HELMHOLTZ MUNICI)

Cabin Air Quality – CAQ3 Project

Animal Study in WP3

January 2023

How do we test if oilrelated fume events are harmful?



Within a team





National Institute for Public Health and the Environment Ministry of Health, Welfare and Sport





Sanitätsakademie der Bundeswehr



Animal experiments are only carried out:



- if no alternatives are available to answer the scientific question
- after the competent authority has approved the animal experiment with the involvement of a commission (according to §15 German Animal Welfare Act)
- in accordance with the European Communities' Council Directive 2010/63/EU

Quality control standards at the German Mouse Clinic (GMC)

TUV

TÜV Süd certified Quality Management ISO 9001 Certified Ű Quality Management System www.tuv-sud.com/ms-cert **Certified Quality Management** The German Mouse Clinic has been approved by TÜV SÜD to follow the **Quality Management System Standard** EN ISO 9001:2015 GMC



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EQIPD certified Quality Management



Enhancing Quality In Preclinical Data EQIPD

Certificate of Compliance with the EQIPD Quality System

German Mouse Clinic Institute of Experimental Genetics (IEG)

Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstr. 1, 85764 Neuherberg

Certificate number: DE - INS - GMC - 06-2021-1

Certificate issue date: June 23rd, 2021 Valid until: June 30th, 2022

Certificate issued by: Thomas Steckler, Project Leader, EQIPD Consortium



TUV

The project leading to this application has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 77364. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.



Study design

- Behavioural and neurological symptoms
- Neurotoxicity and neuroinflammation
- Lung toxicity

Basis for design:

- OECD TG 412: Sub acute inhalation study
- 28 days exposure + follow-up
- 5 days per week inhalation exposure
- Peak exposures per day in 2h window
- 3 dose groups + control + positive control
- Sub acute plus delayed effects

Con

1st part: Range finding study

Finding the optimal dose for main experiment

2nd part: Main Study

A) Sub acute effects

What effects do we see right after exposure phase?

B) Long Term Effects

What are the effects on behavior and after a time of non-exposure?

Study design



Main study: Sub-acute effects	Arrival and acclimatization			SHIRPA	5	Exposi ⁿ 4h/d days pe 4 wee	tion: ay r week eks		SHIRPA	Transfer and sacrifice							
Main study: Long-term effects		Arrival and acclimatization		SHIRPA	5	Exposi [:] 4h/d days pe 4 wee	tion: ay r week eks		SHIRPA	Transfer and acclimatization	Open Field	CatWalk	ASR/PPI	Y-Maze	Social	Discrimination	Sacrifice
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H Sub-acute effects?



SHIRPA – systematic behavioural observation using defined rating scales

General health and autonomous functions

- Weight
- Urination
- Defecation

Reflexes

- Startle reflex
- Contact righting reflex
- Pinna reflex

Behavioral aspects

- Touch escape
- Locomotor activity
- Transfer arousal
- Body position

Posture and movement

- Tail elevation
- Trunk curl
- Gait

Additional observations

- Tremor
- Limb grasping
- Biting
- Vocalisation





DVC racks





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DVC racks – data analysis

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Long-term effects?

Long-term effects on locomotion and cognition?





Open Field, TSE

GMC



CatWalk, Noldus



Prepulse Inhibition, Med Ass.







Social Discrimination



Open Field



- locomotion:
- exploration:
- **anxiety**-related behavior:
- habituation:
- Conditions:





Calculating the center of gravity



www.TSE-Systems.com

distance travelled (cm)

vertical = rearings; horizontal = distance

time spent in center (%) – more centre time = less anxiety

time course

length: 45.5 x 45.5 cm, walls: 39.5 cm 150 – 200 lx, Center: 42% Time: 20 min Infrared beam breaks



Catwalk® gait and motor coordination analysis



- Quantify locomotion and motor coordination parameters during walkway crossing by visualising foot-floor contacts in time
- Subjects walk across an illuminated glass platform while a video camera records from below.
- Gait related parameters are reported for each animal.





Catwalk parameters measured



- Dynamic paw parameters
 - Step cycle time in seconds between two consecutive initial contacts of the same paw with the glass floor consisting of a stand phase and a swing phase
 - Stand phase duration in seconds of contact of a paw with the glass floor
 - Swing phase duration in seconds of no contact of a paw with the glass floor
 - Cadence how many steps are taken in one minute
 - Coordination
 - Stand index speed at which a paw loses contact with the glass floor at the initiation of the swing phase
 - **Print position** distance in cm between the position of the hind paw and the position of the previously placed front paw on the same side of the body and in the same step cycle.
 - Initial dual stance this is the first time in a step cycle of a front paw or hind paw that the contralateral front or hind paw also makes contact with the glass plate.
 - **Phase dispersion** this is a measure of inter limb coordination and describes the temporal relationship between placement of two paws within a step cycle.
- Static paw parameters
 - **Stride length** distance between successive placements of the same paw
 - Print length length in cm of the complete print
 - Print area surface area in cm² of the complete print



Prepulse inhibition of acoustic startle reflex



Startle Pulse

Startle Pulse

Prepulse

Prepulse



- Tests sensorimotor gating and recruitment
- Sensitive to alterations in sensorimotor integration

Y maze spontaneous alternation test – working memory





- Alternate arm returns (AARs): ABA, ACA, BCB, BAB, CAC, CBC
- Same arm returns (SARs): AAA, BBB, CCC

- ↑ % SPAs = enhanced spatial working memory
- ↓ % SPAs = impaired spatial working memory







Social Discrimination - social & olfactory memory

2 hours habituation



Sample phase: familiar subject (2) is introduced to the tested animal (1)

2 1

Test phase: unfamiliar subject (3) together with mouse 1 and 2

Tested Parameters: contact with familiar subject, contact with unfamiliar subject.

Assesses olfactory-based social memory

2 hours break -



Chronic neuroinflammation in the brain?

Stereological analysis of brain-region specific astrocyte and microglia







(Olesen et al., 2017)

Advanced characterization of glial cells

GMC









Blood	 Targeted Metabolome – ITEM Inflammation acute phase – NRCWE
Brain	- Neuroinflammation – HMGU
Lung	 Histopathology on lung toxicity – RIVM Inflammation acute phase – NRCWE

Liver - Inflammation acute phase – NRCWE

All other organs conserved and stored for further analysis if needed

Acknowledgements



<u>*</u>

for Arbejdsmiljø

National Institute for Public Health and the Environment Ministry of Health, Welfare and Sport





InstPharmToxBw



Det Nationale Forskningscenter





Honeywell













Thank you.

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