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Monitoring plan for assessing Ecosystem, Plant, Animal and Human health at Case Study Sites

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1 Introduction

What/Why: Task 2.1 involves the development of a harmonized monitoring plan to assess PPP distribution and related ecosystem, plant, animal, and human (EPAH) health in 11 CSS (*Table 1*). **CSS work will involve collection of data and samples, in situ determinations and preparation, storage, and shipment of samples to different labs**. This monitoring plan will ensure that sampling will be done on a harmonized way, and that samples are stored and sent to the labs in appropriate conditions. The methodology relies on appropriate and standardized methods, allowing comparisons across CSS. This document covers also CSS questionnaires related to farm management, PPP applications, and ecosystem, livestock and human health.

The general site and monitoring information will be used for analytical interpretation and as input parameters in WP3 exposure models. A file will be soon provided by WP3, on the additional samples to collect in the reference CSS (NL and PT) in order to improve and verify/validate models. The health data and PPP distribution data from CSS will be used as input data for (eco)toxicological tests in WP4. The questionnaires data will also serve as basis for some WP6, WP7 and WP8 activities.

Table 1. Overview of the parameters assessed in the environment, livestock and human matrices.

	Matrix	PPP distribution	Health state
Environment	Soil	In soil	Soil microbiome, SOM, Cmin, Nmin, PLFA analyses, enzyme activities, functional gene analyses
	water	In water	Water quality
		In sediment	SOM, Benthic macro-invertebrates diversity
	air	In air and dust outdoor	-
		In dust indoor	
	Plant	In crop	Yield data, pest incidence, weed infestation (questionnaire), Diseases
	Earthworm	In earthworm	Earthworm diversity, Earthworm gut microbiome
	Fish	In fillet and in liver	Fish diversity, gut microbiome
	Bats	In bat faeces	Bat diversity, gut microbiome
	Insects	-	Biodiversity of ground-dwelling insects and of flying insects
Livestock	urine	In urine	Biomarkers* in urine
	faeces	In faeces	Microbiome in faces
	blood	In blood	Biomarkers* in blood
	wristbands	In wristbands	-
	milk (if applicable)	In milk (if applicable)	-
	feed	In feed	-
	-	-	Reproductivity and diseases (questionnaire)
Human	urine	In urine	Biomarkers* in urine
	faeces	In faeces	Microbiome in faces
	blood	In blood	Biomarkers* in blood
	Wristbands	In wristbands	-
	Nasal swabs	-	Microbiome
	-	-	Reproductivity and diseases (questionnaire)

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*Biomarkers for which associations with pesticide exposures have been reported in scientific literature can potentially be used in the context of pesticide studies. This type of studies can be done in study populations of limited size and may result in inferences regarding a predicted outcome related to certain exposure. The evidence to support an associative relationship between exposure and magnitude of the effect will be further explored in scoping and/or systematic reviews performed in WP2 and WP3 of the SPRINT project. Some biomarkers have been reported to specific adverse outcome pathways related to adverse outcomes related to pesticide exposure. Most of these biomarkers have a link to exposure such as the pesticide residues and their metabolites. These biomarkers have been categorized as 'primary' study outcomes.

For most other biomarkers the link with exposure is not established well (or not at all). Even if biomarkers have shown to be linked to exposures such as inhibition of acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) it depends on the working mechanism of the pesticide if an effect on the level of the biomarker can be expected. Most active ingredients that are known to change AChE and BChE enzyme activity are organophosphate or carbamate pesticides and in the EU most of these have been banned in the past decade. Even when we will detect historic residues levels are likely to be too low to trigger any changes beyond background variability. This means that aforementioned biomarkers will not be tested in samples of most CSS, except for Argentina and other countries in this region where they are much used and in such large quantities that intoxications and chronic health effects can be observed in farmers or even the general population (*Ramírez-Santana et al., 2014 and 2020*[1]).

Especially if exposure levels turn out to be low it is questionable if the biomarkers levels changes can be attributed specifically to exposures also regarding the wide intraindividual and interindividual variability. An example is the hormone levels that could certainly be influenced by pesticide levels but are also in part attributable to intra-individual variability observe within a range that is considered normal background, especially in female participants due to hormonal cycle.

[1] Ramírez-Santana M, Iglesias-Guerrero J, Castillo-Riquelme M, Scheepers PTJ. Assessment of Health Care and Economic Costs Due to Episodes of Acute Pesticide Intoxication in Workers of Rural Areas of the Coquimbo Region, Chile. Value Health Reg Issues. 2014 Dec;5:35-39. doi: 10.1016/j.vhri.2014.07.006. Epub 2014 Aug 28. PMID: 29702785.
Ramírez-Santana M, Zúñiga-Venegas L, Corral S, Roeleveld N, Groenewoud H, Van der Velden K, Scheepers PTJ, Pancetti F. Reduced neurobehavioral functioning in agricultural workers and rural inhabitants exposed to pesticides in northern Chile and its association with blood biomarkers inhibition. Environ Health. 2020 Jul 22;19(1):84. doi: 10.1186/s12940-020-00634-6. PMID: 32698901; PMCID: PMC7374955.

Some biomarkers reflect a health condition like an airway infection and have impact on blood cell counts and blood biomarkers such as interleukins. Other blood biomarkers reflect liver function and some urine biomarkers reflect kidney function. Deviations from values that are often considered to be 'normal' regarding gender and age of a subject may deviation from normal organ failure and only change outside the normal range as a result of chronic disease or chronic exposure to xenobiotics. When observed it is most likely that other causes than pesticide exposures will explain these lab results such as use of alcoholic beverages in the case of liver function and age-related loss of kidney function. This does not mean that such effects have not also been observed related to pesticide exposures.

Due to the current limitations the analysis of biomarkers will be restricted to those cases where we find good reason to include the analysis of clinical biomarkers. Such motives could come from published hypothesis that need testing in field studies. Other reasons may be the finding of considerable high levels of specific substances or mixtures of PPP components in the environment of humans and/or animals supported by reports of clearly elevated pesticide and/or metabolite levels in wristbands, blood, urine and/or stool samples. This selective use of biomarkers will result in the most effective use of allocated laboratory capacity and resources. In line with this the following biomarkers will considered for analysis:

Blood:

- PPP components and metabolites
- Blood cell counts
- General inflammatory markers and markers reflecting immunologic status: hsCRP, IL-1β, IL-2, IL-4, IL-5,IL-6, IL-8, IL-10, IL-13 and TNF-α
- ROS-biomarkers: GSH/GSSG
- Inflammatory markers: SAA, VCAM-1 and ICAM-1
- Neurotoxicity biomarkers: AChE, BchE and GFAP
- Kidney: creatinine
- Thyroid: fT4, T3, T4 and TSH
- Liver: ALT, AST, GGT and protein electrophoresis
- Reproductive system: DHEAS, FSH, GGT, LH, s-DHEA, SHBG, testosterone, estradiol, progesterone and cortisol
- Untargeted exposome analysis (exogenous)
- Untargeted metabolome analysis (endogenous)

Urine:

a. PPP components and metabolites
b. urinary electrolytes potassium and sodium
c. creatinine
d. urinary proteins (OBS)
e. KIM-1,
f. NAG

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When: **middle of growing season (i.e. >60–80% of the PPPs annually used were already applied) and climatic conditions adequate for ecosystem health assessment**; for most of CSS it corresponds to end May-Begin of June 2021. The remaining CSS will have sampling campaigns in autumn due to a later growing season (*Table 2*). Data, environmental and biological samples should be collected with the minimum interval possible.

Table 2. Cropping system, livestock types, and sampling time of SPRINT Case Study Sites, CSS.

CSS	cropping system	livestock	Sampling time
• Cartagena (ES)	Vegetables (broccoli)	Sheep, goat	October 2021
• Bairrada (PT)	Vineyards	No	end May/ begin June 2021
• Bordeaux (FR)	vineyards	Pig, chicken	June 2021
• Central zone Switzerland (CH)	orchards	Diary	end May/ begin June 2021
• Po region (IT)	Vegetables	No	June/July 2021
• Istria (HR)	Olives	Sheep	End May OR September 2021
• Central zone Slovenia (SI)	maize	Dairy and cattle for meat	Middle June 2021
• Central zone Czech Republic (CR)	Oil plants	Diary , chicken	oilseed rape may/June 2021; sunflower/poppy=> July/august 2021
• Groningen region (NL)	Potatoes	Dairy	middle-end May 2021
10-Central zone Denmark (DK)	Cereals	Dairy	May 2021
• Buenos Aires region (AR)	cereals	Dairy, chicken	September 2021

How: The monitoring plan includes detailed instructions for CSS leaders to perform the different tasks, and training will be provided in combination with the stakeholder training conducted in WP1 (march 11-march 25; online). In case of problems in applying the recommended methods, the CSS leaders should contact the respective sampling or analytical experts and WP2 leaders. The experts and their contacts are included in the sampling, storage, and shipment overview tables in sections 3-7. Specific sampling equipment's and consumables will be provided to the CSS teams by WU or SKU (these materials are identified in the overview tables in sections 3-7; CSS addresses in Annex 1). Generic sampling material is provided by the CSS leader.

CSS leaders send their CSS samples to the analytical partner labs directly – the labs analysing the samples are indicated in samples and parameters overview figures below, and in the matrices overview tables in sections 3-7 – all lab shipping addresses were included in Annex 2. CSS teams should discuss the preferred shipping day with respective analytical partner, and inform them once the samples are shipped (including tracking

codes). Combined shipment, i.e. combine different types of samples with the same lab destination, is possible as long as storage conditions are compatible and the shipping conditions are the same (some combinations are suggested in the overview tables in sections 3-7). Packaging and shipping of the samples must conform to all applicable regulations and standards regarding packing, marking and labelling. If possible/applicable, part of the original composite samples should be kept in the freezer at CSS labs, at least until confirmation of safe receipt at the analytical laboratories. A separate SOP on shipment (including sample exchange within the European Union and from non-EU countries to EU countries), and on long-term storage and samples destruction (or other solution) can be prepared once all parameters and analytical requirements are confirmed.

Requirements:

- **human ethics approval before recruitment and sampling**. **All CSS should submit the ethics application before 12 March 2021.** The SPRINT ethics committee can help with your questions, and should be informed in any status change with regards to the application.
- if not already covered by the CSS team, CSS leader should contact a **nurse, veterinarian, a fish and a bat scientist** to collect human, livestock and cat blood, fishes and bat faeces, respectively and to determine biodiversity of fish and bats on CSS level.
- Each CSS should include **20 fields** (i.e. 10 conventional/integrated (C/I) and 10 organic (O) fields). A maximum of two fields can be selected per farm, but at least 6 organic and 6 conventional farms should be selected per CSS. Definitions and guidelines for fields, farm and CSS selection are already addressed in a previous document (Guidelines for Case Study Sites CSS selection[2]). The leaflet[3] and a brochure[4] produced by WP8 can be used to inform CSS population on the study and activities. Note that **human and livestock samples will be collected from/in 12 farmer households/farms/CSS** (6 C and 6 O). We cannot analyse more households; consider this when selecting the farms, and either you restrict to i) 4 C and 4 O farms with two fields + 2 C and 2 O farms with one field and all these are sampled, or ii) you consider more than 6 C and/or 6 O farms as long as some of your farmers are okay with having only environmental sample results.

[2] https://sprint-h2020.eu/index.php/project-documents/registered-users/monitoring-plan-documents

[3] https://sprint-h2020.eu/index.php/project-documents/leaflets-and-brochures/leaflets

[4] https://sprint-h2020.eu/index.php/project-documents/leaflets-and-brochures/brochures

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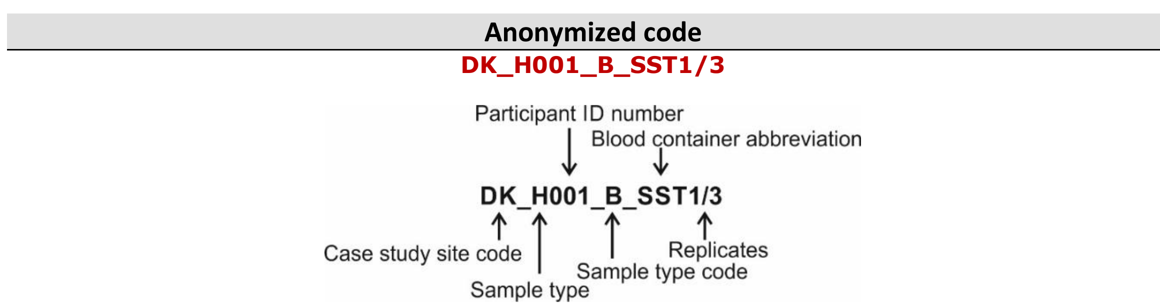
-please guarantee your organic farmers do not operate in conventional farms too. If you don't have enough farmers to choose from, then consider him/her conventional farmer.

-In each farmer households 1 adult female and 1 adult male, i.e. the farmer and his spouse/her husband, or his sister/her brother, or his adult daughter/her adult son "will be sampled" (total 24 "farmers"/CSS).

- A participant ID will be attributed to each study participant. This participant ID code will be used in the interview questionnaires and in the labels of the samples (*Fig. 1*). Only the respective CSS team and the SPRINT data managers will have access to the key code and participant personal data. Any information that would individually or collectively allow to identify someone directly or indirectly will be encrypted. Furthermore, for blind analyses purposes, the labels of the samples to send to the labs should not mention the farming system the samples originate form. These labels should only cover the general information such as the **country** (e.g. DK), the **sample type** (e.g. Human), **participant ID number** (e.g. 001), **sample type code** (Blood/Nasal/Stool/Urine/Wristband), **container abbreviation** (e.g. SST for blood), and **number of replicates** (e.g. 1/3) (Fig. 1, example of Denmark, human sampling). A code system is provided for CSS leaders, in the SPRINT website[5]. Further details on the used pseudonymization and anonymization techniques can be found in the project Data Management Plan.



Legend:

Sample type:	**Sample type code:**	**Blood container abbreviation:**	
E=Ecosystem	B=Blood	SST :	Serum separation test tube
P=Plant	N=Nasal		
A=Animal	S=Stool	PST :	Plasma separation tube
H=Human	U=Urine	K2-EDTA :	Potassium-Ethylenediaminetetraacetic acid blood tube
	W=Wristband		

Figure 1. Participant code used for human sampling; additional detailed personal information corresponding to the ID should be kept by the CSS leaders.

[5] https://sprint-h2020.eu/index.php/project-documents/registered-users/monitoring-plan-documents/codesystem

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- livestock: optimal in the same field where crop, soil, earthworms etc are sampled; if you don't have livestock in that field, then livestock may be sampled from an adjacent field. This if the adjacent field belongs to your farmer or if the owner of the neighbour field agrees to the livestock study; ideally, this adjacent field have a related or similar pesticide use than the initial crop field.

- **human samples will be also collected from 12 neighbours households and from 12 consumers households/CSS.** In each of these households 1 adult female and 1 adult male, i.e. the neighbour/consumer and his spouse/her husband, or his sister/her brother, or his adult daughter/her adult son "will be sampled" (total 24 individuals/population/CSS). Note that neighbours must not be farmers (even if you have to look > 250 meters from the farm), and that consumers are not necessarily consuming the products of the farms, but rather have a preference for conventional or organic food.
- **meteo stations**, ideally < 5 Km from each field. These should cover wind speed and direction at two (or more) heights, rainfall, and air temperature.

Remarks:

- CSS should take the **coordinates** and pictures of all sampling locations (fields, water bodies, bat roosts, insects transects, TIEM devices installation points)

- **Argentinian CSS**: most of sampling consumables and equipment will be acquired locally and most of analyses performed *in situ*. Adaptions to the normal EU monitoring plan must be defined with the project coordinator Violette Geissen.

- minor changes to the monitoring plan can be expected after testing the proposed procedures in the field, or to cope with the coronavirus pandemic guidelines at the countries of the CSS, at the sampling campaign time. CSS leaders must guarantee that the field work follow all the safety rules in force for Covid-19.

1.1 SPRINT questionnaires overview (Farming system, Environment, Livestock, Human)

CSS SPRINT work covers four questionnaires. A short description of these questionnaires is provided below and a detailed overview is provided in the *Table 3*. The questionnaires are provided as monitoring plan appendices, and can be found in the SPRINT website[6]. We recommend to conduct the questionnaires face-to-face, but if conducted via phone, please email or send per post the questionnaire to the person in advance. This will make it easier to respond the questions.

Humans:

1. Questionnaire about exposure & health

   Appendix 1 - Performed once at sampling time to farmers, neighbours and consumers [Appendix_1_SPRINT_Personal interview questionnaire.docx]

2. Questionnaire about behaviour and information with regards to pesticides

   Appendix 2 - Performed once at sampling time to farmers, neighbours and consumers [Appendix_2_SPRINT_WP7_WP8_questions.docx]

Crops:

3. Questionnaire Farm and crop characteristics, agronomic management practices, pesticide and fertilizer records, cost and benefits of the crop.

   Appendix 3 - Performed twice (the first time is the sampling campaign, the second 6 months after that to confirm land and application records), to farmers. [Apendix_3_SPRINT_Monitoring_General_Info_WP2-WP6_2021.xlsx].

Livestock:

4. Questionnaire on livestock characteristics.

   Appendix 4 - Performed once (at sampling time), to farmers and veterinarian [Appendix_4_Livestock_WP2.xlsx]

[6] https://sprint-h2020.eu/index.php/project-documents/registered-users/monitoring-plan-documents/questionnaires

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Table 1. Overview of the CSS SPRINT questionnaires including links with the different project tasks.

WP	Responsible	Time period	Description	Task	Will feed into
Questionnaire i					
2	Abdallah, Vivi	At same time of sampling campaign	Collection of data to support an integrated risk management tool	Task 2.2 / D2.2(M12)	WP3, WP4, WP6
	Abdallah	At same time of sampling campaign	monitoring pest and disease pressure on plants (crops) through interviews with farmers	Task 2.1	WP3, WP4
	Vivi	At same time of sampling campaign	information about overall health status from semiquantitative interviews	Task 2.1	WP3, WP4
Questionnaire ii					
7	Ana	At same time of sampling campaign	Attitude towards PPP	Task 7.1-7.2	WP7
8	Jane		Communication preferences	Task 8.2 – 8.3	WP8
Questionnaire iii					
6	Abdallah	At same time of sampling campaign	Natural and human environment	Task 6.1 – 6.3	WP2
	Lucius, Jenifer & Team		Farm characterization Field characterization Log book of the crop Pesticide record Fertilizer record Cost and benefits		
		6 months after sampling campaign	Log book of the crop Pesticide record Fertilizer record Cost and benefits		
Questionnaire IV					
	Abdallah, Vivi	At same time of sampling campaign	Farmers/veterinaries will be interviewed regarding their perception of animal health and diseases occurring in the CSS	Task 2.1	WP3, WP4

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1.2 Samples overview (Environment, Livestock and Human)

The tables 4-6 compile the type and number of samples to collect per farming system and CSS. These samples will be used to assess both PPP distribution and health in Environment, Livestock and Human. As water, sediment, fishes, and bats are hardly connected to fields, their figures are presented at CSS level only. Please note that some samples have to be divided into aliquots for determination of different parameters, see parameters overview figures below and matrix overview tables in sections 3-7 for details.

Table 2. type and number of to Environment related samples to collect per CSS.

CSS																
#	country	location	system	soil	water	sediment	air-dust outside	dust house	Plant	earthworm	benthic macro-invertebrate	fish	bat (feces)	insects- traps	insects-flying	max No samples per CSS
1	ES	Cartagena	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
2	PT	Bairrada	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
3	FR	Bordeaux	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
4	CH	Central zone	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
5	IT	Po region	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
6	HR	Istria	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
7	SI	Central zone	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
8	CZ	All country	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
9	NL	Groningen	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
10	DK	Central zone	C/I O	10 10	6	6	1 1	10 10	10 10	10 10	3	6	5	15 15	15 15	168
11	AR	Buenos aires region	C I	10 10	6	6	NA NA	10 10	10 10	10 10	3	6	5	15 15	15 15	166
			max No of samples all CSS	220	66	66	20	220	220	220	33	66	55	330	330	1846
			PPP analyses	MU	CIEMAT	WR	HZG	CIEMAT	MU	MU		WR	WR			
			health - microbiome	WU						WU		WU	WU			
			helath - other	MU+HU+CSS lab						WU	UAVR			WU	WU	

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Table 5 - Type and number of livestock related samples to collect per CSS.

#	country	location	system	sheep	goat	dairy	cattle meat	pig	chicken	urine	faeces	blood	wristbands	milk	feed	faeces	blood	wristbands	max No samples per CSS
1	ES	Cartagena	C/I	x	x					18	18	18	6	6	6	2	2	2	156
			O	x	x					18	18	18	6	6	6	2	2	2	
2	PT	Bairrada	C/I					x	x	18	18	18	6	na	6	2	2	2	144
			O					x	x	18	18	18	6	na	6	2	2	2	
3	FR	Bordeaux	C/I													2	2	2	12
			O													2	2	2	
4	CH	Central zone	C/I			x				18	18	18	6	6	6	2	2	2	156
			O			x				18	18	18	6	6	6	2	2	2	
5	IT	Po region	C/I													2	2	2	12
			O													2	2	2	
6	HR	Istria	C/I	x						18	18	18	6	6	6	2	2	2	156
			O	x						18	18	18	6	6	6	2	2	2	
7	SI	Central zone	C/I			x	x			18	18	18	6	6	6	2	2	2	156
			O			x	x			18	18	18	6	6	6	2	2	2	
8	CZ	All country	C/I			x			x	*18*	*18*	*18*	6	6	6	2	2	2	max 156
			O			x			x	*18*	*18*	*18*	6	6	6	2	2	2	
9	NL	Groningen	C/I			x				18	18	18	6	6	6	2	2	2	156
			O			x				18	18	18	6	6	6	2	2	2	
10	DK	Central zone	C/I			x				18	18	18	6	6	6	2	2	2	156
			O			x				18	18	18	6	6	6	2	2	2	
11	AR	Buenos aires	C			x			x	*18*	*18*	*18*	6	6	6	2	2	2	max 156
		region	I			x			x	*18*	*18*	*18*	6	6	6	2	2	2	
			max No of samples all CSS							*324*	*324*	*324*	*108*	*96*	*108*	44	44	44	1416
			PPP analyses							WR	WR	SKU	SKU	WR	WR	WR	SKU	SKU	
			health - microbiome								WU					WU			
			helath - other							SKU		SKU							

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Table 3. Type and number of human related samples to collect per CSS.

CSS				Human - urine			Human- faeces			Human- blood			Human- wristbands			Human - nasal swap			
#	country	location	system	farmers	rural	consumer	farmers	rural	consumer	farmers	rural	consumer	farmers	rural	consumer	farmers	rural	consumer	max No samples per CSS
1	ES	Cartagena	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
2	PT	Bairrada	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
3	FR	Bordeaux	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
4	CH	Central zone	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
5	IT	Po region	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
6	HR	Istria	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
7	SI	Central zone	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
8	CZ	All country	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
9	NL	Groningen	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
10	DK	Central zone	C/I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
			O	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
11	AR	Buenos aires	C	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	360
		region	I	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	
max No of samples all CSS				792			792			792			792			792			3960
PPP analyses				WR			WR			SKU			SKU						
health - microbiome							WU									WU			
helath - other				SKU						SKU									

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1.3 Parameters overview (Environment, Livestock and Human)

The figures 2-4 compile the parameters to be analyse per sample. Different box colours were attributed to different labs; combined shipment, inclusively across environment, livestock and human related samples is possible as long as the storage conditions of the different samples/aliquots are compatible and their shipping conditions are the same.

CSS (10 Conventional/Integrated + 10 Organic Fields)

- 1 soil sample/field (20 per CSS)
  - Aliquot 1 -> FQ parameters (CSS leaders)
  - Aliquot 2 -> PPP (MU)
  - Aliquot 3 -> Cmin & Nmin (MU)
  - Aliquot 4 -> microbiome (WU)
  - Aliquot 5 -> OTHERs (UH)
- 6 water samples per CSS
  - PPP (CIEMAT)
- 6 sediment samples per CSS
  - Aliquot 1 -> FQ parameters (CSS leaders)
  - Aliquot 2 ->PPP (WR)
- 1 air dust outdoor sample in 1 C and 1 O field (2 per CSS)
  - KWALIS Lab Fulda
- 1 house dust sample/ farmer household (20 per CSS)
  - PPP (CIEMAT)
- 1 plant sample/field (20 per CSS)
  - PPP (MU)
- 1 earthworm sample/ field (20 per CSS)
  - Monolith2 -> PPP (MU)
  - Monolith 3 -> microbiome (WU)
  - Monolith 1 -> diversity (WU)
- 3 macro-Invertebr. samples per CSS
  - Diversity (UAVR)
- 6 fish samples per CSS
  - - fillets - liver -> PPP (WR)
  - gut -> microbiome (WU)
- 5 bat faeces per CSS
  - Aliquot 1 -> PPP (WR)
  - Aliquot 2 ->microbiome (WU)
- 1 insect trap In 5 C and 5 O fields *3 times (30 per CSS)
  - Diversity (WU)
- 1 insect transect In 5 C and 5 O fields *3 times (30 per CSS)
  - Diversity (WU)

Figure 2. Parameters overview per environment related sample. Water, sediment, fishes, macro-invertebrates and bats are not mandatory matrices as they depend on the availability in the CSS. These matrices are also hardly linked to field or a farming system, so their figures correspond to CSS level. The order of the matrices are in line with the monitoring plan overall presentation order, not mandatory and no-mandatory matrices.

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6 Conventional/Integrated farms + 6 Organic farms/CSS

CATS: 2 C & 2 O /CSS

If: chicken

3 urine-faeces samples/ farm → PPP (WR); SS2 microbiome (WU)

3 blood samples/ farm → PPP (SKU); biomarkers (SKU/other labs)

3 writsbands /farm → PPP (SKU)

1 feed sample/ farm → PPP (WR)

If: Sheep, goat, dairy , or cattle

3 urine samples/ farm → PPP (WR); biomarkers (SKU/other labs)

3 faeces samples/ farm → PPP (WR); microbiome (WU)

3 blood samples/ farm → PPP (SKU); biomarkers (SKU/other labs)

3 wristbands /farm → PPP (SKU)

1 milk sample/ farm → PPP (WR)

1 feed sample/ farm → PPP (WR)

1 faeces sample/ cat (4 per CSS) → PPP (WR); microbiome (WU)

1 blood samples/ cat (4 per CSS) → PPP (SKU); biomarkers (SKU/other labs)

1 Wristband /cat (4 per CSS) → PPP (SKU)

Figure 3. Parameters overview per livestock and cats related sample. Please note that 3 samples/farm means 3 animals, not 3 time measurements. A CSS might sample more than one type of livestock (in line with CSS description table of the Grant Agreement), but only one type of animal is sample per farm.

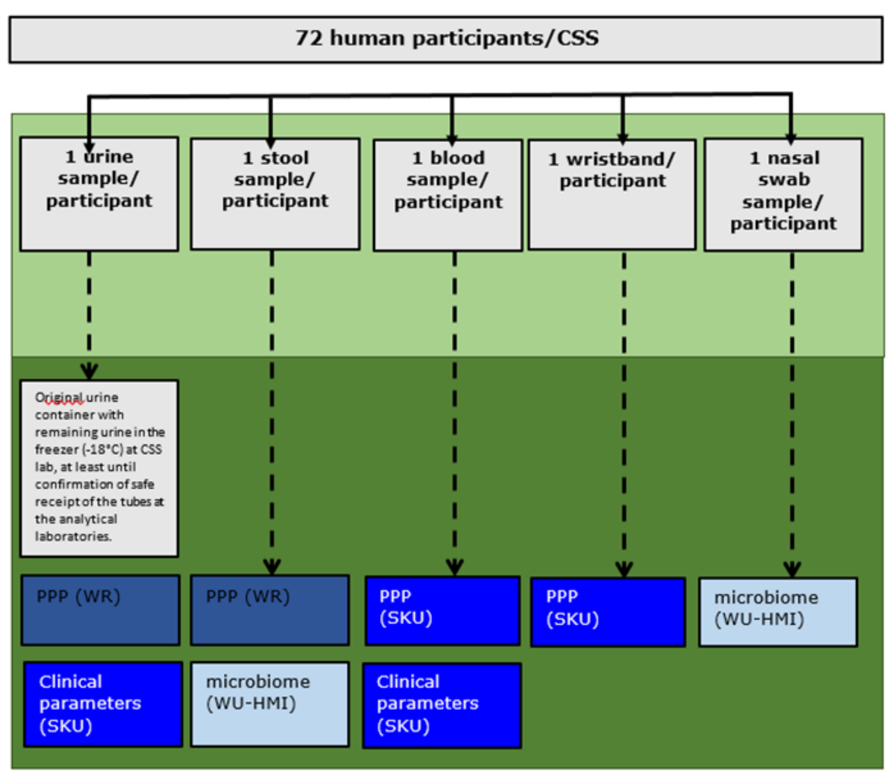

Figure 4. Parameters overview per human related sample. Human population = farmers, neighbors and consumers

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2 General CSS and fields information

To be able to properly analyze environmental and biological data, and to put them in context, general site, crop and monitoring information is needed. This general information covers the natural and human context of the CSS, and farms, field and crop characteristics. **Information on the natural and human context** encompasses information about the climate, topography, soil and water, land ownership; and land and water use rights. **Farm and crop characterization** includes: identification of farms/fields (e.g., coordinates, size), information on livestock and crops on the farm; agricultural management practices for a selected crop (log book) and its pesticide and fertilizer record. It includes also the cost and benefits of the selected crop; Land operations; compilation of relevant baseline data; and stakeholders engagement data are also approached in this study.

The CSS leader is requested to fill this general information in Appendix 3 [Apendix_3_SPRINT_Monitoring_General_Info_WP2-WP6_2021.xlsx]. The excel has the following structure:

Table 4. Environmental, agronomic and economic aspects considered in CSS description.

Natural and human environment	Climate of the study area
	Topography
	Soil information of the farms
	Soil threats
	Water body
	Water management
	Land ownership, land use rights, and water use rights
	Sampling information
Farm characterization	Company identity
	Company profile
	Details farm type
	Details animal
	Animal husbandry
Crop characterization	Considered fields in the farm
	Crop selected
	Soil analysis
	Open questions to disease management
Log book/ crop	Details on management practices (before sowing activities until harvest)
Fertilizer record	Details on fertilizers (type of fertilizers, rates)
Effective pesticide records	Details on pesticides (active substances, dates, applications and methods, commercial product name)
Cost and benefits	Details on incomes and outcomes

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3 Monitoring Environment

3.1 SOIL

3.1.1 Basic soil characterization

The table 8 presents the baseline and the minimum soil data set required **per field**. These parameters should be analyzed by CSS teams, according to the method and conditions summarized in the table. Any deviation should be recorded. The respective methods are provided in Annex 3A-C. If needed, CSS teams must seek the help of a soil scientist for the description of soil profile and the undisturbed soil sampling for laboratory analysis. These data is going to be specially relevant for WP3 on modelling and exposure assessment.

Table 5. Basic soil characterization per field.

Property	Method	Depth	Frequency	Time of assessment
Soil profile description[a]	visual analysis	At least 1 m depth	once	Soil not too wet and not too dry[b]
Texture per soil horizon	Hydrometer or laser diffraction	2 horizons: topsoil and subsoil	once	Soil not too wet and not too dry[b]
Bulk density / total porosity	Core method (ISO 11272)	topsoil: 0–10 cm	once	Soil not too wet and not too dry[b]

[a]If not possible in some fields/farms, soil profile description can be derived from existing soil maps, or previous measurements of the CSS team.
[b]not necessarily at the sampling campaign time.

3.1.2 Sampling, storage, shipment of soil samples for pH, SOC, texture, PPP, Cmin, Nmin, microbiome, PLFA, enzyme activities, and functional gene analyses

Table 6. Overview of sampling, storage, shipment for soil samples.

Sampling	
Sampling design	Material: auger/spade, plastic bags. 1 composite sample per field (this sample is the the combination of 5 sub-samples, randomly sampled across the field; each sub-sample should be ~400g). Sampling should be made in areas of the field that are representative of soil and crop, and should be at least 5 m away from the edges of the field. Sampling depth:0–5 cm in permanent crops, OR 0–20 cm in arable land with tillage. Before collecting the sub-samples, big stones, vegetation residues, grass and litter should be removed from soil surface. The field sub-samples should be placed in a bucket,

	where they should be mixed. Vegetation residues and stones should be hand-picked (do not sieve the soil). *Clean the auger between fields.*
Amount to collect	~2 Kg composite sample
By whom	CSS leader/team
Contact person in case of doubts/changes required	Abdallah, abdallah.alaoui@unibe.ch (+41 31 631 54 44)
Storage and shipment of samples	
Once in the CSS team lab, each soil composite sample should be divided into 5 aliquots	
aliquot 1: ~300 grams, parameter: pH, SOC, and Texture (if not measured from table 8)	
Storage conditions	Air conditions
Shipping	Not applicable; Responsible for the analysis: CSS leader see Annex 3B, D, E. send data to Abdallah, abdallah.alaoui@unibe.ch
aliquot 2: ~100 grams, parameter: PPP	
Storage conditions	Sub-sampled placed into a labelled plastic bag; Shipment should be done right after sampling. If needed, the CSS composite sample can be stored under cold (4ºC) and dark conditions, for a maximum of 24h, until shipping. For longer periods between sampling and shipping, deep freezing (-20º) is recommended.
Shipping *	To Jakub Hofman (MU) By Fast Courier Service/ Express post, in containers with ice, dry ice, or frozen ice packs.
aliquot 3: ~400-500 grams, parameter: Cmin, Nmin	
Storage conditions	The soil is prevented from any drying. Soil should be placed into a labelled plastic or glass container large enough (~1L) to contain also free air space of similar volume like the soil. The sample must be stored under cold (4ºC) and dark conditions immediately after sampling. The shipping should follow within 24h after sampling. For longer time storage, its needed to use lids with small holes or loosely fitting lids to enable little air exchange for soil (before shipping, replace the lids, tape the holes, close the lids tightly).
Shipping *	To Jakub Hofman (MU) By Fast Courier Service/ Express post, in containers with ice, dry ice, or frozen ice packs.
aliquot 4: ~50 grams, parameter: microbiome	
Storage conditions	Individual sample placed into a labelled plastic bag; And frozen (dry ice preferred also on the CSS as slowly freezing samples will influence the activity of microbes) .
Shipping *	To Vera Silva/Paula Harkes (WU) By Fast Courier Service/ Express post, in containers with ice, dry ice, or frozen ice packs.
aliquot 5: ~200 grams, parameter: PLFA analyses, enzyme activities, functional gene analyses	
Storage conditions	Individual sample placed into a labelled plastic bag; Storage at -20°C.
Shipping *	To Sabine Rudolph (UH) Shipping in a frozen state.

* Not applicable to Argentina, analyses will be done locally

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3.2 WATER

No mandatory matrix; depends if available in the CSS.

Surface water bodies = small rivers or stream channels of about 2 m large, small lakes, and ponds. If there are surface water bodies in the CSS area, then CSS teams should sample 6 water bodies/CSS (minimum 3-maximum 6). Riverine ecosystems (small rivers or stream) located in the vicinity of the organic and/or conventional agricultural fields, if possible connected to them, and known/likely to have fishes would be preferred.

CSS teams should (besides take coordinates and pictures):

- indicate connectivity of the water body to agricultural lands (if applicable, indicate if the water is used for field irrigation)
- indicate existence of riparian buffers (i.e. a vegetated "buffer-strip" near the water body, that reduces the impact of adjacent fields or urban areas).
- indicate the size per water body (width/depth)
- measure water temperature, pH, electrical conductivity, dissolved oxygen, and total suspended solids (Annex 4).

3.2.1 Sampling, storage, shipment of water samples for PPP analysis

Table 7. Sampling, storage, shipment for water samples.

Sampling	
Sampling design	Material: plastic bottles 1 sample per water system 3- 6 water systems per CSS. The water sampling must be collected sub-superficially. The bottle must be used to directly collect the water sample. It must be synchronised with sediment, fish, and macroinvertebrates sampling. Detailed protocol Annex 5.
Amount to collect	~2 L sample
By whom	CSS team Any scientist or technician with some experience in water and sediment sampling on aquatic systems who should be supported by at least one more person for better efficiency and overall safety.
Contact person in case of doubts/changes required	Nelson, njabrantes@ua.pt (+351 965611486)
Storage and shipment of samples	
Storage conditions	Shipment should be done right after sampling. If needed, the CSS sample can be stored under cold (4ºC) and dark conditions, for a maximum of 24h, until shipping. For longer periods between sampling and shipping, deep freezing (-20º) is recommended.
Shipping*	To María Ángeles Martínez (CIEMAT) By Fast Courier Service/ Express post, in containers with ice, dry ice, or frozen ice packs .

* Not applicable to Argentina, analyses will be done locally.

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3.3 SEDIMENT

3.3.1 Sampling, storage, shipment of sediment samples for SOM, granulometry and PPP analyses

No mandatory matrix; should be collected in the water sampling locations.

Table 8. Sampling, storage, shipment for sediment samples.

Sampling	
Sampling design	Material: coring device or a stainless-steel scoop, plastic containers 1 composite sample per water body (this composite sample is of 3 sub-samples collected at sites distancing at least 10 m from each other) 3- 6 sediment samples per CSS 0-10 cm deep Detailed protocol Annex 6
Amount to collect	1L/kg of composite sediment sample
By whom	CSS team
Contact person in case of doubts/changes required	Nelson, njabrantes@ua.pt (+351 965611486)
Storage and shipment of samples	
Once in the CSS team lab, each sediment composite sample should be divided into 2 aliquots	
aliquot 1: ~250 grams, parameter: SOM and granulometry	
Storage conditions	Organic matter and granulometry – the sample must be placed in a plastic box (500ml) lined with aluminium foil.
Shipping	Not applicable; Responsible for the analysis: CSS leader see Annex 3B, 3E. send data to Abdallah, abdallah.alaoui@unibe.ch
aliquot 2: ~250 grams, parameter: PPP analysis	
Storage conditions	Once the sediment sample has been taken, you may want to let it settle and then decant the bulk (not all) of clear water above the sediment layer to reduce the weight. Transfer that sediment (still with same water) into a 250ml polypropylene container with a screw cap. Shipment should be done right after sampling. If needed, the sample can be stored under cold (4ºC) and dark conditions, for a maximum of 24h, until shipping. For longer periods between sampling and shipping, deep freezing (-20º) is recommended. Guarantee the container is not completely full in case of freezing explansion.
Shipping*	To Hans Mol, WR By Fast Courier Service/ Express post, in thermal boxes with ice, dry ice, or frozen ice packs.

*Not applicable to Argentina, analyses will be done locally.

3.3.2 Sampling, storage, shipment of Benthic macroinvertebrates for diversity assessment

Table 9. Characterization of the Benthic macroinvertebrates' community.

Sampling	
Sampling design	Material: hand net, plastic containers **Only applicable to CSS with riverine (small rivers or stream) ecosystems.** The biodiversity of benthic macro-invertebrates will be assessed in 3 samples sent to our SPRINT expert Nelson Abrantes njabrantes@ua.pt. These samples originate from 3 riverine systems or from 3 sites within a selected riverine system (in this later case, the sites should be at least 500 m apart from each other). These riverine system(s) should be within the set of water bodies selected for water and fish sampling. Detailed protocol annex 7.
Amount to collect	Not applicable; 3 samples/CSS
By whom	CSS teams
Contact person in case of doubts/changes required	Nelson, njabrantes@ua.pt (+351 965611486)
Laboratory analysis	
Storage conditions	preservation in alcohol
Shipping	To Nelson (UAVR) No special requirement

3.4 AIR OUTDOOR

3.4.1 Sampling, storage, shipment of air and dust outside for PPP analysis

Table 10. Sampling, storage, shipment for air and dust outdoor samples.

<table>
<tr><th colspan="2">Sampling</th></tr>
<tr><td>Sampling design</td><td>Material: 2 TIEM devices + 2 filter collection kits + 1 box to send the filters (Provided by WU)*
=> one to be installed in an organic field and one in a conventional field.=> (dont forget to take the coordinates)=>These devices cover also long distance transport, so better to select 2 fields with no clear barriers, or on a top of hills.

WU will send the head device already assembled but without the foot/pole (to save shipping space). We ask you to arrange this yourself. The head should be at 1.5 height, and at least 0.5m above crop. A maximum distance between trees in the field is adviced.

The devices should be installed one month before the mid growing season/sampling campaign. They stay in the field for 8 weeks, with no need of maintenance. After these 8 weeks you remove the head of the devices, and with nitrile gloves and tweezers, you remove the 4 PEF filters and the 1 PUF filter per device => these filters should be involved in aluminum fold and frozen for at least 24 hours before shipping.

A video will be provided on the installation of the device in the field, disassemble the head to collect the filters, and protection of filters for analyses.

 </td></tr>
</table>

Amount to collect	Not applicable; 4 loaded PEF filters in a and PUF disk/ device
By whom	CSS leader/team
Contact person in case of doubts/changes required	Violette Geissen, violette.geissen@wur.nl (+31 610883790)
Storage and shipment of samples	
Storage conditions	the filters should be involved in aluminum fold and frozen for at least 24 hours before shipping. shipment performed on cool conditions , in the box provided by WU.
Shipping*	**The filters=>** Carolin Schleicher KWALIS Qualitätsforschung Fulda GmbH Fuldaer Straße 21 D-36160 Dipperz, GERMANY Phone: +49-6657-608990 FAX: +49-6657-6592 E-Mail: office@kwalis.de **Return the disassembled heads of the devices =>** Ulrich Schlechtriemen TIEM Integrierte Umweltüberwachung Hohenzollernstr. 20 44135 Dortmund fon +49 231 22395933 fax +49 231 22396548 tiem-technic@arcor.de

* Not applicable to Argentina; Argentina will have BSNE devices; work must be coordinated with Dirk Goossens dirk.goossens@kuleuven.be

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3.5 DUST INDOOR

3.5.1 Sampling, storage, shipment of dust indoor samples for PPP analysis

Table 11. Sampling, storage, shipment for dust indoor samples.

Sampling	
Sampling design	Material: bags, vacuum cleaner from the farmer <u>1 sample per farmer household</u> The farmer will be asked to replace the vacuum bag with a new one, one month before the house visit for questionnaires and human sampling. During this month the participant does the vacuum cleaning as he/she would normally do, without changing the bag. The participant should indicate the following: • The day and time of (the one month before) bag replacement • The frequency of cleaning (ideally at least4 times) • What areas have been vacuum cleaned after that (e.g. House, Car). At house visit by the CSS team, the content of the vacuum cleaner bag should be transferred into a ziplock bag (close it tightly). In case there is not enough dust material in the bag, a last vacuum cleaner exercise should be done in the house, including the indoor entrance doormat which typically contains higher amounts of dust. In this case, avoid inclusion of moist dust/sand. The sample may also contain sand, dirt, hair, fibers - no need to remove them. Dust samples will be handled in the lab.
Amount to collect	all content of the bag, at least 50-100 grams
By whom	CSS team
Contact person in case of doubts/changes required	Hans, hans.mol@wur.nl (+31 317480318)
Storage and shipment of samples	
Storage conditions	Sample covered with aluminum film and placed into a labelled polyethylene bag. Shipment should be done right after sampling. If needed, the CSS sample can be stored under cold (4ºC) and dark conditions, for a maximum of 24h, until shipping. For longer periods between sampling and shipping, deep freezing (-20º) is recommended.
Shipping *	To María Ángeles Martínez (CIEMAT) By Fast Courier Service/ Express post, in containers with ice, dry ice, or frozen ice packs

* Not applicable to Argentina, analyses will be done locally.

3.6 PLANT/CROP

3.6.1 In situ health assessment (questionnaire / diseases)

Plant (meaning crop) health assessment should be performed once per field, and includes crop yield data, pest incidence, weed infestation (all covered in Appendix 3- "Apendix_3_SPRINT_Monitoring_General_Info_WP2-WP6_2021.xlsx") and

- for non-permanent crops only, a root diseases evaluation – at plant collection time, according to Annex 8 scoring systems.

- For permanent crop, refer to https://www.agr.gc.ca/eng/agriculture-and-the-environment/agricultural-practices/agricultural-pest-management/agricultural-pest-management-resources/identification-guide-to-the-major-diseases-of-grapes/?id=1544449361476

VSA Field Guides are available for eight different land uses:
https://www.bioagrinomics.com/visual-soil-assessment

For permanent and non-permanent crops, CSS leaders are requested to report other visual plant damages.

3.6.2 Sampling, storage, shipment of plant samples for PPP analysis

Table 12. Sampling, storage, shipment for plant samples.

Sampling	
Sampling design	Material: paper/plastic bags 1-2/3 composite samples /field When: close to harvest, ideally vegetative and reproductive parts are present; the exact part(s) to samples is(are) crop specific - depends on what is used for the final product (leaves/root, edible parts: grain/grapes/etc) A composite sample(s) is prepared per part of plant (leaves, grain, etc); each composite sample should result of 5 plants/trees. Sampling should be made in areas of the field that are representative of soil and crop, and should be at least 5 m away from the edge of the field. Each plant should be equally represented (amount) in the composite sample. Exchange with experts will enable us to refine sampling procedure. An update of all varieties of plants existing at the CSS will help for this purpose. If multiple plant types are considered within the CSS crop class (Oil plants mostly, but likely cereals)=> please sample type 1 in 5 C and in 5 O field, and type 2 in the other 5 C and O fields.
Amount to collect	~100 grams dried weight equivalent (composite sample)
By whom	CSS team

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Contact person in case of doubts/changes required	Jakub, jakub.hofman@recetox.muni.cz (+420775140071)
Storage and shipment of samples	
Storage conditions	Deep freezing directly after sampling stalk and leaves; root samples can be stored with frozen cooling solutions in thermal boxes to keep low temperature.
Shipping*	To Jakub Hofman (MU) Freeze the samples and send frozen as soon as possible Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.

* Not applicable to Argentina, analyses will be done locally.

3.7 EARTHWORMS

3.7.1 Sampling, storage, shipment of earthworm samples for PPP, microbiome and diversity analysis

Table 13. Sampling, storage, shipment for earthworm samples.

Sampling	
Sampling design	Material: plate, shovel, plastic bottles/flasks, 70% alcohol, plastic sheet, mustard, preferably Hot mustard Aroma solution (150g/L), or Allyl isothiocyanate (AITC) synthetic grade (94% to 97% volume fraction). 3 Monoliths (of 25x25x25cm)/field (according to ISO23611) (1 for diversity, 1 for PPP and 1 for microbiome) See annex 9 for detailed procedure
Amount to collect	all earthworms found in the monoliths
By whom	CSS leader/team
Contact person in case of doubts/changes required	Esperanza Huerta Lwanga, esperanza.huertalwanga@wur.nl (+31 616650961)
Storage and shipment of samples	
Monolith 1 – diversity	
Storage conditions	Earthworms should be preserved at 70% alcohol immediately after collected.
Shipping*	To Vera Silva/Esperanza Huerta Lwanga (WU) No special requirement
Monolith 2 – PPP	
Storage conditions	Earthworms should be immediately frozen after collected
Shipping*	To Jakub Hofman (MU) Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.
Monolith 3 – microbiome	
Storage conditions	Earthworms should be immediately frozen after collected
Shipping*	To Vera Silva/Paula Harkes (WU) Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.

* Not applicable to Argentina, analyses will be done locally.

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3.8 FISH

CSS leaders should find a local fish expert (scientist or fishermen) with license for fish collection. A fish scientist, with certificate in animal experimentation, would be preferred. Otherwise a second person, with a certificate in animal experimentation (scientist, vet), needs to be contacted for euthanasia of fishes.

3.8.1 In situ health assessment (biodiversity)

Biodiversity (richness and abundance) is assessed in situ (in the water sampling locations) by the local CSS fish expert. An inventory will be established to identify which CSS has surface water bodies. Sampling plan and effort is CSS dependent and should therefore be discussed a priori with our SPRINT fish expert Nelson Abrantes (njabrantes@ua.pt).

3.8.2 Sampling, storage, shipment of fish samples for PPP and microbiome analyses

Table 14. Sampling, storage, shipment for fish samples.

Sampling	
Sampling design	Material: electrofisher equipment, seines, angling or passive nets 1 composite sample/water body 1 composite sample = 5 fish of the same species*; the 5 fishes should have similar size (not juveniles). *See annex 10 for Recommended species. The species may differ among CSS water bodies.
Amount to collect	Not applicable
By whom	Local fish expert
Contact person in case of doubts/changes required	Nelson njabrantes@ua.pt (+351 965611486)
Storage and shipment of samples	
By a local person with a certificate in animal experimentation => fishes are euthanized, and the fillets, the livers and the gut contents the 5 fishes per water body are aggregated per tissues type => 3 sub-samples/water body Since all sub-samples have the same storage and shipping conditions, and are destined to Wageningen => only one shipment is needed=> sub-samples are distributed in Wageningen to WU and WR teams	
Storage conditions	Store in the freezer (-20C).
Shipping*	To Hans Mol (WR) Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

3.9 BAT

No need of ethics approval - we will not collect blood or tissues (we are only collecting faeces) and we will not be catching/handling bats. **CSS leaders should find a local bat scientist though.** We advise to collect the bat faeces in/at bat roosts[7];

Sampling inside a roost requires a license for working with endangered species. Bat scientist have this license. If you need help finding a bat scientist, please contact our SRINT bat experts Marlot Jonker marlot.jonker@zoogdiervereniging.nl and Herman Limpens herman.limpens@zoogdiervereniging.nl. If you already have already contacted a local bat scientist, please inform and Marlot Herman.

The local bat scientist and Herman will select the sampling time (should be during the active season, for Netherlands it means May – June, which coincides with sampling campaign), bat species (First choice is *Pipistrellus* sp.) and the roosts to sample. The bat scientists will do the sampling and give the samples to the CSS leaders, whom will forward the samples to the lab, for pesticide and microbiome analyses.

3.9.1 In situ health assessment (biodiversity)

Biodiversity is assessed by the local CSS bat expert. Each CSS sampling plan should be discussed a priori with our SRINT bat experts Marlot and Herman.

3.9.2 Sampling, storage, shipment of bat faeces samples for PPP and microbiome analyses

Table 15. Sampling, storage, shipment for bat faeces samples.

Sampling	
Sampling design	Material: flasks/plastic bags 1 composite sample faeces/roost If possible 3 to 5 roosts/CSS Roosts located in the vicinity of the organic and/or conventional agricultural fields, if possible connected to them, would be preferred.
Amount to collect	Minimum 5 g; preferably 10-25 gram Each composite sample should be divided into 5 tubes (1 scoop if ~1 gram each)
By whom	Local Bat scientist
Contact person in case of doubts/changes required	Herman, herman.limpens@zoogdiervereniging.nl (+31 620736614)

[7] Roosting habitat: bats roost in caves, hollows and crevices in rocks and trees, (stone and wooden) built structures such as houses, stables, sheds, churches, castles, monasteries, bridges.

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Storage and shipment of samples	
Since PPP and microbiome tubes have the same storage and shipping conditions, and are destined to Wageningen for PPP and microbiome analyses => only one shipment is needed=> sub-samples are distributed in Wageningen to WU and WR teams.	
Storage conditions	Store sample in the freezer (-20C).
Shipping*	To Hans Mol (WR) Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.

* Not applicable to Argentina, analyses will be done locally.

3.10 INSECTS

3.10.1 Sampling, storage, shipment of ground-dwelling organisms for diversity assessment

Table 16. Sampling, storage, shipment for ground-dwelling organisms samples.

Sampling	
Sampling design	Materials: pitfall traps/plastic boxes with lid, plastic strip; small plastic flasks to send the samples In 5 conventional and 5 organic fields per CSS (ideally all from different farms) 2. Plot (i.e. group of 12 traps)/field 3 sampling times/plot trap installation: 31 days before the middle of the growing season (most CSS end April); 1st measurement: 21 days before the middle of growing season, 2nd measurement – middle of growing season (coinciding with the start of sampling campaign of other matrices), 3rd measurement – 21 days after 2nd measurement) 1 pooled sample per plot per sampling time See Annex 11 for further details on the procedure
Amount to collect	Not applicable; all the insects in the traps
By whom	CSS leader
Contact person in case of doubts/changes required	Frank van Langevelde (WU) frank.vanlangevelde@wur.nl
Storage and shipment of samples	
Storage conditions	Store the insects in 70% ethanol
Shipping	Preferably shortly after sampling (or can be send all together after the 3 sampling periods). To Vera Silva/Frank (WU) No special requirement

3.10.2 Sampling, storage, shipment of flying insects for diversity assessment

Table 17. Sampling, storage, shipment for flying insects samples.

Sampling	
Sampling design	Materials: nets; small plastic flasks to send the samples In the same fields with the pitfall traps (5C & 5O/CSS) • transect/field

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	3 sampling times (in the same days as the insects in the pitfall traps are collected) 1 sample per transect per sampling time See Annex 12 for further details on the procedure
Amount to collect	Not applicable; all the insects in the net
By whom	CSS leader
Contact person in case of doubts/changes required	Frank van Langevelde (WU) frank.vanlangevelde@wur.nl
Storage and shipment of samples	
Storage conditions	Store the insects in 70% ethanol
Shipping	Preferably shortly after sampling (or can be send all together after the 3 sampling periods).=> can be combined with ground-dwelling samples To Vera Silva/Frank (WU) No special requirement

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4 Monitoring livestock - sheep, goat, diary, cattle for meat, & pig

4.1 IN SITU HEALTH ASSESSMENT

A set of question on livestock health are already covered in Appendix 4. These questions are destined to the 6 C farmers and 6 O farmers/CSS where livestock "will be sampled", but if possible, an overview on livestock health status from the veterinarians would be useful too.

4.2 URINE

4.2.1 Sampling, storage, shipment of urine samples for PPP and clinical parameters/biomarkers analyses

Table 18. Sampling, storage, shipment of urine samples - livestock.

Sampling	
Sampling design	Materials: Measuring beaker with handle (1000mL), collection bottles (>100 mL), nitrile gloves, sealable safety bags, coolbox powered by electricity (12V/220V) with blue ice packs, centrifuge tubes (12 mL), tubes for clinical parameters, pen 3 animals/selected farm*; 1 urine sample/animal *6 C farms and 6 O farms/CSS **If two livestock types are present in the CSS** (i.e. CSS1 sheep & goat, CCS2 pig and chicken, CCS 7 dairy and cattle for meat, CSS8 and CSS11 dairy and chicken) => sample one type of livestock in 3 C and in 3 O farms, and the other type of livestock in the other 3 C and O farms (Only one livestock type is sampled per farm). Measuring beaker with handle (1000mL), collection bottle (250 mL), sealable safety bag with SPRINT participant ID, and coolbox with blue ice packs are provided to the farmer a priori. Ideally the urine is sampled in the **morning of the day of the blood collection**, **and hh:mm of collection should be noted** on a diary form. (When cows are rising after a rest, they usually defacate and urinate. Thus, it is recommended to get a resting cow up and then be ready with buckets/containers for urine and faeces)
Amount to collect	~100 ml urine/animal Follow instructions to aliquoting urine samples – annex 13-B (1 sample=7 tubes, this set of tubes cover PPP and clinical parameters/biomarkers).
Contact person in case of doubts/changes required	Farmer or CSS team
Contact person in case of doubts/changes required	Martin T Sorensen, martint.sorensen@anis.au.dk
Storage and shipment of samples	

Storage conditions	Same as for human urine: Store sample at approx. +4 °C in cool box powered by electricity (12V/220V) with added ice packs until arrival at distribution point for the CSS. After aliquitation the samples should be stored at -18°C until transport to the analytical laboratories.
Shipping*	Same as for human urine: **tubes for pesticide analyses: Jonatan Dias, WR** Wageningen Food Safety Research (WFSR) SPRINT project Akkermaalsbos 2 6708 WB Wageningen The Netherlands Contact details: Jonatan Dias, jonatan.dias@wur.nl, +31317486918 **tubes for determination of clinical parameters** Att: Mr. Maurice van Dael, SKU 133 HEV Centrale Ontvangst Goederen Radboudumc Geert Grooteplein Zuid 30 6525 GA Nijmegen The Netherlands Contact: maurice.vandael@radboudumc.nl, +31619622869 Both types of tubes: Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

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4.3 FAECES

4.3.1 Sampling, storage, shipment of faeces samples for PPP and microbiome analysis

Table 19. Sampling, storage, shipment of faeces samples – livestock.

Sampling	
Sampling design	Materials: collection tubes, nitrile gloves, zip-lock bag (labelled), cool box powered by electricity (12V/220V) with blue ice packs, pen **Ideally from the same animals considered for urine collection,** and in in the morning of the day the blood collection by the veterinarian.
Amount to collect	5 tubes/ animal (this set of tubes cover PPP and microbiome); 1 scoop per tube => see human SOP stool collection, storage and transportation for details (Annex 14)
By whom	Farmer or CSS team
Contact person in case of doubts/changes required	Martin T Sorensen, martint.sorensen@anis.au.dk
Storage and shipment of samples	
Since PPP and microbiome tubes have the same storage and shipping conditions, and are destined to Wageningen for PPP and microbiome analyses => only one shipment is needed=> sub-samples are distributed in Wageningen to WU and WR teams.	
Storage conditions	Same as for human stool samples: store sample in cool box powered by electricity (12V/220V) with added ice packs. Upon arrival of the samples at the CSS collection point, immediately transfer to -18 °C and keep frozen from this moment onwards.
Shipping*	Same as for human stool samples: **Jonatan Dias, WR** Wageningen Food Safety Research (WFSR) SPRINT project Akkermaalsbos 2 6708 WB Wageningen The Netherlands Contact details: Jonatan Dias, jonatan.dias@wur.nl, +31317486918 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

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4.4 BLOOD

4.4.1 Sampling, storage, shipment of blood samples for PPP and clinical parameters/biomarkers analysis

Table 20. Sampling, storage, shipment of blood samples – livestock.

Sampling	
Sampling design	Materials: 2 Plasma separation tubes (PST), 3 Potassium-Ethylenediaminetetraacetic acid (K_2-EDTA) blood tubes, and 3 Blood tube serum separation (SST)/animal. **In the same animals considered for urine collection.**
Amount to collect	1 sample (9 tubes)/animal (this set of tubes cover PPP and clinical parameters/biomarkers) => see human blood SOP for details 3 mL per PST and K_2-EDTA tube and 5mL per SST tube (Annex 15)
By whom	Veterinarian
Contact person in case of doubts/changes required	Martin T Sorensen, martint.sorensen@anis.au.dk
Storage and shipment of samples	
The sets of 9 tubes cover PPP and biomarker analyses. Since all tubes have the same storage and shipping conditions, and are destined to SKU => only one shipment is needed.	
Storage conditions	Store sample in cool box powered by electricity (12V/220V) with added ice packs until fractionation (within <2 hours of sampling). After that, store at -80 ºC until shipment for analysis in NL.
Shipping*	same as for human blood samples: Mr. Maurice van Dael 133 HEV Centrale Ontvangst Goederen Radboudumc Geert Grooteplein Zuid 30 6525 GA Nijmegen The Netherlands maurice.vandael@radboudumc.nl, +31619622869 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.

* Not applicable to Argentina, analyses will be done locally.

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4.5 DIFFUSIVE SAMPLERS (WRISTBANDS)

4.5.1 Sampling, storage, shipment of wristbands for PPP analysis

Table 21. Sampling, storage, shipment of diffusive samplers (wristbands) – livestock.

Sampling	
Sampling design	Materials: pre-cleaned wristbands and zip-lock bag (all provided by SKU) => CSS leaders must inform Paul S. and Maurice on the effective number of livestock, and type of livestock in their CSS In the same animals considered for urine and blood collection. A small silicon wristband should be attached to the animal collar or to an ear label=>See cat SOP for details (Annex 18) The wristband should be should attached to the animal at the day of urine, faces, and blood samples collection, and collected 1 week after that. It is ok for this period to be longer, however it should not be longer than 1 month. Record time and date of application and removal of the wristband.
Amount to collect	Not applicable
By whom	CSS team
Contact person in case of doubts/changes required	Martin T Sorensen, martint.sorensen@anis.au.dk Paul Scheepers, Paul.Scheepers@radboudumc.nl
Storage and shipment of samples	
Storage conditions	After having animals with the wristband for 7 consecutive days, put the wristband inside the zip-lock bag and place it the freezer- either farmer or CSS team. The wrists bands will be collected by the SPRINT CSS team, stored at -20°C and sent to the analysis laboratory as one batch.
Shipping	To Maurice van Dael (SKU); maurice.vandael@radboudumc.nl When you are ready to mail it, take the plastic container out of the fridge and place the plastic container inside the envelope provided. Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport. *You can combine shipment with blood samples.*

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4.6 MILK

4.6.1 Sampling, storage, shipment of milk samples for PPP analysis

Table 22. Sampling, storage, shipment of milk samples.

Sampling	
Sampling design	Materials: one 100-150 mL plastic tubes Milk= fresh/raw milk Not applicable to pig **1 milk sample/selected farm** (i.e. 6 C and 6 O farmers/CSS); it could be either a composite sample from the milk of the 3 animals sampled for urine and blood OR a sample from the milk container of the farm (whichever is possible). If the first option=> milk should be collected manually into the collection tube, at the day of blood collection; a similar amount of milk should be collected from the 3 animals, The collection tube is provided to the farmer a priori. If the second option=> ask when the milk was collected and check if the material is homogeneous, i.e. that there is no separated fat layer on top, before filling the tube. If there is, stir or mix to get it homogeneous again and directly after that, fill in the collection tube.
Amount to collect	50-100 mL
By whom	Farmer or CSS team (if option 2)
Participant coordinating sampling	Martin T Sorensen, martint.sorensen@anis.au.dk
Storage and shipment of samples	
Storage conditions	Store in the freezer (-20C)
Shipping*	To Hans Mol (WR) Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.

* Not applicable to Argentina, analyses will be done locally.

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4.7 FEED

4.7.1 Sampling, storage, shipment of feed samples for PPP analysis

Table 23. Sampling, storage, shipment of feed samples – livestock.

Sampling	
Sampling design	Materials: plastic or paper bags Feed = homegrown energy source (e.g. cereal/corn), homegrown protein source (e.g. rape seed/peas/beans), or roughage (hay, silage, pasture,) **1 feed sample/selected farm** (i.e. 6 C and 6 O farmers/CSS)
Amount to collect	100-500 grams, depending on how homogeneous the feed is. If very inhomogeneous feed material (e.g. silage),=> 500 g; if some mixing/homogenization done on-site less material is needed.
By whom	CSS team
Participant coordinating sampling	CSS leaders, in case of doubts, contact: Martin T Sorensen, martint.sorensen@anis.au.dk
Storage and shipment of samples	
Storage/shipping conditions	If the sample material is dry, then storage in the fridge or ambient temperature (in the dark). Shipment can be done at ambient temp. If the sample is perishable/wet, then store in the freezer and keep frozen or at least cooled during transport. Ship by courier. Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport.
Shipping*	To Hans Mol (WR) – see storage

* Not applicable to Argentina, analyses will be done locally.

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5 Monitoring Chicken

5.1 Sampling, storage, shipment of chicken related samples for PPP, microbiome, and clinical parameters/biomarkers

Table 24. Sampling, storage, shipment for chicken related samples.

Sampling	
Sampling design	3 animals/selected farm; i.e. 6 C farms and 6 O farms/CSS chickens are expected only in CSS2, CSS8 and CSS11, in all these CSS, there are two types of livestock, so consider point raised also above=> sample one type of livestock in 3 C and in 3 O farms, and the other type of livestock in the other 3 C and O farms. Only one livestock type is sampled per farm, and in total 36 animals are sampled per CSS. For • Urine-Faeces – 3 per farm (refer to Table 26. 27)** • Blood – 3 per farm (refer to Table 28)** • wristbands (refer to Table 21) • Feed sample – 1 per farm (refer to Table 23) ** Consider to buy and kill 3 hens/chickens for blood sampling to avoid the risk of subsequent cannibalism. Then faeces (colon content) can also be sapled from the same animals.
Amount to collect	same as in corresponding matrix of sheep, goat, diary, cattle for meat, pig section above
By whom	Farmer or CSS team
Participant coordinating sampling	Martin T Sorensen, martint.sorensen@anis.au.dk
Storage and shipment of samples	
Storage conditions	same as in corresponding matrix of sheep, goat, diary, cattle for meat, pig section above
Shipping*	same as in corresponding matrix of sheep, goat, diary, cattle for meat, pig section above

* Not applicable to Argentina, analyses will be done locally.

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6 Monitoring Cats

4 cats per CSS, 2 from Conventional farmers households, 2 from Organic farmers households.

6.1 Sampling, storage, shipment of cats related samples for PPP, microbiome, and clinical parameters/biomarkers

Table 25. Sampling, storage, shipment cat related samples.

Sampling	
Sampling design	4 cats/CSS; 2 from C and 2 from O farmer household Information to collect from the farmer • basic cat health information • age of the animal • Range of the cat environment (km^2) • Type of food of the cat 1 faeces sample per cat (refer to Table 27) 1 blood sample per cat (refer to Table 28) 1 wristband per cat (refer to Table 21, and Annex 18)
Amount to collect	same as in corresponding matrix of sheep, goat, diary, cattle for meat, pig section above
By whom	Farmer or CSS team + Veterinarian for blood
Participant coordinating sampling	Paul Scheepers, Paul.Scheepers@radboudumc.nl
Laboratory analysis	
Storage conditions	same as in corresponding matrix of sheep, goat, diary, cattle for meat, pig section above.
Shipping	same as in corresponding matrix of sheep, goat, diary, cattle for meat, pig section above.

7 Monitoring Human

7.1 QUESTIONNAIRE ON HEALTH

A questionnaire on exposure and health was designed to farmers, neighbours and consumers [Appendix_1_SPRINT_Personal interview questionnaire.docx] in order to supplement the results of the analyses presented below (refer to section 1.1).

7.2 URINE

7.2.1 Sampling, storage, shipment of urine samples for PPP and clinical parameters/biomarkers analysis

Table 26. Sampling, storage, shipment for urine samples - human.

Sampling	
Sampling design	Materials: Measuring beaker with handle (1000mL), collection bottle (250 mL), sealable safety bags, coolbox powered by electricity (12V/220V) with blue ice packs, centrifuge tubes (12 mL), tubes for clinical parameters, pen 1 urine sample /participant (10 % of the participants will be asked to collect follow-up spot samples on the day of collection of the morning urine sample and the next day) => see SOP human urine collection, storage and transportation (Annex 13). Measuring beaker with handle (1000mL), collection bottle (250 mL), sealable safety bag with SPRINT participant ID, and coolbox with blue ice packs are provided to the farmer a priori; the urine sample will be sampled directly after awakening and getting up (first morning void) and hh:mm will be noted on a diary form.
Amount to collect	~50-125ml urine/participant
By whom	Participant
For questions contact:	Vivi Schlünssen. vs@ph.au.dk
Storage and shipment of samples	
Storage conditions	Store sample at approx. +4 °C in cool box powered by electricity (12V/220V) with added ice packs until arrival at distribution point for the CSS. After aliquitation the samples should be stored at -18°C until transport to the analytical laboratory in Wageningen, The Netherlands. Shipment will be done on dry ice.
Shipping*	**Urine samples for pesticide analyses:** Wageningen Food Safety Research (WFSR) SPRINT project Akkermaalsbos 2 6708 WB Wageningen The Netherlands

	Contact details: Jonatan Dias, jonatan.dias@wur.nl, +31317486918 **Urine samples for clinical parameters** Att: Mr. Maurice van Dael 133 HEV Centrale Ontvangst Goederen Radboudumc Geert Grooteplein Zuid 30 6525 GA Nijmegen The Netherlands Contact: maurice.vandael@radboudumc.nl, +31619622869 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

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7.3 STOOL

7.3.1 Sampling, storage, shipment of stool samples for PPP and microbiome analysis

Table 27. Sampling, storage, shipment for stool samples - human.

Sampling	
Sampling design	Materials: 2 collection papers, 5 pre-labelled collection tubes, 2 pairs of nitrile gloves, 1 zip-lock bag with sticker (labelled), 1 cool box powered by electricity (12V/220V) with blue ice packs, 1 pen 5 tubes/ participant (4 empty tubes and one tube with stabilization buffer) => see human SOP stool collection, storage and transportation for details (Annex 14) The stool sample should be sampled in the morning of the day the urine and blood collection
Amount to collect	1 scoop per tube
By whom	Participant
For questions contact:	Vivi Schlünssen. vs@ph.au.dk
Storage and shipment of samples	
The 5 tubes cover PPP and microbiome analyses. Since all tubes have the same storage and shipping conditions, and are destined to Wageningen => only one shipment is needed=> sub-samples are distributed in Wageningen to WU and WR teams.	
Storage conditions	• Store sample in cool box powered by electricity (12V/220V) with added ice packs. Upon arrival of the samples at the CSS collection point, immediately transfer to -18 °C and keep frozen from this moment onwards. Shipment on dry-ice.
Shipping*	**Stool samples for pesticide analyses:** Wageningen Food Safety Research (WFSR), SPRINT project, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands Contact details: Jonatan Dias, jonatan.dias@wur.nl, 31317486918 **Stool sample for microbiome** Prof. dr. Jerry M. Wells and Dr Jos Boekhorst Host Microbe Interactomics Group, Department Animal Science Campus, Building 122, De Elst 1 6708 WD, Wageningen. The Netherlands Contact details: Loes Bugter, loes.bugter@wur.nl; +31317486125/ Jerry Wells +31620362084 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

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7.4 BLOOD

7.4.1 Sampling, storage, shipment of blood samples for PPP and clinical parameters/biomarkers analysis

Table 28. Sampling, storage, shipment for blood samples – human.

Sampling	
Sampling design	Materials: 2 Plasma separation tubes (PST), 3 Potassium-Ethylenediaminetetraacetic acid (K_2-EDTA) blood tubes, and 3 Blood tube serum separation (SST). 9 tubes/participant => see human SOP Blood collection, storage and transportation (Annex 15) for details The faces should be sampled in the morning of the day the blood collection by the veterinarian.
Amount to collect	3 mL per PST and K_2-EDTA tube and 5mL per SST tube
By whom	Laboratory technician (SPRINT CSS team)
For questions contact:	Vivi Schlünssen. vs@ph.au.dk
Storage and shipment of samples	
The 9 tubes cover PPP and biomarker analyses. Since all tubes have the same storage and shipping conditions, and are destined to SKU => only one shipment is needed	
Storage conditions	Store sample in cool box powered by electricity (12V/220V) with added ice packs until fractionation (within <2 hours of sampling). Store at -80 ºC until shipment for analysis in NL. Samples should be shipped for NL on dry ice.
Shipping*	To Maurice van Dael (SKU). To this shipment wristbands can be added Att: Mr. Maurice van Dael 133 HEV Centrale Ontvangst Goederen Radboudumc Geert Grooteplein Zuid 30 6525 GA Nijmegen The Netherlands Contact details: maurice.vandael@radboudumc.nl, +31619622869 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

7.5 WRISTBANDS

7.5.1 Sampling, storage, shipment of wristbands for PPP analysis

Table 29. Sampling, storage, shipment for wristbands.

Sampling	
Sampling design	Materials: pre-cleaned wristbands and zip-lock bag (all provided by SKU) 1 wristband/ participant worn for 7 consecutive days=>See SOP for assessment of exposure of humans to pesticides using silicone wristbands for details (Annex 16) The wristband is provided to the participant one week before the visit
Amount to collect	Not applicable
By whom	SPRINT CSS team
For questions contact	Vivi Schlünssen. vs@ph.au.dk
Storage and shipment of samples	
Storage conditions	After wearing the wristband for 7 consecutive days, the participant should put the wristband inside the zip-lock bag and place it the freezer. Human wrists bands will be collected by the SPRINT CSS team, stores at -20°C and sent to the analysis laboratory as one batch.
Shipping	To Maurice van Dael (SKU). Combine shipment with blood samples Att: Mr. Maurice van Dael 133 HEV Centrale Ontvangst Goederen Radboudumc Geert Grooteplein Zuid 30 6525 GA Nijmegen The Netherlands Contact details: maurice.vandael@radboudumc.nl, +31619622869 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

7.6 NASAL SWABS

7.6.1 Sampling, storage, shipment of nasal swab samples for microbiome analysis

Table 30. Sampling, storage, shipment nasal swab samples.

Sampling	
Sampling design	Materials: ESwab™ 480C and zip-lock bag 1 nasal swab/ participant (left nostril) => See SOP for human nasal swabs (Annex 17) for details
Amount to collect	Nasal swab
By whom	Laboratory technician (SPRINT CSS team)
Participant coordinating sampling	Vivi Schlünssen. vs@ph.au.dk
Storage and shipment of samples	
Storage conditions	Store cooling box (with ice packs) central sample distribution point for the CSS. Thereafter store at -20°C in a freezer until shipment
Shipping*	**Nasal swab sample for microbiome analysis** **Wageningen University** Prof. dr. Jerry M. Wells and Dr Jos Boekhorst Host Microbe Interactomics Group, Department Animal Science Campus, Building 122, De Elst 1 6708 WD, Wageningen. The Netherlands Contact details: Loes Bugter, loes.bugter@wur.nl; +31317486125/ Jerry Wells +31620362084 Fast Courier Service using frozen cooling solutions in thermal boxes to keep low temperature during transport

* Not applicable to Argentina, analyses will be done locally.

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3- Bordeaux (France)	isabelle.baldi@u-bordeaux.fr, +33 6 72 83 92 67	Isabelle Baldi : 31 chemin du rouquet – 33700 MERIGNAC – France
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MU	Jakub Hofman	jakub.hofman@recetox.muni.cz +420 775 140 071	Jakub Hofman, RECETOX, Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic
SKU	Maurice van Dael (Paul S.)	Maurice.vanDael@radboudumc.nl +31-24-3655729; +31619622869	Maurice van Dael. 133 HEV, Centrale Ontvangst Goederen Radboudumc, Geert Grooteplein Zuid 30, 6525 GA Nijmegen, The Netherlands
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WR	Hans Mol	hans.mol@wur.nl +31317480318	Hans Mol, Wageningen Food Safety Research, Akkermaalsbos 2, (building 123), 6708 WB, Wageningen, The Netherlands
WU	Vera Silva (& on behalf of Paula Harkes, Esperanza Huerta Lwanga, Frank van Langevelde & Jerry Wells)	Vera.felixdagracasilva@wur.nl / sprint@wur.nl +3148429887	Vera Silva Wageningen University, SLM group, P.O. Box 47, 6700 AA Wageningen, the Netherlands
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Annex 3. Basic soil characterization

Annex 3A – Soil description

Method: visual analysis

Dig a hole that allows you to examine top- and subsoil; time and location should be defined with the farmer/land worker to don't affect the crop and harvesting. One soil profile is sufficient to describe the soil in the field. Depending on soil characteristics the depth may be between about 50 and 100 cm. As baseline, we recommend considering the following:

Define the limits of the A, B, and C horizons according to the FAO guidelines (FAO, 2006). Refer to description below

Soil classification according to the World Reference Base (WRB, International Union of Soil Sciences, see reference below);

Remark: Once the hole for soil description is excavated, for the sake of commodity, it is advisable to assess soil structure and soil texture.

Soil classification according to FAO (2006):

Master horizons and layers

The capital letters H, O, A, E, B, C, R, I, L and W represent the master horizons or layers in soils or associated with soils. The capital letters are the base symbols to which other characters are added in order to complete the designation. Most horizons and layers are given a single capital letter symbol, but some require two.

Currently, ten master horizons and layers and seven transitional horizons are recognized. The master horizons and their subdivisions represent layers that show evidence of change and some layers that have not been changed. Most are genetic soil horizons, reflecting a qualitative judgement about the kind of changes that have taken place. Genetic horizons are not equivalent to diagnostic horizons, although they may be identical in soil profiles. Diagnostic horizons are quantitatively defined features used in classification.

H horizons or layers

These are layers dominated by organic material formed from accumulations of undecomposed or partially decomposed organic material at the soil surface, which may be underwater. All *H* horizons are saturated with water for prolonged periods, or were once saturated but are now drained artificially. An *H* horizon may be on top of mineral soils or at any depth beneath the surface if it is buried.

O horizons or layers

These are layers dominated by organic material consisting of undecomposed or partially decomposed litter, such as leaves, needles, twigs, moss and lichens, that has accumulated on the surface; they may be on top of either mineral or organic soils. O horizons are not saturated with water for prolonged periods. The mineral fraction of such material is only a small percentage of the volume of the material and is generally much less than half of the weight. An O layer may be at the surface of a mineral soil or at any depth beneath the surface where it is buried. A horizon formed by illuviation of organic material into mineral subsoil is not an O horizon, although some horizons formed in this manner contain much organic matter.

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A horizons

These are mineral horizons that formed at the surface or below an O horizon, in which all or much of the original rock structure has been obliterated and which are characterized by one or more of the following:

- an accumulation of humified organic matter intimately mixed with the mineral fraction and not displaying properties characteristic of E or B horizons (see below);
- properties resulting from cultivation, pasturing, or similar kinds of disturbance;
- a morphology that is different from the underlying B or C horizon, resulting from processes related to the surface.

If a surface horizon (or epipedon) has properties of both *A* and *E* horizons but the dominant feature is an accumulation of humified organic matter, it is designated an A horizon. In some places, where warm and arid climates prevail, the undisturbed surface horizon is less dark than the underlying horizon and contains only small amounts of organic matter. It has a morphology distinct from the C layer, although the mineral fraction may be unaltered or only slightly altered by weathering. Such a horizon is designated A because it is at the surface. Examples of epipedons that may have a different structure or morphology owing

to surface processes are Vertisols, soils in pans or playas with little vegetation, and soils in deserts.

However, recent alluvial or aeolian deposits that retain fine stratification are not considered to be an A horizon unless cultivated.

E horizons

These are mineral horizons in which the main feature is loss of silicate clay, iron, aluminium, or some combination of these, leaving a concentration of sand and silt particles, and in which all or much of the original rock structure has been obliterated.

An E horizon is usually, but not necessarily, lighter in colour than an underlying B horizon. In some soils, the colour is that of the sand and silt particles, but in many soils coatings of iron oxides or other compounds mask the colour of the primary particles. An E horizon is most commonly differentiated from an underlying B horizon in the same soil profile: by colour of higher value or lower chroma, or both; by coarser texture; or by a combination of these properties. An *E* horizon is commonly near the surface, below an O or A horizon and above a B horizon. However, the symbol E may be used without regard to position in the profile for any horizon that meets the requirements and that has resulted from soil genesis.

B horizons

These are horizons that formed below an A, E, H or O horizon, and in which the dominant features are the obliteration of all or much of the original rock structure, together with one or a combination of the following:

- illuvial concentration, alone or in combination, of silicate clay, iron, aluminium, humus, carbonates, gypsum or silica;
- evidence of removal of carbonates;
- residual concentration of sesquioxides;
- coatings of sesquioxides that make the horizon conspicuously lower in value, higher in chroma, or redder in hue than overlying and underlying horizons without apparent illuviation of iron;
- alteration that forms silicate clay or liberates oxides or both and that forms a granular, blocky or prismatic structure if volume changes accompany changes in moisture content;
- brittleness.

All kinds of B horizons are, or were originally, subsurface horizons. Included as B horizons are layers of illuvial concentration of carbonates, gypsum or silica that are the result of pedogenetic processes (these

layers may or may not be cemented) and brittle layers that have other evidence of alteration, such as prismatic structure or illuvial accumulation of clay.

Examples of layers that are not B horizons are: layers in which clay films either coat rock fragments or are on finely stratified unconsolidated sediments, whether the films were formed in place or by illuviation; layers into which carbonates have been illuviated but that are not contiguous to an overlying genetic horizon; and layers with gleying but no other pedogenetic changes.

C horizons or layers

These are horizons or layers, excluding hard bedrock, that are little affected by pedogenetic processes and lack properties of H, O, A, E or B horizons. Most are mineral layers, but some siliceous and calcareous layers, such as shells, coral and diatomaceous earth, are included. The material of C layers may be either like or unlike that from which the solum presumably formed. A C horizon may have been modified even where there is no evidence of pedogenesis. Plant roots can penetrate C horizons, which provide an important growing medium. Included as C layers are sediments, saprolite, and unconsolidated bedrock and other geological materials that commonly slake within 24 hours when air dry or drier chunks are placed in water and when moist can be dug with a spade. Some soils form in material that is already highly weathered, and such material that does not meet the requirements of A, E or B horizons is designated C. Changes not considered pedogenetic are those not related to overlying horizons. Layers having accumulations of silica, carbonates or gypsum, even if indurated, may be included in C horizons unless the layer is obviously affected by pedogenetic processes; then it is a B horizon.

References:

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- Zak, D.R. 2003. Soil Profile descriptions. NRE 430 / EEB 489: http://www.umich.edu/~nre430/PDF/Lab02_Handout.pdf
- World Reference Base (WRB) classification, FAO: http://www.fao.org/soils-portal/soil-survey/soil-classification/world-reference-base/en/ and http://www.iuss.org/

Annex 3B – Texture per soil horizon

Preferred method: Hydrometer

Materials

• 2mm- Sieved soil (50 g fine-textured, 100 g if sandy)	• Bouyoucos hydrometer
• Electric mixer and cup	• Thermometer (-20° - 110°C)
• Sedimentation cylinder (1000 mL)	• Sodium hexametaphosphate, 1N

Procedure

- Place 50-100 g of dry soil (50 g if fine-textured, 100 g if sandy) into a soil dispersing cup. The soil should be sieved first to remove any fragments larger than sand (>2mm). Record the weight to at least 0.1g.
- Fill cup to within 5 cm of the top with distilled water at room temperature
- Add 5 ml of 1N sodium hexametaphosphate.
- Allow to soak for 15 minutes (high-clay soils only).
- Attach cup to mixer; mix 5 minutes for sandy soils, 15 minutes for fine-textured soils.
- Transfer suspension to sedimentation cylinder; use water to get all of sample from mixing cup.
- Fill cylinder to 1000-mL mark with distilled water.
- Insert plunger into cylinder, carefully mixing suspension thoroughly by pulling plunger upwards in short jerks. After removing plunger, begin timing. Carefully place hydrometer into suspension; note reading at 40 seconds. Repeat this procedure 2-3 times to obtain an average of 40 sec reading.
- After final 40-second reading, remove hydrometer, carefully lower a thermometer into the suspension and record the temperature (°C). Mixing raises temperature by 3-5°C, so it is important to record the temperature for both hydrometer readings (40 sec and 2 hr).
- After the final reading, do not disturb the cylinder. It should stand undisturbed for two hours.
- Make up a blank cylinder with water and sodium hexametaphosphate. Record the blank hydrometer reading.
- Take a hydrometer reading at 2 hours, followed by a temperature reading.

Calculations

- Temperature correction factor, **T** (may be different for each reading):
  T = (Observed temperature - 20°C) * 0.3
- Corrected 40-second reading:
  40-sec (corr) = 40-sec reading - Blank + **T**
- Corrected 2-hour reading:
  2-hr (corr) = 2-hr reading - Blank + **T**
- $\%\mathrm{Sand} = \frac{\text{Dry soil weight} - \text{corr 40 sec reading}}{\text{Dry soil weight}} * 100\%$
- $\%\mathrm{Clay} = \frac{\text{corr 2 hr reading}}{\text{Dry soil weight}} * 100\%$
- $\%\mathrm{Silt} = 100\% - (\%\mathrm{Sand} + \%\mathrm{Clay})$

The Textural classification should be made according to the USDA soil taxonomy using for example the free software:
https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167
For more details on the assessment, refer to ASTM D2488-06, USDA, USCA (2017), and Soil Survey Staff (2014).

Alternative method

The texture can be assessed using the hydrometer described above or by using the laser diffraction method (LDM) (see: Ryzak and Bieganowski, 2011).

Laser analyzer Mastersizer 2000 (Malvern Instruments) with Hydro MU adapter was used to determine the PSD of soil samples. The measurement range of the apparatus is 0.02–2000 lm. The Hydro MU adapter is equipped with:

- a stirrer; to prevent sedimentation of particles in the beaker, by circulating the sample in the measuring system and facilitating flow through the measuring cell. The speed of rotation of the stirrer ranges from 0 to 4000 rpm and can be regulated in increments of 50 rpm.
- an ultrasonic probe; with a maximum power of 35 W and a frequency of 40 kHz. The amplitude ranges from 2 to 20 µm and can be regulated in increments of 0.5 lm (defined by the manufacturer as 2–20 units in increments of 0.5 units).

For the determination of PSD, the Mastersizer apparatus uses two sources of light: red (wavelength 633 nm) and blue (wavelength 466 nm).

For more details, refer to Ryzak and Bieganowski (2011).

References

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- Manual for the Soil Chemistry and Fertility Laboratory-Analytical Methods for Soils and Plants, Equipment, and Management of Consumables. NUGI 835, Ghent, Belgium (ISBN 90-76603-01-4), 1999, 243 pp.
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- USDA, United States Department of Agriculture: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/ref/?cid=nrcs142p2_054253#fragments

Annex 3C – Bulk density / total porosity

Core method (ISO 11272)

Soil samples should be taken from the topsoil between 0 and 20 cm, in 3 plots/field. We recommend taking 3 to 5 samples per depth and per field plot.

Sample holders: thin-walled metal cylinders with a volume varying between 100 cm^3 and 400 cm^3, a steel cap for driving into the soil, and a driver (ISO 11272). It is preferable to use cylinders with a diameter $d \geq 75$ mm and a high $h \leq d$ (Lal et al., 2001) to avoid compaction of soil sample. If not available, use cylinders with 50 mm diameter and 51 mm height. Since the cylinders are different in diameter, appropriate hammering head needs to be purchased as well.

Care should be taken to avoid compaction during sampling: in case the level of the soil surface inside the cylinder is lower than the one of the soil outside the cylinder, we recommend repeating sampling when soil is dryer.

Material

i. Ring core of known volume (see sizes above)	ii. Wooden plank
iii. Putty knife	iv. Trowel
v. Plastic Hammer	vi. Air tight seal plastic bags

Steps for bulk density measurement

- Remove organic layer (leaves, twigs, moss, etc.) by carefully scraping this material aside, so that the top of the mineral soil is exposed and level (flat).
- Place the ring, sharpened side down, on top of the soil.
- Push the ring into the soil as much as you can with your hands. Do not twist the ring.
- Once you have pushed the ring in as much as possible with your hands, place a piece of hard wood (or the putty knife if wood is unavailable) over the ring and gently and evenly hammer it into the soil until the top is flush with the soil surface. Use caution to prevent compaction. If you meet strong resistance due to rocks or large roots, start over in a new spot.
- With your trowel and/or soil knife, dig a small trench on one side of the ring slightly deeper than the ring. The excavated area needs to be large enough that you can place the putty knife horizontally below the base of the ring.
- Push the putty knife under the ring until it lies under the entire ring.
- Gently remove the ring from the soil, holding the putty knife in such way, so that no soil is lost from the bottom of the sample.
- Clean the surfaces of the ring so that the soil sample is clean and cut the excessive soil with a sharp knife, and proceeding from the centre of the ring towards the edges peel the soil off gently.
- Push the soil out of the ring into the plastic bags, being careful not to spill any soil outside of it.
- Keep the samples in a chamber at a relative humidity of 100% to avoid desiccation.

Soil core samples should be taken in appropriate moisture conditions. Too wet or too dry soil at sampling can lead to erroneous values (e.g. for clay soils). Shortly after sampling, soil samples should be conserved at a temperature of around 5°C in a cool box or refrigerator. It is important to avoid disturbing soil samples during transport.

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Laboratory analysis

1. Weigh an ovenproof container in grams (W_1).
2. Carefully remove the all soil from the bag into the container. Dry the soil for 1 night in a conventional oven at 105ºC.
3. When the soil is dry weigh the sample on the scales (W_2).
4. Dry soil weight (g) = $W_2 - W_1$

Bulk density (g/cm^3) = Dry soil weight (g) / Soil volume (cm^3)
Bulk density is usually expressed in g/cm^3.
Total porosity (%) = (1 – BD/PD) x 100
Where PD of most mineral soils = 2.65 g/cm^3. In the case of peat soils, average PD = 1.45 g/cm^3 (Redding and Devito, 2006), while average PD of clay minerals = 2.70 (Reeves et al. 2006). More details on the assessment can be found in the Manual for the Soil Chemistry of Belgium, and Soil Survey Staff, 2014.

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- https://en.eijkelkamp.com/products/augering-soil-sampling-equipment/st-steel-soil-sample-60-x-56-number-73568.html

Annex 3D – pH

We recommend using *KCl* solution for the measurements of pH since it is more appropriate for differences in agricultural land

Protocol (Van Reuuwijk, 2002):

I) Transfer 10.0 g air-dried fine soil (< 2mm) into 50-ml centrifuge tubes with screw lids. Include two blanks. Register sample weight (resolution 0.01 g)

II) Add 25 ml 1 M KCl and close the flasks.

III) Place the flasks in the rotor shaker and extract for two hours.

IV) Dismount the flasks from the shaker. Before opening the flasks for measurement, shake by hand once or twice.

V) Immerse pH electrode in upper part of suspension.

VI) Read pH when the reading has stabilized. (NOTE: The reading is considered stable when it does not change more than 0.1 unit per 30 seconds)

Materials and reagents

Only use acid washed glass and plasticware.

i. Scale (resolution 0.01 g)

ii. *M* KCl. Dissolve 373 g KCl in distilled water and make up to 5 litre

iii. Rotor shaker

iv. Centrifuge tubes, 50 ml, with screw caps (OBS: acid washed)

v. pH-meter with glass-calomel electrode

vi. Conductivity meter

References

- Van Reeuwijk, L.P. 2002. Procedures for Soil Analysis, International Soil Reference and Information Centre (ISRIC) Technical Paper, No. 9, ISRIC, p. 19.
- USDA-United States Department of Agriculture. Soil pH – Soil Quality Kit, Guides for Educators. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_053293.pdf

Annex 3E – SOC determination

1. **Elemental analysis** (preferred)

With this technique, small quantities of soil are weighted on a precision balance and placed in an automatic sampler. The sampler feeds the samples one by one in an elemental analyser where they are combusted at high temperature (>1000°C) with addition of oxygen (Dumas combustion). The gaseous combustion products go into an inert gas carrier and after various traps to remove undesired components and after applying separation techniques, the quantity of CO_2 from the sample is quantified to determine the total carbon content of the soil sample. If inorganic carbon is present in the sample, it can be removed by acidification before combustion. The elemental analysis procedure in fact allows the simultaneous analysis of the content of C, H, N and S in the sample, so these instruments are also called CHNS analysers (or CN- or CHN-analysers depending on which elements can be analysed simultaneously). Elemental analysis has become the routine method for SOC used by most soil test laboratories.
General information can be found under:

http://www.rsc.org/images/CHNS-elemental-analysers-technical-brief-29_tcm18-214833.pdf

2. **SOC determination by loss on ignition**

If elemental analysis is not possible, loss on ignition (LOI) can be used. But it is important to know that the basic assumption is that LOI is due only to combustion of soil organic matter (SOM), and that the commonly used conversion factor of 0.58 can be used to convert SOM to SOC. The disadvantage of the method is that LOI is dependent on combustion temperature and duration, and that some structural water is also lost during combustion. A constant conversion factor of 0.58 is therefore not justified and the conversion factor is moreover soil dependent (Jensen et al., 2018). That is why elemental analysis should be used if possible.

Procedure

	i. Turn muffle furnace on and set control dial at obvious mark. Do so approximately two hours before you want to start procedure in order to bring temperature of furnace up to desired temperature of 950°C. ii. Turn on electronic balance and calibrate it at beginning of the workday.
	iii. Remove wire basket from large desiccator near furnace. Using a forceps to move crucible, not your fingers, weigh the clean and dry porcelain crucible directly. Note and record its name from the outside bottom of the crucible. Also record sample powder name which is being weighed into crucible. No weighing paper is required. As usual, record all weighing to four decimal places.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

<table>
<tr>
<td> </td>
<td>iv. Add approximately 1 gram of finely ground rock powder.
v. Record weight of crucible plus sample. Make sure bottom of crucible has some identifying mark; if not, make one by scratching bottom of crucible.
vi. Do steps 3 through 5 for each of your sample powders. A maximum of 8 samples can be done at one time.
vii. Place in muffle furnace for 60 minutes at 950° C.</td>
</tr>
<tr>
<td colspan="2">viii. Remove basket from furnace and place in desiccator as quickly as possible. Let cool to room temperature and weigh. (Keep in mind the demonstration of this procedure).
ix. Weight of sample before heating minus the weight after heating is "initial" loss on ignition.
5. (NOTE: if sample has lost weight, the loss is positive; if it has gained weight, the loss is negative).
x. Determine % loss on ignition by dividing "initial loss" by initial weight of sample and multiply by 100.
xi. To obtain the true loss on ignition for the sample:
6. a) Multiply 0.111348 times the weight % FeO (determined by titration).
7. b) Add this value to the loss on ignition (whether + or -). This sum is the true loss on ignition.
xii. Empty each crucible, clean them, and finally rinse them with acetone and set on a paper towel to air dry. Load them back into the wire basket using forceps and put basket back into furnace for several minutes. Remove and place basket in the large desiccator. Now they are ready for immediate use by the next person.</td>
</tr>
</table>

References

- Jensen, J.L., Christensen, B.T., Schjönning, P., Watts, C.W., Munkholm, L.J., 2018. Converting loss-on-ignition to organic carbon content in arable topsoil: pitfalls and proposed procedure. European Journal of Soil Science 69, 604-612.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 4. Total Suspended Solids by Gravimetric Determination

Standard Operating Procedure AMBL-105-D

METHOD SUMMARY

This SOP describes the procedure for measuring total suspended solids in water and wastewater. This method is based on Method 2540 D of *Standard Methods for the Examination of Water and Wastewater*, 23rd Edition.

ENVIRONMENTAL HEALTH AND SAFETY

Hazards Assessment: This method involves the use of a convection oven and optionally a muffle furnace, the handling of natural waters or untreated wastewaters that potentially contain pathogenic organisms. The specific hazards associated with this method are as follows.

Burns: Burns to the hands or arm are possible if the sides of the convention oven or muffle furnace are touched when placing the sample into or removing it from the oven or furnace. Burns will also occur if the hot porcelain evaporating dish itself is touched.

Biological Hazard: The presence of pathogenic organisms must be assumed, regardless of the water sample source. Natural waters, sewage and wastewater all contain bacteria, funguses, parasites, and viruses that can lead to intestinal or other infections, including but not limited to diarrhea, fever, nausea, cramps, vomiting, headaches, conjunctivitis (pink eye) and Hepatitis A.

Safety Equipment and Engineering Controls: This method requires that you wash your hands with soap when finished handling samples and that an eye wash station be located nearby.

Personal Protective Equipment (PPE): This method requires the use of the following PPE.

Gloves (nitrile, PVC or neoprene)
Safety goggles or glasses
Laboratory coat

Analysis-derived Wastes and Disposal:

Waste Generated	Hazardous (Y / N)	Disposal
This procedure generates a dried solid residue on the surface of a glass-fiber filter.	**N**	The solid residue is considered

METHOD DESCRIPTION

1. Introduction and Applicability

Total suspended solids is a measure of the undissolved solid matter in a water that remains on the surface of a filter after all the water has been evaporated. Suspended solids affect water quality by making it unfit or unsafe to drink, aesthetically unacceptable for recreational use and aquatic habitats, and unsuitable for use in many industrial or other applications. A known volume of a well-mixed sample is filtered through a standard glass- fiber filter, collecting the solid residue on the surface of the filter. The filter and residue is evaporated to a constant weight condition in an oven maintained at a temperature of 103-105°C. The mass of the dried residue is determined and used to calculate the concentration of total suspended solids in the sample.
This method is applicable for measurement of total suspended solids in all natural waters, in raw, process and treated agricultural, municipal and industrial wastewaters. This method is not considered applicable to wastewater slurries behaving as a Newtonian fluid, non-Newtonian fluids or treated drinking water.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

2. Apparatus

a. Glass-fiber filter, with a 47 mm diameter, nominal pore size ≤ 2.0 μm and ≥ 1.0 μm, and no binders.
b. Graduated cylinder, Class A
c. Wide-bore pipet, Class B
d. Forceps capable of lifting and holding a filter without tearing or puncturing it.
e. Filter pans, aluminum or other inert material, to hold filters.
f. Convection oven operated at 103-105°C for drying samples to a constant weight condition.
g. Muffle furnace operated at 550 ± 50°C.
h. Desiccator containing a desiccant that responds (color change) to moisture or a hygrometer that measures moisture.
i. Analytical balance capable of weighing to the nearest 0.1 mg or less.
j. Magnetic stirrer and stir bar (optional).
k. Blender or homogenizer (optional)
l. Beaker, low-form Class B or Class A having a volume sufficient enough to fully contain the sample and prevent sample loss from spillage or splattering when mixing.
m. Filtration funnel assembly for a 47 mm size diameter filter.
n. Vacuum suction flask, 1000 mL capacity.

3. Reagents

a. Distilled or deionized water.

4. Procedure

a. Read Method 2540D Total Suspended Solids Dried at 103-105°C (*Standard Methods*).
b. Prepare a glass-fiber filter by placing and centering a filter disk onto the filter support screen of the filtration apparatus and attach the funnel.
Apply a low to moderate vacuum and rinse the filter with three successive volumes of ≥30 mL deionized or distilled water. Leave the vacuum on until all traces of water have been removed from the filter.
Turn off the vacuum and use forceps to carefully remove the filter from the filtration apparatus support screen by lifting and holding the filter only by the outer edge and transfer the filter to a filter pan. Place the filter and pan into a drying oven operated at a temperature of 103-105°C. Dry the filter at this temperature for no less than 60 minutes if measuring only total suspended solids or alternately, ignite the filter in a muffle furnace at a temperature of 550 ± 50°C for no less than 15 minutes if volatile suspended solids will be determined (see SOP 105E). Cool the rinsed and dried filter to room temperature, remove the filter from the filter pan, weigh and record its weight - this is the tared weight of the filter. Replace the pre-rinsed and pre-weighed filter in the filter pan and store it in a desiccator until used.
c. Equilibrate the sample's temperature to that of the room's temperature and use a pipet or graduated cylinder to transfer a volume of well-mixed sample onto the filter with the vacuum applied. Use a graduated cylinder for samples having solids that clog the wide bore pipet tip. Select a sample volume that will result in a dried residue ranging from 2.5 to 200 mg. Avoid filtration times exceeding 10 minutes. Rinse the entire surface area of the exposed filter with three successive volumes of ≥10 mL deionized or distilled water. Allow the water to completely drain between each rinsing and leave the vacuum on until all traces of water have been removed from the filter. Turn off the vacuum and using forceps carefully remove the filter from the filtration apparatus support screen by lifting and holding the filter only by the clean outer edge without solids. Transfer the filter to a filter pan.

d. Dry the sample in a convection oven at a temperature of 103-105°C for no less than 60 minutes. Drying samples overnight is acceptable and an appropriate procedural step for the AMBL. In most circumstances, this ensures that constant weight has been achieved.
e. Remove the filter pan containing the sample from the oven, cooling it to room temperature and then using the forceps carefully remove the filter from the filter pan without touching the dried residue and weigh it. Record this as the first 103°C weight.
f. Repeat the drying cycle for no less than 60 minutes, and again cool, weigh and record the second 103°C weight.
g. Calculate the weight change between the first and second weights, and if the change is >0.5 mg, repeat the drying cycle until the change in weight between the final weight and the previous weight is ≤0.5 mg.
   Record and use this final 103°C weight.

5. Calculation and Reporting

a. Calculate the concentration of total suspended solids
   Total Suspended Solids, as mg_TSS/L

$$mg\ total\ suspended\ solids/L = \frac{(A - B)\ .\ 1000}{Sample\ volume, mL}$$

   where A = final 103°C weight of the dried residue + the tared filter, mg,
   F = tared filter weight, mg, and
   S = mL of sample volume.

b. Report as "Total Suspended Solids (TSS) = ____ mg/L" or as "____ mg/L TSS"
c. Identify any sample that yields a residue mass < 2.5 mg or > 200 mg and report the results as an "estimate" because the mass has exceeded the criteria of this analysis.

6. Quality Control

The suspended material in the sample's liquid medium is considered relatively non-homogeneous and, in addition the filtration step and inconsistent or incomplete sample drying, can lead to variable results and thus quality control is considered to be an important part of this method.

a. Analyze a method blank (a clean, dried, and tared filter) with each batch of 20 or fewer samples. If a single sample is being analyzed, a method blank must also be analyzed.
b. Analyze at least one sample in duplicate with each batch of 20 or fewer samples. If a single sample is being analyzed, this sample must be analyzed in duplicate.
c. Each analyst must analyze a laboratory-fortified blank and laboratory-fortified blank duplicate sample set (LFB/LFBD) to demonstrate initial capability and thereafter analyze a LFB/LFBD sample set for each 20 samples analyzed, not including method blanks, to demonstrate ongoing capability. The analyst may analyze their initial LFB/LFBD sample set at the same time they analyze their first sample, but then after measuring 20 samples, including duplicate samples, must analyze another LFB/LFBD sample set. Prepare a LFB control sample for total suspended solids by weighing 100 mg Celite 545 (record the actual weight) to the nearest 0.1 mg. Dilute in distilled water to a volume of 1 liter. Measure the total suspended solids of this standard LFB sample.
d. Evaluate the results obtained from QC data as follows: The method blank results must demonstrate that the initial tared dish weight does not differ by more than ± 0.5 mg. The relative percent difference (RPD) of duplicate samples should not exceed an absolute value of 10%. The RPD of the LFB/LFBD analyses should not exceed an absolute value of 10%. Additionally, the percent recoveries for the LFB samples should be plotted on a control chart for an overall laboratory evaluation of capability associated with each new LFB material prepared.

7. Bibliography

Rodger B. Baird, Andrew D. Eaton, and Eugene W. Rice (2017) *Standard Methods for the Examination of Water and Wastewater*. APHA, Washington, DC, 23nd Edition.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 5. Detailed procedures for water sampling

I - Field procedures

By whom:
• Any scientist or technician with some experience in sampling on aquatic systems who should be supported by at least one more person for better efficiency and overall safety.

When:
• It must be synchronised with water, fish and macroinvertebrates sampling.

How:
• Following the recommendations described in sections I-II.

II - Water body selection and characterization

1 The water bodies should be located within the same catchment as the agricultural fields in each CSS, ideally connected to them.

2 The selected water bodies (river, stream, channel, lake or pond) must be coarsely characterized in terms of hydromorphology, using the River Habitat Survey (for that please fill the RIVER HABITAT SURVEY 2003 VERSION: SITE HEALTH AND SAFETY ASSESSMENT. Please download the form here:

https://mega.nz/file/61M0AD7I#t_Tof488RDopoDExCG9C5ZMjAlhhvW5Nexe7VlQ7E7o

3 Always respect health and safety guidelines while working in the field. Especially important when sampling in riverine ecosystems is the following: sampling should be carried out in a safe section of the river or stream - select easily accessible sites, avoiding steep or slippery banks, deep sections (water levels should be below the knees) and ensuring that you can see the bottom while sampling; never sample alone; wash your hands after sampling and decontaminate with alcohol.

III – Water sampling:

Sampling Equipment

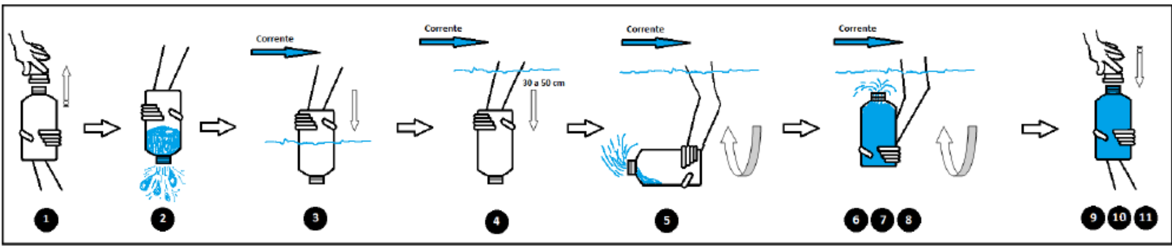
• Waders
• Gloves
• sample bottles
• Multiparameter equipment (pH, O2, EC and TDS)
• G Flow meter
• Ethanol, 70% v/v
• Copies of field protocols
• Pencils, clipboard
• First aid kit
• Global Positioning System (GPS) unit
• Applicable topographic maps

Sampling procedures:

4 The water sampling must be collected sub-superficially. The bottle must be used to directly collect the water sample.

5 Collect one single sample per water system. Maximum of 6 water samples per CSS (one per water body).

<u>Operating procedures for direct surface water sampling:</u>



1. The sample bottles should only be open immediately before the water sampling.
2. Pre-rinse the bottle with sample water before sampling (if applicable);
3. Hold the bottle by the base;
4. Dip the bottle with mouth facing down about 10-30 cm;
5. The mouth of the bottle should be placed against the current;
6. Under water, slowly invert the position of the bottle, turning the mouth up (or to top) so that the neck is slightly higher than the base;
7. Fill the bottle completely (if applicable);
8. Bring the bottle to the surface;
9. Screw the cap immediately after sampling and close tightly;

Field Record Form

The following information should be included on the field record for each sampling site:

- Project number
- Sampling date and time
- Sampling site location (including site name and number, county/parish, latitude/longitude, waterbody name)
- Sampling site description - waterbody type, type bed, presence of macrophytes, shadowing and average flow velocity.
- Any particular observation that may constrain sample representativeness or feasibility.
- Collectors' names and institutions

Sample Packaging

1. Samples should be labelled appropriately. Please place the label shown in Figure 2 in the lateral surface of the glass bottle.

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CSS (name/nº)______________________/___

Sampling site

Sampling date_____/____/_________

WATER SAMPLE

Operator (name)_________________

2. **The sample must be placed and transported in a thermal bag with frozen accumulators to the lab.**

3. The samples must be stored under cold (4ºC) and dark conditions, for a maximum of 24h, until shipping.

Annex 6. Detailed procedures for sediment sampling

This annex provides guidance on the sampling, transport and shipment of sediment for PPP analysis. It was based on the USEPA procedures for sediment sampling: LSASDPROC-200-R4.
https://www.epa.gov/sites/production/files/2015-06/documents/Sediment-Sampling.pdf

Field procedures

By whom:
• Any scientist or technician with experience in sampling on aquatic systems who should be supported by at least one more person for better efficiency and overall safety.

When:
• It must be synchronised with water, fish and macroinvertebrates sampling.

How:
• Following the recommendations described in sections I-II.

I - Water body selection and characterization

- The water bodies should be located within the same catchment as the agricultural fields in each CSS, ideally connected to them.
- The selected water bodies (river, stream, channel, lake or pond) must be coarsely characterized in terms of hydromorphology, using the River Habitat Survey (for that please fill the RIVER HABITAT SURVEY 2003 VERSION: SITE HEALTH AND SAFETY ASSESSMENT. Please download the form here:
- https://mega.nz/file/61M0AD7I#t_Tof488RDopoDExCG9C5ZMjAlhhvW5Nexe7VlQ7E7o
- The sediment at each sampling site should be briefly characterized by providing granulometry (classes of particles) analysis and organic matter content (see Annex 3B, 3E)
- Always respect health and safety guidelines while working in the field. Especially important when sampling in riverine ecosystems is the following: sampling should be carried out in a safe section of the river or stream - select easily accessible sites, avoiding steep or slippery banks, deep sections (water levels should be below the knees) and ensuring that you can see the bottom while sampling; never sample alone; wash your hands after sampling and decontaminate with alcohol.

II – Sediment sampling:

Sampling Equipment

• Waders
• Gloves
• Coring Device (optional: a Stainless-steel scoop) *
• Stainless steel homogenization container
• Mixing rod (or any other tool that allows mixing)
• Sampling container (e.g. 250ml polypropylene containers with a screw cap)
• Plastic box (500ml) covered with aluminium foil
• Ethanol, 70% v/v
• Sodium hypochlorite

***Figure 5-1.** Example of a coring device and a stainless-

- Copies of field protocols
- Pencils, clipboard
- First aid kit
- Global Positioning System (GPS) unit
- Applicable topographic maps

Sampling procedures:

•Collect one composite sample per water system. Maximum of 6 composite sediment samples per CSS (one per water body).

• The collection of sediment must be carried out in stretches with fine-grained material, avoiding gravels. For the composite sample please collect the sediment in 3 sites distancing 10 m from each other.

• Collect the sediment using a Core sampler. Core samplers are used to sample vertical columns of sediment. They are particularly useful when a historical picture of sediment deposition is desired since they preserve the sequential layering of the deposit. They are also particularly useful when it is desirable to minimize the loss of material at the sediment-water interface.

NOTE: In case you do not have a core sampler, you can use a simple stainless-steel scoop, excess water may be removed/drained from the scoop. However, this may result in the loss of some fine-grained particle size material associated with the substrate being sampled. Care should be taken to minimize the loss of this fine-grained material.

• Place the 3 samples into a stainless-steel homogenization container and mix thoroughly with a rod to obtain a composite sample.

The composite samples should be divided for:
1 . PPP analysis – the sample must be packed into a 250ml polypropylene container with a screw cap. The sample container must be filled quickly and completely to eliminate head space;
2. Organic matter and granulometry – the sample must be placed in a plastic box (500ml) lined with aluminium foil.

• Do not forget to decontaminate (sodium hypochlorite), rinse (distilled water) and dry the material before moving into a new sampling site.
The sample container must be filled quickly and completely to eliminate head space.

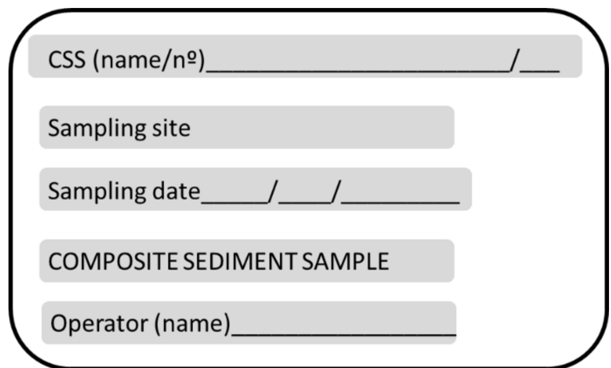
If you are not experienced with the procedures, please familiarise yourself with this sampling method. There are several video sources available on the web for the purpose. We recommend the following link, **https://www.youtube.com/watch?v=So17DAsYak8**

Field Record Form
The following information should be included on the field record for each sampling site:

- Project number
- Sampling date and time
- Sampling site location (including site name and number, county/parish, latitude/longitude, waterbody name)
- Sampling site description - waterbody type, type bed, presence of macrophytes, shadowing and average flow velocity.
- Any particular observation that may constrain sample representativeness or feasibility.
- Collectors' names and institutions

Sample Packaging

Samples should be labelled appropriately. Please place the label shown in Figure 2 both in the lid and in one lateral surface of the glass container and in the plastic box. The glass container as well the plastic box should be secured in a plastic bag.



The sample must be immediately stored in an ice chest and cooled.

he samples must be stored under cold (4ºC) and dark conditions, for a maximum of 24h, until shipping. For longer periods between sampling and shipping, deep freezing (-20º) is recommended.

Shipping

Composite sediment samples for PPPs analysis must be shipped on ice (ideally dry ice) to:

Wageningen Food Safety Research (WFSR)
SPRINT project
Akkermaalsbos 2
6708 WB Wageningen
The Netherlands

Contact details: Jonatan Dias, jonatan.dias@wur.nl, +31317486918

By Fast Courier Service/ Express post, in thermal boxes ideally dry-ice, but in at the minimum ice, or frozen ice packs.

Annex 7. Detailed procedures for benthic macroinvertebrates sampling

This annex provides guidance on the sampling of benthic macroinvertebrate communities for the purpose of analysing the ecological status of freshwater ecosystems, in particular *sensu* the European Water Framework Directive (EU-WFD) hence for the calculation of Ecological Quality Ratios (EQR) and the consequent standard classification of the waterbody. For an overview of the bioassessment under the scope of the WFD and its premises, the official guidance available at each EU Member-State and/or available scientific publications (e.g. Santos et al. 2021[8]) can be relevant sources. Therefore, standard field sampling procedures are recommended and any deviations should be discussed with the UAVR team to avoid unexpected inconsistences in the data analysis stage. Recommendations regarding the processing of the samples, preservation and shipping of the organisms are also given.

Field procedures

By whom:
• Any scientist or technician with some experience in sampling on riverine ecosystems who should be supported by at least one more person for better efficiency and overall safety.

• If applicable, by someone to whom the collection of biological samples from freshwater ecosystems has been permitted. For example, in Portugal, there is the need to ask for a permit to collect any organism from nature, regardless of the type of organism or its status of conservation, which is issued by the national Institute for the Conservation of Nature and Forests. There can be similar requirements for other partners.

When:
• Ideally during Spring, as this is the growth season for most benthic macroinvertebrates; it can be synchronised with fish sampling if more convenient.
• After the approval of a detailed sampling plan developed by the partner in charge of the sampling, taking into account the recommendations described in section I below.

How:
• Following the recommendations described in sections I-III.

I - Water body selection and characterization

- The water bodies should be located within the same catchment as the agricultural fields in each CSS, ideally connected to them.
- The selected waterbodies MUST be riverine ecosystems since typical benthic macroinvertebrate communities for the type of assessment that will be made can hardly be found in lentic ecosystems such as ponds or lakes. Small rivers or creeks, easily accessible and that can be walked with no boat assistance are suitable. Artificial channels with a limited sediment bed (e.g. bottom of concrete, as typical in some irrigation systems) are not appropriate as the benthic communities would hardly develop therein.
-

[8] Santos JI, Vidal T, Gonçalves FJM, Castro BB, Pereira JL (2021). Challenges to water quality assessment in Europe – is there scope for improvement of the current Water Framework Directive bioassessment scheme in rivers? Ecological Indicators 121: 107030. DOI 10.1016/j.ecolind.2020.107030

- No more than 3 water bodies or 3 sites within a selected waterbody (in this later case, the sites should be at least 500 m apart from each other) can be selected per CSS, and these should be within the set selected for fish sampling.

- The water at each sampling sites should be briefly characterized by measuring temperature, pH, dissolved oxygen, conductivity, total dissolved solids (alternative: colored dissolved organic carbon) and total suspended solids (loss-on-ignition of the residue from vacuum filtration through GF/C filters). Additional parameters such as Chl a concentration, total organic carbon, nutrient (NO_3^-, NO_2^- e PO_4^{3-}) contents are welcome for a better characterization of the water.

- The sediment at each sampling site should be briefly characterized by providing granulometry analysis and organic matter content.

- The selected water bodies or sites within a selected waterbody must be coarsely characterized in terms of hydromorphology, using the River Habitat Survey (for that please fill the RIVER HABITAT SURVEY 2003 VERSION: SITE HEALTH AND SAFETY ASSESSMENT. Please download the form here:

- https://mega.nz/file/61M0AD7I#t_Tof488RDopoDExCG9C5ZMjAlhhvW5Nexe7VlQ7E7o
- Always respect health and safety guidelines while working in the field. Especially important when sampling in riverine ecosystems is the following: (i) macroinvertebrate sampling should be carried out in a safe section of the river or stream - select easily accessible sites, avoiding steep or slippery banks, deep sections (water levels should be below the knees) and ensuring that you can see the bottom while sampling; never sample alone; wash your hands after sampling and decontaminate with alcohol.

II – Macroinvertebrates sampling:

Sampling Equipment

a. Appropriate scientific collection permit(s) b. Standard hand net (1 mm - 500 µm mesh; square frame, e.g 0.33 x 0.33 m, or at least with a flat bottom) – see Figure 1. c. Chest waders d. One bucket (10 L) or a tray for collecting the sample from the net e. One plastic box with lid per site to collect the macroinvertebrates captured with the net (recommended dimensions: 30 x 15 x 15 cm) f. Ethanol, 70% v/v g. Waterproof box labels h. Macroinvertebrate Sampling Field Data Sheet i. Applicable topographic maps j. Copies of field protocols k. Pencils, clipboard l. First aid kit m. Global Positioning System (GPS) unit n. Sodium hypochlorite and distilled water for cleaning the net between sites	 **Figure 6-1.** Standard hand net for sampling macroinvertebrates

Sampling method:

• Benthic macroinvertebrates should be collected at each site by kick-sampling, using the standard hand net, in a 10-m long river stretch at each selected sampling site.

• To assure similar effort among sites, sampling should be performed during a similar time period (3-5 min) at all sites, along 3-4 transects covering the diversity of microhabitats (aquatic vegetation, riffles, main canal river bed) and sediment types (rocks, gravel, sand, etc.) of the sampled river stretch.
• The hand net should be placed to collect against the flow, with the flat bottom tightly pressured against the river bed to collect all macroinvertebrates released while kicking. The strength of the kicking should be adjusted depending on the type of river bed (e.g. sandy sediments do not require vigorous kicking).
• If the rived bed is stony, additionally wash stones into the sampler (3 stones randomly picked per microhabitat covered in sampling).
• After completing the sampling, reverse the net bag and wash the contents into the bucket (the water from the sampled river course can be used for this purpose). Carefully inspect the net to ensure that all organisms were washed out. Inspect the bucket and discard large stones and debris, as well as the excess of water.
• For appropriate preservation and transport, transfer the sample from the bucket into the plastic box and add the alcohol (approximately 1:1 v/v, sample:alcohol). Fit the lid tightly.
• Do not forget to decontaminate (sodium hypochlorite), rinse (distilled water) and dry the net, bucket and waders before moving into a new sampling site.

If you are not experienced with the procedures, please familiarise yourself with this sampling method. There are several video sources available on the web for the purpose. We recommend the following link, particularly from frame 2:40 onwards: https://youtu.be/yoFK4hCu42c

Field Record Form

The following information should be included on the field record for each sampling site:

- Project number
- Sampling date and time
- Sampling site location (including site name and number, county/parish, latitude/longitude, waterbody name)
- Sampling site description - waterbody type (river, stream or creek), type of stream bed, presence of macrophytes, shadowing and average flow velocity.
- Sampling method, specifying the microhabitats sampled and whether (and how many) stones were washed additionally.
- Any particular observation that may constrain sample representativeness or feasibility.
- Collectors' names and institutions

Sample Packaging

Preserved samples should be labelled appropriately. Please place the label shown in Figure 2 both in the lid and in one lateral surface of the box containing the sample and cover with transparent adhesive tape.

Note 1: Because alcohol is being used, please make sure that the whole label is covered and not blurred so the information is not lost during sample transport.

Note 2: If the sample is to be shipped rather than hand-delivered to the processing laboratory, field collection staff must ensure that the lid is secure and that the samples are packed properly to avoid any losses.

Laboratory procedures

When:
• For appropriately preserved macroinvertebrate samples: when the laboratory schedule allows.

How:
• Following the recommendations described in sections I-II.

The whole sample processing should be made in an appropriate laboratory facility under cleanroom conditions.

Note: Care must be taken during sample processing to avoid contaminating samples. Potential sources of contamination include dust, instruments, utensils, work surfaces, and containers that may be in contact with the samples. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily. To avoid cross-contamination, all labware used in sample processing should be cleaned thoroughly before each new sample is to be handled.

I- Macroinvertebrate samples processing:

Material

• Sieve (500 µm mesh size)
• Plastic sorting trays
• Dissection tweezers
• Alcohol 70% v/v
• Tightly-lidded vessels for storing and shipping sorted samples

Sample sorting

Depending on the abundance of organisms in each sample and on how free of debris the sample is, run the whole procedure below as many times as necessary using aliquots until the whole sample is processed.

• Wash a portion of the sample through the sieve under running tap water.
• Remove large debris from the sieve.
• Wash the sample retained in the sieve into a tray with tap water. The water level in the tray should be adjusted as it best suits the sorting technique of the operator, but generally we recommend a maximum height of water of 5 mm.
• Sort the sample in the tray to collect organisms into a vessel with alcohol. Cap with the lid to avoid alcohol evaporation.
• After finishing sorting the whole sample, correct for the alcohol level (at least 1:1, biomass: alcohol), cap tightly with the lid and prepare the vessel for shipping (section 10.3) by adding the appropriate label.

Note: if you find parts of organisms, please collect them as well into the vessel. Depending on the taxa and on the integrity of the sample as a whole, parts can be relevant for the final outcome.

Shipping

Macroinvertebrate samples do not have extensive requirements for shipping as long as good preservation in alcohol is ensured as indicated above. Still, the presence of alcohol in the samples must be declared to the shipping company has this is a flammable reagent.

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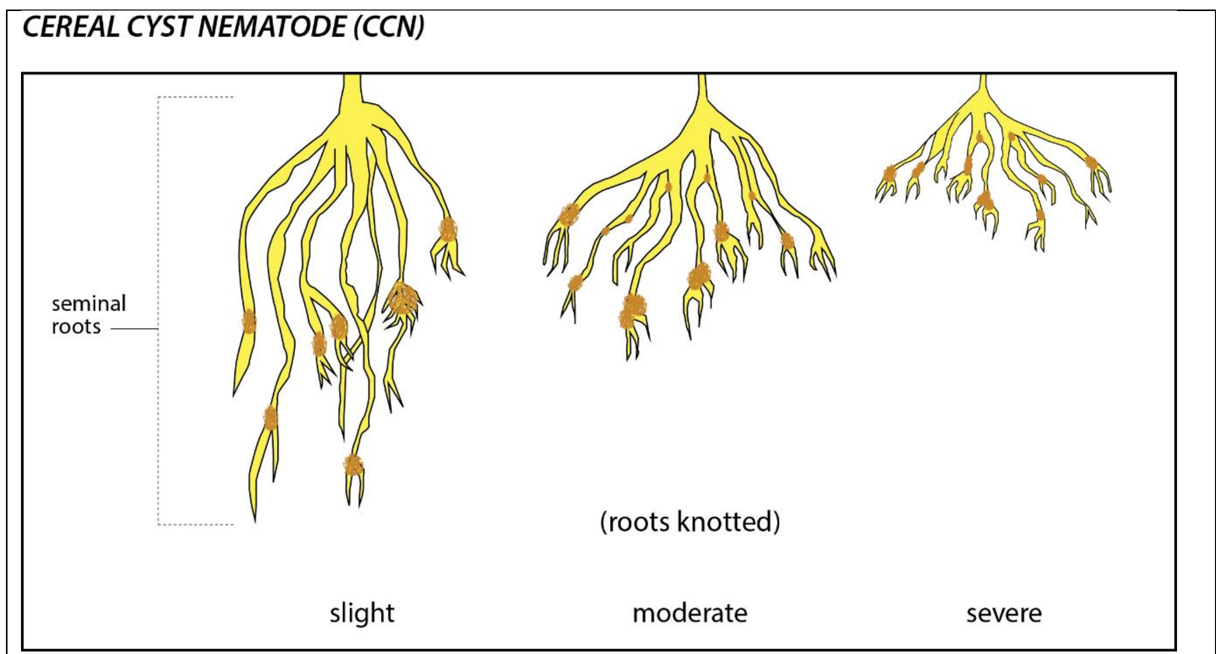
Annex 8. Root diseases assessment

Material

11- A small spade and bucket to place the plants in
12- A bucket of water for washing out the root systems
13- Additional clean water for final examination
14- A flat white tray containing water to be used for identifying and assessing root diseases

Procedure

- Carefully dig up 5 random plants/field, making sure that soil is left intact around the root system of the plant.
- For cereal root diseases, rate the severity of root disease identified. // For other diseases, indicate whether or not they are present in the crop.
- visually assess the area of crop affected by the disease.



Cereal cyst nematode	Score
Root length normal* on a severity scale of 0 - 5, rating is 0	0
Roots are 40 % shorter than healthy roots, on a severity scale on 0 - 5 rating is between 1 - 1.5	1
Roots are 60 % or more shorter than healthy roots, on a severity rating of 0 - 5 scoring 2 or more	2

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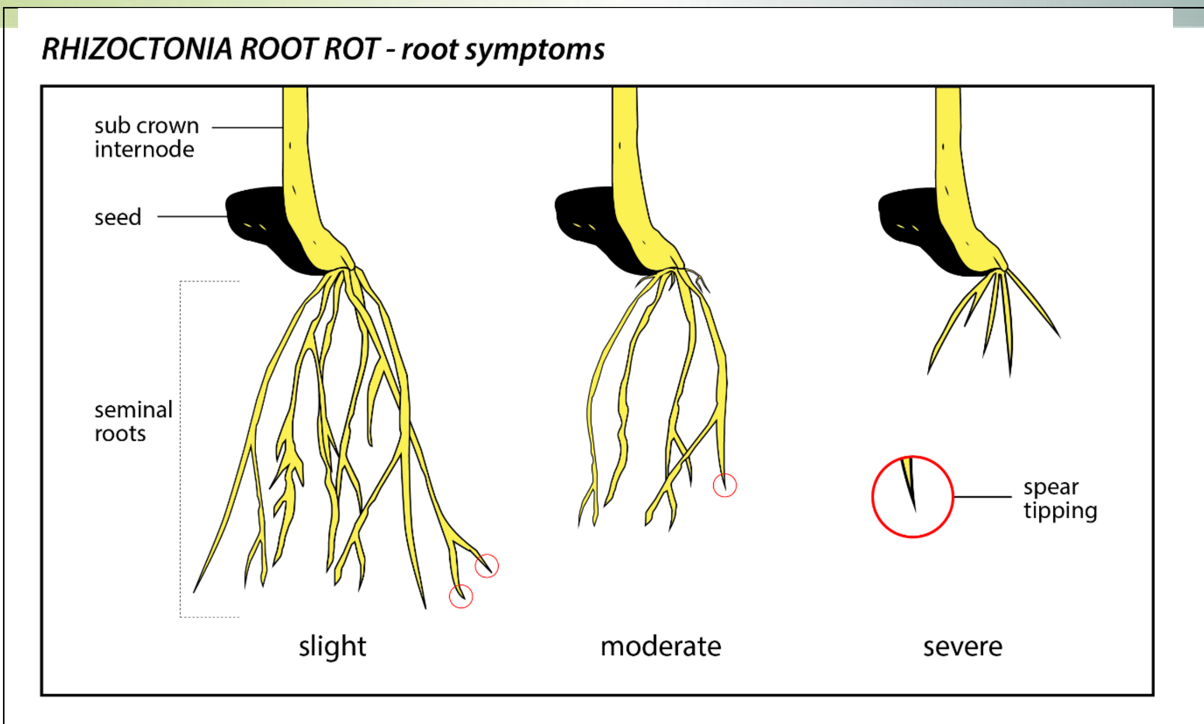
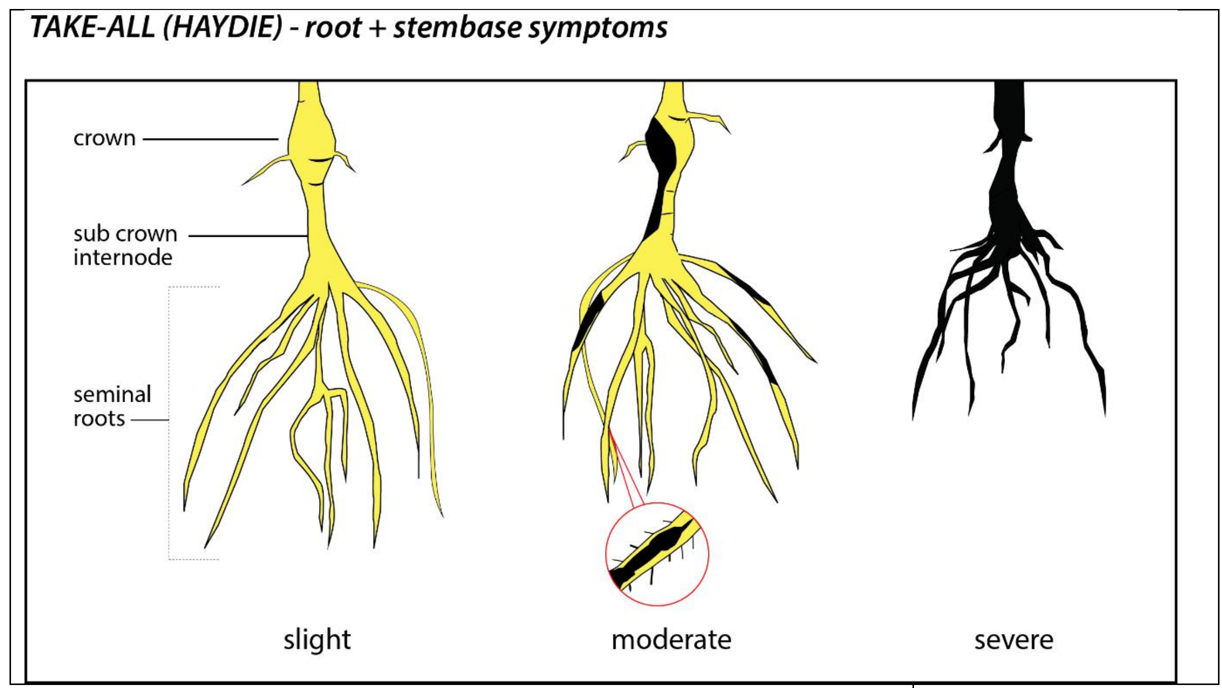


Rizoctonia	Score
No roots are shortened	0
Up to 25% of the roots are shortened	1
More than 25% of roots are shortened	2



Take-all	Score
No lesion visible	0
1 or 2 main seedling roots have lesions	1
3 or more seedling roots have lesions	2

References

- Department of Primary Industries and Regional Development. https://www.agric.wa.gov.au/mycrop/monitoring-root-disease

- Department for Environment Food and Rural Affairs, defra. http://adlib.everysite.co.uk/adlib/defra/content.aspx?doc=202989&id=203609
- Scoring based on Shepherd, T.G. 2000. Visual Soil Assessment. Volume 1. Field guide for cropping and pastoral grazing on flat to rolling country. horizons.mw & Landcare Research, Palmerston North. 84p

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Annex 9. Earthworm extraction

For this assessment, we propose using mustard solution because it is a harmless and low cost product, also it has consistent efficiency. Using mustard solution, earthworms can be effectively sampled from a depth of 25 cm (Valckx et al., 2011).

In this method you dig a 25x25x25 cm monolith for extracting the soil and assessing the earthworms by hand sorting (mainly epigeic and endogeic earthworms), then at the bottom of the hole you will pour a solution of mustard water for allowing it to percolate down. The mustard solution irritates the skin of earthworms and they come to the surface to avoid it, where they can be collected (mainly anecic earthworms), preserved and identified.

Preparation of the mustard solution: To make the solution, mix 2 litres of water with 20 grams ground yellow mustard seed in a container. This is the same powdered yellow mustard you will find in any grocery store (**Fig. 8-1**). You should mix up the mustard solution quite a bit to avoid its solidification on the bottom. A 2L jug of mustard solution is enough to sample a 25 cm x 25 cm sample plot (see for instance Valckx et al., 2011).

Earthworm extraction:

- Choose a representative plot to sample, and do the monolith randomly.
- Cut the vegetation and remove the leaf litter in your sample area.
- Place the frame (25cm x 25 cm) on the ground.
- Slowly pour half of the mustard water solution into the sample area.
- Over a period of 5 minutes, gather any worms that come to the surface being careful to wait until they are completely out of the ground
- After 5 minutes, pour the remaining mustard water into the sample area and again wait 5 minutes gathering any other worms that come to the surface.
- Have a collection tray to put them in until you're done, since they can come up in rapid succession.

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Figure 8-1. **Earthworms extraction (left side) and an example of yellow mustard seed (right side).** ***Add the mustard solution after the soil excavation, to collect the anecic earthworms.***

This technique works well for all species of earthworms, so first do the hand sorting and then must solution application. If it has been very dry, very hot or very cold in the week(s) prior to sampling they may not respond as very well since they may be in aestivation (earthworm version of hibernation). In contrast, if air temperatures have been moderate and it has rained recently they are likely to be active and respond well to the liquid extraction. An exception – if the soil is very compacted and/or has a poor structure (heavy clay, fields, roads, etc.) the extractant doesn't move well through the soil and the earthworms will not respond because the liquid doesn't reach them.

References

- Shepherd, T.G. 2000: Visual Soil Assessment. Volume 1. Field guide for cropping and pastoral grazing on flat to rolling country. horizons.mw & Landcare Research, Palmerston North. 84p
- Valckx J., Govers G., Hermy M., Muys B. 2011: Optimizing Earthworm Sampling in Ecosystems. In: Karaca A. (eds) Biology of Earthworms. Soil Biology, vol 24. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-14636-7_2

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Annex 10. Detailed procedures for fish sampling

Field procedures
By whom: Local fish experts (scientist or fishermen) with a license for fish collection and a person with a certificate in animal experimentation (scientist, vet).

Sample collection activities should be initiated in the field only after a sampling plan has been developed and approved. This section describes the criteria for the selection and characterisation of water body and sites, the target species and the recommended sampling techniques and equipment/material needed.

I - Water body selection and characterization

6 The water bodies should be located within the same catchment as the agricultural fields.

7 At least 3 water bodies may be selected for fish collection. It can consist of small rivers or channels of about 2 m large, or small lakes and ponds located in the vicinity of the agricultural fields (ideally connected to them). Distinct water bodies typologies can be selected per CSS.

8 In each water body, 5 fish must be collected to get one composite sample.

9 The selected water body (river, stream, channel, lake or pond) must be coarsely characterized in terms of hydromorphology, using the River Habitat Survey (for that please fill the RIVER HABITAT SURVEY 2003 VERSION: SITE HEALTH AND SAFETY ASSESSMENT. Please download the form here:

https://mega.nz/file/61M0AD7I#t_Tof488RDopoDExCG9C5ZMjAlhhvW5Nexe7VlQ7E7o

5 fish per water boby - at least 3 water bodies per CSS (max 20)

Figure 9-1 – Illustration of the sampling design in each study area.

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II- Target Species and Sample Type:

After reviewing information on each sampling site, the field collection staff should identify the target species that are likely to be found at the site. The selection of target species in freshwater systems must take into consideration the following criteria: i) avoid threatened and endangered species according the IUCN Red List categories; ii) avoid endemic species; iii) select species widely distributed across European freshwater systems;

Taking into consideration these criteria, we suggest the following species (Table 1):

Table 9-1 – Recommended fish target species.

Common name	Scientific name	Photo	Distribution	IUCN Red List category
Largemouth Bass	*Micropterus salmoides*		https://www.cabi.org/isc/datasheet/74846	Least concern
Common roach	*Rutilus rutilus*		https://www.cabi.org/isc/datasheet/66337	Least concern
Pumpkinseed sunfish	*Lepomis gibbosus*		https://www.cabi.org/isc/datasheet/77080	Least concern
Rainbow trout	Oncorhynchus mykiss		https://www.cabi.org/isc/datasheet/71813	Not evaluated
Common carp	*Cyprinus carpio*		https://www.cabi.org/isc/datasheet/17522	Vulnerable

If you have other species in your CSS that meet the defined criteria, you can choose these.

Organisms used in a composite sample must:

- be of the same species;
- satisfy all legal requirements of harvestable size or weight, or at least be of consumable size if no legal harvest requirements are in effect;
- be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length of the largest individual;

III- Fish sampling:

Sampling Equipment

A basic checklist of field sampling equipment and supplies is shown in Table 2

Table 9-2 - Field equipment/material needed for fish sampling.

- appropriate scientific collection permit(s)
- electrofisher equipment, seines. angling or passive nets (depending on the sampling method (Table 2)
- elbow-length insulated waterproof gloves
- chest waders
- buckets/livewells
- jars for voucher/reference specimens
- waterproof jar labels
- measuring board (500 mm minimum, with 1 mm increments)
- balance (gram scale)
- tape measure (100 m minimum)
- fish Sampling Field Data Sheet
- applicable topographic maps
- copies of field protocols
- pencils, clipboard
- first aid kit
- Global Positioning System (GPS) Unit
- Ice (wet ice, blue ice packets)
- Ice chests
- aluminium foil

Sampling methods:
There are several methods for fish collection. Various types of sampling equipment, their use, and their advantages and disadvantages are summarized in Table 3.
Please also look into the following you tube videos:
https://www.youtube.com/watch?v=Rttu1ZBRyEs&t=78s

Table 9-3 - Summary of Fish Sampling Equipment (USEPA 2000)

Device	Use	Advantages	Disadvantages
Electrofishing	Shallow rivers, lakes, and streams	Most efficiency non selection method. Minimal damage to fish. Adaptable to a number of sampling conditions (e.g.,	Nonselective stuns or kills most fish. Cannot be used in brackish, salt, or extremely soft water.

		boat, wading, shorelines). Particularly useful at sites where other active methods cannot be used (e.g., around snags and irregular bottom contours).	Requires extensive operator training. DANGEROUS when not used properly.
Seines	Shallow rivers, lakes, and streams.	Relatively inexpensive and easily operated. Mesh size selection available for target species.	Cannot be used in deep water or over substrates with an irregular contour. Not completely efficient as fish can evade the net during seining operation
Angling.	Generally species selective involving use of hook and line.	Most selective method. Does not require use of large number of personnel or expensive equipment.	Inefficient and not dependable
Passive nets	Lakes and rivers. Where fish movement can be expected or anticipated	Selectivity can be controlled by varying mesh size	Nets prone to tangling or damage by large and sharp spined fish. Gill nets will kill captured specimens, which, when left for extended periods, may undergo physiological changes.

Notes: Purchasing fish and shellfish from commercial fishers is acceptable; however, field sampling staff should accompany the fishers during both the deployment and collection operations to ensure that samples are collected and handled properly and to confirm the sampling site location. The field sampling staff can then ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination.

Field Record Form

The following information should be included on the field record for each sampling site:

- Project number
- Sampling date and time
- Sampling site location (including site name and number, county/parish,

latitude/longitude, waterbody name/segment number, waterbody type, and site

description)

- Sampling depth (specify units of depth)
- Collection method
- Collectors' names and institutions

Preservation of sample integrity

Between sampling sites, the field collection team should clean each measurement device by rinsing it with sodium hypochlorite and distilled water and rewrapping it in aluminium foil to prevent contamination

IV- Sample handling:

Species Identification

- As soon as fish are removed from the collection device, they should be identified to the species level.
- Nontarget species or specimens of target species that do not meet size requirements (e.g., juveniles) should be returned to the water. (**Note:** Please verify the national guidelines for returning captured invasive alien species and comply with those guidelines above this recommendation.)
- Since the objective is to determine the magnitude of contamination in specific fish, species, it is necessary that all individuals used in a composite sample are of a single species.

Note: Correct species identification is important and different species should never be combined in a single composite sample.

Initial Inspection, sorting and euthanization

i. Individual fish of the selected target species should be rinsed in ambient water to remove any foreign material from the external surface.

ii. Selected fish must be euthanized with an overdose of Ethyl 3-aminobenzoate methanesulfonate (MS222; CAS Number: 886-86-2). Guidelines for the Preparation and Use of MS222 can be found here: https://www.fau.edu/research-admin/comparative-medicine/files/guidelines-for-the-preparation-and-use-of-ms222-final.pdf

iii. Fish may be placed on ice immediately after capture to stun them, thereby facilitating processing procedures.

iv. Once stunned, individual specimens of the target species should be grouped by species and general size class and placed in clean holding trays to prevent contamination.

v. All fish should be inspected carefully to ensure that their skin and fins have not been damaged by the sampling equipment, and damaged specimens should be discarded.

Note: All procedures for handling and euthanasia of animals must follow the national recommendations.

Length or Size Measurements

Each fish within the selected target species should be measured to determine maximum body length (Figure 9-2). The maximum body length is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally).

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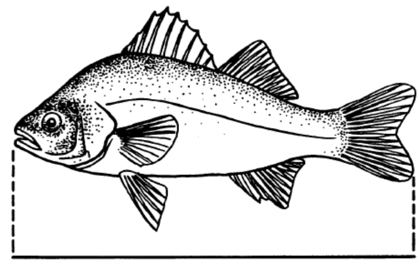
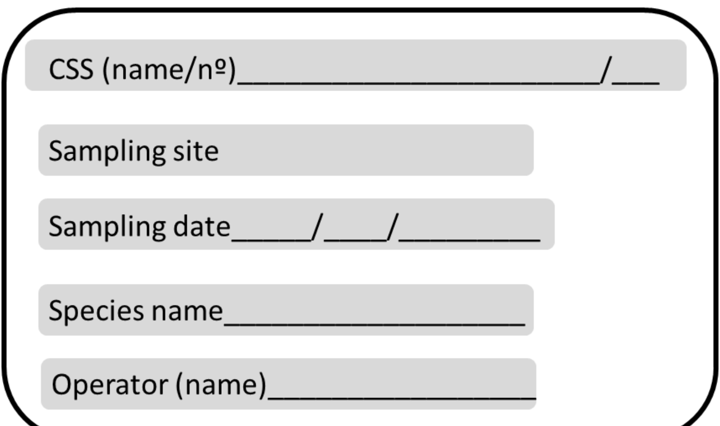


Figure 9-2 - Recommended measurements of body length for fish.

Sample Packaging

- After initial processing to determine species, size, and morphological abnormalities, each fish should be individually wrapped in extra heavy duty aluminium foil.
- The sample identification label shown in Figure 9-3 should be taped to the outside of each aluminium foil package, each individual fish should be placed into a waterproof plastic bag and sealed, and the same label should be attached to the outside of the plastic bag.
- All of the packaged individual specimens in a composite sample should be kept together (if possible) in one large waterproof plastic bag in the same shipping container (ice chest) for transport.
- Once packaged, samples should be cooled on ice immediately.


CSS (name/nº)_______________________/___

Sampling site

Sampling date_____/____/_________

Species name___________________

Operator (name)________________

Figure 9-3 - Example of a sample identification label.

Note: If the sample is to be shipped rather than hand-delivered to the processing laboratory, field collection staff must ensure that the samples are packed properly with adequate ice layered between samples so that sample degradation does not occur.

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Laboratory procedures

By whom: A person with a certificate in animal experimentation (scientist, vet).
This section includes recommended procedures for preparing composite samples of fish fillets, livers and gut contents.

All sample processing (i.e., filleting, removal of other edible tissue, homogenizing, compositing) should be done in an appropriate laboratory facility under cleanroom conditions.

Note: Care must be taken during sample processing to avoid contaminating samples. Potential sources of contamination include dust, instruments, utensils, work surfaces, and containers that may be in contact with the samples. All instruments, work surfaces, and containers used to process samples must be of materials that can be cleaned easily and that are not themselves potential sources of contamination. To avoid cross-contamination, all equipment used in sample processing (i.e., resecting, homogenizing, and compositing) should be cleaned thoroughly before each composite sample is prepared.

Filleting and the removal of the liver should be done on glass or PTFE (polytetrafluoroethylene) cutting boards that are cleaned properly between fish or on cutting boards covered with heavy duty aluminum foil that is changed after each filleting. Tissue should be removed with clean, high quality, corrosion-resistant stainless steel or quartz instruments or with knives with titanium blades and PTFE handles. Fillets or tissue homogenates may be stored in heavy duty aluminum foil.

Processing Fish Fillet and Fish Liver Samples for PPPs analysis and Fish gut content for microbiome analysis

Please note that fish fillet, fish liver and gut content samples must be removed from the same individual fish.

Processing in the laboratory to prepare fish fillet, fish liver and gut content composite samples for analysis involves (Figure 9-4):

- Inspecting individual fish
- Weighing individual fish
- Removing scales and/or otoliths for age determination (optional)
- Determining the sex of each fish (optional)
- Examining each fish for morphological abnormalities (optional)
- Scaling all fish with scales (leaving belly flap on);
- Filleting (resection) (Figure 9-5)
- Removing the liver (Figure 9-6)
- Removing the gut content (Figure 9-7)
- Weighing fillets
- Weighing liver

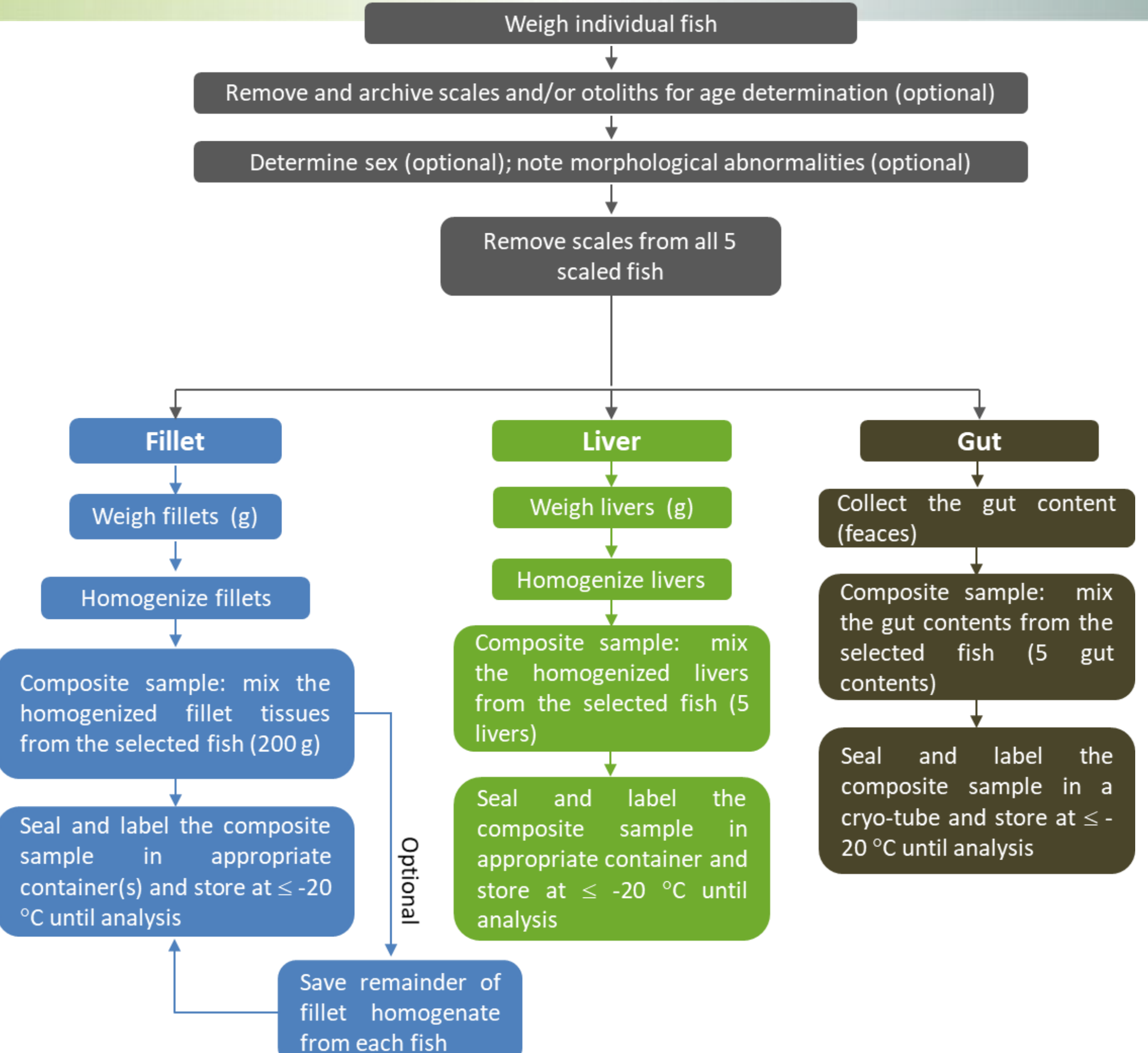

Figure 9-4 - Preparation of fish fillet and fish liver composite homogenate samples. Source: U.S. EPA, 1991d. (There is no need to remove fins)

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1 **Scaled Fish**

After removing the scales (by scraping with the edge of a knife) and rinsing the fish:

1b **Scaleless Fish**

Grasp the skin at the base of the head (preferably with pliers) and pull toward the tail.

Note: This step applies only for catfish and other scaleless species.

2 Make a shallow cut through the skin (on either side of the dorsal fin) from the top of the head to the base of the tail.

3 Make a cut behind the entire length of the gill cover, cutting through the skin and flesh to the bone.

4 Make a shallow cut along the belly from the base of the pectoral fin to the tail. A single cut is made from behind the gill cover to the anus and then a cut is made on both sides of the anal fin. Do not cut into the gut cavity as this may contaminate fillet tissues.

5 Remove the fillet.

Figure 9-5 - Illustration of basic fish filleting procedure. Source: U.S. EPA, 1991d.

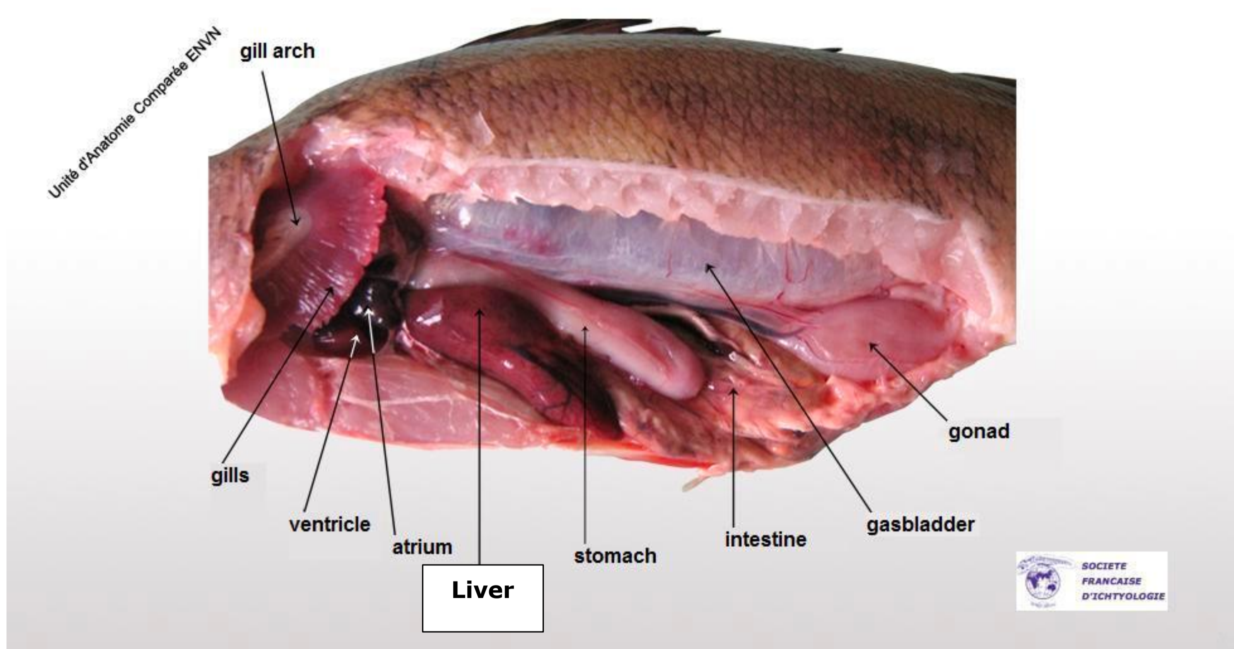

Figure 9-6 – Fish morphology.

Preparation of fish fillet and fish liver homogenates

To ensure even distribution of contaminants throughout tissue samples and to facilitate extraction and digestion of samples, the fillets and livers must be ground and homogenized prior to analysis.

i. Fish fillets as well as fish livers should be ground and homogenized using an automatic grinder or highspeed blender or homogenizer. Large fillets may be cut into 2.5-cm cubes with high-quality stainless steel or titanium knives or with a food service band saw prior to homogenization.

ii. The fillet and liver samples should be ground until it appears to be homogeneous.

iii. The ground samples should then be divided into quarters, opposite quarters mixed together by hand, and the two halves mixed together. The grinding, quartering, and hand-mixing steps should be repeated at least two more times.

Preparation of Composite Homogenates of fish fillet

- Composite homogenates of fish fillets should be prepared from equal weights of ideally 5 individual homogenates (corresponding to 5 fish). In the case of 5 fish, each individual homogenate must weight 40 g.
  - It is essential that the weights of individual homogenates yield a composite homogenate of adequate size to perform all necessary analyses. The recommended sample size of **200 g** is intended to provide sufficient sample material.

iv. Seal and label (200 g) composite homogenate in heavy duty aluminum foil.

v. The sample identification label shown in Figure 9-8 should be taped to the outside of each aluminium foil package, each composite sample should be placed into a waterproof plastic bag and sealed.

vi. Store at -20 °C until analysis.

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Preparation of Composite Homogenates of fish liver samples

- Composite homogenates of fish liver should be prepared from 5 individual homogenates (corresponding to 5 fish).
- Weight the composite homogenate.
- Seal and label (5 livers) composite homogenate of fish liver in heavy duty aluminum foil.
- The sample identification label shown in Figure 8 should be taped to the outside of each aluminium foil package, each composite sample should be placed into a waterproof plastic bag and sealed.
- Store at -20 °C until analysis

Processing composite gut samples for microbiome analysis

- Gut samples must be removed from the same fish used for the fillet and liver homogenates.
- After fish dissection, remove the fish gut (Figure 9-7) and collect the gut contents.
- To prepare a composite sample mix the individual gut contents in one plastic cryo-tube.
- The sample identification label shown in Figure 8 should be taped to the outside of each plastic cryo-tube. Put the three composite gut samples into a waterproof plastic bag and sealed.
- Flash frozen and stored samples at −80 °C before they will be transported to the laboratory for microbiome analysis (Ye a al 2014).

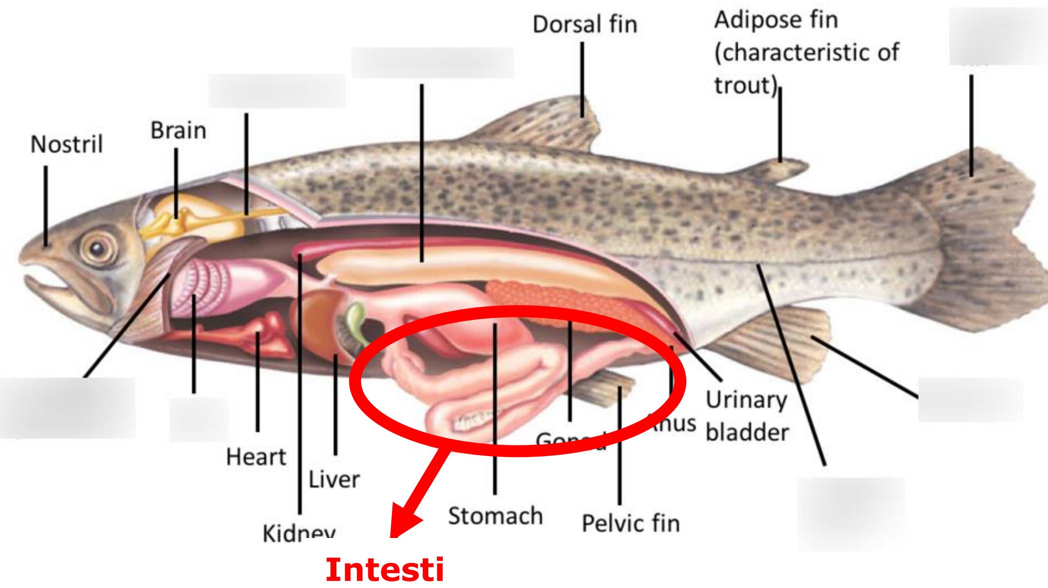


Figure 9-7 – Illustration of fish gut.

Composite Homogenates of fish liver

CSS (name/nº)_______________________/___

Sampling site number:

Species name_________________

Sampling date_____/____/________

Operator (name)________________

Processing date_____/____/________

Mass_________

Composite of fish gut contents

CSS (name/nº)_______________________/___

Sampling site number:

Species name_________________

Sampling date_____/____/________

Operator (name)________________

Processing date_____/____/________

Composite Homogenates of fish fillet

CSS (name/nº)_______________________/___

Sampling site number:

Species name_________________

Sampling date_____/____/________

Operator (name)________________

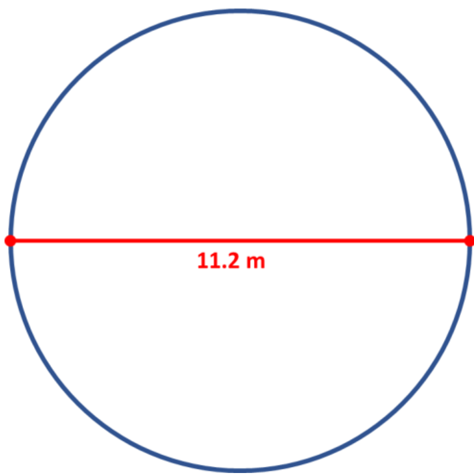
Processing date_____/____/________

Mass_________

Figure 9-8 - Example of an identification label for the fish fillet, fish liver and gut content composite samples.

Annex 11. Detailed procedures for ground dwelling insects sampling

A plot will be installed in 5 Conventional fields and in 5 organic fields per CSS. Each plot should be a circle of 5.6 m in radius (100 m^2):

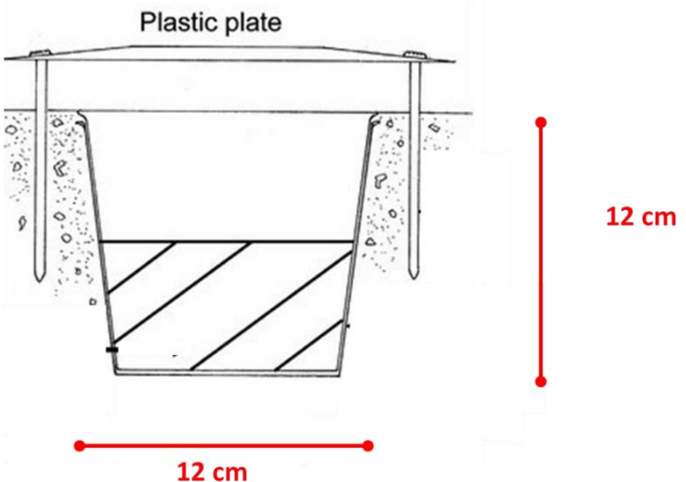


To be able to compare between results of all CSS, it is advisable to follow the setup described here (i.e. if the farmers allows it and there are no land operations during the almost 3 months it should be in the field). Each plot should be surrounded by a 30 cm high plastic enclosure, of which 5 cm is buried (the photo gives an example of a plot in a forest):

The plot areas should be treated as the remaining field by the farmer (including pesticide applications).

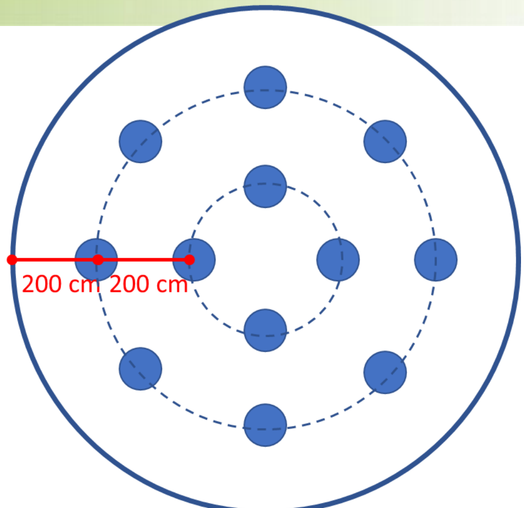
Per plot, 12 pitfall traps will be placed. The dimensions of the pitfall traps should be: radius 6 cm and 12 cm deep, with 20x20 cm white plastic roof 5 cm above trap. The cup should contain 2 cm of salt-saturated

water as a preservative. A simple adapted plastic container could work as trap. The dimensions of the containers should be the ones mentioned above, or as close to that as possible. The dimensions of the boxes should be send to Frank van Langevelde. All the 120 containers per CSS should have the same dimensions.



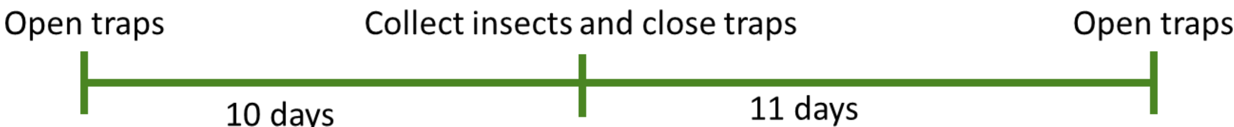
(The photo is an example of roof on a pitfall trap in the forest floor)

The 12 pitfall traps in each plot are placed according to two rings: 2 m and 4m from the border. Pitfall traps of each ring are evenly distributed:



The pitfall traps are open for the first 10 days of the collection event. After 10 days, the pitfall samples are sieved into a strainer and rinsed to eliminate the salted water (only 10 days to prevent that insects deteriorate in the trap and cannot be recognized anymore). Then, store the insects in 70% ethanol for long-term preservation (pool all samples per plot per collection event) the insects should be fully submerged in the ethanol. 2 cm of salt-saturated water is added into the traps and their lit closed. 11 days after collection, the traps are opened and a new cycle starts. (so opening every 3 weeks).

1 collection event of 3 weeks is thus:



If possible, CSS teams can involve the farmers in the cycles. Ideally farmers open the traps, CSS team collect insects and close the traps (and do flying insects measurements – see below).

Record per sample (and write on sample that will be sent to Wageningen): plot ID and date.

Make a photo of every plot and record GPS position.

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Annex 12. Detailed procedures for flying insects sampling

We will collect flying insects (i.e. bees, butterflies, wasps, flies, etc.) in 5 C and 5 O fields per CSS. In each of these fields we will do 1 transect, and will have 3 collection events.

The transects should be done in the fields were the pitfall are, and performed preferably in the same days as the insects in the pitfall traps are collected (so transepts every 3 weeks). If the weather is bad, than postpone collection until the weather is appropriate. Collecting flying insects should be done 3 times (every 3 weeks) using the same transects due to environmental variation.

The transects should be 150 m in a straight line. Use a sweep net to collect all flying insects during 15 min (i.e. the transects should be walked and insects collected in 15 minutes). Emptying the sweep net only after 15 minutes.

The dimensions of the sweep net:

- Head width: 25cm
- Head length: 37cm
- Bag depth: 50cm
- Handle length: 50cm
- Overall length: 103cm
- Mesh size (~0.9 x 0.3mm)

All CSS leaders should use similar nets for their assessment.

If this should be done on the land I foresee trouble with the farmer. We cannot sweep the land any time of the season. Some plants are quite vulnerable when they are young.

Do not touch the plants with the sweep net. So the pesticides are then not swept in the net. The pesticide concentration from the insect will not be affected by the sweeping as long as the plants are not touched.

Collecting the flying insects should be carried out between 10:00 and 17:00 h on days with dry weather, low wind speeds and temperatures above 15 °C.

Then store the insects in 70% ethanol for long-term preservation (pool all samples per transect per collection event).

Record per sample (and write on sample that will be sent to Wageningen): plot ID and date. Make a photo of every transect and **record GPS position of the start and end of each transect**.

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 13. SOP human urine collection, storage and transportation

Authors: Hans Mol, Karsten Beekmann (WR, Wageningen, The Netherlands)

Version: 2.0

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Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Background

The aim of this SOP is to provide information for collection of human urine samples according to the SPRINT "Research Protocol case study sites" (File no. XXX), and their aliquoting, storage and transportation. The samples will be analysed for the following endpoints:

a) Pesticides and/or their metabolites
b) Clinical parameters: Urinary electrolytes (Na/K), Kidney Injury Molecule-1 (KIM-1), N-acetyl-β-d-glucosaminidase (NAG), creatinine, and urinary proteins

A urine sample is going to be collected from each participant on a single occasion (the day of the visit from SPRINT research team with interview, blood sampling, and stool sample) during the field study. Per participant, the first morning void will be collected. 10 % of the participants will be asked to collect follow-up spot samples on the day of collection of the morning urine sample and the next day. Prior to laboratory analyses, samples need to be appropriately stored and transported. Do not discard (but instead safely store) any residual urine until you have verified that the shipment was successful. Also some CSS might want to consider secondary use of biomaterials for other purposes as long as this is arranged in the ethics approval (both purpose and timeline of storage after SPRINT project stops)

The procedures are described in this document. They were based on SOPs drafted in previous projects on pesticides biomonitoring [1,2].

Schematic procedure steps

At home subjects

CSS

Wageningen, NL

Distribution materials to CSS

Preparation and distribution materials to subjects

First morning void

Collect urine in measuring beaker and transfer portion into urine container

Store sample in cool box powered by electricity (12V/220V) with added ice packs

Collection of samples from subjects, transport to CSS facility
Aliquoting of urine into multiple test tubes. Temporary storage (-18°C)
Transport on dry-ice by 24-h delivery service to WR (WFSR)
Keep residue!

Store any remaining urine in original container at -18°C

Register, storage of tubes at -18°C, one tube at -80°C (back-up), forward designated tubes to lab(s) for clinical analysis

Materials

Subjects are provided with the following materials:

- instructions (see Annex A)

- Bottles, wide neck, with screw cap (LDPE, 250 mL, VWR cat.no. 215-5632P) https://nl.vwr.com/store/product/545717/flessen-met-brede-hals-en-schroefdop
-
- Measuring beaker with handle (Lamaplast, Polypropylene, VWR 1000 mL, VWR cat.no. 215-4356) https://nl.vwr.com/store/catalog/product.jsp?catalog_number=215-4358

- Centrifuge tubes, 12 mL (Greiner bio-one, PP with HDPE screw cap, round bottom, VWR cat.no. 391-3471) https://nl.vwr.com/store/product/575205/centrifuge-tubes
  Or Greiner Bio One Polypropylene Tubes, Round Bottom, Sterile, with White Screw Cap 12 mL, 900/Pk Disposable Plastic Test Tubes | Fisher Scientific

- 2 x 9.0 mL Greiner Urine tube (https://shop.gbo.com/en/row/products/preanalytics/venous-blood-collection/vacuette-tube/z-no-additive/455001.html) for Sodium, Potassium, creatinine, and urinary proteins.
- 2 x 9.0 mL Greiner Urine tube for KIM-1-1 and NAG analyses

- Sealable safety bag with sticker (see *Labelling of the samples* below)
    - https://www.daklapack.nl/medische-verpakkingen/vloeistofdichte-safetybags/safetybag-internationaal-165-mm-x-285-mm-transparant/8712963008178
- 1 coolbox with sufficient blue ice packs (e.g. MOBICOOL Mirabelle MM24 AC/DC elektrische koelbox, 20 liter, 12/230 V voor auto, vrachtwagen en stopcontact: Amazon.nl)
- 1 pen

Procedures

Collection of urine samples by participants

Preparation: the urine will be collected in the morning directly after awakening and getting up (first morning void). In case the participant needs to go to the toilet at night, we will not advise to collect the urine then. The participant is asked to collect the urine in the measuring cup and then pour a portion into the urine container (very important to report the time of urine sampling on the diary form (Annex C)).

It will be asked on the diary form to note the last time of toilet visit before urine collection.

Collection: it is important that participants wash their hands before the collection to prevent contamination. For the subjects' convenience, the urine will be collected first in the provided 1000 mL measuring beaker. It does not matter if only part of the urine is collected, for this research the minimum required volume is 50 ml which is 1/5 the volume of the container with the screw cap. After collection in the measuring beaker, the participant needs to pour the urine into the 250 mL container until it is approximately half full. Remaining urine can be discarded. The container will be closed securely with the screw cap.

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After collection: the participant is asked to put the urine container into a safety bag with his/her SPRINT participant ID and write date/time of sampling on the label of the safety bag. The plastic bag will be placed in the provided cool box with ice packs.

Transport to CSS facility, and aliquoting of urine

Blood sampling is done at the subjects home. The laboratory technician taking the blood sample will pick up the cool box with the urine and stool sample and transport it together with the blood samples to the central sample collection/distribution point for the CSS.

Aliquoting of the urine.

From the 50-125 mL urine, aliquots will be prepared in multiple tubes. Detailed instructions for this are provided in Annex B. This will be done at the central sample collection/distribution point for the CSS. Aliquoting of the urine samples will be done on the day of urine sampling. If not possible due to unforeseen circumstances, perform aliquoting before 10 AM of the day following the day the urine was produced, which is roughly within 24h of sample collection by the subject. In case samples are not immediately aliquoted, then temporarily store them in the refrigerator (2-10°C) [do not freeze at this stage].

The content of the urine container will be aliquoted into tubes according to the following scheme (do not overfill as the stopper may come off when freezing at destination):

- 3x 10-11 mL in 12 mL tubes for pesticide biomarkers.
- 2 x 7-8 mL in 9mL Greiner Urine tube for Sodium, Potassium, creatinine, and urinary proteins.
- 2 x 7-8 mL in 9mL Greiner Urine tube for KIM-1-1 and NAG analyses
- Total minimum required: 58 mL (see aliquoting for instructions what to do if the volume is less)

After attaching the corresponding labels (see *Labeling of the samples* below) to each tube, all tubes are put into a sorbent pouch (which can absorb excess liquid during transportation) and placed into a labeled safety bag and stored in the freezer (-18°C) until transport to the analytical laboratory in Wageningen, The Netherlands.

Store the original urine container with remaining urine in the freezer (-18°C), at least until confirmation of safe receipt of the tubes at the analytical laboratories. After that, they can be discarded unless agreed otherwise with the CSS leader.

Transport from CSS to analytical laboratories

To send the package with a courier service and follow EU regulations regarding the sending of biomaterials and dry-ice (see https://europepmc.org/article/med/30539463)

Arrange shipping details, prepare and label shipping containers appropriately.

- Addresses, responsible person and contact details, hazard label dry ice
- Shortly before pickup by carrier, combine samples in styrofoam box and fill with sufficient amount of dry ice.

- For each box, prepare a list of the content (origin, sample type, sample codes) and include it in the box.
- Prepare an e-mail announcing the day of shipment, and the courier tracking code. Attach an excel file listing the content (sample list with codes) of each box included in the shipment and send that to the WFSR contact person.

- Ship at the beginning of the week (preferably Monday, Tuesday) to ensure arrival before the weekend.

It is of utmost importance that the samples remain frozen during the shipment.

Addresses for shipment for analyses:

Urine samples for pesticide analyses:

Wageningen Food Safety Research (WFSR),
SPRINT project,
Akkermaalsbos 2,
6708 WB Wageningen,
The Netherlands

Contact details: Jonatan Dias, jonatan.dias@wur.nl, +31317486918

Urine samples for clinical parameters

Att: Mr. Maurice van Dael
133 HEV
Centrale Ontvangst Goederen Radboudumc
Geert Grooteplein Zuid 30
6525 GA Nijmegen
The Netherlands

Contact details: maurice.vandael@radboudumc.nl, +31619622869

WFSR will check the content of the boxes received with the information supplied by email from the sender. The three 12 mL tubes will be registered in the WFSR-LIMS system. Two tubes will be stored in the freezer at -18°C, one tube will be stored in the ultra-freezer at -80°C. WFSR will analyse the urine for pesticide biomarkers of exposure.

The tubes for determination of clinical parameters will be temporarily stored at -18°C.

Upon arrival samples are processed according to the assigned laboratory SOPs

Labeling of the samples

SPRINT participant label, for accurate specimen labeling. To track and report the result of the urine samples it is important that a simple and unique labelling procedure is used. The printed stickers used must be suitable for storage of the samples at -80°C.

Since urine samples will be obtained from all participants in the participating counties the following uniform tracking code is proposed for the urine samples:

[ISO-Alpha county code]_[Human][unique SPRINT participant identification number]_[Urine indicator]_[Tube count/total tubes]

E.g.: NL_H000_U_1/5, NL_H000_U_2/5, NL_H000_U_3/5, NL_H000_U_4/5, NL_H000_U_5/5

The sealable safety bags will be labelled before distribution to the subjects in the following manner:

[ISO-Alpha county code]_[Human][unique SPRINT participant identification number]_[Urine indicator]

E.g.: NL_H000_U

The bags should be labelled with a sticker that is suitable for storage of the samples at -80 °C, and have sufficient empty space for participants to add date and time of collection of sample.

References

[1] A. Oerlemans, PTJ Scheepers. SOP for collection of urine samples. Original title: OBO-3B-01. SOP voor het verzamelen van ochtendurine, v1.0 24 February 2016.

[2] HBM4EU Joint survey of pesticides: details of approach and contributions. Additional Deliverable Report AD15.7 WP15 - Mixtures, HBM and human health risks Deadline: April 2019. Re-upload by Coordinator: 30/09/2019. https://www.hbm4eu.eu/deliverables/

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex A instructions sample collection urine

You are participating in the research project SPRINT. As part of this project, we kindly ask you to collect a urine sample in the (early) morning. The sample will be analysed for pesticides. Please read the instructions below carefully.

The day before urine collection, you have received the following materials from the SPRINT CSS research team:

- A cool box with blue ice packs directly from the freezer
- 1 large measuring beaker with handle
- 1 urine container with screw cap
- 1 sealable safety bag with label
- 1 pen

Before collection:

- Upon receipt of the sampling materials, please place the ice packs from the cool box in your home freezer until you collect the urine sample the next morning.
- *Place the cool box at a place outside sun/heat and with access to electrical power, either 12V or 220V. Connect to power supply and switch on the cool box active cooling.*
- Place the measuring beaker and the urine container in or near the lavatory the evening before the morning of sample collection.

> Tip: in order not to forget you need to collect the urine sample in the (early) morning, you could place the urine container on the closed toilet lid.

Time of collection:

- Collect the first morning urine, this is the first time you need to urinate after getting up in the morning, preferably before having breakfast.

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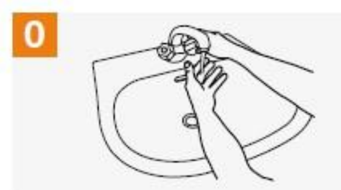
Figure 1: Instructions for hand washing

Duration of the entire procedure: 40-60 seconds



Wet hands with water;

Apply enough soap to cover all hand surfaces;

Rub hands palm to palm;

Right palm over left dorsum with interlaced fingers and vice versa;

Palm to palm with fingers interlaced;

Backs of fingers to opposing palms with fingers interlocked;

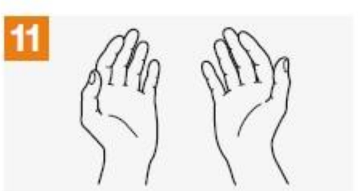
Rotational rubbing of left thumb clasped in right palm and vice versa;

Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

Rinse hands with water;

Dry hands thoroughly with a single use towel;

Use towel to turn off faucet;



Your hands are now safe.

Figure copied from WHO guidelines on hand hygiene in health care (2009).

Collection of the urine sample:

- Wash your hands before collection of the urine sample (see **Figure 1**).
- Collect the urine in the large measuring beaker. Then pour a portion into the urine container until it is approximately half to three quarters full. The remaining urine from the measuring beaker can be discarded in the toilet.
- Close the urine container with the screw cap, and tighten.
- Place the urine container in the sealable safety bag. Do not seal the bag.
- Please wash your hands when completing your bathroom visit.
- Please fill in diary form (Annex C) with respect to date and time of the urine sample, last time of previous toilet visit before urine collection, and additional questions about duration of sleep, coffee intake, physical activity, smoking, medication use, and bladder infections.
- Place the safety bag in the cool box.
- Get the ice packs from your freezer and put the ice packs in the cool box.
- Please keep the cool box connected to the power supply and out of the sun/heat until is picked up by the SPRINT CSS research team.

We thank you for your participation in the study and for following the study procedures.

If you forgot to collect the first morning urine sample, please inform the SPRINT CSS research team and your date will be rescheduled.

Add contact information of CSS:

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Annex B instructions aliquoting of urine samples

Each subject will collect a urine sample of approx. 56-125 mL (container half full). The cool box with the urine (and stool) sample will be picked up by the SPRINT CSS research team.

The urine needs to be aliquoted into different tubes/portions. This annex describes how aliquoting of the urine needs to be done.

Where: Aliquoting is done at the central point of collection/distribution of samples of the CSS.

When: Aliquoting needs to be done preferably on the day the urine sample was collected by the participant, and in any case before 10h in the morning following the day of collection by the participant. Until the moment of aliquoting, the sample is stored cooled/refrigerated (2-10°C), but not frozen/in the freezer. After aliquoting, all tubes are put into a sorbent pouch (which can absorb excess liquid during transportation) and placed into a safety bag labelled with SPRINT participant ID and date and stored in the freezer (-18°C or lower) until transport to the analytical laboratory in Wageningen, The Netherlands.

Materials:

- 1 pair of nitrile gloves (M and XL)
- Optional: variable pipet 1-10 mL e.g. Single-channel pipettes, mechanical, fixed / variable volume, Eppendorf Research (VWR, 1,000-10,000 µL, cat.no. 613-0868) https://nl.vwr.com/store/product/en/2173752/mechanische-eenkanaalspipetten-vast-of-variabel-volume-eppendorf-research-plus-ivd-gemarkeerd?languageChanged=en
  with Pipette tips, epT.I.P.S.® Standard (VWR, 1,000-10,000 µL, cat.no. 613-4269).

- Centrifuge tubes, 12 mL (Greiner bio-one, PP with HDPE screw cap, round bottom, VWR cat.no. 391-3471) https://nl.vwr.com/store/product/575205/centrifuge-tubes
  Or Greiner Bio One Polypropylene Tubes, Round Bottom, Sterile, with White Screw Cap 12 mL, 900/Pk Disposable Plastic Test Tubes | Fisher Scientific

- 2 x 9.0 mL Greiner Urine tube (https://shop.gbo.com/en/row/products/preanalytics/venous-blood-collection/vacuette-tube/z-no-additive/455001.html) for Sodium, Potassium, creatinine, and urinary proteins.

- 2 x 9.0 mL Greiner Urine tube (https://shop.gbo.com/en/row/products/preanalytics/venous-blood-collection/vacuette-tube/z-no-additive/455001.html) for KIM-1-1 and NAG analyses

- Sealable safety bag
  - https://www.daklapack.nl/medische-verpakkingen/vloeistofdichte-safetybags/safetybag-internationaal-165-mm-x-285-mm-transparant/8712963008178

- Pre-printed stickers with subject code (print ink/stickers need to be water and freezer resistant)
- Sorbent pouches

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Procedure:

- Put on gloves.
- Take the containers with the urine out of the cool boxes (or refrigerator when temporarily stored until aliquoting).
- Prepare for each container:
-
    - 3x 12 mL tube with white cap
    - 2 x 9.0 mL Greiner Urine tube (https://shop.gbo.com/en/row/products/preanalytics/venous-blood-collection/vacuette-tube/z-no-additive/455001.html) for Sodium, Potassium, creatinine, and urinary proteins.
    - 2 x 9.0 mL Greiner Urine tube for KIM-1 and NAG analyses
    - Sealable safety bag

- Put the stickers with the corresponding unique SPRINT participant identification number on the tubes and the sealable safety bag.
- Important: homogenise the urine by gently shaking/turning the container upside down a few times each time before taking a portion (to ensure inter-comparability of lab results). Do not overfill the tubes to prevent the stopper from coming of when freezing in the laboratory of final destination.
- Transfer* 10 mL of urine in each of the three 12 mL tubes.
- Transfer* 7-8 mL of urine in each of the two 9mL Greiner tubes for Sodium, Potassium, creatinine, and urinary proteins.
- Transfer * 7-8 mL of urine in each of the two 9mL Greiner tubes for KIM-1-1 and NAG analyses

  Note-1: transfer can be done by carefully directly pouring the urine from the container into the tubes, or, alternatively by using a 1-10 mL volume adjustable pipette. In case of pipette, use a new pipette tip for each new urine sample.

  Note-2: in case the volume of urine is insufficient, fill the tube for clinical analysis first, and distribute the rest of the urine approx. equally into the three 12 mL tubes.
- Close the tubes with the screw cap and tighten.
- Place the tubes into the sorbent pouch.
- Close the container with the remaining urine.
- Put the pouch containing the tubes into the corresponding labelled sealable safety bag.
- Put the bag into the freezer (-18°C or lower) for storage until shipment to the analytical laboratory.
- Also store the urine container with remaining urine in the freezer (-18°c or lower) at least until confirmation of safe receipt by the analytical laboratory, and longer when requested by the CSS leader.

Annex C Diary form

DIARY FORM			
Date/year			
SPRINT participant ID			
Sex: Male ☐ Female ☐			
URINE SAMPLE			
	First day of sampling	Second day of sampling	Third day of sampling
Date (dd/mm/yyyy)			
Clock time of urine collection (hh:mm)			
Clock time of **previous** toilet visit before you collected			
Duration of sleep (hh:mm)			
Coffee/alcohol intake			
Physical activity			
Smoking			
Medication use			
Do you suffer from bladder infection?			
Other remarks			
STOOL SAMPLE			
Date (dd/mm/yyyy)			
Clock time of stool sample collection (hh:mm)			

Investigator name:

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 14. SOP stool collection, storage and transportation

Authors: Karsten Beekmann, Hans Mol (WR, Wageningen, The Netherlands)

Version: 2.0

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Background

The aim of this SOP is to provide information for collection of stool samples according to the SPRINT "Research Protocol case study sites" (File no. XXX), and their storage and transportation. The samples will be analyzed for the following endpoints:

c) Pesticides and/or their metabolites
d) microbial composition

A stool sample is going to be collected from each participant on a single occasion during the field study. Per subject, 5 scoops of stool sample will be collected (i.e. 3 for pesticide analysis, 1 for analysis of microbial composition, and 1 as backup to be kept at -80 °C at WUR-Wageningen Food Safety Research (WFSR)).

Prior to laboratory analyses, samples need to be appropriately stored and transported.

The procedures are described in this document.

Schematic procedure steps

At home subjects	CSS	Wageningen, NL
		Distribution materials to CSS
	Preparation and distribution materials to subjects	
Bowel movement		
Collect 5 tubes of stool sample (1 scoop per tube, 4 empty tubes and one tube with stabilization buffer)		
Store samples in cool box with ice packs	Collection of samples from subjects Transport to CSS collection point Temporary storage (-18°C) Transport on dry ice to WUR-WFSR by 24h courier service	Register Store 3 tubes at -18°C (pesticide analysis) and 1 tube at -80°C (back up) Forward 1 tube to WUR-HMI (microbiome analysis)

Materials

Subjects are provided with the following materials:

- instructions (see Annex I)
- 2 collection papers
    - https://nl.vwr.com/store/product/26835737/faecal-collection-paper-fe-col
- 4 pre-labelled collection tubes for analysis of pesticides and their metabolites (3 for pesticide analysis, 1 backup) (see *Registration and Labelling of Samples* below)
    - https://www.alphalabs.co.uk/sample-collection-un3373-packaging/primary-sample-containers/primaries-road-transport/30ml-universal-containers/rc2145
- 1 pre-labelled collection tubes for analysis of the microbiome (composition and metagenome) (see *Registration and Labelling of Samples* below)
    - https://www.baseclear.com/lab-products/zymo-research/sample-collection-preservation/faecal-collection-preservation/
- 2 pairs of nitrile gloves (M and XL)
- 1 zip-lock bag with sticker, labelled (see *Registration and Labelling of Samples* below)
- https://nl.vwr.com/store/product/22557739/slider-zip-bags
- 1 cool box with active cooling and sufficient frozen blue ice packs
- 1 pen

Procedures:

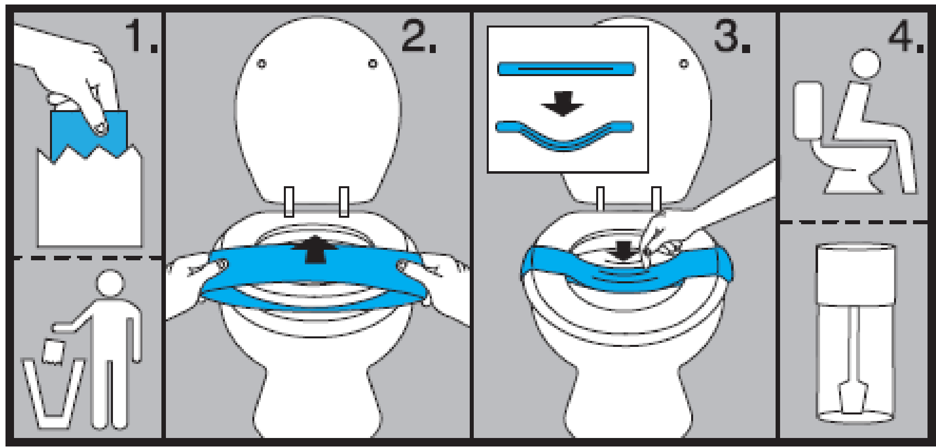
Collection of stool samples by study participants

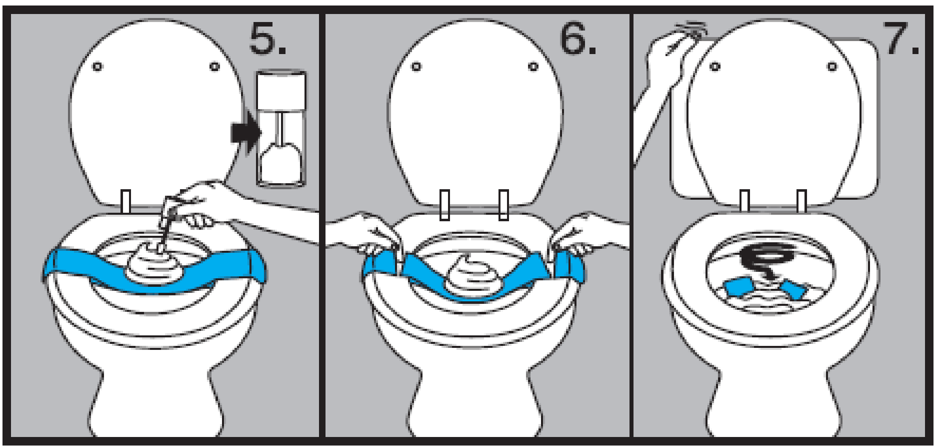
Participants are asked to collect a sample of the first bowel movement of the day using the provided collection materials (see Figure 1) as follows (See Annex A Instructions of sample collection for the participant)

- Participants are asked to not have the stool sample get into contact with water in the toilet, urine, or toilet paper before collection of the samples.
- Participants take the collection paper from its packaging (Figure 1, picture 1).
- Participants place the collection paper over the toilet seat (Figure 1, picture 2) and gently push its center down by hand (Figure 1, picture 3).
- After producing the bowel movement on the collection paper, subjects put on the provided gloves and take out the collection tubes. Subjects place one spoon (as large as reasonably possible, about the size of a glass marble) of stool matter in each tube, using the spoons attached to the lids of the collection tubes (Figure 1, picture 5). One of the five collection tubes (the one with a brown cap) contains a preservation fluid that must stay in the tube.
- The spoon remains on the lid and is placed with the sample back in the tube. Tubes are closed tightly.
- Participants shake the tube containing preservation fluid for 10 seconds to mix the stool matter with the fluid. Some foaming may occur, this is normal.
- The collection tubes are placed in the zip-lock bag, and the bag is closed and placed in the cool box.
- Participants take off the gloves and dispose of them in the waste bin.
- By tearing the collection paper at its sides (Figure 1, picture 6), the remaining bowel movement will fall into the toilet with the paper. Subjects will be advised to wait one minute and let the paper get wet before flushing the toilet (Figure 1, picture 7).

- Participants should wash their hands.

- Participants write down the date and time of the collection of the bowel movement on the safety bag.
- Participants place the zip-lock bag with their samples into the cool box with active cooling also containing frozen blue ice packs.
- Participants inform the study assistant that the samples are collected and ready for pick up. Samples should be picked up as soon as possible on the same day of collection of samples.





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Registration and labelling of samples

SPRINT participant label, for accurate specimen labeling. To track and report the result of the obtained stool samples it is important that a simple and unique labelling procedure is used. Tubes will be labelled before distribution to subjects. Labels need to be used that are suitable for prolonged storage at -80 °C. Since stool samples will be obtained from all participants in the participating counties the following uniform tracking code is proposed for the stool samples:

[ISO-Alpha county code]_[Human][unique SPRINT participant identification number]_[Stool indicator]_ [Tube count/total tubes]

E.g.: NL_H000_S_1/5, NL_H000_S_2/5, NL_H000_S_3/5, NL_H000_S_4/5, and NL_H000_S_5/5

The sealable safety bags will be labelled before distribution to the subjects in the following manner:

[ISO-Alpha county code]_[Human][unique SPRINT participant identification number]
E.g.: NL_H000_S

The bags should be labelled with a sticker that is suitable for storage of the samples at -80 °C, and have sufficient empty space for participants to add date and time of collection of sample.

Transport and packaging

Additional material required by CSS for safe shipment of samples

- Sealable safety bags
    - https://www.daklapack.nl/medische-verpakkingen/vloeistofdichte-safetybags/safetybag-internationaal-165-mm-x-285-mm-transparant/8712963008178
- Absorbent sheets
    - https://www.alphalabs.co.uk/sample-collection-un3373-packaging/secondary-packaging-solutions/secondary-air-or-road-transport/absorbent-sheets/ab001-500

Pickup of samples from subjects, storage, and shipping

- Check that the labelling on the bags is complete with date and time of collection; ask if there were any deviations from the protocol and take note.
- If needed, add additional ice packs to cool boxes, or transfer samples to other mobile cooling equipment if available.
- Upon arrival of the samples at the CSS collection point, immediately transfer to -18 °C and keep frozen from this moment onwards.
- To send the package with a 24h delivery service and follow EU regulations regarding the sending of biomaterials (see https://europepmc.org/article/med/30539463)
    - Place the zip-lock bag provided by the participants (containing the stool samples) together with an absorbent sheet into a sealable safety bag labelled with SPRINT participant ID and date of collection
        - sealable safety bags https://www.daklapack.nl/medische-verpakkingen/vloeistofdichte-safetybags/safetybag-internationaal-165-mm-x-285-mm-transparant/8712963008178
        - absorbent sheets https://www.alphalabs.co.uk/sample-collection-un3373-packaging/secondary-packaging-solutions/secondary-air-or-road-transport/absorbent-sheets/ab001-500
    - Arrange shipping details, prepare and label shipping containers appropriately.

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    - Addresses, responsible person, warning label infectious substances (Shipping Category B Biological Substances), hazard label dry ice.
  - Shortly before pickup by carrier, combine samples in styrofoam boxes filled with dry ice.
  - For each box, prepare a list of the content (sample type, sample codes) and include it in the box.
  - Prepare an e-mail announcing the day of shipment and the courier tracking code. Attach an excel file listing the content (sample list with codes) of each box included in the shipment and send that to the Wageningen Food Safety Research contact person.
  - Ship at the beginning of the week (preferably Monday, Tuesday) to ensure arrival before the weekend.
- It is of utmost importance that the samples remain frozen during the shipment.

Address for shipment for analyses:
Stool samples for pesticide analyses:

Wageningen Food Safety Research (WFSR),
SPRINT project,
Akkermaalsbos 2,
6708 WB Wageningen,
The Netherlands

Contact details: Jonatan Dias, jonatan.dias@wur.nl, +31317486918

Stool sample for microbiome
Prof. dr. Jerry M. Wells and Dr Jos Boekhorst
Host Microbe Interactomics Group, Department Animal Science,
Campus, Building 122, De Elst 1
6708 WD, Wageningen.
The Netherlands

Contact details: Loes Bugter, loes.bugter@wur.nl; +31317486125/ Jerry Wells +31620362084

Upon arrival samples are processed according to the assigned laboratory SOPs

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex A Instructions of sample collection for the participant

You are participating in the research project SPRINT. As part of this project, we kindly ask you to collect a stool sample. The sample will be analysed for pesticides and the microbial composition of your stool. Please read the instructions below carefully.

Prior to sample collection, you have received the following materials from the SPRINT CSS research team:

- a cool box with active cooling and frozen blue ice packs
- 2 collection papers
- 5 collection tubes
  - 4 for analysis of pesticides and their metabolites (blue cap)
  - 1 for analysis of microbial composition (brown cap, containing liquid)
- 2 pairs of nitrile gloves (sizes M and XL)
- 1 zip-lock bag
- 1 pen

Before collection

- Please read the instructions below carefully, and place the ice packs from the cool box in your home freezer immediately upon receipt until sampling of bowel movement.
- Place the cool box at a place outside sun/heat and with access to electrical power, either 12V or 220V. Connect to power supply and switch on the cool box active cooling.

Time of collection

- Please collect the first bowel movement on the day you collect the morning urine sample. If your stool is diarrheal (unusually watery or runny) this day please refrain from collecting a sample and inform your CSS contact.

Sampling of bowel movement (day of visit from CSS research team)

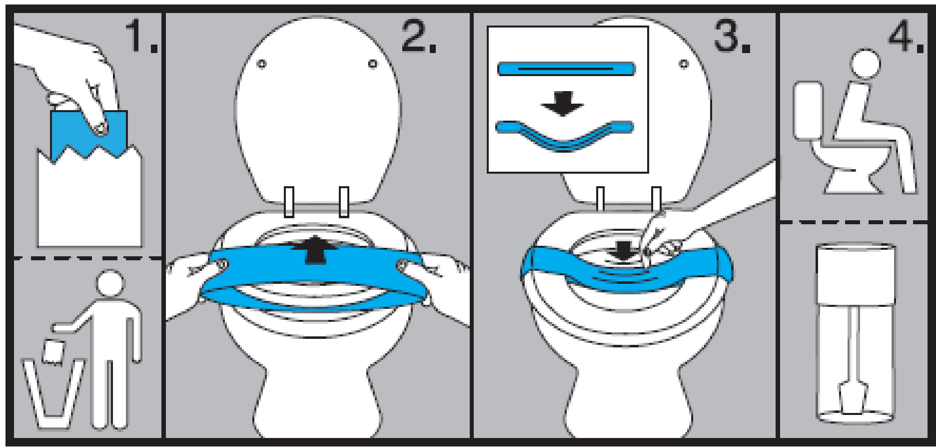
- If you also have to urinate, please do so before producing your bowel movement to avoid mixing of the faeces with urine. Please also avoid contact of the samples you collect with toilet paper and water from the toilet.
- Before you produce your bowel movement, take the collection paper from its packaging (Picture 1 below).
- Place the collection paper over the toilet seat (Picture 2 below) and gently push its center down by hand (Picture 3 below).
- Produce your bowel movement on the collection paper. Put on the provided gloves and take out the 5 collection tubes from the bag. Please collect one spoon (as large as reasonably possible, about the size of a glass marble) of stool matter per tube, using the spoons attached to the lids of the collection tubes (Picture 4 below). The spoon remains on the lid and is placed with the sample back in the tube. Be careful not to contaminate the outer surface of the tube with feces. Close the lids on the tubes tightly.

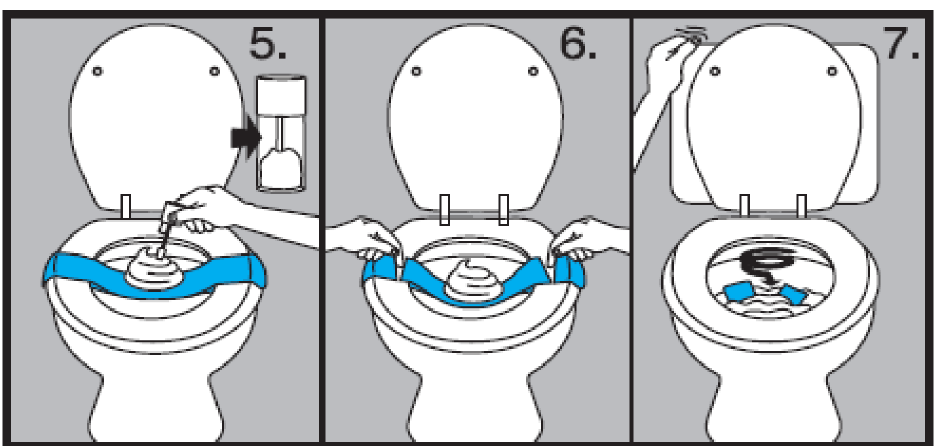
One of the five collection tubes (the one with the brown cap) contains a fluid that must stay in the tube, please be careful not to spill it when collecting that sample.

- After having taken all samples and closed the tubes tightly, shake the tube containing preservation fluid for 10 seconds to mix the stool sample with the fluid. Some foaming may occur, this is normal.
- Place all five collection tubes in the zip-lock bag, and close it (with as little extra air in as possible).
- Take off the gloves and dispose of them in the waste bin, not the toilet.
- By tearing the collection paper at its sides (Picture 6 below), the remaining bowel movement will fall into the toilet with the paper. It is advised to wait one minute and let the paper get wet before flushing the toilet (Picture 7 below).
- Please wash your hands when completing your bathroom visit.
- Please write down the date and time of the collection of the bowel movement on the zip-lock bag, and place the bag in the cool box with frozen blue ice packs along with the urine container.
- Please keep the cool box out of the sun/heat until it is picked up by a research assistant.

We thank you for your participation in the study and for following the study procedures.

Figure 1. Steps stool sampling.





Inform researcher contact that samples can be collected at earliest convenience: <Add contact information of CSS>

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 15. SOP Blood collection, storage and transportation

Authors: *Martien H.F. Graumans, Maurice F.P. van Dael, Paul T.J. Scheepers, and Anne Vested*

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List of abbreviations

AChE	Acetylcholinesterase
ALT	Alanine transaminase
AST	Aspartate transaminase
BChE	Butyryl cholinesterase
DHEAS	Dehydroepiandrosterone sulphate
FSH	Follicle-stimulating hormone
fT4	Functional Thyroglobulin 4 (biomarker of iodine status)
GFAP	Glial fibrillary acidic protein
GGT	Gamma-glutamyl transferase
GSH	Glutathione
GSSG	glutathione disulphide
hsCRP	high sensitivity C-reactive protein
ICAM-1	Intercellular Adhesion Molecule 1
IL	Interleukin
MN	Micronuclei (MN)
LH	Luteinizing Hormone
RPM	Revolutions per minute per rotor
RCF	Relative centrifugal force
SAA	Serum amyloid A
s-DHEA	Dehydroepiandrosterone sulphate
SHBG	Sex hormone-binding globulin (SHBG)
SOP	Standard Operating Procedure
SCE	Sister chromatid exchanges
T3	Triiodothyronine
T4	Thyroxine
TNF-α	Tumor necrose factor alpha
TSH	Thyroid stimulating hormone
VCAM-1	Vascular Cell Adhesion Molecule-1

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Background

To harmonise the phlebotomy procedure, information for this standard operating procedure (SOP) has been adapted from the WHO guidelines on drawing blood (WHO, 2010), EFLM recommendation for venous blood sampling report (EFLM, 2017), and the HBM4EU SOP for blood sampling (HBM4EU, 2018).

Aim:

The aim of this SOP is to provide information for safe collection of venous blood samples, and their storage and transportation. A blood sample is going to be collected from each participant by vena puncture on a single occasion during the field study. Prior to laboratory analyses, samples need to be appropriately stored and transported.

Pre-analytical phase

Whole blood is composed of cellular matter (45%) and blood plasma (55%). Cellular matter does contain erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets). Transport of oxygen (O_2) and carbon dioxide (CO_2) is performed by the erythrocytes, where the leukocytes are important for the body's immune response. Blood plasma is an aqueous solution that contains nutrients, proteins (including fibrinogens), hormones, vitamins, trace elements, metabolites, exogenous substances and electrolytes (sodium (Na^+), calcium (Ca^{2+}) chloride (Cl^-), potassium (K^+) and magnesium (Mg^{2+})). When whole blood is centrifuged and clotted, blood serum will remain. Blood serum is comparable to blood plasma, where the erythrocytes, leukocytes and clotting proteins are settled. Blood serum is therefore also useful for diagnostic blood testing since all other constituents are still present and detectable (Koolman et al. 2009). According to the SPRINT "Research study Protocol – see ethics aplication documents" several parameters are going to be tested in blood matrix:

Primary endpoints in blood:

a. pesticides
b. blood cell counts
c. general inflammatory/immunologic status: hsCRP,
d. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13 and TNF-α
e. ROS-biomarkers: GSH/GSSG

Secondary endpoints in blood:

f. Other inflammatory markers: SAA, VCAM-1 and ICAM-1
g. Neurotoxicity biomarkers: AChE, BChE and GFAP
h. Kidney function: creatinine
i. Thyroid: fT4, T3, T4, and TSH
j. Liver function: ALT, AST, GGT and protein electrophoresis
k. Reproductive: DHEAS, FSH, GGT, LH, s-DHEA, SHBG, testosterone, estradiol, progesterone, and cortisol
l. Untargeted exposome analysis (exogenous)
m. Untargeted metabolome analysis (endogenous)

Due to wide variety of endpoints, it is recommended to <u>draw (*n*=8) blood vacutainers.</u> Depending on the laboratory test required, whole blood, serum or plasma might be used. To stop clotting or separate the plasma from the cellular matrix, different tube types with additives will be used, see Table 1. To avoid cross contamination of blood container additives, it is important to mention that a correct order need to be used during phlebotomy. Table 1 provides the suitable consecutive order according to WHO guidelines on drawing blood (WHO, 2010).

Table 1 General overview frequently used laboratory blood tubes with additives

Tube type and additive	Usage	Colour	Draw Sequence	Number of tubes (n)

Plasma separation tube (PST), Li-heparin (Plasma)	Plasma, clinical chemistry		1	3
Potassium-Ethylenediaminetetraacetic acid (K_2-EDTA) blood tube (Whole blood)	Whole blood, haematological tests		2	3
Blood tube serum separation (SST), clot activator (Serum)	Immunological markers		3	3

Schematic procedure steps

Further information about the laboratory analysis is going to be determined in agreement with the selected clinical laboratory, see SOP: XXX. According to the Radboud University medical center diagnostic laboratory application form, vena puncture blood must be sampled in PST, K_2-EDTA, or SST containers:

Phlebotomy

- Participant identity confirmation
- **Labeling**
- Blood **container selection**

3 x 3 mL PST (Li-Heparin)

3 x 3 mL K_2-EDTA

3 x 5 mL SST

- **Invert each tube:** 8 to 10x
- Place upright in a tuberack
- Store samples (Annex B)
- **Transport to laboratory ASAP**

Centrifugation

Centrifugation

Pesticides kidney and liver function, and Neurotoxicity biomarkers

Haematology, Exposome-scan, and metabolomics

Inflammatory markers, reproductive hormones, ROS biomarkers

- **Laboratory Analysis**
  Clinical diagnostics
  Radboudumc

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Requirements for the SOP

- Certified/registered healthcare practitioner who is authorized to perform the blood drawing
- Appropriate location with a comfortable room/area with privacy
- Chair with armrest
- Swing bucket centrifuge
- Cooling device for transportation of the samples

A. Health and Safety

Bear the regional local COVID19 restrictions regarding use of personal protective equipment related to not keeping distance in an indoor environment (use of proper respiratory protective equipment, gloves and protective clothes)

- Explain the procedure to the study participant
- Hand-hygiene of the phlebotomist
- Minimise the contamination of interfering chemicals/pathogens
- Reusable tourniquets need to be clean (potential MRSA source)
- Keep sufficient distance from the puncture site
- Use disposable needles (potential HIV, or hepatitis B sources)
- Disinfect the skin with alcohol (70%)
- In case of blood spillage, clean the surface appropriately (e.g. sodium hypochlorite solution 5.25% v/v, or equivalent). Dispose the paper towels into the infectious waste bin
- Dispose-off used needles in a needle safety box with biohazard label

B. Materials

- Hand-hygiene => sink with medical soap
- Single-use towels
- 70% ethanol for hand rub
- Talc-free gloves
- Single-use appropriate personal protective equipment in line with COVID-19 protocols
- Sterile disposable needles (Use standardized and accepted products for phlebotomy)
- Blood containers => **BD Vacutainer (see https://www.bd.com/)**
    - Plastic whole blood tube with spray-coated K_2EDTA (3.0 mL Draw volume)
        - BD Catalog no.: 367835
    - BD SST tube with silica clot activator, polymer gel, silicone-coated interior (3.5 mL Draw volume)
        - BD Catalog no.: 367983
    - Plasma tube, 75 USP of lithium heparin, spray coated (4.0 Draw volume)
        - BD Catalog no.: 367880
- Tourniquet
- 70% ethanol swabs for skin disinfection
- Bandages
- Puncture-resistant sharps safety for safe disposal of used needles (with biohazard label)
- Tube collection tray

For storage conditions see Annex B (Storage prior analysis/transportation)

C. Registration and labelling of samples

Use the SPRINT participant label, for accurate specimen labeling. To track and report the result of the obtained blood samples it is important that a simple and unique labelling procedure is used. Since blood samples will be obtained from all participants in the participating counties the following uniform tracking code is proposed for the blood samples:

[ISO-Alpha county code]_[Human][unique SPRINT participant identification number]_[Blood indicator] [Blood container abbreviation]_[Tube count/total tubes]

E.g.: NL_H000_B_EDTA1/3, NL_H000_B_EDTA2/3, NL_H000_B_EDTA3/3…

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To minimise confusion and create a simple tracking number, use the [tube count/total tubes] to specify the number of tubes taken per used blood container. E.g. If there are 3 PST tubes drawn, use the following ascending labelling for the tubes: NL_H000_B_PST1/3, NL_H000_B_PST2/3, NL_H000_B_PST3/3

D. Phlebotomy Instructions

Step 1 Equipment checklist:

- ☐ Perform the procedure in an appropriate clean and confined room with a chair
- ☐ Preferably perform the phlebotomy on a Monday, Tuesday, Wednesday or a Thursday between 7 and 9 AM.
- ☐ Single-use appropriate facial mask (<u>in case of COVID 19 restrictions</u>)
- ☐ Sterile needles including tube holder
- ☐ Presence of the laboratory test tubes (Total: 9 tubes per participant)
    - Avoid cross contamination of the tube additives, draw the blood samples in the correct order (**1:** PST *n=3*, **2:** EDTA *n=3,* and **3:** SST *n=3*)
- ☐ Clean tourniquet
- ☐ 70% ethanol hand rub, 70% ethanol swabs for skin disinfection
- ☐ Single-use paper towels
- ☐ Well-fitting gloves
- ☐ Complete labeling, writing equipment
- ☐ Bandage for the vena puncture site
- ☐ Sample tube tray
- ☐ Puncture-resistant sharps container

Step 2 Laboratory test tube usage and storage

1. Label every tube that will be filled. Blood container usage per laboratory analysis at Radboudumc see Tabel 2
2. The blood containers need to be separately filled and gently mixed as soon as possible to minimise blood clotting
    a. Directly invert each tube 8 to 10 times to mix the blood with the additive
    b. Minimise contamination or spillage

Table 2 Blood collection and laboratory endpoints

Draw	Tube type	Total blood (mL)	Colour	Laboratory analysis endpoints	
				Primary	**Secondary**
1 ↓	(PST) *n=3*	6		Pesticides (for glyphosate and AMPA in separate tube	ALT, AST, GGT, creatinine, AChE, BChE and GFAP
2 ↓	(K_2-EDTA) *n=3*	9		Hemocytometry (blood cell counts)	Metabolomics, Exposome scan
3	(SST) *n=3*	15		hsCRP, ROS-biomarkers: GSH/GSSG, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13 and TNF-α	SAA, VCAM-1, ICAM-1, T3, T4, ft4, TSH, DHEAS, FSH, LH, SHBG, Estradiol, Testosterone, Progesterone, cortisol

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3. Place all the tubes in a rack, check the labeling and corresponding forms.
4. Send one K_2-EDTA sample for complete blood count (CBC) analysis as soon as possible to a central laboratory (within 24 hours) or use point of care devise (e.g. HemoCue® WBC DIFF) to count CBC.
5. Store the blood samples refrigerated at a temperature of 4-8 ºC until fractionation which needs to be performed <2 hours subsequent to collection.
    a. Unless other specified by the laboratory, samples need be stored on their recommendations

Step 3 The phlebotomy procedure (See Annex A):

1. The phlebotomy is going to be performed by a certified healthcare practitioner
    a. The general written procedure can be found on ***p.:11***

E. Sample pre-treatment

Fractionation of the blood samples need to be performed <2 hours following collection by the health care practitioner on the CSS at the participant's home if >2 hours transportation (using a transportable swing bucket centrifuge). The gel present in SST and PST tubes need to be well mixed first:

PST tubes:

1. Invert the tubes 8 to 10 times to mix the blood with the additive
2. Centrifuge for 10 minutes at 1300 RCF (or equivalently 4200 RPM (1900 x *g*))
    a. Depending on the swing bucket centrifuge use **Eq. 1** to convert to RPM

   Pipette 1ml in each cryo-tube for plasma analyses. Store at -80 ºC until shipment for analysis in NL. Samples should be shipped for NL on dry ice.

$$(\sqrt{RCF})/(r \bullet 1.118) \bullet 1000 \quad \textbf{Eq. 1}$$

RCF = Relative centrifugal force
r = radius of the centrifugal rotor (cm)

K_2-EDTA tubes:

- Blood from EDTA tubes is aliquoted in 6 cryo-vials of 2 mL (1 mL per vial). 2 vials for CBC, 4 vials are immediately stored at -80 °C.

SST tubes:

1. Invert the tubes 5 times to mix the blood with the additive
2. Let the blood clot for 30 minutes
3. Centrifuge for 10 minutes at 1300 RCF (or equivalently 4200 RPM (1900G))
    a. Depending on the swing bucket centrifuge use **Eq. 1** to convert to RPM
    b. Pipette 1ml in each 2mL cryo-vials for serum analyses. Store at -80 ºC until shipment for analysis in NL. Samples should be shipped for NL on dry ice.

F. Transportation and packaging

It is advisable is to use Cryo-Babies® stickers for a laser printer. Laser printed labels are desirable to minimise sample error or loss, additionally these stickers are temperature resistant and useful for long-term storage (-196 – 100 ºC).

As previously mentioned, samples need to be transferred to the assigned laboratory as soon as possible. To obtain an accurate laboratory result, it is important to follow their guidelines to ensure that samples arrive in a fit condition. It is advisable to keep the samples cooled during the shipment.

Transportation checklist:

- ☐ Label with dispatching organisation
    - o Add details of the responsible person
- ☐ Tubes placed upright in a tube rack
- ☐ Leakproof transportation box
- ☐ Filled in forms and all tubes labelled
    - o Additional information if needed
- ☐ Shipping Category B Biological Substances
    - o Warning label (infectious substances)
- ☐ Post-paid package
    - o Avoid sending the package on Friday

Address of the assigned clinical laboratory:

Att: Mr. Maurice van Dael
133 HEV
Centrale Ontvangst Goederen Radboudumc
Geert Grooteplein Zuid 30
6525 GA Nijmegen
The Netherlands

Contact: maurice.vandael@radboudumc.nl, +31-619622869

Transportation of the samples:

1. Note clearly the when the blood samples are collected and shipped
    a. Follow guidelines of the assigned laboratory
2. Place the samples in a tube rack
3. Pack the samples in leak-proof box
4. Add sorbent (paper toweling against any leakage)
5. Add a logfile with date of packaging, temperature and add additionally details if necessary
    a. Note the date and time of shipment **(Phlebotomy site)**
    b. Note the date and time upon arrival **(receiving laboratory)**
6. Send the package with a 24h delivery service and follow EU regulations regarding the sending of biomaterials (see https://europepmc.org/article/med/30539463)
7. Inform Maurice van Dael by e-mail about the time and date of dispatchment

Upon arrival samples are processed according to the assigned laboratory SOPs

Reference list

Ana-Maria Simundic, K. B., Janne Cadamuro, Stephen Church, Michael P. Cornes, Edmée C. van Dongen-Lases, Pinar Eker, Tanja Erdeljanovic, Kjell Grankvist, Joao Tiago Guimaraes, Roger Hoke, Mercedes Ibarz, Helene Ivanov, Svetlana Kovalevskaya, Gunn B.B. Kristensen, Giuseppe Lippi, Alexander von Meyer, Mads Nybo, Barbara de la Salle, Christa Seipelt, Zorica Sumarac, Pieter Vermeersch. (2017). *EFLM Recommendation for venous blood sampling*.

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Transport of samples to Viapath (Guy's and St Thomas's NHS, King's College Hospital NHS). *2021*(12 January). Retrieved from https://www.kch.nhs.uk/gps/gp-pathology-guide/sample-transport

Annex A

The phlebotomy procedure (Step 3):

1. Welcome the participant (Bear the COVID19 restrictions in mind of the concerned country).
2. Introduce yourself and confirm the identity of the SPRINT participant.
3. Explain verbally or with the leaflet the vena puncture procedure and receive the consent form.
   a. Ask the participant whether he/she might faint.
4. Wash your hands and rub with alcohol for at least 30 seconds.
5. Use 1 pair of well-fitting talc-free gloves.
6. Relax and extend the participant's arm.
   a. Inspect the antecubital fossa or forearm (certified healthcare practitioner).
   b. Locate the vein, it needs to be visible without applied tourniquet.
7. Fasten the tourniquet above the site which is used for phlebotomy.
8. Clean the site of phlebotomy with 70% ethanol using swab.
   a. Allow to dry completely ~ 30 seconds.
9. Ask the participant to make a fist (veins become more prominent).
10. Perform the puncture rapidly (angle of 30 degrees), take ~ 15 mL of blood.
    a. Distribute the blood directly into the selected tubes – once the blood flows, release the tourniquet
11. When all nine blood tubes has been collected, take the needle gently out and apply directly pressure to the puncture site with a clean bandage. Ask the participant to not bend the arm yet, and keep pressure on the puncture location to minimise the formation of a haematoma.
12. Check whether the participant is feeling well
    a. If not, look after the participant and try to make the situation comfortable.
13. Dispose the used needle in the puncture-resistant sharps container.
14. Perform again the hand hygiene as on the start of the procedure
15. Thank the participant, and ask whether there are any questions left.

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Annex B

Storage conditions according to: *Radboudumc Diagnostic laboratory*

Biomarker	Blood type	~ 21 °C	2-8°C	-20 °C
Hemacytometry	K_2-EDTA	24h	-	-
ALT	Heparine plasma	3 days	7 days	3 months
AST	Heparine plasma	4 days	7 days	3 months
GGT	Heparine plasma	7 days	7 days	-
Creatinine	Heparine plasma	7 days	7 days	3 months
T3	Serum	2 days	8 days	3 months
TSH	Serum	8 days	14 days	1 month
fT4	Serum	8 days	14 days	3 months
DHEAS	Serum	5 days	14 days	12 months
FSH	Serum	14 days	14 days	12 months
LH	Serum	5 days	14 days	12 months
SHBG	Serum	5 days	7 days	12 months
Testosterone	Serum	5 days	14 days	12 months
Estradiol	Serum	1 day	3 days	6 months
Progesterone	Serum	-	-	12 months
Cortisol	Serum	7 days	7 days	12 months

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 16. SOP for assessment of exposure of humans to pesticides using silicone wristbands

Authors: Daniel Figueiredo / Revision by: Anke Huss and Paul Scheepers

Version: 2.0

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Background

In our research, we assess pesticide exposure through a safe and relatively new method: silicone wristbands (the famous Livestrong wristbands). Next to their popular appearance as 'awareness wristbands' in charity projects, silicone bracelets have a very useful property: they are able to absorb pesticides from their surroundings. By analyzing the wristbands, we can determine with which pesticides the wearer had come in contact.

In our study, besides cats and cattle, humans will wear a wristband for one week. After the study period, we will measure the pesticide contents of this wristband. This SOP describes the human wristband procedure.

Content of this standard operating procedure

- Transport of wristbands from origin to participants
- Deployment and collection
- Shipment for laboratory analysis

List of abbreviations

HW: wristbands, human (to distinguish between other used wristbands in this study)
RUMC: Radboud university medical center, Nijmegen
CSS: Case study site

The procedure

Materials

- Pre-cleaned silicone wristbands (one size, 20cm) will be supplied directly to CSS leaders by RUMC.
- Zip-lock bag for containing the wristband
- Instruction brochure for the participant
-

Transport of human wristbands from origin to deployment

- RUMC Nijmegen will ship a designated amount of human wristbands (HWs) to a mail/delivery address provided by the CSS leader (in a big envelope).
    - HWs will be sent as one batch in an envelope/box containing:
        - Smaller envelopes corresponding to number of HWs
        - Inside each smaller envelope -> Each HW inside a separate airtight zip-lock bag and a small brochure with instructions on how to wear, store and ship HWs after the sampling period
        - The zip-lock bag will be labeled with unique SPRINT participant identification number and also the sampler on and off dates and times.
        - The ID will be a number including the following:

          [ISO-Alpha county code]_[Human][unique SPRINT participant identification number]_[Wristband indicator]
          e.g. NL_H000_W

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IMPORTANT!

EACH CSS leader is responsible for adding a SPRINT participant identification number on the smaller envelope!

Note: During transport from origin to deployment the HW's are always kept in the dark. These should only be taken out from the dark place (example, closed box) once starting "Deployment and collection".

After the participant has worn the wristband for 7 consecutive days, HWs should be collected by the SPRINT CSS team and sent to the analysis laboratory as one batch. Try to combine shipments (e.g. combine with blood shipment. Any questions contact Maurice van Dael at maurice.vandael@radboudumc.nl.

Deployment and collection

This section is addressed to the responsible(s) for field work in each CSS. Please read these instructions carefully.

Step 1: Unpack the HW and give it to the participant. The HW should be worn around the wrist of the dominant hand, continuously during the complete experiment. This includes also sleeping and taking showers.

Step 2: Do not dispose the zip-lock bag of the HW. They will be used for transportation of the HW after the study.

Step 3: The participant should register the start date and time when he/she starts wearing the HW (write this on the label of the zip-lock bag). The participant should store the empty zip-lock bag and brochure in the provided envelope for returning the wristband.

Step 4: Give the envelope to the participant and inform him/her of the following:

- There is a brochure (a brief instruction in the spoken language of the CSS) inside the envelope explaining the next steps.
- The HW should only be collected after a period of 1 week (7 consecutive days).
- After this period, the participant puts the HW inside the zip-lock bag and place it in his/her own freezer. Write down, on the zip-lock bag, the end date and time.

  SPRINT CSS research team is responsible for collecting the HW at the end of the study period and sending them to the lab in The Netherlands. Mail the HWs to the analyzing lab as early as possible, preferably in one batch with other HW's.

Shipment for laboratory analysis

Return the HWs in a combined shipment of HW and blood samples to the following address:

Att : Mr. Maurice van Dael
133 HEV
Centrale Ontvangst Goederen Radboudumc
Geert Grooteplein Zuid 30
6525 GA Nijmegen
The Netherlands

Contact: maurice.vandael@radboudumc.nl, +31-619622869

Analysis will be performed by RUMC.

Responsibilities per procedure (within SPRINT)

Materials

Responsible for wristbands: RUMC.
Responsible for number of HWs needed for each CSS: CSS leaders.

Transport of HW from origin to deployment

Responsible for shipment to CSS leaders: RUMC.
Each CSS leader can request the wrist bands to be sent by mail

Deployment and collection

Responsible: Each CSS leader and respective field workers.

Shipment for laboratory analysis

Responsible: CSS leader and respective field worker will collect wristbands from all participants and send it off in one shipment along with the human blood samples.

Laboratory analysis

Responsible: RUMC

Important! During long-term storage at −20°C, all chemical levels are stable for up to 6 months for SVOCs (Anderson et al. 2017) -> Should be analysed within the period of reception and month 6.

References

Anderson, K. A., Points III, G. L., Donald, C. E., Dixon, H. M., Scott, R. P., Wilson, G., Tidwell, L. G., Hoffman, P. D., Herbstman, J. B., and O'Connell, S. G. 2017. "Preparation and Performance Features of Wristband Samplers and Considerations for Chemical Exposure Assessment." J Expo Sci Environ Epidemiol 27 (6):551

Hollmann S, Frohme M, Endrullat C, Kremer A, D'Elia D, Regierer B, et al. (2020) Ten simple rules on how to write a standard operating procedure. PLoS Comput Biol 16(9): e1008095. https://doi.org/10.1371/journal.pcbi.1008095

Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Annex 17. SOP for human nasal swabs

Authors: Jerry Wells, Anne Vested

Version: 1.0

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Disclaimer: This report is part of a project that has received funding by the European Union's Horizon 2020 research and innovation program under grant agreement number 862568.

Background

In the SPRINT study microbiome analyses will be performed on nasal swab samples from the anterior nare (left nostrils) using E-swabs from Copan.

The nasal swab procedure will be performed by a trained SPRINT CSS laboratory technician at the participants' home and is a quick and safe method for collecting material from the anterior nare with very little discomfort.

This SOP describes the human nasal swab procedure.

Materials and methods

The nasal swab procedure will be performed by a trained laboratory technician of the SPRINT CSS team at the scheduled visit at the participants households in order to increase consistency of the collection process.

E-swabs from Copan will be used for the human nasal swab procedure. See https://www.copanusa.com/sample-collection-transport-processing/eswab/ including training guides for collection of human samples. ESwab™ combines a COPAN-invented flocked swab with 1mL of liquid in a plastic screw cap tube. The innovative system elutes over 90% of the patient specimen into the liquid medium.

The E-Swab plastic applicator is sprayed with Nylon® fibers forming a soft, velvet brush that allows for improved collection of cell samples. Capillary action between the Nylon® fiber strands facilitates strong hydraulic uptake of liquid samples. The sample stays close to the surface allowing fast and complete elution.
ESwab™ provides 1mL of homogenous liquid sample and provides up to 10 aliquots of identical sample to perform multiple tests.

Materials

- ESwab™ 480C
- Zip-lock bag for containing the ESwab™

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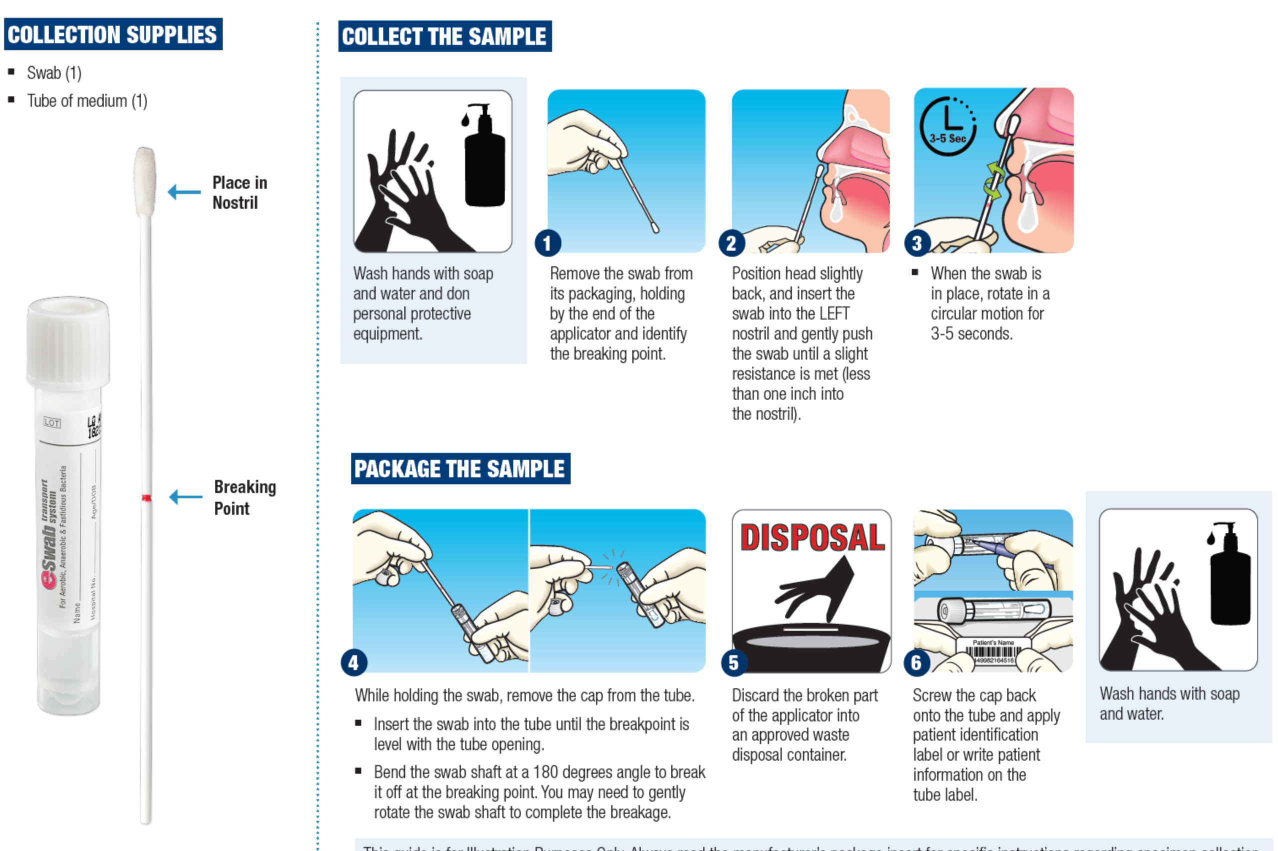
Instruction for anterior nares sample collection using ESwabTM

Figure 1: Instructions for trained SPRINT CSS team laboratory technician



Procedure (to be performed by the SPRINT CSS team laboratory technician)

-See instructions in **Figure 1**-

1. Nasal swabbing is performed from the left nare (the outermost part of the nare) by rotating the swab three time. Be aware not to hold the swab under the red marking as the sample will be contaminated.

2. The swab should be returned in the tube containing transport media and should be broken at the red marking by "bending" the swab at the tip of the tube. Avoid getting media on your hands as this may cause irritation on the skin.

3. Close the lid on the tube. Apply label with date and unique SPRINT participant identification number and transfer tube immediately to the cooling box (with ice packs) during transport to the central sample distribution point for the CSS. Thereafter store at -20°C in a freezer until shipment. Store sample at -20°C until further use.

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Labelling of nasal swab tubes:

[ISO-Alpha county code]_[Human][unique SPRINT participant identification number]_[Nasal swab indicator]

e.g. NL_H000_N

Transport from CSS to analytical laboratories

To send the package with a courier service and follow EU regulations regarding the sending of biomaterials and dry-ice see https://europepmc.org/article/med/30539463

Arrange shipping details, prepare and label shipping containers appropriately.

- Addresses, responsible person and contact details, hazard label dry ice
- Shortly before pickup by carrier, combine samples in styrofoam box and fill with sufficient amount of dry ice.
- For each box, prepare a list of the content (origin, sample type, sample codes) and include it in the box.
- Prepare an e-mail announcing the day of shipment, and the courier tracking code. Attach an excel file listing the content (sample list with codes) of each box included in the shipment and send that to the contact person, Loes Bugter, loes.bugter@wur.nl; +31317486125, Host Microbe Interactomics Group, Department Animal Science. Ship at the beginning of the week (preferably Monday, Tuesday) to ensure arrival before the weekend.

It is of utmost importance that the samples remain frozen during the shipment.

Address for shipment for nasal swab microbiome analyses:

Att: Prof. dr. Jerry M. Wells and Dr Jos Boekhorst
Host Microbe Interactomics Group, Department Animal Science
Campus, Building 122, De Elst 1
6708 WD, Wageningen.
The Netherlands

Contact details: Loes Bugter, loes.bugter@wur.nl; +31317486125/ Jerry Wells +31620362084

Upon arrival samples are processed according to the assigned laboratory SOP

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Annex 18. Wristbands SOP for Animal

In our research, we assess pesticide exposure through a safe and relatively new method: silicone wristbands (the famous Livestrong wristbands). Next to their popular appearance as 'awareness wristbands' in charity projects, silicone bracelets have a very useful property: they are able to absorb pesticides from their surroundings. By analyzing the wristbands, we can determine with which pesticides the wearer had come in contact. Thus far, silicone wristbands have only been worn by humans, so in order to make the silicone wristband wearable for cats we tightly bound them to cat collars. In our study, **cats will wear a wristband fit to a collar in their daily lives.** After the study period, we will measure the pesticide contents of this collar.

Content of this standard operating procedure

- Adapting silicone wristbands to collars
- Transport of collars from origin to participants
- Deployment and collection
- Shipment for laboratory analysis

List of abbreviations

CW – modified collars, i.e. wristband sewed to a cat collar

The procedure

Materials – Wristbands, collars

- Precleaned silicone wristbands will be supplied directly to CSS leaders. Collars should be provided by local team.
- Collars can be bought in bulk online or at a pet shop, as long as this are Nylon collars. Nylon collars proved to be the most efficient collar material for silicone attachment. Unlike (synthetic) leather, nylon is easily penetrated with thread and needle.
- Thread and needle can be acquired in any shop

Adapting silicone wristbands to collars

1. In order to fit the silicone rubber on the collars, smooth segments of silicone wristbands are to be carefully cut into pieces of 7cm.
2. Before silicone attachment, collars and needle are immersed in a glass vial with n-Hexane and subjected to an ultrasonic bath at 25.000 Hz for 10 minutes. The solution then is decanted, and the collars are placed in a 37 degrees incubator for one hour to evaporate the remaining hexane.
3. Attach the silicone to the collars with thread and needle, as cotton thread minimizes coverage of the silicone's air-exposed surface, compared to cable ties or rivets.
4. A single piece of silicone (7cm) has to be tightly secured to each collar with thread and needle.
5. The modified collars (CW) are then placed in plastic airtight containers and kept in dark until deployment.

Transport of collars from origin to deployment

CWs will be sent to CSS leader post address in 1 big envelope, the envelope will have on it:

- Smaller envelopes = number of CWs
- Inside each smaller enveloped -> Each CW inside a separate plastic airtight container and a small brochure with instructions on storing and shipping CW as well as (point 1.4, step 4)

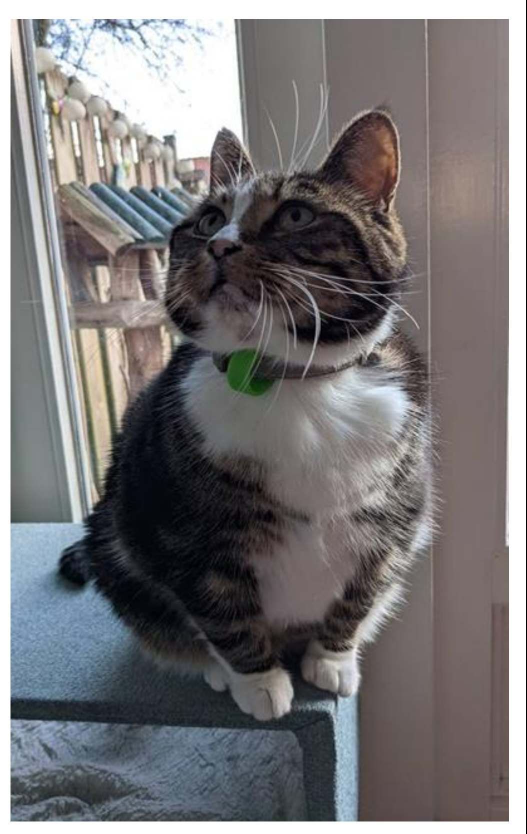
- The plastic containers will be labeled with an ID (sample identification number) and also the sampler on and off dates and times.
- The ID will be a number that includes: CSS specific ID + ID for House + ID for cat.

IMPORTANT! EACH CSS Leader should collect the wristbands and send them as a badge. Try to combine shipments (e.g. combine with blood shipment. Any questions contact Maurice van Dael at maurice.vandael@radboudumc.nl (point 1.5).

Note: During transport from origin to deployment the CW are always kept in the dark. These should only be taken out from the dark place (example, closed box) once starting procedure 1.4.

Deployment and collection

This section is addressed to the responsible(s) for field work in each case study site. Please read these instructions carefully.

<table>
<tr><td>Step 1: Unpack the CW and give to the owner of the cat, so he can put it gently around the cat's neck, preferably with the silicone region on the front-side of the cat's neck (see below). The collar should fit relatively tight. Ideally, two fingers should fit between the neck and the collar (safety procedure).</td><td rowspan="4"></td></tr>
<tr><td>Step 2: Do not dispose the plastic containers of the CW. They will be used for transportation of the equipment after the study.</td></tr>
<tr><td>Step 3: Notify the date and time that cat starts wearing the CW (write this in the label of the plastic container). Ask the owner what is the average time the cat spends outdoors and note this number down in the brochure. Please store the empty plastic container and brochure back in the envelope.</td></tr>
<tr><td>Step 4: Give the envelope to the participant and inform him of the following:
- There is a brochure inside the envelope explaining the next steps.
- The CW should only be collected after a period of 1 week. It is ok for this period to be longer, however it should not be longer than 1 month.
- After this period, take the CW from your cat, put it inside the plastic container and place it in your own fridge. Write down, in the plastic container, the date and time you took the CW from your cat.
- Mail the CW as early as possible. When you are ready to mail it, take the plastic container out of the fridge and place the plastic container inside the envelope we provided. Seal the envelope and put it in the mail box.</td></tr>
</table>

Shipment for laboratory analysis

Envelopes will already have the return address on it. Envelopes with CWs will be sent to Maurice van Dael, 133 HEV, Centrale Ontvangst Goederen Radboudumc, Geert Grooteplein Zuid 30. 6525 GA Nijmegen Analysis will be performed by SKU

References

- Anderson, K. A., Points III, G. L., Donald, C. E., Dixon, H. M., Scott, R. P., Wilson, G., Tidwell, L. G., Hoffman, P. D., Herbstman, J. B., and O'Connell, S. G. 2017. "Preparation and Performance Features of Wristband Samplers and Considerations for Chemical Exposure Assessment." J Expo Sci Environ Epidemiol 27 (6):551
- Hollmann S, Frohme M, Endrullat C, Kremer A, D'Elia D, Regierer B, et al. (2020) Ten simple rules on how to write a standard operating procedure. PLoS Comput Biol 16(9): e1008095. https://doi.org/10.1371/journal.pcbi.1008095