OBSERVATION SHEET

PROTOCOL NO: 25-OUMD-25LS

OBSERVER: (b) (6)

PROCEDURE DATE: 9FEB22

START TIME: 0621

PROCEDURE PROFILE: Doxy

ANIMAL # 57015

OBSERVATIONS/PROCEDURES

BASELINE WT:	BASELINE SPO2	BASELINE HR	BASELINE TEMP	BASELINE RR
74.3Kg	95	140	97.5	20

COMMENTS: 50mL 0.9g Doxycycline

	Time	RR	HR	SPO2	TEMP	SAMPLE	COMMENTS	INT
0621	0 min	20	140	95	97.5	✓	Flushed	(b) (6)
0626	5 min	21	108	97	100.0	✓		(b) (6)
0631	10 min	40	124	98	101.0			(b) (6)
0636	15 min	23	109	96	101.5	✓		(b) (6)
0641	20 min	47	110	95	100.5			(b) (6)
0646	25 min	30	105	92	101.0			(b) (6)
0651	30 min	38	109	91	100.0	✓		(b) (6)
0701	40min	42	92	96	101.5			(b) (6)
0711	50 min	16	107	96	102.0			(b) (6)
0721	1 hr	16	109	96	102.5	✓		(b) (6)
0731	+ 10 min	16	117	93	102.0			(b) (6)
0941	+ 20 min	24	130	82	100.5			(b) (6)
0951	+ 30 min	20	80	97	100.5			(b) (6)

	TIME	RR	HR	SPO2	TEMP	SAMPLE	COMMENTS	INT
0801	+40 min	200	121	98	103.5		Standing	(b) (6)
0811	+ 50 min	28	131	95	101		Restless	(b) (6)
0821	2 hr	24	140	98	101.5	✓	Bld drawn (early)	(b) (6) 0811
0831	+10 min	60	142	99	100.5		Medicated ↑ Vocalizing	
0841	+20 min	60	140	93	98.5		Medicated Vocalizing	
0851	+ 30 min	36	134	96	100.5		Resting	
0901	+ 40 min	56	118	99	101.5		VOCALIZING, RESTLESS	(b) (6)
0911	+ 50 min	56	180	93	103.5		ATTACKING CAGE	
0921	3 hr	44		91	104.5		Thrashing	
0931	+ 10 min	56	148	94	104.5		LYING DOWN, STRESS (b) (6) (b) (6)	(b) (6)
0941	+ 20 min	48	142	91	102.5		Thrashing — Medicated	(b) (6)
0951	+ 30 min	65	131	84	103.5		Vocalizing —	(b) (6)
1001	+ 40 min	54	163	94	102.5		Laying Flat → Resp	
1011	+ 50 min	37	157	96	106		Blanket of Cold water (wet)	
1021	4 hr	46	153	91	105	✓	↓ 1° Bld cBK drawn	(b) (6)
1031	+ 10 min	60	130	88	106		Cold Cloths on back	
1041	+ 20 min	60	120	96	106		New IV ↑ 500ml #3 (b) (6) (b) (6) (b) (6) (NSS)	
1051	+ 30 min	100	126	96	104		↑ Resp	
1101	+ 40 min	120	117	89	107.0		↑ temp, feels bloated	(b) (6)
1111	+ 50 min						Euthanized 1107	
1121	5 hr							
1131	+ 10min							
1141	+ 20 min							
1151	+ 30 min							
1201	+ 40 min							
1211	+ 50 min							
1221	6 hr							
	24 hr							

Procedure Notes:

No more than 5 mL's of blood will be taken during any blood draw. The sampled blood will be kept as serum and frozen stored in the -80 freezer.

Animal euthanized (Yes)/No — Temp↑ noted. wet Cold towels Applied for comfort

Animal transferred Yes/No New protocol number: _____ (b) (6) (b) (6)

Additional Comments: 10.37 Blue Padding to avoid injury Animal thrashing ↑ Temp.

Euthanized at 1107 due to bloat, fever

Animal Number: _____ Lab Superv (b) (6) _____

73.7°F 49.2 humidity

MEDICAL RECORD	CHRONOLOGICAL RECORD OF MEDICAL CARE

PRIVACY ACT STATEMENT: This information is subject to the Privacy Act of 1974 (5 U.S.C. Section 552a). This information may be provided to appropriate Government agencies when relevant to civil, criminal or regulatory investigations or prosecutions. The Social Security Number, authorized by Public Law 93-579 Section 7 (b) and Executive Order 9397, is used as a unique identifier to distinguish between employees with the same names and birth dates and to ensure that each individual's record in the system is complete and accurate and the information is properly attributed.

DATE	SYMPTOMS, DIAGNOSIS, TREATMENT, TREATING ORGANIZATION *(Sign each entry)*
	CC: Swine received at building 503.
02 FEB 22	S/O: Weight: 63 Kg T: 103.2°F P: 200 bpm R: 60 bpm
	A: Swine appears fit for protocol
	P: Place in stabilization. Will monitor for medical problems.
	Fecal Test: negative Positive
	When receiving Pig there was scratches all around the face (b) (6)(b) (6) (b) (6)(b) (6) 68T ANIMAL CARE-PFC WRAIR VSP
7 Feb 22	Animal released from quarintine - placed Animal on protocol 21-OLMD-25L - prepped for CVL placement - CVL placed in ® Jugular by (b) (6) - Vetted received Animal 4 Feb 22
9 Feb 22	Animal transported to Cdw 88 for Dexy Infusion and Blood draws IAW protocol - Vitals and physiological Samples taken PI Notified of rising temperature - Euthanized w/ 10 ML Euthasol @ 9 Feb 22

HOSPITAL OR MEDICAL FACILITY	STATUS	DEPARTMENT/SERVICE	RECORDS MAINTAINED AT
Walter Reed Army Institute of Research			
SPONSOR'S NAME	SOCIAL SECURITY/ID NUMBER	RELATIONSHIP TO SPONSOR	

PATIENT'S IDENTIFICATION *(For typed or written entries, give: Name - last, first, middle; ID NUMBER or Social Security Number; Gender; Date of Birth; Rank/Grade.)*

ID #: 57015
Gender: MALE
Species: Yorkshire Cross

REGISTER NUMBER	WARD NUMBER

CHRONOLOGICAL RECORD OF MEDICAL CARE
Medical Record

STANDARD FORM 600 (REV. 8/2018)
Prescribed by GSA/ICMR
FIRMR (41 CFR) 201-9 202-1

PREVIOUS EDITION IS NOT USABLE

FOR OFFICIAL USE ONLY
When Filled Out

AUTHORIZED FOR LOCAL REPRODUCTION

OBSERVATION SHEET

PROTOCOL NO: 25-OUMD-25LS **OBSERVER:** (b) (6)

PROCEDURE DATE: 2MAR22 **START TIME:** 0627

PROCEDURE PROFILE: Doxy **ANIMAL #** 57117

OBSERVATIONS/PROCEDURES

BASELINE WT:	BASELINE SPO2	BASELINE HR	BASELINE TEMP	BASELINE RR
70.6Kg	91	88	100.5	32

COMMENTS:

0.9g Doxycycline 50mL given at 0617-0627

temp 104.5 0805 Euthanized 0853 by PI Malignant hyperthermia

	Time	RR	HR	SPO2	TEMP	SAMPLE	COMMENTS	INT
0627	0 min	32	88	91	100.5	✓		(b) (6)
0632	5 min	32	122	92	100.0	✓		(b) (6)
0637	10 min	32	124	93	100.0			(b) (6)
0642	15 min	32	136	92	100.5	✓		(b) (6)
0647	20 min	25	72	91	99.5			(b) (6)
0652	25 min	36	66	92	99.5			(b) (6)
0657	30 min	50	75	95	99.5	✓		(b) (6)
0707	40min	24	82	95	100.5			(b) (6)
0717	50 min	28	98	96	101.0			(b) (6)
0727	1 hr	28	95	92	101.0	✓	Thrashing	(b) (6)
0739	+ 10 min	42	111	93	99.5		Thrashing	(b) (6)
0747	+ 20 min	46	110	96	96 985		Thrashing	(b) (6)
0757	+ 30 min	44	150	82	102.5		Thrashing, temp increase	(b) (6)

	TIME	RR	HR	SPO2	TEMP	SAMPLE	COMMENTS	INT
0807	+40 min	40	172	88	104.0		Flushed	(b) (6)
0817	+ 50 min	59	173	88	104.0		Rectal temp. Ice packs	(b) (6)
0827	2 hr	50	150	82	105.2		Ice packs, Flushed	(b) (6)
0837	+10 min	34	207	90	103.8		Spraying alcohol	(b) (6)
0847	+20 min							
0857	+ 30 min							
0907	+ 40 min							
0917	+ 50 min							
0927	3 hr							
	+ 10 min							
	+ 20 min							
	+ 30 min							
	+ 40 min							
	+ 50 min							
	4 hr							
	+ 10 min							
	+ 20 min							
	+ 30 min							
	+ 40 min							
	+ 50 min							
	5 hr							
	+ 10min							
	+ 20 min							
	+ 30 min							
	+ 40 min							
	+ 50 min							
	6 hr							
	24 hr							

Procedure Notes:

No more than 5 mL's of blood will be taken during any blood draw. The sampled blood will be kept as serum and frozen stored in the -80 freezer.

Animal euthanized (Yes)/No

Animal transferred Yes/No New protocol number: _____

Additional Comments:

Ice packs and rectal temp used

104.6 0832

103.7 0838 Euthanized 0853 AAggMalignant hyperthermia

Final temp: 103.7 Euthanized by PI

Animal Number: _____ Lab Supervisor: _____

Summary Sheet

21-OUMD-25LS

Animal #: 57117

Procedure Date: 2MAR22

Pre Dive Wt.: 70.6Kg

Trial: Doxycycline/Control

Group: 0.9g Doxy

Med Dose: 30 mg/kg

Case Description: **Assessment of the Efficacy of High Dose Doxycycline in Preventing Decompression Sickness in Swine (*Sus Scrofa*) Subjected to Accelerated Decompression from Saturation**

Comments:

Euthanized 0853 2MAR22 by PI due to malignant hyperthermia / post sedation distress - suspected reaction to ketamine. repeat dose of xylazine did not affect sedation + abnormal respiration was noted (inspiratory stridor and abdominal breathing)

AV cannot with humane euthanasia. (b) (6) (b) (6)

Lab Supervisor: _____

DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
503 ROBERT GRANT AVENUE
SILVER SPRING, MD 20910-7500

FCMR-UWS-AR (40-33c) 5 December 2022

MEMORANDUM FOR IACUC, Animal Research Compliance Office, Water Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500

SUBJECT: Animal Welfare Inspection, Mechanical Failure Rat 19-OUMD-34.

1. Background: On June 15th there was an equipment misconfiguration which resulted in a single rat fatality. The misconfiguration caused inaccurate chamber gas composition information to be reported to the chamber operator leading to a hypoxic condition in the chamber. When animal technicians noted an alteration of animal disposition, the chamber operator followed the emergency operating procedure (surfacing the chamber) which exacerbated the hypoxia leading to the animal being rendered unconscious. On surface the chamber was flooded with 100% oxygen and multiple attempts to resuscitate the animal were conducted but were ultimately unsuccessful. An examination self-conducted by the investigative team identified there was an exhaust hose connected to a vacuum pump that was not operating and thus prohibiting exhaust air from leaving the chamber.

2. Action: IACUC team member (b) (6) conducted an interview with investigative team members and reviewed laboratory records and self-report. An investigation of the equipment set up was not conducted as it had been taken apart and moved into storage.

3. Findings: The IACUC members found events described by the investigative team to be accurate. The investigative team has taken the appropriate action in developing a checklist to verify proper set up and operation of the equipment to include a test run prior to permitting animals to be placed the chamber.

4. Recommendation: The PI team acted responsibly in identification and mitigation of root cause. There is no further action required at this time.

5. The points of contact for this action can be reached through the WRAIR/NRMC ARCO.

(b) (6)(b) (6)
(b) (6)(b) (6)
(b) (6)

Digitally signed by (b) (6)(b) (6)(b) (6)(b) (6)
(b) (6)
Date: 2022.12.05 14:30:55 -05'00'

IACUC Inspection Team
Member 1 (Chair): (b) (6)

DEPARTMENT OF THE ARMY
WALTER REED ARMY INSTITUTE OF RESEARCH
503 ROBERT GRANT AVENUE
SILVER SPRING, MD 20910-7500

REPLY TO
ATTENTION OF

FCMR-UWZ

4 January 2023

MEMORANDUM FOR Director, Division of Compliance Oversight, Office of Laboratory Animal Welfare (OLAW), National Institutes of Health, Bethesda, MD 20892

SUBJECT: Final Report to OLAW

1. The Walter Reed Army Institute of Research/Naval Medical Research Center (WRAIR/NMRC) Animal Care and Use Program (Assurance A4117-01) in accordance with PHS Policy IV.F.3., provides this final report regarding mechanical failure in a dive chamber as a serious noncompliance. The preliminary report was first conveyed to OLAW (b) (6)(b) (6)(b) (6) on 15 August 2022 via an e-mail from (b) (6) (b) (6) IACUC Chair/Director, Animal Research Compliance Office.

2. On 8 July 2022, a Principal Investigator self-reported that on 15 June 2022 there was an equipment misconfiguration which resulted in a single rat fatality. The misconfiguration caused inaccurate chamber gas composition information to be reported to the chamber operator leading to a hypoxic condition in the chamber. When animal technicians noted an alteration of animal disposition, the chamber operator followed the emergency operating procedure (surfacing the chamber) which exacerbated the hypoxia leading to the animal being rendered unconscious. The chamber was flooded with 100% oxygen and there were multiple attempts to resuscitate the animal which were ultimately unsuccessful. An examination, self-conducted by the research team, identified there was an exhaust hose connected to a vacuum pump that was not operating that prohibited exhaust air from leaving the chamber.

3. Through analysis of the self-report and interviews conducted of the research team, it was determined that appropriate action was taken during the event and in subsequently developing a checklist to verify proper set up and operation of the equipment, including a test run prior to permitting animals to be placed the chamber.

4. The findings and actions taken were presented to the IACUC for concurrence. The PI was notified of the outcome and has already made the necessary procedural changes. The PI and research team members were fully compliant. The IACUC determined that this was an isolated incident and not a programmatic failure.

5. The funding for this protocol is not NIH and there was no impact on PHS-supported activities. Reporting to the funding agency is non-applicable.

6. The WRAIR/NMRC Animal Care and Use Program is committed to protecting the welfare of animals used in research and appreciates the guidance and assistance

provided by OLAW in this regard. Should you have any questions regarding this report, please contact [redacted] or [redacted]. Thank you for your consideration of this matter.

[redacted]
Digitally signed by [redacted]
Date: 2023.01.05 14:34:38 -05'00'

[redacted]
Chair, WRAIR/NMRC Institutional Animal Care and Use Committee

Reviewed by WRAIR/NMRC Institutional Official

[redacted]
Digitally signed by [redacted]
Date: 2023.03.03 16:52:34 -05'00'

[redacted]

CF:
NMRC CO ([redacted])

PROTOCOL TITLE: **The impact of carbon dioxide on simulated DISSUB survival and decompression sickness at 5ATA air in a 70kg swine *(Sus scrofa)* model**

PRINCIPAL INVESTIGATOR: (b) (6)

CO-INVESTIGATOR(S):
(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)

I. NON-TECHNICAL SYNOPSIS:

This study will assess the impact of increased carbon dioxide (CO_2) on survival in a hyperbaric air exposure designed to mimic a disabled submarine (DISSUB). This study will consist of three aims. In the first aim, we will compare survival time of swine exposed to 1.5% CO_2 vs trace CO_2 in 5 Atmospheres Absolute (ATA) hyperbaric air for up to 8.5 days. This aim will allow us to confirm and expand on our previous findings. In the second aim, we will expose swine to 0% CO_2, 0.5% CO_2, 1.0% CO_2, and 1.5% CO_2 in a 5ATA hyperbaric air atmosphere for up to 8.5 days. This aim will allow the development of a model for the effect of CO_2 level on survival. In the third aim, we will surgically implant telemeters to measure blood gas parameters, cardiovascular function, and end-tidal CO_2. These swine then will be exposed to the conditions in aim 2 that had the greatest variability in survival time. The purpose of this aim is to identify the cause of death observed in the previous two aims.

II. BACKGROUND

II.1. Background:

The submarine force supports US Navy missions including strategic deterrence, intelligence gathering, and freedom of movement on the seas, requiring sailors to endure potentially dangerous operations in an austere environment. The submarine force's training requirements and operational tempo necessitate risks, which may lead to DISSUB and loss of life (1). Internationally, from 2001 to 2014, there have been 13 near miss events, occurring at a rate of nearly 1 per year. Many of these were minor mechanical failures stemming from navigational errors, but several led to significant morbidity and/or mortality (2). Most recently, the ARA San Juan disappeared on 15 November 2017, and 44 submariners perished. As long as the submarine community remains committed to current missions, DISSUB events for the US and our allies remain a real possibility. The US Navy has established strategies to reduce lives lost during future DISSUB events. However, recent experience demonstrates that the plans contain incompletely tested concepts of operations (3, 4).

A normally-operating submarine's internal pressure is maintained at one atmosphere absolute pressure (1 ATA). A DISSUB's internal pressure may rise secondary to flooding and the use of air banks. According to the May 2014 version of the United States' Submarine Rescue Diving and Recompression System (SRDRS), rescue is possible for submariners at internal pressurization up to 5 ATA (equivalent of 132 feet of Sea Water (fsw)). However, there is a lack of data to support survival of submariners with prolonged exposure to the expected high partial pressures of oxygen (PO_2), which could induce pulmonary oxygen toxicity (5, 6). Additionally, new swine data suggest that CO_2 may further exacerbate lung injury and increase mortality risk. We will briefly discuss submariners' O_2 and CO_2 exposures during a DISSUB scenario and then consider evidence for the potential consequences of prolonged crewmember exposure to high PO_2 with rising CO_2 levels.

According to the submarine rescue system decompression plan (SRSDP), the last crewmembers rescued would remain at 5 ATA for 172 hours with initial oxygen content of near 21% or 1.05 ATA (i.e. 5ATA5 ATA x 0.21), placing them at extremely high risk for pulmonary oxygen toxicity

(5, 7-10). PO_2 levels up to 0.5ATA generally are regarded as safe and higher levels can induce pulmonary oxygen toxicity. The risk of pulmonary injury correlates with the exposure duration and PO_2 level (11, 12). To minimize lung injury, the SRSDP recommends that submariners breathe down the PO_2. Following assumptions suggested in the SRSDP, at an internal pressurization of 5ATA (pO_2=1.05ATA), crewmembers would need 177.4 hours to reach aa pO_2 of 0.5ATA and 327 hours to reach aa pO_2 of 0.18ATA (SRSDP target).. During the oxygen breathe down, CO_2 will replace O_2, and PCO_2 will rise unless LiOH curtains absorb at a rate greater than or equal to CO_2 production. At an internal pressure of 5ATA, PCO_2 will start at 0.2% surface equivalent value (SEV) and begin to increase as a result of normal human respiration. If CO_2 rises above 3% SEV without sufficient atmospheric control to reduce it, then crewmembers should initiate DISSUB escape via SEIE suit vice waiting for rescue via PRM or SRC (SSN688 Guard Book) (13). Altogether, DISSUB survivors in a DISSUB with internal pressure of 5ATA, will breathe high PO_2 and PCO_2. The impact of this DISSUB exposure is unknown.

The existing literature and our recent data suggest that prolonged exposure to elevated PO_2 and PCO_2 portends a poor prognosis for DISSUB crewmembers. Prolonged normobaric 100% oxygen exposures (96-110 hours) in non-human primates (14) and swine (20) demonstrate significant functional decrements and pulmonary edema from pulmonary oxygen toxicity. Humans have also had shorter documented exposures. Eckenhoff et.al, compared a control group (n=6), breathing 30% SEV O_2 at 5 ATA and an exposure group (n=12) breathing 105% SEV O_2 at 5 ATA for 48 hours. They found significant decrements in pulmonary function as measured through vital capacity and diffusion capacity. Symptoms (e.g., cough, chest pain, dyspnea, exercise intolerance) in the exposure group lasted up to a month; but most resolved within 7 to 10 days (9). Together, these data suggest that pulmonary edema from oxygen toxicity will decrease gas exchange and increase decompression sickness-associated morbidity and mortality compared to expected outcomes from the accelerated decompression strategies developed in healthy volunteers (5, 15).

Oxygen is not the only culprit to consider with respect to morbidity and mortality in DISSUB trapped submariners. In a recent study, we exposed a human surrogate swine model (70 kg, n=6) to 132fsw (air, 105% SEV O_2) with intentions of simulating DISSUB rescue for the last sortie from this internal pressure using the

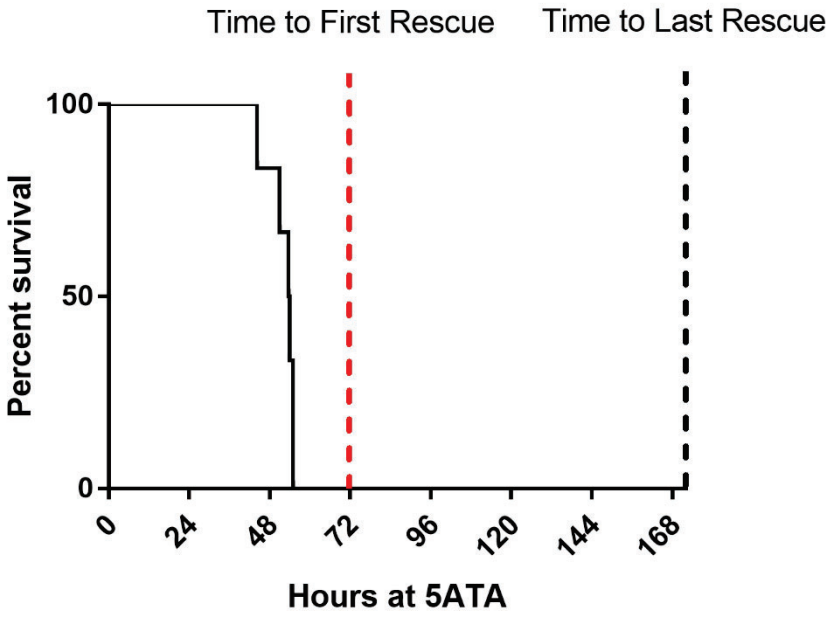

Figure 1. Kaplan Meier plot of survival latency for swine exposed to 5ATA air. Time to first rescue and Time to Last Rescue derived from SRS rescue plan are annotated on the graph.

accelerated decompression tables in the SRSDP (2). All animals died within 54 hours (mean latency 52±1.69 hours), which is less than the theoretical time to first rescue (72 hours), let alone last rescue (172 hours) (Figure 1). The exposure in this simulated DISSUB environment had two major differences from the previous prolonged, high-partial pressure oxygen exposures in animals: increased total atmospheric pressure and increased CO_2. And, at least one major difference from the human work at 5 ATA for 48 hours: increased CO_2.

Table 1: Chamber Environmental Conditions

Time points (hrs.)	CO_2 (%SEV)	O_2 (%SEV)	H_2O	Temp
0	1.07	104.33	67.20	106.60
12	1.19	104.67	76.20	76.60
24	1.33	104.17	85.20	76.60
36	1.33	104.50)	95.40	76.00
48	1.58	103.50	88.40	76.80

During our DISSUB simulation study, CO_2 levels steadily increased without exceeding 2% SEV CO_2 (Table 1). In the aforementioned human and animal studies, CO_2 levels were maintained below 0.02% SEV. Numerous animal studies suggest that CO_2 potentiates both CNS and pulmonary oxygen toxicity though the mechanism remains unclear (16). We suspect that the increased levels of CO_2 synergized with the hyperoxia will increase pulmonary injury in the swine, at least partially explaining the difference among findings. Animal studies also suggest that increases in PCO_2 increase mortality, independent of pulmonary injury (16). We believe that CO_2 contributed to unexpectedly early mortality in our DISSUB rescue study.

This study aims to test the hypothesis that rising CO_2 levels potentiate pulmonary oxygen toxicity and increase mortality, limiting DISSUB rescue survivability at 5 ATA. We propose to compare survival rates of swine exposed to a 5 ATA DISSUB rescue according to SRSDP to determine if there is a CO_2 threshold at which DISSUB rescue from 5 ATA internal pressure is physiologically feasible. These data will support the feasibility of the SRSDP and provide critical knowledge products for potential revisions to the SRSDP and Disabled Submarine Survival Guides (Guard Books). These data may also provide necessary information to develop medical and/or engineering solutions to improve DISSUB morbidity and mortality and operational solutions to remove excess CO_2.

II.2. Literature Search for Duplication

II.2.1. Literature Source(s) Searched:

PubMed, DTIC, NIH RePorter and ProQuest
(EMBASE, BIOSIS, Global Health, MEDLINE and NTIS). FedRIP is part of NTIS

II.2.2. Date of Search: 1/27/2021

II.2.3. Period of Search:

No limits set

II.2.4. Key Words of Search:

DISSUB, swine, decompression sickness, pulmonary oxygen toxicity, carbon dioxide

II.2.5. Results of Search:

DTIC: 1 result,

1) "PROLONGED EXPOSURE OF ANIMALS TO PRESSURIZED NORMAL AND SYNTHETIC ATMOSPHERES" Workman, Robert D., Bond, George F., Mazzone, Walter F.
This technical report described animal studies which did not involve exposures to oxygen above sea level equivalent and therefore is not relevant to the proposed studies.

Pubmed: 1 result,

1) "Effects of treatment with Pluronic F-68 during continuous venous air embolism in swine." Jenssen BM, Vik A, Brubakk AO. Undersea Hyperb Med. 1993 Mar;20(1):17-26. PMID: 8471956.
This study did not involve hyperoxia or hyperbaric exposures and therefore is not relevant to the proposed studies

NIH RePorter: 0 results

ProQuest: 10 results

1) Pasternak, Jeffrey J. MD Neuroanesthesiology Update, Journal of Neurosurgical Anesthesiology: April 2020 - Volume 32 - Issue 2 - p 97-119doi: 10.1097/ANA.0000000000000676
This review is a summary of the literature pertaining to the perioperative care of neurosurgical patients and patients with neurological diseases. This work is not relevant or a substitution to the proposed studies.

2) Olson, KR, Gao, Y, DeLeon, ER, et al. Extended hypoxia-mediated H2S production provides for long-term oxygen sensing. Acta Physiol. 2020; 228:e13368. https://doi.org/10.1111/apha.13368
This manuscript evaluates the role of H2S as a cellular oxygen sensor. These studies were conducted in cell culture and do not replace the proposed studies in any way.

3) Reovirus strain R-92 in in vitro generation of tumor-specific T-lymphocytes. Anastasia O. Sitkovskaya, Elena Yu. Zlatnik, Sergey A. Kolpakov, Elena P. Kolpakova, Irina V. Mezhevova, Elena S. Bondarenko, Inna A. Novikova, Andrey Dashkov, Dmitry O. Kaymakchi, Gapiz M. Chupanov, Nairi B. Oganyan, and Liubov Yu Vladimirova Journal of Clinical Oncology 2019 37:15_suppl, e14216-e14216
This study assessed the potential of the reovirus strain R-92 for in vitro generation of tumor-specific T-lymphocytes in cell culture. This study has no relevance to the proposed work.

4) Forbes AS, Regis DP, Hall AA, Mahon RT, Cronin WA. Propranolol effects on decompression sickness in a simulated DISSUB rescue in swine. Aerosp Med Hum Perform. 2017; 88(4):385–391.
This study evaluated the effect of propranolol on DCS rates during decompression from saturation. This study did not evaluate survivability during long term pressurization and therefore is not a substitution for the proposed work.

5) Lundgren CE, Bergoe GW, Tyssebotn I. The theory and application of intravascular microbubbles as an ultra-effective means of transporting oxygen and other gases. Undersea Hyperb Med. 2004 Spring;31(1):105-6. PMID: 15233165.
This study is not relevant to any aspect of the proposed work.

6) Jenssen BM, Vik A, Brubakk AO. Effects of treatment with Pluronic F-68 during continuous venous air embolism in swine. Undersea Hyperb Med. 1993 Mar;20(1):17-26. PMID: 8471956.
This study evaluates the efficacy of a surfactant at preventing vascular injury due to air embolism. This study is not relevant to the proposed work.

7) Lillo RS, Maccallum ME, Caldwell JM. Intravascular bubble composition in guinea pigs: a possible explanation for differences in decompression risk among different gases. Undersea Biomed Res. 1992 Sep;19(5):375-86. PMID: 1355314.\
This study evaluates the impact of different breathing gas mixes on decompression risk in small animals. This study is not relevant to the proposed work.

8) Masurel G. Intérêt de la détection ultra-sonore des bulles circulantes chez l'animal et chez l'homme--apport à la physiopathogénie de l'accident de décompression [The value of ultrasonic detection of circulating bubbles in animal and man--the contribution to physiopathogenesis of a decompression accident]. Schweiz Z Sportmed. 1989 Apr;37(1):41-4. French. PMID: 2499041.
This study evaluated the predictive relationship between intravascular bubbles and decompression sickness. This study is not relevant to the proposed work.

9) Lauten, A. Some aspects of the decompression diseases, hypercapnia and oxydosis, in diving. Medsport 10.7 (1970): 221-224.
This study evaluated risk factors for decompression sickness, hypercapnia, and oxygen toxicity in divers. This study is of limited relevance and does not replace any of the proposed studies.

10) CAMPBELL, SPENCER D.; SPENCER, MERRILL P. M.D. Pharmacologic Agents in the Prevention of Decompression Sickness, Journal of Occupational Medicine: May 1969 - Volume 11 - Issue 5 - p 252-256
This study evaluated the effect of inhaled bronchodilator/smooth muscle relaxant on inert gas elimination in a guinea pig model of decompression sickness. This study is not relevant to the proposed work.

After review of the findings of the literature search below; the proposed studies were not found to be duplicative

III. OBJECTIVE/HYPOTHESIS:

AIM 1:

Hypothesis 1: We hypothesize that the 0% CO_2 (Atmospheric Control System (ACS)) exposed group will show an increased latency to mortality when compared to the 1.5% CO_2 (ACS) exposed group.

Null hypothesis: There is no difference in survival latencies between the 0% CO_2 (ACS) and 1.5% CO_2 (ACS) groups.

Hypothesis 2: We hypothesize that there will be no difference in survival latency between the 1.5% CO_2 (ACS) and 1.5% CO_2 (Flow Controlled) groups.

Null hypothesis 2: There is a significant difference in survival latency between the 1.5% CO_2 (ACS) and 1.5% CO_2 (Flow Controlled) groups.
AIM 2: We hypothesize that latency to mortality will decrease as an inverse function of atmospheric CO_2 concentration.
Null hypothesis: There is no correlation between atmospheric CO_2 concentration and latency to mortality.
AIM 3: We hypothesize that exposure to elevated CO_2 in the setting of hyperbaric air (5 ATA) will cause changes in cardiopulmonary function incompatible with life.
Null hypothesis: Mortality associated with exposure to hyperbaric air (5 ATA) and elevated CO_2 is caused by factors not associated with cardiopulmonary physiological endpoints.

IV. MILITARY RELEVANCE:

The US Navy has a robust and active submarine force that patrols and hides in all types of oceanic environments. Despite an excellent safety record, history suggests that submarines will intermittently become disabled, jeopardizing the lives of the DISSUB crewmembers. The ARA San Juan served as a tragic reminder of the dangers associated with submarine operations. The US Navy has invested heavily in DISSUB rescue capabilities to reduce mortality and morbidity associated with future DISSUB scenarios. While aspects of SRSDP operations are man tested, many of the required exposures would be unethical in humans. Animal studies have filled in some of these gaps, but many portions of the SRSDP remain untested. For example, the survivability of rescue from a DISSUB with an internal pressure of 132fsw is unknown. Recent swine data suggests that DISSUB crewmembers will not survive until time to first rescue (72 hours) at an internal pressure of 5ATA. These swine are thought to have died from pulmonary insufficiency, likely due to prolonged exposure to high PO_2 and PCO_2. This follow-on study will further characterize the role of PO_2 and PCO_2 in a DISSUB simulation at an internal pressure of 132fsw to improve DISSUB rescue planning and operations.

Current DISSUB scenarios rely on the SRSDP, various versions of the Guard Book, and coordinated expert opinion. While we cannot predict the outcomes of the proposed study, the project has the potential to greatly impact each of these. The findings may change the rescuable parameters in the SRSDP and guidance about escape versus rescue. The findings may change the CO_2 guidance provided in the Guard Book, which also impact the decision for Emergency Air Breathing System (EABS) use, rescue, escape, or some combination. The findings may invite creative engineering solutions to increase the suitability of the DISSUB environment. The findings will inform future coordinated expert opinion through platforms such as ISMERLO. Ultimately, the outcomes of this study will help to reduce the morbidity and mortality associated with future DISSUB events.

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures:

AIM 1: Swine (n=9; n=3/group) will be randomly assigned to 0% CO2 (ACS), 1.5% CO2 (ACS), or 1.5% CO2 (Flow Controlled) groups. Swine will be exposed singly in the Multiple Large Animal Chamber (MLAC).

This first aim is designed to confirm the unexpected findings in 18-OUMD-13L. Immediately prior to the conduct of 18-OUMD-13L, the Atmospheric Control System (ACS) system (environmental control) for the MLAC was taken offline due to renovations of (b) (6) . To

conduct the study, we controlled CO_2 buildup by increasing flow of fresh hyperbaric air into the boxes. This approach, while functional, is less effective than using the system as designed which allows reduction of CO_2 to trace levels. While unlikely, it is possible that the previous findings were a function of the modified system and not representative of the underlying physiology. With the ACS system back online, it is in the best interest of the science and animal welfare to use it. This study has three groups designed to control for reintegration of the ACS loop (0% CO_2, 1.5% CO_2) and compare it to the flow control (1.5% CO_2) condition.

Day 0 (Wednesday): One 65-75 kg swine will arrive at NMRC. Veterinary medicine will evaluate the swine for infection, congenital abnormalities, or other health problems that may compromise the study. The swine will be introduced to its run and begin its acclimation phase. He will be slowly transitioned from normal feed to high fiber feed required for the chamber dive to reduce food volume during hyperbaric exposure. This transition will happen over 3-5 days.

Day 1-4 (Thursday – Sunday): The swine will continue its acclimation phase.

Day 5 (Monday): The swine will have baseline function testing, dive preparation, and dive initiation. This will include the following: Recording Electrode Attachment: While in his transport cage, the swine will receive an intramuscular injection of ketamine and xylazine. Once sedated, each electrode attachment site will be washed with soap and water, clipped using an electric shaver, shaved using shaving cream and a single blade disposable razor. The site will then be disinfected with 70% isopropyl alcohol and a combination of electrodes to enable ECG (Electrocardiogram), Respiratory band, and EEG (electroencephalogram) monitoring will then be attached. A pulse oxygen saturation probe may also be placed on the tail. These electrodes will be secured through a combination of mechanisms – Vet Bond, tape, nylon sutures, jacket/harness, reinforced wires and a single subdural stitch using 2.0 prolene suture. The swine will don a vest, which will cover the electrodes. The swine will be then be recovered within the transport cage. Dive Initiation: Once recovered, the swine will be led to the temporary housing within the Multiple Large Animal Chamber (MLAC) hyperbaric chamber using a series of ramps and Plexiglas side walls. A ration of high fiber food will be dispensed from the automatic feed hopper for consumption. Subsequently the electrodes will be connected to the appropriate outputs. After at least 1 hour of acclimation and at least 4 hours since administration of xylazine and ketamine to allow for minimum of five half-lives for each agent, we will begin to record 1 hour of baseline vitals with two goals. (1) Collect baseline vitals for comparison. (2) Ensure that the recording equipment is functioning and troubleshoot prior to initiating dive. Between 1800 to 1900, the swine will begin its dive. It will descend at 60 fsw/min to 132 fsw.

Day 6 – 12 (Tuesday – Monday): The swine will remain at pressure. There will always be at least one person physically present with knowledge of chamber operation. There will always be at least one chamber technician on call (if not physically present) with more extensive chamber knowledge. Environmental conditions including but not limited to Temperature, humidity and oxygen content will be measured remotely via sensors mounted to the Plexiglas housing chamber. There will always be one investigator on call (if not present). The swine will have two meals daily of high fiber feed administered via an automatic feeding system and water available ad libitum using a lixit style water dispenser. His excrement will fall between the grated floor and be hosed out with high pressure water hosing. This will be drained from the hyperbaric chamber at the same interval. Every 4 hours, the swine will be evaluated for signs of

pain/distress, vitals, and habitat. The "Habitat" environmental assessment will focus on feces/waste accumulation and availability/access to food and water. Feces accumulation will be reduced by utilization of a sprayer system to wash waste out of the box where it can be evacuated from the hyperbaric chamber. The checklist for the evaluation is attached as Enclosure C. If at any point during the dive, the swine is identified as dead (loss of vitals without evidence of equipment failure) or no movement visualized for 12 minutes, then the dive will be aborted. The time of death will be recorded as last heart beat (if ECG functioning appropriately rounded to the next whole minute) or the start of the 12-minute monitoring period assessing movement (rounded to the next whole minute).

Day 13 (Tuesday): After 172 hours of bottom time, it will be early on day 13 (between 0300 to 0400). The swine will begin decompressing following the oxygen accelerated decompression plan in the SRSDP, shown in Enclosure2.

Day 14 (Wednesday): After 205 hours of hyperbaric exposure, the swine will reach surface (between 0655 to 0800). The swine will then have the attached electrode leads disconnected from the chamber wiring harness and secured to the jacket in a coil. The swine will be led down the ramp and into his transportation cage if possible. If not, at least two individuals will carry the swine to its transportation cage using a blanket as a sling. The swine will be returned to his run. The electrode leads will be connected to a mobile recording suite and the swine will be observed for at least 1 hour with regular monitoring of vital signs. Should there be any signs of pain or distress, the swine will receive an intramuscular injection of xylazine/ketamine as necessary for sedation, placement of an ear vein catheter, and administration of diazepam (0.125mg/kg)and Ketorolac (1mg/kg) intravenously. After the 1hr monitoring period, the swine will be returned to its run overnight. Buprenorphine (0.01-0.05mg/kg, SC) will be administered to provide pain relief during overnight monitoring.

Day 15 (Thursday): The swine will be recovered from its run and start its post-dive assessment, which will include a vital sign measurement. These procedures will be the same as described for the pre-dive assessment. After finishing the post-dive assessment, the swine will be infused with heparin and humanely euthanized with Euthasol ® in preparation for perfusion/fixation.

If there is no difference observed between 0% and 1.5% SEV CO_2 in latency to mortality (i.e., the null hypothesis is proven), then Aim 2 and Aim 3 will not be conducted. Additionally, if no mortality in observed in the 1.5% CO_2 group (ACS) then Aims 2 and 3 will not be conducted.

Statistical Analysis: We expect a mean survival time at 5 ATA air with SEV CO_2 1.5% of 51.25 hours with a standard deviation of 5.7 hours or less. Using a one-way ANOVA with 80% power and an alpha of 0.05, 3 animals per group (N=9) will sufficiently address the primary endpoint of survival.

AIM 2: Swine (n=16; n=4/group) will be randomly assigned to 0%, 0.5%, 1.0% or 1.5% SEV CO_2 groups. Swine will be exposed singly in the MLAC.

The purpose of this study is to identify whether CO_2 concentration is inversely correlated with mortality in swine exposed to 5 ATA hyperbaric air. The information provided by this study is important in two ways: first it will establish whether the reduced latency of mortality due to

hypercapnia is continuous in nature or if there is a threshold of effect; second, it will provide a data curve which can be used to model pulmonary oxygen toxicity in the setting of increasing CO_2 levels.

Day 0 (Wednesday): One 65-75 kg swine will arrive at NMRC. Veterinary medicine will evaluate the swine for infection, congenital abnormalities, or other health problems that may compromise the study. The swine will be introduced to its run and begin its acclimation phase. The swine will be slowly transitioned from normal feed to high fiber feed required for the chamber dive to reduce food volume. This transition will happen over 3-5 days.

Day 1-4 (Thursday – Sunday): The swine will continue its acclimation phase.

Day 5 (Monday): The swine will have baseline function testing, dive preparation, and dive initiation using the same procedures described in in AIM 1

Day 6 – 12 (Tuesday – Monday): The swine will remain on bottom. There will always be at least one person physically present with knowledge of chamber operation. There will always be at least one chamber technician on call (if not physically present) with more extensive chamber knowledge. Environmental conditions including but not limited to Temperature, humidity and oxygen content will be measured remotely via sensors mounted to the Plexiglas housing chamber. There will always be one investigator on call (if not present). The swine will have two meals daily of high fiber feed administered via an automatic feeding system and water available ad libitum using a lixit style water dispenser. Its excrement with fall between the grated floor and be hosed out with high pressure water hosing. This will be drained from the hyperbaric chamber at the same interval. Every 4 hours, the swine will be evaluated for signed of pain/distress, vitals, and habitat. The "Habitat" environmental assessment will focus on feces/waste accumulation and availability/access to food and water. Feces accumulation will be reduced by utilization of a sprayer system to wash waste out of the box where it can be evacuated from the hyperbaric chamber. The checklist for the evaluation is attached as Enclosure C. If at any point during the dive, the swine is identified as dead (loss of vitals without evidence of equipment failure) or no movement visualized for 12 minutes, then the dive will be aborted. The time of death will be recorded as last heart beat (if ECG functioning appropriately rounded to the next whole minute) or the start of the 12-minute monitoring period assessing movement (rounded to the next whole minute).

Day 13 (Tuesday): After 172 hours of bottom time, it will be early on day 13 (between 0300 to 0400). The swine will leave bottom following the oxygen accelerated decompression plan in the SRSDP, shown in Figure 1.

Day 14 (Wednesday): After 205 hours of hyperbaric exposure, the swine will reach surface (between 0655 to 0800). The swine will then have the attached electrode leads disconnected from the chamber wiring harness and secured to the jacket in a coil. The swine will be lead down the ramp and into his transportation cage if possible. If not, at least two individuals will carry the pig to his transportation cage using a blanket as a sling. The swine will be returned to its run. The electrode leads will be connected to a mobile recording suite and the animal will be observed for at least 1 hour with regular monitoring of vital signs. Should there be any signs of pain or distress, the swine will receive an intramuscular injection of xylazine/ketamine as

necessary for sedation, placement of an ear vein catheter, and administration of diazepam (0.125mg/kg) and Ketorolac (1mg/kg) intravenously. After the 1hr monitoring period, the swine will be returned to its run overnight. Buprenorphine (0.01-0.05mg/kg, SC) will be administered to provide pain relief during overnight monitoring.

Day 15 (Thursday): The swine will be recovered from its run and start its post-dive assessment, which will include a Vital sign measurement. These procedures will be the same as described for the pre-dive assessment. After finishing its post-dive assessment, the swine will be infused with heparin and humanely euthanized with Euthasol ® in preparation for perfusion/fixation.

Statistical Analysis: We expect a mean survival time at 5 ATA air with SEV CO2 1.5% of 51.25 hours with a standard deviation of 5.7 hours or less. Using a one-way ANOVA with 80% power and an alpha of 0.0125, 4 animals per group (N=16) will sufficiently address the primary endpoint of survival.

AIM 3: Swine (n=5) will have surgery to implant telemeters and indwelling catheterization of the carotid artery, jugular vein, and trachea. The swine will then be allowed to recover, then be exposed to 5 ATA air with the CO_2 concentration selected which had the widest confidence intervals from AIM 2.

The objective of this study is to characterize the mechanism(s) of mortality from a 5 ATA air DISSUB exposure. In this phase, we will repeat the exposure from a selected group from Aims 1&2 based on the confidence interval of latency to death. Briefly, the groups with the widest confidence interval is predicted to have the greatest likelihood of variety in physiological parameters that can be identified as correlated to mortality. The primary outcome of Aim 3 will be changes in cardiopulmonary physiologic measurements [end-tidal CO2, ECG, respiratory rate, cardiac output, electromyography (EMG), and Arterial Blood Gas (ABG), and Venous Blood Gas (VBG)]. We plan to correlate these changes in physiologic measurements with mortality.

Day 0 (Wednesday): One 50-55 kg swine will arrive at NMRC. Veterinary medicine will evaluate the swine for infection, congenital abnormalities, or other health problems that may compromise the study. The swine will be introduced to its run and begin its acclimation phase. He will be slowly transitioned from normal feed to high fiber feed required for the chamber dive to reduce food volume. This transition will happen over 3-5 days.

Day 1-4 (Thursday – Sunday): The swine will continue its acclimation phase.

DAY 5 (Monday): Swine will be transported to the surgical suite, sedated with Ketamine/Xylazine and transferred to the surgical table. The swine will then be induced with 4% isoflurane, have an ear vein catheter placed and intubation with ventilation initiated. Once stably ventilated, the swine will be positioned in the dorsal recumbent position. The neck and pectoral region surgical sites will then be clipped for sterilization. The pectoral region will then be cleaned using the aseptic technique. Bupivacaine/epinephrine (0.25-5%, SC) will be infiltrated at the incision site. An incision will be made for the DSI L04 telemeter to be implanted subcutaneously in the pectoral region of the chest with leads tunneled to the diaphragm (EMG) and the chest wall (ECG). Once the telemetry is properly implanted, secured and the leads tunneled, the pectoral surgical site is closed. The neck will then be cleaned using the aseptic technique and surgically draped. Bupivacaine/epinephrine (0.25-5%, SC)

will be infiltrated at the incision site. An incision will then be made in the neck to expose the common carotid artery and the jugular vein. CVC catheters (Teleflex) will be placed in the internal carotid artery and jugular vein. The trachea will then be exposed and a fenestrated cannula will be placed into the airway. The swine will then be repositioned onto its left side and the nape will be shaved and sterilized for exit sites for the catheters. The catheters (n=3) will then be tunneled subcutaneously through the nape of the neck using a trochar as a guide. Each catheter will be terminated with a three-way stopcock. The throat incision will be closed with sutures and be covered with Tegaderm and elasticon. The animal will then be extubated, recovered, and returned to its run. A jacket will be fitted to the swine to further protect the incision sites and catheters during the animal's recovery.

DAY 6-11 (Tuesday-Sunday): Each swine will recover for one week. The arterial and venous catheter will be checked BID for patency and heparin locked. The tracheal catheter will be flushed twice per day with a small (2ml) volume of normal saline followed by a 5cc air bolus to prevent mucus plugging. The swine will be gradually transitioned to high fiber feed during this time.

DAY 12 (Monday): The swine will be transported from its run to the hyperbaric suite. The swine will then be placed in a plethysmograph chamber for baseline pulmonary function testing along with baseline blood and tracheal gas samples. Next, the swine will receive an intramuscular injection of ketamine and xylazine. Once sedated, the swine will be transported by a minimum of two personnel to the hyperbaric suite for shaving and electrode attachment as in AIM 1. The swine will don a vest, which will cover a combination of electrodes to enable ECG, Respiratory band, and EEG monitoring. A pulse oxygen saturation probe may also be placed on the tail. These electrodes will be secured through a combination of mechanisms – Vet Bond, tape, jacket/harness, and reinforced wires. The swine will be then be recovered within the transport cage. Dive Initiation: Once recovered, the swine will be led to the temporary housing within the Multiple Large Animal Chamber (MLAC) hyperbaric chamber using a series of ramps and Plexiglas side walls. A ration of high fiber food will be dispensed from the automatic feed hopper for consumption. The swine will enter the Plexiglas housing and the electrodes will be connected to the appropriate outputs. After at least 1-hour of acclimation and at least 4 hours since administration of xylazine and ketamine to allow for minimum of five half-lives for each agent, we will begin to record 1 hour of baseline vitals with three goals. (1) Collect baseline vitals for comparison with the telemeter data. (2) Ensure that the recording equipment is functioning and troubleshoot prior to initiating dive. (3) establish catheter patency and functional operation of the through-hull sampling system. Between 1800 to 1900, the swine will begin its dive. It will descend at 60 fsw/min to 132 fsw. We will continually monitor physiologic measurements (end-tidal CO_2, ECG, respiratory rate, EMG,) and perform periodic (at every 24 hours) blood draws (VBG and ABG).

Day 13 – 19 (Tuesday – Monday): The swine will remain on bottom. There will always be at least one person physically present with knowledge of chamber operation. There will always be at least one chamber technician on call (if not physically present) with more extensive chamber knowledge. Environmental conditions including but not limited to Temperature, humidity and oxygen content will be measured remotely via sensors mounted to the Plexiglas housing chamber. There will always be one investigator on call (if not present). The swine will have two meals daily of high fiber feed administered via an automatic feeding system and water available ad libitum using a lixit style water dispenser. His excrement with fall between the grated floor and be hosed out with high pressure water hosing. This will be drained from the hyperbaric

chamber at the same interval. Every 4 hours, the swine will be evaluated for signed of pain/distress, vitals, and habitat. The "Habitat" environmental assessment will focus on feces/waste accumulation and availability/access to food and water. Feces accumulation will be reduced by utilization of a sprayer system to wash waste out of the box where it can be evacuated from the hyperbaric chamber. The checklist for the evaluation is attached as Enclosure C. If at any point during the dive, the swine is identified as dead (loss of vitals without evidence of equipment failure) or no movement visualized for 12 minutes, then the dive will be aborted. The time of death will be recorded as last heart beat (if ECG functioning appropriately rounded to the next whole minute) or the start of the 12-minute monitoring period assessing movement (rounded to the next whole minute). We will continually monitor physiologic measurements (end-tidal CO2, ECG, respiratory rate, EMG) and perform periodic (at every 24 hours) blood draws (ABG). Should swine show signs of cardiopulmonary decline, additional ABG/VBG draws may be conducted at the discretion of the Investigators or the attending veterinarian.

Day 20 (Tuesday): After 172 hours of bottom time, it will be early on day 13 (between 0300 to 0400). The swine will leave bottom following the oxygen accelerated decompression plan in the SRSDP, shown in Enclosure 1.

Day 21 (Wednesday): After 205 hours of hyperbaric exposure, the swine will reach surface (between 0655 to 0800). The swine will then have the attached electrode leads disconnected from the chamber wiring harness and secured to the jacket in a coil. The swine will be led down the ramp and into his transportation cage if possible. If not, at least two individuals will carry the pig to its transportation cage using a blanket as a sling. The swine will be returned to its run. The electrode leads will be connected to a mobile recording suite and the animal will be observed for at least 1 hour with regular monitoring of vital signs. Diazepam (0.125mg/kg) and Ketorolac (1mg/kg) will be administered intravenously via jugular catheter for pain relief during the 1hr monitoring period. After the 1hr monitoring period, the swine will be returned to its run overnight. Buprenorphine (0.01-0.05mg/kg, SC) will be administered to provide pain relief during overnight monitoring.

Day 22 (Thursday): The swine will be recovered from its run and start its post-dive assessment, which will include a vital sign measurement. These procedures will be the same as described for the pre-dive assessment. After finishing its post-dive assessment, the swine will be infused with heparin and humanely euthanized with Euthasol ® in preparation for perfusion/fixation.

Statistical Analysis: We plan to use a repeated measures ANOVA to assess significant changes from baseline for each physiological parameter. Given the novel approach and new technologies included in Aim 3, we plan to use a sample size of 5 to quantify the standard deviation and biological effect size for each parameter during the exposure. These data will support generation of *a priori* power analyses in future studies.

References:

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered:

The objective of this study is to determine the mortality associated with prolonged exposure to high pO_2 while exposed to varying concentration of CO_2, while also exposed to 5ATA. Computer modeling was considered as an alternative. However, given the lack of data in human or animal models, there is a complete void in data from which to generate a computer based prediction model. Any computer model developed at this point would be purely speculative at the extremes of pulmonary oxygen toxicity and, therefore, operationally useless for addressing the feasibility of the current SRSDP. Additionally, pulmonary oxygen toxicity directly affects the lungs, and may indirectly affect the cardiovascular system, nervous system, and perfusion to every other organ system. The interplay of these systems can be addressed only with a complete animal model. As a result, *in vitro* organ, tissue, or cell culture models do not provide adequate interplay or system information to be useful or effective.

V.3.2. Animal Model and Species Justification:

The goal of this project is to evaluate the feasibility of an existing operational execution plan. This plan is designed to save the lives of trapped submariners. The extremes of the operation of this plan will never be tested in humans due to the ethical considerations. As a result, it is our duty to provide the best established human surrogate animal model to evaluate this plan. The 70 kg swine model is an excellent human surrogate for decompression sickness and cardiopulmonary manipulation. It is critical for the generalizability of the study to the submariner population thatwe use larger swine.

V.3.3. Laboratory Animals

V.3.3.1.	Genus and Species:	Sus Scrofa
V.3.3.2.	Strain/Stock:	Yorkshire
V.3.3.3.	Source/Vendor:	Per VSP procurement procedures
V.3.3.4.	Age:	6-9months
V.3.3.5.	Weight:	Aim 1 & 2, 70kg Aim 3, 55kg
V.3.3.6.	Sex:	Male
V.3.3.7.	Special Considerations:	Castrated

V.3.4. Number of Animals Required (By Species): 30 swine

Aim 1: 9
Aim 2: 16
Aim 3: 5

V.3.5. Refinement, Reduction, Replacement (3 Rs)

V.3.5.1. Refinement:

The swine will be anesthetized with ketamine and xylazine during pre- dive preparation to

minimize distress during active handling. The swine will be acclimated to the new feed and jacket (in Aim 3) prior to diving. No acclimatization to the plexiglass enclosure will be conducted due to the inherent safety risks to staff and subject associated with transport of large swine inside the chamber The swine will be observed every four hours during the dives and evaluated for pain, distress, or discomfort. In all cases where significant pain or distress occurs, we will document the animal's behavior, attempt to identify the source of the discomfort/distress, and consider non-pharmacologic interventions that may prevent this discomfort or distress in future swine. All personnel on the protocol will be familiar with the assessment occurring every four hours. In aim 3 additional refinement is utilized in the form of catheter and telemeter placement. Telemetry allows for continuous collection and monitoring of physiologic data in awake animals, minimizing stress from handling, restraint, or use of sedation. Catheters allow of serial blood collections without having to manipulate or sedate the animals for venous blood draws.

V.3.5.2. Reduction:

The experimental design used historical data collected from 18-OUMD-13L in order to conduct power analysis for group size selection.

V.3.5.3. Replacement:

The current SRDRSP was created using sophisticated computer models derived from historical human and animal data. The CONOPS requires rescue capability which exceeds the data parameters the models were created from to such an extent that additional animal research is required. The intent of this study is to provide actionable operationally relevant models of pulmonary oxygen toxicity in the setting of hypercapnia in order to inform future human and animal exposures (research or operationally oriented).

V.4. Technical Methods

V.4.1. Pain/Distress Assessment:

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of Animals (By Species): 30 Swine.
- V.4.1.1.1.1. Column C: 0
- V.4.1.1.1.2. Column D: 0
- V.4.1.1.1.3. Column E: 30

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization:

Jugular vein, carotid artery, and tracheal catheterization: Ketamine (10-20 mg/kg, IM) and Xylazine (1-2 mg/kg, IM) for induction. Isoflurane (4%) will be used for induction then titrated to maintain surgical plane of anesthesia. Bupivacaine/epinephrine (0.25-5%, SC) to reduce incision site discomfort.

Ear vein catheterization: Ketamine (10-20 mg/kg, IM) and Xylazine (1-2 mg/kg, IM).

DCS: Immediate post-dive monitoring, Diazepam infusion (0.125 mg/kg, IV), Ketorolac (1mg/kg, IV).

DCS: 24 hour recovery, Buprenorphine (0.01-0.05%, SC Max 6mg/kg), Diazepam boluses (25mg/bolus, IV/IM).

V.4.1.2.2. Pre- and Post-procedural Provisions:

V.4.1.2.3. Paralytics:

None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures

V.4.1.3.1. Source(s) Searched:

ProQuest, PubMed

V.4.1.3.2. Date of Search: 1/27/2021

V.4.1.3.3. Period of Search:

No limit set

V.4.1.3.4. Key Words of Search:

(("Disease Models, Animal"[Mesh] OR "Models, Biological"[Mesh] OR "Models, Theoretical"[mh] OR In-Vitro OR "Cell line"[mh] OR "Cells, Cultured"[mh] OR tissue-culture* OR virtual-cell* OR "Computer Simulation"[Mesh] OR "Cadaver"[mh] OR ex-vivo OR "Animal Use Alternatives"[mh] OR "Animal Testing Alternatives"[mh]) OR ((reduction AND refinement AND replacement) OR "Handling (Psychology)"[Mesh] OR 3-Rs OR 3Rs OR "Stress, Psychological"[mh] OR stress OR stressful OR distress* OR discomfort OR "Pain"[Mesh] OR pain OR painful OR "Analgesics"[mh] OR "Analgesia"[mh] OR "Anesthesia and Analgesia"[mh] OR environmental-enrichment OR "Animal Technicians/education"[Mesh] OR "Animal Husbandry"[Mesh])) AND ("Models, Animal"[Mesh] OR "Animal Experimentation"[Mesh] OR "Ethics, Research"[Mesh] OR "Animal Care Committees"[Mesh] OR "Laboratory Animal Science"[Mesh] OR "Bioethical Issues"[Mesh] OR "Ethics Committees"[Mesh] OR "Animal Welfare"[mh] OR "Animal Rights"[Mesh] OR Institutional Animal Care and Use Committee OR IACUC) AND ("Decompression Sickness"[MAJR] OR "Carbon Dixide "[MAJR] OR "Lung Injury"[MAJR] OR "Lung/injuries"[MAJR] OR "Oxygen/toxicity"[MAJR] OR "Electrodes"[MAJR] OR telemeter OR telemeters OR "Catheters"[MAJR] OR "Catheterization"[MAJR] OR "Euthanasia, Active"[MAJR] OR "Injections"[MAJR] OR "Phlebotomy"[MAJR] OR "Euthanasia, Animal"[MAJR] OR "Immobilization"[MAJR] OR "Restraint, Physical"[MAJR]) AND ("Swine"[MAJR])

V.4.1.3.5. Results of Search:

Pubmed: There were 15 results, none of which were relevant to the proposed study

1) Wild boar behaviour during live-trap capture in a corral-style trap: implications for animal welfare. Fahlman Å, Lindsjö J, Norling TA, Kjellander P, Ågren EO, Bergvall UA. Acta Vet Scand. 2020 Nov 10;62(1):59. doi: 10.1186/s13028-020-00557-9. PMID: 33168032 Free PMC article.
   We are using domesticated swine and not performing any of the capture methods described here. This study is not relevant to the proposed work.

2) Swine model of in-stent stenosis in the iliac artery evaluating the serial time course. Ishikawa O, Tanaka M, Konno K, Hasebe T, Horikawa A, Iijima A, Saito N, Takahashi K. Exp Anim. 2018 Nov 1;67(4):501-508. doi: 10.1538/expanim.18-0027. Epub 2018 Aug 1. PMID: 30068792 Free PMC article.
   This study sought to evaluate the time course of in-stent stenosis in swine. No aspect of this study is relevant to the proposed work.

3) Effects of confinement duration and parity on stereotypic behavioral and physiological responses of pregnant sows. Zhang MY, Li X, Zhang XH, Liu HG, Li JH, Bao J. Physiol Behav. 2017 Oct 1;179:369-376. doi: 10.1016/j.physbeh.2017.07.015. Epub 2017 Jul 11. PMID: 28705536.
   This study evaluated the effects of increased confinement time in pregnant swine. The study found that there was no effect of confinement on multiple metrics of swine health (behavioral, cytokine, and cortisol) until confinement persisted beyond 8 days. The maximum confinement in the hyperbaric chamber is 192hours (8.5days) and all swine in the protocol are castrated males. Due to these facts this study is not relevant to the experimental protocol.

4) Safety assessment of epidural wire electrodes for cough production in a chronic pig model of spinal cord injury. Kowalski KE, Kowalski T, DiMarco AF. J Neurosci Methods. 2016 Aug 1;268:98-105. doi: 10.1016/j.jneumeth.2016.05.002. Epub 2016 May 7. PMID: 27168496 Free PMC article.
   This study used electrodes implanted in the spinal cord to induce a cough reflex by means of an electrical stimulation. No aspect of this study is relevant to the proposed work.

5) Porcine survival model to simulate acute upper gastrointestinal bleedings. Prosst RL, Schurr MO, Schostek S, Krautwald M, Gottwald T. Lab Anim. 2016 Jun;50(3):217-20. doi: 10.1177/0023677215600946. Epub 2015 Aug 25. PMID: 26306615
   This publication described the creation of a novel model of gastro-intestinal bleeding in swine. No aspect of this study is relevant to the proposed work.

6) A preterm pig model of lung immaturity and spontaneous infant respiratory distress syndrome. Caminita F, van der Merwe M, Hance B, Krishnan R, Miller S, Buddington K, Buddington RK. Am J Physiol Lung Cell Mol Physiol. 2015 Jan 15;308(2):L118-29. doi: 10.1152/ajplung.00173.2014. Epub 2014 Nov 14. PMID: 25398985 Free article.
   This study evaluated the time course of pulmonary injury in ventilated neonatal pigs. No aspect of this study is relevant to the proposed work.

7) Evaluation of intraosseous pressure in a hypovolemic animal model. Frascone RJ, Salzman JG, Adams AB, Bliss P, Wewerka SS, Dries DJ. J Surg Res. 2015 Jan;193(1):383-90. doi: 10.1016/j.jss.2014.07.007. Epub 2014 Jul 7. PMID: 25091338
This study evaluated the utility of measuring vascular pressures by implanting the pressure transducers intraosseously in a swine model of hypovolemic shock. No aspect of this study is relevant to the proposed work.

8) Delayed endoluminal vacuum therapy for rectal anastomotic leaks after rectal resection in a swine model: a new treatment option. Rosenberger LH, Shada A, Ritter LA, Mauro DM, Mentrikoski MJ, Feldman SH, Kleiner DE. Clin Transl Sci. 2014 Apr;7(2):121-6. doi: 10.1111/cts.12140. Epub 2014 Jan 23. PMID: 24456480 Free PMC article.
This study evaluated the utility of endoluminal vacuum therapy for sealing rectal anastomotic leaks in a swine model of rectal resection. While interesting, there is no aspect of this study which is relevant to the planned studies.

9) Effects of Natural versus Synthetic Surfactant with SP-B and SP-C Analogs in a Porcine Model of Meconium Aspiration Syndrome. Salvesen B, Curstedt T, Mollnes TE, Saugstad OD. Neonatology. 2014;105(2):128-35. doi: 10.1159/000356065. Epub 2013 Dec 14. PMID: 24356240
This study was evaluating the resistance of synthetic vs natural surfactant to inactivation by meconium in a neonatal swine model. None of the methods in this publication are relevant to the proposed studies.

10) Corticosteroid treatment ameliorates acute lung injury induced by 2009 swine origin influenza A (H1N1) virus in mice. Li C, Yang P, Zhang Y, Sun Y, Wang W, Zou Z, Xing L, Chen Z, Tang C, Guo F, Deng J, Zhao Y, Yan Y, Tang J, Wang X, Jiang C. PLoS One. 2012;7(8):e44110. doi: 10.1371/journal.pone.0044110. Epub 2012 Aug 29. PMID: 22952892
This study used a rodent model of H1N1 induced acute lung injury to assess the efficacy of corticosteroid therapy on outcomes. No aspect of this publication is relevant to the proposed work.

11) Evaluation of methods of rapid mass killing of segregated early weaned piglets. Whiting TL, Steele GG, Wamnes S, Green C. Can Vet J. 2011 Jul;52(7):753-8. PMID: 22210939 Free PMC article.
This study evaluated various methods of euthanasia of large numbers of weanling piglets in a factory farm setting. This study found that physical methods (primarily gunshot) were superior to injection (IP) of pentabarbitol for the euthanasia of piglets. This study is not relevant to the proposed study due to the use of small numbers of swine, the use of large adult swine, and a more controlled research setting.

12) A surgical device for minimally invasive implantation of experimental deep brain stimulation leads in large research animals. Ettrup KS, Tornøe J, Sørensen JC, Bjarkam CR. J Neurosci Methods. 2011 Aug 30;200(1):41-6. doi: 10.1016/j.jneumeth.2011.06.011. Epub 2011 Jun 22. PMID: 21723320
This study evaluated a new method and device to implant leads into the brain for deep brain stimulation in swine. This study is not relevant to the proposed studies in any way.

13) A model of hemorrhagic shock and acute lung injury in Landrace-Large White Swine. Xanthos TT, Balkamou XA, Stroumpoulis KI, Pantazopoulos IN, Rokas GI, Agrogiannis GD, Troupis GT, Demestiha TD, Skandalakis PN. Comp Med. 2011 Apr;61(2):158-62. PMID: 21535927 Free PMC article.
This study reported an experimental protocol of acute hemorrhagic shock and fluid resuscitation in Landrace-Large White swine. This study is not relevant to the proposed studies in any way.

14) A porcine model of acute lung injury by instillation of gastric fluid. Meers CM, De Wever W, Verbeken E, Mertens V, Wauters S, De Vleeschauwer SI, Vos R, Vanaudenaerde BM, Verleden GM, Van Raemdonck DE. J Surg Res. 2011 Apr;166(2):e195-204. doi: 10.1016/j.jss.2010.10.015. Epub 2010 Nov 13. PMID: 21109258
This study evaluated the impact of gastric juice aspiration on lung structure and function. This study is not relevant to the proposed studies.

15) A porcine model to study ex vivo reconditioning of injured donor lungs. Meers CM, De Wever W, Verbeken E, Wauters S, Vos R, Vanaudenaerde BM, De Vleeschauwer SI, Verleden GM, Lerut TE, Van Raemdonck D. J Surg Res. 2011 Apr;166(2):e175-85. doi: 10.1016/j.jss.2009.09.028. Epub 2009 Oct 4. PMID: 20034636
This publication reported on the development of a swine model of lung injury to study mechanisms to improve lung quality for transplant. This study is not relevant to the proposed studies.

Proquest: There were 18 results, 4 of which were relevant to the proposed study.

1) Conceptual and methodological issues relating to pain assessment in mammals: The development and utilisation of pain facial expression scales. McLennan KM, Miller AL, Dalla Costa E, Stucke D, Corke MJ, Broom DM, Leach MC, Department of Biological Sciences University of Chester, Parkgate Rd, Chester, CH1 4BJ, United Kingdom; Newcastle University, School of Natural and Environmental Sciences, Newcastle upon Tyne, United Kingdom; Newcastle University, School of Natural and Environmental Sciences, Newcastle upon Tyne, United Kingdom; Università degli Studi di Milano, Dipartimento di Mediciana Veterinaria, Milan, Italy; Havelland Equine Clinic, Beetzsee-Brielow, Germany ; Centre for Animal Welfare and Anthrozoology, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, United Kingdom. Applied animal behavior science. 2019 Apr; 217: p. 1-15. doi: 10.1016/j.applanim.2019.06.001. Epub 2019 Jun 12.
This is a novel way to assess pain in swine and other large animals. We have elected to utilize the Reyes scale as it provides information on the animal's functional status in addition to pain status.

2) Noninvasive, in-pen approach test for laboratory-housed pigs. Hulbert LE, Becker GY, Bortoluzzi EM, Coffin MJ, Khaing ZZ, Luo Y, McNeil EM, Mumm JM, Vandevord PJ, Walilko T, Zai L, Animal Sciences and Industry, Kansas State University; DynaSim Technical Services, INC 2 DynaSim Technical Services, INC 3 Animal Sciences and Industry, Kansas State University 4 Lucent Research, LLC. Department of Neurological Surgery, University of Washington 5 Center for Injury Biomechanics; Virginia Polytechnic Institute and State University Applied Research Associates, Inc.; Applied Research Associates, Inc.. Lucent Research, LLC. Journal of visualized experiments. 2019 Jun; 148: p. e58597. doi: 10.3791/58597. Epub 2019 Jun 5. PMID: 31233023

This study developed a standardized behavioral test called HAT (human approach test) to diagnose a pig's motivation to receive stimulus before and after TBI (traumatic brain incidence) treatment. This technique provides information regarding the animal's cognitive state. This test requires access to the animal which will be blocked during the hyperbaric exposure.

3) On-farm pig dispatch methods and stockpeople attitudes on their use. Dalla Costa FA, Coldebella A, Faucitano L, Neville GG, Dalla Costa OA, Steffan EOO, Gibson TJ, Embrapa Swine and Poultry, BR 153, Km 110, Concórdia 89700-991, Brazil 2 Agriculture and Agri-Food Canada, 2000 College Street, J1M 0C8 Sherbrooke, Quebec, Canada; Royal Veterinary College, University of London, United Kingdom 4 Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, 13635-900. Livestock Science. 2019 Mar; 221: p. 1-5. doi: https://doi.org/10.1016/j.livsci.2019.01.007. Epub 2019 Jan 7.

This study constructed a survey for dispatching methods for swine by Brazilian stockpeople and their comfort in such techniques. Most of their methods do not meet legal and welfare requirements and should be discontinued. This study is not relevant to the proposed studies in any way.

4) Oleic acid-injection in pigs as a model for acute respiratory distress syndrome. Kamuf J, Garcia-Bardon A, Ziebart A, Thomas R, Rümmler R, Möllmann C, Hartmann E, Department of Anesthesiology, University Medical Center of the Johannes Gutenberg-University; Department of Anesthesiology, University Medical Center of the Johannes Gutenberg-University. Journal of visualized experiments. 2018 Oct; 140: p. e57783. doi: 10.3791/57783. Epub 2018 Oct 26. PMID: 30417861.

This study researched oleic acid injection (OAI) lung injury in various animal models to study acute respiratory distress syndrome (ARDS). This study is not relevant to our proposed studies.

5) Evaluating environmental enrichment as a method to alleviate pain after castration and tail docking in pigs. Backus BL, McGlone JJ; Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX, United States. Applied animal behaviour science. 2018 Jul; 204: p. 37-42. doi: https://doi.org/10.1016/j.applanim.2018.04.009. Epub 2018 Apr 17.

This study evaluated the effect of enrichment on pain after castration and tail docking procedures in pigs. This study is not relevant to our proposed studies in any way.

6) Thermo-sensitive injectable glycol chitosan-based hydrogel for treatment of degenerative disc disease. Li Z, Kwon B, Shim H, Ik Sung C, Lee JH, Huh KM, Cho MO, Sun-Woong K; Wooridul Hue Brain, Seoul 137-701, Republic of Korea; Department of Polymer Science and Engineering, Chungnam National University, Daejeon 305-764, Republic of Korea; Polymer Technology Institute, Sungkyunkwan University, Suwon, Gyeonggi 440-746, Republic of Korea; Department of Polymer Science and Engineering, Chungnam National University, Daejeon 305-764, Republic of Korea; Predictive Model Research Center, Korea Institute of Toxicology, Daejeon 305-343, Republic of Korea; Predictive Model Research Center, Korea Institute of Toxicology, Daejeon 305-343, Republic of Korea; Department of Human and Environmental Toxicology, University of Science and Technology, Daejeon, 305-343 Republic of Korea. Carbohydrate polymers. 2018 Mar; 184: p. 342-353. doi: 10.1016/j.carbpol.2018.01.006. Epub 2018 Jan 2. PMID: 29352928.

This study found that injecting hydrogel formulations into rat and pig models, reduced lower back pain and may be a potential treatment for disc herniation. This study is not relevant to our proposed studies in any way.

7) Laminotomy for lumbar dorsal root ganglion access and injection in swine. Unger MD, Maus TP, Puffer RC, Newman LK, Currier BL, Beutler AS; Departments of Anesthesiology and Oncology, Mayo Clinic, Translational Science Track, Mayo Graduate School, Department of Radiology (Section of Interventional Pain Management), Mayo Clinic; Department of Radiology (Section of Interventional Pain Management), Mayo Clinic; Department of Neurologic Surgery, Mayo Clinic; Departments of Anesthesiology and Oncology, Mayo Clinic, Translational Science Track, Mayo Graduate School; Department of Orthopedic Surgery, Mayo Clinic. Journal of visualized experiments. 2017 Oct; 128: p. e56434. doi: 10.3791/56434. Epub 2017 Oct 10. PMID: 29053676.

This study used a different method of laminotomy on swine to research dorsal root ganglia in the neurons below the head. This study is not relevant to our proposed studies in any way.

8) Effects of Shenfu injection on macrocirculation and microcirculation during cardiopulmonary resuscitation. Wu J, Li C, Yuan W. Journal of ethnopharmacology 2016 Mar; 180: p. 97-103. doi: 10.1016/j.jep.2016.01.027. Epub 2016 Jan 19. PMID: 26806577.

This study examined the effects of Shenfu injections (SFI) on macro and microcirculation on pigs during ventricular fibrillation and cardiopulmonary resuscitation. This study is not relevant to our proposed studies in any way.

9) Surgical placement of catheters for long-term cardiovascular exercise testing in swine. De Wijs-Meijler DPM, Stam K, van Duin RWB, Verzijl A, Reiss IK, Duncker DJ, Merkus D; Experimental Cardiology and Neonatology, Erasmus MC, Experimental Cardiology, Erasmus MC; Experimental Cardiology, Erasmus MC ; Experimental Cardiology and Neonatology, Erasmus MC; Neonatology, Erasmus MC. Journal of visualized experiments 2016 Feb; 108: p. e53772. doi: 10.3791/53772. Epub 2016 Feb 9. PMID: 26889804.

This study described the surgical procedure to monitor cardiopulmonary dysfunction while pigs were on a treadmill. This study is not relevant to our proposed studies in any way.

10) Nitrous oxide as a humane method for piglet euthanasia: Behavior and electroencephalography (EEG). Rault JL, Kells N, Johnson C, Dennis R, Sutherland M, Lay DC; Animal Welfare Science and Bioethics Centre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN, USA; AgResearch Ltd., Ruakura Research Centre, Hamilton, New Zealand. Physiology & behavior. 2015 Nov; 151: p. 29-37. doi: 10.1016/j.physbeh.2015.06.026. Epub 2015 Jun 27. PMID: 26129686.

This study compared the methods of humanely euthanizing piglets via CO_2 and N_2O. This study is not relevant to our proposed studies.

11) Welfare implications of invasive piglet husbandry procedures, methods of alleviation and alternatives: a review. Sutherland, MA. New Zealand veterinary journal. 2015 Jan; 63.1: p. 52-57. doi: 10.1080/00480169.2014.961990. Epub 2014 Dec 11. PMID: 25204203.

This study reviewed the current swine husbandry procedures and how relevant they are with new technology available to negate such procedures. This study is not relevant to our proposed studies.

12) Pain management in the neonatal piglet during routine management procedures. Part 2: Grading the quality of evidence and the strength of recommendations. O'Connor A, Anthony R, Bergamasco L 3 ; Coetzee, J 4 ; Dzikamunhenga, R S 4 ; Gould, S 4 ; Johnson, A K 4 ; Karriker, L A 4 ; Marchant-Forde, JN, Martineau GS, McKean J, Millman ST, Niekamp S, Pajor EA, Rutherford K, Sprague M, Sutherland M, von Borell E; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa, USA; Philosophy Department, University of Alaska Anchorage, Alaska, USA; Philosophy Department, University of Alaska Anchorage, Alaska, USA; Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia, USA; Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa, USA; USDA-ARS, Livestock Behavior Research Unit, West Lafayette, Indiana, USA; National Veterinary School of Toulouse, Toulouse, France; National Pork Board, Des Moines, Iowa, USA; Production Animal Health, University of Calgary, Calgary, Canada; Scotland'd Royal College (SRUC), Edinburgh, United Kingdom; American Association of Swine Veterinarians (AASV), Perry, Iowa, USA; AgResearch Ltd, Ruakura Research Centre, Hamilton, New Zealand; Institute of Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Halle, Germany. Animal health research reviews. 2014 Jun; 15.1: p. 39-62. doi: 10.1017/S1466252314000073. Epub 2014 Jun 15. PMID: 25605278.

This study made recommendations to reduce pain for piglets undergoing standard swine production procedures (tail docking, ear notching, etc). This is not relevant to the proposed studies, as piglets will not be used.

13) Tissue engineering of a human 3d in vitro tumor test system. Moll C, Reboredo J, Schwarz T, Appelt A, Schürlein S, Walles H, Nietzer S; Department of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg. Journal of visualized experiments. 2013 Aug; 78: p. e50460. doi: 10.3791/50460. Epub 2013 Aug 6. PMID: 23963401.

This study compared the 2D and 3D culture models for tumor cells to mimic the complexity of real-life cancer cells. This is not relevant to our proposed studies.

14) Assessment of unconsciousness in pigs during exposure to nitrogen and carbon dioxide mixtures. Llonch P, Dalmau A, Jospin M, Manteca X, Rodríguez P, Velarde A; IRTA, Animal Welfare Subprogram, Finca Camps i Armet s/n, Monells, 17121 Girona, Spain; Department of Automatic Control, Biomedical Engineering Research Center, Technical University of Catalonia (UPC), 08028 Barcelona, Spain; Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Campus Bellaterra, edifici V. Cerdanyola del Vallès, 08193 Barcelona, Spain. Animal. 2013 Mar; 7.3: p. 492-498. doi: https://doi.org/10.1017/S1751731112001966. Epub 2020 Dec 7.

This study assessed unconsciousness in pigs during and after exposure to gas mixtures of CO_2 and N_2. The concentrations of CO_2 used are far higher than in the proposed studies and thus this publication is not relevant.

15) A surgical technique for a terminal intracranial hypertension model in pigs. Janda M, Bajorat J, Simanski O, Nöldge-Schomburg G, Hofmockel R, Schütze M; Department of Anaesthesiology and Intensive Care Medicine, University of Rostock, Schillingallee 35, D-18057 Rostock, Germany; University of Applied Sciences Technology, Business and Design Engineering Sciences, Wismar, Germany; Department of Neurosurgery, University of Rostock, Rostock, Germany. Laboratory animals. 2012 Jul; 46.3: p. 258-260. doi: 10.1258/la.2011.011149. Epub 2012 Apr 20. PMID: 22522418.

This study described an efficient and reliable surgical method for temporary elevation of intracranial pressure in research pigs. This study is not relevant to our proposed studies.

16) Aversion to the inhalation of nitrogen and carbon dioxide mixtures compared to high concentrations of carbon dioxide for stunning rabbits. Llonch P, Rodríguez P, Velarde A, Abreu de Lima V, Dalmau A. Animal welfare. 2012 Jun; 21.2: p. 123-129. doi: 10.7120/096272812X13353700593923.

This study assessed the aversion of inhaling 90% CO_2 and 80% N_2/20% CO_2 mixture in rabbits. This study is not relevant to our proposed studies.

17) Coronary angiography and percutaneous coronary intervention in the porcine model: a practical guide to the procedure. Williams PD, Malik N, Kingston PA; The University of Manchester, Manchester Academic Health Science Centre, School of Biomedicine, Vascular Gene Therapy Unit, Core Technology Facility, 46 Grafton Street, Manchester M13 9NT, UK. Animal. 2012 Feb; 6.2: p. 311-320. doi: 10.1017/S1751731111001650. Epub 2012 Feb 6. PMID: 22436190.

This study described how to safely and effectively do a porcine coronary artery procedure. This study is not relevant to our proposed studies.

18) Behavior of piglets after castration with or without carbon dioxide anesthesia. Van Beirendonck S, Driessen B, Verbeke G, Geers R. Journal of animal science. 2011 Oct; 89.10: p. 3310-3317. doi: 10.2527/jas.2010-3104. Epub 2011 Apr 29. PMID: 21531848.

This study discussed the behavior of piglets after castration with and without carbon dioxide anesthesia. This study is not relevant to our proposed studies.

After review of the findings of the literature search below; no alternatives to the proposed studies were found

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification:

Discussions were held with the attending veterinarian regarding the painful/distressful procedures. The goal of this project is to evaluate the feasibility of an existing operational execution plan. This plan is designed to save the lives of trapped submariners, which if in a DISSUB, historically have a high probability of death. The extremes of the operation of this plan will never be tested in humans due to the ethical considerations and, yet, physiologically, the current plan may not be feasible as described in the background section. To understand the gaps in the plan and increase the probability of success for the future rescue operations to save the lives of submariners, we need to evaluate the feasibility. These submariners will be in a life or death situation and, therefore, to stop at an end-point short of death will be a disservice to the evaluation of this current plan and more importantly the lives of the future submariners that we are working to save. Using death as an endpoint will allow us to identify the gaps in feasibility for future research and DISSUB rescue plan development.

V.4.2. Prolonged Restraint:

N/A

V.4.3. Surgery

V.4.3.1. Pre-Surgical Provisions:

After receipt and proper time for acclimatization (WRAIR SOP-UWN-216-D), swine will be fasted for 12 hours prior to surgery. Water will be withheld from all animals on the morning of surgery.

V.4.3.2. Procedure:

DSI L04 Transducer, Carotid/Jugular Catheter and Trachea Cannula placement: The animal will be surgically scrubbed and draped using the aseptic technique for transducer placement. Bupivacaine/epinephrine (0.25-5%, SC) will be infiltrated at the incision site. A 5-7 cm skin incision will be made over the lateral/dorsolateral portion of the neck between the caudal aspect of the ear and the cranial aspect of the scapula using a #10-15 scalpel blade and electrocautery will be available to control bleeding. Using incision and blunt dissection, the cutaneous trunci muscle fibers will be exposed to form a pocket slightly larger than the size of the implant. The implant will be seated between the cutaneous trunci and the underlying cervical muscle orienting the biopotential leads away from the antenna (e.g., leads cranially and antenna ventrally). The leads should not cross the antenna, as this could interfere with signal transmission. The implant is then secured to the underlying fascia or muscle using non-absorbable sutures through the suture straps. Three incisions, one on the left side between the 5th and 6th intracostal space; the second on the left side at the level of the diaphragm will be made to tunnel the positive lead and the EMG leads subcutaneously to the incision on the left side between the 5th and 6th intracostal space. Leave the ECG positive electrode at the incision then further tunnel the EMG electrodes to the diaphragm incision. Suture the positive ECG electrode in place to the musculature using an absorbable suture. At the diaphragmatic incision, using an 18- gauge needle, tunnel through approximately 1-2 cm of diaphragmatic muscle tissue perpendicular to the long axis of the fiber bundles. Pass the exposed EMG wire into the lumen of the needle so that, as the needle is withdrawn, the wire is left embedded in the muscle with the tip of the wire coming out the other side. Repeat for the other EMG lead. Place both leads approximately 5-10 mm apart within the same group of muscle fibers. Ensure that the exposed portion of the leads from each channel do not make contact. The negative ECG lead will be tunneled to the incision at the jugular notch and the lead sutured to the superficial muscle using absorbable suture. After the transducer is secured and the incision site is closed, the jugular vein and carotid artery area will be cleaned aseptically and draped for catheter insertion. With the animal in the supine position, bupivacaine/epinephrine (0.25-5%, SC) will be infiltrated at the incision site. A 5-7cm craniocaudal incision will be made in the ventral neck over the right jugular furrow using a #10-15 scalpel blade with electrocautery available to control bleeding. An incision will be extended from the region over the external jugular vein superficially down to the level of the sternomastoid muscle. The EJV will be identified and isolated via blunt dissection of muscle and SQ tissue. A 0- silk ligature will be placed around the vessel cranially and distally to secure catheter in place. A primed (0.9% NS) 16g x 12” CVC catheter kit (Teleflex) will be used. Using iris scissors, a small cut will be made on top of the vessel. The 16g x 12” catheter will be introduced into the right external jugular vein at least 12-15 cm leaving enough catheter to be tunneled. Placement will be confirmed via free flow of venous blood. Once confirmed, the catheter will be secured with the 0 silk ligatures. The caudal end of the catheter will be tunneled to the nape of the neck using a trochar and exteriorized. Next the carotid artery will be isolated and ligated in the same manner as the jugular vein. Once exposed, a primed (0.9%) 16g x 12” CVC catheter (Teleflex) will be inserted into the vessel in the same manner as the jugular catheter leaving enough catheter to tunnel and exteriorize at the same location as the jugular catheter. Confirm catheter patency on both catheters. All subcutaneous tissues can be closed with 2-0 to 3-0

Prolene suture in a simple continuous pattern. Skin incisions can be closed with 2-0 non absorbable in a continuous intradermal pattern. Tissue glue (Vet Bond, 3M, St Paul, MN) can be used to close any gaps in the skin incision that remained after suturing. Once the incision site is closed, the neck will be prepped and draped for tracheal cannulation. Bupivacaine/epinephrine (0.25-5%, SC) will be infiltrated at the incision site. A 3-4cm vertical incision will then be made sharply in the midline at least 3-4 cm below the cricoid but well above the sternal notch. This incision will be carried down via sharp and blunt technique, separating strap muscles and retracting thyroid tissue when necessary. With the anterior trachea exposed, a small horizontal stab incision between the tracheal rings (preferably between the 3rd and 4th rings to allow adequate distance from laryngeal structures) will be made. The incision will be small enough to allow passage of a 1.8-5.0 mm outer diameter tracheal cannula without creating a surrounding air leak. Once verified in place, a 2-0 or 3-0 Prolene suture will be used to secure the tube to the anterior trachea. The incision will be closed in multiple layers if needed. A small lateral neck stab incision can be used to externally tunnel the catheter or the midline incision can be closed around the catheter. In either setting, an additional skin stay-suture of 2-0 Prolene will be used to secure the catheter from being dislodged. Skin glue will be applied to any primarily closed incisions.

V.4.3.3. Post-Surgical Provisions:

Following the transducer and catheter placement procedures, swine will be observed continuously until fully recovered by surgeon and animal technicians. Body temperature, heart rate, and SPO_2 will be monitored every 15 min for the first hour postoperatively, at 30 minute intervals for the following hour, and then hourly thereafter until the pig exhibits complete recovery from anesthesia. The animal swine then will be returned to its pen and undergo routine veterinary medicine care (VSP staff).

V.4.3.4. Location:

(b) (6)(b) (6)

V.4.3.5. Surgeon:

(b) (6)(b) (6)(b) (6)(b) (6)(b) (6)(b) (6) (Alternate)

V.4.3.6. Multiple Major Survival Operative Procedures

V.4.3.6.1. Procedures:

N/A

V.4.3.6.2. Scientific Justification:

N/A

V.4.4. Animal Manipulations

V.4.4.1. Injections:

Pre-operative anesthesia for induction):
Ketamine (10-20 mg/kg, IM) and Xylazine (1-2 mg/kg, IM) delivered using 20-23 gauge needle and syringe at the nape of the neck.
Bupivacaine (0.25-5% SC Max 6mg/kg) injected peri-incision site using 20-23 gauge needle.
Heparinized Saline Flush (5cc) delivered intravenously via central venous line following catheter placement.
Diazepam infusion (0.125 mg/kg, IV.) delivered via ear vein catheter during post-dive monitoring period.
Ketorolac infusion (1mg/kg, I.V, single bolus) delivered via ear vein catheter during post-dive monitoring period.
Diazepam Bolus (25mg, I.V.) delivered via ear vein or central line during overnight recovery period.
Buprenorphine (0.01-0.05mg/kg, SC) administered using a 20-23 gauge needle during overnight recovery period at the nape of the neck.
Euthanasia will be consistent with the current AVMA Guidelines on Euthanasia using a commercially available, pentobarbital based, euthanasia solution (1-1.5 ml/10kg) administered IV via ear vein or jugular catheter.

V.4.4.2. Biosamples:

AIM 3: Arterial and venous blood samples for blood gas analysis. Exhalate gas samples from tracheal catheter.

V.4.4.3. Adjuvants:

None

V.4.4.4. Monoclonal Antibody (MAbs) Production:

None

V.4.4.5. Animal Identification:

Animals are identified by cage cards and ear tags.

V.4.4.6. Behavioral Studies:

None

V.4.4.7. Other Procedures:

None

V.4.4.8. Tissue Sharing:

None

V.4.5. Study Endpoint:

Death, or euthanasia after surfacing.

V.4.6. Euthanasia:

All animals will be euthanized by the PI/AI IAW the current AVMA Guidelines on Euthanasia using Euthasol (1-1.5 mL/10kg IV). Also, any animal becoming moribund during post-dive experimental procedures will be injected via central line with Euthasol 1-1.5ml/10kg.

V.5. Veterinary Care

I.1.1. V.5.1. Husbandry Considerations:

Care and husbandry of animals will be according to the most recent VSP SOPs. Animals will be housed for a 5-day minimum acclimation period in appropriately sized cages in (b) (6)(b) (6) (b) (6). Each animal will be weighed upon arrival and the day prior to experimental procedures. They will be observed daily for general health, humane treatment, and husbandry consideration. Animals will be housed in (b) (6)(b) (6).

V.5.1.1. Study Room:

(b) (6)

V.5.1.2. Special Husbandry Provisions:

Transition to High Fiber feed formulation

V.5.1.3. Exceptions:

The plexiglass boxes in the MLAC have dimensions of 48”x36”x40” (LxWxH). This equates to 12ft2 and is the maximum size which can be accommodated in the chamber in its current configuration. Table 3.6 of the guide for the care and use of laboratory animals (Recommended Minimum Space for Agricultural Animals) has minimum ft2 for swine housing broken out by weight. Extrapolating from the data in the table, a 70kg swine should have 18.7ft2of floor space for housing. This number is meant to allow the animals to turn around and move freely and rest away from urine and feces. In the conduct of the previous protocol (18-OUMD-13L) the 70kg swine were able to turn around and were observed to be able to comfortably rest. Exposure to feces was managed by a sprayer system and food and water were available ad libitum. During the protocol, animal distress was not observed until shortly before death. The exposure during that protocol was limited to 54hrs due to mortality associated with the environmental exposure. During the current study the maximum exposure to the enclosed space will be 205hrs. Should animal distress be observed during the longer study intervals that may be encountered during Aim 1 (in the 0% CO2 group), then an engineering assessment will be conducted to enlarge the enclosure prior to Aim 2. No acclimatization to the plexiglass enclosure will be conducted due to the inherent safety risks to staff and subject associated with transport of large swine inside the chamber

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care:

I.1.1.1. Animals will be monitored for health, humane treatment, and husbandry considerations twice daily by VSP personnel during routine weekday rounds and at least once daily during weekends and holidays. This is in addition to, and not in place of, monitoring by the PI and UMD staff. In the event of a debilitating illness or adverse reaction, the decision to treat or euthanize an animal will be made by either the veterinarian and/or the PI.

V.5.2.2. Emergency Veterinary Medical Care:

In an emergency or an animal health problem, if the responsible person (PI/AI) is not available, or if the investigator or veterinary staff cannot reach consensus on treatment, the veterinarian has the authority to treat the animal, remove it from the experiment, institute

appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy:

Environmental enrichment will be in accordance with the most recent VSP SOP.

V.5.3.2. Enrichment Restriction:

While in the hyperbaric chamber, stainless steel toys will be used for enrichment (to reduce fire risk).

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

Investigator/ Technician	Name of the activity (procedure, observation or manipulation) to be performed in first species	Training	Experience	Qualifications
[redacted]	Surgery, data acquisition, animal handling, observation, euthanasia	Swine Waiver 31 MAR 2016 Aseptic Tech: Waiver 30 Mar 2016 AALAS: 10 OCT 2018 ACUP: 12 Jan 2016	12 years, including swine experimentation, bronchoalveolar lavage	Post graduate training
	Data acquisition, Tissue biopsies/ harvesting of tissues/blood	Swine handling: Jan 2019 Aseptic technique: Jan 2019 AALAS: ACUP:	Physician new to animal research and will assist with data acquisition and tissue harvesting	Post graduate training, interna medicine, cardiology
	Data acquisition, animal handling, observation, euthanasia	, Swine Handling: Feb 2009 Aseptic Tech: 30Mar2016 AALAS: 10Dec2012 ACUP: 23Nov2013	20+ years. Swine experimental work since 2005 including all aspects in this protocol	Post graduate training and practice in internal medicine; pulmonary and critical care
	Data acquisition, model development, animal handling, observation, euthanasia Tissue biopsies/ harvesting of tissues/blood	Swine Handling: 06 Jan 2018 Aseptic technique: 15 Dec 2017 AALAS: 08 Jan 2018 ACUP: 09 Jan 2018	3 years. Well versed in swine work including current protocols with rodent cranial windows, tracheostomy	Post graduate training
	Surgery, data acquisition, model development, animal handling, observation, euthanasia Tissue biopsies/ harvesting of tissues/blood	Swine Handling: 06 Jan 2018 Aseptic technique: 15 Dec 2017 AALAS: 08 Jan 2018 ACUP: 09 Jan 2018	3 years. Well versed in swine work including current protocols with rodent cranial windows, tracheostomy	Post graduate training

(b) (6) (b) (6) (b) (6) (b) (6)	Data acquisition, animal handling, observation. euthanasia Tissue biopsies/ harvesting	Swine Handling: Jan 2021 Aseptic technique: Jan 2021 AALAS: Dec 2020 ACUP: Dec 2020		
(b) (6) (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) (b) (6)	Animal handling, observation, euthanasia, Tissue biopsies/ harvesting of tissues/blood	Surgery, data acquisition, animal handling, observation, euthanasia, Tissue biopsies/ harvesting of tissues/blood/Swine Swine Handling: Aseptic Technique: Waiver 30 Mar 2014 AALAS: 14 Jan 2016 ACUP: 12 Jan 2016	25+ years. Of which she has a large body of rodent handling experience and tissue harvesting	Swine, sheep, rats, dogs
(b) (6)				
(b) (6) (b) (6) (b) (6) (b) (6)	Animal handling, observation, euthanasia, Tissue biopsies/ harvesting of tissues/blood/Swine, , data acquisition	Swine Handling: 02 Oct 2019 Aseptic Technique: 21 Nov 2019 AALAS: 23 Sep 2019 ACUP: 03 Dec 2019	1year swine handling, 1 year rodent handling	Swine, rats
(b) (6) (b) (6) (b) (6) (b) (6)	Surgery, animal handling, observation, euthanasia, Tissue biopsies/ harvesting of tissues/blood/swine	Swine Handling: Dec 2018 Aseptic technique: Dec 2018 AALAS: Dec 2018 ACUP: 12 Jan 2016	25 years, including rodent handling, observation and tissue harvesting. 10 years swine handling including surgical placement of catheters.	Swine, sheep, rats

(b) (6) (b) (6) (b) (6)	Animal handling, observation, blood collection, tissue collection euthanasia	Swine Handling: Jan 2019 Aseptic technique: Jan 2019 AALAS: 1988 ACUP: Jan 2019	20+ Years including extensive experience with rodent handling, observation and	Swine, sheep, rats

VII. BIOHAZARD/SAFETY:

1. **Hyperbaric Chamber Use:** The use of hyperbaric chambers and compressed gases is potentially hazardous. Emergency procedures have been established for each chamber, thus reducing hazard potential. Chambers will be operated by the following trained personnel:

Name	Position	Training, certification	Chamber Experience
(b) (6)	Chamber operator; supervisor; instructor	Naval Medical Research Center	20 years
	Chamber operator	Naval Medical Research Center	8 years
	Chamber Operator	Naval Medical Research Center	1 Year

2. **Sharp Instruments:** All investigators and research technicians have been trained in the proper use and disposal of sharp instruments and needles.

3. **Animal handling:** All personnel participating in this protocol will be advised of the potential for zoonotic disease transmission. Investigators and Division of Veterinary Medicine personnel follow safety guidelines IAW the current version of the WRAIR/NMRC Joint Safety, Health, and Environmental Compliance Manual and relevant VSP SOPs. All personnel working with animals will wear protective clothing following current VSP SOPs at a minimum. Regulated medical waste is deposited in appropriate waste receptacles prior to departing the laboratory. All sharps are handled and disposed IAW WRAIR/NMRC Joint Safety, Health, and Environmental Compliance Manual.

Isoflurane: An inhaled anesthetic agent. Will be stored and used in accordance with WRAIR/NMRC policy. All anesthetic gases will be properly scavenged following VSP Policy.

VIII. ENCLOSURES:

Enclosure A: Literature Cited
Enclosure B: Oxygen accelerated decompression profile
Enclosure C: Evaluation checklist

Literature Cited:

1. Sontag S, Drew C. Blind Man's Bluff: The Untold Story of Cold War Submarine Espionage. 2011.
2. Jacoby T. Navigational Submarine Accidents. Information Design. 2014.
3. Van Liew H, Flynn E, Liew VH. Direct ascent from air and N 2 -O 2 saturation dives in humans: DCS risk and evidence of a threshold. Direct ascent from air and N 2 -O 2 saturation dives in humans: DCS risk and evidence of a threshold. UHM. 2005;32:409-19.
4. Bielawski A. Lessons Learned from ARA San Juan. Garmish-Partenkirchen, Germany 2018.
5. (b) (3) (A) [redacted]
6. Blatteau JE, Hugon J, Castagna O, Meckler C, Vallée N, Jammes Y, et al. Submarine Rescue Decompression Procedure from Hyperbaric Exposures up to 6 Bar of Absolute Pressure in Man: Effects on Bubble Formation and Pulmonary Function. PLoS ONE. 2013;8:1-11.
7. Operational Requirements Document for Submarine Rescue, Diving, and Recompression System (SRDRS), Revision 22006; Washington, DC.
8. Harabin AL, Homer LD, Weathersby PK, Flynn ET. An analysis of decrements in vital capacity as an index of pulmonary oxygen toxicity. J Appl Physiol. 1987;63:1130-5.
9. Eckenhoff RG, Dougherty Jr JH, Messier AA, Osborne SF, Parker JW. Progression of and Recovery From Pulmonary Oxygen Toxicity in Humans Exposed to 5 ATA Air. Aviation Space and Environmental Medicine. 1987;58:658-67.
10. Comroe J, Dripps R, Dumke P, Deming M. Oxygen toxicity: The effects of inhalation of high concentrations of oxygen for twenty-four hours on normal men at sea level and at a simulated altitude of 18,000 feet. J Am Med Assoc. 1945;128:710-7.
11. Harabin AL, Survanshi SS, Homer LD. A Model for Predicting Central Nervous System Oxygen Toxicity from Hyperbaric Oxygen Exposures in Humans. Toxicology and applied pharmacology. 1995;132:19-26.
12. Wright W, editor Use of the University of Pennsylvania Institute for Environmental Medicine Procedure for Calculation of CUmulative Pulmonary Oxygen Toxicity1972; Washington, DC.
13. SSN 688 Class Guard Book Disabled Submarine Survival Guide, (2014).
14. Fracica PJ, Knapp MJ, Piantadosi CA, Takeda K, Fulkerson WJ, Coleman RE, et al. Responses of baboons to prolonged hyperoxia: physiology and qualitative pathology. Journal of Applied Physiology. 1991;71:2352-62.
15. (b) (3) (A) [redacted]
16. Clark JM, Lambertsen CJ. Pulmonary oxygen toxicity: a review. Pharmacological reviews. 1971;23(2):37-133.
17. Schaefer KE. Environmental Physiology of Submarines and Spacecraft; Atmospheric Requirements of Confined Spaces. Arch Environ Health. 1964;9:320-31.
18. Katalan S, Falach R, Rosner A, Goldvaser M, Brosh-Nissimov T, Dvir A, et al. A novel swine model of ricin-induced acute respiratory distress syndrome. Dis Model Mech. 2017;10(2):173-83.
19. Petersen K, Soutiere SE, Tucker KE, Dainer HM, Mahon RT. Oxygen breathing accelerates decompression from saturation at 40 msw in 70-kg swine. Aviation Space and Environmental Medicine. 2010;81:639-45.
20. Cronin WA, Forbes AS, Wagner KL, et al. Exhaled Volatile Organic Compounds Precedes Pulmonary Injury in a Swine Pulmonary Oxygen Toxicity Model. Front Physiol. 2019;10:1297. Published 2019 Dec 3. doi:10.3389/fphys.2019.01297

Decompression Stop Time (min)

Equivalent Air Depth		85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	Total Stop Time (hrs)
70 fsw	Air								85	170	185	200	215	240	260	290	325	140	35.2
	Oxygen								**25**	**55**	**60**	**65**	**70**	**80**	**85**	**95**	**150**		11.4
80 fsw	Air						20	145	160	170	185	200	220	235	260	285	325	155	39.3
	Oxygen						**10**	**45**	**50**	**55**	**60**	**65**	**75**	**75**	**85**	**90**	**155**		12.8
90 fsw	Air					95	140	150	160	170	185	200	215	235	260	290	325	165	43.2
	Oxygen					95	140	**50**	**55**	**55**	**60**	**65**	**70**	**75**	**85**	**95**	**160**		16.8
100 fsw	Air			35	130	130	145	150	165	170	180	200	215	235	265	290	325	165	46.7
	Oxygen			35	130	130	145	150	165	**55**	**60**	**65**	**75**	**80**	**85**	**95**	**160**		23.8
110 fsw	Air		100	120	125	135	140	150	160	170	185	200	220	235	260	290	325	175	49.8
	Oxygen		100	120	125	135	140	150	160	**60**	**60**	**65**	**75**	**80**	**85**	**95**	**160**		26.8
120 fsw	Air	150	115	120	130	130	145	150	160	175	180	200	220	235	260	295	330	175	52.8
	Oxygen	150	115	120	130	130	145	150	160	**60**	**60**	**70**	**75**	**80**	**85**	**95**	**165**		29.8
132 fsw	Air	305	115	125	130	135	145	150	165	175	190	205	225	245	275	305	345	215	57.5
	Oxygen	305	115	125	130	135	145	150	165	**60**	**65**	**70**	**75**	**80**	**90**	**100**	**180**		33.2

Figure 1. U.S. NAVY SUBMARINE RESCUE SYSTEM (SRS) DECOMPRESSION PLAN, Table 4.3, showing the accelerated decompression schedule for DISSUB rescuees.

DIVE ASSESSMENT SHEET

Protocol # : _____ Animal #: _____ Leave Surface Date/Time: _____

References/Instructions:

- **PAIN / DISTRESS EVALUATION:**
  - **Score animals pain: 0 No evidence of pain/Distress, 1 Mild discomfort, 2 Moderate Discomfort, 3 Severe Discomfort**
  - **Examples include:**
    - **Mild: Rapid respiratory rate or heart rate; Reduced appetite (based on food consumption)**
    - **Moderate: Mild, plus increased movement or vocalization**
    - **Severe: Moderate, plus excessive vocalization, movement, or any posturing**
  - **If in severe pain/distress call investigator on call. If moderate pain/discomfort, re-assess in 1 hour. If no or mild pain/distress, reassess in 4 hours.**
- **HABITAT**
  - **Satisfactory – Access to food and water. Cleaned within last 24 hours.**
  - **Unsatisfactory – No access to food or water. Housing not cleaned within last 24 hours.**
  - **If Unsatisfactory, correct problem if possible. If not, call investigator on call.**
- **VITALS**
  - **Rate vitals: Satisfactory, Okay, Concerning**
  - **Record vitals available vitals: HR, RR**
  - **Ratings:**
    - **Satisfactory: HR 70 – 120, RR 20 – 60**
    - **Okay: HR 120 – 200, RR 60 – 100**
    - **Concerning: HR > 200, RR > 100**
  - **Actions: Re-check vitals within 2 hours if "Okay." If stable, then return to every 4 hour checks. Call investigator on call if vitals are "Concerning." If no vitals available, monitor for 12 minutes for any signs of movement, including respirations. If no signs, call investigator on call.**
- **Questions or Concerns: Call (b) (6) @ (b) (6) or on-call PI.**

DATE	TIME	INITIALS	DOMAIN	STATUS	DETAILS & COMMENTS
			Pain/Distress	0 1 2 3	
			Habitat	Satisfactory Unsatisfactory	
			Vitals	Satisfactory Ok Concerning	
			Pain/Distress	0 1 2 3	
			Habitat	Satisfactory Unsatisfactory	
			Vitals	Satisfactory Ok Concerning	
			Pain/Distress	0 1 2 3	
			Habitat	Satisfactory Unsatisfactory	
			Vitals	Satisfactory Ok Concerning	
			Pain/Distress	0 1 2 3	
			Habitat	Satisfactory Unsatisfactory	
			Vitals	Satisfactory Ok Concerning	
			Pain/Distress	0 1 2 3	
			Habitat	Satisfactory Unsatisfactory	
			Vitals	Satisfactory Ok Concerning	
			Pain/Distress	0 1 2 3	
			Habitat	Satisfactory Unsatisfactory	
			Vitals	Satisfactory Ok Concerning	