



Minor Procedures (general principles)

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Introduction

- Learning outcomes
 - In handout
 - More information in practical sessions
- References:
 - Procedures with care www.procedureswithcare.org.uk
 - BVA-AWF tutorials <http://www.ahwla.org.uk/site/tutorials/BVA/BVA01-Title.html>
 - Norwegian Reference Centre for Laboratory Animal Science and Alternatives (<http://film.oslovet.veths.no/>)
 - AALAS learning library - <https://www.aalaslearninglibrary.org/>

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LO: 1.1

Before You Begin - preparation

Is the procedure permitted:

- ...by the Project Licence?
- ...by your Personal Licence?
- ...by the Establishment Licence?

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LO: 3.1.9

Record Keeping

Standard condition 20 to a PIL

1. "The personal licensee shall maintain a record of all animals on which procedures have been carried out...
2. ...including details of supervision and declarations of competence by the PPL holder as appropriate.
3. This record shall be retained for at least five years and shall... be submitted to the Secretary of State or made available to an Inspector."

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LO: 7.6

Standard operating procedures?

- SOPs or quality assurance schemes such as GLP/GMP?
- Improves consistency
- May be required for some (regulatory) studies
- Allows for acceptance of results in other countries
- Applies to conduct of procedures and storage of drugs and compounds

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Environment and equipment

- Ensure clean uncluttered environment
 - No storage in procedure room
- Get equipment ready
 - Make sure you can use it!
 - Appropriate for the species/procedure
 - Seek advice as required



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Prepare yourself

- Appropriate protective clothing
- Practise makes perfect
 - Cadavers, models, observe others
 - Online resources
 - http://www.theodora.com/rodent_laboratory/injections.html
- Check dose/sample volume
 - Determined by route, compound and size/species
 - As small as practical
 - Frequent dosing may be needed for small animals

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Calculating dose volumes

- Dose (ml) = $\frac{\text{Dose rate (mg/kg)} \times \text{weight (kg)}}{\text{Concentration (mg/ml)}}$
- **Example:**
 - Rat weighs 250g
 - Dose rate for ketamine = 10mg/kg
 - Ketamine concentration = 100mg/ml
 - Dose volume = $\frac{10 \times 0.25}{100} = 0.025\text{ml}$
 - May need to dilute with sterile diluent if volumes are small

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LO: 7.3

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Refinement

- Scientific procedures can cause pain and distress
- This must be minimised, otherwise
 - Welfare implications
 - Science may suffer
- You have a responsibility to check you are complying with the 3 Rs
- Seek advice (NVS, NACWO, PPLH, NIO etc)
- Check the literature
- **Do not start unless you are confident that you have chosen the most refined method available**

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Important steps in refinement

- Acclimatisation
 - Transportation and movement into a new facility can cause stress
 - Animals can take 1-2 weeks to settle after transport
 - An acclimatisation period allows animals time to stabilise in a new environment and promotes both animal welfare and good science.
- Training of animals
 - Even mice can be trained!
 - Positive reinforcement training
- Careful animal handling

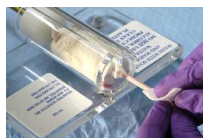
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Performance of procedures

- Sterile technique required
 - New, sterile needle for each animal
 - Compounds for administration should be sterile
 - May need to disinfect injection site – care not to drown the animal!
Use warmed fluids, use alcohol sparingly



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Common Methods of Dosing

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ADME – pharmacokinetics

- Absorption – depends on blood flow, solubility of cpd, presentation
 - First pass effect for oral compounds
- Distribution – depends on blood flow, solubility/nature of cpds
- Metabolism – mostly in liver, affected by many factors
- Excretion – usually kidneys, can be via bile or lungs
- These four determine how the compound behaves in the body = pharmacokinetics
- Must be understood before administration of the compound
- Bioavailability = fraction of administered cpd reaching bloodstream. High if iv, lower for other routes

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Oral Dosing

- Tablets or oral paste
- Food or water – all group
- Gavage/stomach tube
- (Nose/mouth)



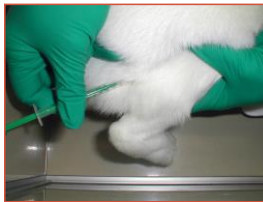
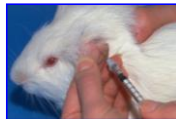
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Injections

- Intraperitoneal - SA only
- Subcutaneous
- Intravenous
- Intradermal
- Intramuscular - painful



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Injections in farm animals

- <https://www.youtube.com/watch?v=hV5KVSu9vD8>
- Sc and id
- <https://www.youtube.com/watch?v=CF5y92DijZQ>
- im
- <https://www.youtube.com/watch?v=op6zcThF7BY>
- Bovine Tail vein

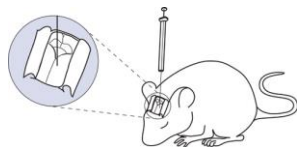
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Less common routes

- Topical
- Inhalation
- Transdermal
- Intracranial



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Common Methods of Sampling

Blood, DNA, Urine, Faeces

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Blood sampling - Potential Adverse Effects

For animal -

- Pain
- Haemorrhage
- Bruising
- Thrombosis
- Phlebitis
- Infection
- Hypovolaemia – if too much too quickly

For sample –

- Clotting
- Haemolysis

All can be minimised by skilled, aseptic technique and proper handling

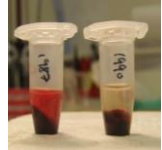
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Before you start...

- Samples must be good quality and preserved appropriately. Must determine requirements before you take the sample.....
- Choose the right anticoagulant (if needed)
 - Heparin, EDTA, oxalate/fluoride
- Think about storage and shipment
- Fresh, chilled, frozen



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Venipuncture Technique

- Choose device
 - Needle, butterfly, catheter
- Choose container
 - capillary tube, syringe, Vacutainer
- Consider topical aids
 - Local anaesthetic, vasodilator
 - Tourniquet?

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Blood Collection

- Choose site
 - Small samples
 - Needle puncture, peripheral blood vessels
 - Large samples
 - Central veins, arteries
 - Cardiac puncture, exsanguination
- Prepare site
 - Aseptic technique
 - Clip, clean with antibacterial cleanser
 - Alcohol

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Peripheral Veins

- Tail vein
- Saphenous vein
- Cephalic vein
- Marginal ear vein
- Wing vein



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Central veins

- Jugular
- Anterior vena cava



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Venipuncture technique

- Identify location of vein
- Immobilise and raise vein
- Insert needle –bevel uppermost
- Advance needle until blood appears in hub, then hold securely
- Obtain sample – care to avoid haemolysis. Suction, or allow to drip
- Volume – depends on experiment and animal
- Withdraw needle and ensure haemostasis – pressure!

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How Much Blood to Take?

- Total blood volume (TBV) is around 60-70ml/kg
- Take minimum for scientific need
- Impact depends on other factors
 - Age, health, physiological status of animal
 - Volume replacement
- For mild severity, 10% blood volume is typical (6-7ml/kg)

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DNA Sample Collection

- Blood
- Ear notch skin
- Tail tissue
- Cheek cells
 - Mouth cell collection device for newborn mice. Y.-H. Zhang, B.-L. Huang, K. Eastman, L.L. McCabe, N.K. MacLennan and E.R.B. McCabe. Molecular Genetics and Metabolism, September-October 2006



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Collection of Urine and Faeces

- As voided naturally
- Metabolism cage (PPL may be needed)
- Home cage with non-absorbent bedding
- Cystocentesis
- Urinary catheter
- Rectal swab
- Manual



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Summary

- Check authorities
- Get everything prepared first
- Take minimum sample or administer minimum volume necessary for science
- Minimise adverse effects by careful technique
- Practise, practise, practise!

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