



CropLife
EUROPE

A harmonized approach for the assessment of treatments and analytics according to EFSA/ECHA GD on water treatment

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Harmonization purpose (1) – General



Goal:

The objective of this harmonized approach is to provide more precise and practical guidance for implementing and conducting the procedures described in the EFSA/ECHA guidance document on drinking water treatment.

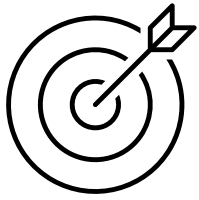
Harmonization:

This document seeks to harmonize the procedures outlined in the EFSA/ECHA guidance document, ensuring full alignment with all relevant requirements. Establishing a harmonized approach represents an important first step in supporting industry stakeholders and CROs. The approach has been presented in several webinars and broadly shared across the stakeholder community.

This document highlights specific sections of the guidance in which, in our view, critical parameters are described and where harmonization can meaningfully enhance comparability between experiments—for example, test design, incubation periods, dosing concentrations, and other essential methodological elements. It is intended as a living document and will be updated based on feedback from stakeholders and CROs. Furthermore, it is expected to serve as a foundation for future ring-testing, which is typically a prerequisite for establishing a new study design.

This document serves as supporting material and should always be used in conjunction with the EFSA/ECHA guidance document on drinking water treatment, including its descriptions and abbreviations (see also [slide 48](#)).

Harmonization purpose (2) - Outline



- **General information on experiments**
- **Treatment procedures**
 - Chlorination
 - Hypochlorite
 - Monochloramine
 - Oxidation
 - Chlorine dioxide
 - Combined approach (Oxidation + Chlorination)
 - Ozonation
 - UV-Treatment
 - Biodegradation using sand filtration
- **Analytcs**
 - Analytical Workflow
 - Use of 14C-labeled test items
 - Method qualification and quantification strategies
 - Non-target screening
 - TP quantification and Structure elucidation
 - Positive Control

General information on experiments (1)



Samples to be tested:

We recommend the following scope of samples for each experimental condition:

Mandatory samples:

1. **High concentration samples:** drinking water with t.s. in high concentration, “treated”, as duplicate (2)
2. **Low concentration samples:** drinking water with t.s. low concentration, “treated”, as duplicate (2)
3. **Nominal sample:** aliquot of the background control after treatment + t.s. in high and low concentration, single replicates (2)

Optional samples:

4. **Background control:** drinking water + dose vehicle (e.g. MeCN) – single replicate (1)
5. **Stability control:** drinking water with t.s., “untreated”, single replicate (1)
6. **Positive control:** control substance in high concentration, “treated”, single replicate (1) [direct or indirect, [see slide 36](#)]

It should be noted that this sample size is necessary for each individual test (e.g. for each pH value if tests are to be carried out at several pH values).

“**Nominal sample**”: the samples for the single-point calibration for the purpose of quantifying the t.s. are also prepared from the background controls. For this purpose, the background control is aliquoted after the treatment and applied with t.s. so that the starting concentration of the t.s. is reached (t.s. in matrix). This must be carried out for high and low concentration. The t.s. should be applied as shortly as possible before the measurement.

“**Treated**” or “**untreated**” in this context refers to the addition or non-addition of the corresponding disinfectant (e.g. hypochlorite, UV dose)

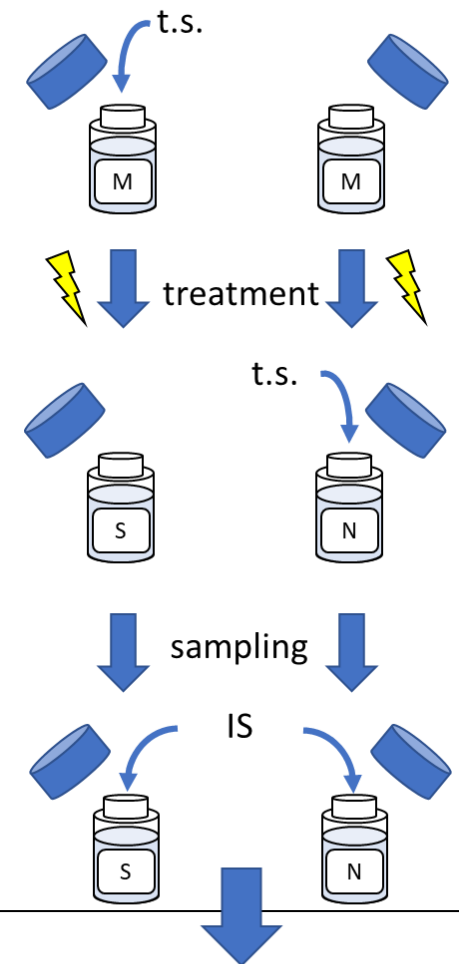
“**Drinking water**”: Consists of CIPAC Water C with 3 mg C/L of DOC. The Guidance specifically calls for Suwannee River DOC, but the fraction is not specified (Fulvic, Humic, NOM). We recommend to use the fulvic acid (FA) standard due to its good solubility. (For further Information: [International Humic Substances Society 2025 Products – IHSS](#))

General information on experiments (2)

Samples and nominal samples:

- Nominal samples can be used for the quantification and also for the non-target screening workflow
- The analytical methods for quantification and qualitative analysis should fulfill the requirements of the guideline.
- **Recommendations for the quantification are discussed on [slides 31-32](#)**
- **Recommendations for the qualification of the used analytical methods are discussed on [slides 31-32](#)**
- Non-target and Suspect Screening
 - Statistical evaluation against the nominal sample is crucial for feature reduction and evaluation
 - We recommend to use a “treated” drinking water (matrix) for the preparation of a nominal sample
 - **Further recommendations on non-target and suspect screening are discussed on [slide 33](#)**

M: matrix (drinking water)
S: sample (high/low concentration)
N: nominal sample (high/low conc.)
t.s.: test substance
IS: internal standard



LC/MS quantification

Example

General information on experiments (3)



Application checks:

In some cases, the guidance requires the concentration of the oxidant in the test system to be experimentally derived e.g., 'at the start of the reaction' or at 't = 0' in/or in the test system. The exact time is not defined.

According to our interpretation, this refers to the moment immediately after the oxidant has been added to the drinking water, which already contains the test substance.

However, detection methods for quantifying the amount oxidant present generally require an incubation time (see DPD method). After the oxidant has been added to the test system, there is a reaction between the oxidant, DOC and test item. This results in differences between the "measured" concentration and the "nominal" dosed concentration of oxidant.

For this reason, in addition to the concentration control in the test system, we recommend application controls in ultrapure water to show that the correct nominal concentration has been applied.

The dosing rates and all relevant concentrations are described in detail in the dedicated sections for each treatment in this document (e.g. [slide 9](#) and [slide 10](#) for hypochlorite)

General information on experiments (4)



4.2.4.1. Chlorination with NaOCl; description of experiments

Experimental procedure

Add NaOCl to 800 mL of drinking water to a free chlorine concentration with a molar ratio free chlorine/[AS or eTP] > 10 at the start of the experiment. This concentration has to be experimentally confirmed, and the residual concentration should be measured after a reaction time of 12 h.

Carry out the experiment at a starting pH of 6.5, 7.5 and 8.5 at room temperature.

Add the compound under investigation (the active substance and/or its eTP) to a concentration of 1,000 × the LOQ (also see https://ec.europa.eu/food/system/files/2021-02/pesticides_mrl_guidelines_2020-12830.pdf).

Stir for at least 12 consecutive hours, with a maximum of 24 h.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.

Test vessel volume: 800 mL is stated in GD as suggested volume. We deem this to be unnecessarily high – it is expensive and not sustainable. **Sample concentrations** are relevant not sample volume.

- Test vessel volume should be reduced to a reasonable volume e.g. 10 or 50 mL, as long as stirring is ensured and low-adsorbing vials are used. Scale up approaches can be conducted in higher vessel volumes if needed.

Reaction time and oxidation agent residues:

- Reaction time can be variable in the GD. We recommend to harmonize the reaction time for each treatment. Oxidant residue concentrations should be determined at the end of the reaction to ensure oxidant excess during the whole reaction. Our proposal would be to standardize this as **24 hours for chlorination, chloramination and ClO₂**.

Test concentration: The relevant low concentration needs to be decided case-by-case, considering PECgw and PECsw. 1000 x LOQ is only relevant for the identification of potential features/TPs (scale-up exp.)

- We recommend to define the LOQ as the **target LOQ of 0.075 µg/L for any given tTP**. 1000 × LOQ should be set at minimum of 75 µg/L.



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4.2.4.1. Chlorination with NaOCl; description of experiments

Experimental procedure

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Stir for at least 12 consecutive hours, with a maximum of 24 h.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.

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Test item concentration and chlorine determination: The GD gives no information on how to calculate chlorine concentration. Chlorine concentrations are usually determined by the DPD test.

- Using a molar ratio of >10, in low concentration testing, would lead to chlorine concentrations below the LOQ of chlorine determination with the DPD test.
- Calculation of molar ratios could lead to different dosing in chlorine due to differences molar masses of test substances.
- To avoid different treatment scenarios for each test substance, a harmonized approach should be considered for the treatment as long the excess of chlorine (molar ratio >10) is ensured.
- The added chlorine concentration needs to be experimentally confirmed which is difficult to achieve for each test vessel. After spiking the hypochlorite to the drinking water hypochlorite will be consumed which makes it impossible to confirm the calculated concentration after spiking.





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4.2.4.1. Chlorination with NaOCl; description of experiments

Experimental procedure

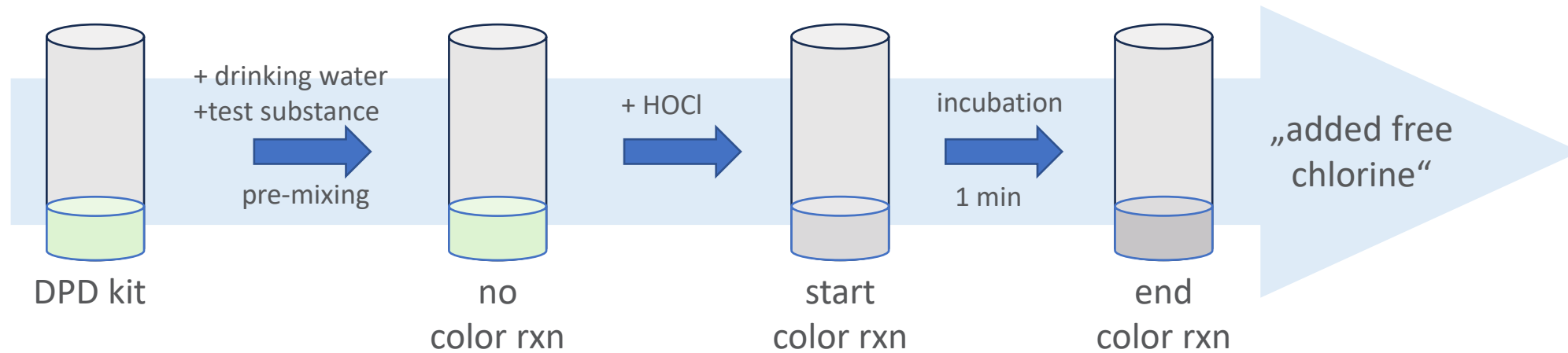
Add NaOCl to 800 mL of drinking water to a free chlorine concentration with a molar ratio free chlorine/[AS or eTP) > 10 at the start of the experiment. This concentration has to be experimentally confirmed, and the residual concentration should be measured after a reaction time of 12 h.

Test item concentration and chlorine determination:

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- The aim is to use treatment concentrations which are realistic and independent on the molar mass of the test item. German Umwelt Bundesamt (UBA) published the [§20 List](#) which regulates the allowed dosing concentrations of hypochlorite. The maximum allowed dosing for sodium hypochlorite is 1.2 mg/L free chlorine (Cl₂). **We suggest to always dose 1.2 mg/L free chlorine (Cl₂) in accordance with the §20 List.**
 - For reproducibility reasons we recommend to conduct the treatment and determination of free chlorine using the DPD method as described on [slide 10](#).
 - For the definitive experiments, standard water will be prepared. The test item will be spiked to the drinking water. The treatment starts after adding the hypochlorite solution to the test vessel which includes the drinking water + test substance.
 - According to the GD, a measurement of chlorine concentration should be made at the “start of the experiment”. This is likely to be < dosing chlorine concentration due to reaction with DOC/ t.s.
 - The amount of chlorine dosed should be confirmed by dosing the hypochlorite in **ultrapure water** to serve as a treatment control ([slide 4](#)). Hypochlorite dosing into standard water with DOC will lead to direct consumption of the free chlorine and concentrations obtained will not be reflective of the amount dosed into the test system.
 - A measurement should also be made from the “test system” to satisfy the >10 molar ratio at the start of the experiment.

Chlorination: Hypochlorite

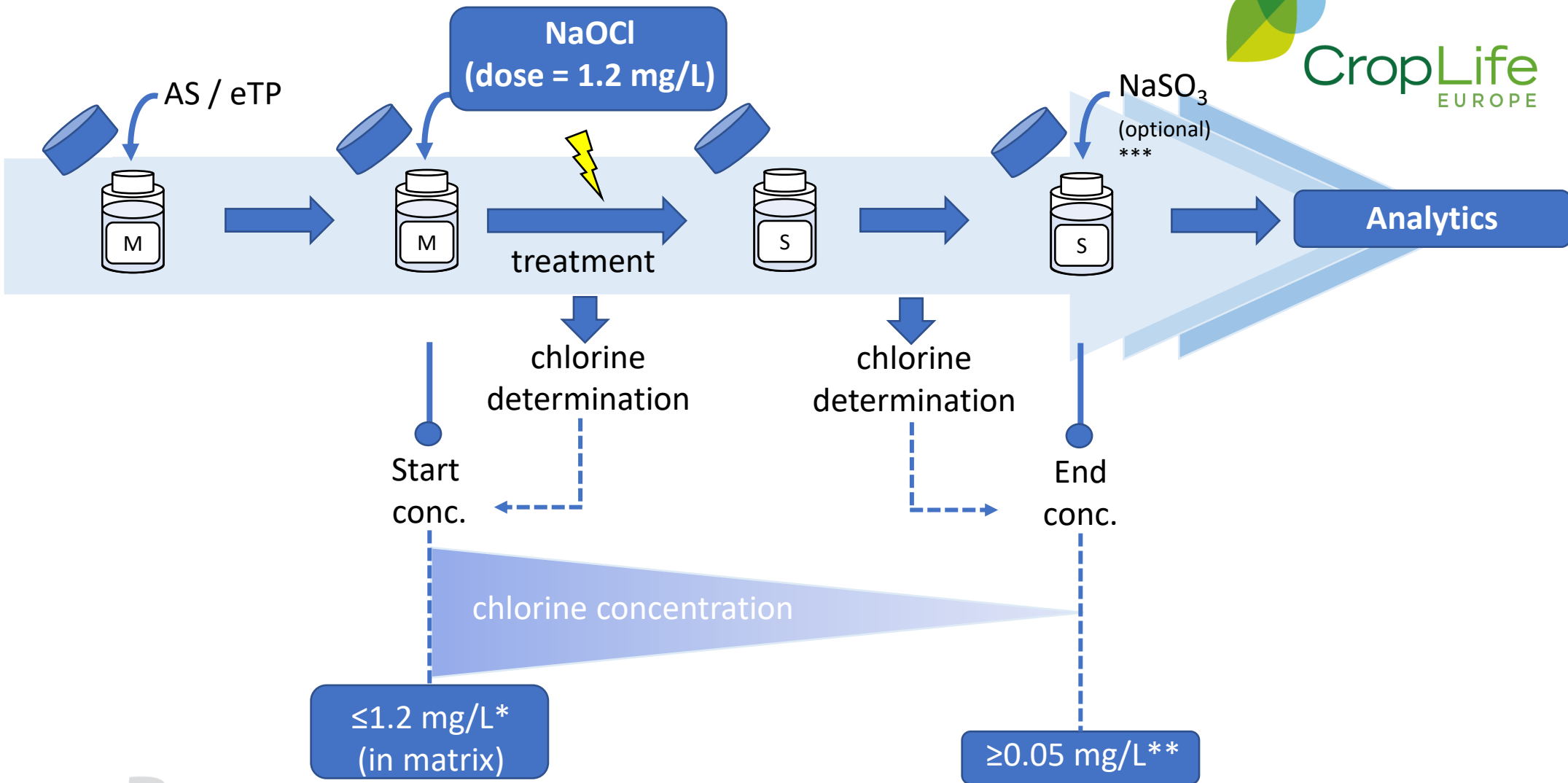
Direct determination* of chlorine concentration in drinking water matrix



* Color reaction starts immediately after oxidant addition, no delay time, more realistic free chlorine concentration at the start of the treatment



Chlorination: Hypochlorite



Abbreviations: M = Matrix; S = Sample; AS = active substance; eTP = environmental transformation product
 * 1.2 mg/L is the spiking concentration. Confirmation of the achieved application dose of NaOCl in test system should be obtained by dosing into ultra-pure water and measuring free chlorine concentration.
 ** LOQ of DPD Method
 *** quenching can lead to artefacts

4.2.4.2. Chloramination with NH_2Cl ; description of experiments

Experimental procedure

First, the NH_2Cl stock solution (with a concentration of NH_2Cl of about 50–250 mg $\text{H}_2\text{Cl}/\text{L}$) has to be prepared by slowly adding NaOCl into a rapidly stirred NH_4Cl solution adjusted to $\text{pH} = 8.5$ at a Cl_2 : N molar ratio of 1: 1.2 (procedure described by Le Roux et al. (2012)). Experiments are carried out at room temperature.

Add NH_2Cl to 800 mL drinking water to a concentration $> 5\text{--}10$ mol/mol.

Carry out the experiment at a pH between 6.5 and 7.0.

Add the compound under investigation to a concentration of $1,000 \times$ the LOQ.

Stir for at least 24 h, measuring the NH_2Cl concentration at $t = 0$ and $t = 24$ h.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.

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Preparation of NH_2Cl stock solution: We recommend the protocol by Le Roux (cited in the GD).¹

- The GD gives no information on how exactly to calculate the monochloramine concentration.
- A calculation using molar ratios would lead to a different concentration of monochloramine for each test item due to differences in their molar masses.
- To avoid different treatment scenarios for each specific substance, a generic and realistic approach should be considered for the treatment as long the excess of monochloramine (molar ratio $>5\text{--}10$) is ensured.
- While the concentration of monochloramine should be determined at “ $t = 0$ h” (start of the experiment) and “ $t = 24$ h” (end of the experiment, respectively), it is not exactly described what $t = 0$ means. Therefore, it is recommended that application checks will be performed (described in general procedures) as well as monochloramine concentration after application to prove, that $n/n >5\text{--}10$ is reached in the test vessel.

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1) Le Roux, J. et. al. *Environ. Sci. Technol.* **2012**, 46, 11095–11103 (link: [see supporting information](#)) DOI: [dx.doi.org/10.1021/es3023094](https://doi.org/10.1021/es3023094)

4.2.4.2. Chloramination with NH_2Cl ; description of experiments

Experimental procedure

First, the NH_2Cl stock solution (with a concentration of NH_2Cl of about 50–250 mg $\text{H}_2\text{Cl}/\text{L}$) has to be prepared by slowly adding NaOCl into a rapidly stirred NH_4Cl solution adjusted to $\text{pH} = 8.5$ at a Cl_2 : N molar ratio of 1: 1.2 (procedure described by Le Roux et al. (2012)). Experiments are carried out at room temperature.

Add NH_2Cl to 800 mL drinking water to a concentration > 5–10 mol/mol.

Carry out the experiment at a pH between 6.5 and 7.0.

Add the compound under investigation to a concentration of $1,000 \times$ the LOQ.

Stir for at least 24 h, measuring the NH_2Cl concentration at $t = 0$ and $t = 24$ h.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.

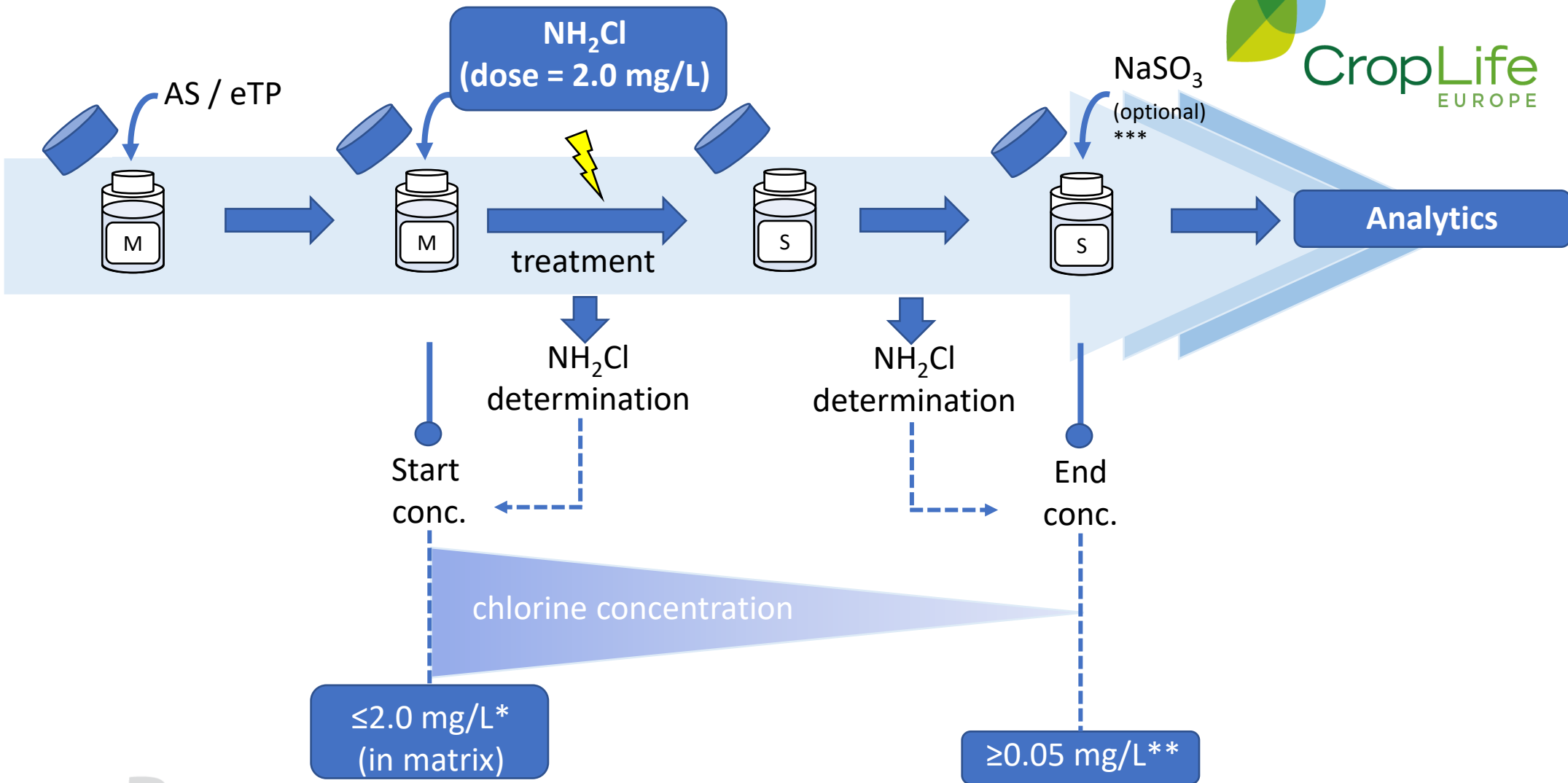
NH_2Cl determination and treatment concentration:

The aim is to use treatment concentrations which are realistic and independent on the molar mass of the test item. The WHO published the **Guideline for drinking water quality** (fourth ed., 2022, pg. 359), where a typical concentration between 0.5 – 2.0 mg/L is mentioned, if used as primary disinfectant or to provide a chlorine residual in the distribution system. It is recommended to not exceed a monochloramine-concentration of 3.0 mg/L in drinking water, as a health precaution.

We suggest to always dose 2.0 mg/L NH_2Cl in accordance with the WHO Guidelines.

For reproducibility reasons we recommend to conduct the treatment and determination of monochloramine using the a colourimetric method such as Indophenol, DPD or similar. For the treatment standard water will be prepared. The test item will be spiked to the drinking water. The treatment starts after adding the monochloramine solution to the test vessel which includes the drinking water + test substance.

Chlorination: Monochloramine



Abbreviations: M = Matrix; S = Sample; AS = active substance; eTP = environmental transformation product
 * 2.0 mg/L is the spiking concentration. Confirmation of the achieved application dose of NH_2Cl in test system should be obtained by dosing into ultra-pure water and measuring monochloramine concentration.
 ** LOQ of colorimetric method used
 *** quenching can lead to artefacts



4.2.4.3. Oxidation with ClO_2 ; description of experiments

For all experiments, apply the required safety measures for handling ClO_2 .

The ClO_2 solution can be prepared from gaseous ClO_2 by slowly adding dilute H_2SO_4 to a NaClO_2 -solution following the standard method APHA, 1998. The ClO_2 concentration can be determined, e.g. using a Hach DR2800 portable photometer (Hach, Loveland, CO, USA) by the diethyl-p-phenylene diamine colourimetric method (see APHA, 1998). Experiments should be carried out applying a ClO_2 /compound (parent compound or eTP) ratio of 2.5 (at least $\text{ClO}_2/\text{DOC} = 0.5\text{--}1.5$).

After at least 12 h of reaction time at room temperature, the residual ClO_2 concentration should be measured before removing the excess ClO_2 by bubbling N_2 through the solution. In this way, it is proven that an excess of oxidant has been applied.

Analyse the added compound(s) and mark the signals of possibly relevant TPs. The presence of nitrogen has a strong effect on the TP formed (see Appendix B).

Preparation of ClO_2 stock solution:

- A general recommendation for the preparation of chlorine dioxide solution cannot be given directly, as it depends on the relevant laboratory equipment and local safety requirements. The APHA method cited in the guidance has been successfully reproduced and is suitable, however, it is generally not recommended to purchase commercial chlorine dioxide solutions/tablets, as these are unstable and usually not sufficiently concentrated.
- **We recommend to conduct the treatment with a chlorine dioxide concentration of 1.5 mg/L.**
 - Applied concentration is $>2.5 \text{ ClO}_2/\text{compound}$ ratio – this will satisfy both high- and low-test item concentration experiments.
 - Applied concentration also is in the range of $0.5 - 1.5 \text{ ClO}_2/\text{DOC}$ ratio. DOC set at 3 mg C/L, therefore dosing at 1.5 mg/L will give a harmonized ratio of 0.5 for ClO_2/DOC .
- Although, the §20 list states a max. dosing of 0.4 mg/L, this is below the GD requirements. **The dose of 1.5 mg/L ensures that we meet guideline requirements.**





4.2.4.3. Oxidation with ClO_2 ; description of experiments

For all experiments, apply the required safety measures for handling ClO_2 .

The ClO_2 solution can be prepared from gaseous ClO_2 by slowly adding dilute H_2SO_4 to a NaClO_2 -solution following the standard method APHA, 1998. The ClO_2 concentration can be determined, e.g. using a Hach DR2800 portable photometer (Hach, Loveland, CO, USA) by the diethyl-p-phenylene diamine colourimetric method (see APHA, 1998). Experiments should be carried out applying a ClO_2 /compound (parent compound or eTP) ratio of 2.5 (at least $\text{ClO}_2/\text{DOC} = 0.5\text{--}1.5$).

After at least 12 h of reaction time at room temperature, the residual ClO_2 concentration should be measured before removing the excess ClO_2 by bubbling N_2 through the solution. In this way, it is proven that an excess of oxidant has been applied.

Analyse the added compound(s) and mark the signals of possibly relevant TPs. The presence of nitrogen has a strong effect on the TP formed (see Appendix B).

Chlorine dioxide determination:

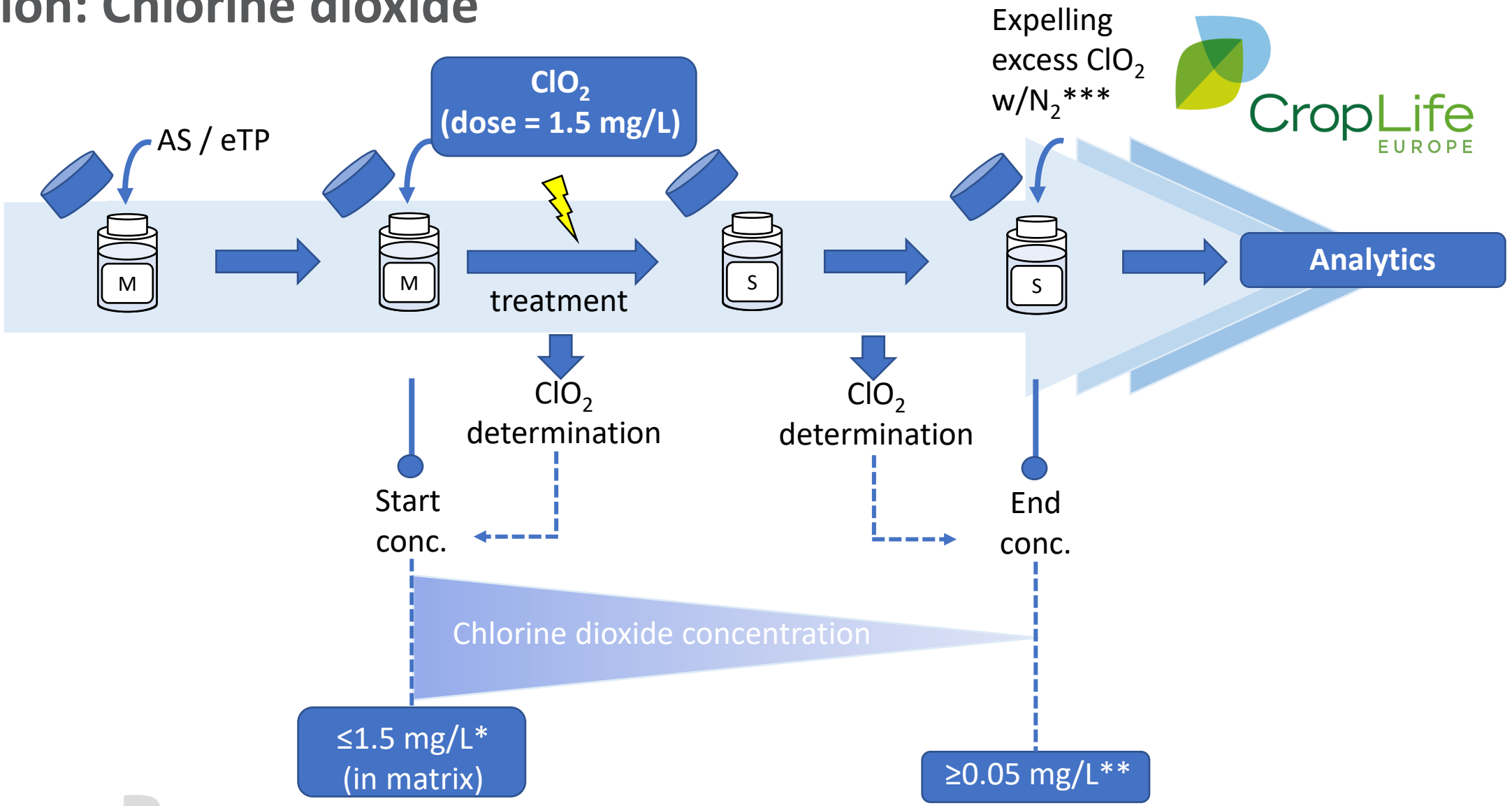
- ClO_2 determination should be carried out in ultrapure water as it degrades rapidly after dosing into the drinking water consisting DOC. Colorimetric methods such as DPD method should be used

Reaction time and oxidation agent residues:

- We recommend to harmonize the reaction time to 24 hours as for the other oxidation-based treatments.



Oxidation: Chlorine dioxide



Abbreviations: M = Matrix; S = Sample; AS = active substance; eTP = environmental transformation product
 * 1.5 mg/L is the spiking concentration. Confirmation of the achieved application dose of chlorine dioxide in test system should be obtained by dosing into ultra-pure water and measuring chlorine dioxide concentration.
 ** LOQ of DPD Method
 *** if substantial amounts of ClO_2 are left

Combined processes:

4.2.4.4. Combined processes, pre-oxidation followed by chlorination or monochloramination; description of experiments




For all experiments, apply the required safety measures for handling ClO_2 .

ClO_2 often is applied as pre-oxidation, followed by chlorination by either NaClO or NH_2Cl . Therefore, it is necessary to also carry out the experimental procedures for ClO_2 oxidation followed by NaClO treatment and for ClO_2 oxidation followed by NH_2Cl treatment, according to the procedures described for the separate processes.

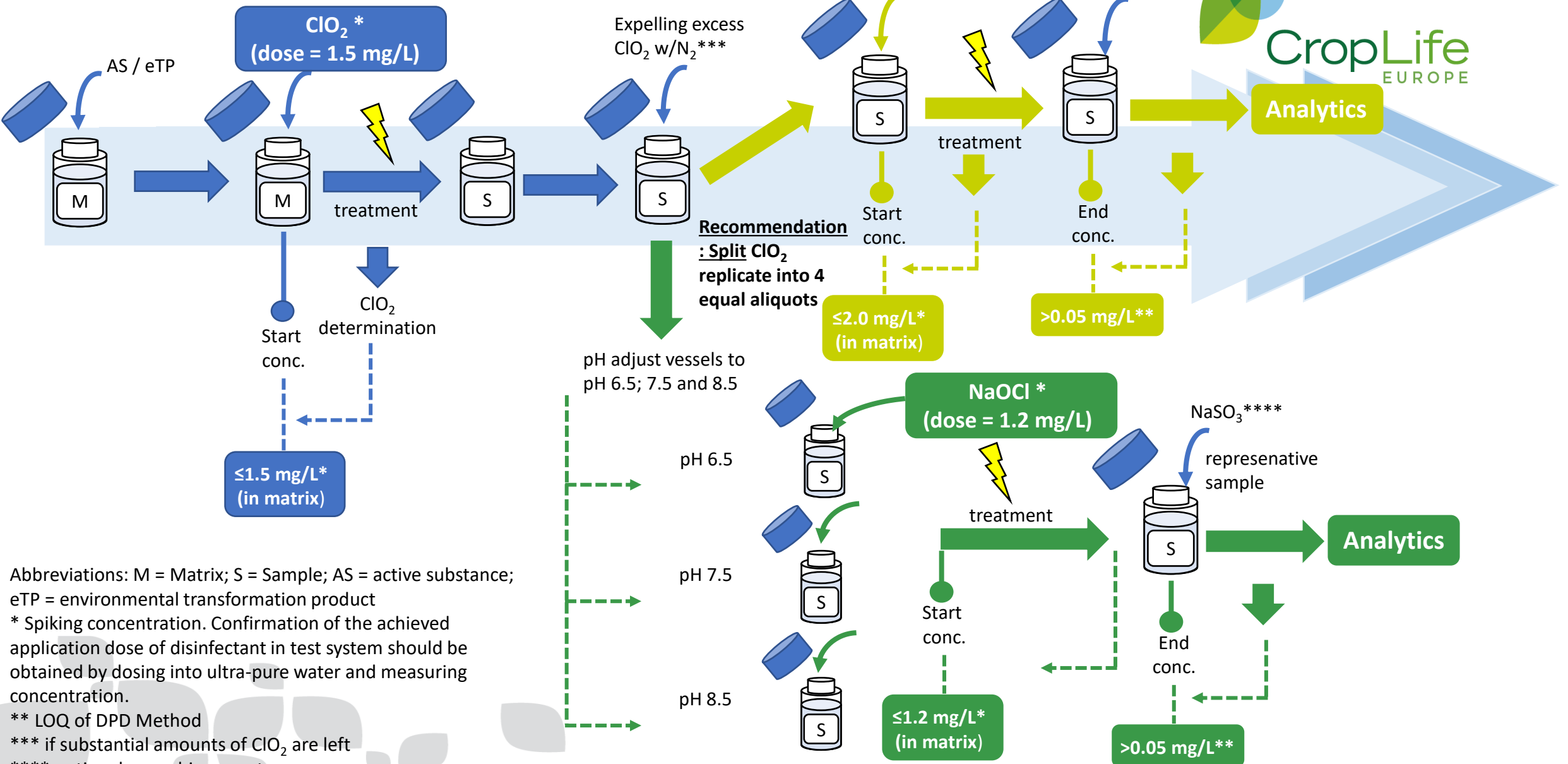
Analyse the added compound(s) and mark the signals of possibly relevant TPs. The presence of nitrogen has a strong effect on the TP formed.

Testing approach:

In general:

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- It is assumed that if no degradation occurs in each of the three individual tests, **the combination test in cascade will also not lead to any conversion and can therefore be omitted.**
 - If no degradation of test item occurs under ClO_2 oxidation regime, **read-across of combined processes** can be made by evaluating $\text{NaOCl}/\text{NH}_2\text{Cl}$ treatment data only.
 - If degradation occurs in ClO_2 testing, the impact of follow-on treatment with NaOCl and NH_2Cl is **required to be assessed.** Although the test substance may be stable to these follow-on treatments, tTPs originating from oxidation may be further degraded.
 - Initial ClO_2 reactions should be split into aliquots for further testing under the various conditions, therefore ensure that suitable aliquots are taking forward for preliminary oxidation.
 - **Proposed experimental workflow outlined in following slide.**

Combined processes



Abbreviations: M = Matrix; S = Sample; AS = active substance; eTP = environmental transformation product
 * Spiking concentration. Confirmation of the achieved application dose of disinfectant in test system should be obtained by dosing into ultra-pure water and measuring concentration.
 ** LOQ of DPD Method
 *** if substantial amounts of ClO_2 are left
 **** optional quenching agents

4.2.4.5. Ozonation; description of experiments

Experimental procedure

For all experiments, apply the required safety measures for handling O₃.

Prepare a solution of the compound under investigation in standard water, preferably at a concentration of 1,000 × the LOQ. In a subsequent stage, if certain TP will have been identified, this may be decreased to an environmentally relevant concentration in the order of about 1–10 µg/L. O₃ can be dosed as a gas in the solution by means of a bubble column and by using either air or oxygen in an ozone generator. For water treatment, ozone is typically generated from ambient air or oxygen using the DBD method (dielectric barrier discharge). A preferable method is to dose directly from a concentrated, freshly prepared O₃ solution in ultrapure water. The ozone concentration to be dosed is 1.5 mg/L.

Stir the solution for at least 1 h, both at a pH of 6.5 and 9.5 at room temperature and at low alkalinity, to prevent radical scavenging by bicarbonate ions. In principle, the solution can be quenched with H₂SO₃ to remove the excess ozone. However, since this compound may interfere with the analyses and ozone reacts very quickly, it will not be necessary to quench the solution before analysis.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.

Testing approach:

- The description of experiments for ozonation is well described.
- However, the preparation of the ozone stock solution and dosing is not straight-forward. A general recommendation for the preparation of ozone solution cannot be given directly, as it depends on the relevant laboratory equipment and local safety requirements. Ozone has a very short half-life in ultra-pure water (~20 min at rt). Therefore, **we recommend to dose directly from the stock solution after determination of the ozone concentration of the stock solution.**
- Defined ozone dosage (DOD) can support ozone dosing using a re-circulating system that produces an ozone stock solution with a constant and uniform ozone concentration in ultra-pure water. The ozone stock solution is always prepared in ultra-pure water. To avoid a too high dilution of the drinking water we recommend to dose maximum 10% of ozone stock solution compared to the final test volume. **Therefore, we recommend an ozone concentration of the ozone stock solution of >15 ppm.**



UV treatment

4.2.4.6. UV disinfection; description of experiments

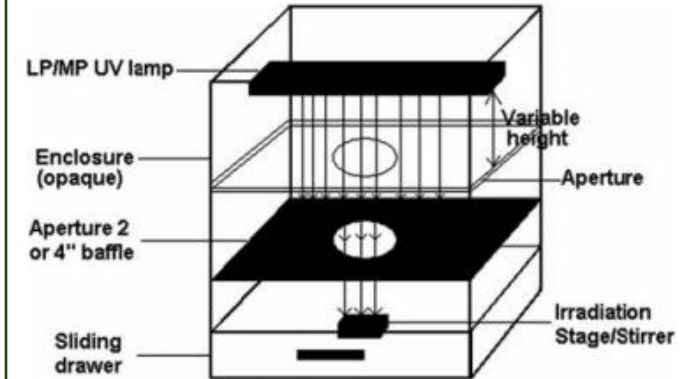
Experiments are carried out in a continuously stirred volume of 150 mL, with a water depth of 1.6 cm.

Prepare a solution of the compound under investigation in the defined standard water, preferably at a concentration of 1000x the LOQ. Later, if certain TPs require identification, this may be decreased to a concentration in the order of 1–10 µg/L.

Make a UV absorption scan by measuring the UV-transmission of the solution at every wavelength from 200 to 300 nm.

Apply a UV dose of 100 mJ/cm², using a medium pressure (MP) UV lamp (equipped with quartz sleeves to prevent radiation < 240 nm to be involved)¹⁶ and room temperature. The irradiation time required can be calculated using the spreadsheet of J. Bolton (Bolton and Cater, 1994, Bolton and Stefan, 2002, Bolton and Linden, 2003, Bolton, 2010). Low pressure (LP) lamps can also be applied for disinfection, but MP lamps have a higher chance for TP formation, and therefore should be used in this test.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.



Testing equipment:

The experiments for UV treatments are well described:

- It is clearly stated that a **medium pressure UV lamp** should be used instead of a monochromatic low pressure lamp.
- The cabinet design is described by Bolton *et. al.* but also in DIN 19294-1. Devices with a **collimated beam and a geometry as described by Bolton *et. al.* are required.**
- The cabinet need to equipped with quartz sleeves to prevent radiation <240 nm and avoid ozone formation.
- **Commercial systems are available**, one example is the BSM-03 CBD (Opsytec Dr. Groebel).

UV treatment

4.2.4.6. UV disinfection; description of experiments

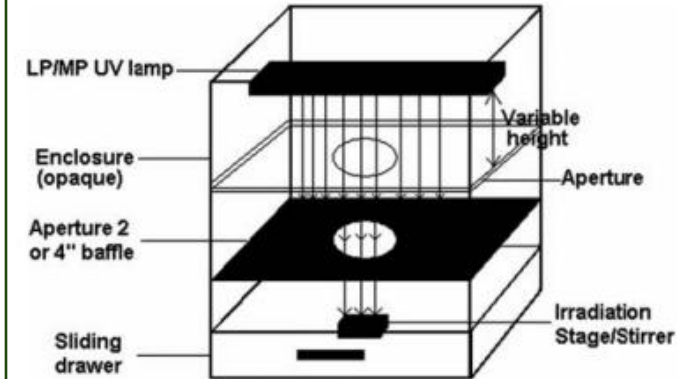
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Analyse the added compound(s) and mark the signals of possibly relevant TPs.



Testing approach:

- The average dose in relation to the irradiation time can be calculated as described by **Bolton**.
- An average dose of 100 mJ/cm² should be applied. To calculate the average dose the transmission of the sample needs to be determined. Since an irradiation of $\lambda > 240$ nm is not applied **we recommend to measure the transmission of UV λ 240-300 nm**. The wavelength range which is used for measuring the dose/fluence is the germicidal UV range from λ 200-300 nm (as described in Bolton 2003) which is also in accordance to §20 of German drinking water disinfection ordinance (200-290 nm).
- **For UV treatment the water depth is important not the diameter of petri dish. Therefore, it is important to ensure that the depth of the water is 1.6 cm (for robust disinfection), rather than total volume.**

UV treatment

4.2.4.6. UV disinfection; description of experiments

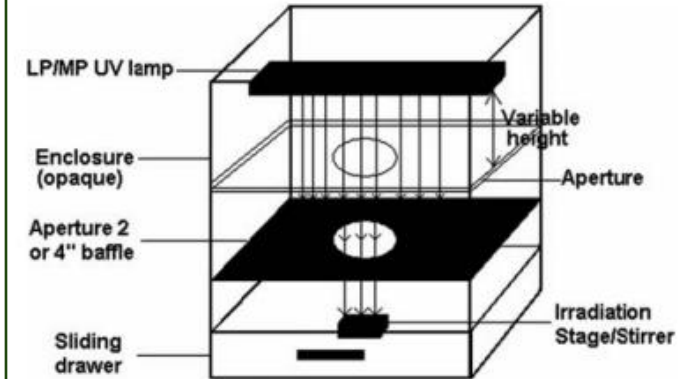
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Apply a UV dose of 100 mJ/cm², using a medium pressure (MP) UV lamp (equipped with quartz sleeves to prevent radiation < 240 nm to be involved)¹⁶ and room temperature. The irradiation time required can be calculated using the spreadsheet of J. Bolton (Bolton and Cater, 1994, Bolton and Stefan, 2002, Bolton and Linden, 2003, Bolton, 2010). Low pressure (LP) lamps can also be applied for disinfection, but MP lamps have a higher chance for TP formation, and therefore should be used in this test.

Analyse the added compound(s) and mark the signals of possibly relevant TPs.



UV dose/fluence:

Medium pressure lamps are not covered by the cited Bolton spreadsheet, which applies only to low pressure lamps. **Bolton has acknowledged the complexity of medium pressure cases, with no formal publication available.**

- Effective disinfection requires sufficient UV transmission of a solution.
- The UBA §20 list mandates a disinfection efficiency of 40 mJ/cm² based on 254 nm.
 - A UV dose of 100 mJ/cm² represents an overdosing when compared to the 40 mJ/cm² mandated by the UBA §20.
 - No formal calculation available for MP lamps, **therefore a pragmatic approach is recommended to dose an uncorrected 100 mJ/cm² irradiation to samples.**
 - This dose exceeds both the standard doses for disinfection and dose required for samples with low UV-transmission.

Biodegradation using sand filtration

4.2.4.7. Biodegradation during sand filtration

Biodegradation can occur both in the environment and in filter beds applied in drinking water treatment processes (sand filtration and filtration over activated carbon). Biodegradation in the environment is addressed by four OECD guidelines:

- OECD Guideline test no. 307: aerobic and anaerobic transformation in soil.
- OECD Guideline test no. 308: aerobic and anaerobic transformation in aquatic sediment systems.
- OECD Guideline test no. 309: Aerobic mineralisation in surface water – simulation biodegradation test.
- OECD Guideline test no. 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater.

It is difficult to test the occurrence of biodegradation in a sand (or GAC) filter, as biodegradation will only start after the filter has been operated for some time. The microbial community needs time to adapt to the water composition. This would severely complicate the experiments, require long-term processes and still might not show what kinds of TP may be formed. It can be assumed that, in general, the microbially mediated transformation products in rapid sand filters will be comparable to those already identified in OECD 307, 308 and 309 when appropriately radiolabelled test substance was used. If all these tests are not available in the dossier (which can be the case for some PPP or biocide product uses), another test has to be carried out. For this purpose, OECD 314 may be used.

Test requirement:

- The requirement to carry out such testing depends on the availability of existing **OECD 307, 308, 309 or 314** data for the test item in question.
- BUT how can we translate existing OECD study data into relevant data for biodegradation during sand filtration practices in a typical water treatment process?



Biodegradation using sand filtration



It is difficult to test the occurrence of biodegradation in a sand (or GAC) filter, as biodegradation will only start after the filter has been operated for some time. The microbial community needs time to adapt to the water composition. This would severely complicate the experiments, require long-term processes and still might not show what kinds of TP may be formed. It can be assumed that, in general, the microbially mediated transformation products in rapid sand filters will be comparable to those already identified in OECD 307, 308 and 309 when appropriately radiolabelled test substance was used. If all these tests are not available in the dossier (which can be the case for some PPP or biocide product uses), another test has to be carried out. For this purpose, OECD 314 may be used.

Technical position

- GD emphasises that tTPs arising from microbially mediated processes in rapid sand filters would be comparable to those observed in OECD 307, OECD 308 and OECD 309 studies, but gives no indication on how these data points can be used in relation to the water treatment GD.
- **Two main assumptions can be used to derive a technical position:**
- **Rapid sand filtration processes are not more biologically active than soil** – rapid sand filtration is essentially a physical process to remove suspended particles and other turbidity.
- **The time available for potential substance biotransformation is defined by the water residence time** – given operational high rates of water throughput for rapid sand filters, typical residence times are of the order of 10-60 minutes.

***CLE technical position on biodegradation during sand filtration** for the EFSA/ECHA guidance document on the impact of water treatment processes on residues of active substances or their metabolites in water abstracted for the production of drinking water*



CLE sand filtration document

Biodegradation using sand filtration



It is difficult to test the occurrence of biodegradation in a sand (or GAC) filter, as biodegradation will only start after the filter has been operated for some time. The microbial community needs time to adapt to the water composition. This would severely complicate the experiments, require long-term processes and still might not show what kinds of TP may be formed. It can be assumed that, in general, the microbially mediated transformation products in rapid sand filters will be comparable to those already identified in OECD 307, 308 and 309 when appropriately radiolabelled test substance was used. If all these tests are not available in the dossier (which can be the case for some PPP or biocide product uses), another test has to be carried out. For this purpose, OECD 314 may be used.

Technical position - Tier 1 Assessment

- An equation can be derived to calculate the theoretical maximum concentration of a tTP from a rapid sand filtration process:



$$[tTP]_{max} = [C]_0 \left(1 - \exp \left(\frac{-\ln 2}{DT_{50}} \times t \right) \right)$$

$[tTP]_{max}$ = predicted concentration of a single transformation product in treated water (units of $\mu\text{g/L}$).
 $[C]_0$ = concentration of substance being evaluated (AS or eTP) in raw water, equivalent to PEC_{dwa} (units of $\mu\text{g/L}$). This is equivalent to the selected concentration for experimental simulations.
 DT_{50} = first-order biodegradation half-life (e.g., in soil, total system water/sediment, aerobic mineralisation) (units of days^{-1}). This is equivalent to the $DT_{50,modelling}$ of the substance being evaluated (AS or eTP) from OECD 307 (OECD 308 or OECD 309, respectively) studies.
 t = water residence time in the rapid sand filtration apparatus (units of days). Rapid sand filtration occurs with a residence time of <60 minutes (equivalent to 0.04167 days).

- Proposal that choice of DT_{50} to be left up to individual applicants (modelling SFOs / geomeans etc.)
- In using OECD rate of degradation study endpoints:
 - If theoretical maximum concentration of a tTP is $<0.075 \mu\text{g/L}$, **no further assessment is required.**
 - However, if theoretical maximum concentration of a tTP is $>0.075 \mu\text{g/L}$, **then the nature of the tTP should also be addressed via Tier 2 assessment, using both available rate and route data.**

Biodegradation using sand filtration

Technical position - Tier 2 Assessment – required where Tier 1 assessment results in theoretical maximum concentration of tTP >0.075 µg/L.

- Expected tTP concentrations can be derived from OECD study data, based on the occurrence of the tTP at the first available timepoint after 0 DAT, and a correction of the molecular weight of the tTP.

$$[tTP]_{max} = [C]_0 \left(1 - \exp\left(\frac{-\ln 2}{DT_{50}} \times t\right) \right) \times \text{Max. Occ.}_{tTP} \times \frac{Mw_{tTP}}{Mw_{AS \text{ or } eTP}}$$

[tTP]_{max} = predicted concentration of a single transformation product in treated water (units of µg/L). **[C]₀** = concentration of substance being evaluated (AS or eTP) in raw water, equivalent to PEC_{dwa} (units of µg/L). This is equivalent to the selected concentration for experimental simulations.

DT₅₀ = first-order biodegradation half-life (e.g., in soil, total system water/sediment, aerobic mineralisation) (units of days⁻¹). This is equivalent to the DT_{50,modelling} of the substance being evaluated (AS or eTP) from OECD 307 (OECD 308 or OECD 309, respectively) studies.

t = water residence time in the rapid sand filtration apparatus (units of days). Rapid sand filtration occurs with a residence time of <60 minutes (equivalent to 0.04167 days).

Occ._{tTP} = maximum occurrence of the tTP at first available timepoint after 0 DAT from the OECD 307, 308 and 309 studies (units of %). This is equivalent to % AR in the OECD studies.

Mw_{tTP} = Molecular weight for the tTP (units of g/mol).

Mw_{AS or eTP} = Molecular weight for the substance being evaluated (AS or eTP) (units of g/mol).

- Timepoint used from OECD study data should be as close to 0 DAT as possible – ideally 1 HAT, to match water residence time from kinetic assessment used in Tier 1.
- Metabolites present in the corresponding OECD studies – **levels of metabolites can be taken (% AR) and translated into theoretical concentrations (µg/L) that are expected to be observed after a rapid sand filtration process.**



Biodegradation using sand filtration



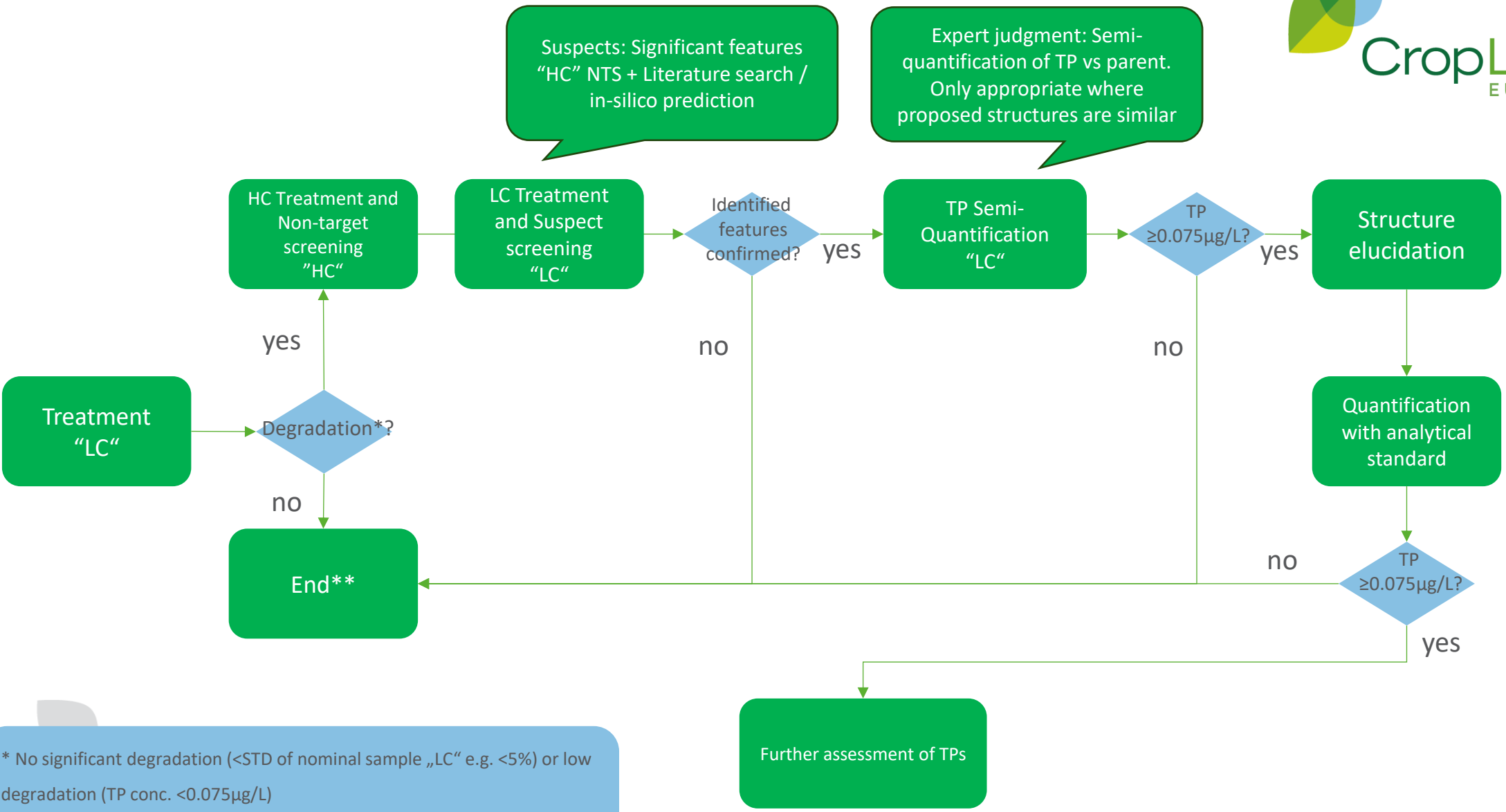
It is difficult to test the occurrence of biodegradation in a sand (or GAC) filter, as biodegradation will only start after the filter has been operated for some time. The microbial community needs time to adapt to the water composition. This would severely complicate the experiments, require long-term processes and still might not show what kinds of TP may be formed. It can be assumed that, in general, the microbially mediated transformation products in rapid sand filters will be comparable to those already identified in OECD 307, 308 and 309 when appropriately radiolabelled test substance was used. If all these tests are not available in the dossier (which can be the case for some PPP or biocide product uses), another test has to be carried out. For this purpose, OECD 314 may be used.

Biodegradation using sand filtration – absence of OECD study data

- Proposed technical position, and approach outlined in the GD, requires the use of existing OECD rate / route study data to establish the magnitude and nature of tTPs arising from a sand filtration process.
- Potential for some eTPs not to have full OECD 307, OECD 308, OECD 309 study data – how can assessment be finalised?
- Approach for each AS or eTP under investigation will have different set of circumstances – choice should be left up to applicant.
- **Absence of OECD rate data** – rate of degradation data, derived from an upstream precursor (e.g. parent), is appropriate for the assessment of whether total degradation is expected to be $\geq 0.075 \mu\text{g/L}$.
- **Absence of OECD route data** – only data derived from [^{14}C]-labelled OECD studies where the substance being investigated was dosed as the test item to the test system can be used to assess the route of transformation and the formation amounts of any TP formed. Route data should be established *or* suggestions in proposal below.
- **In the absence of OECD rate and route study data** – typical cases for aqueous photolysis specific metabolites, no OECD 307, OECD 308 or OECD 309 rate/route data will be available to conduct the assessment.
 - **Proposal:**
 - *Either* conduct the biodegradation assay described in the guidance document
 - *Or* conduct OECD 314 assay. Although the OECD 314 guidance is multifaceted, the choice of tests employed in an OECD 314 should be down to the applicant and be based on the release scenarios and anticipated properties of the AS / eTP in question.



Analytical Workflow



* No significant degradation (<STD of nominal sample „LC“ e.g. <5%) or low degradation (TP conc. <0.075µg/L)
 ** No further investigation: no TP formed

4.2. Identification of transformation products

which corresponds to concentrations that may actually be observed in the environment. Only if at low concentrations tTPs can still be observed in significant concentrations ($> 0.075 \mu\text{g/L}$) the results of the high concentration experiments should be used for identification of the relevant tTPs. Analyses of TP can be done using radiolabelled AS to track the TP by the labelling, but this is not strictly required. If AS has different functional groups and radiolabelled AS is used, consider a different radiolabel for each functional group. It is also possible to perform suspect analysis or non-target analysis, to study which TP may be formed. As these chemical analyses are not straightforward, a scheme is proposed to facilitate the process (see Figure 5). As a first step, we suggest applying modelling, e.g. based on QSARs

...
Apart from the suspect and non-target methods described above, also radio labelling (e.g. using radiolabelled ozone) may be applied to facilitate analyses. It will depend on the individual cases whether radio labelling or suspect/non-target analysis methods is preferred.

¹⁴C material:

- Although [¹⁴C]-labeled material is the gold standard in metabolism studies the GD recommends the use of cold non-target and/or suspect screening
- If [¹⁴C]-labeled test items will be used for the treatment experiments **each functional group must be labeled** (multi-label studies needed and labeling position in many cases different to metabolism studies)
- **0.1 – 10 $\mu\text{g/L}$ which is below the LOD of radio-detection (incl. MicroBeta), which is the relevant test conc.**, especially without concentration steps which should be avoided according to the GD. TP quantification as we are used to do it in typical metabolism studies is not applicable
- **Mass balancing** is in most cases **not applicable**, and mineralization depends on the label position
- **Non-target or suspect screening** is the method of choice and is **also required for [¹⁴C]-material**
- **We recommend to use non-target and suspect screening using high-resolution mass spectrometry for the tTP identification. To support structure elucidation [¹⁴C]- or stable isotopes can be used.**

Method qualification and quantification strategies (1)



For the detection of TPs, a non-target screening approach using liquid chromatography-mass spectrometry (LC-MS) and/or gas chromatography-mass spectrometry (GC-MS) will be used. To cover a wide polarity range of TPs, multiple chromatographic techniques should be used (e.g. reversed phase and hydrophilic interaction chromatography) as well as varying detection techniques (e.g. positive and negative mode mass spectrometry, UV/Vis detection). Besides variation in chromatographic techniques, sample preparation should be minimised to prevent loss of TPs. Nowadays, most water samples containing micropollutants are analysed by direct injection (LC-MS). To cover a wide polarity range of TP, multiple pre-treatment steps like solid phase extraction (SPE) can be used (for instance, lipophilic/hydrophilic affinity, cation exchange, anion exchange). A concentration step like SPE is only used when the sensitivity for a compound is low, or very low detection limits are required (e.g. < 1 ng/L) or for clean-up in case of a complex matrix. A detection limit of ≤ 0.075 $\mu\text{g/L}$ is required. For GC-MS analysis, sample pre-treatment (e.g. SPE) is still necessary.

Method qualification:

- We recommend to fulfil the analytical requirements of the **guidance document**
 - **LOQ ≤ 0.075 $\mu\text{g/L}$**
 - **Avoid sample preparation if possible**
- For quantification, linearity must be given in the concentration area between the low-test concentration and if possible, the LOD
- The qualification should be conducted in drinking water matrix
- Depending on the quantification method the qualification can demonstrate the suitability and precision of the method

Method qualification and quantification strategies (2)




Quantification:

- All common quantification strategies can be used for either degradation evaluation or TP quantification using HPLC/MS
 - e.g. **external calibration using a calibration curve**
 - e.g. **internal calibration using a representative standard**
- Linearity and sufficient sensitivity should be ensured
- Quantification can be carried out using HPLC-TQ-MS/MS or HPLC-HRMS as long selectivity is given
- To avoid matrix effects, we recommend a matrix-matched calibration curve for quantification
- If available, we recommend using an internal standard (preferable labelled with stable isotopes e.g. ^{13}C or ^2H (D) with a total mass shift of +3 Da minimum compared to the t.s.)
the use of an internal standard can compensate errors during sample preparation and measurements

Non-target and suspect screening



4.2.4. Step 3: Experimental procedures at high concentrations

Analytical procedures




Two types of analyses can be applied: suspect or non-target screening. This depends on whether or not it was possible to predict the formation of certain TP (suspects). For the analyses, the initial concentration should be $1,000 \times \text{LOQ}$, to facilitate the identification of relevant TPs. If some TPs are found, concentrations can be decreased to e.g. 1–10 $\mu\text{g/L}$, which should be in the relevant order of magnitude for contaminants in surface water (see Chapters 2 and 3). In this way, it can be checked whether the TP that may be formed are detectable at environmentally relevant concentrations. If the limit of detection of a compound is so low that $1,000 \times$ the LOD would be in the same order of magnitude as the environmentally relevant concentration, both experiments will coincide. In that case, it should be substantiated whether this concentration will give sufficient information to continue, or whether a higher concentration should be applied to be able to identify possible tTPs.

Non-target and suspect screening:

- 
- 
- Those techniques require **high-resolution mass spectrometry data** (e.g. using Orbitrap or qToF systems)
 - **Various vendor and open-source software packages** for data mining and feature detection, identification and interpretation **are available**.
 - It is **not possible to recommend a dedicated procedure**, however, **analytical requirements from GD needs to be fulfilled** (e.g. positive/quality control for validation of analytical method)
 - **Statistical analysis for feature evaluation** (e.g. via volcano plot) can be used for the identification of relevant features **against a nominal sample. We recommend to check feature quality (e.g. peak-shape, data points etc.) manually after the non-target screening.**
 - **We recommend to check the data acquisition/processing and feature identification by means of a positive control and/or an analytical standard mixture with representative (or “tTP-like”) analytes**
 - Relevant features but also tTPs from literature and in silico prediction are the basis for additional suspect screening

Non-target and suspect screening



4.2.4. Step 3: Experimental procedures at high concentrations



chromatographic techniques, sample preparation should be minimised to prevent loss of TPs. Nowadays, most water samples containing micropollutants are analysed by direct injection (LC-MS). To cover a wide polarity range of TP, multiple pre-treatment steps like solid phase extraction (SPE) can be used (for instance, lipophilic/hydrophilic affinity, cation exchange, anion exchange). A concentration step like SPE is only used when the sensitivity for a compound is low, or very low detection limits are required (e.g. < 1 ng/L) or for clean-up in case of a complex matrix. A detection limit of ≤ 0.075 $\mu\text{g/L}$ is required. For GC-MS analysis, sample pre-treatment (e.g. SPE) is still necessary.


Apart from the suspect and non-target methods described above, also radio labelling (e.g. using radiolabelled ozone) may be applied to facilitate analyses. It will depend on the individual cases whether radio labelling or suspect/non-target analysis methods is preferred.

Analytical requirements:

- 
- 
- The GD recommends to use a holistic approach with minimal sample preparation which is not applicable and suitable for the needed high sensitivity
 - A LOQ of ≤ 0.075 $\mu\text{g/L}$ is required, therefore we recommend to ensure this LOQ for the used test items and evaluate the linearity of the method for quantification purposes if needed
 - Very low quantification limits needs to be assured for “relevant substances” or substances of concern. Such LOQs are related to suspects e.g. from literature. This is a case-by-case decision and related to (literature)-known suspects such as CMR substances. For such cases, a dedicated method should be utilized to fulfill GD requirements.
 - We recommend to use RP-LC/ESI-HRMS as a standard approach. A high polarity range is covered by this technique, however if (literature) known tTPs need to be addressed, techniques such as HILIC, APCI, GC/HRMS or others should also be considered. An expert judgement is required. See also [Guideline NTS EN.pdf](#) of the German Water Society.



TP quantification and Structure elucidation

4.2.7. Step 6: Hazard and risk assessment



Hazard and risk assessments (see Chapter 5) have to be carried out for the relevant tTPs identified in step 5. In practice, a structure/identity is needed for all tTPs that are present at a concentration exceeding 0.075 µg/L (level above which screening for genotoxicity is necessary, see paragraph 5.2.1). It can be difficult to determine the concentration of unknown compounds. An estimation can be made in these cases based on reference compounds. These reference compounds depend on the laboratory involved, the type of compounds under investigation and the matrix composition. Examples are atrazine D5 and bentazone D6, PFOA-13C8 and isotope labelled pharmaceuticals to study their metabolites.¹⁷ After identification, the real concentration observed after treatment should be established.

TP quantification and Structure elucidation:

- 
- 
- Quantification using radio-detection is in most cases not applicable, in addition quantification by mass spectrometry is also not applicable without an analytical standard.
 - Structure elucidation is required for all tTPs with a concentration ≥ 0.075 µg/L in the low concentration experiments, but structural information is a pre-requisite for quantification.
 - The GD suggests an estimation based on reference material (i.e., stable isotopic-labeled standards)
 - A “semi-quantification” or “estimation” is possible if the structure of the tTP is comparable to the test item – i.e. there is an expectation that the MS response for both analytes will be similar.
 - **We recommend a good argumentation (expert judgement) whether a semi-quantification with available standards is applicable. It is always a case-by-case decision based on the acquired data, test item or reference material.**

Positive Control / Quality control (1)



Depending on the purpose there is the option to differentiate between positive control and quality control.

Positive control:

- Should show that the test system has the potential for transformation of the test item.
- For most of the treatments the quantification of the oxidation agent via a coloring reaction, e.g. via DPD or indophenol method can serve as a positive control.
- A positive control can support the reliability of the experimental result e.g. “no degradation”.

Quality control:

- Control that the analytical quality is guaranteed, and data procession was conducted as required. e.g., the use of a standard mixture can ensure the chromatographic separation over a high polarity range.
- A positive control may also serve as a quality control.
- In any case the results of a positive and quality control must be robust and reproducible.

Positive Control / Quality control (2)



Positive control:

- **Indirect:** For all treatments (except UV) we consider the use of the DPD/Indophenole reaction to prove the transformation potential of the test system.
- **Direct:** Compounds can be used as positive controls (e.g. sulfamethoxazole), which show degradation under the selected test conditions.
- **Please note:** From our knowledge there is no single compound currently available, which can be used as a positive control for all treatments. We promote to use the indirect method where applicable.

Test System	Indirect	Direct
Chlorination Hypochlorite	DPD	
Chlorination Monochloramine	DPD/Indophenole	
Oxidation Chlorine Dioxide	DPD	
UV	-	e.g., sulfamethoxazole
Ozone	DPD	

Positive Control / Quality control (3)



Quality control:

- To verify the quality of the analytical methods (e.g. reproducibility of retention times, signal intensity,...) and/ or data procession we recommend to use a quality control
- We recommend to use drinking water matrix spiked with an analytical standard mix consisting of substances covering a broad structural variety and with this a broad polarity range and ionization behavior (e.g. commercially available Pharma/Pesticide mix (stable isotope labels are optional) 10-20 compounds)
- A quality control can support the reliability of the non-target and suspects screening workflow and can also support data processing (definition of processing criteria-based quality control data)
- The use of a quality control is also well described in the [Guideline NTS EN.pdf](#) of the German Water Society

Non-target analysis: Introduction

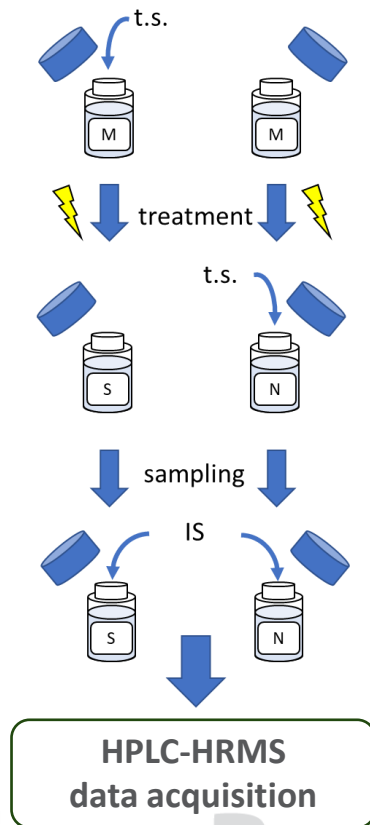


Targeted, non- or untargeted and suspect analysis or screening – What is the difference?

- All techniques are related to mass spectrometry typically hyphenated with separation techniques (HPLC, GC, SFC,...)
 - Non-target and suspect analysis requires high-resolution mass spectrometry (Orbitrap or Time-of-Flight (ToF))
 - Target analysis requires a reference standards, therefore also low-resolution mass spectrometry is possible (e.g. TQ-MS)
- Non-target and suspect screening does not require an analytical standard or reference material
 - Suspect analysis searches for predicted compounds (e.g. in silico prediction) or targets without analytical standard “suspects”
 - For suspect screening identifiers are needed, such as sum formular / exact mass or MS² data, ...
 - Non-target analysis searches for anything in any possible way
- Goal of non-target screening is to identify a peak/feature of interest identified by means of specific filter, for example
 - Statistical evaluation of two (or more) different experiments e.g. volcano plot or principal component analysis
 - Patterns like isotopic patterns or MS² similarities (e.g. molecular networking)
- A detailed description of the different techniques can be found in [Hollender et al. 2023](#)

Test design and sample preparation

Test design and sample preparation



positive and negative mode mass spectrometry, UV/Vis detection). Besides variation in chromatographic techniques, sample preparation should be minimised to prevent loss of TPs. Nowadays, most water samples containing micropollutants are analysed by direct injection (LC-MS). To cover a wide polarity range of TP, multiple pre-treatment steps like solid phase extraction (SPE) can be used (for instance, lipophilic/hydrophilic affinity, cation exchange, anion exchange). A concentration step like SPE is only used when the sensitivity for a compound is low, or very low detection limits are required (e.g. < 1 ng/L) or for clean-up in case of a complex matrix. A detection limit of ≤ 0.075 $\mu\text{g/L}$ is required. For GC-MS analysis, sample pre-treatment (e.g. SPE) is still necessary.

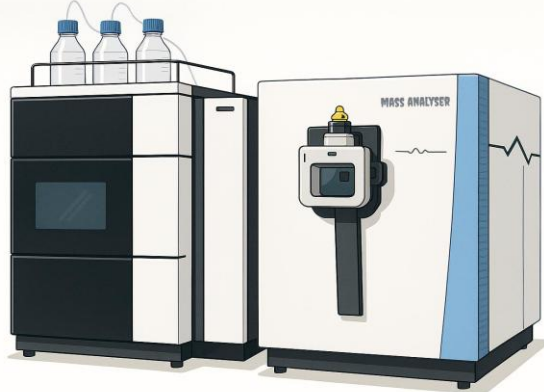
- The test design is crucial for a reliable and robust non-target screening and should be always related to the data analysis intended to use
- We recommend using a test design consisting of control samples and background samples in addition to the test sample. Recommended sample types are described on [slide 4](#)
- Test and control samples should be as similar as possible
- The addition of internal standards or quality control standards can increase robustness
- According to the GD additional sample prep e.g. for concentration should not be considered or minimized. The analysis via direct injection is recommended to prevent the loss of TPs.
- In exceptional cases sample prep e.g. using solid phase extraction needs to be considered if the method lacks of sensitivity or GC-MS is required
- If possible, we recommend to analyze the samples immediately after treatment to avoid artificial TP formation/degradation
- If storage is necessary, we recommend freezing the samples for a short period at $\leq -20^\circ\text{C}$ and gentle thawing before analysis



M: matrix (drinking water)
S: sample (high/low concentration)
N: nominal sample (high/low conc.)
t.s.: test substance
IS: internal standard

HRMS data acquisition

HRMS data acquisition



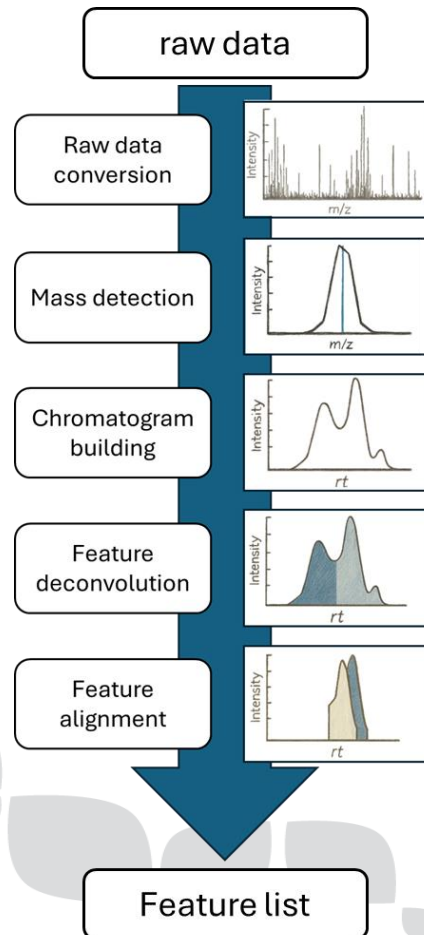
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- Based on the test item we recommend to use RP-LC/ESI-HRMS in positive and negative ionization mode as a standard approach. Other techniques such as HILIC, APCI, GC/HRMS can be used analogously if expected tTPS require more data.
- High-resolution mass spectrometric data is needed for NTA. The measurements can be carried out using an Orbitrap, a time-of-flight mass analyzer (TOF), or another high-resolution mass spectrometer (FT-ICR, sector field MS), depending on the available instrument. Further identification of relevant TPs require MS² spectra with accurate mass for individually selected (MS/MS or ddMS²) or, if possible, simultaneously for all precursor ions (MS/MS^{all}).
- The data acquisition needs to fulfil a lot of quality criteria to deliver reliable, robust and reproducible data. Resolution, mass range, mass accuracy and sensitivity of the mass spectrometer must be ensured. Minimum requirements are well described in the [Guideline NTS EN.pdf](#) of the German Water Society or in [Hollender et al. 2023](#).




Software assisted data pre-processing (feature list creation)

Data pre-processing / Feature list



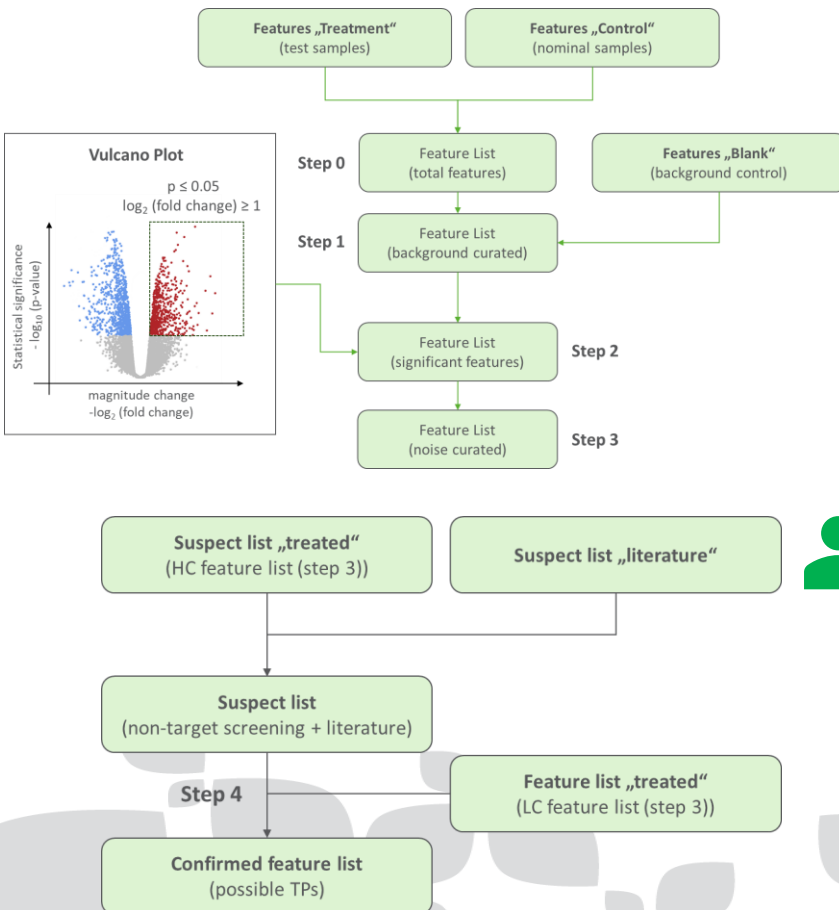
4.2. Identification of transformation products

carried out. The principles of non-target analyses have been described by Hollender et al. (2017 and 2019), Bletsou et al. (2015) and Béen et al. (2021). The specific application of non-target analyses for the identification of TPs has been described by Wang et al. (2020), Schollée et al. (2018), Di Marcantonio et al. (2020) and Brunner et al. (2019). According to the procedures described there, it is possible to determine the molecular structures of TPs that have been formed. This type of experiment

- 
- Non-target analysis creates large data sets which requires automated data processing.
 - Software, including compound discoverer provide efficient and accurate tTP determination. [Bletsou et al. 2015](#) and [Hollender et al. 2023](#) suggest a number of open-source and commercial software options.
 - It is important to exclude unrelated peaks by comparing to the nominal and/or background control samples.
 - It is recommended that processing criteria is selected based on your knowledge of your data.
 - Tools such as minimum intensity thresholds (e.g. method LOQ) , peak shape, MS/MS, feature alignment and gap filling are some of the suggested criteria to include.
 - Experience and a substantial knowledge of the experiment and data is required!
 - [Kind & Fiehn 2007](#) validated “seven golden rules” for filtering molecular formulas

Feature evaluation and statistical methods

Feature prioritization and statistical methods



4.2. Identification of transformation products

Marcantonio et al. (2020) and Brunner et al. (2019). According to the procedures described there, it is possible to determine the molecular structures of TPs that have been formed. This type of experiment will enable the identification of tTPs where in the chromatograms, the signals of any tTP are to be expected. At this stage, it will not be necessary to identify the tTPs themselves, although it is expected that this should be possible in this concentration range. Not all tTPs will have to be identified in this manner, nor will have to be considered in the hazard assessment, as they may not be formed under realistic conditions, where lower concentrations of the AS or eTP can be expected. In the next step, the experiment can thus be repeated at an environmentally relevant concentration (see Section 4.2.5),

- Once a feature list is created (Step 0), features must be reduced and filtered to focus on the relevant ones
- Feature reduction can be done using different refinement/filtering steps
- Step 1: Background subtraction
 - Ubiquitarian features identified in background samples are excluded
- Step 2: Statistical evaluation
 - Vulcano plot \log_2 fold change ≥ 1 (feature formation compared to control of factor 2) and a p-value of ≤ 0.05
- Step 3: Manual curation
 - Manual evaluation of feature list after Step 1-2; noise, false positive features, artefacts can be filtered out)
- Step 4: Comparison of suspect list (literature, high conc. experiments) with low concentration feature list
- Features after Step 4 are considered for further evaluation as possible TPs

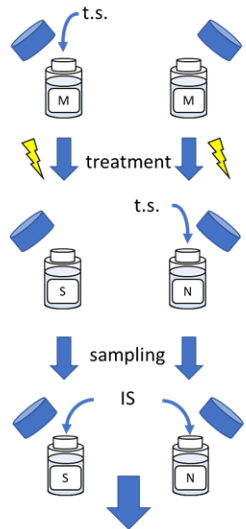
Non-target analysis workflow

Test design and sampling

HRMS data acquisition

Data pre-processing / Feature list

Feature prioritization and statistical methods

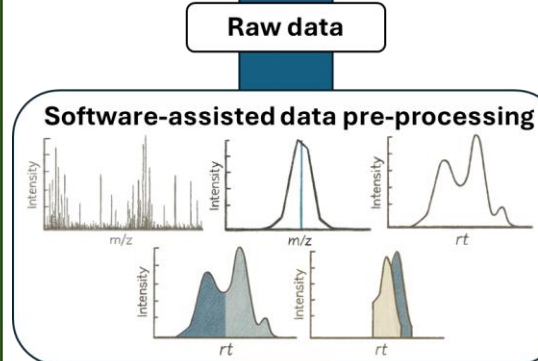


HPLC-HRMS
data acquisition

- Robust test design
- Minimal sample prep
- Minimal storage

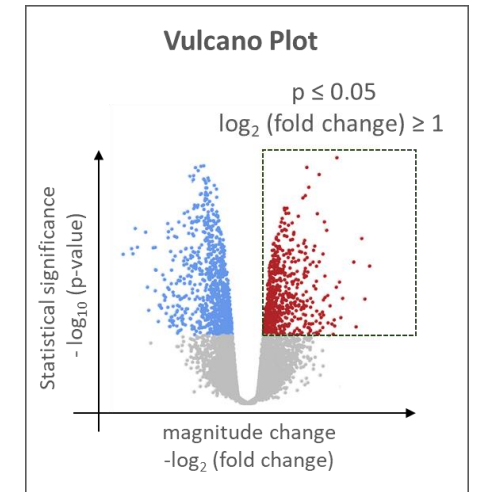


- HRMS data required
- RP-LC/ESI-HRMS (+/-)
- Quality criteria defined



Feature list

- Software-assisted
- Experience required
- Know your data!



- Statistical evaluation
- Manual curation
- Confirmation in low conc.

References (1)



EFSA/ECHA guidance: European Food Safety Authority (EFSA). EFSA Guidance Document on the Impact of Water Treatment Processes on Residues of Active Substances or Their Metabolites in Water Abstracted for the Production of Drinking Water. EFSA J. 2023, 21 (8), 1–108.

<https://doi.org/10.2903/j.efsa.2023.8194>.

UBA §20 List: German Environment Agency (UBA). List of Permitted Treatment Substances and Disinfection Processes according to §20 of the Drinking Water Ordinance (Liste zulässiger Aufbereitungsstoffe und Desinfektionsverfahren nach § 20 der Trinkwasserverordnung); German Environment Agency: Dessau-Roßlau, Germany, 2023. Available online:

https://www.umweltbundesamt.de/sites/default/files/medien/13195/dokumente/liste_zulaessiger_aufbereitungsstoffe_und_desinfektionsverfahren_nach_ss_20_trinkwv.pdf (accessed July 17, 2025).

Monochloramine (Slide 11): Le Roux, J. *et. al. Environ. Sci. Technol.* **2012**, 46, 11095–11103 DOI: [dx.doi.org/10.1021/es3023094](https://doi.org/10.1021/es3023094)

Supporting information: https://pubs.acs.org/doi/suppl/10.1021/es3023094/suppl_file/es3023094_si_002.pdf

WHO Guideline for drinking water quality: WHO (World Health Organization) (2022) Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda; ISBN 978-92-4-004506-4; <https://iris.who.int/bitstream/handle/10665/352532/9789240045064-eng.pdf?sequence=1>

Bolton et al. (slide 20): Bolton JR, Linden KG, Kuo J, Chen CL and Nellor M, 2005. Discussion of "Standardized collimated beam testing protocol for water/wastewater ultraviolet disinfection" by Jeff Kuo, Ching-lin Chen, and Margaret Nellor. *Journal of Environmental Engineering*, 131, 827–829.

References (2)



Bolton Spreadsheet (slide 22): Spreadsheet:

[https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fcdn.wildapricot.com%2F197216%2Fresources%2FResource%2520Documents%2FGermicidal%2520Fluence%2520\(UV%2520Dose\)%2520Calculations%2520for%2520a%2520Low%2520Pressure%2520UV%2520Lamp.xlsx%3Fversion%3D1588162140000%26Policy%3DeyJTdGF0ZW1lbnQiOiBbeyJSZXNvdXJzSI6Imh0dHBzOi8vY2RuLndpbGRhcHJpY290LmNvbS8xOTcyMTYvcmVzb3VyY2VzL1Jlc291cmNIJTlwRG9jdW1lbnRzL0dlcm1pY2lkYWwIMjBGBHVlbnNIJTlwKfVWJTlwRG9zZSkIMjBDYWxjdWxhdGlbnMIMjBmb3IlMjBhJTlwTG93JTlwUHJlc3N1cmUIMjBVViUyMEExbXAueGxzeD92ZXJzaW9uPTE1ODgxNjlxNDAAwMDAiLCJDb25kaXRpb24iOnsiRGF0ZUxlc3NUaGFuLjpw7IkFXUzpFcG9jaFRpbWUiOjE3NzlxMzQ3MzF9LCJJeFkZHIjlc3MiOnsiQVdTOlNvdXJzUlwljoiMC4wLjAuMC8wLn19fV19%26Signature%3DA4Zj4-lrstDFL1ehtD6S7laB~yypFpQZUeYNwjXSmndFI9NiVDv6hSaPQfdT9teaus1m8sxu1aV2197qGboFtLngnDalgGXgLgtqKSW3I6A0yyhraup0B6ZrL-6j3KaPUI5wplb0JpKpAaNNfdCGQt84M-szwfHa4e-4D6YxID9QYExNBpfQPkLRi~4XGDxl1qL~q5z8vdjT~o4iCrXEyjfjb-Leb~tgMI2BCX9wyyAllDV7MrofuXW6wFuLoXzw-jVvkewrfnpX5yd~N76XyDeuk7EkftvqQycSFUv2KG2OSPhJ5gmXyaX2zNH3yHgo3piwiUTiajQdO1UqJ~k28w__%26Key-Pair-Id%3DK27MGQSHTHAGGF&wdOrigin=BROWSELINK](https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fcdn.wildapricot.com%2F197216%2Fresources%2FResource%2520Documents%2FGermicidal%2520Fluence%2520(UV%2520Dose)%2520Calculations%2520for%2520a%2520Low%2520Pressure%2520UV%2520Lamp.xlsx%3Fversion%3D1588162140000%26Policy%3DeyJTdGF0ZW1lbnQiOiBbeyJSZXNvdXJzSI6Imh0dHBzOi8vY2RuLndpbGRhcHJpY290LmNvbS8xOTcyMTYvcmVzb3VyY2VzL1Jlc291cmNIJTlwRG9jdW1lbnRzL0dlcm1pY2lkYWwIMjBGBHVlbnNIJTlwKfVWJTlwRG9zZSkIMjBDYWxjdWxhdGlbnMIMjBmb3IlMjBhJTlwTG93JTlwUHJlc3N1cmUIMjBVViUyMEExbXAueGxzeD92ZXJzaW9uPTE1ODgxNjlxNDAAwMDAiLCJDb25kaXRpb24iOnsiRGF0ZUxlc3NUaGFuLjpw7IkFXUzpFcG9jaFRpbWUiOjE3NzlxMzQ3MzF9LCJJeFkZHIjlc3MiOnsiQVdTOlNvdXJzUlwljoiMC4wLjAuMC8wLn19fV19%26Signature%3DA4Zj4-lrstDFL1ehtD6S7laB~yypFpQZUeYNwjXSmndFI9NiVDv6hSaPQfdT9teaus1m8sxu1aV2197qGboFtLngnDalgGXgLgtqKSW3I6A0yyhraup0B6ZrL-6j3KaPUI5wplb0JpKpAaNNfdCGQt84M-szwfHa4e-4D6YxID9QYExNBpfQPkLRi~4XGDxl1qL~q5z8vdjT~o4iCrXEyjfjb-Leb~tgMI2BCX9wyyAllDV7MrofuXW6wFuLoXzw-jVvkewrfnpX5yd~N76XyDeuk7EkftvqQycSFUv2KG2OSPhJ5gmXyaX2zNH3yHgo3piwiUTiajQdO1UqJ~k28w__%26Key-Pair-Id%3DK27MGQSHTHAGGF&wdOrigin=BROWSELINK)

CLE technical position on biodegradation during sand filtration (slide 24): <https://croplifeeurope.eu/wp-content/uploads/2026/03/CLE-harmonized-approach-Sand-Filtration.pdf>

Non-target screening guideline (German water society):

https://www.gdch.de/fileadmin/downloads/Netzwerk_und_Strukturen/Fachgruppen/Wasserchemische_Gesellschaft/Guideline_NTS_EN.pdf

Hollender et al. 2023 (slide 38-41): Hollender, J., Schymanski, E. L., Ahrens, L. et al. NORMAN guidance on suspect and non-target screening in environmental monitoring; Environ Sci Eur., 2023, 35, 1-61. <https://doi.org/10.1186/s12302-023-00779-4>

References (3)



Bletsou et al. 2015: Bletsou AA, Jeon J, Hollender J, Archontaki E and Thomaidis NS, 2015. Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment. *TrAC- Trends in Analytical Chemistry*, 66, 32–44. <https://doi.org/10.1016/j.trac.2014.11.009>

Kind and Fiehn 2007: Kind, T., Fiehn, O. Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. *BMC Bioinformatics* 8, 105 (2007). <https://doi.org/10.1186/1471-2105-8-105>



List of Abbreviations



APCI: atmospheric pressure chemical ionization

AS: active substances

CLE: CropLife Europe

CRO: Contract Research Organization

Da: Dalton

DOC: dissolved organic carbon

DOD: defined ozone dosage

DPD: N,N-Diethyl-p-phenylendiamin

DWT: drinking water treatment

EC: European Commission

ECHA: European Chemicals Agency

EFSA: European Food Safety Authority

ESI: electrospray ionization

eTP: environmental transformations product

GC: gas chromatography

GD: guidance document

HC: high concentration

HILIC: hydrophilic interaction chromatography

(HP)LC: (high-performance) liquid chromatography

HRMS: high-resolution mass spectrometry

IS: internal standard

LC: low concentration

LOQ: limit of quantification

M: Matrix

MP: medium pressure

MS: mass spectrometry

MS/MS: tandem mass spectrometry

N: nominal sample

NTA: non-target analysis

NTS: non-target screening

PECgw: predicted environmental concentration ground water

PECsw: predicted environmental concentration surface water

ppm: parts per million

PPP: plant protection product

rt: room temperature

RP: reversed-phase

S: sample

SPE: solid-phase extraction

t: time

TP: transformations product

tTP: (water) treatment transformation product

TQ: triple quadrupole

t.s.: test substance

UBA: Umwelt Bundesamt

UV: ultra violet



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