SOP - 8
STUDIES INVOLVING MEDICAL AIR AND (UP TO) 7.5% CARBON DIOXIDE INHALATIONS

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Definitions/Abbreviations	
SOP	Standard Operating Procedure
CO_2	Carbon Dioxide
Air	Medical Air
CRF	Case Report Form
UoB	University of Bristol

1. PURPOSE:

- To provide step-by-step instruction to all persons conducting research studies which require modelling the effects of anxiety using inhalations of CO_2-enriched air (referred to as CO_2 in text) and inhalations of medical air as a placebo or sham treatment.
- These guidelines provide standards for the following procedures; i) participant recruitment, ii) obtaining informed consent, iii) communicating risks of CO_2 administration, iv) the procedure for a typical CO_2 experiment, v) how to submit an ethics application, and vi) who to contact if assistance is needed.
- This SOP has been reviewed by the School of Psychological Science Human Research Ethics Committee.
- THIS SOP COVERS UP TO 20 MINUTES EXPOSURE TO 7.5% CO_2, PLUS MEDICAL AIR CONTROL. If you wish to administer CO_2 in a way which is not covered by this SOP (e.g. concentration exceeding 7.5% or for longer than 20 minutes), you must submit a separate ethics application to the committee.

2. REFERENCES:

Bailey, J. E., Argyropoulos, S. V., Kendrick, A. H., & Nutt, D. J. (2005). Behavioral and cardiovascular effects of 7.5% CO_2 in human volunteers. *Depression and Anxiety*, *21*(1), 18–25. http://doi.org/10.1002/da.20048

3. PARTICIPANT ELIGIBILITY AND SCREENING

3.1 INCLUSION/EXCLUSION CRITERIA

To be eligible for a CO_2 study, the participant must meet all of the inclusion criteria and none of the exclusion criteria:

3.1.1 Inclusion criteria

- Aged between 18-50 years
- Good physical and psychiatric health
- English as first language (or equivalent level of fluency)

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3.1.2 Exclusion criteria

- Alcohol consumption within 36 hours of the study (self-report and zero breath alcohol concentration (BrAC) reading)
- Recent or ongoing use of illicit drugs (including cannabis within 7 days; self-report and urine screen)
- Systolic/diastolic blood pressure higher than 140/90 mm/Hg respectively
- Heart rate lower than 50 or higher than 90 beats per minute
- Pregnancy or current breast feeding (urine screen for pregnancy)
- Body mass index (BMI) less than 17 or greater than 30 kg/m^2
- Significant current or past medical or psychiatric illness
- Personal or family history of mood disorder, including panic disorder
- Ongoing physical illness or abnormality (e.g., history of cardiac or respiratory problems, including asthma)
- Personal strong history of migraine (i.e. frequent and requiring medication)
- Drinking more than 35 alcoholic units/week*
- Daily cigarette smokers
- Recent smoking (measured by carbon dioxide breath test: >10 ppm)
- Drinking more than eight caffeinated drinks per day
- Personal history of alcoholism or drug dependence
- Medication use (except local treatment, aspirin or paracetamol) within past 8 weeks
- Impaired or uncorrected vision
- Uncorrected visual or auditory impairment that would compromise informed consent or ability to complete test procedures
- Potential, in the opinion of the investigator, to be non-compliant with the study or unable to provide written informed consent

* One unit equals one 25 ml single measure of spirit (ABV 40%), or a third of a pint of beer (ABV 5-6%) or half a standard (175 ml) glass of red wine (ABV 12%).

Note: if there is doubt relating to any of these criteria (particularly in relation to any reported health issues), the researcher will discuss with the study doctor before making an inclusion/exclusion decision.

3.2 SCREENING PROCEDURE

Participants must be screened to confirm eligibility based on the criteria listed above. Screening usually proceeds through two phases. Participants first complete a telephone-based screening to assess basic eligibility (e.g. age, caffeine and alcohol use, smoking, GP registration, basic health check – self-report). Note: the telephone screening can be completed via an online form or on the study day if preferred or if the participant is uncontactable by phone. However, a pre-study day screening of basic eligibility should be attempted to negate unnecessary laboratory booking.

At the start of the session, the second phase of screening should be completed comprising physical assessments (heart rate, blood pressure, height, weight, urine screens – for recent drug use and pregnancy) and a neuropsychiatric interview to assess psychiatric health.

For all screening procedures, participants should be allocated a unique numeric *screening* identifier. When participants successfully complete a screening, they are officially enrolled onto the study and at this point they are additionally assigned a separate unique numeric *study* identifier. All screened participants should be added to the screening log, but screening CRFs of failed screenings are immediately disposed of using UoB's confidential waste facility.

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4. OBTAINING INFORMED CONSENT

Participants should be sent the study information sheet before the study day and given the opportunity to contact the research team if they have any questions. On arrival at the session, participants must be given the opportunity to read the information sheet again and ask any questions. When they are ready to do so, they should sign two identical consent forms, one of which they will be able to keep and the other will be stored in the study master file.

5. POTENTIAL RISKS OF CO_2 administration

- Carbon dioxide inhalation may cause feelings of suffocation, anxiety, unpleasantness or fear. Other physiological effects that may occur include racing of heart, dizziness, pins and needles, and breathlessness. Some people also experience a headache afterwards. The more severe symptoms are more common to higher concentrations (e.g. 35% CO_2).
- People experience and describe the effects of inhaling 7.5% CO_2 gas in different ways. Most people will notice some effects, and all participants must be told that they can stop the inhalation at any time. The effects of the gas are transient and begin to wear off immediately (when breathing of normal air resumes).
- Prior to running a CO_2 study, the researcher must read “SOP 14 Dealing with panic attacks” which instructs how to recognise, and deal with, a panic attack. The likelihood of panic attacks is low (particularly for 7.5% CO_2 inhalations), but possible and therefore researchers should be clear on how to recognise and deal with them prior to running any inhalations.
- A senior researcher trained in CO_2 administration and CPR should be available to attend the session at any point (i.e., should be present in building and in possession of a TARG Lab Phone for duration of the session).
- A researcher must stay with the participant throughout the inhalations and recovery periods.

6. PROCEDURE FOR A CO_2 EXPERIMENT

6.1 PERSONNEL REQUIRED AND LEVEL OF EXPERTISE:

- Investigator and/or research team.
- Completed training in cylinder use, CO_2 administration and study screening.
- A senior researcher trained in CO_2 administration and CPR who will remain in the building and hold the TARG lab phone for duration of the session. They will provide telephone support for the session and provide emergency support if required.

6.2 MATERIALS AND EQUIPMENT REQUIRED

- Medical air and/or CO_2 (up to 7.5%) cylinders
- Mask or mouthpiece
- Douglas bag
- Plastic tubing
- Cleaning materials (see SOP 30 Mask Cleaning)
- Timer
- Nose clip (required if using mouthpiece)
- Scripted CRF explaining inhalation procedure
- SOP 10 – Urine collection for pregnancy test or drugs of abuse screen
- SOP 13 – Dealing with a positive pregnancy test
- SOP 14 – Dealing with panic attacks
- SOP 18 – Dealing with multiple drug consumption
- SOP 22 – Screening for psychiatric health
- SOP 30 – Mask cleaning
- Cylinder handling risk assessment

- Administering hypercapnic challenges risk assessment

6.3 HOW:

- Studies covered by this SOP can consist of a single experimental session or multiple experimental sessions, but total exposure to 7.5% CO_2 (i.e. across all sessions) would not exceed 20 minutes. Each session can deliver a matched amount of medical air (control).
- An experimental session that consists of a single inhalation (of CO_2 or medical air) will last approximately 45 minutes.
- An experimental session that consists of two inhalations (one of CO_2 and one of medical air) will last approximately 2.5 hours. When CO_2 and medical air are administered during the same experimental session, there must be a 30-minute "wash-out" between inhalations. The second inhalation should follow the identical procedure to inhalation one. As there can be gas order (medical air, CO_2), it is advised that gas order should be counterbalanced across participants. At the end of the final inhalation, there must be a 20-minute recovery period where participants sit quietly. After this blood pressure and heart rate must be checked to ensure they have returned to baseline levels.
- Reimbursement will be determined on a study-by-study basis, but unless otherwise justified, the general rule is £8/per hour (e.g., £20 for 2.5 hour sessions).

6.3.1 Pre-Inhalation checks:

- Ensure you have enough air and/or CO_2 for the session. If not, you are advised to ask trained staff 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).
- ONLY TRAINED STAFF CAN MOVE OR CHANGE CYLINDERS. DO NOT MOVE OR CHANGE A CYLINDER UNLESS YOU HAVE BEEN GIVEN AUTHORITY TO DO SO. IF YOU ARE UNSURE, ASSUME YOU ARE NOT AUTHORISED AND CONTACT YOUR STUDY PI WHEN YOU NEED TO MOVE OR CHANGE A CYLINDER. To change cylinders, firstly close all valves. Then use the relevant spanner/spanner key to undo the bolt and remove the regulator. Attach the regulator to the new cylinder and tighten using the appropriate 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).
- ALL USERS MUST REQUEST AND READ THE CYLINDER USAGE RISK ASSESSMENT. ALL USERS MUST HAVE COMPLETED TARG CYLINDER TRAINING BEFORE USING OR MOVING CYLINDERS.
- Air and CO_2 can be ordered from BOC via central Research Support. CO_2 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.

6.3.2 Inhalation of medical air or CO_2:

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- 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.
- Read the standardised 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.
- 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 stopcock 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.
- 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% CO_2**.
- The participant should never be left alone during the inhalations.
- 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 SOP 30 – Mask Cleaning. 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 the room air. 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 study doctor. The study doctor will give advice and reassure the participant, but they will not attend the session. In any medical emergency, immediately call an ambulance.
- If a participant requests to stop an inhalation, they should not be put back on the gas even if they report feeling better.

6.3.3 Post-Inhalation:

- After the inhalation(s), the participant should be instructed to remain in the laboratory for 20 minutes to ensure good recovery. At the end of the 20-minute period, blood pressure and pulse rate should be measured to ensure that they have returned to a normal level. The participant should be informed that they may remain longer in the laboratory if they feel that the effects of the gas have not worn off. At the end of the session, participants will be

debriefed, given a safety card describing the inhalation and giving contact details of the research team, and reimbursed. Approximately 24 hours after the session, the participant should be telephoned to assess whether there are any residual symptoms or if the participant experienced any adverse events or reactions after the study session. The participant's responses should be recorded in a post-session CRF. Up to three call attempts should be made (all logged in CRF), and if there is still no answer, the researcher should send the participant a follow-up email.

7. REIMBURSEMENT IF PARTICIPANTS WITHDRAW FROM THE STUDY

- Participants should be informed that they are able to withdraw from the study at any time without having to give a reason. Participants should be reminded at the start of each inhalation that they can stop the inhalation at any time. If a participant withdraws from the study during the study session, they should be partially reimbursed with the amount of reimbursement being dependent on the time spent in the study. If participants withdraw due to an adverse event, they should receive full reimbursement.

8. INCIDENTAL FINDINGS

- For failed screenings, relay the reason for failure to the participant (e.g., "You are not eligible to take part in this study as you have tested positive for a drug on the urine test").
- If a positive pregnancy result is obtained, refer to "SOP 13 Dealing with a positive pregnancy test" and relay this information directly to the participant. Female participants should be advised via the information sheet that a pregnancy test will be performed and that they will be told if a positive result is obtained.
- If a suspected abnormality is revealed during the screening procedure, the findings (e.g., blood pressure, heart rate, responses on the neuropsychiatric interview) should be disclosed to the participant. Emphasize that we are not clinicians, and therefore are not providing a clinical diagnosis, but that we need to prioritize participant safety. For example, "You are ineligible for this study as your blood pressure is higher than our eligibility criteria for this study. We are not clinicians and therefore this is not a clinical diagnosis. Our criteria are set to protect our participants safety, and all this means is that your blood pressure is too high for us to enrol you on this study. If you are concerned, then you should make an appointment with your GP." This standardised phrasing should be included in the Experimenter CRF.
- The participant should also be advised of who to contact if they are concerned by the findings. "If you are concerned, we advise you to contact your GP." For findings related to mental health, in additional to their GP, you can tell the participant about relevant support groups (e.g., Student health service, Student counselling service, Residential Life Services, Bristol Wellbeing Therapies, Off the Record, The Bridge, International suicide hotlines, Samaritans, etc.). These should be listed in the Experimenter CRF.

9. ETHICS APPLICATIONS

- When completing ethics applications for experiments covered by this SOP, fill in the ethics checklist as usual.
- When completing question 5 "Will drugs, placebos, or other substances (e.g., drinks, foods, food or drink constituents, dietary supplements) be administered to the study participants?" select "yes". However, underneath, write that the medical air/CO_2 administration and is covered by the Tobacco and Alcohol Research Group "SOP 8 Studies Involving Medical air and CO_2 inhalations" (ethics number: 83982).
- This SOP cannot be used in place of the full ethics application process if the study requires administration of CO_2 in a way which is not covered by this SOP (e.g. concentration exceeding 7.5%), or if any other components of the research study do not meet the requirements of a

checklist application. In those cases, a full ethics application must be submitted to the committee.

10. TROUBLE SHOOTING:

Problem	Solution
There is no spanner, tubing, or bags.	Contact Angela Attwood (0117) 33 17450
There are no cylinders.	Contact Angela Attwood (0117) 33 17450
Participant becomes unwell or too panicky.	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. After the inhalation, tell the participant to take long slow 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 the participant is examined by a Doctor as soon as possible. Register this as an adverse event and follow up participant if possible.
Participant wishes to speak to a doctor	Call the lab phone first and the supervisor/PI 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.
Medical Emergency	Contact the on-call first-aider via the lab phone 07957 334265. Office telephone numbers for all first-aiders are also listed below. Angela Attwood (0117) 33 17450 Olivia Maynard (0117) 92 89943 Jennifer Ferrar (0117) 92 89707 Anna Blackwell (0117) 92 88011
Any other problems or problem persists.	**TARG Laboratory phone:** 07957 334265 **Prof Marcus Munafò** (0117) 95 46841 internal 46841 Marcus.Munafo@bristol.ac.uk **Dr Angela Attwood** (0117) 33 17450 internal 17450 Angela.Attwood@bristol.ac.uk