Guideline for Sampling and Sample Processing

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

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UMWELTPROBENBANK DES BUNDES

Umwelt Bundesamt

Contents

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects ecologically representative environmental and human samples and stores and investigates them for environmentally relevant substances.

Specific operating procedures as well as the conception of the ESB are the basis of the program. (Umweltbundesamt 2008, 2014)

The long-term storage is carried out under conditions which, as much as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigations of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Guideline Objective

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based, standardized methods to avoid contamination and inhibit loss of chemical information. The need for an exceptionally high level of quality assurance results from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison

The present sampling guideline is in accordance with methods of the “emission-ecological forest vitality study" (Knabe 1981, 1982, 1984) and the VDI Guideline 3957 sheet 11 (Kommission Reinhaltung der Luft im VDI und DIN 2007). The guideline is an update of Bartel *et al*. (2009) version and is applied to both, spruce and pine, with slight differences.

Transport, further sample treatment and storage as well as chemical analysis have to be carried out according to the current guidelines of the ESB.

3 Function of the Specimen Type

Evergreen conifers are of particular importance as bio-indicators, among other reasons, due to their:

- wide distribution,
- importance to forestry,
- great capacity to filter substances out of the air flow,
- year-round exposure of the assimilation organs.

The Norway spruce (*Picea abies L*.), as an important forest tree, shows a wide distribution and great abundance with economic and ecological relevance (BMEL 2016).

Eastwards, as the climate becomes increasingly continental, the Norway spruce is replaced by the Scots pine (*Pinus sylvestris L.).* The selection of both species for the ESB is based on their prominent role as primary producers in numerous near-natural and anthropogenically influenced ecosystems throughout Central, Eastern and Northern Europe and far beyond their original natural range.

The following criteria underline their use as accumulation indictors:

- availability of comprehensive baseline and comparative data, e.g. due to forest research programs, as well as the forest decline inventories (Knabe 1981, 1982, BMELF 1990, Weiss and Trimbacher 1998, Haußmann *et al.* 2000, BMJ 2000, Schröter-Kermani *et al.* 2006, Tarricone *et al.* 2015),
- for more than 100 years spruce and pine have been used both as sensitive effect indicators and as accumulation indicators (Wagner and Müller 1979, Höpker 1991, Umlauf *et al.* 1992, 1994a, b, Baur *et al.* 1998, Trimbacher and Weiss 1999, Viskari 2000),

- wide distribution throughout Central, East and Northern Europe where they can also be found in areas with a high pollution burden (Schmidt-Vogt 1977).

4 Target Compartments

One-year-old shoots of spruce and pine best reflect the overall situation of an entire year because they are physiologically most active. Depending on pollutant type and age group, varying accumulation rates can arise, so that the predefinition of one age group becomes essential.

In several monitoring programs (i.e. VDI 3957 Bl. 11, 2007) shoots of spruce and pine from different age groups are dried before the analysis. Thereafter, the needles are removed and analyzed separately. This procedure contradicts ESB's concept of storing samples without modification. A mechanical separation of the needles would also change the samples by squeezing them, leading to a loss of substances in and on the needles (Tarricone *et al.* 2015). In addition, the low-volatility substances bound to particles are largely deposited on glandular hairs of the entire shoots, which is why they accumulate airborne substances more effectively (Wyttenbach *et al.* 1988, Umlauf *et al*. 1994a, b, Tarricone *et al.* 2015).

5 Predefinitions for the Sampling

5.1 Selection and Definition of Sampling Sites

In order to determine the sampling site(s) and sampling size, a screening according to the stratified random sampling principle (Green 1979) must be carried out before the first sampling in a sampling area.

In the first step, homogeneous screening areas in the respective sampling area are determined with regard to the following criteria, for example:

- geology
- soil
- exposure
- gradient of slope
- height
- sufficient distance from busy roads and other local emitters
- Availability of spruce or pine populations that are a minimum of 40 years old.

Care must be taken to ensure that the selected stands are also suitable long-term locations from the point of view of forest planning and forest protection.

An appropriate number of screening areas shall then be determined at random, on which at least 30 trees should be examined. At least three (preferably six) trees should be selected from each area.

After the chemical characterization analysis has been carried out, the dispersion range of the pollutant contents and the spatial pattern of the pollution burden are examined. On the basis of these results, the sampling site is determined as the sum of the suitable screening sites (= future sampling points).

Access to the designated sampling points should, as much as possible, be secured by contracts.

5.2 Selection of Individuals and Sample Size

After evaluation of the screening results, the sample size for the annual routine sampling is determined. The minimum sample size is 15 trees per sampling site.

For a sample collective of 15 trees, a minimum of 150 g fresh weight (one-year-old shoots) per tree should be collected, in order to adequately represent the respective tree and to reach the required total sample quantity of 2,200 g.

The trees are randomly selected within the locations and should comply with the following criteria:

- more than 40 years old, predominant, dominant or co-dominant (Fig. 1),
- free from intense biological (e.g. bark beetle infestation) or mechanical damage (e.g. top rupture).

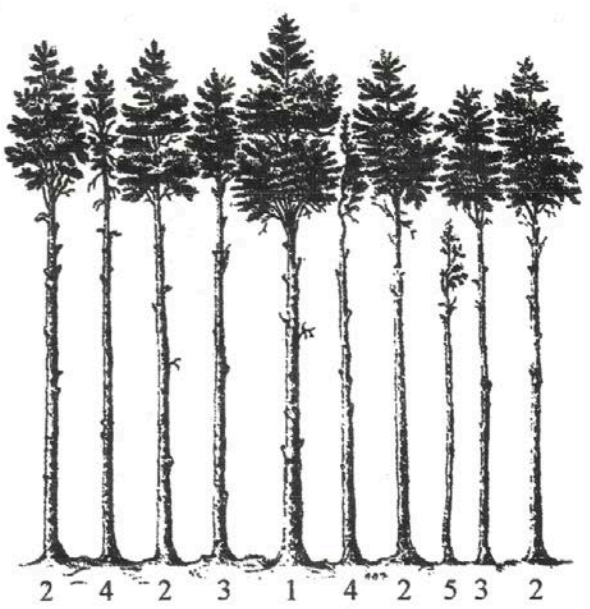

Fig. 1: Tree Categories by Kraft (1884) (1 = pre-dominant, 2 = dominant, 3 = co-dominant, 4 = dominated, 5 = complete epigenous) (from BMJ 2000))

5.3 Sampling Period and Frequency

In long-term programs such as the ESB, sampling should be carried out annually.

The period chosen should be characterized by physiological stability of the trees. In accordance with the Environmental Specimen Bank, a period at the end of the dormancy is chosen to measure the emission situation of winter as well as that of the entire year.

Depending on the altitude and weather conditions, sampling is carried out from March to May (between snowmelt and new spring flushing).

5.4 Area-Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area-related sampling scheme. These include, but are not limited to:

- location and demarcation of the sampling sites,
- required sample size,
- time frame for sampling,
- appropriate authorities.

Here it is important to consider how to ensure a long-term sampling continuity. If changes are made, the document must be updated.

6 Sampling Procedure

All data collected during sampling and biometric sample characterization must be documented in the corresponding specimen data sheets (see appendix). In addition, a protocol must be prepared for each sampling with the following information:

- persons that participated in the sampling,
- chronological sequence of the sampling,
- the underlying version of the sampling guideline and the area-related sampling scheme for the current sampling as well as,
- deviations from the sampling guideline and the area-related sampling scheme.

Collecting specimens from the crown area of standing trees is performed by and exclusively permitted by persons suitably qualified (“cone pickers”) with respect to the safety regulations of the professional association. Their health condition also must be regularly checked. If special requirements exist, e.g. to protect the trees from which samples are taken against damage, appropriate tree climbing equipment has to be used.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets,
- stainless steel scissors,
- stainless steel trough to catch the cut shoots,
- stainless steel containers (3.5 l or 5.5 l) with lids and fasteners,
- waterproof pen for labeling the paper bags and stainless steel containers,
- paper bags (1 bag per tree),
- disposable gloves,
- scales (weighing range up to at least 3 kg, reading 1 g),
- air thermometer,
- soil thermometer,

- camera for documentation,
- liquid nitrogen,
- protective clothing for liquid nitrogen handling,
- cooling devices (dewar vessel) for the immediate deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN), corresponding to the number of required stainless steel containers.

Laboratory:

- specimen data sheets for the biometric sample description,
- cabinet dryer (80°C),
- ruler / scale paper (reading 1 mm),
- precision scales (reading 0,001 g),
- weighing pans,
- stainless steel tweezers.

Sample containers and all equipment are cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (90 – 95°C) rinsing, neutralization using 30% phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure the containers are dried in a cabinet dryer at 130°C (± 10°) for a minimum of an hour (sterilization). The containers remain in the closed cabinet dryer while they are left to cool. Sterilization is not applied to synthetic materials.

6.2 Sampling Technique

Sampling is only carried out in dry weather and stopped if it starts raining. Morning dew must have evaporated from the shoots in the tree crown prior to starting or continuing the collection. Inevitable deviations must be precisely noted in the sampling record.

At least four branches are taken from the crown of each tree (approx. 7-12 whorls with spruce, the outer crown with pines). When branches are thrown down onto the ground, care must be taken that they are not contaminated by fresh soil damage (e.g. wheel tracks, wild boar rummaging etc.). At least three branches must have a complete number of the last seven needle years for the spruce, at least the last five for pines.

After the sample characterization, according to specimen data sheets 3 and 4, the one-year shoots of each branch, with the exception of the branch tip shoot, are cut off with stainless steel scissors so that they fall into a stainless steel trough (see Fig. 2).

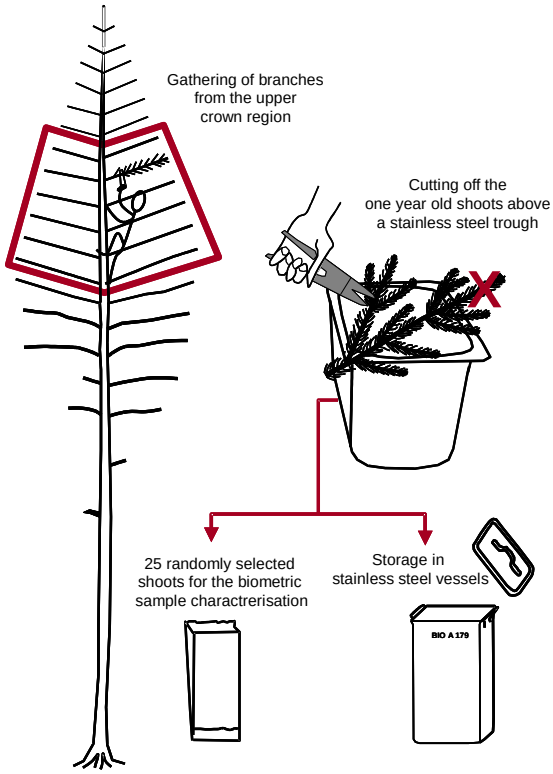

Fig. 2: Schematic representation of the sampling (altered according to Wagner 1995)

For further sample characterization, 25 shoots are randomly selected out of the total sample amount and collected in a paper bag labeled with the respective tree number.

The remaining shoots are transferred from the stainless steel trough into the storage containers.

The samples are immediately rapid-frozen on-site in a dewar vessel for the further storage and transportation of the samples in the gas phase above liquid nitrogen (LIN).

7 Biometric Sample Characterization

Using 25 randomly selected one-year-old shoots per tree, the following parameters are determined in the laboratory:

- shoot length including buds (reading 1 mm),
- dry weight of the needles (reading 0.01 g),
- dry weight of the stems with buds (reading 0.01 g),
- 1000-needle weight (reading 0.001 g).

Paper bags containing the shoots are laid in a cabinet dryer (approx. 80°C) – directly upon returning from sampling – for drying (not too densely packed to prevent overheating), and dried until weight stability is reached (approx. 2 days).

Needles and stems are separated from each other by shaking the paper bags. Remaining needles are removed by hand from the stem. The stems are weighed together with all buds; flower remains etc. that have come off.

Shoot lengths (stem length including buds) are measured to a reading of 1 mm by using mm-paper. With curved stems, the length is estimated as precisely as possible, e.g. by straitening the stems.

To determine the 1000-needle weight, exactly three times 100 whole, randomly selected needles are counted and weighed (N.B.: for pine needles, 100 intact double needles). From these individual weights, the 1000-needle weight is multiplied.

Additionally, the weight ratio of dried needles to dried stems is assessed.

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Checklist to Prepare and Conduct the Sampling

Specimen Type:	**Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)**
Target Compartments	one-year-old shoots from at least four branches out of the upper, outer exposed crown region, of the spruce tree's 7th-12th whorl of the branches
Individual Specimens	predominant, dominant or co-dominant trees (tree category 1, 2 or 3 by Kraft) older than 40 years
Random Sample Number	at least 15 trees, mind. 150 g fresh weight
Sample Quantity for the ESB	150 g fresh weight from 15 trees must be sampled to gain the needed quantity of 2,200 g
Sampling Period	March until May (after snow thaw and prior to spring flushing)
Sampling Frequency	1 sampling per annum
Required Equipment for Field Work	• specimen data sheets • stainless steel scissors • stainless steel trough to catch cut shoots • • waterproof pen to label the paper bags and stainless steel containers • paper bags (1 bag per tree), • disposable gloves • scales (weighing range up to at least 3 kg, reading 1 g) • air thermometer, soil thermometer (reading 1°C) • camera for documentation
Sample Packing until Further Processing	stainless steel containers (3.5 or 5.5 l) with lids and fasteners (1 container per tree), paper bags (1 bag per tree)
Transport and Interim Storage	cooling device (dewar) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN)
Required Equipment for Laboratory Work	• specimen data sheets for the biometric sample description • cabinet dryer (approx. 80°C • ruler / scale paper (reading 1 mm) • precision scales (reading 1 mg) • weighing pans • stainless steel tweezers
Sample Characterization	• stand type • needle coverage, yellowing (chlorosis) and necrosis, fructification • one-year-old shoots (see specimen data sheets) • damage to needles and shoots (feeding on needles/shoots, chlorosis, necrosis), contamination • for 25 shoots: o shoot length (reading 1 mm) o dry weight of the needles (reading 0.01 g) o dry weight of the stems including buds (reading 0.01 g) o 1000-needle weight (reading 0.001 g) o weight ratio between needles and stems

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

	Specimen Type
	Specimen Condition
	Collection Date (MM/YY)
	Sampling Area (SA)
	Sampling Region (SR)
	Sampling Site (SS)
	Additional information

Sampling Site (plain text) _____

Sampling Location (number) _____

Sampling Location (plain text) _____

Sampling Leader _____

Remarks _____

_____

_____

_____

_____

Notes _____

_____

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Specimen Data Sheet 2: Weather Conditions

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Numbers: from __ __ to __ __

Last Precipitation Date Preceding the Sampling: __ __ . __ __ . __ __ __ __

Type of Precipitation:
(see table below) ___

Start of the Sampling:		**End of the Sampling:**
__ __ . __ __ . __ __	Sampling Date	__ __ . __ __ . __ __
__ __ : __ __	Time	__ __ : __ __
__ __	Air Temperature at 1.5 m Height (°C)	__ __
__ __	Soil Temperature at 10 cm Depth (°C)	__ __
__ / 8	Cloud Covering	__ / 8
__	Type of Clouds (see table below)	__
__ __ __	Wind Direction	__ __ __
__	Wind Force in Degree Beaufort (see table below)	__
__	Type of Precipitation (see table below)	__

Type of Clouds:
0 = unclouded
1 = Cirrus
2 = Stratus
3 = Cumulus
4 = Fog
5 = High Fog
6 = Stratocumulus




Cirrus | Stratus | Cumulus | Stratocumulus




Type of Precipitation:
0 = no Precipitation
1 = Rain
2 = Drizzle
3 = Snow
4 = Dew
5 = Rime
6 = torrential Rain
7 = Hail

Wind Force (according to Beaufort):
0 = Calm
1 = Very Slight Breeze
2 = Slight Breeze, moves leaves
3 = light Breeze, moves twigs
4 = moderate Breeze, moves thin branches
5 = bright Breeze, moves medium sized branches
6 = strong Wind, moves thick branches
7 = stiff Wind, shakes trees

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Specimen Data Sheet 3: Description of the Tree

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Number: __ __

Stand Type: ☐ Dense Stand ☐ Marginal Stand Zone ☐ Sparse Stand ☐ Forest Aisle ☐ Free Standing Solitary Trees

Tree Height (estimation in 5 m-intervals): __ __ m

Needle Coverage: __ __ __ % (* automatic calculation in IS ESB)

(percentage estimate per needle age group in 5 %-intervals)

Needle Age Group	1	2	3	4	5	Summation Pine Trees	6	7	Summation Spruce Trees
1st branch						__ __ __*			__ __ __*
2nd branch						__ __ __*			__ __ __*
3rd branch						__ __ __*			__ __ __*

All yellowish to whitish discolorations (Chlorosis): __ __ __ % (* automatic calculation in IS ESB)

(percentage estimate per needle age group in 5 %-intervals)

Needle Age Group	1	2	3	4	5	Summation Pine Trees	6	7	Summation Spruce Trees
1st branch						__ __ __*			__ __ __*
2nd branch						__ __ __*			__ __ __*
3rd branch						__ __ __*			__ __ __*

All brownish to reddish discolorations (Necrosis): __ __ __ % (* automatic calculation in IS ESB)

(percentage estimate per needle age group in 5%-intervals)

Needle Age Group	1	2	3	4	5	Summation Pine Trees	6	7	Summation Spruce Trees
1st branch						__ __ __*			__ __ __*
2nd branch						__ __ __*			__ __ __*
3rd branch						__ __ __*			__ __ __*

Fructification: (related to cones and visible scars of current cone losses)

☐ none/little ☐ moderate ☐ heavy

Water sprouts: (Spruce only):

☐ none/little ☐ moderate ☐ heavy

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 4: Description of the one-year-old Shoots

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Number: __ __

Damage to Needles:	**Biotic Damage:** (percentage of the needles surface) __ __ __ % (percentage estimate per 5 %-	**Damage Type** ☐ nonexistent ☐ feeding on Needles ☐ sucking Spots of Insects ☐ other:
	Chlorosis: (all yellowish to whitish discolorations) __ __ __ % *(percentage estimate perin 5 %- (* automatic calculation in IS ESB)	**Chlorosis Type** ☐ nonexistent ☐ blotchy, skewbald ☐ at the Needle Base ☐ at the Needle Top ☐ on the Entire Needles
	Necrosis: (all brownish to reddish discolorations) __ __ __ % (percentage estimate per (* automatic calculation in IS ESB)	**Necrosis Type** ☐ nonexistent ☐ blotchy, skewbald ☐ at the Needle Base ☐ at the Needle Top ☐ on the Entire Needles
Damage to Shoots:	☐ hollow Shoots ☐ unopened Top Buds ☐ Galls, Pineapple Galls ☐ other:	
Description of Conta-mination:	_____ _____ _____	

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 4: Sample Description and Storage

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Number: __ __

Storage condition:	☐ dry Samples (standard)	☐ humid Samples

Number of Stainless Steel Vessel	Weight Empty [g]	Weight Filled [g]	Weighted Sample [g]	Remarks
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	

Specimen Description (related to 25 shoots)

Separation of needles and stems

dry weight of the needles: __ __ . __ __ g

dry weight stems with buds: __ __ . __ __ g

Weight ratio of needles to stems: : ☐ . ☐☐ automatic calculation in IS ESB

1000-needle weight:

dry weight of 3 x 100 needles

__ . __ __ __ g __ . __ __ __ g __ . __ __ __ g

1000-needle weight: ☐ . ☐☐☐ g automatic calculation in IS ESB

shoot length of 25 one-year-old shoots:

1 __ __ __ mm	11 __ __ __ mm	21 __ __ __ mm
2 __ __ __ mm	12 __ __ __ mm	22 __ __ __ mm
3 __ __ __ mm	13 __ __ __ mm	23 __ __ __ mm
4 __ __ __ mm	14 __ __ __ mm	24 __ __ __ mm
5 __ __ __ mm	15 __ __ __ mm	25 __ __ __ mm
6 __ __ __ mm	16 __ __ __ mm	
7 __ __ __ mm	17 __ __ __ mm	**Mean of shoot length**
8 __ __ __ mm	18 __ __ __ mm	☐☐☐ mm
9 __ __ __ mm	19 __ __ __ mm	automatic calculation in IS ESB
10 __ __ __ mm	20 __ __ __ mm	

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Protocol

Norway Spruce (*Picea abies*) / Scots Pine (*Pinus sylvestris*)

Sampling Area: _____ Identification: __ __ __ __ __

Underlying Version of the Sampling Guideline __ __ . __ __ . __ __

Underlying Version of the Sampling Scheme __ __ . __ __ . __ __

1. Objective of the Sampling: _____

_____

2. Actual Timeframe of the Sampling:

Start		End		Sample no.		Sampling Leader	Remarks
date	time	date	time	from	to		

3. Participants: internal _____

_____

external _____

4. Checklist Referring to Sampling Scheme and Sampling Guideline: ☒ as prescribed

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Point (selection/definition)
- ☐ 4.3 Selection of the Individual Specimens
- ☐ 4.4 Technical Preparations
- ☐ 4.5 Cleaning Procedure for the Packages
- ☐ 4.6 Sampling Technique/Method of Capture
- ☐ 4.7 Sample Amount
- ☐ 4.8 Data Collection
- ☐ 4.9 Transport and Interim Storage

Number, kind and reason for deviation (clear text):

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_____

_____

Remarks: _____

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_____ __ __ . __ __ . __ __ _____

Recorder Date Signature