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1. **PURPOSE:**
   - To provide step-by-step instruction to all persons performing an inhalation of air or CO\textsubscript{2} in clinical trials or research studies.

2. **REFERENCES:**
   - None.

3. **PERSONNEL REQUIRED AND LEVEL OF EXPERTISE:**
   - Investigator and/or research team.
   - Training required in cylinder use, CO\textsubscript{2}-enriched air administration and medical screening.
   - *A senior researcher trained in CO\textsubscript{2} administration who will be available for telephone support for inhalation session.*
   - *An individual trained in CPR who will remain in the same building as the session in order to provide emergency support if required.*

   *Note: this can be the same person.

4. **MATERIALS AND EQUIPMENT REQUIRED**
   - Medical air and/or CO\textsubscript{2} cylinders
   - Mask or mouthpiece
   - Douglas bag
   - Plastic tubing
   - Cleaning materials (see relevant SOP)
   - Timer
   - Nose clip (required if using mouthpiece)
   - Scripted CRF explaining inhalation procedure
   - SOP 14 – Dealing with panic attacks
   - Cylinder handling risk assessment
   - Administering hypercapnic challenges risk assessment

5. **PROCEDURE:**

5.1 **When:**

When administering inhalations of CO\textsubscript{2}-enriched air (referred to as CO\textsubscript{2} in text) to model the effects of anxiety, using medical air as a placebo or sham treatment.
5.2 Pre-Inhalation:

- Ensure you have enough air and/or CO₂ for the session. Air and CO₂ can be ordered from BOC via central Research Support. CO₂ mixtures take at least two weeks to arrive. Spare and empty cylinders must be stored in the school gas cage.
- Any cylinders that are stored in the labs MUST be in a cylinder trolley. They should never be left unsecured.
- Any empty cylinders or cylinders not in use should not be stored in labs – these should be removed to the gas cage. Regulators must be removed when storing cylinders in gas cage.
- Any labs with cylinders inside should have a compressed gas sticker on the door and the School Manager should be made aware that the lab contains cylinders.
- When taking a cylinder from the gas cage, check the label to ensure you are taking the correct gas mixture. Check label and ensure gas is still in date.
- Do not remove the label. This informs emergency services of the content of cylinder in case of an emergency.
- ALL USERS MUST REQUEST AND READ THE CYLINDER USAGE RISK ASSESSMENT. ALL USERS MUST HAVE COMPLETED TARG CYLINDER TRAINING BEFORE USING OR MOVING CYLINDERS.

5.3 Inhalation:

- Prior to running an inhalation the researcher must read SOP 14 which instructs how to recognise, and deal with, a panic attack. The likelihood of panic attacks is low (particularly for 7.5% CO₂ inhalations), but possible and therefore researchers should be clear on how to recognise and deal with them prior to running any inhalations.
- You should ensure that you have appropriate phone support: a person trained in CPR should be available to attend the session at any point (i.e., should be present in building for duration of the session and you should have a means of contacting them directly).
- Before fitting inhalation equipment to the participant, ensure you have completed any other tasks specified in the protocol or CRF, such as attaching heart rate monitors or completing questionnaires.
- A participant should never be put on an inhalation until they have completed and passed all screening procedures.
- The participant should never be left alone during the inhalations.

A. 20 MINUTE INHALATION OF MEDICAL AIR OR 7.5% CO₂:

Note: our current protocol stipulates a maximum inhalation of 7.5% CO₂ for a maximum of 20-minutes. Unless your protocol is approved to do otherwise, you must never exceed 20 minutes for any inhalation of 7.5% CO₂.

- Before the inhalation, check the cylinder you will be using contains enough gas. If not, you are advised to fit a new cylinder. Alternatively, you will have to switch cylinders mid-inhalation. If you only have one regulator, this will need to be moved to the new cylinder, so ensure you have the required tools (spanner/spanner key).
- To change cylinders, firstly close all valves. Then use the relevant spanner/spanner key to undo the bolt and remove the regulator. Attach to the regulator to the new cylinder, and tighten using the spanner. Once fixed you should turn the regulator on and check for leaks (you will hear gas escaping and moisture will become visible on regulator if there is a leak). **You should only attempt to change a cylinder regulator if you have had training in doing so.**
- To fill the bag, open the cylinder at the neck. All subsequent in-session gas flow control should be controlled via the regulator. Ensure that the three-way stop-cock is closed at the bag, otherwise gas will leak through the mask. **Do not overfill the bag.**
Check all rigging for gas leaks. You will hear gas hissing at any point of leakage or may see moisture appear.

Read the CRF script relating to the inhalation to the participant. This will explain what will happen during the inhalation and what the participant should expect. Give the participant the opportunity to ask any questions. Any task instructions should be given prior to the fitting of the mask. Participants must be told that they can stop the inhalation at any time.

When fitting the mask ensure that the stopcock is open at the room (closed at the bag). This ensures participants are not breathing the contents of the bag while you are fitting the mask. Ask the participant to hold the mask over his/her mouth and nose, and attach the Velcro straps around the back of his/her head, being careful not to trap ears or pull hair. The top strap should pass over the crown (top) of head.

Pull the Velcro straps tight so the fit of the mask is tight around the participant’s face. Ensure the participant is comfortable. Different size masks are available, but a medium will fit most people. Always ask the participant if the mask is comfortable. If the mask is fitted correctly, it will not drop or sag when it is not being held.

If using a mouthpiece instead of a mask, the piece should be placed in mouth per the manufacturer’s instructions. A nosepiece should be used to avoid participants breathing through their nose.

When you are ready to start the inhalation, turn the stopcock of the Douglas bag so that the arrows point in the direction from the bag to the mask. Start your timer and instruct the participant to breathe normally. NEVER EXCEED 20 MINUTES FOR A SINGLE INHALATION OF 7.5 CO₂.

To end the inhalation, turn the stopcock so that the bag outlet is closed/room outlet is open, so that the participant is now breathing room air. Remove the mask from the participant.

The mask and breathing valve should be cleaned thoroughly between inhalations. See relevant SOP. All equipment should be rinsed thoroughly and left to dry. NEVER USE WET MASKS OR VALVES FOR AN INHALATION.

**EMERGENCY STOP:** If participant wants to stop the inhalation, immediately turn the stopcock at the bag so that the bag is closed and participant is now breathing to the room. Do not stop via the cylinder as this will not cease the inhalation for the participant. Encourage the participant to describe how they are feeling and reassure them that the effects of the gas will soon wear off. If the participant would like to speak to a doctor, telephone the on-call medical doctor. The doctor will give advice and reassure the participant but they will not attend the session. In any medical emergency, immediately call an ambulance.

### B. SINGLE BREATH INHALATION OF 35% CO₂:

- Fill the bag and close the valve.

- Read the CRF script that explains what the participant is expected to do before you start the inhalation. Ask participant if he/she has any questions.

- Ask the participant to insert the mouthpiece into their mouths like a boxers gum shield (i.e. inside their lips in front of their teeth, and bite on the inner rubber parts). Support the bag by holding it. A nose clip should be used to avoid participants breathing through their nose.

- Ask the participant to relax and breathe normally for a few breaths. The participant should be seated.
When you are ready for the inhalation, ask the subject to breathe out as much as they can and empty their lungs. You may need to prompt them to do twice as participants often do not fully empty lungs.

Turn the valve to open and ask the participant to breath in, one big breath, completely filling their lungs. They should hold for 4 seconds. Count these seconds out loud.

Remove the mouthpiece immediately and ask the participant to breathe normally. Check the participant is OK.

To clean the mouthpiece, allow to rest in Milton sterilising fluid for 15 minutes. Also wipe over with alcohol sterilising wipes and rinse thoroughly.

6. TROUBLE SHOOTING:

<table>
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<th>Problem</th>
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<tbody>
<tr>
<td>There is no spanner, tubing, or bags.</td>
<td>Contact Angela Attwood (0117 331 7450)</td>
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<tr>
<td>There are no cylinders.</td>
<td>Contact Angela Attwood (0117 331 7450)</td>
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<tr>
<td>Participant becomes unwell or too panicky.</td>
<td>The participant may feel unwell, but do not stop the inhalation without asking if the participant would like to stop. It may be the case that they are just experiencing the desired effects of the gas and they are happy to continue. If this is the case, monitor the participant closely, and stop immediately if requested. Tell the participant to take long slow deep breaths and assure them what they are feeling is normal and that they will recover soon. Offer a glass of water and a lie-down. If the participant continues to feel unwell (identified as the follow up call) suggest that they participant is examined by a Doctor as soon as possible. Register this as an adverse event and follow up participant if possible.</td>
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<td>Participant wishes to speak to a doctor</td>
<td>Call the lab phone first and the supervisor will arrange a call with the study doctor. Tim Williams – 07812 244443 Dr Williams is available for over-the-phone medical advice. If symptoms persist, call an ambulance. Ensure you have the named physician’s direct contact number prior to commencing study.</td>
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<td>Any other problems or problem persists.</td>
<td><strong>TARG Laboratory phone:</strong> 07512 135 242 <strong>Prof Marcus Munafò</strong> (0117) 954 6841 internal 46841 <a href="mailto:Marcus.Munafo@bristol.ac.uk">Marcus.Munafo@bristol.ac.uk</a> <strong>Dr Angela Attwood</strong> (0117) 331 7450 internal 17450 <a href="mailto:Angela.Attwood@bristol.ac.uk">Angela.Attwood@bristol.ac.uk</a></td>
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