MUNI | RECETOX

Faculty of Science, Brno, Czech Republic

Fundamentals in Environmental Processes Research

Laboratory course

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ISBN 978-80-210-9685-1

Content

1 General information and course objectives

The laboratory course, Fundamentals of Environmental Processes Research (E1230), is intended for students of the first semester of the follow-up master's degree program, Environment and Health, at the Faculty of Science, Masaryk University, taught in the autumn semester.

The course is directly associated with computational seminar E6050, and lecture E6051 The fate of toxic substances in the environment. The main aim of these courses is to acquaint students with the chemical and physical laws that allow a qualitative and quantitative description of the fate of chemicals in the environment, including the transport and balance of substances between compartments, and their chemistry in matrices and biomes. The aim of the course Fundamentals of Environmental Processes Research is to transfer theoretical knowledge about the behaviour of chemicals in the environment and processes at phase interfaces to the level of practical skills. The course includes laboratory experiments focused on environmental processes, transport and balance of substances between compartments, following the content of the new field of study Environment and Health.

At the end of the course, the student will be able to:

- understand the transport, distribution and fate of chemicals based on the properties of individual substances and matrices
- interpret the laboratory results from the point of view of their fate in the environment
- predict the environmental behaviour of the chemicals based on simulation in laboratory conditions
- develop laboratory skills and principles of good laboratory practice

During the exercises, students will gain valuable practical skills with currently used trends, techniques and instrumentation of modern environmental chemistry. Also, students will proceed as independently as possible, to learn as much as possible, using their own practical laboratory experience, and be able to independently implement all stages of laboratory procedures, including their perception of context. In addition, they will consolidate their knowledge of English terminology, which is crucial for the international nature of environmental chemistry.

Recommended literature:

SCHWARZENBACH, René P., P. M. GSCHWEND a Dieter M. IMBODEN. Environmental organic chemistry. Third edition. Hoboken, New Jersey: Wiley, 2016. xvii, 1005. ISBN 9781118767238. info

SCHWARZENBACH, René P., P. M. GSCHWEND a Dieter M. IMBODEN. Environmental organic chemistry. 2nd ed. Hoboken, N.J.: Wiley-Interscience, 2003. xiii, 1313. ISBN 0471357502. info

1.1 Organisation of exercises

The philosophy of the laboratory course is the individual work of students as much as possible. The annual course schedule is unique. The schedule may vary according to the number of course days and the number of student groups.

The course takes place in a one-week block. In the case of a more significant number of students, it will be held regularly each week according to the schedule, in well-equipped student laboratories, enabling the practical implementation of even more complex laboratory tasks.

Students will be trained in the safety of laboratory work, and for working with chemicals, at the beginning of the course (see chapter 2). To prepare for laboratory work and an entrance safety test, read chapter 2 carefully. You cannot complete the course without passing the test.

Read all the instructions carefully, and answer the preliminary questions. At the beginning of the course, you will take an entrance test, in which you must obtain 80% to pass. All materials and instrumentation will be prepared for you in the laboratories.

Take notes and write down, in particular, all the details of the procedures performed and the results. Whenever you don't know something, ask the teachers. Some parts are done as a group, but most of the methods will be done individually or in pairs.

Each student will prepare protocols for all exercises individually. The protocols contain a brief theoretical introduction, including citations, a description of the goal of the task, a report of any deviations from the instructions, precise results, including graphical form, commentary on the results and answers to the questions. Pay particular attention to the results and the subsequent discussion. The protocol is submitted in electronic form no later than 14 days after the completion of the task

The course ends with 4 ECTS credits.

Conditions for obtaining credit:

1. 100 % attendance in all laboratory exercises
2. entrance test - obtain a minimum of 9 points (max. 15 points)
3. laboratory work - each of the tasks is evaluated individually (include interest in the issue, preparation for the task, good laboratory practice, diligence (max. 4 points for each task)
4. laboratory protocols - each protocol is evaluated individually - for each protocol, a minimum of 3 points is required (max. 5 points). Each student will prepare a separate protocol for each task.

The condition for granting the credit is to obtain a minimum of 36 points* (max. 60 points). Points will be awarded by each lecturer based on the successful completion of the respective task.

* for each evaluation item, it is necessary to obtain at least the minimum number of points listed. Failure to meet any of the evaluation items will result in non-award of credit.

1.2 Protocol

Name and surname:	
Study programme, semester:	
Laboratory course:	
Date of laboratory work:	
Date of protocol elaboration:	
Task:	

Introduction: Describe the subject (goal) of the experimental work. Briefly summarise the sources of specific substances, and why we are interested in the matrix. Indicate possible methods of sampling and subsequent processing of the sample. Provide citations to the facts.

Workflow: The work procedure is included in the study materials, and therefore it is not necessary to copy it to the protocol. Emphasise the principle of the sample processing and indicate any deviations from the procedure.

Results and evaluation: In this section, provide calculations, tables, graphs, or other graphical representations of the results. When processing data, do not forget to round. Follow the notes and questions of the individual task.

Discussion and conclusion: The lecturer will inform you of each task about the specific requirements for this crucial section. In general, provide a verbal assessment, and a summary of the task. Discuss what the results indicate, for example, in comparison with measured values from other studies. Answer the questions at the end of the task.

Reference: Be sure to cite the sources used.

2 Laboratory work safety

- Operation at all workplaces where substances or preparations are harmful to health must be designed in such a way that these substances cannot endanger workers in these workplaces or in the vicinity of the workplace. So as not to endanger groundwater and surface water, or to escape into the air in harmful concentrations and thus damage health, i.e. the maximum permissible concentrations for the working environment must not be exceeded.
- Remedies must also be provided in the event of an accident. If the harmful substance is spilt, it must be disposed of immediately.
- The main principle when working with harmful substances and preparations is to prevent all possibilities of intoxication (avoid direct contact of workers with these substances), use all necessary protective equipment (work clothes, goggles, a suitable type of gloves, face shields, masks, etc.) and comply with all safety regulations.
- Eating, drinking and smoking should not be allowed when working with chemicals. Before eating, drinking and smoking during work breaks and after work, workers must wash their hands and face thoroughly, and, depending on the nature of the work; they must perform a thorough cleansing of the entire body after work. If the worker is working in protective clothing, he must not eat or drink while he wearing it.
- Corrosives must not be stored at a height higher than the height of the shoulders of the worker handling them (and at the same time at a maximum height of 165 cm).
- When diluting, always pour the acid into the water and never the other way around. The acid is poured slowly and carefully, especially sulphuric acid.
- When dissolving solid hydroxide, the hydroxide must be poured into the water in small amounts, stirring constantly. Water is never poured onto the hydroxide.
- Spilt nitric acid must not be removed with sawdust, rags or other organic substances. It must be neutralised before disposal and, if this is not possible, at least diluted as much as possible. Utensils contaminated with organic substances must not be cleaned with nitric acid (danger of violent reactions, development of nitrogen oxides and spontaneous combustion).
- Spilt acids, especially concentrated ones, must first be carefully diluted with water, slightly neutralised by sprinkling with a carbonate (e.g. soda, chalk, etc.) or pouring dilute alkali solutions, followed by careful rinsing with water, or soaking the liquid in sawdust, rags, etc. Care must be taken not to contaminate too large an area.
- Any handling of smoky, irritating, foul-smelling and toxic gases must only be carried out in a fume hood.

- Solid chemicals must never be handled without hand protection (gloves).
- Corrosive, toxic and infectious liquids should only be pipetted using safety pipettes or a balloon.
- For all handling of substances in test tubes and open containers, the mouth of the containers must be facing away from workers, into open space.
- Bottle caps with a stained surface must not be placed on tabletops (to reduce the possibility of intoxication and contamination).
- Perchloric acid must be stored in ground-necked bottles and separate from other chemicals, especially organic ones. Perchloric acid cylinders must not be placed on wooden shelves, only on glass, porcelain, ceramic or other refractory, or other non-absorbent pads, so that traces of spillage can be easily removed.
- Chemical laboratory equipment that has been used to work with toxic substances or corrosives must be thoroughly rinsed before further use. Similarly, all toxic bottles must be free of residual contents before disposal.
- Laboratory tasks are performed according to instructions, under the supervision of laboratory staff. Manipulation with cylinders and pressure reducing valves is only permitted in the presence of, and under the control o the supervisor.
- Any injury, accident, damage or another defect must be reported immediately to the laboratory supervisor.
- When working with chemicals and biological materials, it is necessary to follow the related articles of the RECETOX Operating Rules, including appendices 1 and 2.

I confirm with my signature that I have understood the trained topic, and I am aware of the responsibility for any non-compliance or deliberate violation of these rules.

Date and duration of the training		30 min
Method of knowledge verification	oral	
Training materiál:	This document; Related articles of the valid version of the RECETOX Operating Rules, including Annexes No. 1 and 2; Operating rules of laboratories	
Name and signature of the trainer		

	UCO (personal identificati on number)	**Name and Surname**	**Field of Study**	**Sem/Year**	**Signature**
1.					

3 Introduction

The environment is contaminated with many chemicals, mainly due to human activity. To predict the fate of persistent organic pollutants in the environment, several aspects have to be considered carefully: their basic physical and chemical properties, distribution, transport within and among compartments, biotic and abiotic transformation processes, as well as the effects on living organisms including humans.

Today, organic chemical pollutants can be found widespread over the whole planet, even in places thousands of miles away from their origin. They get into individual components of the environment from various sources, both natural and anthropogenic. In most cases, inputs from various anthropogenic technologies dominate today. Chemicals can be transported in the components to which they were primarily emitted; they can cross the interfacial interface to other components of the environment. During this transport, they can be chemically transformed and create secondary pollution. Due to their properties, they can accumulate both in abiotic components of the environment and in living organisms, including humans. After entering living organisms, they can adversely affect health. It is necessary to understand the external and internal concentrations of these substances to assess exposure routes.

Laboratory simulation of the processes that control the chemical behavior of organic compounds in the environment is often desirable for deeper understanding, as well as for determination of basic characteristics required for successful environmental modelling. However, laboratory exercises targeted on the simulations of environmental processes are very rare.

The course includes experiments to determine phase equilibria, partition and distribution coefficients, transport of substances between compartments, bioaccumulation and degradation. The first experiments are focused on understanding the basic principles, which are then applied in the determination of organic pollutants in commonly sampled matrices.

1. Partition coefficient n-octanol/water Kow
2. Henry's Law Constant
3. Adsorption and volatilisation of chemicals from the soil
4. Photochemical degradation
5. Polychlorinated biphenyls, organochlorine pesticides and lipid determination in a butter
6. Determination of persistent organic pollutants in water by passive sampling
7. Extraction of microcystins from water using SPE method
8. Extraction of pesticides from the soil by QuEChERS method

9. PAHs analysis in the air using GC-MS/MS
10. Needles as a passive air sampler
11. Determination of flame retardants in a dust sample
12. Determination of endocrine disruptor metabolites in urine samples

4 Partition coefficient n-octanol/water

Theory

If we want to use a specific substance in the industry, we need to quantify how dangerous the substance is (for example, if someone comes with anew product, we need to have some measures to determine that it is safe to use on a large scale). For such a decision, we have different properties that we can measure, and based on this measurement; we can estimate if the product can be used safely. There are many such properties; for example, we can examine corrosivity of the substance by measuring pH, volatility by boiling temperature, and many others.

However, from the point of view of environmental chemistry, we also need to know how the compound will behave in the real environment. We are not able to give precise numbers due to the complexity of real systems (there are too many factors in nature). Even if we could somehow be precise, this precision can be problematic in most situations. It is like a map with scale 1:1; we cannot use it. So, we need to make an estimation based on modelling.

There are various models with various uses. Nevertheless, one of the simplest is based on the partition coefficient between n-octanol and water. Imagine this situation. We have a substance, and we pour it into the water accidentally. Now we want to know if fish living in the water are in danger. So, firstly, we need to estimate the dose which will pass into fish.

Moreover, that is what K_{ow} does. We can take fish and water and experiment to see what amount will ultimately be in fish. However, a much safer approach is to take octanol, use it as a substitute for the fish, and do the testing. It is cheaper, faster, and doesn't harm fish [1].

So, we put octanol and water together. We add our potentially dangerous substance. Then we determine K_{ow} from the concentration of the examined substance in water and octanol after dynamic equilibrium is achieved. So, mathematically speaking, we have this formula [2].

$$K_{ow} = \frac{c_{oktanol}}{c_{water}}$$

From the formula, we can see that K_{ow} is a Dimensionless quantity because concentration is divided by concentration. Also, we can say that with the increasing value of $K_{ow,}$ the $c_{oktanol}$ is increasing (or c_{water} is decreasing). Thus, we can conclude with the statement higher K_{ow} = higher $c_{oktanol}$ = higher lipophilic character = lower hydrophilic character. Because the K_{ow} of different substances differs by several orders of magnitude in the literature, log(K_{ow}) is commonly used.

Principle:

K_{ow} is determined by adding a measured substance into the emulsion of octanol and water. After shaking intensively and achieving the equilibrium, we can quantify the concentration in water and

octanol. If we plot the concentration in one phase as a function of the second phase, we will obtain a linear function in the low concentration region. The slope of the function is equal to K_{ow}.

Task:

Determine partition coefficient n-octanol/water by using shaking flask

1A Extraction of sample

2B Instrumental analysis

3C Processing of results

1A Extraction of sample

We will use:

- stand
- separatory funnel (shaking flask)
- measuring cylinders (50 ml; 500 ml)
- funnel
- flask (100 ml; 500 ml)
- Pasteur pipets
- vials (5x 22 ml and 2x 2ml)
- pipets, tips
- examination gloves
- evaporation equipment

Chemicals:

- distilled water
- dichloromethane (DCM)
- anhydrous sodium sulfate
- solution of naphthalene (naphthalene K_{ow} will be analysed)

Sample preparation and processing

Put 5 ml of octanol with naphthalene and 500 ml of distilled water into shaking flask

Shake intensively 10 minutes (! It is necessary to release emerging gases!)

After shaking put flask into the stand, wait 5 minutes for equilibrium then separate the phases

Water

Put water phase back to shaking flask

Add 20 ml of DCM

Repeat snaking process (do not forget to release gases)

Wait for equilibrium

Separate phases

Dry organic phase by Anhydrous sodium sulfate

Reduce the volume to 1 ml (by flowing nitrogen)

Octanol

Put octanol into a vial

Add Anhydrous sodium sulfate

Put a little piece of cotton wool into Pasteur pipet

Use prepared pipet as a filter and clean octanol from the solid phase

Take 100 µl of sample and dilute is 10x into micro-vial

Figure 1 Shaking and simultaneously releasing gases

Instrumental analysis

Will be conducted on gas chromatograph with flame ionisation detector HP6890

Processing of data

From experimental data calculate K_{ow} of naphthalene and log K_{ow} (do not forget about dilution)

Write structured protocol (introduction, principle, workflow, calculation, and summary, add answers to following questions)

Questions

Compare the value of K_{ow} we have obtained experimentally with value in literature. Explain the differences (if there are any).

What are the reasons we cannot use ethanol instead of octanol?

Can we determine K_{ow} of any substance?

Why is it better to determine K_{ow} from slope rather than from one point?

What is the approximate threshold of K_{ow} where we can say a substance is very lipophilic?

References

[1] D. Mackay, A. K. D. Celsie, and J. M. Parnis, "The evolution and future of environmental partition coefficients," *Environ. Rev.*, vol. 24, no. 1, pp. 101–113, 2016.

[2] S. Amézqueta, X. Subirats, E. Fuguet, M. Rosés, and C. Ràfols, "Octanol-Water Partition Constant," in *Handbooks in Separation Science*, C. F. B. T.-L.-P. E. Poole, Ed. Elsevier, 2020, pp. 183–208.

5 Determination of Henry´s law constant

Theoretical background

Partition coefficient

The air/water partition coefficient KAW is used to describe the distribution of substances between water and air, which is defined as the ratio of the equilibrium concentration of a given substance in the air to the equilibrium concentration in water. It is often expressed as Henry's law constant - H. The relationship between KAW and Henry's law constant can be expressed by the law of ideal gas by this equation:

$$K_{AW}=c_A/c_W = H/RT;$$

where c_A is the concentration of the analyte in air, c_W is the concentration in water, H is Henry's law constant, R is gas constant, and T is temperature. If we express the amount of compound in air and water as c_A and c_W, we can obtain the so-called dimensionless Henry's law constant, which corresponds to K_{AW}. The air/water partition coefficient is widely used in models of the fate of pollutants in the environment.

What other coefficients do you know?

Name examples of real situations in the environment for which you would use Henry's constant.

When determining Henry's law constant using headspace analysis, we assume that the equations apply:

$$c_0=c_A V_A+c_W V_W;$$

where c_0 is the concentration of analyte at the beginning of the experiment in mg/ml, c_W is the concentration in the water after equilibration; c_A is the concentration in the gas phase after equilibration, V_W is the volume of the water, and V_A is the volume of the gas phase.

Questions after lab practical:

Compare measured Henry constant with the literature.

Determination of Henry's law constant for benzene by headspace

I. Headspace

Materials:

100 ml volumetric flask

Beaker

6x headspace container (25 ml)

Gas-tight syringe

Chemicals:

Mili-Q water

Benzene

Dichloromethane (DCM)

Workflow:

Prepare 100 ml of a solution of 10 µl of benzene in water

Pipette the prepared solution into 1.5 ml, 2.5 ml, 3 ml, 5 ml, 7 ml and 10 ml headspace analysis vials

Close the containers carefully and allow to equilibrate for 24 hours

II. Gas phase analysis by gas chromatography

The amount of benzene in the gas phase will be determined by gas chromatography.

6 Adsorption and volatilisation of chemicals from the soil

Theoretical background

Adsorption on the soil

Knowledge of the behaviour of substances in soil, sediment or sludge is very important from an environmental point of view. The distribution of chemicals between the soil and the water is a complex process that depends on several factors: chemical properties of the substance, soil character, climatic factors (e.g. temperature, rain, sunlight, airflow). Therefore, the process cannot be simulated in the laboratory in its real condition. However, the following methodology can provide at least an idea of the adsorption characteristics of the chemicals.

The key parameter of the fate of chemicals in these matrices is the adsorption coefficient Kd, which is defined as the ratio between the concentration of the substance in the soil and the concentration of the substance in water in adsorption equilibrium:

$$K_d = \frac{c_{soil}}{c_{water}}$$

What other equilibrium coefficients do you know?

Name examples of real situation in the environment for which you would use this model?

Known volumes of a solution of analytes of known concentration in 0.01 M $CaCl_2$ solution are added to a soil sample of known mass. The mixture is shaken for the specified time. The soil suspension is then separated by centrifugation. Soil and water samples are extracted and analysed.

Volatilisation from the soil

Leaching from the soil is a very complex process that depends on the physicochemical properties of the chemicals (water solubility, vapour pressure, Henry's constant, n-octanol-water partition coefficient Kow or sorption coefficient for soil organic component Koc, photolytic stability), environmental conditions (soil surface size, bare soil vs. vegetation-covered soil, soil structure and porosity, air and soil temperature, soil moisture, air humidity, airflow, soil organic matter content, amount of precipitation, etc.), state of an applied substance (solid, liquid or gaseous) and type of application (application to soil surface vs. incorporation into the soil, the amount applied). Some substances can be present in both neutral and ionic form in soil solution. Depending on the pKa of the pesticide, volatilisation may be affected by soil pH.

The direction and magnitude of the diffusion gradient are controlled by the concentration (fugacity) of the substance in air and in the soil, and by the equilibrium soil/air partition coefficient Ksa. Chemical could start leaching from the soil when its concentration (fugacity) in the soil exceeds its concentration (fugacity) in the ambient air. The Ksa coefficient is a critical parameter of this process.

Leaching from the soil could be affected by soil pH. What types of soils do we have in the Czech Republic, and what is their pH? What can we conclude from this?

Air "free" of the monitored analytes passes to the evaporation chamber via two pre-cleaned polyurethane filters. The air then flows over a soil surface contaminated with a known concentration of the analytes and through another polyurethane filter located at the chamber outlet. The analytes evaporated from the soil are captured in this polyurethane foam,

Questions after lab practical:

Discuss the main differences between previously used pesticides (e.g. DDT or lindane) and currently used pesticides (CUPs).

6.1 Determination of phenol adsorption on the soil

I. Adsorption on the soil

Materials:

- 4 centrifuge tubes (50 ml)
- Shaker
- Centrifuge
- Cylinder
- 4 mini vials (2 ml)
- Filter paper

Chemicals:

- Soil (clay up to 5.5 %)
- Solution of phenol in methanol (100 mg/l)
- 0.01 M $CaCl_2$ solution

Workflow:

- Weigh approximately 5 g of sieved soil into 4 centrifuge tubes
- Add 36 ml of 0.01 M $CaCl_2$ solution and 4 ml of phenol solution to the soil samples
- Place tubes to the shaker and start shaking
- Collect one tube in 0, 3, 6 and 24 hours
- separate the soil and aqueous suspension by centrifugation (4000 rpm, 10 min)

- at all times, take 1 ml of the aqueous phase into a pre-prepared mini-vial and store in the refrigerator until analysis
- at 0, 6 and 24 hours, separate the soil suspension and let it dry on the filter paper until the next day

II. Soil extraction

Materials:

- Ultrasonic bath
- 2 beakers
- Cylinder
- Vials (2x 40 ml a 1x 2 ml)
- Nylon filter

Chemicals:

- Methanol

Workflow:

- Weigh the dried soil and pour it into a beaker
- Pour 15 ml of methanol into the soil sample
- Extract 3 times for 15 minutes in an ultrasonic bath
- Always take the extract into a pre-prepared beaker after each 15-minute extraction cycle
- Filter the extract into a vial
- Evaporate the volume of solution in the vial to a volume of 1 ml under a stream of nitrogen
- Quantitatively transfer the concentrated extract to a pre-prepared mini vial
- If necessary, filter the sample through a nylon filter
- Close the vial tightly and store it in the fridge until further processing

III. Determination of phenol by HPLC

Phenol concentrations in soil and water samples will be determined by HPLC.

6.2 Determination of coefficient K_{sa} for phenol

I. Air sampling

Materials:

- Evaporating chamber
- Low volume pump
- Polyurethane foams (PUFs) – cleaned
- Aluminium foil

Chemicals:

- Soil (clay up to 5.5 %)
- Solution of phenol in methanol (100 mg/l)

Workflow:

- Place 5 g of sieved soil into the evaporation chamber
- Apply 1 ml of a solution of phenol in methanol dropwise to the soil using a Pasteur pipette
- Allow to equilibrate for 24 hours
- Place PUF at the inlet of the evaporation chamber
- Place a low volume pump together with the second PUF at the outlet of the evaporation chamber
- Start a 24-hour subscription
- Record the timer status when the experiment starts
- After 24 hours, switch off the pump and record the timer status
- Remove the exposed filter and wrap it in two layers of aluminium foil and store in a freezer

II. Soil extraction

Materials:

- Ultrasonic bath
- 2 beakers
- Cylinder
- Vials (2x 40 ml a 1x 2 ml)
- Nylon filter

Chemicals:

- Methanol

Workflow:

- Weigh the dried soil and pour it into a beaker
- Pour 15 ml of methanol into the soil sample
- Extract 3 times for 15 minutes in an ultrasonic bath
- Always take the extract into a pre-prepared beaker after each 15-minute extraction cycle
- Filter the extract into a vial
- Evaporate the volume of solution in the vial to a volume of 1 ml under a stream of nitrogen
- Quantitatively transfer the concentrated extract to a pre-prepared mini vial
- If necessary, filter the sample through a nylon filter
- Close the vial tightly and store it in the fridge until further processing

III. PUF extraction

Materials:

- Ultrasonic bath
- 2 beakers
- Cylinder
- Vials (2x 40 ml a 1x 2 ml)
- Aluminium foil

Chemicals:

- Methanol

Workflow:

- Place the exposed PUF into the beaker
- Add 100 ml of methanol and cover withaluminium foil
- Extract in an ultrasonic bath for 45 minutes
- Remove the extract into a vial
- Evaporate the volume of solution in the vial to a volume of 1 ml under a stream of nitrogen
- Quantitatively transfer the concentrated extract to a mini vial
- Close the vial tightly and store it in the refrigerator until further processing

IV. Determination of phenol by HPLC

Phenol concentrations in soil and PUF samples will be determined by HPLC.

7 Photochemical degradation

Theoretical background

Photochemical reactions have played a crucial role in the evolution of the atmosphere and life on Earth. Life on Earth is completely dependent on sunlight, not least due to photosynthesis of plants. On the one hand, photochemical reactions can mean the formation of new and often undesirable compounds of natural or atropogenic origin, on the other hand, the elimination of environmental pollutants because the photodegradation - photolysis. Photolysis is one of the most important natural degradation processes. For example, in the atmosphere, direct photolysis is an important reaction, the most efficient elimination process in the atmosphere for most substances is the reaction with photochemically generated reagents such as OH radicals, ozone or nitrate radicals.

Name photochemistry situations from everyday life.

Why should you not to go on sunlight, when some drugs are taken?

Consider how photochemistry could be used in medicine.

8 Polychlorinated biphenyls, organochlorine pesticides and lipid determination in a butter

Introduction:

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent, toxic, bioaccumulative and lipophilic compounds undergoing long-range transport ("The Stockholm Convention" 2019). Despite the use of these compounds being banned by the Stockholm convention, we can still find them in the environment.

Think about how it is possible that despite the ban, these substances are still in the environment?

In the middle of the last century, OCPs were applied on fields as a pest control. Crops raised on these fields were used as feed for stock animals, or further processed into food. If the crop was not processed immediately after harvest, it was necessary to store the crop in silos. In the 1970s, paints containing PCBs, used as an adhesive agent, were applied in silos or animal feeders. As a result, the PCBs and OCPs went up the food chain through stock animals to humans (Langenbach 2013).

Think about the possible reasons for the use of these substances, despite their known toxicity, which was confirmed in the in the 1970's Do you think something similar is happening now?

What would you choose as a suitable matrix for sampling PCBs and OCPs in relation to human exposure?

For monitoring, it is therefore appropriate to use animal products, which contain a high amount of lipid. Upon consumption of such a product, i.e. butter, the compounds are transferred into the human body and re-accumulated in adipose tissue. If the product would contain PCBs or OCPs in quantity more significant than the legal limit and be eaten daily, adverse health effects may occur (Santillo et al. 2003).

What are your eating habits? How much butter do you eat per week? And how many other animal products?

Do you see the advantage of organic or animal products from small farms?

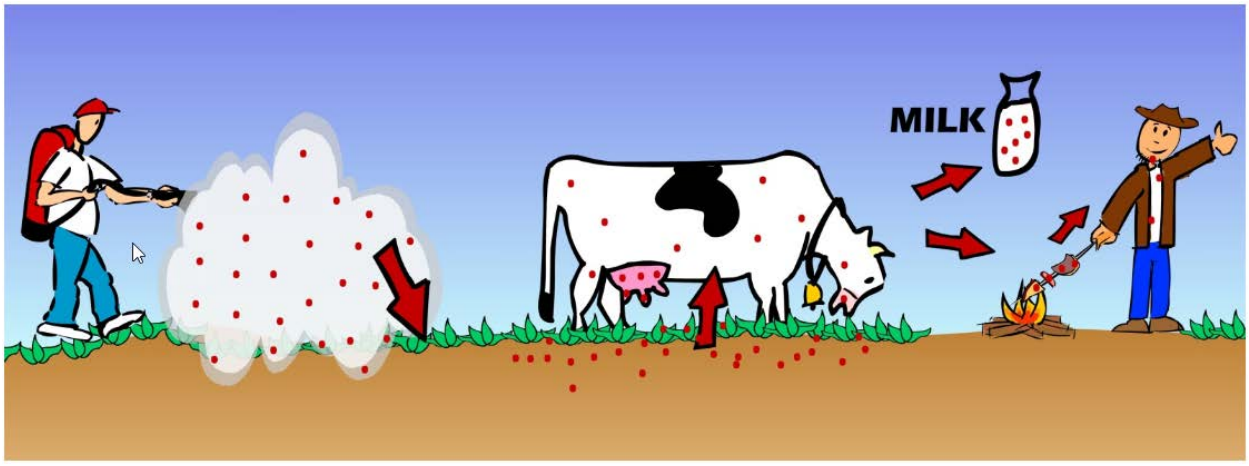

Figure 1: POPs transport by biomagnification through cattle into a dairy products (Langenbach 2013).

Principle:

The principle of this method is to transfer the butter into an organic phase, dividing the organic phase into two. One part is used for determination of lipid content. The other part is used for the analysis of PCBs and OCPs.

Equipment:

- Glassware
- Stainless steel spatula
- Analytical balances
- Aluminium cups
- Horizontal shakers
- Centrifuge
- Pasteur pipets
- Automatic pipets
- Rotary vacuum evaporator
- Heated nitrogen evaporation unit
- Oven

Chemicals:

- Dichloromethane (DCM)
- Anhydrous sodium sulphate
- Internal recovery standards (^{13}C-labelled PCBs mixture)
- Internal standard (^{13}C-PCB 95)
- Pre-cleaned silica gel (DCM, 8 h)

- Activated silica gel (oven)
- 44% H_2SO_4 modified silica gel
- Sulphuric acid, 98%
- Hexane
- Cyclohexane
- Isopropanol
- Water, MQ purity
- Nonane

Method:

1. Weigh ~2 g of butter (m_1) into a glass jar.
2. Add 50 µL of internal recovery standard directly onto the butter. Add aliquot to a conical mini vial as a reference.
3. Add 32 mL of isopropanol and 40 mL of cyclohexane (use half the volume for a blank sample), shake for 1-2 min byhand until the butter dissolves.
4. Add 44 mL of MQ water (22 mL for blank sample). Shake all samples for 10 minutes on a horizontal shaker. In the meantime, prepare cyclohexane:isopropanol mixture (87:13, V:V).
5. Centrifuge samples to obtain good separation of both layers. Then, transfer the organic phase into a 250 mL round bottom flask.
6. Add 40 mL of cyclohexane:isopropanol (20 mL to the blanks) to the aqueous phase and shake for 10 minutes on a horizontal shaker. Use a centrifuge if necessary. Remove the organic phase and add it to the first extract. Repeat this step once more.
7. Weigh the aluminium cups for the sample containing the butter (m_2) and 22 mL vials for all samples and blanks (m_3) on the analytical balance.
8. Evaporate the extracts on a rotary vacuum evaporator down to ~10 mL, add ~50 mL of hexane and evaporate down again to ~10 mL.
9. Quantitatively transfer samples in pre-weighed 22mL vials.

   Lipid determination in butter

10. Split the samples containing butter in half by weight. Transfer one half to the pre-weighed aluminium cups, keep the other half in 22mL vials.
11. Evaporate the solvent in the aluminium cups to dryness under a stream of nitrogen at 80 °C and weigh the cups afterwards (m_4).
12. Place the aluminium cups in the oven (105 °C/ 12h) to dry the samples completely and weigh again (m_5).

Clean up for analysis of PCBs and OCPs

13. Column preparation: pre-clean cotton/glass wool, 1 g activated silica gel, 8 g (44% w.) H_2SO_4 modified silica gel, 1 g non-activated silica gel and 2 g anhydrous sodium sulphate.
14. Evaporate down the aliquot in 22mL vials down to ~2 mL and quantitatively transfer to prepared columns.
15. Elute the samples with 40 mL of hexane/DCM (1:1; V:V).
16. Evaporate the samples under a stream of nitrogen at 35 °C down to less than 1 mL and quantitatively transfer to conical mini vials.
17. Add 50 µL of internal standard ^{13}C-PCB 95 and 50 µL nonane. Evaporate the samples down to final volume 100 µL.

Results:

1. Calculate the lipid percentage in the butter and compare with declared amounts at the packing.
2. Recalculate the results from ng/sample to ng/g lipid.
3. Create overview charts for PCBs and OCPs.

Questions:

What are the sources of PCBs and OCPs in soils and plants? (provide reference)

Are there any limits on PCBs content in butter? If so, compare the measured concentration with the limits. (provide reference)

Give examples of other existing methods for lipid extraction. (provide reference)

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9 Determination of persistent organic pollutants in water by passive sampling

Persistent organic pollutants (POPs) are hydrophobic, nearly insoluble in water, bioaccumulative with a long half-life ("The Stockholm Convention" 2019). Even though they are challenging to determine accurately in water samples, it is important to know their concentrations in the water environment because they are connected to various health issues. It is mainly biomagnification in the food chain, through fish up to humans, who are at the top of the food chain (Deribe et al. 2011). POPs can cause a number of problems in the human body, such as allergies, hormonal disruption, or cancer (El-Shahawi et al. 2010).

Describe how the POPs are transported through aquatic environment up to oceans.

The question is how these substances get into the aquatic environment. If POPs from the primary source enter rivers, these substances are then carried into the oceans, or may evaporate into the atmosphere. There are several processes that are associated with the transport and distribution of POPs in water. In connection with the atmosphere, POPs can be brought into the water through wet and dry deposition, or, on the other hand, they can volatilise from the water back to the atmosphere (Holoubek and Klánová 2007). Furthermore, in connection with sediments, POPs bound to particulate matter can settle to the ocean floor, or they can be transported by ocean currents. Also, the sedimentation in colder regions is supposed to be a "well" for POPs on a global scale (Dachs et al. 2002).

What are the differences and principles of active and passive sampling?

What are the main advantages and disadvantages of active and passive sampling?

So, how can we measure these compounds? Routine sampling of water using spot sampling provides information about the quality of whole water (particles, freely dissolved compounds, and colloids) only temporarily, and concentrations of POPs are often below the limit of quantification. However, passive sampling has the advantage to give a time-integrated sample (continuous diffusive sampling during exposure) due to its long exposure time (months). The passive sampling can be used for screening evaluation of presence, or absence, of freely dissolved POPs at immensely low water concentrations (fg/L) (Greenwood, Mills, and Vrana 2007). It is also possible to use only one type of passive sampler for diverse water types – seawater with different salinity, freshwater, or brackish water. Exposure is also possible in remote areas with difficult access and without a source of electricity – Arctic regions,

high elevation lakes, ocean buoys. It is, therefore, suitable for POPs monitoring in the aquatic environment (Lohmann et al. 2017).

Do you think that different conditions at sampling sites affect the sampling? Take into account salinity and water warmed by unlight.

Figure 1: Left – standard size of passive sampler sheet used for exposure in the aquatic environment. Middle – Sampling cage used for exposures of passive samplers in the ocean buoys. Right – BBC grid used for exposure of passive samplers in freshwater bodies (rivers, lakes)

Principle:

Exposed silicone rubbers are Soxhlet extracted in an organic solvent. After transfer to a suitable solvent for cleaning, the extract is cleaned-up on H_2SO_4 modified silica gel column, where most of the matrix is destroyed. Afterwards, the samples are measured on GS-MS/MS.

Equipment:

- 70mL Soxhlets
- 250mL point-flasks
- Boiling stones
- Cooling system
- Kuderna-Danish distiller
- Laboratory balance
- Laboratory spoon
- Pasteur pipets
- Pre-cleaned cotton wool (8h, Dichloromethane, Soxhlet)

- Silicone rubbers 5.5×9.5×0.05 cm (homogeneously dosed with performance reference compounds)
- Tweezers
- Water bath

Chemicals:

- Acetone
- Dichloromethane (DCM)
- Distilled water
- Hexane
- Internal standards (^{13}C-labbeled PCB 95, ^{13}C-labbeled BDE 77, 138)
- Methanol
- Nonane
- Performance reference compounds (PRCs): D_{10}-Biphenyl, PCB-1, PCB-2, PCB-3, PCB-10, PCB-14, PCB-21, PCB-50, PCB-55, PCB-78, PCB-104, PCB-145, PCB-204
- Pre-cleaned (Activated) Silica gel (Cleaning: 12h, DCM, Soxhlet; Activation: 12h, 150 °C, Furnace)
- Recovery internal standards (^{13}C-labelled mixture of PCBs and OCPs, ^{13}C-labelled PBDEs)
- Sulphuric acid, 98%

PCBs = Polychlorinated biphenyls; OCPs = Organochlorine pesticides; PBDEs = Polybrominated diphenyl ethers

Method description:

1) Take the exposed samples out of the freezer and let them heat up to room temperature.
2) Put a piece of alufoil in the fumehood with a paper towel on top. Distribute the silicone rubber on the paper towel.
3) Clean the samples with a paper towel to remove any remaining water (moisture), if the samples are covered with biofilm or rust, moisten the paper towel with distilled water.
4) Prepare 250mL point-flask with boiling stones, fill with ~150mL of Methanol.
5) Put six silicone rubber sheets, accordion folded, into 70mL Soxhlet.
6) For solvent blank use an empty 70mL Soxhlet.
7) For fabrication blanks use six sheets of non-exposed silicone rubber (dosed with PRCs).

8) Add recovery internal standards onto the sheets in Soxhlet (add aliquot to standard reference vials):

Recovery standards	Concentration (ng/mL)	Spike (µL)	Amount ng/sample
^{13}C-labelled PBDEs	20	50	1
^{13}C- labelled PCBs and OCPs	200	50	10

Figure 2: Left – Apparatus for extraction of silicone rubbers – 250mL point flask, Soxhlet with accordion folded silicone rubbers, cooler. Right – Uncleaned silicone rubbers after exposure.

9) Extract the samples for 1 hour on a water bath at 85°C.
10) After extraction, distribute the sheets by samples on a paper towel to dry out.
11) Put the Kuderna-Danish distiller on top of the point-flasks with extracts.
12) Evaporate the extract on a water bath (85°C) to minimum volume (1 mL).
13) Add 30 mL of hexane to transfer the extract to hexane and reduce the volume to ~2 mL.
14) Destructive clean up:
    a. Column ø1cm filled from bottom: a piece of cotton/glass wool, 1 g activated silica, 8 g H_2SO_4 (44% w.) modified silica gel, 1 g non-activated silica gel, 2 g of Na_2SO_4.
    b. Quantitatively (3× rinse) transfer the extract with Pasteur pipet.
    c. Elute with 40 mL of mixture DCM:hexane (1:1, V/V)
15) Reduce the volume of samples in a water bath (Kuderna-Danish distiller; 55 °C, after the DCM is evaporated, heat up to 80 °C) and transfer to the mini vial (If necessary, evaporate down under a gentle stream of nitrogen).
16) Add 50 µL of nonane as a keeper.
17) Add internal standards

Standard	Concentration (ng/mL)	Spike (µL)	Amount ng/sample
^{13}C-labelled BDE 77, 138	100	10	1
^{13}C-labelled PCB 95	200	50	10

18) Reduce the volume of the sample to 100 µL under N_2.
19) Measure the samples for PCBs, OCPs, PBDEs on GC-MS/MS.
20) Weigh the dried silicone rubber on a laboratory balance.

Protocol tasks:

PRC calculation

1) Calculate the recovery of PRCs for model calculation and normalise for PCB 104, PCB 145 and PCB 204. (always use the data from the previous step)
    a. Recalculate values ng/sample into a ng/g of silicone rubber (use the values provided in Excel, sheet *info* for every sampler where the value is stated).
    b. Calculate an *exposed sample* relative recovery on an average of all *field blanks* and *fabrication blanks* (without the solvent blank)
    c. Normalise on the PCB 104, PCB 145 and PCB 204, always for a specific sample.

PCBs, OCPs and PBDEs calculation

2) Correct the raw data for blanks.
    a. Subtract the value of a *field blank* with corresponding *exposed sample* (if the value of the *solvent blank* is higher than the value of the *field blank* subtract the *solvent blank* value). If the values are below the limit (sign <), do nothing.
3) You will receive sample-specific values (parameters FA) calculated by the model, and compound-specific distribution coefficients between polymer and water. Calculate concentrations in water using given equations. Firstly, calculate the sampling rate (R_s; eq. 1), secondly the degree of equilibrium (DEQ; eq. 2) and finally the water concentration (c_w; eq. 3).

Requirements for the protocol:

1) Introduction – one paragraph about general statements of passive sampling (2× reference)
2) Short method description – maximum four lines of text
3) Results:
    a. QA/QC
        i. Data modification (Subtraction of blanks, compounds below limit)

ii. Write down If some blanks were over limit of quantification, if so how many times higher values

b. Tables with used data (calculated R_s, DEQ and c_w)

c. Make overview graphs

d. Compare the three highest concentrations for each different compound group PCBs, OCPs and PBDEs with other locations (e.g. polar regions)

e. Compare SUM PBDE concentrations with water framework directive limits (state which congeners are included in the SUM)

4) Conclusion

Send also the Excel sheet with calculations.

Questions:

Do you think passive sampling is suitable for monitoring of POPs in waters? (reference)

Why is it necessary to use the PRCs? And what kind of compounds we can use as PRCs? (reference)

What kind of material can be used as a passive sampler (examples), can we sample polar compounds in water by passive sampling? (reference)

Find and compare the distribution coefficient between n-octanol and water (K_{ow}) with a given distribution coefficient between polymer and water (K_{pw}). (lower, higher, trends?) only for PRCs. – make a chart.

Equation 1:

$$R_s = FA \times M^{-0.47}$$

Where R_s (L/d) is the compound and sample-specific sampling rate, FA (L/d) is sample-specific value obtained from the model, M is a molar mass of a compound.

Equation 2:

$$DEQ = 1 - \exp\left(-\frac{R_s t}{m_p K_{pw}}\right)$$

Where DEQ is the degree of equilibrium, t is a time of sampling (d), m_p is the mass of the sampler used for the extraction (kg), and K_{pw} is the compound-specific distribution coefficient between polymer and water (L/kg)

Equation 3:

$$c_w = \frac{N_t}{m_p K_{pw} DEQ}$$

Where c_w is the water concentration (pg/L), N_t is the amount in the extracted sampler (pg/sample)

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10 Extraction of microcystins from water using SPE method

Introduction:

Cyanobacteria occur naturally in the aquatic environment as a part of phytoplankton. If there are enough nutrients in the water (content of nitrogen and phosphorus is important), and good conditions for the growth of biomass, cyanobacteria multiply quickly and form cyanobacteria bloom (Babica, Maršálek, and Bláha 2005). The development of cyanobacterial bloom occurs in warm and sunny climates, i.e. mainly in summer and early autumn. During the cell death and decomposition of cyanobacteria, toxins are released into the aquatic environment.

From what sources can excessive amounts of nutrients enter the aquatic environment? What do we name the phenomenon associated with the excessive supply of nutrients to the aquatic environment? Under what conditions will cyanobacteria die and decompose?

Microcystins are widely distributed cyanotoxins that are produced by several genera of cyanobacteria including *Microcystis*, *Planktothrix*, *Anabaena* and *Oscillatoria* (Babica, Maršálek, and Bláha 2005). Microcystins are cyclic hepatopeptides with seven amino acids. Microcystins are named according to various amino acids in their structures. One of the most common and most studied microcystin is microcystin-LR which contains leucine (L) and arginine (R).

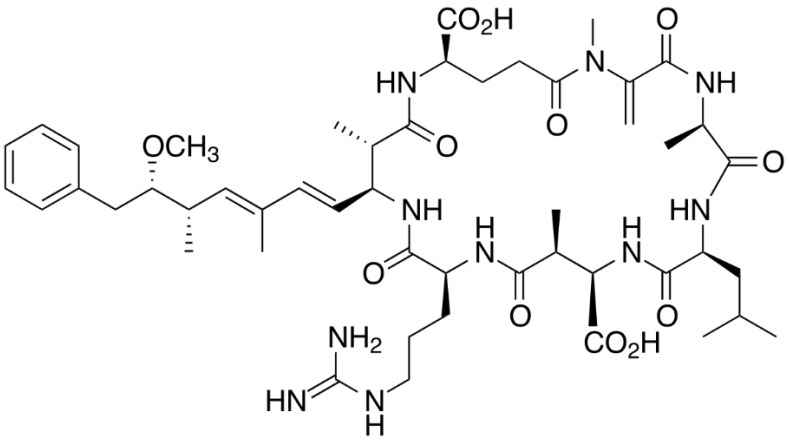

Fig. 1: Structure of microcystin-LR.

Microcystins are nonvolatile, hydrophilic compounds which are stable in sunlight, and stable over a wide temperature and pH range (USEPA, 2006a). In the aquatic environment, toxins may persist for weeks or months (USEPA, 2006b). Microcystins are, in general, hepatotoxins. While the liver is the primary target of microcystins, it is also a skin, eye and throat irritant (World Health Organization,

2003). Thus, microcystins can pose a health risk, mainly when reservoirs with excessive cyanobacteria occurrence are used for recreation or as a source of drinking water.

How can we be exposed to cyanobacterial toxins most often? What will be the main exposure routes?

Extraction of microcystins from water using SPE method

Principle:

Microcystins in a water sample are sorbed onto solid sorbent and are then eluted into a small amount of solvent. This method of extraction and concentration of analytes is known as solid-phase extraction (SPE). The extract in the solvent is then analysed using HPLC-MS (high-performance liquid chromatography combined with mass spectrometry).

Chemicals and consumables:

- methanol
- 1% hydrochloric acid
- Solution of sodium thiosulfate (c = 10 g/L)
- GF (glass fiber) filters (0,45µm)
- SPE columns with C18 sorbent
- clean 1.5mL vials, caps, septa
- bottle with distilled water

Equipment:

- filtration apparatus Nalgene DS0320
- SPE apparatus
- electric oil (membrane) pump
- nitrogen evaporation system
- pH meter

Procedure:

Preparation of sample

- 100 mL of water sample filter through GF filter (0,45µm)

Note: In the case of drinking water it is not necessary to filter the sample.

- add methanol to 10 % of total volume (v/v) of the sample
- add a few drops of HCl to achieve pH~6, check pH with a pH meter
- add 1 mL of sodium thiosulfate solution

Activation and equilibration of SPE column

- instructions for specific types of column are listed on the manufacturer's package leaflets
- in the case of Supelclean LC-18 3 mL Tubes use follow these steps:
  - activation with 5 mL of 100 methanol (without vacuum)
  - equilibration with 5 mL of water (vacuum can be used)
- in the process of activation, equilibration and application of the sample, the column must not dry out!

Application of the sample

- load the sample through the column using vacuum, flow 5 mL/min
- keep the air going through the column until dry (1 min)
- wash the dry column with 5 mL of 20% methanol

Analytes elution

- elute the analytes with 5 mL of 100 % methanol using vacuum
- if necessary store eluates in freezer at -18°C

Eluate concentration

- evaporate the eluate under nitrogen stream
- dissolve the residue in 500 µL of 50% methanol and transfer to a vial
- store the samples in vials in freezer until analysis (t = -18 °C)

Results:

Calculate the measured concentration of analytes to the original volume of the sample, and report the results in mg/L.

Find out if there is a limit for microcystins in drinking water. If yes, compare with your results.

Questions:

Why are bodies of water affected by water blooms and not flowing rivers?

How can cyanobacterial overgrowth in water reservoirs be prevented?

How can cyanotoxins be removed from water when it is treated into drinking water?

Analysis of microcystins in lyofilized cyanobacteria biomass

Principle:

Lyofilized cyanobacteria biomass is solvent extracted using ultrasonic homogeniser. Cell debris is separated by centrifugation, the supernatant is filtered and analysed for microcystins.

Chemicals and consumables:

- 50% methanol
- 1.5mL Eppendorf microtubes
- plastic syringes 2mL
- syringe nylon filters (0.45 µm)
- minivials, caps, septa

Equipment:

- analytical scales
- automatic pipette
- vortex
- ultrasonic homogeniser or bath
- centrifuge

Procedure:

- weigh 5 mg of lyofilized cyanobacteria biomass into Eppendorf tube (record the exact weight)
- add 1 mL of methanol using pipette and vortex properly
- extract the sample using ultrasonic homogeniser (2 x 20 s, cycle 0,8 x 10%, power 90%), leave the sample to get cold between the two extractions
- separate cell debris using centrifugation (maximum rotates, 10 min)
- filter the supernatant through nylon syringe filter into glass minivial
- dilute the filtered sample 10 times with 50 % methanol into another glass minivial to the final volume of 1 mL
- store the samples at -18 °C until analysis

Results:

Measured amount of microcystins in the sample recalculate to the original weight of cyanobacteria biomass.

Questions:

Why is it better to use an ultrasonic homogeniser for extraction than an ultrasonic bath?

What other cyanotoxins do cyanobacteria produce?

Reference:

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11 Extraction of pesticides from soil by QuEChERS method

Pesticides are necessary to ensure food production for mankind and have been used since ancient times. Pesticides are a wide group of compounds with different chemical structures and properties. Many were banned because of their hazardous properties, such as persistence, toxicity for non-target organisms, and long-range transport.[1,2]

Which substances were used as pesticides in the past?

Are pesticides measured in food? If yes, how can this be prevented?

Pesticides enter the environment by application or unsuitable storage. Once they are in the environment, they can negatively influence ecosystems and organisms. Even though pesticides are primarily applied on plants, they can get into the atmosphere, water and soil. Soil can act as a reservoir for these compounds and they can then be released into the air or water.[2]

How can different application influence the pesticides environmental fate?

QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe) is currently one of the most used methods for soil sample preparation. Analytes of interest are extracted from the sample through the addition of an organic solvent and a blend of salts. This type of extraction is quick and suitable for a wide spectrum of compounds. QuEChERS has less preparation steps, requires less materials and no chlorinated solvents are needed. QuEChERS has two steps – extraction and cleaning. The cleaning step can be skipped in case of soil samples without plant remains. The use of suitable sorbents reduces the effect of the matrix on the final instrumental analysis and increases the robustness of the method.[3]

Why are chlorinated solvents considered problematic?

How can the matrix influence the final instrumental analysis?

Table 1 – Types of sorbents and absorbed compounds

Sorbent	**Absorbed compounds**
Magnesium sulphate	Water
PSA (mixture of primary and secondary amines)	Fatty acids, polar pigments and carbohydrates
Silica gel (Silica-C18)	Hydrophobic compounds
Graphitized non-porous carbon (EnviCarb)	Non-target compounds

Working procedure

Equipment:

- polypropylene test tubes
- analytical scales
- ultrasonic bath
- centrifuge
- laboratory glassware
- automatic pipettes, Pasteur pipettes

Chemicals:

- magnesium sulphate
- acetonitrile
- sodium chloride
- mixture of trisodium citrates
- PSA (mixture of primary and secondary amines)
- silica gel-C18 (silica gel modified by hydrocarbon chains C18)
- mixture of pesticide standards
- 5% solution of formic acid in acetonitrile
- MilliQ water

Sample processing:

- weigh 5 g of soil and add it to 50ml polypropylene test tubes
- add mixture of pesticide standards (prepared by teacher)
- shake fortified soil in shaker for 1 hour and store for 1 week in fridge to reach equilibrium
- add 5 ml of MilliQ water and 10 ml of acetonitrile to fortified soil
- shake mixture for 1 minute and then extract for 15 minutes in ultrasonic bath (see Fig. 1 and 2)

Figure 1 and 2 – Extraction of soil sample in ultrasonic bath

- add these compounds as follows 4 g $MgSO_4$ + 1 g NaCl + mixture of citrates (1 g $Na_2Citr.2H_2O$
- + 0.5 g $Na_2HCitr.1.5H_2O$), shake for 1 minute after each addition

  Note: *After the addition of magnesium sulphate, shake vigorously to prevent formation of lumps. After the addition of citrates, cool the samples during shaking to prevent degradation of less stable pesticides.*
- centrifuge test tube for 5 minutes (3000 rpm) (see Fig. 3)

Figure 3 – Centrifugation of samples

- take 5 times 1 ml from upper layer to clean test tube
- to individual extract aliquots add following sorbents:
    1. –
    2. 150 mg $MgSO_4$
    3. 50 mg PSA + 150 mg $MgSO_4$
    4. 50 mg Silica gel-C18 + 150 mg $MgSO_4$
    5. 50 mg EnviCarb + 150 mg $MgSO_4$
- close test tubes, shake for 1 minute and centrifuge them for 5 minutes (3000 rpm)
- take 0,5 ml of extract to 2 ml vials, add 0,5 ml of MilliQ water, 10 µl of 5% formic acid in acetonitrile and internal standard
- analysis will be done by means of LC-MS/MS (see Fig. 4)

Figure 4 – Liquid chromatography coupled with tandem mass spectrometry

Questions

What other methods can be used for soil extraction?

Why is important to sieve the soil samples before extraction?

For which other matrices is QuEchERS extraction a suitable method?

List 3 examples of pesticides, sort them to categories based on the structure, and state how they are used?

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12 PAHs analysis in the air by means of GC-MS/MS

Polycyclic aromatic hydrocarbons (PAHs) are lipophilic, ubiquitous, organic compounds consisting of at least two condensate aromatic rings[1, 2]. Even though there are many individual compounds, usually only 16 EPA PAHs are measured[3]. These 16 EPA PAHs are called priority EPA PAHs, and one of them is benzo(a)pyrene which is carcinogenic to humans[3]. Individual PAHs have different toxicity, but generally they are irritants. They can also damage the kidney and liver, and are also carcinogenic, mutagenic and teratogenic[1].

Are 16 EPA PAHs really representative? What about their derivatives or PAHs with more rings which can be even more toxic?

Even though PAHs are not persistent, they can undergo long-range atmospheric transport[3]. PAHs are semi-volatile, meaning they can be present in the atmosphere in both phases, particulate and gaseous, and and are divided between them based on their properties and meteorological conditions (e.g. temperature) [3]. PAHs are products of incomplete combustion of organic matter[4]. They are rarely produced intentionally, mostly for scientific reasons[4]. They are emitted to the environment by human activities (traffic, local heating,…) but also from natural sources (volcanos, natural fires,…) [4]. They are primarily emitted into the air, and therefore they are most often measured or monitored there. However, they can freely transfer between each component of the environment[5].

What sources are significant for different localities? What are the sources of PAHs in indoor air?

Air can be sampled by two basic approaches – active and passive[6]. Active samples contain pumps and require a constant supply of electricity. They are also expensive[6]. These reasons lead to the usage of passive samplers. In the passive samplers, compounds are sorbed into the sampling media by diffusion[6]. The sampling medium can be, for example, a disc from polyuretane foam or XAD rasin[7].

Do you think that sampling media differ? If yes, how can it influence sampling?

When using passive samples we cannot determine exact air concentration of targeted compounds, different models or extrapolation is used for these purposes[8]. In this course you will be using the sampler shown in picture 1. This sample is not as effective for atmospheric particles and it is used for comparing localities or as screening of pollution at a sampling site[9].

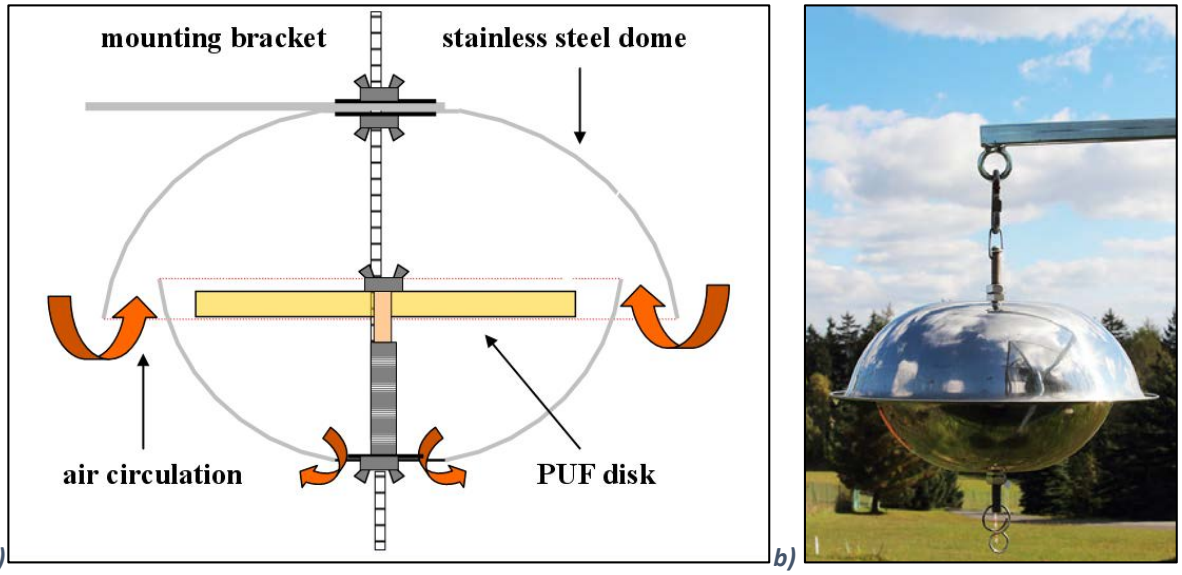
Why is it important to sample atmospheric particles? Which information we can underestimate if we are sampling only one of the phases, gaseous or particulate?

Working procedure

Demonstration of passive air sampling:

Equipment:

- passive sampler
- pre-cleaned polyurethane filter (8h acetone, 8h dichloromethane), covered in two layers of aluminium foil
- protective gloves
- aluminium foil, zip lock
- sampling protocol



a) *b)*

Picture 1 Passive air sampler a) scheme and b) photo (www.monairnet.eu)

Sampling:

- hold the middle rod by hanging hook
- add two nuts to secure upper part of sampler
- add flat washer and upper part of sampler
- add flat washer, nut and stainless-steel tube
- place pre-clean polyurethane filter with stainless-steel middle ring
- add flat washer, stainless-steel tube, nut, flat washer, lower part of sampler, flat washer and two security nuts
- add security hook
- usual sampling period is 28 days

Workflow:

Equipment:

- automatic extractor Büchi, extraction flask with boiling stones, extraction cartridge
- vials
- automatic pipettes and Pasteur pipettes
- glass tube
- glass columns
- mini-vials
- pre-cleaned cotton
- laboratory glassware

Chemicals:

- dichloromethane (DCM), *n*-hexane, nonane
- recovery standards (isotopically mass labelled PAHs)
- activated silica gel (12 h, 150 °C)
- internal standard

Sample processing:

- fill ¾ of extraction flask with DCM and add boiling stones, remove exposed polyurethane filter and place it together with glass tube into extraction cartridge
- add recovery standards and place extraction cartridge into automatic extractor
- make sure that everything is well-sealed, open water valve and turn on extractor, choose DCM extraction programme (40 minutes extraction, 20 minutes rinsing)
- after extraction, remove extraction cartridge with filter and concentrate extract to 5 ml
- quantitatively transfer extract into the vial

Picture 2 Photo of automatic extractor

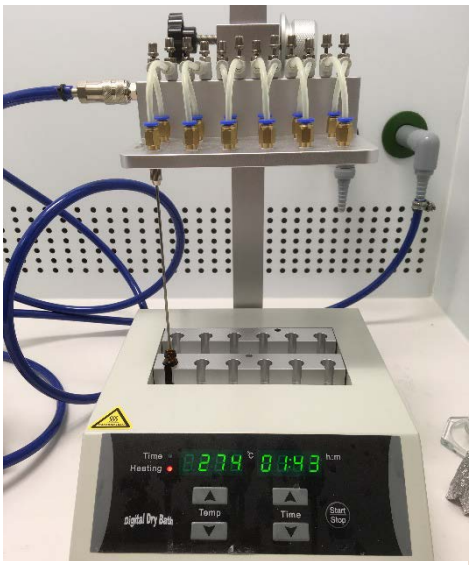
prepare cleaning column:

- add pre-cleaned cotton to the bottom
- add 5 g of activated silica gel and gently tap the column

Picture 3 Photo of cleaning columns

- add sample and elute with 10 ml of hexane and 20 ml of DCM
- collect the eluates into the vials
- concentrate the eluates under a gentle stream of nitrogen to 1 ml
- transfer the samples to mini-vial and add 50 µl of nonane
- add internal standards

- close the mini-vial and give it to teacher
- analysis will be done by means of GC-MS/MS



Picture 4 Photo of concentration system

Questions

What are the seasonal changes of PAHs air concentration?

What are the differences in PAHs air concentration between city and village?

Are there any other air sampling media to be used?

What are the units of your result concentration? Can they be converted to ng m^{-3}?

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13 Needles as passive air sampler

The concept of persistent organic pollutants (POPs), was introduced by The Stockholm Convention on Persistent Organic Pollutants, which was adopted by the Conference of Plenipotentiaries on 22 May 2001 in Stockholm, Sweden[(1)]. POPs are one of the most problematic organic compounds in the environment. These compounds are persistent, lipophilic, toxic, bio-accumulative and generally semi-volatile. They can undergo long-range transport, and therefore they are present in areas where they have never been used or produced[(2)].

Can you explain the long-range atmospheric transport?

The examples of POPs are organochlorinated pesticides (OCPs, e.g. DDT), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-dioxins and furans (PCDDs, PCDFs). Polycyclic aromatic hydrocarbons are often associated with POPs even though they are not persistent. However, PAHs share other problematic properties with POPs so they are often monitored together.

What are the differences in structure of POPs and PAHs? Which properties do these two groups share?

All these compounds are measured in all areas of the environment, and they are monitored in many monitoring networks[(3)]. POPs and PAHs can be also measured in biological matrices. For example, RECETOX measure them in blood serum and needles. In this task, you will process needles which are useful tools to evaluate contamination of the ambient air[(4)]. The big advantage of conifers is long life. They can effectively sorb POPs and PAHs during long time periods because the needles have a wax layer on their surface[(4)], which means that they can be used as passive samplers[(4)].

What is the advantageof needles compared to a traditional air sampler? Which pathways are used by pollutants to get into needles? What are the most important parameters influencing the different pathways?

Working procedure

Equipment:

- aluminium foil, zip lock
- mill
- analytical scales
- automatic extractor Büchi
- extraction cartridges
- pre-cleaned cotton
- automatic pipettes, Pasteur pipettes
- glass column
- laboratory glassware
- turbovap vial
- Turbovap

Chemicals:

- dichloromethane (DCM), *n*-hexane, nonane
- recovery standards (isotopically mass labelled PAHs, corresponding compounds for PCBs and OCPs)
- pre-cleaned silica gel
- activated silica gel (12 h, 150 °C)
- activated silica gel modified by sulphuric acid (22 ml of sulphuric acid and 50 g of activated silica gel)
- sulphuric acid
- internal standards

Sample processing:

- weigh 10 g of dried needles and, wearingprotective gloves, separate needles and fascicles

Picture 2 Photo of separated needles and fascicles

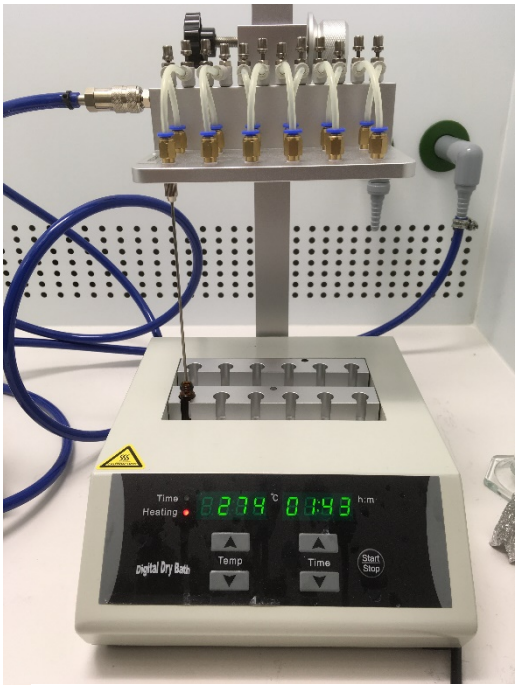
- grind needles in mill and then weigh exact mass of the needles and fascicles

Picture 3 Photo of the mill

- put needles and fascicles into extraction cartridges and cover them with a piece of cotton
- add recovery standards and place extraction cartridge into automatic extractor
- fill ¾ of extraction flask with DCM and add boiling stones
- make sure that everything is well-sealed, open water valve and turn on extractor, choose DCM extraction programme (40 minutes extraction, 20 minutes rinsing)
- after extraction, remove extraction cartridge with filter and concentrate extract to 5 ml
- quantitatively transfer extract into the vial
- concentrate the eluates under gentle stream of nitrogen to 1 ml and split sample to two aliquots
- add small amount of pre-cleaned silica gel to create homogeneous mixture
- prepare cleaning column for PAHs samples:
    - add pre-cleaned cotton to the bottom
    - add 5 g of activated silica gel and gently tap the column

Picture 4 Photo of automatic extractor

- add sample and elute with 10 ml of hexane and 20 ml of DCM
- collect the eluates to the vials
- prepare cleaning column for POPs samples:
    - add pre-cleaned cotton to the bottom
    - add 5 g of activated silica gel and 25 g of activated silica gel modified by sulphuric acid and gently tap the column
- add sample and elute with 100 ml mixture of *n*-hexane and DCM (1:1)
- collect the eluates to the turbovap vials
- use gel permeation chromatography to clean PAHs samples
- concentrate PAHs samples under gentle stream of nitrogen and POPs samples under stream of nitrogen in Turbovap
- transfer the samples to mini-vial and add 50 µl of nonane
- add internal standards
- close the mini-vial and give it to teacher
- analysis will be done by means of GC-MS/MS



Picture 5 Photo of concentration system

Questions

Compare the final concentrations in the needles and in the fascicles. What could explain their differences?

Compare the final concentrations of PAHs, PCBs and OCPs to each other. What are possible sources of these compounds in the environment?

Describe the long-range transport of these compounds.

How can the preparation of the needles influence the final results? Would there be any differences if the needles were ground, cut or whole?

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14 Determination of flame retardants in dust samples

Theoretical background

Indoor environment

Spaces, which we call indoor environments, are e.g. our houses, offices, schools, public spaces or transport vehicles. In our modern era, people spend more than 90 % of their time indoors,[1] therefore the indoor environment is crucial for human health.

How much time do you spend indoors? Do you have any behavioural differences between work and non-work days or between summer and winter?

Dust

In an indoor environmentwe can sample air, material itself, or settled dust. Dust is ubiquitous in all indoor environments and it is made of e.g. fibres, deposited atmospheric particulate matter, hair, ash, soil, crumbs, bacteria, pollen or mould.[2] In addition, dust also contains a wide range of chemicals (semi- and non- volatile) reflecting the composition of the surrounding environment and also due to that, dust is important for human exposure. Chemical exposure via dust is dictated by human behaviours contributing to dust exposure and the levels of contaminants in the dust. For some chemicals (e.g. flame retardants), dust is the major exposure matrix for human exposure.[3] Another important aspect of dust is, that it is easy and cheap to collect. Because of these properties , dust is an important matrix for indoor environment research.[4]

How much dust have you eaten during your life? Onaverage, babies up to 1 year eat 30mg/day, children from 1 to 6 years old 60 mg/day ,and peopleolder than 6 years eat 30 mg/day.

Sampling possibilities

Two main sampling techniques are used for collecting dust: (1) wipes - Fig. 1a; and (2) vacuuming – Fig. 1b.[2]

Wipes could be dry or wet (e.g. use of isopropyl alcohol). Cleaned kimwipes or special equipment, which controls pressure to surface, are used for wiping.[2] Wipe methods are used especially for smooth surfaces of furniture, electro-devices or windows.

Different approaches to vacuum sampling can be chosen depending on the type of information that we want to obtain,. There is wide variation in vacuuming methods – we may collect the whole dust bag from household vacuum cleaners, vacuum a whole house in one sampling session, vacuum separate rooms in a single house, or choose one representative location in the house. For a specific space, we can use nylon socks, which we insert to the nozzle and collect dust there. The other option

is to use a special adapter with a QUARTZ filter for dust collection. This method is regularly used for floors, carpets and upholstered furniture.

What limitations of these two methods do you see? In which units would you present your results?

Wipes could be wet or dry. Are there any differences in results due to the method used? If yes, which?

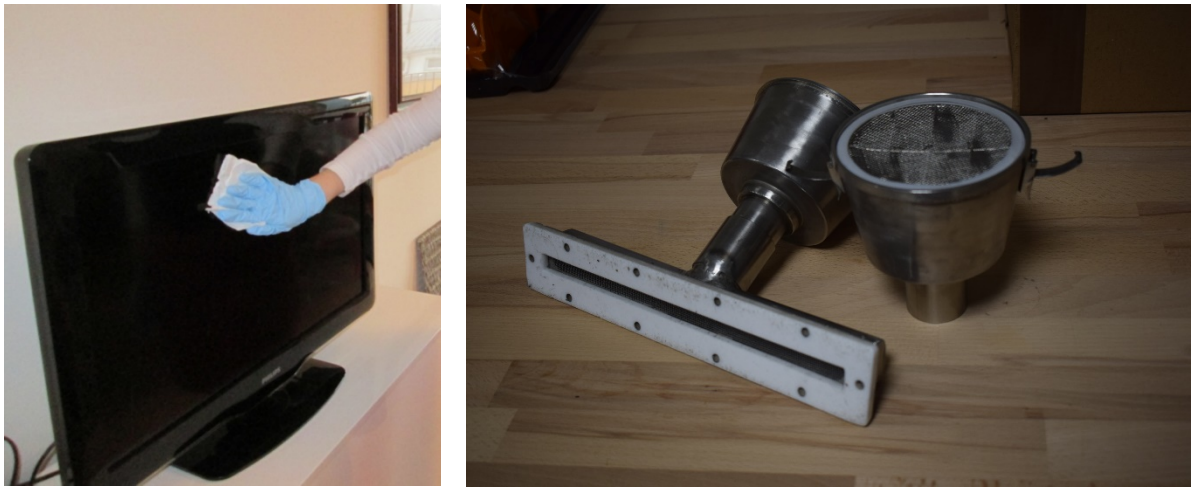

Fig. 1: a – wet wipe of smooth surface of TV, b – adapter to vacuum cleaner

Dust homogenisation

The dust is a heterogenous mixture of particulate matter. For better reproducibility and for estimation of human exposure, specific size fractions are used. However, nowadays, no specific fraction for analysis is established. You can see dust fractions for particular matter from >2 mm to <0.25 mm.

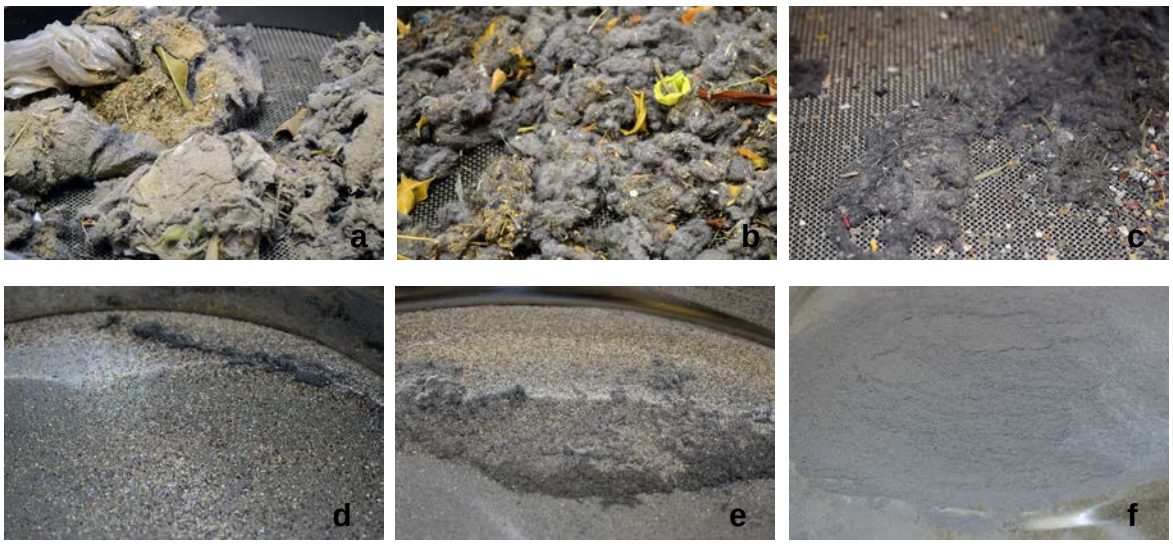

Fig. 2: Vacuum cleaner bag dust a) before sieving; and according to size fractions b) dust > 2 mm, c) fraction 2 mm > x > 1 mm, d) fraction 1 mm > x > 0.5 mm, e) 0.5 mm > x > 0.25 mm and f) 0.25 mm > x.[5]

Which fraction is the most relevant for human exposure?

Do you think, that no established fraction for dust analysis is a problem? Why? Which fraction would you choose?

Flame retardants

Flame retardants are one group of the chemicals which we can identify and measure in dust.[4] Flame retardants are used in many combustible materials (e.g. in electric-devices, plastics or textiles) to reduce their flammability and prolong the time for escape in case of fire.[6] Flame retardants consist of various molecules and could be inorganic or organic. Organic flame retardants are divided into two main groups, which are different in physical-chemical properties, and also used in different materials. The first group is halogenated flame retardants[7] (e.g. polybrominated diphenyl ethers (PBDE) or hexabromobenzene); and the second group is organophosphate esters[8], which are used also as plasticisers. The examples are in Fig. 3. Research into these chemicals is needed because flame retardants are in a lot of different environments and matrices, and because of the evidence of their possibility to be toxic,

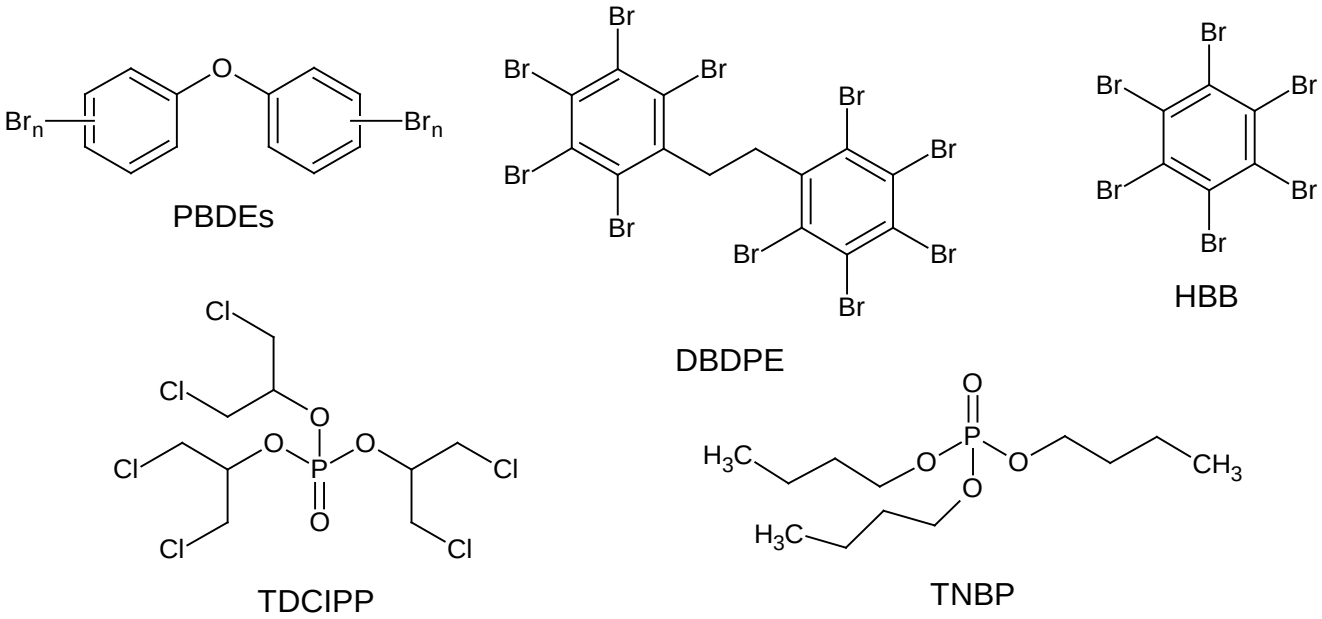

Fig. 3: Examples of flame retardants: general structure of polybrominated diphenyl ethers (PBDEs); two examples of novel flame retardants (DBDPE a HBB); and two examples of organophosphate esters with chlorine (TDCIPP), and without chlorine (TNBP).

How are flame retardants toxic?

Questions after lab practical:

Describe flame retardancy mechanism of PBDEs.

Which organic flame retardants are more polar? Would it influence the sample preparation?

We mentioned equilibrium several times during lab practical. During which processes did we use equilibrium?

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14.1 Determination of flame retardants in dust samples

I. Soxhlet extraction

Materials:

- dust sample
- extraction cartridges
- clean cotton wool
- Büchi automatic Soxhlet extractor
- extraction container
- boiling stones
- vials EPA 20 ml
- glass Pasteur pipettes
- scale

Chemicals:

- recovery standards
- dichloromethane (DCM)

Workflow:

- put the dust (approx. 0.1 g) into the extraction cartridge
- add recovery standards
- cover the sample with a piece of clean cotton wool and close it
- pour solvent (DCM) into the extraction container, approx. 150 ml, add boiling stones
- start extraction programme for DCM (40 minutes warm Soxhlet, 20 minutes solvent dripping)
- after completion of the extraction, start programme to evaporate the volume to less than 10 ml
- quantitively transfer the sample to a 20 ml vial (wash the original extraction container at least 2x with 1-2 ml DCM and add to the vial extract)
- evaporate the extract under a stream of nitrogen to a volume of 1-2 ml

II. Cleaning

Materials:

- glass column, internal diameter 1 cm
- 20 ml vial
- Pasteur pipette, cotton
- 1 ml minivial

Fig. 4: Büchi automatic Soxhlet extractor

Chemicals:

- cleaned activated silica gel (activation for 12 hours at 150 ° C)
- cleaned non-activated silica gel
- sulphuric acid modified silica gel (22 ml concentrated H2SO4 + 50 g activated silica)
- hexane, DCM, nonane
- internal standards

Workflow:

- prepare the separation column:
    - put cotton on the bottom of the column
    - add about 1 cm high layer of cleaned activated silica gel, then 5 g of activated sulphuric acid-modified silica gel
    - gently tap the column with the stick
    - load 1-2 cm layer of non-activated silica gel at the top of the column, and then tap again
- transfer the sample to the column, wash the original vial at least 2x with 1 ml dichloromethane, add to the sample in the column
- elute with 30 ml of 50% dichloromethane in hexane into a 20 ml vial
- concentrate the sample under a gentle stream of nitrogen to 500 µl

- transfer the sample to a minivial, add 40 µl of nonane and evaporate to the final volume of 40 µl
- add internal standards
- carefully close the minivial and store it in the refrigerator

Fig. 5: Column chromatography with sulphuric acid-modified silica gel

III. Determination of analytes by GC-MS

Analyte determination will be done by GC-MS.

14.2 Determination of organophosphate esters in dust

I. Methanol extraction

Materials:

- dust sample
- vial or beaker for the extraction
- vials EPA 20 ml
- glass Pasteur pipettes
- scale

Chemicals:

- recovery standards

- methanol

Workflow:

- put approximately 100 mg of dust to the vial
- add recovery standards
- extraction repeat three times
    - add 3 ml of methanol to the vial
    - do the extraction in ultrasonic bath for 20 min
    - leave the dust to settle for 10 min
    - transfer the extract to another vial
- outcome: 9 ml extract in vial

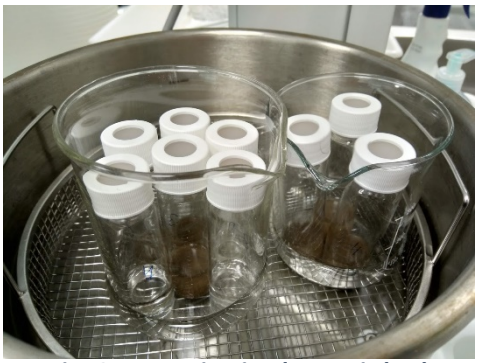

Fig. 6: Extraction in ultrasonic bath

II. Cleaning

Materials:

- syringe 2 ml
- syringe nylon filters (0,45 µm)
- minivials (2 ml)
- glass Pasteur pipettes
- scale

Chemicals:

- methanol
- Mili-Q water

Workflow:

- concentrate the extract under a stream of nitrogen to 0.5-1 ml
- filtrate the extract through syringe filter
- concentrate the filtrate under a stream of nitrogen <0.5ml
- add methanol to exactly 0.5 ml
- add 0.5 ml Mili-Q water

Fig. 7: Filtration through syringe filter

III. Determination of analytes by LC-MS

Analyte determination will be done by LC-MS.

15 Determination of endocrine disruptor metabolites in urine samples

Humans are exposed to many compounds, which are known or suggested for negative health effects. Compounds known as endocrine disruptors (ED) are responsible for hormonal disbalance and therefore can lead to other undesirable health effects. These compounds are heavily used in industry and are found in many products around us/in our environment. Therefore, information about their levels in human organisms is necessary. ED are easily metabolised in the human body, thus it is possible to monitor their concentrations in form of metabolites, for example in urine. However, despite their easy degradation, their levels in human bodies do not decrease. On the contrary, because of the continuous exposure to ED, concentrations in humans still increase. That is why these compounds are often described as pseudopersistent.

What are ED exposure pathways into the human body?

Phthalates

Phthalates are industrially used as plasticisers, solutions or stabilisers. There are various products with phthalate content, such as automotive plastics, medical devices etc. Phthalates are often part of personal care products, for example fragrances, hair sprays, nail polishes etc. They are also used while producing PVC (polyvinylchloride) or PVC products, such as plastic bags or cosmetic containers. Therefore, phthalates are used in personal care products intentionally (as solutions), or unintentionally, by migrating from plastic containers. Due to the fact that phthalates are not chemically bonded into the plastic, they can easily get out from the product, become present in the environment, and migrate between the matrices.

Alternative plasticisers

Considering the legislative restriction of some phthalates, the industrial usage of alternative plasticisers began. These alternative plasticisers have similar properties to phthalates, and are currently considered safe. However, particularly due to the similar properties in products, we can suppose similar effects also in organisms. Therefore, it is useful to monitor these compounds as well. In our case it is a compound called DINCH (1,2-cyclohexane dicarboxylic acid diisononyl ester). It is currently widely used, mainly in sensitive applications such as baby products or medical devices.

Bisphenols

Bisphenols are synthetic compounds which are widely used in consumer products. They are defined as non-persistent chemicals with short half-lives in humans, around 6 hours (Sakhi et al., 2018). Non-

conjugated bisphenols in urine samples are often used as biomarkers to determine the whole bisphenol exposure (Moos et al., 2014). The most common representative is Bisphenol A (BPA), which is used in various products, such as dental sealants, cans, thermal receipts, personal care products and food packaging materials (Geens et al., 2014). It is well known, that BPA has effects of estrogen activity and reproductive toxicity. Therefore, its use in production of feeding bottles is regulated in Europe since 2011 (EU, 2011). It is also included in the REACH because of its endocrine disrupting properties and toxicity (ECHA, 2013). BPA usage in production of polycarbonate feeding bottles for infants has even been prohibited by the European Commission (ECHA, 2016). Compounds suitable for substitution of BPA in some consumer products are bisphenol S (BPS) and bisphenol F (BPF).

Pesticides

Pesticides are widely used compounds, having many types, such as herbicides, insecticides and fungicides. These compounds are heavily used and applied in agriculture, but also in residential situations, which leads to the spreading of pesticides into the environment in general. Their widespread use makes them very difficult to avoid completely. Most of the acute effects of exposure are well known, but chronic low-dose exposure is the issue which is lacking information, and which can cause serious problems. There are many groups of pesticides, containing carbamate insecticides, organophosphorus insecticides, organochlorine pesticides and pyrethroids. In this case, the focus will mainly be on metabolites of organophosphorus insecticides and pyrethroids, because of their higher polarity and the greater expected occurrence of their metabolites in urine.

Think about your common day. How many times a day are you in contact with these chemical compounds? Can you think of possible steps to eliminate the exposure to these compounds?

The following procedure of sample preparation is suitable for all mentioned groups of compounds. The only limitation is availability of specific isotopically labelled standards.

Workflow

Equipment

- 2,5 ml Epperdorf tubes
- pipettes
- glass Pasteur pipettes
- manifold for SPE
- SPE columns Oasis HLB (60 mg, 3cc)
- minivials

Chemicals

- ammonium acetate

- β-glucuronidase
- acetic acid
- acetonitrile
- methanol
- Milli-Q water
- mixture of isotopically labelled standards of phthalate metabolites

Sample preparation

Add 0.5 ml of urine (0.5 ml of Milli-Q water to blank sample), isotopically labelled mixture (10 ng/ml or 5 ng/sample) and 127.5 µl of β-glucuronidase (6.25 ml 1mM ammonium acetate with 125 µl of β-glucuronidase) into the clean 2.5 ml Eppendorf tube. Close the tube, vortex for 10 seconds and incubate at 37 °C for 90 minutes.

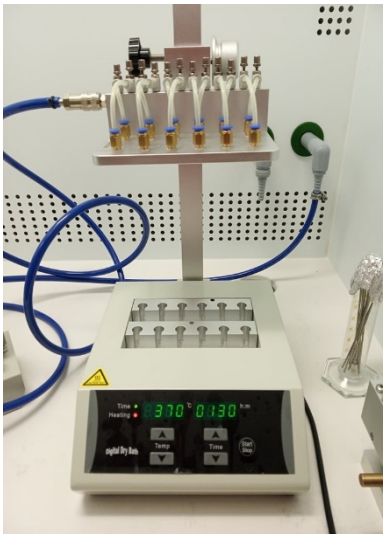

Figure 1 Equipment for enzymatic incubation (37 °C, 90 minutes)

Why do we incubate the target analytes with the enzyme (β-glucuronidase) at 37 °C?

Sample clean-up

For clean-up step and extraction of the target analytes at the same time, solid-phase extraction (SPE) will be used. Place the SPE columns with C18 sorbent (Oasis HLB, 60 mg, 3 cc) into the manifold. Activate the column with 1 ml of methanol, equilibrate with 1 ml of 0.1 % acetic acid. Then, pass the sample through the column. Wash the column with 1 ml of 0.1 % acetic acid and 1 ml of Milli-Q water. Finally, elute the sample with 1 ml of methanol. Transfer 500 µl into the minivial. These samples will then be analysed using HPLC-MS/MS.

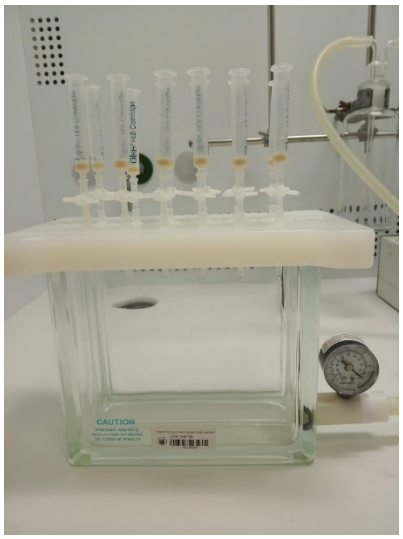

Figure 2 SPE Equipment

Questions

Describe the principle of the SPE method.

Which other compound would you expect to occur in urine samples and why?

What are the other methods of sample preparation used for urine samples?

What other undesirable compounds can be found in urine samples, what is the pre-clean step good for?

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This work was carried out with the support of MUNI/FR/1160/2019 project and RECETOX Research Infrastructure (LM2018121, MEYS CR).