ANNEXES TO THE SUMMARY REPORT: PROMOTE THE ADOPTION OF COVID-19 WASTEWATER MONITORING (ACTIVITY 1.4.2)

Funded by the European Union

EU4Environment
Water and Data in Eastern Partner Countries

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ANNEX 1 – SYNOPSIS ON SARS-COV-2 WASTEWATER BASED EPIDEMIOLOGY

Preface

This paper provides an overview about the scientific development over the last years and summarizes useful cornerstones to help planning and coordinating an effective wastewater-based epidemiology (WBE) monitoring system. Basis are guidance documents issued by the European Commission[1] and WHO[2]. This document is split into a general part (chapters 1 - 6.) and a country specific part (chapter **Fehler! Verweisquelle konnte nicht gefunden werden.**), which includes facts and figures elaborated during EU4Environment Water & Data (EU4WD) missions. The synopsis shall help explain the benefits of wastewater-based epidemiology monitoring at the higher political level in order to reach an agreement or national regulation for operating a continuous and sustainable system in support of national health authorities. The document illustrates the following benefits of wastewater-based monitoring of epidemiology:

- **Early detection**. WBE can detect the presence of the virus in wastewater even before clinical cases are identified. As a result, it can provide an early warning system for outbreaks in communities as well as an early de-warning due to a progressing breakdown.
- **Pathogens beyond SARS-CoV-2**. Among others, poliovirus, influenza virus and other emerging pathogens as well as antimicrobial resistance (AMR) can be additionally implemented as targets for WBE.
- **Surveillance of infection spread**. WBE can be used to monitor the spread of infection in a community over time and survey trends in epidemiology. By analysing the viral load in wastewater, health officials can assess the need and effectiveness of control measures.
- **Identification of asymptomatic cases**. Many individual persons infected with SARS-CoV-2 are asymptomatic, meaning they are not aware of their infection. The virus can be detected in wastewater also of this group of people, allowing for earlier identification and control of outbreaks.
- **Large-scale testing**. WBE allows surveying entire populations without the need for individual testing. This makes it an efficient and cost-effective way to monitor the spreading of the virus in a large community.
- **Non-invasive testing**. As non-invasive testing method, it does not require many individuals to provide samples, making it more acceptable and efficient.
- **Cost-effective testing**. One single wastewater sample can provide information about the entire wastewater catchment, instead of testing all individuals therein. In a continuous data chain, valuable information for decision-making can be obtained at a minimum of expenses.
- **Variant detection**: WBE allows to get information on the abundance and the development of virus variants spread in a population without sequencing a high number of human samples.

[1] European Commission Communication C(2021) 1925 final on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU (2021)

[2] World Health Organisation (2022): Environmental surveillance for SARS-CoV-2 to complement public health surveillance. 14 April 2022.

Overall, wastewater-based epidemiology can provide valuable and representative insights into the spread of SARS-CoV-2 and other pathogens in a community.

1. General introduction

According to Article 168(7) of the Treaty on the Functioning of the European Union, the 'definition of health policy', as well as the 'organisation and delivery of health services and medical care' remain at national power. Member States are therefore responsible for deciding on the strategies to track the presence of SARS-CoV-2 (and infectious diseases in general) in their populations, taking into consideration their epidemiological and social situations. The European Commission therefore established in 2021 the Health Emergency Preparedness and Response Authority (HERA) to enable cross-border and EU-wide preparedness and response to human health threats and to rapidly deploy the most advanced medical and other counter measures. The swift detection of current and future SARS-CoV-2 variants of concern by the surveillance in wastewaters has now proven to be a cost effective, rapid and reliable source of information on the spread of SARS-CoV-2 in the population. Further, it can form a valuable part of an increased genomic and epidemiological surveillance.

The European Commission's (DG NEAR) multi-year action "**EU4Environment – Water Resources and Environmental Data**" aims at further supporting Eastern Partner countries in the preservation of natural resources in line with the European Green Deal and a post-COVID-19 green recovery. This action contributes to longer-term environmental, climate, and socio-economic resilience, and to improved human health and wellbeing, as well as to the achievement of the Sustainable Development Goals (SDGs) in the Eastern Partnership region. One activity promotes the adoption of Covid-19 wastewater monitoring, as outlined in the EC communication for EU Member States.

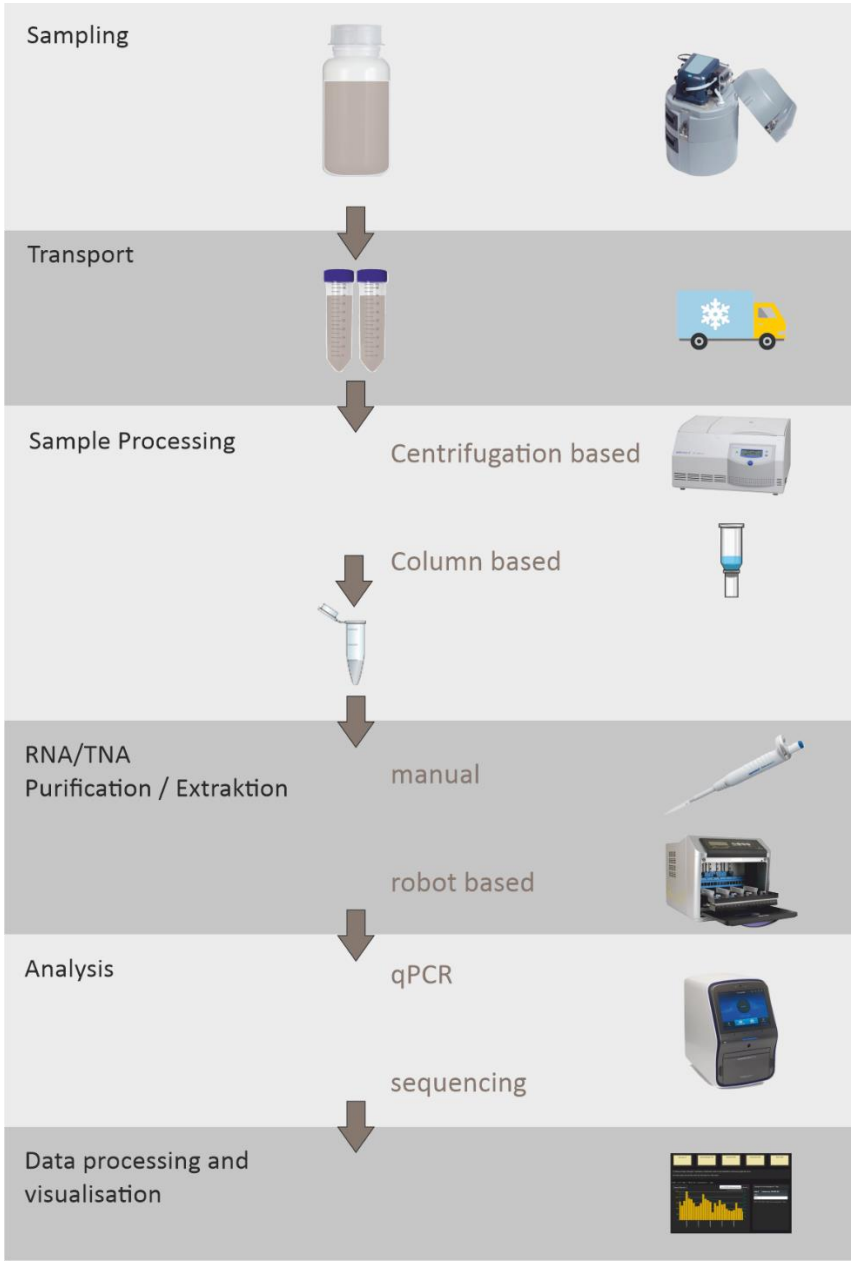
Austria has been one of the first countries, which elaborated and implemented this concept since spring 2020 on a national scale. The same scientists and practitioners, who successfully supported the establishment of such a surveillance system are engaged to transfer their experience and knowledge to EU4Environment partners. Depending on the interest and capacity of each EaP country, their available support includes:

- Enhance cooperation between relevant health and environmental authorities and raise stakeholder awareness;
- Transfer the experience for use by the public health sector as an early warning and de-warning system;
- Assess and identify needed capacities, skills, equipment, sampling logistics and analytic procedures, selection of catchment areas and installations via dedicated bilateral collaborative working groups (e.g. via webinars);
- Provide training on methods for COVID-19 wastewater monitoring (e.g. via e-learning), including genome extraction from wastewater, PCR analysis, calibration and normalisation, sequencing, statistical analysis for forecasts, data handling, data interpretation, data visualisation;
- Promote a collaborative design of a required wastewater based epidemiological surveillance system for practical implementation, including agreed responsibilities and funding;
- Jointly implement pilot surveillance activities.

2. Principle

Wastewater represents a collective sample of the society residing in the catchment area. It has been proven that wastewater surveillance for COVID-19 well reflects the development of the pandemic and can in the best case predict its development. Wastewater samples should be 24 hours flow or time proportionate composite samples, which ideally are taken twice a week as part of a routine sampling procedure for the assessment of wastewater treatment plant efficiencies, and is handed over to the laboratory doing the WBE analysis. After sample processing , the virus RNA is extracted and purified and the resulting extract can be integrated in the “normal” q-PCR routine analyses as used for human samples. After the calculation of the amount of genome copies per sample volume, the average virus loads per capita per day and other information can be calculated and the share of variants be detected. Results will be processed for trend analysis and visualised in order to follow and predict the course of the pandemic. An adapted workflow that is based on equipment typically available in molecular biological laboratories can easily be implemented without additional investments, as it has been found during assessment and training missions. This has been concluded in a Standard Operating Procedure (SOP) for the processing of samples, which can easily be linked then to the normal human PCR testing procedures.

Figure 1: Simplified workflow from taking the sample to analysis and reporting.



3. Key requirements

Commission document C(2021) 1925 final sets some key requirements for the concept of wastewater surveillance as stated below and are herewith recommended to be applied, also outside the EU. Several pieces of additional information are added where applicable (*in italic*).

WASTEWATER SURVEILLANCE

- The monitoring system should cover a significant part of the population
- It should include at least wastewaters from large cities with over 150,000 inhabitants (*e.g in Austria 48 treatment plants covering roughly 58% of the population*)

- Samples should be taken twice a week in form of 24h composite samples
    - *Recommendation: sample every Monday morning (i.e. the Sunday composite) and Wednesday morning (= Tuesday composite) to integrate samples into the weekly routine laboratory work cycle*
    - When necessary, additional sites (airports, hospitals, seasonal touristic regions etc.) can be selected to better cover specificities like movements of large groups of people
- The minimum sampling frequency and geographical coverage should be adapted according to the epidemiological situation:
    - When the competent public health authorities assess that, based on the local epidemiological situation, the pandemic is no risk to the local population, the minimum sampling frequency should be reduced to one sample per week;
    - When the disease is only present in some parts of the territory, the minimum sampling frequency should be either decreased or increased depending on local circumstances.
    - The samples should be taken at inlets of wastewater treatment plants or, where relevant, upstream in the wastewater collecting network.
- The presence of SARS-CoV-2 variants should be regularly analysed, ideally twice a month.
- When more specific information is required to better map the presence of the virus and its variants, including among vulnerable communities, additional timely sampling and analysis should be carried out in targeted locations of the wastewater collecting network that corresponds to the population centre of concern. The definition of the locations and of the sampling frequencies should be adapted to the local needs (e.g. main sewer catchments and sub-systems of interest connected for instance to parts of the cities, hospitals, schools, university campuses, airports, other transport hubs, retirement centres, prisons, etc.).
- Member States *and other participating countries* should ensure that the results of the wastewater surveillance are promptly sent by electronic means to the competent public health authorities and then to the European exchange platform once the platform is operational. For early warning surveillance purposes, the results for each sample should be recorded as soon as possible and preferably no later than 48 hours following sample collection.
- To ensure an appropriate interpretation of the results but also to adapt the surveillance system to public health needs, Member States *and other participating countries* are encouraged to put in place adequate structures involving health and wastewater competent authorities with the objective to merge and link relevant datasets and to coordinate the interpretation and communication of results.
- Member States *and other participating countries* should pay particular attention to ethical considerations: wastewater surveillance is an integral part of public health surveillance and therefore should comply with the same ethical principles, as set out in the 2017 WHO guidelines on ethical issues in public health surveillance.

SAMPLING AND ANALYSIS METHODS

- To ensure that sampling and analysis methods are comparable and reliable, Member States *and other participating countries* should ensure that:
    - the samples are taken over a period of 24 hours, using a flow or time composite sample - during dry periods where at all possible or corrected for the influence of

meteorological events - through normalisation, using the 24 hours wastewater flow during the time of sampling and the given population size of the sewer shed: this allows to calculate virus loads per capita per day;
    - analyses are carried out in laboratories operating with appropriate RT-PCR methods under standard quality management conditions;
    - variant detection is made based on duly documented gene-sequencing methods;
    - laboratories participate in appropriate proficiency testing, as organised by accredited providers and use, where available, (certified) reference materials;
    - the specific quality standards in Annex are respected.

SUPPORT TO UNION COORDINATION

- Member States and participating countries are encouraged to participate in the efforts made by the Commission, in close collaboration with the European Centre for Disease Prevention and Control (ECDC) and other Union agencies to ensure that best practices and results, enabling appropriate and timely public health responses, as well as interpretation or use of such results are shared. To this end, Member States *and other participating countries* are strongly encouraged to participate in a European exchange platform *(e.g. regular "townhall meetings")* set up by the Commission and which will focus on:
    - gathering and sharing best practices, from Member States and outside the EU;
    - collecting results from wastewater surveillance activities;
    - publishing and regularly updating sampling and analysis methods;
    - creating a voluntary list of experts involved in wastewater surveillance and disease prevention and control using wastewater surveillance;
    - organising a collaborative environment, promoting the inter-calibration of approaches and sharing best practices.
- Member States *and other participating countries* are invited to send feedback on their experience in that field to support the work of the Commission to define relevant health-related parameters to be regularly monitored in wastewaters. In this context, a broader surveillance going beyond public health should be considered. Member States *and other participating countries* are encouraged, in particular, to inform on the results of the monitoring in wastewater of emerging pollutants, emerging pathogens, drugs, pharmaceuticals, microplastics or, consumption of antimicrobials.

INTERNATIONAL DIMENSION

- Member States are strongly encouraged to:
    - share best practices at international level by promoting further harmonisation in the surveillance of SARS-CoV-2 in wastewater; *(for this reason, the European Commission set up two formats to encourage coordination and exchange: Townhall Meetings for sharing progress and experience, National Contact Point Meetings to coordinate with the national coordination teams)*
    - assist third countries having limited access to other sources of information to track virus presence in their population through wastewater monitoring;
    - foster permanent cooperation in close coordination with the WHO but also with other advanced partners having put in place their own surveillance systems.

REPORTING - SHARING BEST PRACTICES

- To coordinate the responses to this Recommendation, Member States *and other participating countries* are encouraged to designate by 1 of April 2021 not more than two contact points – representing public health and wastewater competent authorities.
- Member States are encouraged to report to the Commission by 15 May 2021 the actions taken under the present Recommendation.

ANNEX TO THE COMMISSION RECOMMENDATION

The Annex comprises several details for PCR analysis and is added as chapter 7.1 to this document.

4. Recast of the EU Urban Wastewater Directive

Article 17 of the recast of the Urban Wastewater Treatment Directive proposes to establish monitoring of WBE in general and specifically mentioning SARS-CoV-2 and its variants, poliovirus, influenza virus, emerging pathogens, contaminants of emerging concerns and "any other public health parameters that are considered relevant by the competent authorities of the Member States for monitoring". Whereas the detailed content of the new directive still is in discussion, Article 17 is already agreed on.

Based on the Commission document C(2021) 1925 (see above) Member States shall set up a national system for permanent cooperation and coordination between competent authorities responsible for public health and urban wastewater treatment. It shall identify other relevant health parameters, determine the locations and frequencies of sampling and organise timely and appropriately communication of the results to the competent authorities and Union platforms. For agglomerations of 100.000 p.e. and more, at least one sample per week shall be taken and at 70% of the population shall be covered.

Any recognised candidate country for membership of the European Union will be obliged to implement the Directive and thus also urban wastewater surveillance.

5. Specific steps towards a sustainable surveillance system

Wastewater epidemiology is a powerful tool to support public health decision making. The tool, however, requires multidisciplinary cross-sector coordination of key stakeholders such as national health authorities, environment agencies, regional and local authorities, wastewater operators and laboratories. WHO recommends the initiation, coordination and leadership by health authorities and lists detailed procedural steps towards establishment of a continuous surveillance system. Among others, it specifies

- Identification of the relevant stakeholders, their needs, expectations and willingness and ability to participate and establish sustainable structures.
- Identification of a lead entity (ideally public health agency) that will be responsible for the surveillance system and the coordination of participants / stakeholders.

- Understand the technical, organisational and financial capacity of the participants and beyond. Funding needs to be committed for setting up and maintaining WBE and need to be reviewed in response to changing circumstances.
- Agreement on sampling, requirements on the sample, its transport, analysis and interpretation. Guidance documents and operational SOPs are needed for all of the steps involved and training of staff should be carried out.
- Clarification and coordination of data sharing arrangements for end use of data.
- Set up of databases to collate and communicate relevant data and information, with specific requirements on formats and content, considerations on backups, etc.
- Development of means to communicate with stakeholders and the public, by public reporting systems, dashboards etc.
- Ensure ongoing sustainability and reliability of the programme. **Gain formal commitment from relevant actors and ensure adequacy of resourcing (human resources, technical capability and competency, required facilities and funding)**. Ensure ongoing training and maintenance of capacity, sourcing of revenue, and management of the data by the health and COVID-19 incident management and control agency. Ensure reliability of supplies and equipment (suppliers and supply chain). Ensure that results will be shared in a timely manner and will be used to inform public health action.
- *EU4WD could already contributes to this process in EaP countries with promoting sampling, elaborating a SOP, calibration and normalisation of data. Further trainings on data processing, statistical analysis, sequencing etc. are foreseen and depend on continuous data elaboration from wastewater samples in each participating country*.

6. Conclusion and recommendation

Wastewater based epidemiology (WBE) is a complementary system to support the national public health authorities and is the only means to provide epidemiological information when no or little information from human testing is available. This not only applies for SARS-CoV-2 but all health relevant microorganisms. Further to this, structures and methods promoted for SARS-CoV-2 targeted WBA easily can be adapted to other health relevant targets, such as antibiotic resistance or monitoring of illicit drugs.

- There is a correlation between the quantities of the SARS-CoV-2 virus found in wastewaters and the number of persons infected in the corresponding area. The same applies for influenza.
- Wastewater surveillance can be effectively used as:
    - preventive or early warning tool, as detection of the virus in wastewater should be taken as a signal of a possible (re)emergence of the pandemic;
    - early detection tool, which can help guide decisions about allocation of clinical and public health resources
    - analysis of trends, in order to proof the effectiveness of the measures put in place;
    - providing of data for communities, where timely clinical testing is underutilized or unavailable;
    - follow of the spread of emerging virus variants,
    - detection and prevention of other diseases and health threats, for which fingerprints exist in wastewater.
- Tracking wastewater is a cost-effective preparedness and resilience tool: securing effective surveillance of one treatment plant requires approx. 25.000 €/year (without sequencing).
- Tracking viruses' presence at regular intervals in wastewaters can also be useful to anticipate possible new pandemics next to future waves of the current one.
- It is a cost-effective tool to generate information about the entire wastewater catchment, rather than individual testing, at a minimum of costs.

It is therefore highly recommended to EaP countries to institutionalise WBE according to the steps described above. All cited guidance documents can be useful to create a system tailored to the needs and opportunities in the countries. In particular, the following (political) decisions and arrangements should be done:

- Establishment of a **national steering group** with representatives from the health and environment sectors with the aim to coordinate their collaboration, e.g.
    - Health sector – one person each from pandemic management, political decision-making level
    - Environment sector – one person each from wastewater management, environmental monitoring
    - One representative of the reference laboratory.
- Nomination of one health and one environment representative in the **EU National Contact Point group (NCP)**
- Installation of a **head of operations** (by the steering group), with the tasks of operatively supporting the steering group (coordination, organisation, administration), operational contact to the EU, coordination of logistics, contact point for donations and equipment, assessment of needs (equipment, training, personnel,…), reporting, maintenance of dashboards and databases etc.

- Drafting of a **national regulatory document** to define responsibilities of the key actors and allocate necessary budget funding. The regulatory document should encompass the collaboration of
    - wastewater treatment plants by providing the samples according to the protocols and providing meta-data (discharge, COD, NH4-N, etc.) (see also chapter 4. , Urban Wastewater Treatment Directive and its obligations);
    - defining the sample logistics from the wastewater collection sites to the laboratory;
    - extension of the scope of the reference laboratory with WBE and nomination of a contact person;
    - definition of responsible entities for data maintenance, evaluation and reporting and the planned pathways for data and information transfer to different end-users and stakeholders;
    - **allocation of necessary funding**, depending on the costs in each country. Roughly, effective surveillance of one treatment plant requires approx. 25.000 €/year (without sequencing) and approx. 10.000 EUR for every additional parameter per surveillance site. It is highly recommended to foresee also one full person equivalent for the operational coordination and a buffer budget for unforeseeable circumstances.

7. Appendix to the Synopsis Paper

7.1. Appendix to the Commission recommendation

Specific Quality Standards, as annexed to the Commission recommendation on a systematic SARS-CoV-2 surveillance in wastewaters

Standards for PCR/Digital-PCR (polymerase chain reaction)

(a) Threshold cycle value of real-time polymerase chain reaction (RT-qPCR) should be below 40 to report a sample as positive either for qPCR (quantitative polymerase chain reaction) analysis or to use for sequencing.

(b) Alternative quantification approaches to RT-qPCR (as digital polymerase chain reaction - dPCR) could be used provided that they achieve results equivalent to RT-qPCR and apply quality requirements equivalent to RT-qPCR.

(c) All samples should be run at least in duplicates to avoid false positive or false negative results.

(d) The analytical procedure of real-time polymerase chain reaction used should include adequate controls to assess at least the efficiency of the concentration/extraction steps and the absence of significant reaction inhibition.

(e) Each run should include appropriate standards (at least 3 point serial dilutions in triplicate employing synthetic SARS-CoV-2 RNA) and positive and negative controls to determine if the PCR/qPCR run produced reliable results.

(f) A quantification cycle (Cq) cut-off value for positive samples should be set [at] 5 cycles before the termination of the amplification protocol to avoid misattribution of late fluorescence signals.

(g) A negative extraction control should be used to account for any contamination during the RNA extraction.

Standards for Next Generation Sequencing

(a) At least 1 million reads-per-sample should be generated and the read length should be above 100 base pair.1

(b) At least 3 genetic markers per variant should be reported for better characterization of mutations for High Throughput Sequencing analysis of wastewater.

Standards for normalisation

(a) Viral number of gene copies should be normalised by the population number served by the sewer system and using the wastewater flow for better comparability of measurements between different locations.

(b) Additional normalisation controls using cross-assembly phage (c) or pepper mild mottle virus are recommended for this purpose*. (not of core importance)*

(c) If data for none of viruses referred to in point (b) can be obtained, alternative parameters could be used, provided they deliver equivalent corrections for meteorological or other influences causing fluctuations of the viral load, which are not related to the pandemic such as precipitation or other meteorological effects. *In that regard it has proven suitable to use conventional chemical wastewater parameters, such as COD (chemical oxygen demand), TN (total Nitrogen) and NH_4-N (Ammonium) that should be readily available from routine wastewater analysis.*

ANNEX 2 - CONCLUSION OF HIGH LEVEL WORKSHOP ON 3 JULY, 2023

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EU4Environment
Water and Data in Eastern Partner Countries

High-level Regional Workshop on Wastewater-based Epidemiology (WBE) – follow up notice

Monday 3 July, 2023 at 11:00 – 13:30 CEST

Dear Participant!

The team of EU4Environment – Water Resources and Environmental Data (EU4WD) would like to thank all participants for attending the high-level regional workshop on wastewater based epidemiology! In particular, we thank all presenters for their contributions and all EaP country representatives for their valuable interventions!

The main conclusions of the workshop can be summarized as follows:

- WBE has global dimension, the interest and activity is steadily growing. The European Commission runs several support actions in coalition with international partners. The international conference "Towards a Global Wastewater Surveillance System for Public Health" is organised by the European Commission and will take place on **November 15-17, 2023**.
- The recast of the European Urban Wastewater Treatment Directive will **oblige EU Member States** to implement WBE.
- Benefits and advantages of WBE were summarized in various documents, one of which addresses the institutionalization of WBE specifically at national level of the countries of the Eastern Partnership. Coordination and sustainable budgeting should be provided as soon as possible to capitalize on all the benefits of WBE. Progress has been reported.
- Further trainings on data management and statistics as well as sequencing under EU4WD will commence in September; preparations are in progress.
- The final declaration of **WHO 7th Ministerial Conference on Environment and Health** refers to WBE in its commitment 24: https://www.who.int/europe/publications/i/item/EURO-Budapest2023-6

Next steps

As promised during the workshop, we are happy to provide the presented material. Please use the following link to download the presentations and additional materials:

https://docs.umweltbundesamt.at/s/AnHLqjjJCMHi5e2

In case of questions or requests related to the workshop on 3 July please do not hesitate to contact philipp.hohenblum@umweltbundesamt.at.

This message is sent to all invitees and participants of the event as well as to all persons who were additionally nominated in response to the invitation. Please feel free to forward this email to other persons interested in the topic.

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