FACTS Task 3A.3: Bioassay assessment and limited mice inhalation study using RIVM mini-BACS

Public report & Confidential Annex

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Introduction

The central question in the FACTS project was whether exposure to neurotoxic substances formed during fume events in aircrafts can be the cause of neuronal damage as observed in cases of 'aerotoxic syndrome'. One of the main problems in the risk assessment of cabin air quality is the lack of neurotoxicity hazard data for the majority of substances present in fumes. Even more, in an aircraft there is potential exposure to a complex mixture of a large number of different substances during a fume event, in highly variable concentrations. To reliably study the influence of a chemical substance or mixture of compounds on function of the nervous system integrated measures of neurological function are required.

Therefore, an in vivo study was foreseen in which animals would be exposed to the complex mixture and tested for neurotoxicity using several behavioural and histopathological parameters. In addition, the in vivo study was foreseen to contribute to the search for biomarkers of exposure and effect.

For proper toxicity screening, integrated cellular testing approaches combining different organ systems, as well as whole organism test systems are needed, to allow more realistic simulation of organ interaction, and to include metabolic competence in the test system. In the context of fume exposure, only few experiments have been done studying potential neurotoxicity.
It is necessary to study biomarker formation, stability and half-life under conditions of controlled exposure, allowing investigating the relationship between fume mixtures and internal exposure doses, without interference of other sources influencing the biomarker levels. It is therefore needed to screen for, and semi-quantify, selected/targeted biomarkers in animals exposed to realistic and characterized fumes. The proposed study allowed for this approach.

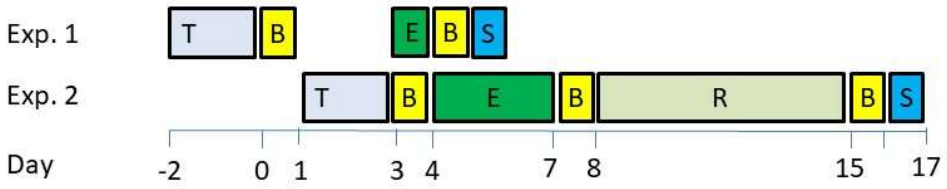
Materials and methods

To test whether exposure to oil fumes exerts a neurotoxicologogical effect, an in vivo study was designed and prepared. The study involved the inhalation exposure of mice to oil fume followed by dedicated behavioural neurotoxicity testing and biomarker analysis.
In this study, groups of healthy mice (WT C57Bl6, n = 12 per group) would be exposed to clean air, or oil fumes. Based on the in vitro/in vivo screening assays (MEA and Zebrafish) the most appropriate fume, i.e. the fume with the highest neurotoxic potential, would be selected to be tested in in vivo inhalation experiments. The clear benefits of using a whole organism combined with realistic exposure conditions and the possibility to use more complex read-outs for neurological effects (e.g. behavioural effects and correlating biomarkers, both generic and specific for neurological damage) justified the proposed use of animals.

Fume generation for the in vivo study was to be performed using the same BACS-setup used for the in vitro studies under the same conditions. Using this setup, mice would have been exposed for 4h,

this is considered a representative duration of an average intra-European flight in which fume exposure may occur. To assess a dose response relationship, the concentrations in the test atmosphere would be varied. The conditions for fume generation were to depend on the outcomes of the chemical analysis of the simulated fume-events performed in task 2. To discriminate between potential effects of single- and repeated exposures, two experiments were designed in which mice would have received either a single 4h exposure or three 4h exposures on consecutive days. In the repeated exposure experiments a recovery period of 7 days was included to assess potential delayed neurotoxicity or reversibility of effects. The latter group would have been tested in the behavior setup before exposure, directly after exposure and a third time following the recovery period.

The study was designed according to the following scheme:



E: Exposure
T: Training
B: Behaviour assessment
R: Recovery
S: Sacrifice, 24h after the last behaviour assessment

To assess the effect of exposure to fumes on neurological functioning, a neurobehavioural test battery was to be used. This test battery was designed in such a way that it would detect effects such as (spatial) disorientation and anxiety using the Barnes maze and open fields test, as well as neuromuscular function via measurements of grip strength and balance in the Rotarod and string suspension test. All behaviour was to be recorded on video for detailed automated analysis.
It was foreseen to test all animals both before exposure and directly after exposure to minimize the effect of inter-animal variation. Reversibility of neuromuscular and neurobehavioural effects as well as the occurrence of delayed toxicity would have been tested for in a third round of neurobehavioural testing one-week post exposure.

Upon sacrifice several organs were to be harvested including brain, liver, and lungs. In addition, body fluids such as blood and urine were to be collected along with samples from muscle tissue and peripheral nerve samples. The latter were to be harvested for the analysis of biomarkers of exposure and biomarkers of neurotoxicological effect.

The preparation for the in vivo study included acquiring approval of the animal ethical committee. For this purpose an application had to be written with a detailed description of the rationale behind the study, the experimental design and the expected outcome (see attachements). The application process consists of a tiered approach in which the proposal is first evaluated by the board for animal welfare, followed by the committee for scientific evaluation and finally the committee for ethics in animal testing. The latter committee decides on the approval. The three-part 14-page application for the in vivo study was submitted for approval by the board for animal welfare on November 15, 2019. On November 28 we received a request for additional information to which we returned a 15-page rebuttal on December 4. Upon return of the application, the second tier of the application process was started handing in the proposal to the committee for scientific evaluation. A request for additional information from the committee for scientific evaluation was received on December 17, 2019. The process for approval was terminated upon receipt of the decision of DG MOVE not to prolong the project.

Annex (confidential):

- Submitted study proposal including rebuttal

Animal application complete.pdf

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