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1 Introduction

1.1 Background and rationale

The EPHOR project aims to investigate the occupational exposome. In two case studies (WP6 Working-life exposome, lung function, and obstructive lung disease among men and women and WP7 Exposome case studies on night shift work and health), a set of sensors, samplers, and questionnaires will be applied to collect information, in a workers day to day life, on a wide range of exposures or behaviours that may affect health outcomes. This will be done for a subset of the case study cohorts. This subset will undergo a more intensive short-term monitoring period, including the external exposome assessment protocol being tested in this feasibility study.

Thorough pilot testing of methods, instruments, protocols and logistical aspects are needed before their application in the actual case studies. We therefore design this feasibility study to test the fieldwork protocol which will then be amended, if needed based on this feasibility study results before being applied to the case study cohorts in a number of European countries. Depending on the aims of the individual case studies, case study leaders may choose to add on additional methods and tools to this base protocol at a later stage.

This is a base protocol for fieldworkers, i.e. it is not material that will be handed out to participants of case studies. Thus this feasibility study will be conducted internally, i.e. team members of WPs 6, and 7 will trial the protocol. For ethics and recruitment processes in the case studies, the case study leaders are responsible and will create their own protocol. Nevertheless, during this feasibility study team members are not only testing the setting up of the various methods but also will be asked to wear the sensors and trial the questions on the app as if they were participants. By doing so, both views, those of the fieldwork staff dealing with logistics and participants and those of participants receiving instructions and sensors/samplers will be considered. The feasibility study will reflect experience of study team members using the protocol and samplers/instruments so that they will understand the potential burden that a case study participant may face. This experience is important so that the study team can evaluate areas where data may be lost and possible reasons for lack of compliance with the protocol.

This internal feasibility study is meant to test the use of these different assessment methods and the protocol together as closely as possible to the way it will be done in the case studies excluding ethics and recruitment as well as data analysis. The aims of this feasibility study are therefore to test the methods and procedures including set up, collection, and feedback on standard operating procedures (SOPs) and the overall protocol to further improve individual methods, processes and the protocol. The final field protocol will be generated based on results from this feasibility study.

As mentioned before, not all methods and tools will be applied in full. Specifically, any methods described in this protocol requiring laboratory analysis (passive samplers and electrostatic dust collection) will not be tested in full as this is not included in the protocol or the current resource plan. These methods are however established methods that have been applied before and SOPs already exist. It is however anticipated that a set of passive samplers will be distributed to all participating teams to familiarise themselves with the samplers and protocol should logistics and time allow to do so. Further the case studies aim to apply a set of standardised (partly already existing and applied) questionnaires of their own choice which will not be trialled in this feasibility study.

The main overall outcome of this feasibility study will be feedback from the study team members about the practicalities, logistics and feasibility of the chosen methods and the clarity of the protocol and individuals SOPs presented in this document. A standardised survey will be provided to collect

feedback, and organised discussions amongst the team will follow the study with the aim of improving both the actual method/samplers/sensor and the protocol including individual SOPs. Before the case studies commence it is anticipated to run training sessions with fieldworkers on the protocol as well.

2 Objectives

The objectives of this internal feasibility study of the working-life exposome are to:

1) Test the feasibility of the EPHOR WP1 fieldwork protocol for data collection on external environmental exposure of the working population with several the respiratory health case study (selection of WP6 partners) and the shift work case study (selection of WP7 partners).

2) Demonstrate feasibility of using new and established sensors, monitoring methods and an app for asking daily questions for collecting exposure data and contextual information, on a wide range of exposures and behaviours that may affect health outcomes.

3) Use lessons learned from the feasibility study, collected via a survey and targeted discussions, to finalise a base fieldwork protocol for application in the EPHOR WP6 and WP7 case studies.

3 Study design

The study aims to characterise environmental exposures of individuals during their day-to-day working life, which contribute to the person's working-life exposome (Figure 1). From each of the participating centres 2-5 team members will take part and trial the protocol.

The study will test the field use of active and passive personal sampling methods for environmental, chemical and biological agents. The full protocol for the case studies would include personal wear of a sensor box, 'pen-like' passive sampler for chemical detection, and physical activity sensor and, in some cases, a heart rate monitor. In some cases, subjects will also be asked to place a dust sampler in their home and workplace. Both the passive samplers and the dust sampler are established methods which will not be tested by each team member and will not be analysed because of the aforementioned reason. Data from the newly developed sensor box however will be checked to evaluate its performance and consistency i.e. if there are any issues with data gaps, timestamps, or outliers for example.

Additionally, the *How am I* app will be tested and trialled for functionality and feasibility. The app is programmed to ask a series of daily contextual questions, answers will however not be analysed in this feasibility study, the data recording will be checked for accuracy.

Standardised questionnaires on exposures, some of which are already established and are already part of the existing cohort studies, will not be tested in this feasibility study. They are generally not part of the short-term intensive study, but part of the larger cohort study.

Once the feasibility testing is finished feedback will be gathered from all participating team members by means of a survey (Survey monkey or a similar software). In a second step a meeting for further discussions based on the feedback will be organised with the aim to troubleshot and improve the protocol.

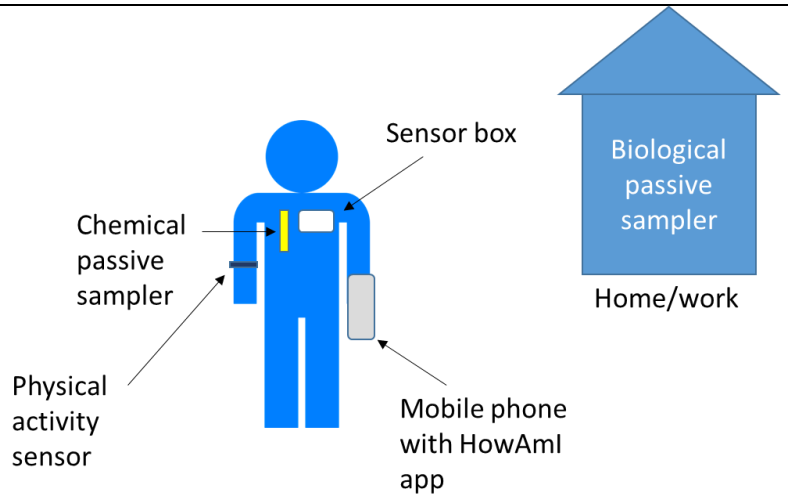


Figure 1: Schematic display of the sampling and monitoring strategy for the case studies.

For this feasibility study, the research staff teams of the case studies who will eventually apply the protocol in the field will undertake the study procedures. This is due to the aim of the study being to test new and established methods and protocols, trouble shoot and improve individual methods and the overall protocol. Further it is anticipated that this exercise will help tailoring the protocol to the specific needs of the case studies.

Each study team will provide feedback to determine potential issues with specific sensors and methods. Fieldwork is due to take place in the first quarter of 2021. Participating team members will be asked to trial the field protocol, app, samplers, and sensors for a period of 5-7 days.

It is estimated that trial of the protocol will start as soon as the sensor boxes have undergone initial testing and are ready to be used in the feasibility study.

4 Methods

4.1 Data collection

A number of different data collection methods will be applied during the feasibility study including active and passive personal monitoring, static passive dust fall sampling as well as an app for asking daily questions. These will be briefly described here, with full SOPs in the appendices.

4.1.1 Sensor box

The sensor box together with the gateway needed to store the data (intermediate storage) is shown in Figure 1. The sensor is a small box to be worn on the body, which includes the following environmental sensors

- Temperature
- Humidity
- Sound
- Light
- UV
- Particulate matter (PM) (PM 1, 2.5 and 10)

Figure 1: Sensor box (right) and gateway (left)

A detailed SOP about setting up and operating the sensor box can be found in Appendix 0.

4.1.2 Passive samplers

In the case studies, passive samplers, in the shape of a pen will be worn by subjects for a period of 5-7 days. During the full study, samplers will then be send to the TNO laboratory for chemical analysis, however for the purpose of this feasibility study the samplers will not be analysed. Detailed instructions on how to use these samplers can be found in Appendix 8.2.

4.1.3 Daily questions (App)

Participants will be asked to download the *How am I* App developed by project partners TNO to their smartphone. Through this app, subjects are asked a set of questions every day, some questions may be asked several times a day, concerning aspects such as sleep quality, commute to and from work, diet and lifestyle for example.

Details on how to install and log into the app are provided in a separate protocol in Appendix 8.3.

4.1.4 Additional/separate sensors

4.1.4.1 Electrostatic dust fall collector (EDC)

In WP6 a passive EDC will be used in addition to the above mentioned sensors and samplers, which will be placed in the participant's home and workplace (this will be done depending on Covid-19 restrictions) for a period of 14 days to allow collection of sufficient material for analysis. For the purpose of this feasibility study the already established method will however not be tested in full, but samplers may be distributed to study teams to familiarise themselves with the method. A detailed description on how to prepare, install and remove the EDC samplers can be found in appendix 8.5.

4.1.4.2 Activity sensors

In WP7 an additional activity sensor may be used which will be separate to the sensor box, however data collection will be integrated with the sensor box system so that all sensor data streams can be made available together. This integration is still work in progress and more details can be found in Appendix 8.1

4.1.4.3 Heart rate monitor

Also in WP7 an additional heart rate monitor will be used that is worn around the chest. While the sensor is separate to the sensor box, data transfer and download may be integrated with the sensor box system, as with the activity sensors (work in progress). More details can be found in Appendix 8.1.

5 Data analysis

Due to the nature of this feasibility study, we are interested in two key aspects.

a) The performance of the sensor box (technical issues)
b) The feasibility of all methods (if tested), accompanying SOPs, and the overall base fieldwork protocol.

The collected sensor data will be checked and evaluated for completeness and consistency. Results from the pre-testing phase with these sensors will be used for further evaluation if needed.

For the second aspect, the study teams will be provided with a standardised survey (Appendix 8.6) to provide feedback and information about the feasibility of above mentioned methods. The information will be collated and targeted discussions will take place to further decide on improvement actions needed before the case studies can go ahead.

6 Risks

Each study team is responsible for the safety of their staff. Appropriate risk assessments are the responsibility of the individual study teams.

There should not be any major risk of harm as long as the equipment is handled appropriately and electrical safety measures are observed. Wearing the devices should not pose any risk of harm to the wearer as long as worn appropriately to avoid danger of getting caught in something. Generally devices should not be worn or used if the situation is not deemed safe. Any incidents involving study equipment or procedures should be reported to all participating teams as soon as possible.

Study teams are advised to consider COVID19 safety and follow to local restrictions.

7 Administrative aspects, monitoring and publications

7.1 Data management and security

Each study team is responsible for the data management and security of information for their study. The feedback survey will organised and run by IOM, who will provide an overview of results, which will function as basis for targeted discussions with the aim to improve the base protocol.

7.2 Device and sample management

The sensor boxes, and its accompanying gateways as well as activity and heart rate monitors will be re-used throughout the study. In all cases, data will be downloaded throughout the monitoring period from the sensor box via Bluetooth onto the accompanying gateway and removed from the device before re-use for another subject. Data from the gateways will be uploaded to the cloud and downloaded by authorised EPHOR personnel for further analysis. Data from the other sensors will also be downloaded and removed before reuse. Each device will be assigned a unique ID, which will be linked with any identifiers, such as a serial number, for the device, and will not be changed during the study. This will allow researchers to track any issues that may come up with certain devices. The device ID used for each subject will be recorded. None of the sensor data contains potentially identifying details (e.g. GPS location, name, age).

Data from the *How am I* app is constantly stored (whenever the phone is connected to the internet) on protected TNO servers. Data gathered during this feasibility study will not be analysed, but will be checked to ensure that data was collected correctly. TNO will share data offline though with participating centres if they wish to access the data. If subjects use a provided study phone, these will be checked over to make sure no personal data is on the phone, before it is handed out to another

subject. Each phone will be assigned a unique ID and the same procedure as for the sensors boxes and gateways will be applied.

7.3 Public disclosure and publication policy

Public disclosure may include:

- Summarized study results issued on the study or institutional website;
- Oral or poster presentation at conferences, symposia or other public meetings;
- Full publication in peer-reviewed scientific journals.

In any case only encoded data will be used and no reference to any information leading to the identification of the subjects will be disclosed.

8 Appendices

8.1 Sensor box and gateway SOP

SOP 1 - sensor measurements in EPHOR case studies

Version: 1
Date: 07-01-2021

1. Purpose:
Describing the sensor equipment and standardizing procedures for their use in the EPHOR case studies.

2. Background:
Sensors can be used to monitor environmental (e.g., particulate matter, sound) and personal (e.g., heart rate, activity) parameters that may be related to exposure in the workplace. These devices are characterized by low costs, which allows for multiple sensors to be integrated into a single device and a higher distribution of devices. This, together with high resolution measurement capabilities, make low-cost sensors promising additions to large-scale occupational exposome studies, such as the EPHOR project.
In the EPHOR project, an environmental multi-sensor (EPHOR sensor, VTEC, Eindhoven, the Netherlands), an activity tracker (Ax3, Axivity Ltd, UK) and a heart rate monitor (H10, Polar, Polar Electro Oy, FInland) will be used to study the occupational exposome in two case studies. These devices will be worn by volunteers for 5-7 days, in parallel with additional measurement methods such as passive samplers, stationary dust samplers and a daily questionnaire app.

3. Measurement principles:

- Quick overview of how the EPHOR sensors, activity tracker and heart rate monitors operate (see also Table 1).
- How is data collected and stored?
    - EPHOR Sensors: stores locally 1440 data points until overwritten.
    - AXIVITY Tracker: ~1 Week of log
    - HRM: data for one period at a time e.g. one cycle ride is logged
    - Gateway: Can store about 6 months of data from an EPHOR sensor downloaded USB drive/cable.
- How often is data uploaded to the cloud? How long can data be stored internally?
    - The gateway is in charge of uploading data to the cloud once it downloads it from the EPHOR devices.
    - The cloud upload happens every two hours, where it checks if there is any data internally stored and uploads them. Data however once uploaded, gets deleted from local storage/database in the gateway. If an upload is not made or fails on a particular data set, it can be stored infinitely. The only limit is on the storage size, currently, around 10GB of data can be properly stored.
- How long do the batteries last? How long does it take to charge?
    - EPHOR sensors: The batteries should last for 10 hours. Charging the device will take 2.5 hours.
    - AXIVITY Tracker: 30 days @ 12.5Hz; 14 days @ 100Hz
    - HRM: 400 hours with Bluetooth Low Energy and 5 kHz transmission active

- How can the internal time of the monitors be adjusted to the current (local) time?
    - The gateways take care of syncing the device times. However, the gateway time gets automatically updated with the nearest NTP (http://www.ntp.org/) from the internet. This time cannot be adjusted by users, however, VTEC can adjust it for you. The current time of the gateways will be shown on the web interface immediately.
    - For the EPHOR devices, they locally store data for 24 hours, and a maximum of 1440 data points, in 1-minute resolution, after which data is overwritten in a FIFO manner. If a person stops the sensor (powers off) then the device continues to store after it is powered on only.

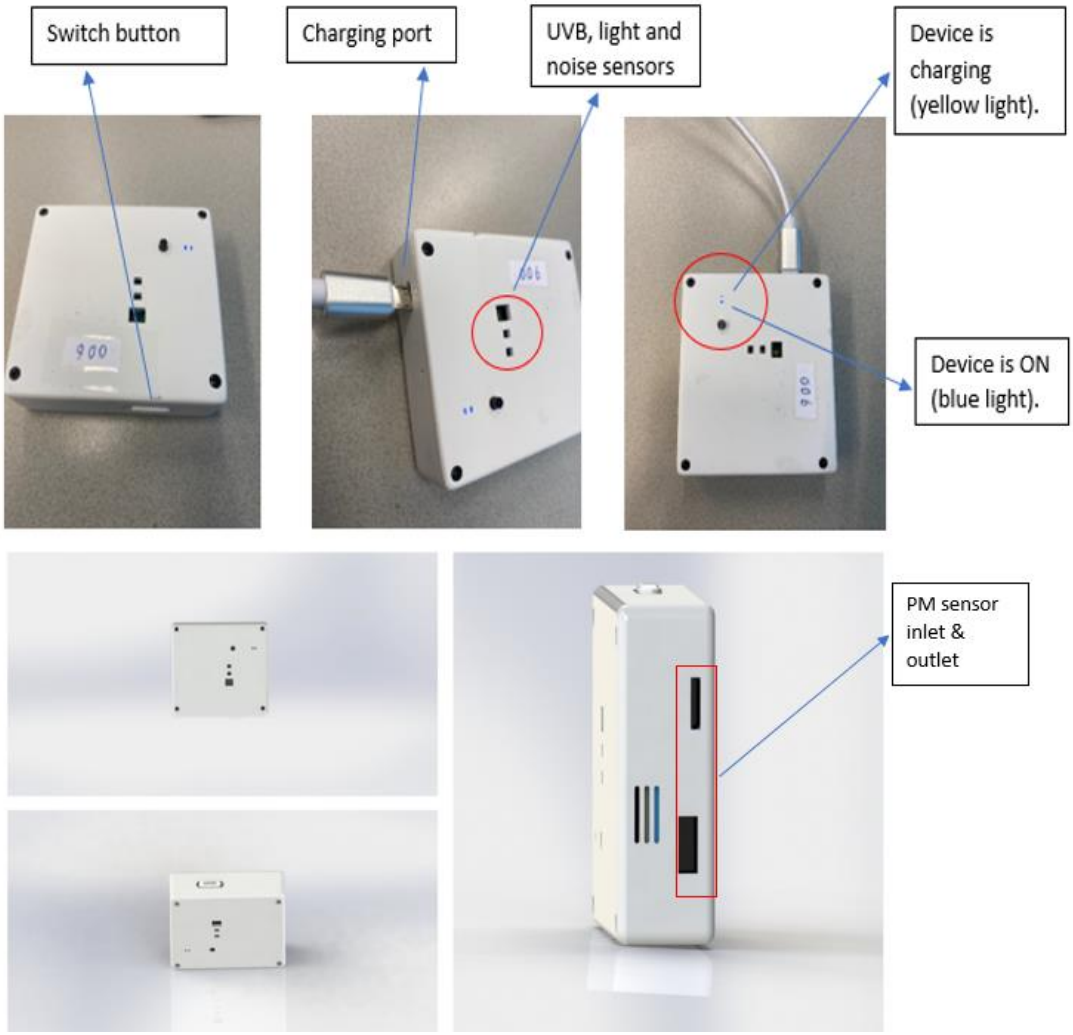
Table 1. Specifications sensor equipment

Device	**Manufacturer**	**Parameters**	**Data storage method**	**Measurement frequency**	**Expected battery life**
EPHOR sensor	VTEC, Eindhoven, the Netherlands	Particulate matter <1, <2.5 and <10 µm (µg/m^3) Light intensity (lux) UV intensity (W/cm^2) Sound (dB(A)) Temperature (°C) Relative humidity (%)	Internal, uploaded to gateway via Bluetooth every 2 hours	1/minute	Run time: 10 hours, charging time: 2 hours 30 mins
Activity tracker	Ax3, Axitivity, UK	Sample rate 12.5-3200Hz Accelerometer range 2/4/8/16g configurable	Internal, query data through USB	Configurable 12.5Hz - 3200Hz	30 days @ 12.5Hz; 14 days @ 100Hz
Heart rate monitor	H10, Polar	Accelerometer data with sample rates of 25Hz, 50Hz, 100Hz and 200Hz, and range of 2G, 4G and 8G. Axis specific acceleration data in mG. Heart rate as beats per minute. RR Interval in ms and 1/1024 format,	Internal, uploaded to gateway via Bluetooth	Configurable 1 or 5s per sample	400 hours

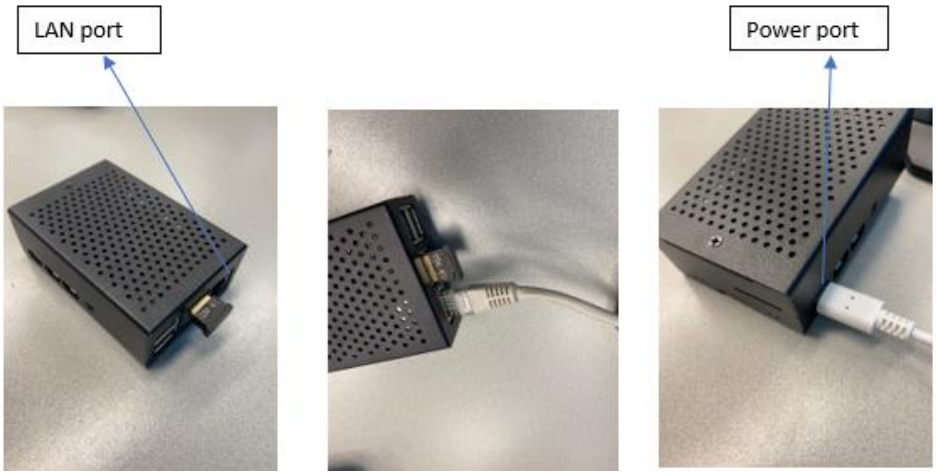
4. Materials:

4.1. EPHOR environmental sensor

- EPHOR sensor v1 (VTEC, Eindhoven, the Netherlands)
- Dimensions:
- Weight:
- EPHOR sensor charging cable, USB-A to USB-micro
- EPHOR sensor mount for personal measurements
- Gateway v1 (VTEC)
- Gateway charger, power plug to USB-C (Pi4)



a)



(b)

Figure 1. EPHOR environmental sensor materials. (a) Sensor module; (b) gateway.

4.2. Activity tracker

- Activity tracker
    - AX3, manufactured by Axitivity Ltd, UK.
    - Dimensions: 23 x 32.5 x 7.6 (mm)
    - Weight: 11g
- Activity tracker charger: Micro USB charger
- Activity tracker mount : to be determined

Figure 2. Activity tracker.

4.3. Heart rate monitor

- Heart rate monitor
    - Polar H10 Heart rate sensor, manufactured by Polar.
    - Dimensions: connector 34x65x10 mm, strap sizes XS-S: 51-66 cm, M-XXL: 65-93 cm
    - Weight: Connector 21 g, strap 39 g
- Heart rate monitor charging cable: battery (CR2025)
- Heart rate monitor mount : to be determined

Figure 3. Heart rate monitor.

5. Procedure:

5.1. Setting up system

- The USB-drive that is supplied with each gateway can be removed before giving the equipment to the participant to eliminate the possibility of the participant tampering with the data.
- Select a place to position the gateway.
    - It is better to have gateways near to the devices, ideally the same room when stationary (< 5 m).
- Install the gateway by plugging the charging cable into mains power.
- Put the charging cables in the gateway and charge the sensors prior to the first measurement day. Confirm that each sensor is charging correctly.
    - EPHOR sensor: Charging light is on (yellow)
    - Activity tracker: Solid green/white LED

    - Heart rate monitor: On the app
- If possible, confirm that data is collected.
    - There will be a Web interface provided by the gateway where users can see the last reading time and also be able to force a read or check the status of a device. This is work in progress and will be packed in the next release automatically and updated in all gateways.

5.2. Daily operation routine

- Remove sensors from chargers and turn on if needed.
- Place sensors on personal mount
    - The sensor is placed in the mount so the PM sensor inlet side is facing upward (side with 3 slots, the side with the charging port is then facing down).
    - EPHOR sensor in breathing zone, on outer layer of clothing
    - Activity tracker on wrist
    - Heart rate monitor on chest
- Put on personal mount and continue the day as planned.

- During the day, personal mount can be taken off for several reasons. For instance:
    - Health and safety reasons e.g. when performing specific tasks at work or doing sports; place the sensors as close as possible to you after taking it off.
    - Personal hygiene e.g. showering; the sensors must be placed in a secure place outside the bathroom where they cannot get wet.
    - Sleeping; place the sensors next to your bed or close by.

- If any error occurs, or if you have any questions or concerns, contact the study supervisor.

- At the end of the day, connect the sensor to the charging cable that is connected to the gateway.
- Confirm that the sensors are charging (blue light is on, see 5.1.)

5.3. Concluding the measurements

- At the end of the measurement period, please disconnect and turn off all the equipment and package the sensors the same way they were received.

6. Data collection and analysis:

- Data should be collected and checked between each deployment of the sensors to avoid potential data losses and to confirm that the sensors are operating as required and can be used for a next participant.
- Collect measurements.
    - EPHOR sensor: place the USB-drive in the gateway. Wait 10-30 minutes for the files to be copied. Retrieve the data files from the USB.
    - Activity tracker: tbd
    - Heart rate monitor: tbd
- Save the data on the YODA WP1 folder.
- Inspect the data and check that measurements have been collected and that values are within the expected range.
- Any cleaning/maintenance of the equipment needed?

- Covid-19 safety measures should be observed and devices cleaned before handed out to the next participant.

8.2 Passive sampler SOP

SOP 2 – Passive samplers

Passive samplers are easy-to-use sampling devices for measurement of chemicals: volatile and semi-volatile organic compounds (VOCs and SVOCs), in the air. The uptake of chemicals is mainly based on molecular diffusion. The passive samplers for EPHOR are specially designed and consist of a small aluminium tube filled with an adsorbent (Tenax TA) to capture VOC. A piece of silicone hose is attached around the tube, which captures SVOCs. Around the silicone hose there is protective mesh cover to avoid contact with clothes and skin (hands).

This is an established method and sample preparation and analysis will be done in the TNO lab. Thus no detailed description of these steps are included in this SOP for fieldworkers.

Preparation

- The passive samplers are sent to the participant in an airtight tube with a screw cap. An envelope for the return will be enclosed. The tube is provided with a label and unique code that is linked to the participant.
- Before application, the samplers have to be kept at room temperature in the airtight tube.

Application

- Upon application the sampler is removed from the tube.
- The passive sampler with the size of a pen (12 x 100mm), is equipped with a clip and a buckle to attach the sampler to clothes. Alternatively, the sampler can also be attached to a necklace.
- Preferably, the sampler should be placed in the breathing zone at chest- or shoulder height (figure 1).

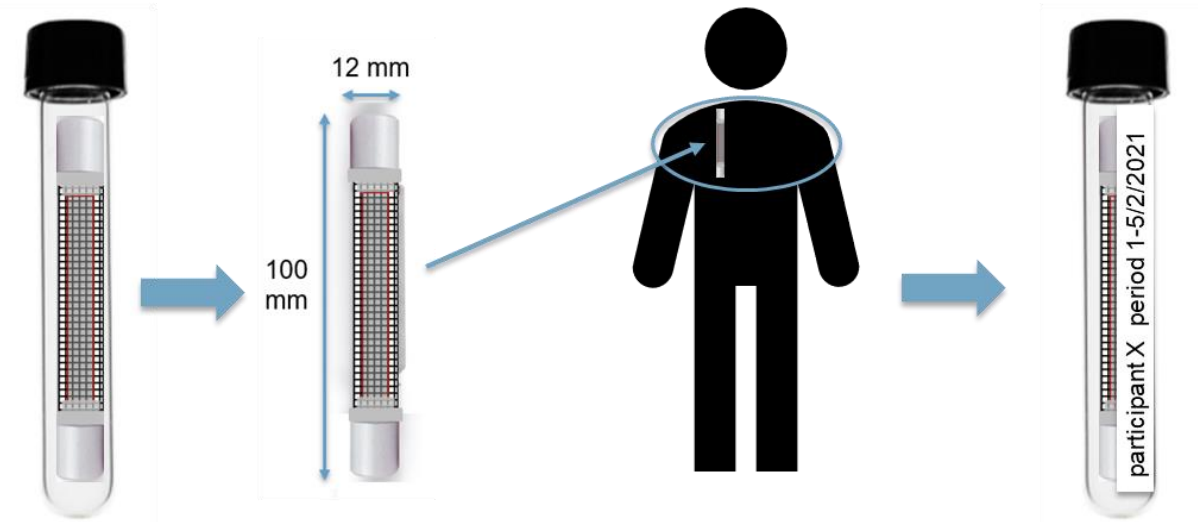


Figure 1: The pen sized passive sampler in its transport tube (left and right) and exposed to ambient air (second from right). The sampler must be worn in the breathing zone as indicated in this figure (second from left).

- The sampler is worn for five days continuously and must be attached to the out layer of clothing (not covered by other clothes).
- Outside, the sampler must be attached to the jacket.
- While sleeping, the sampler must be placed in the bedroom (on the bedside table).
- While showering the sampler must be place in the bathroom outside the shower cubicle.
- While sporting, if it's not possible to attached the sampler on the clothes it can be placed in the vicinity of the participant.

At the end of the sampling period

- After use (which is expected to be ~5 days) the sampler will be put immediately back in the same airtight tube with the screw cap attached.
- The tube is provided with a label where the participant's code and the period of sampling period must be entered.
- After that, the tube will be returned to TNO in the enclosed envelope.

8.3 *How am I* App SOP

SOP 3 - *How am I* app

Setting up the app

1. Please download TNO's *How am I* app from the app store (iOS) or play store (Android) and install it on your phone (figure 1).

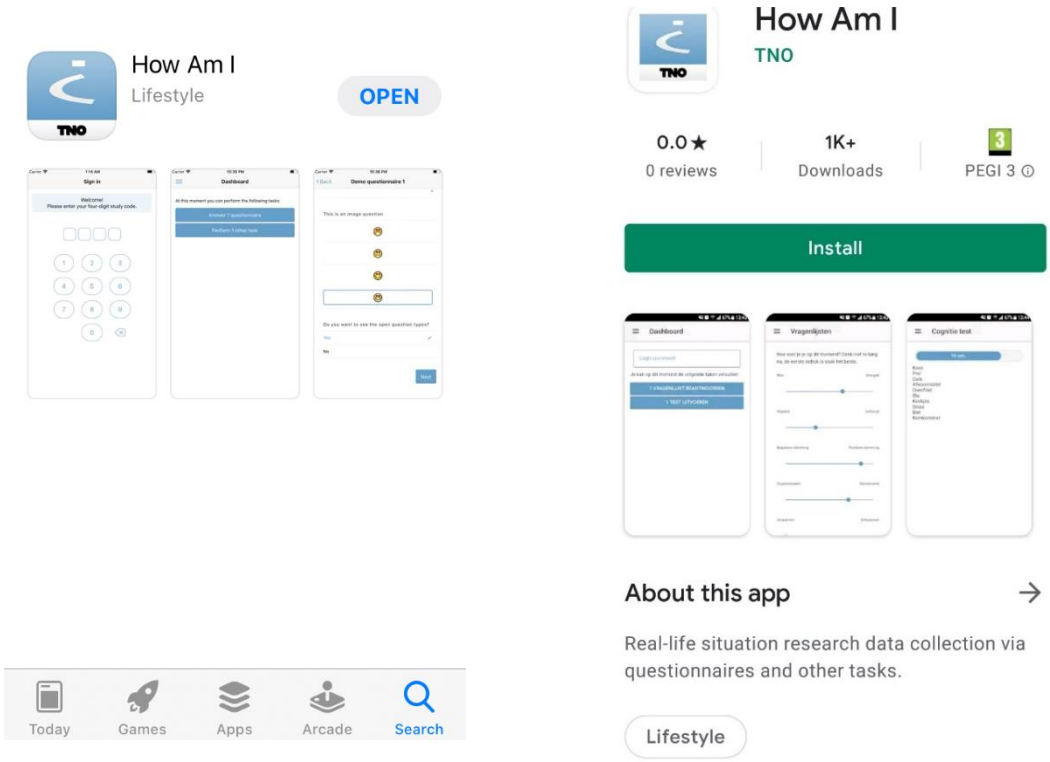


Figure 1: Download the app for iOS (left) or Android (right) in the relevant app/play store and install it on your phone.

2. Open the app. It will ask for a study code. This code and further login credentials will be provided by TNO in an email (figure 2). Note: For the feasibility study this will be coordinated by the developers at TNO. Case studies will need to provide their own data management and recruitment plans incorporating issues like this.
3. Log in with the provided credentials, the app will guide you through the set up process.

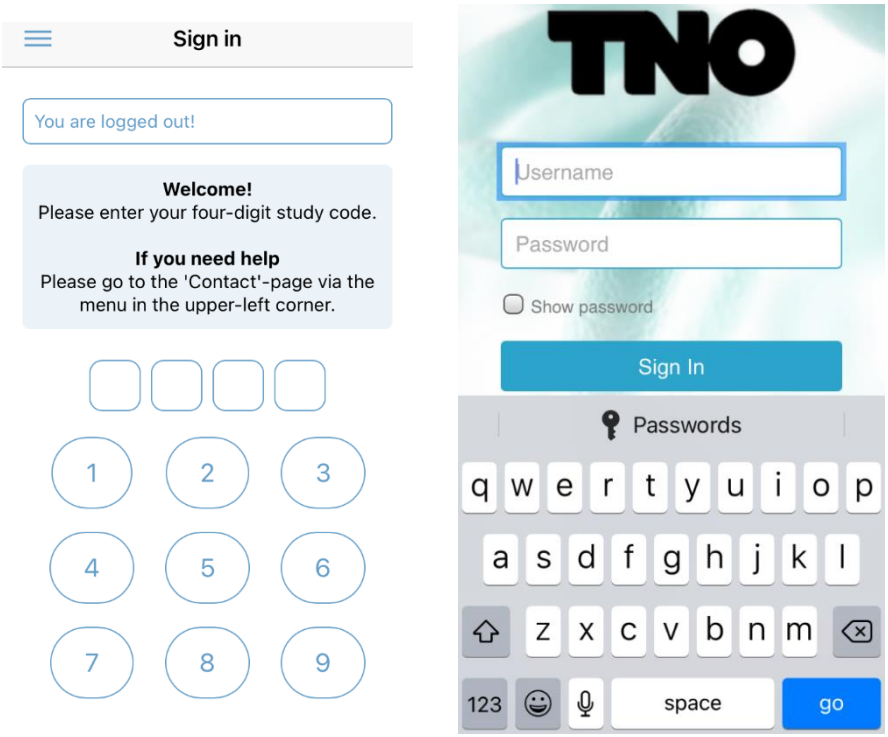


Figure 2: The App will guide you through the set up process. A study code (left) as well as username and password (right) will be provided to login.

4. The App will now ask you about the timing of reminders, please choose a time for each day when you would like to receive text message/app notifications reminding you to fill in the questionnaire (figure 3). Keeping in mind your working hours. If you wish to change the timing of your reminders, go to the main menu (top left) and choose settings which will take you to the same page again and allows you to change the timings.

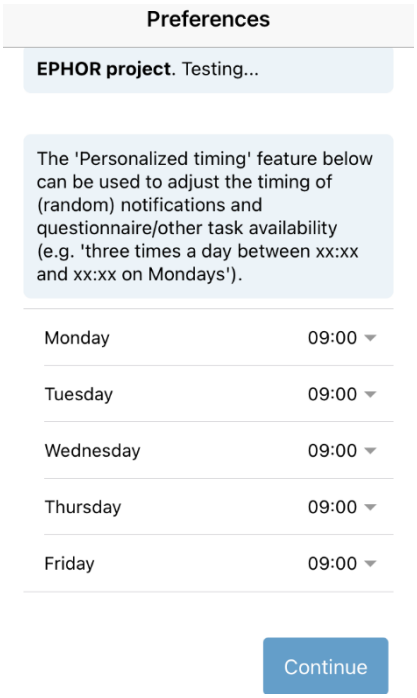


Figure 3: During the set up process the app will ask for preferences regarding the timing of reminders. Choose a time for each when you would like to receive a notification.

5. Also under the main menu is the “contact” page, where you can contact the app developers at TNO about any issues or general questions about the app.

Using the app

6. On your dashboard you can now navigate to the available questionnaires (figure 4). Answer options are available for each question. Once you come to the end of a set of questions, the app will take you back to the dashboard.

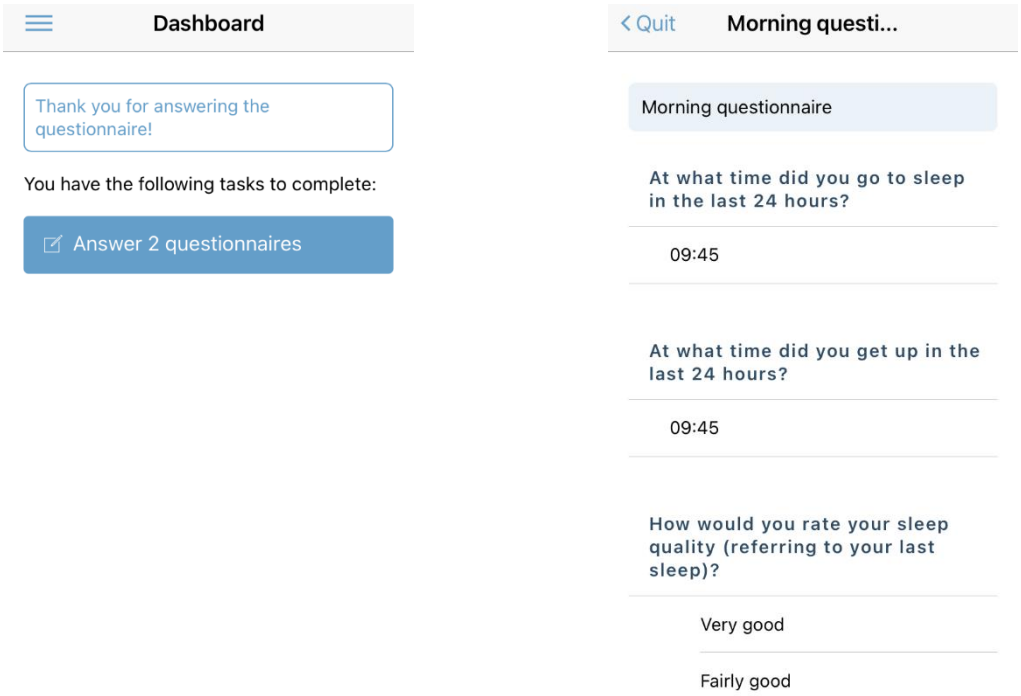


Figure 4: On the dashboard you can see if there are more questionnaires to answer. The main menu can be seen on the top left (left). The app will ask a number of questions with answer options provided (left).

Logging out/ending participation

7. If you wish to log out or end your participation please go the main menu on the top left and choose “account” -> “end participation”/“logout”.

All data collected with the app will be stored on a secure TNO server.

8.4 EDC information sheet

SOP 4 - Information sheet on the passive dust monitor

This sheet is used together with the passive dust sampler

Regarding handling and placing of the passive sampler, please follow the instructions in the enclosed manual.
After placing the sampler, please write the actual date and the precise height the sampler is placed in. After 14 days, please close the sampler and write the date. Please also state if unintended events have occurred (e.g. a cat has stepped on the sampler; something fell on the sampler etc.). Put the sampler and this information sheet in the pre-paid return envelope, and post it.

Information on the participant:

1. First name: _____ 2. Family name: _____

3. Study ECRHS ID: _____ 4. Address: _____

Information on the housing:

5. What type of housing do you live in:

[1]☐ Detached house
[2]☐ Farmhouse without livestock
[3]☐ Farmhouse with livestock
[4]☐ Multifamily house/ terraced house
[5]☐ Apartment
[6]☐ Other _____

6. Size of your house: _____m^2 7. Size of your bedroom: _____ m^2

Information on the dust sampler <u>in the bedroom</u>:

8. The date of opening and placing the sampler (dd/mm/yy): |__|__|/|__||__|/|__||__|

9. At which height the sampler is placed: _____ cm above the floor

10. The date of closing the sampler (dd/mm/yy): |__|__|/|__||__|/|__||__|

11. Unintended events: ……

……

……

Additional questions about the passive dust sampler placed in the bedroom

12. At which floor is the bedroom situated? [1]☐ Basement [2]☐ Ground floor [3]☐ Higher

13. Which flooring does the bedroom have:

[1]❑ Wall to wall carpet [2]❑ Lacquered wooden floor [3]❑ Unlacquered wooden floor [4]❑ Other flooring

What kind of surface does the bedroom walls have? (Mark each line with a cross)

14. Wallpaper [1]❑ *Yes* [2]❑ *No*
15. Painted wallpaper [1]❑ *Yes* [2]❑ *No*
16. Painted fibreglass texture [1]❑ *Yes* [2]❑ *No*
17. Other, what kind_____ [1]❑ *Yes* [2]❑ *No*

18. What kind of windows does the bedroom have?

[1]❑ Single layer [2]❑ Single layer with an extra glazing in front [3]❑ Double glazing [4]❑ Triple glazing

[5]❑ Other

What type of heating is used in the bedroom? (Mark each line with a cross)

19. Hot water radiators [1]❑ *Yes* [2]❑ *No*
20. Electric radiators [1]❑ *Yes* [2]❑ *No*
21. Floor heating [1]❑ *Yes* [2]❑ *No*
22. Woodstove/fireplace [1]❑ *Yes* [2]❑ *No*
23. Other, what kind? _____ [1]❑ *Yes* [2]❑ *No*

What type of ventilation is in the bedroom? (Mark each line with a cross)

24. Sliding windows [1]❑ *Yes* [2]❑ *No*
25. A vent or a grid in the ceiling [1]❑ *Yes* [2]❑ *No*
26. A vent or a grid in external walls or windows [1]❑ *Yes* [2]❑ *No*
27. Exhaust fan in the ceiling or external walls [1]❑ *Yes* [2]❑ *No*
28. Other, what kind?_____ [1]❑ *Yes* [2]❑ *No*

29. Have you noticed visible mould/fungi on the floor, walls or on the ceiling in the bedroom?

[1]❑ *Yes* [2]❑ *No*

30. Have you noticed visible damp patches on the floor, walls or the ceiling in the bedroom?

[1]❑ *Yes* [2]❑ *No*

31. Do you suspect any problems with dampness and/or mould/fungi which is not visible inside in the house i.e. inside the floor, walls and ceilings? [1]❑ *Yes* [2]❑ *No*

Has there been any flooding or other kind of water damage in: (Mark each line with a cross)

32. The bedroom? [1]❑ *Yes* [2]❑ *No* [3]❑ *Don't know*
33. Other places in the house? [1]❑ *Yes* [2]❑ *No* [3]❑ *Don't know*

Thank you for participating!

8.5 EDC SOP

SOP 5 - Method Description of the Electrostatic Dust Fall Collector (EDC)

Gitte Juel Holst 26th of April 2011, updated 04th of January 2021 Vivi Schlünssen

Aim

The electrostatic dust fall collector (EDC) aims to collect airborne dust settling on the surface. The EDC is in particular sufficient for exposure assessment in residential and occupational epidemiological studies due to the low-cost, efficient and manageable nature of the EDC (1-3).

Principale

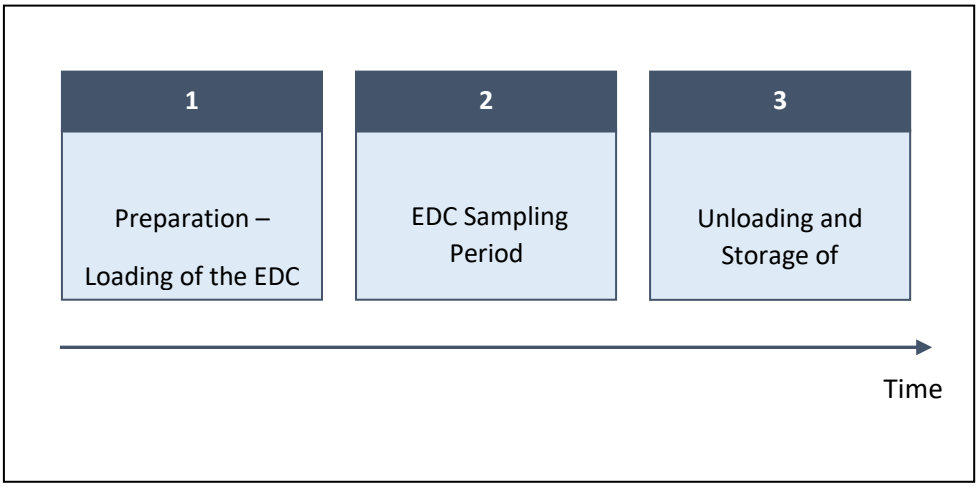
The EDC combines several passive airborne dust sampling methods both the pizzabox/dustfall collector method (4) and the electrostatic cloth sampling method (5). The EDC consists of a costum-fabricated polypropylene sampler that has electrostatic cloths attached to it to provide a sampling surface. Airborne dust settles on this surface and is captured by the electrostatic properties of the cloth. Extraction of dust components from the electrostatic properties allows for assessments of long-term microbial associated molecular patterns (MAMP's), microbial abundancy and diversity, and allergen exposures. The EDC sampler has been validated within both urban and rural homes in respect to reproducibility within homes and between two sampling periods has been investigated showing a reproducibility over time at least as equivalent as for reservoir dust analyses.

Picture 1. The EDC

Process of airborne dust sampling with the EDC

The process of airborne dust sampling with the EDC is illustrated in figure 1 and will be described in the following.

Figure 1. Process of airborne dust sampling with the EDC



1. Preparation – Loading of the EDC

Equipment and material

- Sampler
- 2 electrostatic cloths (Stofwisdoekjes, Zeeman) per sampler, packed in aluminium foil, sterilised at 200°C for at least 4 hours
- Sticker to mark the sampler
- 2 pair of tweezers
- Lab gloves
- Lab coat
- <70% ethanol
- Paper tissues
- 4 split pens per sampler, packed in aluminium foil, sterilised at 200°C for at least 4 hours
- 1 paperclip per sampler

Procedure

Wear lab coat and lab gloves during preparation. Clean workplace and hands thoroughly with ethanol and collect all needed materials. Clean sampler by wiping it with ethanol soaked paper tissues. After sterilizing the electrostatic cloths at 200°C for at least 4 hours in aluminium foil open the package with the cloths. Take the cloth out of the package by using sterile tweezers. Between each step the tweezers are bathed in ethanol and directed through a flame. Cloths may only be touched with a clean tweezer. Position the cloths in sampler, so that it fits under the frame. Fold the frame over the cloths and tighten it with the split pens in the according holes. It is easier to prepare each side of the folder separately. The finished sampler can now be sealed with a paperclip in each corner. A manual for assembly of the EDC is found in appendix 1.

2. EDC sampling period

The EDC is either handed out by instructed personnel or send to the participants by post.

Instructions to participants

The participants may open the EDC carefully without touching the wipes. The participants must be instructed to install the EDC in their bedroom or in their work place location 150 cm above the floor on a flat area where to expect as little air turbulence as possible e.g. away from windows, doors, ventilation and heaters. The participants must be instructed to leave the sampler open for a time period of 14 days without touching and removing the sampler. Date and time of opening and placing the sampler, exact height and in case of unintended events such as a cat has been sitting on the sampler or the wipes have been touched are reported in the questionnaire. After 14 days the sampler has to be closed slowly and carefully to insure that the sampled dust is not disturbed. Seal the sempler with a paperclip and the sealed EDC can be placed in an envelope and send back to the desired laboratory. An instruction on how to place the passive sampler is found in appendix 2.

3. Unloading and storage of EDC wipes

Equipment and material

- The envelop with the exposed EDC sampler
- 2 pair of tweezers
- Lab gloves
- Lab coat
- >70% ethanol
- Paper tissues
- 2 or 4 packaging zipped bags, depending on the number of clothes on the EDC
- 2-4 pre-labled stickers or a permanent marker to mark the minigrip pags
- 2-4 storage boxes, depending on the number of clothes on the EDC

Unloading procedure

Considering potential bacterial growth it is highly recommended to unpack and store the wipes as soon as possible and within a period of just a few days and at the highest up to two weeks when they are returned by mail by the participants to the laboratory. Collect all needed materials and place the materials in close range to your working area. Wear lab coat and lab gloves during the unloading procedure. Handle one wipe at the time. Start by labelling the zipped plastic bags with: the participation identification number, the measurement area (urban/rural), season (summer/winter), sampling location (house/stable/barn), the storage series, or other important characteristics. Clean workplace and hands thoroughly with ethanol. Only handle one EDC at the time. Remove the EDC and any accompanying material from the participant's envelope. Remove the safety pins from each corner of the EDC. Open the safety cuttings. Place the labelled plastic bags next to the sampler. Sterilize the two pair of tweezers by putting them in ethanol and directed through a flame. Each wipe is removed by the sampler

using a pair of sterile tweezers. It is folded twice and then placed carefully on the zipped, pre-labelled (either by a permanent pen or a sticker) bag. The bag is sealed and placed in the storage box. Clean the working area and hands with ethanol soaked paper tissues between each sampler. An instruction on how to unload and store the passive sampler is found in appendix 3.

Storage

Cloths from each sampler must be distributed in different storage boxes that is two to four boxes based on the type of EDC. This allows one box at a time from several participants to be removed from the freezer and used for extraction and analysis. About 40 to 60 cloths can fit in one box. The box should be labelled (e.g. project name. box 1A, 1B, 2A 2B etc.) to allow identification of the parallel project samples. In order to prevent bacterial growth long-term storage should always take place either at -20 or -80 ^{0}C. To reduce dramatically endotoxin activity repeated freeze-and-thaw circles in extracts are not recommended.

References

(1) Noss I, Doekes G, Sander I, Heederik DJ, Thorne PS, Wouters IM. Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. Ann Occup Hyg 2010 Aug;54(6):651-658.

(2) Noss I, Wouters IM, Bezemer G, Metwali N, Sander I, Raulf-Heimsoth M, et al. beta-(1,3)-Glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays. Appl Environ Microbiol 2010 Feb;76(4):1158-1167.

(3) Noss I, Wouters IM, Visser M, Heederik DJ, Thorne PS, Brunekreef B, et al. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Appl Environ Microbiol 2008 Sep;74(18):5621-5627.

(4) Wurtz H, Sigsgaard T, Valbjorn O, Doekes G, Meyer HW. The dustfall collector--a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). Indoor Air 2005;15 Suppl 9:33-40.

(5) Thorne PS, Metwali N, Avol E, McConnell RS. Surface sampling for endotoxin assessment using electrostatic wiping cloths. Ann Occup Hyg 2005 Jul;49(5):401-406.

Appendix 1. Manual for the assembly of the Electrostatic dust fall collector (EDC)

Material:

- Sampler
  - 2 electrostatic cloths (Stofwisdoekjes, Zeeman) per sampler, packed in aluminium foil, sterilised at 200°C for at least 4h
  - Sticker to mark the Sampler
  - 2 pair of tweezers
  - Lab gloves
  - Lab coat
  - <70% Ethanol
  - Paper tissues
  - 4 Split pens per sampler, packed in aluminium foil, sterilised at 200°C for at least 4h
  - 1 Paperclick per Sampler

Adding the electrostatic cloths :

1. Clean workplace and hands (with gloves!) thoroughly with Ethanol and collect all needed materials (Sampler, electrostatic cloths, paper tissues, tweezers and Splitpens). Wear lab coat and lab gloves from now on to prevent contaminations.

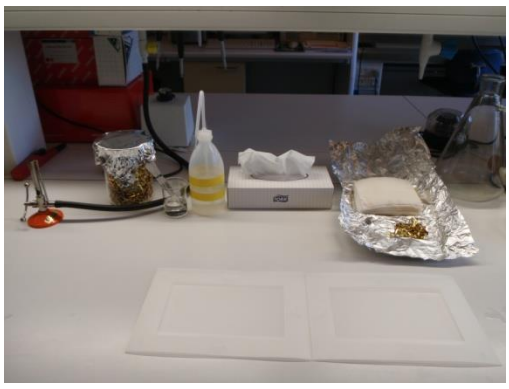


Figure 1

2. Clean sampler (fig 2) by wiping it with Ethanol soaked paper tissues.

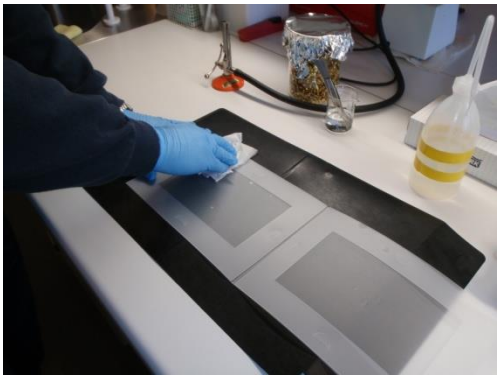


Figure 2

3. Take electrostatic cloths packed in aluminium foil, sterilised at 200°C for at least 4h and open the package (fig 3+4)

Figure 3

Figure 4

4. Take the cloth out of the package by using sterile tweezers (fig 7). Between each step the tweezers are bathed in Ethanol and directed through a flame (fig 5 6). Cloths are only to be touched with a clean tweezer.

Figure 5

Figure 6

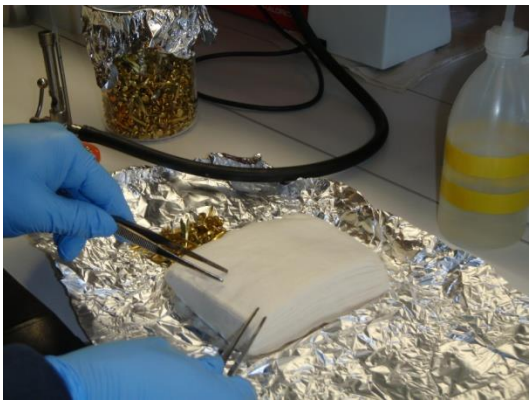


Figure 7

5. Position cloths in sampler, so that it fits under the frame (fig 8 9).

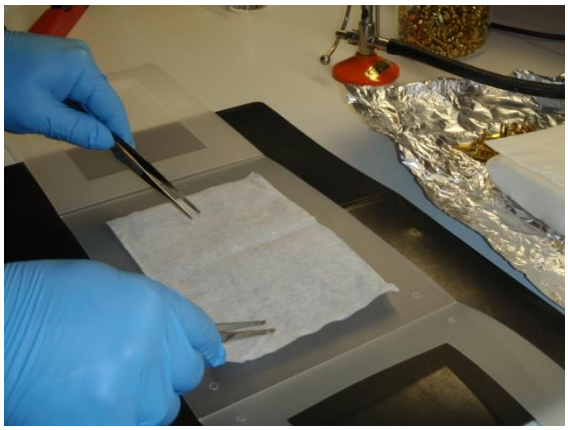


Figure 8

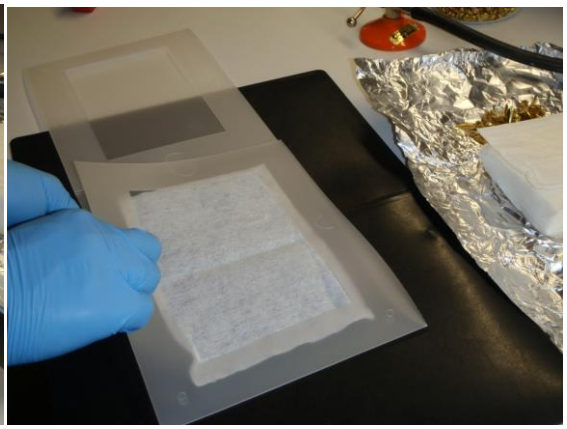


Figure 9

6. Fold the frame over the cloths and tighten it with the split pens in the according holes (fig11 12). It is easier to prepare each side of the folder separately.

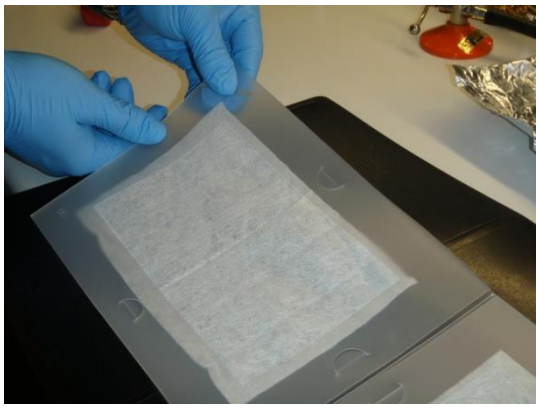


Figure 10

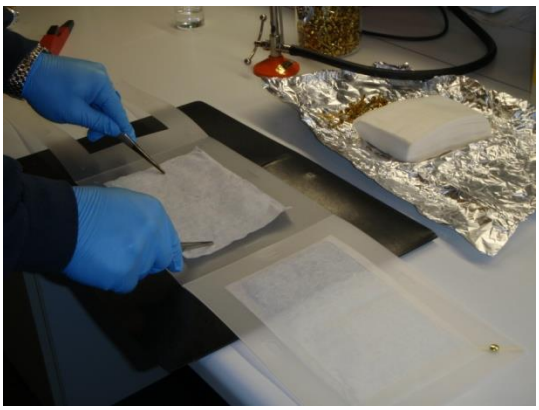


Figure 11

7. The finished sampler (fig 12+13) can now be sealed with a paperclick (fig 14+15) and send by post to the participants of the study.

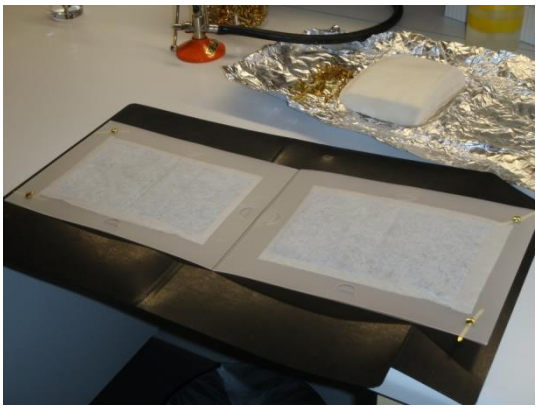


Figure 12

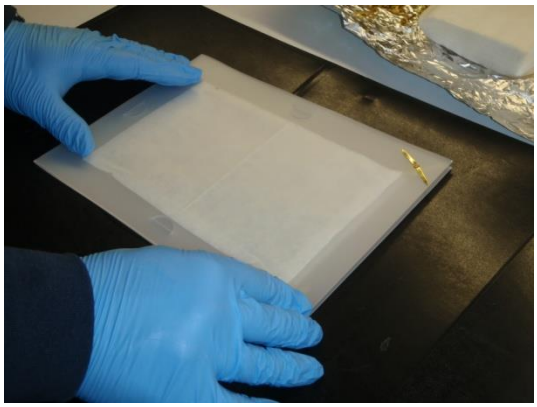


Figure 13

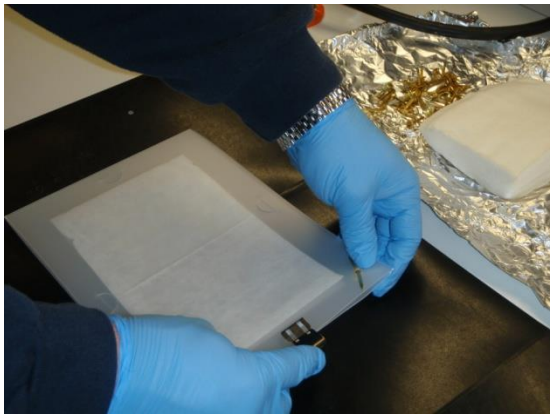


Figure 14

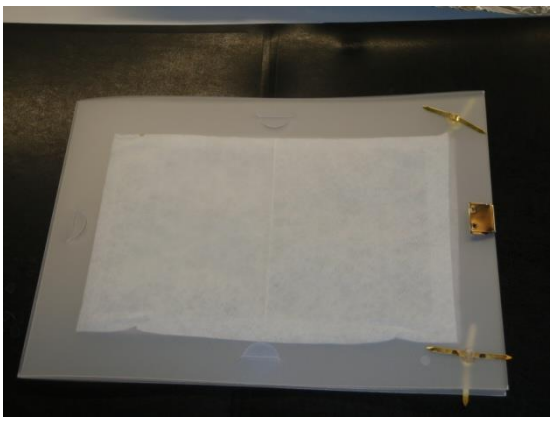


Figure 15

Appendix 2. How to handle the EDC

At first take the sampler carefully out of the envelope. From now on only touch the sampler on the plastic. Open it carefully by sliding of the paperclick. Unfold the folder.

Only touch the sampler on the plastic parts and never touch the electrostatic cloths.

Position the sampler on a place at least 150 cm above ground and with as little tendency to sudden air disturbances as possible (i.E. not close by doors or windows). Sampler should also not be placed in the kitchen, close by a heating system, TV or computer.

Leave sampler in that space for 14 days and do not touch or move it during that time! Please write down: Date and time of opening and placing the folder, exact height and if irregularities such as a cat sitting on it or something fell on it, please also write that down.

After 14 days each single folder has to be closed slowly and carefully so that the sampled dust is not disturbed. Seal the folder with a paperclick and touch again only the plastic parts!

The sealed folder can now be placed in the envelope and send back to the Laboratory.

Appendix 3. Memo on the post-sampling handling and storage of the EDC samplers

Ioannis Basinas (IB), Date: March 3, 2011

Subject: Description and suggestions on the process of unfolding and storing of the EDC samplers.

Introduction

The Electrostatic dustfall collector (EDC) is a newly developed, low-cost, packed and efficient sampling method for the collection of long-term microbial associated molecular pattern (MAMP) exposures. Detailed description of the use and the validity of the sampler can be found by the developers (Noss *et al.*, 2010a; Noss *et al.*, 2010b; Noss *et al.*, 2008). The EDC sampler is available in two versions; a) the old one with 4 sampling windows (wipes; the version described by Noss et al., in publications) and b) the new smaller one with only 2 windows. The EDC was introduced as a sampling method by the Department of Environmental and Occupation epidemiology, Aarhus University for the first time in the framework of the GABRIEL project within the exposure assessment of the SUS study. More, recently EDC has become a main sampling method for many studies performed either solely by the Department of Environmental and Occupation epidemiology, Aarhus University or in collaboration with other external research groups. Based on our experiences our group provided a series of material in order to assist both collaborators and participants in the handling and usage of the sampler. In this framework, instructions on the material preparation and assembly of the sampler have been developed both in the form of a video and as a standard operation manual (SOP). The present memo comes in sequence in order to provide guidance and suggestions for the handling, unfolding and storage of the EDCs following the completion of the sampling period.

1. Handling prior and upon arrival

The EDC are usually posted by the participants on pre-stamped envelopes. Special care should be given in order to make the sampler traceable. The sampler must either be marked with a code by a sticker or a permanent marker. Ideally, the pre-stamped envelope should carry the identity of the participant in order to smooth labeling of the wipes during the unpacking and storage procedure. Administrative users must be aware that according to the published literature the EDC sampled MAMP concentrations are not linearly associated to the period of sampling; meaning that a doubled sampling period (i.e. 4 weeks instead of the advised 2) will not necessarily result in 2-times higher concentrations, as someone should expect (Noss *et al.*, 2010a). The reasons are unknown but it is highly advised by the developers to keep the sampling period uniform in order to avoid variations that cannot be explained.

In most studies the samplers will either be posted or handed out to the participants few at a time. Therefore, the samplers return will be a continuous process. Nevertheless, considering potential bacterial growth it is highly recommended to unpack and store the wipes as soon as possible and within a period of just a few days and at the highest up to 2 weeks upon their arrival. Previous analysis by the developers (Noss *et al.*, 2010a) showed no difference in measured endotoxin and glucan concentrations between samples stored in room temperature and in -20 ^{0}C for a short period (one to few weeks) in concordance with earlier studies on glass fiber of PVC filters (Spaan *et al.*, 2007). Though,

long-term storage should always take place either at -20 or -80 ^{0}C in order to prevent bacterial growth (Noss *et al.*, 2010a).

2. Unpacking

Preparation for the unpacking of the EDCs follows the same rules as their assembly process. The materials needed for the unpacking and storage are:

- The envelop with the used EDC sampler
  - 2 pair of tweezers
  - Lab gloves
  - Lab coat
  - >70% Ethanol
  - Paper tissues
  - 2 or 4 packaging zipped bags, depending on the number of clothes on the EDC
  - 2-4 pre-labled stickers or a permanent marker to mark the minigrip pags (figure 1)
  - 2-4 storage boxes (figure 2)

The workplace and hands (with gloves!) should be cleaned thoroughly between each sampler unfolding with Ethanol and all the needed material must be in close range. A lab coat and lab gloves must be worn in order to prevent further contaminations.

The sampler is removed from the envelope, opened carefully and the folding cuttings and split pens should be also removed from the according holes.

One wipe is handled at a time. Each wipe is removed by the sampler using a pair of sterile tweezers. It is folded twice and then placed carefully on the zipped, pre-labelled (either by perm. pen or sticker) bag. The bag is sealed and placed in the storage box.

Cloths from each sampler must be distributed in different storage boxes (2 to 4 boxes based on the type of EDC). This allows one box at a time from several participants to be removed from the freezer and used for extraction and analysis. About 40 to 60 cloths can fit in one box. The box should be labelled (e.g. project name. box 1A, 1B, 2A 2B etc.) to allow identification of the parallel project samples.

Figure 1. Minigrip bag for storage of the EDC clothes. Details description at: http://www.minigrip.nl/product_detail/1_5_9/minigrip-verpakkingen/1104/ or

http://91.143.113.91/bhhandel/main.pgm?SMURFID=0020282bd92c2d98845241d9d16e7fefe8d92c8adceb444b142f7a34c82703e1 or http://91.143.113.91/bhhandel/main.pgm?SMURFID=0020282bd92c2d98845241d9d16e7fefe8d92c8adceb444b142f7a34c82703e1.

Figure 2. EDC storage boxes. Details at: http://www.emergolab.com/index2.asp?id=4060554001.39

3. Wipe labelling

The zipped-bags/wipes should be labeled with an identity code that allows the identification of the project, participant id and in case of applicability the measurement area, season or other important characteristics. For example in the SUS study measurements were seasonal (summer and winter) performed in the stables and bedrooms of participants working in different farms (occasionally there were 2 or more SUS participants working in the same farm). A labeling code on the following form was developed:

SUS12-FID/SUSID/S/A/D

Were:

- *FID* was a 3 digit number stating the farm identity
- SUSID was a 4 digit number giving the SUS participant identity
- S was the season as a single letter, i.e. S for summer and W for winter
- A was the area, i.e. H for home bedrooms and S for stables and
- D the designation of the wipe stating the storage series (A, B, C and D)

Slashes were omitted in the code and they are just given to discriminate the different characters.

An e-storage database (e.g. an excel file) can also be developed in order to associate wipe codes with boxes.

4. Storage

In most cases the storage order by sampler id is of minor importance. Every box should be filled in and subsequently stored in most cases in a fridge due to the long storing period until extraction (see section 1.). The literature suggests repeated freeze-and-thaw circles in extracts to reduce dramatically endotoxin activity (Douwes *et al.*, 1995). In case of storage by identity order the bags with the newly arrived wipes must be placed in the box fast and without allowing the freezer-stored filters to de-freeze; though storage by id is not necessary for vast majority of the currently ongoing studies. In the context of the SUS and most of the GABRIEL related studies storage of the filters took place in a -80 ^{0}C freezer.

Reference List

Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. (1995) Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol; 61: 1763-9.

Noss I, Doekes G, Sander I, Heederik DJ, Thorne PS, Wouters IM. (2010a) Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. Ann Occup Hyg; 54: 651-8.

Noss I, Wouters IM, Bezemer G, Metwali N, Sander I, Raulf-Heimsoth M, Heederik DJ, Thorne PS, Doekes G. (2010b) beta-(1,3)-Glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays. Appl Environ Microbiol; 76: 1158-67.

Noss I, Wouters IM, Visser M, Heederik DJ, Thorne PS, Brunekreef B, Doekes G. (2008) Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Appl Environ Microbiol; 74: 5621-7.

Spaan S, Heederik DJ, Thorne PS, Wouters IM. (2007) Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. Appl Environ Microbiol; 73: 6134-43.

8.6 Feedback/Survey

Feasibility study feedback

This is not the final format. It is anticipated to utilise a survey software such as Survey Monkey to gather the feedback.

Method	Category	Did it work as expected (Y/N)	How easy or difficult is it to use? (scale 1-5)	If N please describe issues (be as specific as possible)	Suggestions for improvement/Questions (be as specific as possible
Sensor box	Setting up				
	Does it work as it is meant to work? (individual sensors/full box)				
	Wearability				
	Ease of use				
Gateway	Setting up				
	Does it work as it is meant to work?				
	Ease of use				
Axivity sensor	Setting up				
	Does it work as it is meant to work?				
	Ease of use				
Heart rate monitor	Setting up				
	Does it work as it is meant to work?				
	Ease of use				
Data visualisation	Access to data				
	Data output/management				
Passive samplers	Setting up				

	Wearability				
	Ease of use				
	Logistics regarding shipment to/from lab (if tested)				
***How Am I* app**	Setting up				
	Does it work as it is meant to work?				
	Ease of use				
	Data output/management				
EDC (dust fall)	Setting up				
	Ease of use				
	Logistics regarding shipment to/from lab				
Instructions/SOPs	Clarity				
	Completeness				
Overall study protocol	Clarity				
	Completeness				
Study procedure	Logistics				