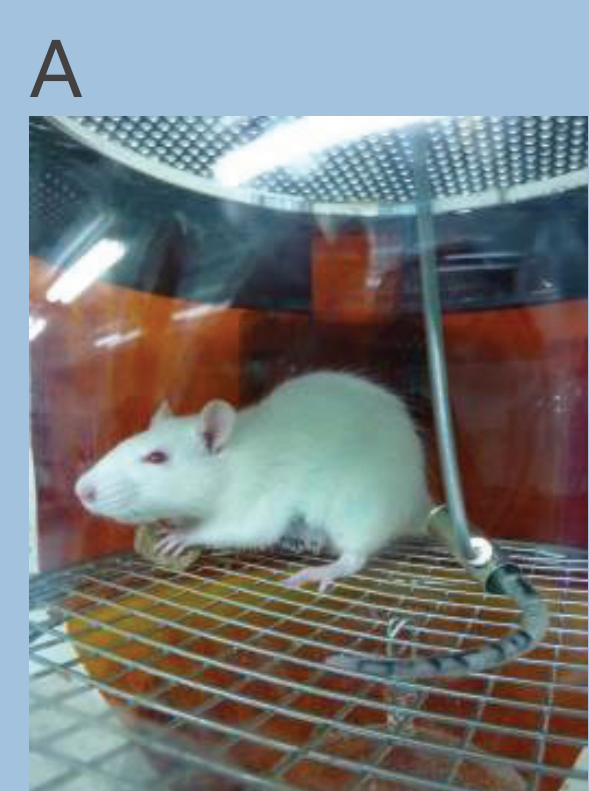
- **Post-operative monitoring and handling**
 - Staff should have the appropriate level of experience with the model and understand the gentle approach required following surgery, and successful troubleshooting of issues that arise with the cannulas or swivel system. The handling of animals following surgery should be minimised so as not to disturb the cannulations, and the flushing of bile duct cannulas should be avoided.
- **Assessment of clinical signs**
 - The decision whether to take forward an animal for use on study depends on the general condition following surgery, whether the animal has retained sufficient body weight (no higher than a 15% loss in weight), and whether there is successful bile flow through the cannula. Clinical signs should be routinely observed and recorded.
- **Assessment of bile flow**
 - Flow should be checked daily following surgery. The colour of the bile should be monitored as a dark colouration can indicate bile stasis.
- **Understanding causes of problems**
 - If an animal cannot enter the data collection phase of the study or it is removed from study during the data collection period a necropsy should be performed, including examination of the cannula positioning.

2. Surgical considerations

- **Aseptic techniques**
 - Surgery should be performed in a serviced surgical facility suitable for aseptic procedures, by personnel trained and experienced in appropriate techniques. The surgical procedure must be performed consistently to deliver a repeatable outcome whilst reducing complications.
- **Positioning of cannula**
 - If sub-optimal success rates are experienced, the positioning of the cannula should be one of the first factors examined. Incorrect positioning of the cannula either during surgery or due to its movement following surgery can lead to leakage from the cannula or complete dislodgment of the catheter.

4. Considerations during dosing/bile collection

- Animals should be placed in their Metabowls overnight the night before study and successful bile flow through the cannula checked at this point.
- If pilot study data or data from other phases of the metabolism study are available these should be used to determine the length of time necessary for bile collection. This will ensure animals are not singly housed for longer than necessary.
- Modifying standard Metabowls can improve the animals' access to food and their overall welfare. Other modifications applicable to all types of metabolism study include the application of red transparent film to the sides of the Metabowl, which has been shown to have a beneficial effect on markers of well-being in rats.



A) Typical Metabowl set-up for animals with tail exteriorisation. B) Example of amended Metabowl enabling easier access to food and water. C) Animal with nape of neck exteriorisation and harness attachment of swivel system.

Concluding remarks

- Consideration and incorporation of the recommendations described here should result in the generation of useable data from >80% of animals that are surgically prepared.
- The ultimate aim is to consistently achieve 100% success rates but in some instances removal of an animal from a study will be unavoidable, for example if the cannula becomes irreversibly twisted after an animal has been placed in a Metabowl.
- Undertaking the measures proposed here will minimise these events as far as possible.