



**SERBIA ACCELERATING INNOVATION AND GROWTH  
ENTREPRENEURSHIP (SAIGE) PROJECT**

**Program PROMIS2023**

**ENVIRONMENTAL AND SOCIAL MANAGEMENT PLAN  
(ESMP)**

*Characterization of cerebral interstitial fluid secretome following intermittent theta burst stimulation - deciphering the neurochemistry of brain stimulation  
(CEREBRO)*

**DRAFT DOCUMENT**

**Belgrade,**

**26/12/2023**

## **ABBREVIATIONS AND ACRONYMS**

CEREBRO – project “Characterization of Cerebral Interstitial Fluid Secretome Following Intermittent Theta Burst Stimulation - Deciphering the Neurochemistry of Brain Stimulation”

ESMF – Environmental and Social Management Framework

ESMP – Environmental and Social Management Plan

FBUB – Faculty of Biology; University of Belgrade

IBF - Interstitial Brain Fluid

iTBS – Intermittent Theta Burst Stimulation

LFS - Life and fire safety

NITRA - The Ministry of Science, Technological Development, and Innovation of The Republic of Serbia

PI – Principal Investigator

PIU – Project Implementation Unit

rTMS - Repetitive Transcranial Magnetic Stimulation

SAIGE – Serbia Accelerating Innovation and Growth Entrepreneurship

SF – Science Fund SRO – Scientific and Research Organization

SOP – Standard Operating Procedure

SRO - Scientific and Research Organization

TM – Team Member

VINS - Vinča Institute of Nuclear Sciences, University of Belgrade

WP – Work Package

## **Table of Contents**

<b>EXECUTIVE SUMMARY .....</b>	<b>4</b>
<b>LEGAL AND ADMINISTRATIVE FRAMEWORK .....</b>	<b>5</b>
<b>Relevant Institutions .....</b>	<b>5</b>
<b>Existing Serbian legislation .....</b>	<b>5</b>
<b>PROJECT DESCRIPTION .....</b>	<b>8</b>
<b>Project location and description of work packages.....</b>	<b>10</b>
<b>ASSESSMENT OF THE POTENTIAL ENVIRONMENTAL AND SOCIAL IMPACTS OF SPECIFIC TASKS WITHIN THE PROJECT .....</b>	<b>12</b>
<b>Potential impact on generated waste .....</b>	<b>12</b>
<b>Potential impact on the health and safety of the project team.....</b>	<b>13</b>
<b>SUMMARY OF ENVIRONMENTAL AND SOCIAL IMPACT .....</b>	<b>13</b>
<b>I MITIGATION PLAN .....</b>	<b>15</b>
<b>II MONITORING PLAN.....</b>	<b>18</b>

## EXECUTIVE SUMMARY

The purpose of the Environmental and Social Management Plan (ESMP) is to highlight the potential negative environmental and social impacts and management issues during the preparation and implementation of the research project “Characterization of Cerebral Interstitial Fluid Secretome Following Intermittent Theta Burst Stimulation – Deciphering the Neurochemistry of Brain Stimulation (hereinafter: CEREBO)”. The draft ESMP document was prepared in accordance with the Environmental and Social Management Framework (ESMF) for the SERBIA ACCELERATING INNOVATION AND GROWTH ENTREPRENEURSHIP (SAIGE) PROJECT. The comprehensive analysis and the result of the screening showed that this project has low risk in terms of ethics and moderate risks related to the environment and society.

This ESMP has been prepared according to the identified potential risks and to determine specific mitigation, monitoring and institutional measures to be taken during the project implementation and duration. These measures are set to eliminate, offset, or reduce any potential negative environmental and society impacts to an acceptable level. No social and ethical risks were identified during this process. Any concerns raised can be easily addressed through mitigation measures presented in the Plan for the mitigation and further monitored using the Plan for monitoring the measures undertaken. All relevant Ethical approvals from participant institutions (FBUB and VINS) have been obtained.

Leading SRO of the project, FBUB, is one of the oldest academic institutions in the region, founded 170 years ago. FBUB is planned to be a part of the new BIO4 Campus in Belgrade – a strategic investment project to concentrate people, knowledge, and infrastructure to accelerate development in biodiversity, biomedicine, biotechnology, and bioinformatics. Alongside, VINS is the National Institute of the Republic of Serbia and one of the oldest institutes with multidisciplinary background in the Balkan region. All these statuses imply already implemented or planned capacity building activities both in scientific and administrative sections. During the project’s lifetime, further activities will be foster to increase environment and social management capacities and any specific missing or incomplete procedures related to the project will be developed and implemented to increase the scientific and administrative capacity of FBUB and VINS.

# LEGAL AND ADMINISTRATIVE FRAMEWORK

## Relevant Institutions

The Ministry of Environmental Protection of the Republic of Serbia is the key relevant institution for the environmental management of all project implementation activities related to environmental impacts. Other relevant institutions are the Institute for Nature Conservation of Serbia and the Institute for the Protection of Cultural Monuments. The Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (NITRA) is responsible for the implementation of the SAIGE project.

## Existing Serbian legislation

All experimental work, procedures involving animals and waste management will be performed in concordance with the relevant laws and/or management strategies of the Republic of Serbia, including specific rulebooks.

### *The most important legal provisions for the implementation of the CEREBRO project*

- ✓ The Constitution of Serbia (“Official Gazette of RS” No. 98/06).
- ✓ The National Strategy for Sustainable Development (“Official Gazette of RS” No. 72/09, 81/09)
- ✓ Law on Science and Research (“Official Gazette of RS” No. 49/19)
- ✓ Law on environmental protection (“Official Gazette of RS” No. 135/04, 36/09, 72/09, 43/11, 14/16, 76/18 and 95/18)
- ✓ Law on waste management (“Official Gazette of RS”, 36/09, 88/10, 14/16 and 95/2018)
- ✓ Law on noise protection (“Official Gazette of RS”, 36/09, 88/10 and 96/2021)
- ✓ Animal Welfare Law, (“Official Gazette of RS” No. 41/09)
- ✓ Regulation on welfare of animal intended for experimental purposes (‘Official Journal of. RS”, No 39/10)
- ✓ Law on Health Care (“Official Gazette of RS”, No. 25/2019)
- ✓ Law on Occupational Health and Safety (“Official Gazette of RS”, 35/2023)
- ✓ Law on protection of personal data (“Official Gazette of RS”, No. 87/2018)
- ✓ The law on data confidentiality (“Official Gazette of RS”, No. 104/2009)
- ✓ Law of gender equality (“Official Gazette of RS”, No. 52/2021).

*Regulations established based on the Law on Health and Safety*

- ✓ Rulebook on preventive measures for safe and healthy work to prevent the occurrence and spread of infectious disease epidemics ("Official Gazette of RS", No. 94/2020),
- ✓ Rulebook on preventive measures for safe and healthy work when exposed to biological hazards ("Official Gazette of RS", Nos. 96/2010 and 115/2020),
- ✓ Rulebook on personal protective equipment ("Official Gazette of RS", No. 23/2020),
- ✓ Rulebook on preventive measures for safe and healthy work at the workplace ("Official Gazette of RS", Nos. 21/20109 and 1/2019),
- ✓ Rulebook on preventive measures for safe and healthy work when exposed to chemical substances ("Official Gazette of RS", Nos. 106/2009, 117/2017, 107/2021),
- ✓ Rulebook on the provision of signs for safety or health at work ("Official Gazette of RS", Nos. 95/2010 and 108/2017),
- ✓ Rulebook on the provision of first aid, the type of means and equipment that must be provided at the workplace, the method, and deadlines for training employees to provide first aid ("Official Gazette of RS", No.109/2016),
- ✓ Rulebook on the provision of signs for safety and health at work ("Official Gazette of RS", Nos. 95/2010 and 108/2017)
- ✓ Rulebook on preventive measures for safe and healthy work when using work equipment ("Official Gazette of RS", Nos. 23/2009, 123/2012, 102/2015 and 101/2018)
- ✓ Rulebook on the procedure for inspecting and checking work equipment and testing working environment conditions ("Official Gazette of RS", No. 15/2023)
- ✓ Rulebook on the procedure for inspecting and testing work equipment and testing working environment conditions ("Official Gazette of RS", Nos. 94/2006, 108/06, 114/2014 and 102/2015),
- ✓ Rulebook on records in the field of safety and health at work ("Official Gazette of RS", Nos. 62/2007 and 102/2015),
- ✓ Rulebook on the manner and procedure of risk assessment at the workplace and in the working environment, ("Official Gazette of RS", Nos. 72/2006, 84/2006, 30/2010 and 102/2015),
- ✓ Guidelines Good laboratory practices ("Off. Gazette of the RS", No. 28/2008).

*Regulations established based on the Law on Waste*

- ✓ Rulebook on the manner of storage, packaging and marking of hazardous waste ("Official Gazette of RS", Nos. 92/2010 and 77/2021)
- ✓ Rulebook on categories, testing and classification of waste ("Official Gazette of RS", No. 56/10 and 93/2019)
- ✓ Rulebook on the form of daily records and annual report on waste with instructions for its completion ("Official Gazette of RS", Nos. 95/10 and 88/2015)

- ✓ Rulebook on the document form on the movement of waste and instructions for its completion ("Official Gazette of RS", No. 72/2009)
- ✓ Law on Fire Protection ("Official Gazette of RS", Nos. 111/2009, 20/2015, 87/2018 and 87/2018)
- ✓ Rulebook on organizing fire protection according to the category of fire hazard ("Official Gazette of the RS", No. 6/2021)
- ✓ Law on Chemicals ("Official Gazette of RS", Nos. 36/2009, 88/2010, 92/2011, 93/2012 and 25/2015)
- ✓ Rulebook on how to keep records on chemicals ("Official Gazette of RS", No. 31/2011),
- ✓ Rulebook on the Register of Chemicals ("Official Gazette of RS", Nos. 16/2016, 6/2017 and 117/2017)
- ✓ Rulebook on chemical advisers and conditions that must be met by a legal entity or entrepreneur who conducts training and knowledge testing of chemical advisers ("Official Gazette of RS", Nos. 13/11, 28/11 and 47/12)
- ✓ Rulebook on the content of the safety data sheet ("Official Gazette of RS", No. 81/10)
- ✓ Instructions on determining preventive measures for safe storage, storage, or use of particularly dangerous chemicals ("Official Gazette of RS", Nos. 94/10 and 6/2017)
- ✓ Rulebook on amendments to the Rulebook on classification, packaging, labelling and advertising of chemicals and specific products in accordance with the Globally Harmonized System for Classification and Labelling ("Official Gazette of RS", No. 21/2019)
- ✓ Law on Inspection Supervision ("Official Gazette of RS", Nos. 36/2015, 44/2018 and 95/2018)

### **Regulations of the participating SROs relevant to the project**

Table 1. Internal regulations relevant to the project

FBUB	VINS
Code of professional ethics of University of Belgrade, (2016)	Code of professional ethics of University of Belgrade, (2016)
Statute of FBUB (2021)	Statute of VINS, (2022)
Gender equality strategy of FBUB, (2022)	Gender equality strategy of VINS, (2021)
Rulebook on prevention and protection against sexual harassment at University of Belgrade, (2021)	Rulebook on prevention and protection against sexual harassment at University of Belgrade, no. (2021)
Rulebook on the internal whistle-blowing procedure of FBUB, (2015)	Rulebook on the internal whistle-blowing procedure of VINS, (2015)
Act on risk assessment of workplaces at FBUB, (2018)	Act on risk assessment of workplaces at VINS, (2012)

Rulebook on the systematization and organization of work at the FBUB, (2023)	Rulebook on the systematization and organization of work at the VINS, (2018)
Rulebook on occupational safety and health of the FBUB, (2010)	Rulebook on occupational safety and health of the VINS, (2020)
Rules on the procedure for ethical determination responsibilities at the university of Belgrade (2021)	Rules of procedure of the Ethics Commission of VINS (2019)
Rulebook on the work of the Ethics Committee for the protection of the welfare of Experimental animals of FBUB, (2017)	Rulebook on the work of the Ethics Committee for the protection of the welfare of Experimental animals of VINS, (2019)
Waste management plan of FBUB, (2012)	Waste management plan of VINS, (2021)
Rulebook on Fire Safety of the FBUB, (2012)	Rulebook on Fire Safety of VINS, (2020)

## PROJECT DESCRIPTION

Basic project information	
Country	Serbia
Project	Serbia accelerating innovation and growth entrepreneurship (SAIGE) project
Sub-component	Science Fund of the Republic of Serbia
Program	PROMIS 2023
Subprogram	Biomedicine
Project title	Characterization of Cerebral Interstitial Fluid Secretome Following Intermittent Theta Burst Stimulation – Deciphering the Neurochemistry of Brain Stimulation
Acronym	CEREBRO
Principal Investigator	Dr Milorad Dragić
Contact email address	milorad.dragic@bio.bg.ac.rs
Participating Scientific and Research Organization (SROs)	Faculty of Biology, University of Belgrade (FBUB) Vinča Institute of Nuclear Sciences, University of Belgrade (VINS)
Project duration	24 months
Number of researchers	3/Three/ (PI + 2 team members)

The basic aim of the CEREBRO project is to characterize and understand the changes in the composition of the interstitial brain fluid (IBF) after application of a specific protocol of repetitive transcranial magnetic stimulation (rTMS) – intermittent theta burst stimulation (iTBS). IBF is a fluid that floods all brain cells, i.e., neurons, astrocytes, microglia, and oligodendrocytes, and represents an important medium through which the cells communicate with each other via neurotransmitters, gliotransmitters, cytokines, chemokines and other signaling molecules. rTMS has been used for more than 10 years to treat several severe psychiatric disorders that do not respond to classical pharmacological approaches, and recent advances in the field of neuromodulation have also supported this type of therapy in other disorders, i.e., mainly neurodegenerative disorders. The basic principle of how rTMS can be beneficial in these disorders is explained by its short- and long-term effects. By applying a magnetic field over the brain tissue, the magnetic waves induce an electric field in the neuronal tissue (Faraday's law of electromagnetic induction), which causes the cells to change the polarity of their cell membranes. This change then leads to the release of various soluble factors from the cells into the IBF so that the cells can communicate with each other. The major gap in the field of neuromodulation is that there is a lack of thorough characterization of what cells release in IBF when stimulated. So far, only a few molecules have been identified, but neuronal tissue can release more than 100 different molecules that can further modulate brain activity and plasticity.

Given the lack of data on this particular topic, CEREBRO will comprehensively characterize the acute, sub-chronic and after-effects of iTBS on the composition of the IBF in the prefrontal cortex of adult male Wistar rats following the principles of discovery-based science. The longitudinal approach gives our project the unique opportunity to distinguish the acute effects of iTBS (i.e., what happens in the IBF minutes after stimulation), but also sub-chronic and after-effects that will give us the answer to the question of which molecules are present in the IBF 7 days after stimulation and even afterward. This is important because rTMS as a therapeutic approach offers the possibility to modulate brain activity non-invasively. The main advantage is that the effect extends beyond the time frame of stimulation and the effects are present in human subjects up to one month after the last stimulation. The understanding of the neurochemistry behind this rTMS would enable the usage of this technique as a more customized tool for treating specific conditions or providing a rationale for why someone is or is not a good candidate for this type of therapy.

## Project location and description of work packages

The research project will take place in two different locations at Laboratory for Neurobiology, Faculty of Biology, University of Belgrade (FBUB) and Laboratory for Molecular Biology and Endocrinology, Vinca Institute of Nuclear Sciences, University of Belgrade (VINS). Furthermore, the research project is organized in two work packages:

1) The PROTEOME work package is the first and crucial activity of the project and will be carried out in both institutions. In this part of the project, all animal experimentation and handling will be performed on forty-eight adult male *Wistar* rats obtained from the Animal Research Facility (Reg. No. 323-07-2541/2017-05/1) in VINS in accordance with European Communities Council Directive (2010/63/EU) for animal experimentation and relevant national/institutional legislation. The VINS animal research facility has three types of rooms – rooms for breeding, rooms for reproduction and rooms for animals which are in experiment. Each room has controlled humidity (40-65%), dark/light cycles (12h/12h) and ambient temperature (21-22°C), while all animals receive commercial food and tap water *ad libitum*. The main pen is divided into two corridors (one clean and one dirty) and at the end there is a special room for handling the animals. Only authorized personnel are allowed to enter the facility (veterinarians and researchers with ongoing experiments), wearing special protective clothing to minimize the animals' exposure to external conditions. The air conditioning system provides an air exchange of 8-12 volumes per hour to ensure macroecological air quality. All staff working with animals in research are highly trained in animal experiments and have more than 5 years of experience in working with laboratory animals as well as all the necessary certificates. All animals will be handled seven days before the start of the experiment for accommodation and stress reduction. Each laboratory and hallway are secured with first aid kits in case of emergency. The animals will be divided into two experimental groups and each group receives iTBS or a sham treatment for 7 consecutive days. The noise that rTMS apparatus produce is below 60 decibels, according to the manufacturer, thus no noise pollution will be generated. On the 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> day after the last stimulation, the animals will be sacrificed, and brains isolated. This experimental step will create a non-hazardous medical waste i.e., the carcasses and tissue remaining, which are stored in accordance with national and institutional regulations and later permanently disposed of by an authorized company. Following tissue preparation, samples from each animal will be tested for the content or presence of 80 pre-selected soluble, releasable factors using special kits in FBUB. The waste generated in this part of the project is non-toxic and non-infectious (mainly left-overs of reagent kits, and plastic,

summarized below in Table 2.) and will be disposed of in accordance with national/institutional regulations.

2) The cOFM work package is the next step which aims to specifically identifies the levels of neurotransmitters and molecules which will be selected based on the results of PROTEOME. Animals handling and collections of IBF will be performed at VINS on forty adult male *Wistar* rats, while biochemical analysis of IBF will be analyzed at FBUB. IBF will be taken from awake, freely moving animals immediately after first stimulation (acute effect) and 1<sup>st</sup>, 7<sup>th</sup>, and 14<sup>th</sup> day after last stimulation. Biochemical analysis will be performed with commercial kits and all the waste generated in this part of the project is non-toxic and non-infectious and will be disposed of in accordance with national/institutional regulations. Tissue for immunohistochemistry will be processed at VINS and no waste will be generated for that part of the project.

In both WP1 and WP2, the transportation of samples for the analysis in FBUB will be carried out with extreme care in special sample boxes using a sealed portable refrigerator on dry ice to avoid accidental spillage and possible contamination of the environment and each transfer will be recorded in the project documentation (please refer to Mitigation measures).

Table 2. Summary of potential waste that will be generated during the CEREBRO Implementation (waste types, substance, and hazard identification)

WP 1 PROTEOME	WP 2 cOFM
<b>General waste and recyclable rubbish</b>	
<b>Non-hazardous medical waste:</b> 1) animal carcasses generated after euthanasia, maximum expected quantities: 27 kg 2) tissue remaining generated after isolation of brain structures, maximum expected quantities: 50 g	
<p style="text-align: center;"><b>Chemical waste</b></p> 1) Buffers used for tissue preparation are regularly used and represent mixture of non-hazardous compounds such as sucrose, (hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), phosphate etc. <i>Hazard Identification.</i> These buffers are stable, and completely non-hazardous. Maximum expected quantities: 500 ml	<p style="text-align: center;"><b>Chemical waste</b></p> 1) Elisa kits; <i>Substances and Hazard Identification.</i> Proclin 300: Classification of the substance or mixture Classification according to Regulation (EC) No 1272/2008 [GHS/CLP]: Skin Corrosion/Irritation - Category 1, H317: May cause an allergic skin reaction. Sulfuric acid (H <sub>2</sub> SO <sub>4</sub> ): Classification of the substance or mixture Classification according to Regulation (EC) No 1272/2008 [GHS/CLP]: Skin Corrosion/Irritation -

<p>2) Proteome Profiler XL Cytokine Array kits containing Rectangular 4-Well Multi-dish, cytokine Array nitrocellulose membranes spotted with 80 different antibodies to rat cytokines, Array Buffer 4 (21 mL of a buffered protein base with preservatives), Array Buffer 6 (2 vials, 21 mL/vial of a buffered protein base with preservatives), Chemi Reagent 1 (2.5 mL of stabilized hydrogen peroxide with Preservative), Chemi Reagent 2 (2.5 mL of stabilized luminol with Preservative), Detection Antibody Cocktail rat XL Cytokine Array (1 vial of biotinylated antibody cocktail; Lyophilized), Streptavidin-HRP (200 µL of streptavidin conjugated to horseradish-peroxidase), Transparency Overlay Template, Wash Buffer Concentrate (25X). <i>Hazard Identification.</i> Classification according to Directive 67/548/EEC: The product is classified as a skin sensitizer according to Directive 1999/45/EC and its amendments. Other hazards: none. Maximum expected quantities: 500 ml</p>	<p>Category 2 Eye Irritation - Category 2A. Hazard Statement(s): H315: Causes skin irritation. H319: Causes serious eye irritation. Carbamide peroxide (CP): Classification of the substance or mixture Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008. 3,3',5,5'-tetramethylbenzidine: Classification of the substance or mixture - Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008. Maximum expected quantities: 150 ml</p> <p>2) Antibodies used for immunohistochemistry will be applied in µl amounts (small drops). <i>Hazard Identification.</i> Classification of the substance or mixture: Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008. Maximum expected quantities: 1-2 ml</p>
--	--

## **ASSESSMENT OF THE POTENTIAL ENVIRONMENTAL AND SOCIAL IMPACTS OF SPECIFIC TASKS WITHIN THE PROJECT**

### **Potential impact on generated waste**

In general, no dangerous, infectious waste will be generated during the project implementation. Three types of waste will be generated during the project: 1) general waste and plastic rubbish, 2) chemical waste, and 3) non-hazardous medical waste (summarized above in Table 2). General waste and plastic rubbish will be regularly disposed by Serbian City Waste Disposal company in accordance with national regulations. Chemical waste will be packed in adequate bags and/or boxes for chemical waste and placed in special room intended for those purposes and periodically removed by specialized companies for handling waste with whom both FBUB and VINS has signed a contract. As for non-hazardous medical waste, the carcasses and waste of animal origin will be stored in special insulated freezer until they are disposed of by an authorized company with which VINS has signed a cooperation agreement. Waste disposal of all three types will follow strict protocol, i.e., the Waste management plan at the FBUB and VINS, therefore its handling and storing will have no adverse impact on human health or environment.

## Potential impact on the health and safety of the project team

The implementation of project activities involving the handling of animals can only have a negative impact on the members of the project team in the event of accidents such as rat bites. In such situations, a first aid kit will be used, and the injured person will be transported to the nearest ambulance to avoid long-term negative effects. All animals in the animal research facility are regularly tested for various parasites and bacteria/fungi, which are mostly transmitted from rodents to rodents, but also for some of the most common pathogens transmitted from rodents to humans. Project team members are all trained personnel who are very familiar with animal handling and with all the risks in the laboratory environment (each member will always wear adequate personal protective equipment). Laboratory work protective clothes and equipment consist of lab coats, latex gloves, and eye protection (goggles) in accordance with the Act on Risk Assessment in written form for all workplaces in accordance with the valid Law on Occupational Safety and Health. Occupational safety and health equipment (first aid, firefighting equipment, etc.) is available to all employees at the location. Also, in the laboratory where scientific research tests related to the Project are carried out, the principles of Good Laboratory Practice are respected in accordance with local law. Also, safe work in the laboratory follows the strict FBUB and VINS protocols given in Table 1. Internal regulations relevant to the project.

## SUMMARY OF ENVIRONMENTAL AND SOCIAL IMPACT

During the preparation and implementation phase of the *CEREBRO* scientific research project there are certain/potential environmental impacts listed below, together with the intensity of their actions.

### Review of the impact on the environment predicted during project implementation

INFLUENCE	SIGNIFICANCE	COMMENT
Ground and surface water	Low	No chemical or other waste will be disposed of in the ground and/or surface water. The low amount of water that can come to the recipient by drainage, the consequential impact is negligible. Waste water from the laboratory is tested in accordance with local law (quarterly).
Air quality	Low	Temporary impact

INFLUENCE	SIGNIFICANCE	COMMENT
Flora and fauna (protected areas and species)	Does not exist	Project activities do not use protected species nor are carried out on protected areas.
Noise	Low	Temporary impact. During the realization of the project activities, no noise of inadequate levels will be produced.
Management of Waste	Low	Ensured through environmental management. In accordance with the existing waste management plan.
Management of hazardous materials, including hazardous waste	Low	Ensured through management of hazardous materials, including hazardous waste, SROs have adopted the Waste management plan that will be implemented during the project activities. However, as described in Table 2. the substances used during CEREBRO implementation have extremely low hazardous potential.
Laboratory work including Life and Fire Safety	Moderate	In accordance with existing procedures on Health and Safety. During the work in the laboratory, members of the project team could be exposed to chemicals. With the application of appropriate protective equipment, personal training, and emergency preparedness and response plan in place the impact is decreased. Also, adequate control measures are aligned with safety procedures related to working with chemicals or infectious agents.
Safe management of biohazards and hazardous materials	Low	Ensured through management of biohazards and hazardous materials – specific management plan for the Project will be prepared and implemented. As described in Table 2. the substances used during CEREBRO implementation have extremely low (bio) hazardous potential.
Use of chemicals	Low	All researchers in the research laboratory are familiar with safety procedures related to working with chemicals and all Safety Data Sheets for Chemicals.
Handling of gases under pressure	Does not exist	During the realization of the project, there will be no handling of gases under pressure.
Health and Safety of the local populations	Does not exist	No field activities are planned during project implementation. In addition, the project does not use infectious substances/agents or other uncontrollable components that could accidentally contaminate the local community.
Cumulative impacts	Moderate/Low	Temporary, laboratory work will produce carcasses, plastic and chemical waste during the work, and members of the project team could be exposed to potentially accidental situations during laboratory work.

ESMP Prepared by:

December 27, 2023

**CEREBRO team**

PI signature:



## I MITIGATION PLAN

Phase	Issue	Mitigating Measure	Cost of Mitigation	Responsibility	Supervision observation and comments (to be filled out during supervision)
Project Preparation	Lack of project specific SOPs related to laboratory work	Creation of appropriate documents related to the Project. Created SOP documents and training manuals.	None	Project team members	PIU/SF/FBUB/VINS
Project Preparation	Outdated Rulebooks on Occupational Safety and Health, Fire Safety and Waste management plan at FBUB	Creation of updated Rulebooks on Occupational Safety and Health, Fire Safety and Waste management plan at FBUB	None	FBUB	PIU/SF/FBUB/VINS
Project Implementation	Occupational Health and Safety	<p>All project participants are familiar with:</p> <ul style="list-style-type: none"> <li>- the health and safety measures to ensure that the project is carried out in a safe and disciplined manner, organized to prevent accidents, and reduce the potential negative impact on employees and the environment, in accordance with the relevant national legislation and the SROs' instructions for safe working. In both SROs there is a certified person who can provide first aid,</li> <li>- the SRO's current evacuation plan and the protection and rescue plan and must comply with the prescribed measures,</li> <li>- the dangers of fire hazards and fire safety measures and are trained to use fire extinguishers, hydrants and other equipment to extinguish fires in accordance with relevant national/institutional legislations (training documentation is available in FBUB/VINS);</li> </ul> <p>All work/laboratory equipment is regularly inspected and checked in accordance with the regulations.</p>	None	FBUB/VINS fire safety officers; FBUB/VINS persons certified to provide first aid; team members	PIU/SF

Project Implementation	Exposure of team members to potential accidents when handling <i>Wistar</i> rats (rat bites or scratches)	<ul style="list-style-type: none"> <li>- All team members have been previously trained and are very experienced in working with laboratory animals.</li> <li>- The veterinarian in charge will control all work related to the animals and the correct handling in order to avoid accidents.</li> <li>- Team members will use the required personal protective equipment when working with animals.</li> <li>- All team members will follow the SOP which is mandatory in the animal research facility and issued by the veterinarian in charge.</li> </ul>	None	Veterinarian in charge of VINS; Ethics Committee for the Use of Laboratory Animals of VINS; team members	PIU/SF
Project Implementation	Exposure of team members to risks associated with working in the laboratory (e.g., exposure to various chemicals)	<ul style="list-style-type: none"> <li>- All team members will use the required personal protective equipment and the experimental work will be carried out in accordance with the principles of good laboratory practice and the laboratory's SOPs established during the project preparation phase.</li> <li>- In the laboratories where the experiments are carried out, the results of the measurement of environmental conditions (microclimate, chemical hazards, physical hazards, lighting, biological hazards) are controlled and recorded in accordance with the relevant regulations.</li> </ul>	None	FBUB/VINS	PIU/SF
Project Implementation	Transfer of samples from VINS to FBUB	<ul style="list-style-type: none"> <li>- The samples will be transported with extreme care in special sample boxes using a portable refrigerator on dry ice from VINS to FBUB. The refrigerator will be properly sealed to avoid accidental spillage and possible contamination of the environment.</li> <li>- Team members will wear all required personal protective equipment during transportation and the transfer will be recorded in the project documentation.</li> </ul>	None	Team members	PIU/SF
Project Implementation	Generation of chemical waste	<ul style="list-style-type: none"> <li>- Disposal in accordance with the waste management plan by contracted registered services for the disposal of chemical waste.</li> <li>- The person responsible for waste disposal has been appointed in accordance with the law (No. of Decision, VINS: 2021, FBUB: 2012) The records for all chemicals used, including the chemical inventory, purchase plans and safety data sheets and other documents are kept in both SROs.</li> <li>- The amount of chemical waste generated will be kept to a minimum, but the exact amount cannot be predicted at this time.</li> </ul>	Costs covered by FBUB/VINS, or Project overhead	Person responsible for the disposal of chemical waste in FBUB/VINS	PIU/SF

Project Implementation	Generation of non-hazardous medical waste -collection and deposition of euthanized experimental animals	After euthanasia, the carcasses and waste of animal origin are stored in special insulated freezer until they are disposed of by an authorized company with which VINS has signed a cooperation agreement (no. and date of signed contract: 21.6.2022.).	Costs covered by VINS, or Project overhead	Authorized contracting company; Veterinarian in charge for VINS; team members	PIU/SF
Project Implementation	Well-being of experimental animals ( <i>Wistar</i> rats)	<ul style="list-style-type: none"> <li>- For the purpose of the project, the Ethical opinions from the Ethics Committee for the Use of Laboratory Animals of FBUB and VINS have been obtained.</li> <li>- Additionally, the decision of the RS Veterinary Administration has been requested which will be given to the SF upon receipt.</li> <li>- All experimental procedures will be conducted according to the guidelines of the EU registered Serbian Laboratory Animal Science Association (SLASA), a member of the Federation of the European Laboratory Animal Science Associations and national relevant legislation.</li> <li>- Additional care will be taken to minimize any pain and discomfort of the animals and all experimental procedures will be carried out in compliance with the European Communities Council Directive (2010/63/EU) for animal experiments.</li> <li>- All animals will be obtained from the Local colony of VINS animal research facility (officially registered at the Serbian Registry for experimental animals, number. of decision: 323-07-2541/2017-05/1). Veterinarian in charge will closely monitor the health of animals twice a day during Project Implementation.</li> <li>- The health of the animals such as parasitological/microbiological testing is conducted minimally once a year according to national/institutional legislations.</li> </ul>	Costs covered by VINS, or Project overhead	Veterinarian in charge for VINS, Ethics Committee for the Use of Laboratory Animals of VINS; team members	PIU/SF

## II MONITORING PLAN

Phase	What parameter is to be monitored?	Where is the parameter to be monitored?	How is the parameter to be monitored/ type of monitoring equipment?	When is the parameter to be monitored/ frequency of measurement or continuous?	Monitoring Cost What is the cost of equipment or contractor charges to perform monitoring?	Responsibility	Supervision observation and comments (to be filled out during supervision with reference to adequate measuring reports)
Project Preparation	Creation of project specific SOPs for work in laboratory	On Project site: FBUB and VINS laboratories and CEREBRO database on Google Drive	On-site visual assessment and checks of documentation (dates and signs).	Once, prior to commencing work	None	PI and team member 1	PIU/SF/FBUB/VINS
Project Preparation	Creation of updated Rulebooks on Occupational Safety and Health, Fire Safety and Waste management plan at FBUB	On Project site: FBUB and FBUB website	On-site visual assessment and checks of documentation (dates and signs).	Once, prior to commencing work	None	FBUB	PIU/SF/FBUB
Project Implementation	Fulfillment of occupational health and safety precautions and procedures	On Project site: FBUB and VINS	Visual assessment and inspection of equipment on site	Once a year during active Project Implementation (animal/laboratory experiments)	None	FBUB/VINS fire safety officers; FBUB/VINS persons certified to provide first aid; team members	PIU/SF/ FBUB

Project Implementation	Health and Safety	On Project site: FBUB and VINS laboratories	Continuously monitor the health and safety protocols	Monthly monitoring	Costs covered by FBUB/VINS	H&S Officer in FBUB/VINS	PIU/SF
Project Implementation	Emergency Response	On Project site: FBUB and VINS laboratories	Review and practice the emergency response protocols, ensuring that all team members are aware of healthcare facilities and contacts	Quarterly emergency response drills and quarterly protocol reviews	Costs covered by FBUB/VINS	FBUB/VINS	PIU/SF
Project Implementation	Life and fire safety (LFS) procedures in laboratory	Laboratory of the institution implementing the project	Visual inspections and checks of the documentation	Before the start of the activities and periodically during the implementation of the project	Costs covered by FBUB/VINS	FBUB/VINS fire safety officers; FBUB/VINS	PIU/SF
Project Implementation	Measures and precautions to alleviate risks of accidents related to animal handling and procedures	On Project site: VINS	Visual on-site assessment of the use of personal protective equipment, implementation of the issued SOP in the VINS animal research facility	Once a year during active Project Implementation (animal experiments)	None	Veterinarian in charge for VINS; Ethics Committee for the Use of Laboratory Animals of VINS; team members	PIU/SF
Project Implementation	Measures and precautions to alleviate risks associated with working in laboratory	On Project site: FBUB and VINS laboratories	Visual assessment of the use of personal protective equipment on site, implementation of the project-specific SOPs created in the project preparation phase	Once a year during active Project Implementation (laboratory experiments)	None	FBUB/VINS	PIU/SF

Project Implementation	Safety measures related to transfer of samples from VINS to FBUB	On Project site: VINS and FBUB	Visual assessment of the portable refrigerator, records of the transfer in project documentation	Regularly, during the project duration	None	Team members	PIU/SF
Project Implementation	Management of chemical waste	On Project site: FBUB and VINS	Visual or indirect (e-mail) assessment of on-site waste management plans, signed contracts with a registered waste management company, records of all chemicals used, including chemical inventories, purchasing plans and safety data sheets and other documentation.	Regularly, during the project duration	Costs covered by FBUB/VINS, or Project overhead	Person responsible for the disposal of chemical waste in FBUB/VINS	PIU/SF
Project Implementation	Management of non-hazardous medical waste (animal carcasses and tissue remaining)	On Project site: VINS animal research facility	Visual assessment of storage conditions on site and contract with registered disposal company, record book of the veterinarian in charge	Regularly, during the project duration	Costs covered by FBUB/VINS, or Project overhead	Authorized contracting company; Veterinarian in charge for VINS; team members	PIU/SF
Project Implementation	Well-being of experimental animals ( <i>Wistar</i> rats)	On Project site: VINS animal research facility	Visual assessment of the conditions in the animal research facilities, results of parasitological/microbiological tests, records of pre/post-operative care and other relevant documentation	Regularly, during the project duration	Costs covered by VINS, or Project overhead	Veterinarian in charge for VINS, Ethics Committee for the Use of Laboratory Animals of VINS; team members	PIU/SF