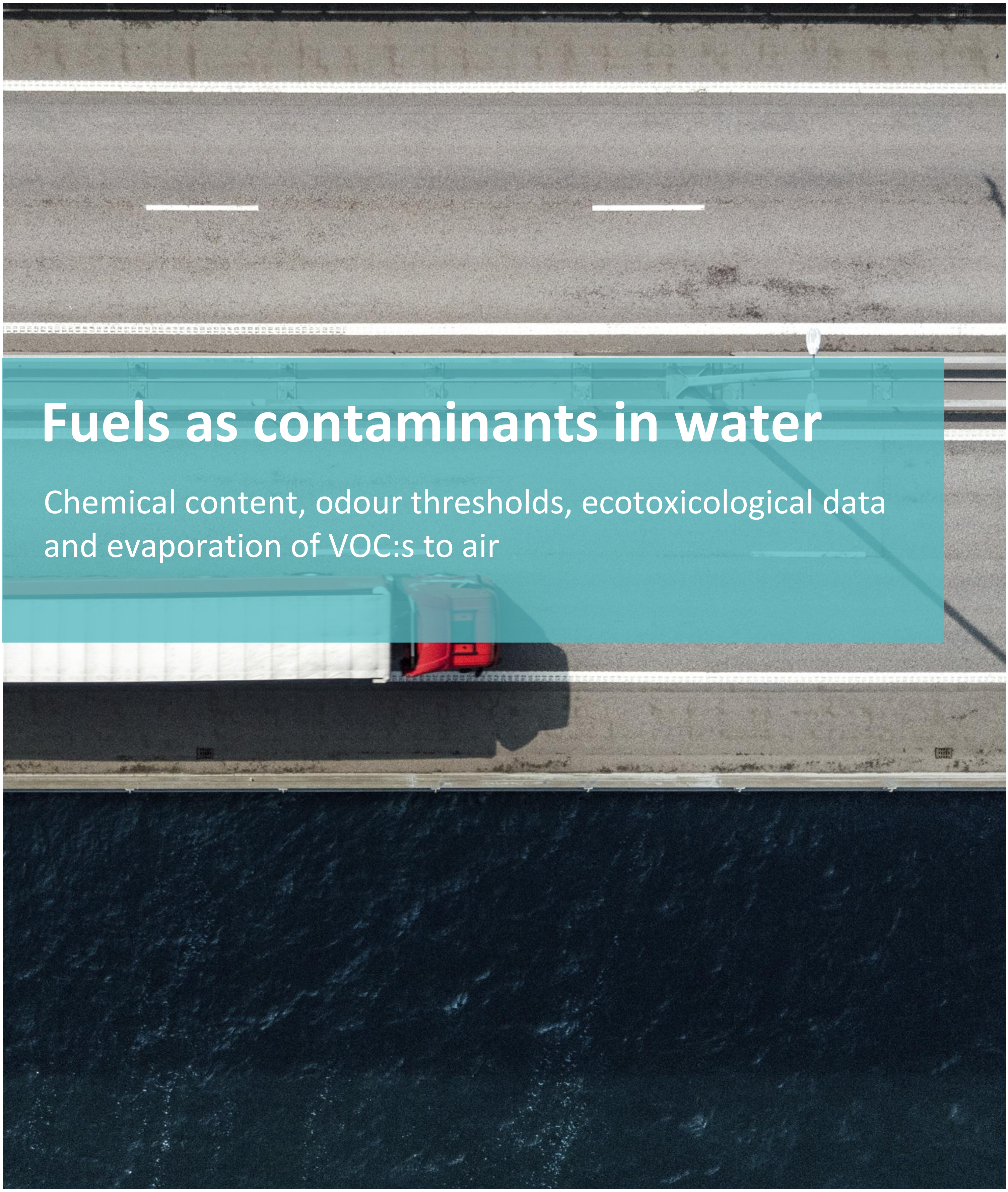

Fuels as contaminants in water

Chemical content, odour thresholds, ecotoxicological data and evaporation of VOC:s to air

Svenskt Vatten
UTVECKLING

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Research Institute

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Fuels, contaminants in water, odour, drinking water, oil spill

Summary

Oil spills, the most frequent environmental incidents in Sweden, have decreased in recent years but still pose risks to drinking water and aquatic ecosystems, with about 600 cases registered annually by the Swedish Fire Protection Association. Yet, detailed information about modern fuels and their environmental and human health impacts remains scarce. Hence, this study focuses on enhancing the understanding of the environmental impact of common fuels.

This study collected thirty fuel samples of different types: petrol, diesel, fuel oil, and marine gas oil. A selected number of substances in the fuels and the water-soluble phase were analysed using GC-MS. A crucial step in the analytical method in this project, since the focus was on the effect on sub-surface aquatic life and drinking water production, was to form a stable water-accommodated fraction (WAF) where non-dissolved fuel elements were separated from the water. Since odour properties were of interest, the mixing was extensive, with limited space allowed for gases, meaning that more volatile organic carbons (VOC:s) would be in solution. The chemical analysis focused on identifying and quantifying 50 substances, including aromatic hydrocarbons, aliphatic hydrocarbons, ethers, and esters, plus 17 polycyclic aromatic hydrocarbons (PAH:s) for eight of the samples. These substances were chosen for their significance in interpreting results related to odour and to illustrate the proportion of light and heavy substances in the fuels.

Twelve of the thirty fuel samples were selected for odour threshold testing, where a dilution series from the WAF was used to evaluate the intensity of odours at different concentrations. Six samples were chosen for ecotoxicological assessments on crustaceans, algae, and bacteria, offering a comprehensive understanding of the ecotoxicity of the fuel-water mixtures. Four samples were used in tailor-made evaporation experiments to study how volatile fuel components evaporate from the water surface under different temperatures and ethanol concentrations.

For odour, three fuels were notably distinguished, namely the fuels containing higher concentrations of ether: 98 Octane petrol and E85 fuel. While there was significant variability in odour thresholds among different panel members, the concentration of MTBE (Methyl Tertiary-Butyl Ether) in the fuel-water mixtures was generally identified as a precise predictor of odour. Conversely, the lack of ether in diesel fuels made them significantly less prone to cause odour in the WAF.

Generally, petrol-specific substances dissolve more readily in water than those in diesel, which only marginally ended up in the water-accommodated fraction. However, ethanol in petrol and RME (rapeseed methyl ester) in diesel favoured the dissolution of hydrocarbons into water. For ether, which is of utmost importance for odour, a strong correlation was observed between the concentration of ether in water and its content in the fuel. Therefore, it is possible to predict the ether concentration in the WAF solely from ether concentration in the fuel, meaning that ethanol did not significantly increase ether solubility.

In the case of a fuel spill into surface water, volatile substances like ether or toluene evaporate into the air, reducing the water concentration. The experimental conditions in this study do not reflect actual real-world conditions. The evaporation experiments showed that the evaporation of ether can be predicted based on the WAF ether concentration, water temperature, and ethanol content. It was found that cold water (5 °C) conditions reduce the evaporation rate of ether to almost negligible levels.

The ecotoxicological tests showed reproduction inhibitions in crustaceans across all fuel samples. However, the inhibiting effect from HVO (hydrogenated vegetable oil) was only marginally greater than that of the control. Fuel oil and some petrol fuels had detrimental effects on the algae growth, while diesel did not. The decrease of luminescence of bacteria, an indicator of toxicity, exhibited a similar trend; petrol fuels inhibited luminescence more than diesel. None of the fuels disturbed activated sludge to the extent that respiration was inhibited at toxic levels. This shows that an active sludge is more robust than single organisms, probably due to the diverse bacteria flora.

For a drinking water producer, fuels containing water-soluble ethers, such as E85 and 98 Octane petrol, are the most prominent risk. If a spill occurs in the drinking water supply, the production disturbance likelihood depends on the dilution prerequisites below the odour threshold of 1.5-4 μg/L. The study also shows that modern diesel has become an issue of marginal concern for surface water-based raw water sources due to very low solubility and regulations that have reduced the amounts of toxic substances in the products.

For freshwater ecosystems, water-soluble petrol-associated substances and hydrophobic toxic substances in fuel oil or EU diesel have the most severe effects during a spill. However, MK1 and HVO diesel only marginally affected the test organisms compared to the control, which represents unaffected organisms.

Sammanfattning

Oljeutsläpp har minskat i antal de senaste åren, men utgör fortfarande den vanligaste miljöskadan, med cirka 600 fall som hanteras årligen av restvärderäddning. Trots detta är information om moderna bränslen och deras påverkan på miljö och människors hälsa fortfarande knapphändig. Därför fokuserar denna studie på att öka kunskapen kring vanliga bränslens kemiska innehåll och miljöpåverkan av dessa vid utsläpp.

Inom ramen för denna studie samlades trettio bränsleprover av olika typer in: bensin, diesel, eldningsolja och MGO (Marine Gas Oil). Bränslenas, såväl som den vattenlösliga fasens kemiska sammansättning analyserades med hjälp av GC-MS (gaskromatografi med masspektrometer). Ett viktigt moment i metoden, eftersom fokus låg på påverkan på vattenekosystem och dricksvattenproduktion, var att skapa en stabil WAF (water-accomodated fraction) genom att blanda vatten och bränsle och sedan låta icke lösta ämnen separera från vattenfasen. I blandningen gavs ett begränsat utrymme för gaser, vilket innebar att mer flyktiga organiska kolväten (VOC) fanns i vattenlösningn. Detta var ett medvetet val, då dessa ämnen förväntades ge utslag i lukttester och att en stor skillnad mellan olika bränslen var önskvärd. Den kemiska analysen fokuserades på att identifiera och kvantifiera 50 ämnen, inklusive aromatiska kolväten, alifatiska kolväten, etrar och estrar, samt 17 polycykliska aromatiska kolväten (PAH:er) i åtta av bränsleproverna. Dessa ämnen valdes dels för att de väntades påverka luktegenskaper, dels för att illustrera fördelningen mellan lätta och tunga ämnen i bränslena.

Tolv av de trettio bränsleproverna valdes ut till lukttester, där en spädserie tillverkades utifrån WAF för bränslena. Denna användes för att utvärdera lukt vid olika spädningar. Sex prover valdes ut för ekotoxikologiska tester av kräftdjur, alger och bakterier, vilket ger en relativt god förståelse för ekotoxiciteten hos bränsle-vattenblandningarna. Fyra prover användes i specialdesignade avdunstningsexperiment för att studera hur flyktiga bränslekomponenter avdunstar från vattenytan vid olika temperaturer och etanolkoncentrationer.

Resultaten med avseende på lukttester gav att tre bränslen stack ut kraftigt från övriga. Dessa bränslen var de som innehåller högre koncentrationer av eter: 98-oktanig bensin och E85-bränsle. Även om variationer med avseende på lukttrösklar mellan olika panelmedlemmar var stor, var sambandet mellan MTBE (metyl-tertiär-butyleter) i bränsle-vattenblandningarna och lukt mycket stark. Omvänt

gjorde avsaknaden av eter i dieselbränslena dem betydligt mindre benägna att orsaka lukt i WAF.

Etanol i bensin och RME (rapsmetylester) i diesel gjorde att mer av respektive bränsle löstes i vatten. Generellt löser sig bensinspecifika ämnen väsentligt lättare i vatten än de i diesel, som bara marginellt finns i vattenfraktionen. För eter, som har stor betydelse för lukt, spelar koncentrationen etanol mindre roll. Eter är så pass vattenlösligt att det kunde observeras ett starkt samband mellan koncentrationen av eter i vatten och dess innehåll i bränslet, utan hänsyn tagen till etanol.

De förhållanden som rådde vid blandning av bränsle och vatten i denna studie återspeglar inte verkliga förhållanden. Vid bränslespill i ytvatten avdunstar en del av de flyktiga ämnena såsom eter eller toluen till luft, vilket minskar koncentrationen i vatten. Experimenten visade att avdunstningen av eter kan förutsägas baserat på WAF-eterkoncentrationen, vattentemperaturen och etanolhalten. De visade också att kallt vatten (5 °C) minskar avdunstningshastigheten för eter till nästan försumbara nivåer.

De ekotoxikologiska testerna visade reproduktionshämningar hos kräftdjur i alla bränsleprover. Den hämmande effekten från HVO (hydrerad vegetabilisk olja) var dock endast marginellt mer än kontrollen. Eldningsolja och vissa bensinbränslen hade skadliga effekter på algtillväxt, medan diesel inte hade det. Resultatet med avseende på bakteriers luminiscens var i samma riktning; Bensinbränslen hämmade luminiscensen mer än diesel. Inget av bränslena hämmade respirationen hos aktivt slam i sådan utsträckning att toxiska nivåer kunde konstateras. Detta visar att ett aktivt slam är mer robust än enskilda organismer, på grund av den mångsidiga bakteriefloran.

För en dricksvattenproducent är bränslen som innehåller vattenlösliga etrar, såsom E85 och 98-oktanig bensin, den mest potenta risken. Om ett utsläpp inträffar i en dricksvattentäkt beror sannolikheten för produktionsstörning på förutsättningarna för utspädning så att eterkoncentrationen går under lukttröskeln på 1,5-4 µg/L. Modern diesel (både svensk och europeisk) har däremot, genom väldigt låg vattenlöslighet och tack vare regleringar som minskat andelen toxiska ämnen i produkterna, blivit en marginell risk för ytvattentäkter.

För sötvattensekosystem har vattenlösliga bensinrelaterade ämnen och hydrofoba giftiga ämnen i eldningsolja eller EU-diesel de allvarligaste effekterna vid ett utsläpp. MK1-diesel eller HVO-diesel påverkade testorganismerna endast marginellt jämfört med kontrollen, som representerar opåverkade organismer.

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1 Introduction

Oil spills are by far the most common environmental damage incident in Sweden. The number has decreased in recent years thanks to better routines, techniques, and, for some products, reduced usage. Despite these advancements, around 600 incidents are still addressed annually by emergency services and salvage teams (Swedish Salvage Rescue, 2021). These incidents pose potential threats to drinking water sources and aquatic ecosystems. Yet, detailed information about modern fuels and their environmental and human health impacts remains scarce. The most extensive and transparent summary available dates to 1999 (ATSDR).

Under the Fuel Act (SFS 2011:319) in Sweden, the chemical composition of petrol and diesel is strictly regulated. For instance, MK1 petrol (environmental class 1 petrol) is allowed to contain up to 35% volume of aromatic hydrocarbons. Aromatic hydrocarbons are a group of chemicals known for their significant environmental impact. From an environmental assessment perspective, it is not just the total volume of aromatic hydrocarbons that matters; the specific types of aromatics present are equally critical. This is because individual aromatic hydrocarbons vary in toxicity and water solubility. Some can be more harmful and more soluble in water sources than others. Hence, understanding not only the total amount but also the precise composition of these aromatics in fuels is essential. Accurate and up-to-date data on these substances are imperative for effectively assessing and managing environmental risks and ensuring water safety.

Some substances in fuels can affect the smell and taste of water at concentrations lower than those that are toxic. For drinking water production, in addition to avoiding unwanted health effects from pollutants, water must be taste- and odourless (LIVSFS 2022:12). While there are known odour thresholds for individual substances like ethers (World Health Organization, 2005), comprehensive studies on the odour properties of fuel products mixed in water are limited. Such studies are necessary to understand how different substances might interact to enhance or reduce each other's effects in water (Scottish Environmental Protection Agency, 2010). In-depth evaluations are needed to comprehend the full impact of fuel spills on drinking water resources.

Besides human health, fuel spills in aquatic environments can affect both the shoreline (beach zone) and open water (pelagic zone). With the recent shift in fuel composition, including adding more ethanol to petrol and biofuels like HVO (hydrogenated vegetable oil) and RME (rapeseed methyl ester) to diesel, these fuels' solubility and toxicity characteristics may change. However, the extent and nature of these changes remain unclear. Understanding the ecotoxicity of these new fuel products is particularly crucial for diesel fuels used by ships in large quantities on freshwater lakes. Thus, this study aims to:

1) Quantify the chemical content of common fuels sold at petrol stations, providing essential data that can inform environmental risk assessments and safety protocols.
2) Determine odour thresholds for common fuels when mixed in water and investigate possible synergies between fuel content and taste/odour properties.
3) Assess the ecotoxicity of selected fuels to freshwater organisms.
4) Develop guidelines for evaluating the influence of ethers, particularly in petrol spills, on surface water sources. This aids in effective response planning and risk mitigation for such environmental incidents.

The results presented in this report were originally published by Strandberg J et al. (2024).

2 Fuels included in the study

A total of 30 fuels were analysed for chemical composition (Table 1). Project group members acquired the fuels at fuel stations or fuel oil incinerators in Sweden. The EU diesels were acquired in Germany and Italy, and the MGO-1 sample was acquired in Trinidad and Tobago by the project group.

From the total 30 fuels, specific fuels were selected for further tests. For the odour test, 12 fuels were chosen to represent a variety of compositions regarding the origin of hydrocarbons, additives, bio-components, and ethanol content. This selection aimed to assess how each substance or group of substances contributed to the overall odour, ensuring a comprehensive evaluation across varying fuel types. For the ecotoxicity test, 6 fuels were selected to represent the major product groups available in the market, providing insights into the ecological impact of different fuel categories. Additionally, 2 standard fuels and one custom-made were used in evaporation tests, chosen to provide a range of ethanol and ether concentrations.

All a priori information available on the composition of fuels was the consumer-available information provided by fuel producers, found in Table 1. More information about the legal framework on fuels in Sweden and Europe is found in Section 7.1.

Table 1. The fuels included in the study and the tests performed on each sample, where WAF stands for Water-Accommodated Fraction. Note that the product names are generic and do not represent the fuel company's consumer-branded names. The claimed content is based on consumer-available information on the websites of the fuel companies.

Sample ID	**Product name**	**Claimed content**	**Analysis of fuel**	**PAH analysis of fuel**	**Analysis of WAF**	**Odour test**	**Ecotoxicity test**	**Evaporation**
P95-1	Petrol 95 Octane	E10 petrol, 95 octane, 10 % ethanol, 68 % bio naphtha	Yes		Yes	Yes		
P95-2	Petrol 95 Octane	E10 petrol, 95 octane, 10 % ethanol	Yes		Yes	Yes		
P95-3	Petrol 95 Octane	E10 petrol, 95 octane, 10 % ethanol	Yes				Yes	
P95-4	Petrol 95 Octane	E10 petrol, 95 octane, 10 % ethanol	Yes	Yes				
P95-5	Petrol 95 Octane	E10 petrol, 95 octane, 10 % ethanol	Yes					
P95-6	Petrol 95 Octane	E10 petrol, 95 octane, 10 % ethanol	Yes					
P98-1	Petrol 98 Octane	E05 petrol, 98 octane, 5 % ethanol	Yes		Yes	Yes		
P98-2	Petrol 98 Octane	E05 petrol, 98 octane, 5 % ethanol	Yes			Yes	Yes	Yes
P98-3	Petrol 98 Octane	E05 petrol, 98 octane, 5 % ethanol	Yes					
P98-4	Petrol 98 Octane	E05 petrol, 98 octane, 5 % ethanol	Yes	Yes				
E85-1	Ethanol 85	E85 fuel, 85 % ethanol and 15 % petrol	Yes		Yes	Yes		
E85-2	Ethanol 85	E85 fuel, 85 % ethanol and 15 % petrol	Yes				Yes	Yes
E85-3	Ethanol 85	E85 fuel, 85 % ethanol and 15 % petrol	Yes					
E85-4	Ethanol 85	E85 fuel, 85 % ethanol and 15 % petrol	Yes					
D-SE1	MK1 Diesel	Diesel, 20% of HVO	Yes		Yes	Yes		
D-SE2	MK1 Diesel	Diesel, 7% of RME	Yes		Yes	Yes	Yes	
D-SE3	MK1 Diesel	Diesel, 6% RME	Yes		Yes	Yes		
D-SE4	MK1 Diesel	Diesel, 7% of RME	Yes					
D-SE5	MK1 Diesel	Diesel, 7% of RME	Yes					
D-SE6	MK1 Diesel	Diesel, 7% of RME	Yes					
D-SE7	MK1 Diesel	Diesel, 7% of RME	Yes					
HVO1	HVO100	100 % Hydrogenated Vegetable Oil	Yes		Yes	Yes	Yes	
HVO2	HVO100	100 % Hydrogenated Vegetable Oil	Yes					
HVO3	HVO100	100 % Hydrogenated Vegetable Oil	Yes					
HVO4	HVO100	100 % Hydrogenated Vegetable Oil	Yes					
MGO-1	Marine Gas Oil	Unknown claim	Yes	Yes				
MGO-2	Marine Gas Oil	Unknown claim	Yes	Yes	Yes	Yes		
D-EU1	EU Diesel	Diesel, 6% RME	Yes	Yes	Yes	Yes		
D-EU2	EU Diesel	Diesel, 6% RME	Yes	Yes	Yes	Yes		
EO1-1	Fuel oil no 1	Unknown claim	Yes	Yes	Yes		Yes	
EO1-2	Fuel oil no 1	Unknown claim	Yes	Yes	Yes			

3 Chemical substance groups analysed in the study

Aromatic hydrocarbons

Aromatic hydrocarbons are a group of chemicals known for their distinct ring-like structure, e.g. based on a phenyl group as the “back-bone” of the molecule. This group includes substances like benzene, toluene, ethylbenzene, and xylene (collectively called BTEX) and others like 3-ethyl-toluene, propylbenzene, and trimethylbenzene. These compounds are notable for their strong, sometimes unpleasant odours. They also have some solubility in water and specific health risks; for instance, benzene is well-known for its carcinogenicity (IARC, 2018).

Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a complex chemical group consisting of multiple aromatic rings fused together. PAHs include numerous substances, each with varying molecular weights and structural complexities. For example, naphthalene, with its two-ring structure, is at the simpler end of the PAH spectrum, whereas benzo[a]pyrene, known for its five-ring structure, is more complex and has been extensively studied for its carcinogenic properties (IARC, 2010). The environmental persistence of PAHs, coupled with their potential to bioaccumulate and induce adverse health effects such as cancer and reproductive issues, underscores the environmental importance of these compounds.

Aliphatic hydrocarbons

Aliphatic hydrocarbons are a diverse group of compounds characterised by straight or branched chains of carbon atoms, as opposed to the ring structures of aromatics. In this study, they ranged from smaller molecules like hexane (with six carbon atoms) to larger ones like tetracosane (with twenty-four carbon atoms). These compounds constitute the bulk of the fuels and have varying properties based on their chain length.

Ethers

Ethers are a class of organic compounds characterised by an oxygen atom connected to two alkyl or aryl groups. Common ethers analysed in this project include methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE), and tertamyl-methyl ether (TAME). These substances are added to fuels to enhance performance. Ethers are noted for their strong odour (WHO, 2005), which is a key reason for their inclusion in the study.

Esters

Esters, in this context, refer to organic compounds produced by replacing the hydrogen of an acid with an alkyl or other organic group. Many esters are derived from carboxylic acids and alcohols. Esters like rapeseed methyl ester (RME) are used as bio-components in fuels. They are known for their less harmful environmental impacts than traditional fuel components. Their presence in the study is relevant for understanding how bio-components interact with traditional fuel substances and contribute to overall fuel properties, including odour and toxicity.

4 Tests performed in the study

This study conducted a series of tests to investigate the interaction between various fuels and water, focusing on the potential impact on odour and ecotoxicity. The methodology was structured to simulate a worst-case scenario regarding the concentration of water-soluble substances. A graphical overview of the execution is shown in Figure 1. Each step is explained in the subsequent text.

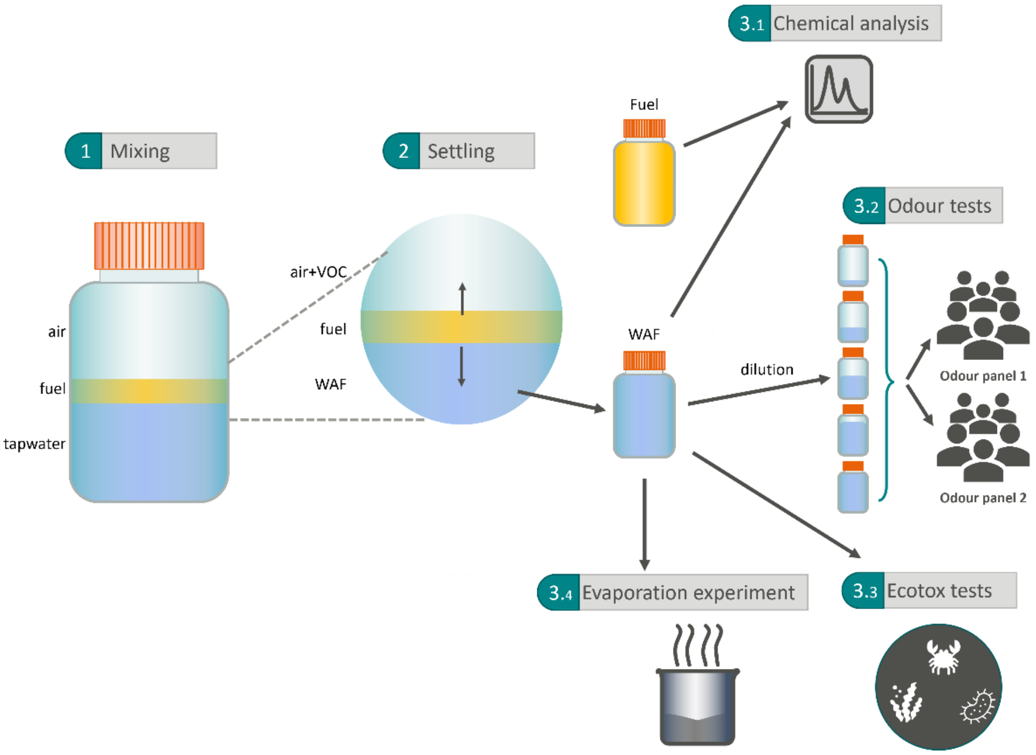

Figure 1. Overview of the execution of tests, including mixing water (1), settling to produce a water/fuel mixture (WAF) (2), which is analysed (3.1), diluted for odour tests (3.2), ecotoxicological tests of crustaceans, algae and bacteria (3.3), and evaporation experiments (3.4), respectively.

Step 1. Mixing Water with Fuel

The initial step involved mixing a large proportion of fuel with water. This ratio was intentionally selected to represent a severe spill scenario. The mixing process was carried out to create a comprehensive blend of fuel and water to ensure that the fuel components were thoroughly dispersed within the water. More detailed information about this step is found in Section 6.1.

Step 2. Settling to produce WAF

After the mixing, the mixture was allowed to settle. This step was crucial for forming a stable water-accommodated fraction (WAF), where non-dissolved fuel components had sufficient time to separate from the water. More detailed information about the method is found in Section 6.1.

Step 3.1 Chemical analysis of the Water/Fuel Mixture

The chemical composition of the WAF was analysed using GC-MS (gas chromatography coupled to mass spectrometry). This analysis was designed to identify and quantify a diverse range of substances, each representing varying numbers of carbon atoms and different substance groups, focusing on those believed to be crucial for interpreting results related to odour and ecotoxicity. More detailed information about the methods is found in Section 6.2.

Step 3.2 Odour Testing

A portion of the WAF was used to produce a dilution series for odour testing. These tests were designed to assess the odour at various dilutions, allowing for an understanding of the threshold levels at which the odours become noticeable. More detailed information about the method is found in Section 6.4.

Step 3.3 Ecotoxicological Testing

Ecotoxicological tests were conducted on selected fuels, targeting three types of organisms: crustaceans, algae, and bacteria. By studying the effects on three different organism trophic levels, the tests provided a comprehensive view of the ecotoxicity of the fuel-water mixtures. More detailed information about these methods is found in Section 6.5.

Step 3.4 Evaporation Experiments

Finally, evaporation experiments were carried out on the WAF. These experiments were designed to understand how volatile fuel components evaporate from the water surface under different temperatures and ethanol concentrations. This part of the study was used to model the fate of fuel spills in natural aquatic environments. More detailed information about this step is found in Section 6.3.

5 Results

5.1 Chemical analysis of fuels

Diesel composition is more homogenous than petrol

A total of 55 selected substances were quantified using GC-MS and then categorised according to the SPIMFAB substance groups (the classification is found in Appendix 2) to analyse the composition of various fuel types. These substances were chosen for their significance in interpreting results related to odour and to illustrate the proportion of light and heavy substances in the fuels. While indicative of the overall chemical makeup of the products, they do not encompass the entire composition of the fuels.

The results are generally in line with the provisions of European standards and the Swedish Fuel Act (specified in Chapter 10), where it is evident that MK1 diesel and HVO fuels have lower concentrations of aromatic compounds compared to EU Diesel, Fuel oil 1, and Marine Gas Oil (MGO), as seen in Figure 2.

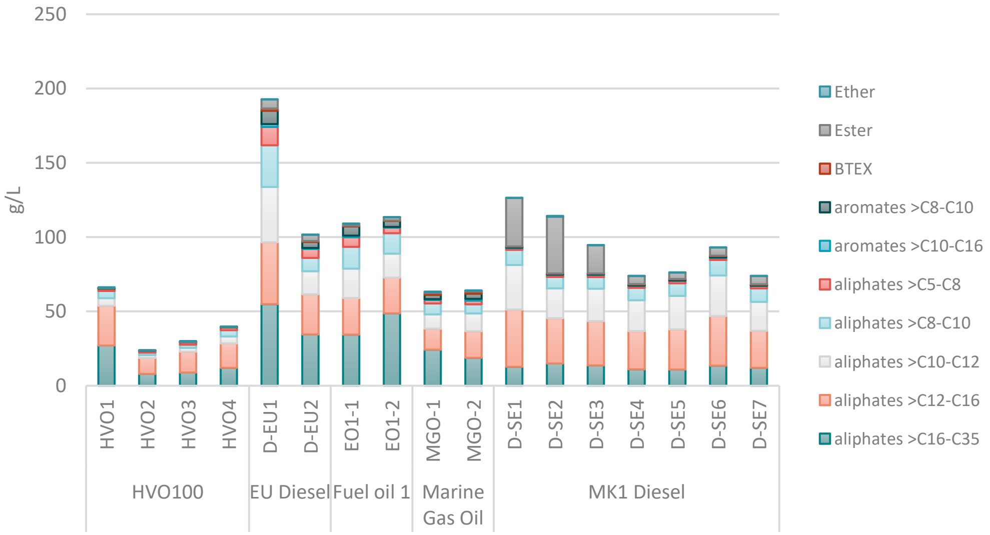

Figure 2. Total concentrations of the SPIMFAB substance groups in diesel and diesel-like fuels. Note that sums of substance groups only contain the quantified substances in this study and not the sum of the entire substance group.

MK1 diesels are noted for containing HVO (Hydrogenated Vegetable Oil) or RME (Rapeseed Methyl Ester) as biocomponents, contributing to the variance in ester concentrations among these fuels. This component is not present in Fuel oil 1 or MGO.

The analysis of petrol types reveals a substantial presence of aromatic compounds of around 120 g/L across all samples (Figure 3). The observation aligns with known petrol compositions and the Fuel Act's allowed limit of 35 vol %. Furthermore, it is observed that fuels like E85 and 98 octane petrol are blended with ethers like MTBE, ETBE, and TAME, with concentrations noted to be well below the Fuel Act's allowance of up to 22 vol % for ethers. It will, in practice, be suppressed by the limit on oxygen content of 2.7%, which means that with a 10% ethanol blend, there is less room to add MTBE/ETBE compared to lower ethanol blends, such as the 5% found in 98-octane fuels.

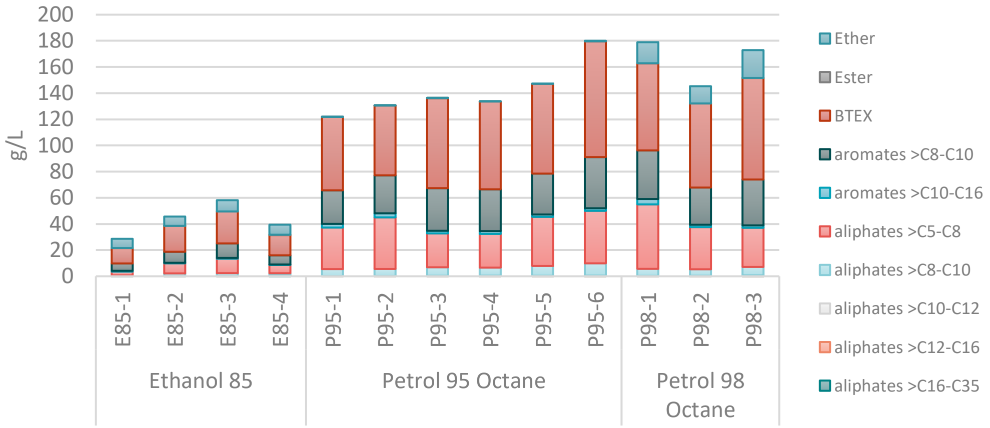

Figure 3. The sum of analysed substances per SPIMFAB group in petrol and petrol-like fuel. Note that sums of substance groups only contain the quantified substances in this study and not the sum of the entire substance group.

Another observation is that petrol, even if divided into the subgroups 98 octane, 95 octane and E85, have more heterogeneity in the fuel's main constituents than diesel. This can be seen in Figure 4, which shows a box and whisker plot including products with three or more samples per product group. The boxes for petrol fuels are generally longer, indicating a greater span from the first to the third quartile within the respective group.

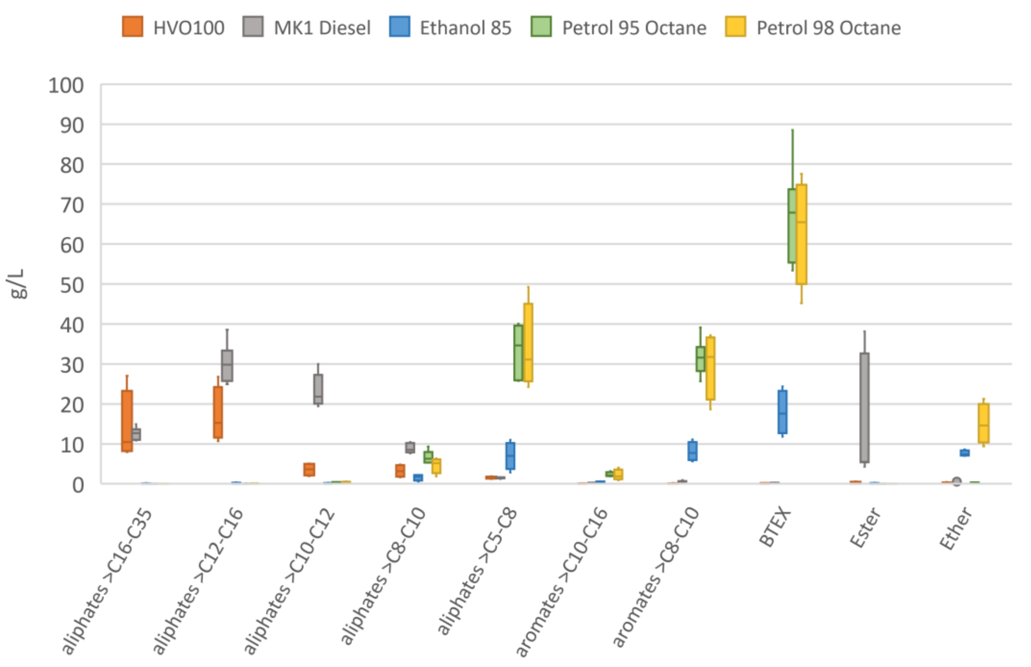

Figure 4. Box and whisker plot of concentrations (g/L) of SPIMFAB compound groups in the study with three or more samples in fuel products. Note that sums of substance groups only contain the quantified substances in this study and not the sum of the entire substance group. The plot features boxes that represent the interquartile range (IQR), extending from the first quartile (Q1) to the third quartile (Q3) and enclosing the median, marked by a line within each box. Whiskers extend from the boxes to the furthest data points within 1.5 times the IQR, capturing the overall range of the data while excluding outliers.

PAH analysis

Eight fuel samples were chosen for a PAH analysis. The selection criteria were informed by suspicions of PAH presence in these fuels, intentionally excluding Swedish MK1 diesel and HVO from the analysis in compliance with stringent legislation prohibiting PAHs in these fuel types. The result of the analysis is shown in Figure 5. European diesel samples revealed a moderate presence of these compounds, with medium molecular weight PAHs like phenanthrene emerging significantly in one of the samples, indicating a uniformity that might reflect regulations governing European fuel standards.

Further insights were gathered from comparing fuel oils produced across different time frames. The EO1-1 sample, a modern fuel oil, contained considerably lower amounts of the PAH:s than the EO1-2 sample produced during the 1970s. Another difference was observed in the Marine Gas Oil samples, where the product from Sweden, MGO-1, contained a higher concentration of naphthalene than the Trinidad (MGO-2) sample. The most striking observation is that 95 and 98-octane fuels displayed considerable amounts of naphthalene, which is suspected to have a toxic effect on aquatic organisms.

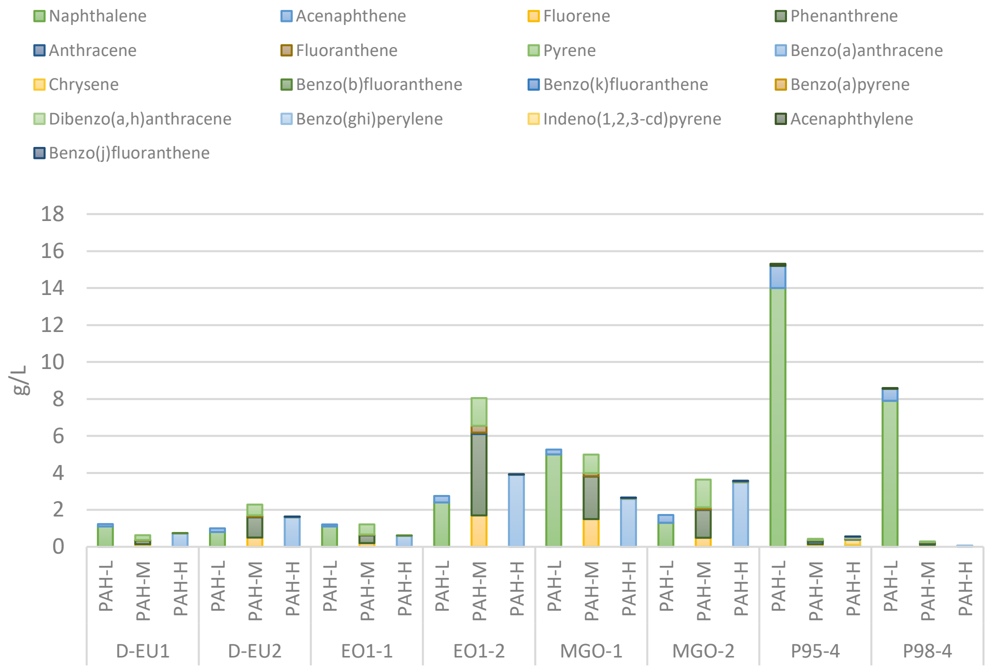

Figure 5. Concentrations of PAH compounds in eight fuel samples grouped in PAH-L (low molecular weight), PAH-M (medium molecular weight) and PAH-H (high molecular weight). The definition is found in annex 2.

A minor proportion of compounds quantified

The concentrations of specific chemicals were measured using standards and readily available standard mixtures containing common constituents of spark ignition fuels. These mixtures do not include every chemical found in the fuels but cover a significant portion relevant to the study. As a result, many hydrocarbons were not accounted for in the findings. The goal was to identify which chemicals contributed to odour, allowing for the exclusion of compounds unlikely to affect odour significantly. Additionally, the relationship between the chemicals' toxicity and their composition was not examined, meaning chemicals that might have harmful environmental effects were not focused on.

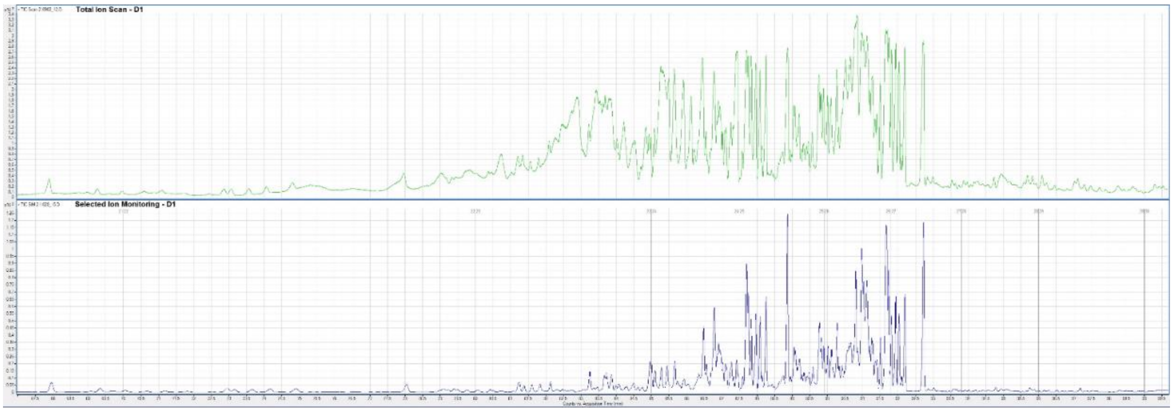

Figure 6. GC/MS chromatograms of HVO-1 fuel in full scan (upper) and Selected Ion Monitoring (lower).

The total concentration of quantified components in the products D-SE3 and HVO1 was lower than the D-SE2 and D-SE1, which can be seen from the heights of the bars in Figure 2. However, the chosen compounds quantified by the analysis represent a wide range of the chemicals existing in the fuels. The compounds were selected based on the availability of standards for quantifying paraffins, isoparaffins, aromatics, naphthenes, and olefins commonly quantified in fuel mixtures. Therefore, they should also provide a representative picture of structurally similar compounds even though the extraordinary complexity of chemical compounds in a fuel can not wholly be covered. Figure 6 shows the same sample, analyzed with the instrument collecting all generated ions at each moment (top) or only the ions used for quantification (bottom). This figure clearly shows that the fuel contains a wide range of hydrocarbons that are not included in the calibration mixture. This includes both compounds having low boiling points, seen in the centre-right part of the upper (green) trace and branched long-chain aliphatic compounds, present in the left part of the upper trace. In HVO-1, this is likely why the proportion of quantified compounds is low. The sheer complexity of the fuel composition limits the possible degree of investigation. An analysis is still performed of representative hydrocarbons in each boiling point fraction representing substances of equal molecular size to those not qualified.

5.2 Chemical analysis of fuels in water

Petrol-specific substances are more soluble in water

When comparing Figure 2, Figure 3, and Figure 7, it can be seen that fewer constituents of diesel fuels are dissolved in the water phase than petrol fuels, mainly due to the dissolved ether and BTEX components from petrol. A branched alkane, 2,3-dimethyl pentane and two unsaturated short-chain alkenes, 1-hexene and 1-heptene, were also found to occur to a greater extent dissolved from the petrol fuels.

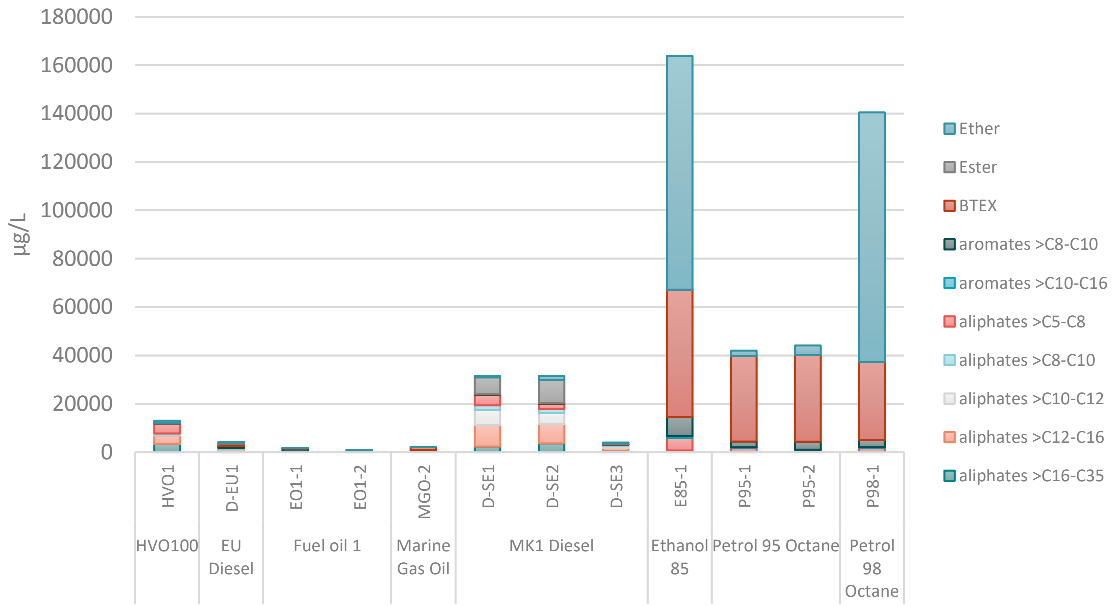

Figure 7. Total concentrations of the determined substances in the WAF per SPIMFAB substance group. Note that sums of substance groups only contain the quantified substances in this study and not the sum of the entire substance group.

Figure 8 shows the concentrations of selected aromatic compounds in fuels and the corresponding concentrations in the resulting WAF. These compounds are predominately occurring in petrol fuels. The less substituted aromatic compounds are dissolved to a greater extent in the water than the more substituted compounds, meaning that proportions between the concentration of compounds in fuel and WAF are affected.

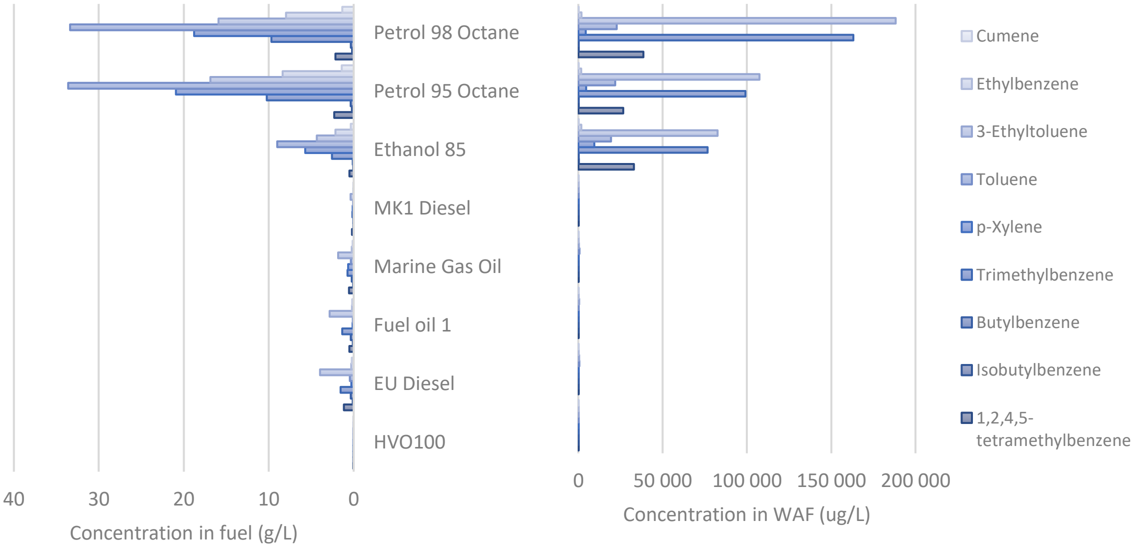

Figure 8. Concentrations of selected aromatic compounds in the fuels, right, and in the WAF, left.

Figure 9 shows the concentrations of selected aliphatic compounds in fuels and WAF. These compounds, present predominately in diesel fuels, do not show any apparent differences concerning the relative proportion between substances in the transfer from the fuel to the water.

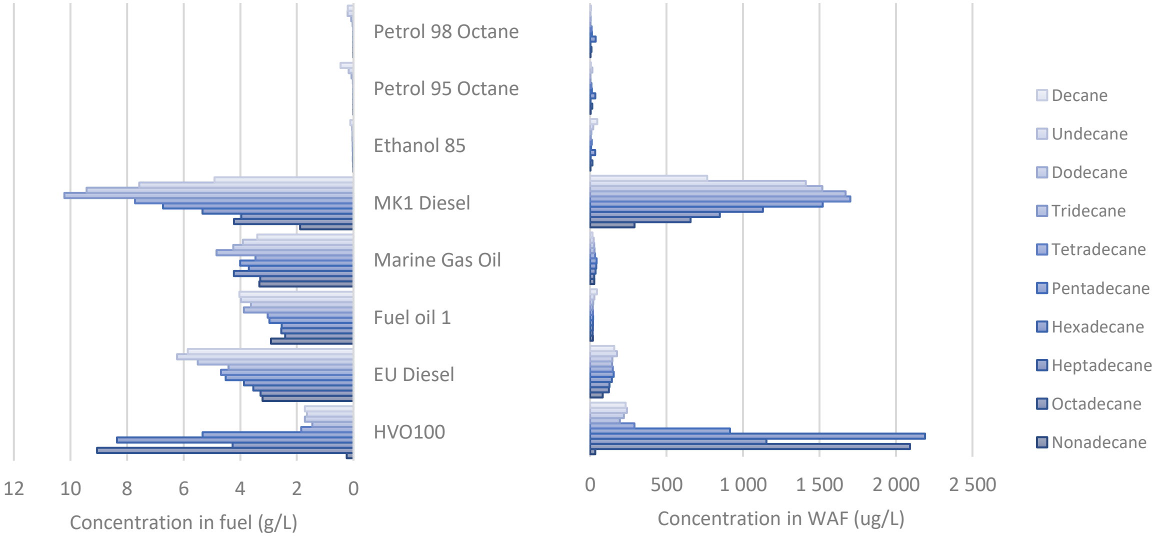

Figure 9. Concentrations of selected aliphatic compounds in fuels and in WAF.

Ethanol and RME enhance hydrocarbon dissolution in water

The most striking observation regarding concentrations in the WAF was that the ethanol fuel (E85-1), containing only 15% of petrol, had the highest concentrations of the dominating petrol components (BTEX, aromatic components and linear-, branched- and cyclic short alkanes and alkenes). This is most likely an effect of the ethanol acting as a solvent, increasing the phase transfer and solubility in the receiving WAF. However, since the test system is a closed system with limited fuel and water phase volumes, the system will likely increase the solubility of petrol compounds compared to a real-case scenario. Nevertheless, the trend is still valid during a spill.

The occurrence of longer chain aliphatic compounds in the water samples appears to follow with the concentration of the RME component (oleic acid methyl ester) in the fuel, even though the initial concentrations of the aliphatic compounds in the fuel were similar. Therefore, the WAF from D-SE2 and D-SE1 contained more dissolved long-chain aliphatics than the WAF from D-SE3. This implies that the methyl ester could act as a surfactant, affecting the surface tension of the water and, thereby, the droplet formation in the emulsion of fuel in water, resulting in a larger contact surface between the two phases. This effect was also evident for the cyclic aliphatic compounds studied: methyl-, ethyl, propyl, butyl- and pentyl cyclohexane.

Strong correlation between ether concentration in WAF and fuel content

Based on the chemical analysis of fuels and the corresponding analysis in the WAF:s, an ether transfer equation was derived between concentrations in fuel and WAF. The most important variable explaining the ether concentration of the WAF was the ether concentration in the raw fuel. Figure 10 shows a scatterplot of the total concentration of ether in the WAF:s vs the total concentration in fuels on a logarithmic scale. It shows the linear relation between the concentrations and the regression equation describing ether transfer from fuel to WAF. Given the low number of observations, the equation still gives a good prediction of the solubility of ether from fuel to water.

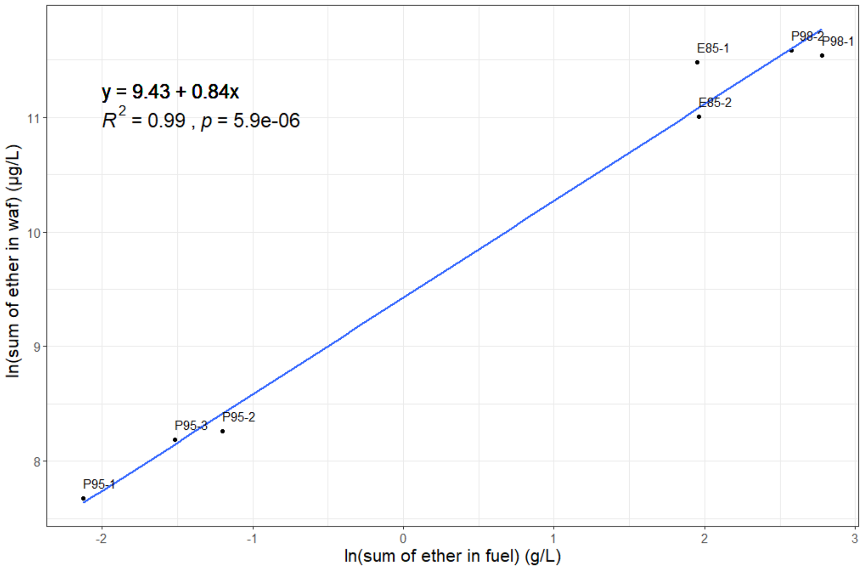

Figure 10. Scatter plot of the sum of ether in WAF vs in fuel on a logarithmic scale and regression equation, where y=ln(sum ether in WAF), x=ln(sum ether in fuel).

5.3 Determination of odour thresholds

Three fuels are notably distinguishable by odour

The threshold odour numbers (TON) in Table 2 is the dilution ratio beyond which a sample has no perceptible odour. For example, if TON=10, the WAF and reference water mixture consisted of 9 parts reference water and 1 part WAF. The dilution ratio is thus 10%. A high TON means that odour was detected at a low proportion of WAF in the sample. i.e., the odour was strong. Table 2 summarises the results of the odour tests. The fuels E85-1, P98-2 and P98-1 stand out with remarkably high TON values, i.e., low odour thresholds. These are the 85% ethanol petrol and the 98-octane petrol, respectively.

Table 2. Threshold odour numbers (TON) averaged over fuel types, the dilution ratio where 50%of the panellists did not sense an odour and the MTBE concentration at that dilution. The rationale for including ether concentrations is explained in section 5.2 Prediction of odour.

Fuel	Threshold odour number (TON)	Dilution ratio (%) [1]	Concentration MTBE (µg/L)
HVO1	62	1.6	13
P95-1	250	0.40	8.5
D-SE3	260	0.39	0.22
D-SE1	300	0.33	1.7
D-SE2	300	0.33	5.5
P95-2	490	0.21	8.1
MGO-2	1 500	0.066	0.0031
D-EU2	1 500	0.066	
D-EU1	9 500	0.011	0.011
E85-1	24 000	0.0042	4.0
P98-2	24 000	0.0042	3.4
P98-1	60 000	0.0017	1.4

(1) Percent of WAF in a sample beyond which an odour could not be detected.

Considerable variability in odour thresholds among panel members

There were substantial variations regarding when an odour was noticed among panel members assessing the same fuel type (Figure 11). In the first round, several panel members detected an odour in the most diluted sample in the series (high dilution factor) for fuels E85-1 and P98-1. In the first round, a dilution factor of 2 was used, and in the second and third round, a dilution factor of 2.5 was used. This means that the steps between each dilution were more significant, resulting in a broader range of concentrations and larger differences in odour threshold between panel members. The TON values in Table 2 were obtained when summarising the data per fuel type.

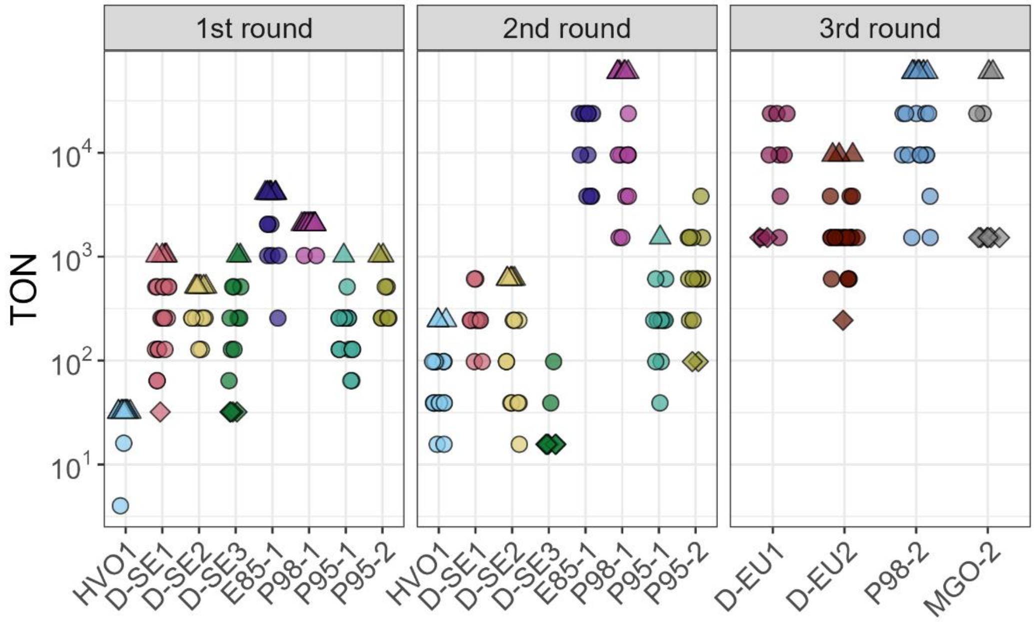

Figure 11. The threshold odour number, on a logarithmic scale, indicates where an odour first was noticed by each panel member. Triangles indicate a panel member noticing an odour in the most diluted sample, and diamonds indicate no odour in the least diluted sample. Dots indicate a panel member noticing an odour in any other bottle. In the 1st round, a dilution factor of 2 was used, and in the 2nd and the 3rd round, a dilution factor of 2.5 was used. One colour per fuel type for better visual separation.

5.4 Prediction of odour

A limited number of substances correlate with strong odour

Of all the quantified chemicals in the fuels, the correlation with threshold odour number (TON) was high for only a handful of chemicals. The substances correlated with TON above 0.6 are shown in a correlation matrix (Figure 12). A preliminary analysis showed that relations between TON and concentrations were best modelled on a logarithmic scale. Hence, the natural logarithm was applied to TON and the concentrations of chemicals.

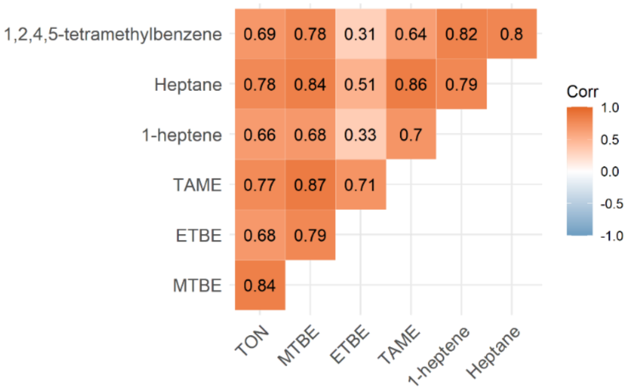

Figure 12. Correlation matrix for selected substances with TON. Natural logarithm was applied to all data before calculating correlations.

The main observation is that the substances correlated to the odour threshold are also positively correlated. The exceptions are the correlations between 1-heptene and ETBE and 1.2.4.5-tetramethyl benzene and ETBE, which have lower correlations.

The MTBE concentration in the samples where 50 % of the odour panel recognised an odour was 1.4 µg/L in P98-1 and 4.1 µg/L in E85-1. These concentrations are lower than the odour threshold reported by WHO (2005), which was 15 µg/L. The concentrations of other ethers in the WAF are too low to be the reason for the difference. Hence, results imply that the low threshold results from MTBE being in a mixture of other substances that add or enhance odour. However, this could not be evaluated statistically due to the limited number of samples.

MTBE concentration as a reliable odour predictor in water

Figure 13 shows that MTBE correlates with TON (R^2 of 0.7) and with every other substance in Figure 12. Including MTBE and other chemicals as explanatory variables would lead to large errors in the estimated model. In statistical terms, this is called multicollinearity and should be avoided if possible. A variable selection technique called Lasso regression (least absolute shrinkage and selection operator, explained further in section 6.3.1), which accounts for multicollinearity, was applied to these data. The Lasso regression suggested using only MTBE as the explanatory variable. Thus, the choice was to select this simple model, which only contains MTBE as the explanatory variable, and thereby avoiding multicollinearity.

The TON and the MTBE-concentration plot in Figure 13 shows the linear relation and the regression equation based on the linear mixed model. The linear mixed model is described further in section 6.3.1.

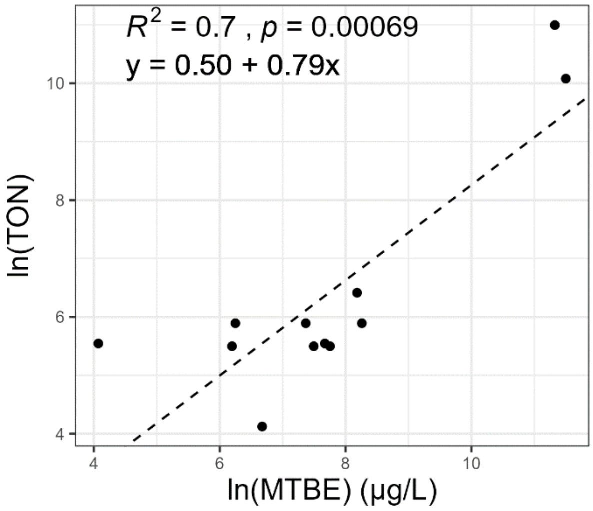

Figure 13. Scatter plot of threshold odour number (TON) vs MTBE on a logarithmic scale and regression equation, where y=ln(TON), x=ln(MTBE)

It should be emphasised that the limited number of observations restricted this model's construction. When constructing linear models, a rule of thumb is to have at least 10-20 observations per estimated parameter (Harrell F., 2015). This condition is met when only one explanatory variable is used.

5.5 Ecotoxicological tests

All ecotoxicological tests were performed on WAF:s attained from mixing fuel and water and extracting the water phase. In some tests, the concentration is expressed as the loading rate, which is the amount of WAF per litre of water, and in some tests, it is noted as a percentage of WAF in the solution. All tests focus on the effective toxicity of the fuel product, which means that the effects of potential solvents in the product, such as ethanol, are desired outcomes.

The results from the analysis of total organic carbon (TOC), Table 3, show the difference in total solubility of fuels, where higher concentrations of ethanol in P95-3 (10%) and E85-3 (85%) are reflected in the increased TOC.

Table 3. Total organic carbon in prepared water/fuel solutions of fuels at test start and termination after 72 hours of alga test. The same method, described in 6.3.1, was used to produce WAF for all ecotoxicological tests.

Test sample	TOC (mg/L). 0 h	TOC (mg/L). 72 h
Control 1	0.77	1.16
HVO1	<0.50	<0.50
D-SE2	0.64	<0.50
Control 2	<1.0	<1.0
P98-2	4.2	3.8
P95-3	6.9	6.6
E85-2	49	48
Control 3	<1.0	<1.0
D-EU1	<1.0	<1.0
EO1-1	1.2	<1.0

The description of methods and results is found in Appendix 3, where additional ecotoxicological parameters, such as EC_{20} and EC_{50}, can also be found.

Growth inhibition in algae and cyanobacteria by fuel oil and certain petrol fuels

The test aims to determine a substance's effects on a freshwater microalgae's growth. Exponentially growing algae cultures are exposed to the WAF of the test items over 72 hours. Despite the relatively brief test duration, effects over several generations can be assessed. The response variable is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations (loading rates) of the test substances. The response is evaluated as the function of the exposure concentration compared to the average growth of an unexposed control culture. Growth and growth inhibition are quantified from measurements of the algal biomass (dry weight per volume) as a function of time. Cell counts (cells/ml) are used as a surrogate parameter for dry weight (OECD TG No. 201). The test endpoint is inhibition of growth, expressed as the logarithmic increase in biomass (average specific growth rate) during exposure.

The tests showed that average specific growth rates of algae significantly differed from their controls (unpaired t-test, *p*>0.05) when exposed to fuels E85-2, P98-2, and Eo1-1. The NOEC (No Observed Effect Concentration) is therefore set at 50 mg/L loading rate, meaning that the WAF had a toxic effect on the test organisms. Conversely, HVO1, D-SE1, P95-3 and D-EU1 showed no significant difference compared to the control (*p*>0.05), establishing their NOEC at 100 mg/L, representing the highest concentration tested, hence no toxic effect. The specific growth rates for all dilutions and replicates are found in Annex 3.

Figure 14 shows algae growth over 72 hours for the most concentrated solutions used in the tests.

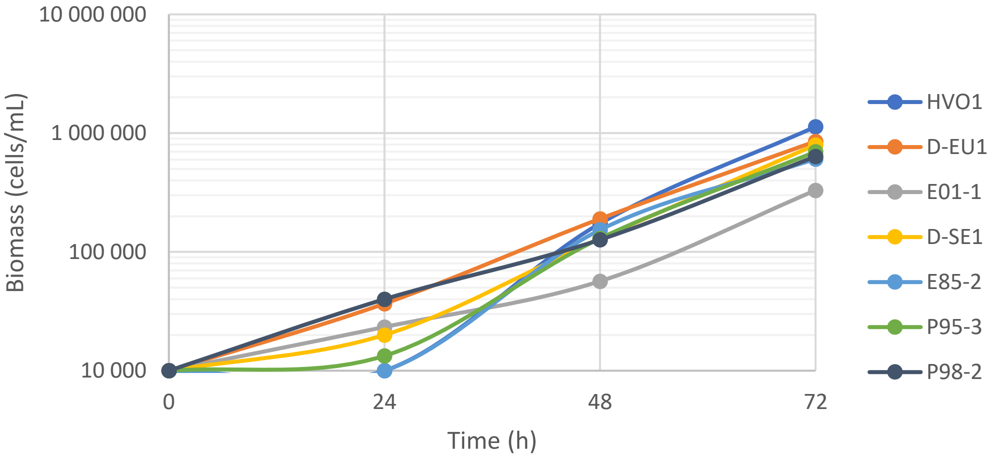

Figure 14. The biomass (cells/mL) in the highest test concentration used (100 mL loading rate) after 0, 24, 48 and 72 hours of exposure to the fuel/water solutions of P95-3, P98-2, E85-2, D-EU1, Eo1-1, HVO1 and D-SE1.

Greater luminescence inhibition in bacteria by petrol compared to diesels

A microtox test indicates general toxicity by inhibiting the activity of bacterial cells genetically modified to provide luminescence. Inhibition of the luminescence indicates a toxic effect on the cells.

Microtox tests indicate that the luminescence of bacteria is inhibited strongly by petrol fuels (Figure 15). For a solution of 50 % WAF, the inhibition was absolute for the E85 and P95 samples, and P98 was close to 100 %. The EU-diesel and the fuel oil inhibited luminescence at 50 % WAF but not when diluted to 4.5%. The HVO and the MK1 diesel (D-SE1) did not cause any inhibition when used as a 90 % WAF solution, except a minor for day 28 after 5 min.

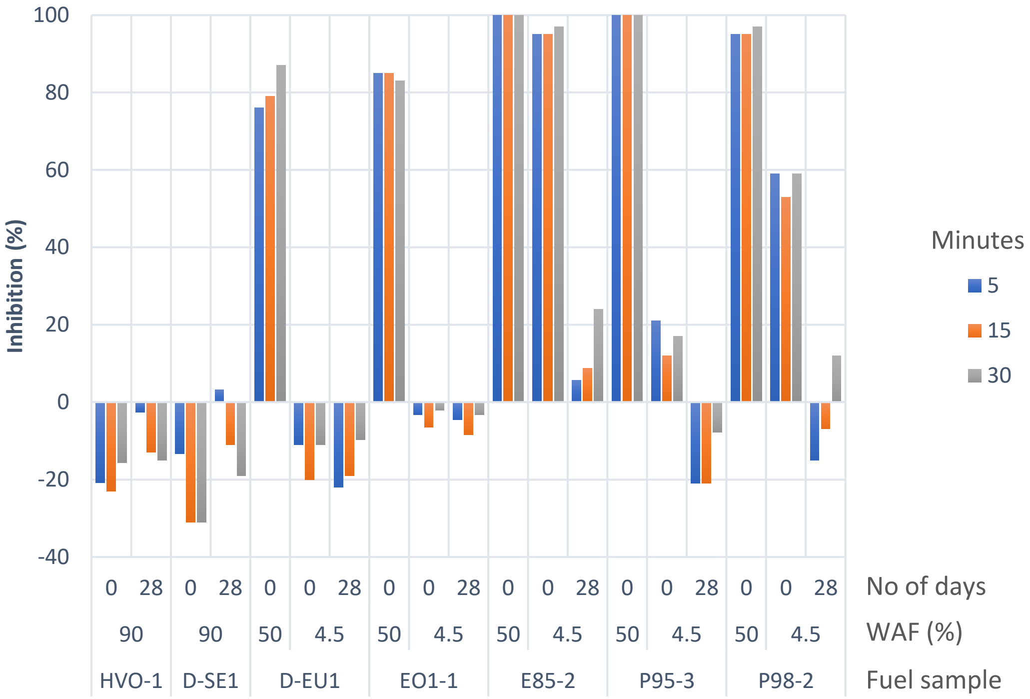

Figure 15. Inhibition of luminescence in a Mictotox test for seven fuel samples. HVO-1 and D-SE1 were tested using 90 % WAF solution, while the others were tested using 50 % and 4.5% WAF solutions. Each solution was tested for 0 and 28 days, and 5, 10 and 15 minutes. The incubation conditions are controlled to maintain the bacterial culture's viability, providing a nutrient-rich medium, ensuring proper aeration, and regular subculturing. Accurate and consistent bacterial cultures are essential for reliable Microtox test results.

Some reproduction inhibitions observed in crustaceans across all fuel samples

The toxicity to *Daphnia magna* from WAF:s of seven fuel samples, expressed as inhibition of reproduction, is shown in Figure 16. For HVO-1, the EC_{10} (the fraction of WAF where 10 % of the organisms are affected) was reached at 11 vol % WAF and EC_{50} at 69 vol %, which means that the HVO fuel was only mildly inhibiting. The EU-diesel D-EU1 reached the EC_{50} already at 1% WAF, revealing very strong toxic properties. The uptake of toxic substances in crustaceans occurs through direct absorption of substances through gills and on body surfaces or through ingesting contaminated food. The D-EU1 sample contained hydrophobic aromatic substances, which might have adsorbed to the food, thereby adding to the exposure of the crustaceans compared to the other fuels.

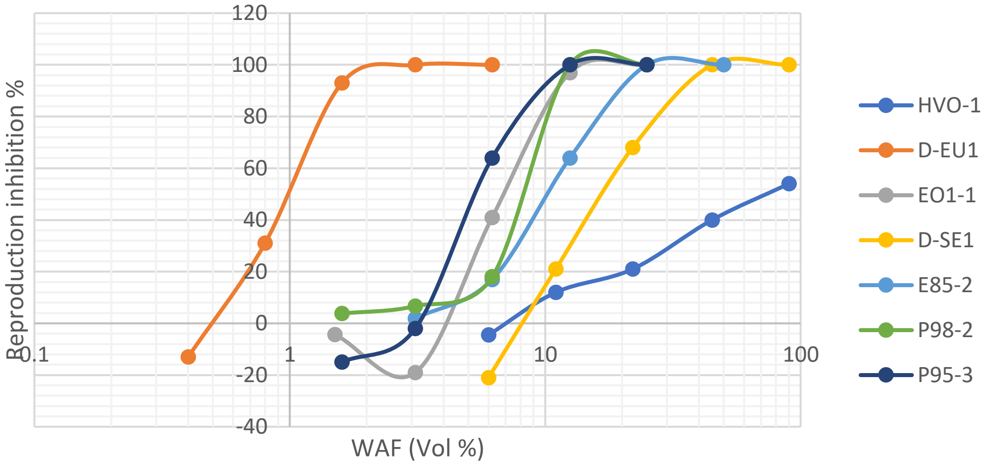

Figure 16. Dose-response curve for seven fuel samples on the reproduction rate of Daphnia magna. The response is given as % of inhibited reproduction, and the dose is given as % of WAF in the test solution.

No disturbance in microbial degradation by any fuel

The microbial degradation test employs wastewater sludge inoculated in Water Accommodated Fraction (WAF) solutions. None of the samples that produced acceptable levels of dissolved organic content (DOC) interfered with the degradation process of DOC (Figure 17). For HVO-1 and D-SE1 samples, the water solubility and, consequently, the DOC levels in the WAF were below the permissible range for our analytical method. These levels were near the lower limit for D-EU1 and EO1-1 samples. The method recommends a DOC range of 10-40 mg/L, but the test material's concentration was below 2 mg/L. Therefore, the results were deemed invalid for evaluation purposes.

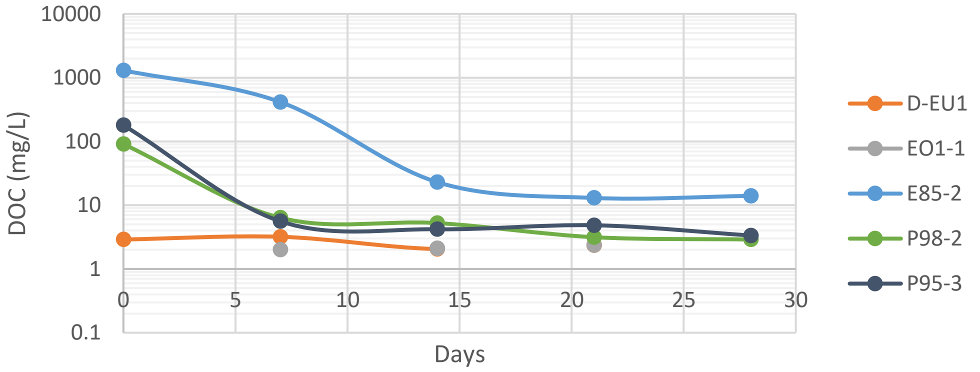

Figure 17. The dissolved organic carbon (DOC) in mg/L from five fuel sample WAF:s during the test execution of 28 days.

Highest toxicity to activated sludge exhibited by petrol fuels

The ISO 8192 method assessed the inhibitory impact of Water Accommodated Fraction (WAF) on the respiration rate of activated sludge microorganisms by exposing the sludge to 0.5, 5, and 50 vol% WAF concentrations and monitoring changes in oxygen consumption. This serves as an indicator of the fuels’ potential toxicity to the microbial community. Notably, at a 50% WAF concentration, all samples except EO1-1 exhibited inhibitory effects, whereas EO1-1 demonstrated negative inhibition, indicating it acted as a growth substrate for the activated sludge (Figure 18).

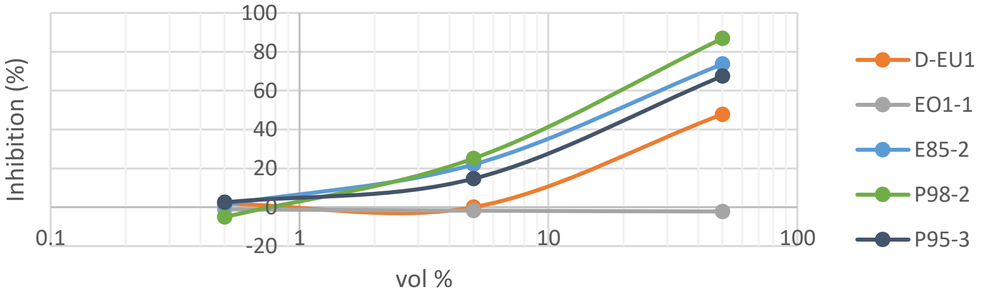

Figure 18. The inhibition of respiration of active sludge from different doses of WAF (vol %) attained from five different fuels.

5.6 Aeration of ether

Ethers determine the odour properties of a water and fuel mixture. They are highly soluble but evaporate, decreasing water concentrations with time. The speed of evaporation during different temperatures and ethanol concentrations was studied in bench-scale tests. Given the substantial variation in the composition of ethers across various fuels, it was decided to study the change in the total sum of ethers in the water-fuel mixtures.

Negligible evaporation of ether in cold water

The test was performed with three different fuels: one E85 (E85-2), one 98-octane petrol (P98-2), and one 98-octane petrol that was diluted with ethanol to reach an ethanol concentration of 45% (P98-4*). Figure 19 shows the decline of the sum of ethers (MTBE, ETBE, and TAME) in the water caused by evaporation. The decline is almost linear on a logarithmic scale, indicating that the decrease in the WAF due to evaporation is exponential. The results also show that MTBE and ETBE have distinctly different evaporation patterns. MTBE slightly increases with increasing ethanol concentration, while ETBE shows a strongly decreasing evaporation rate with increasing ethanol concentration.

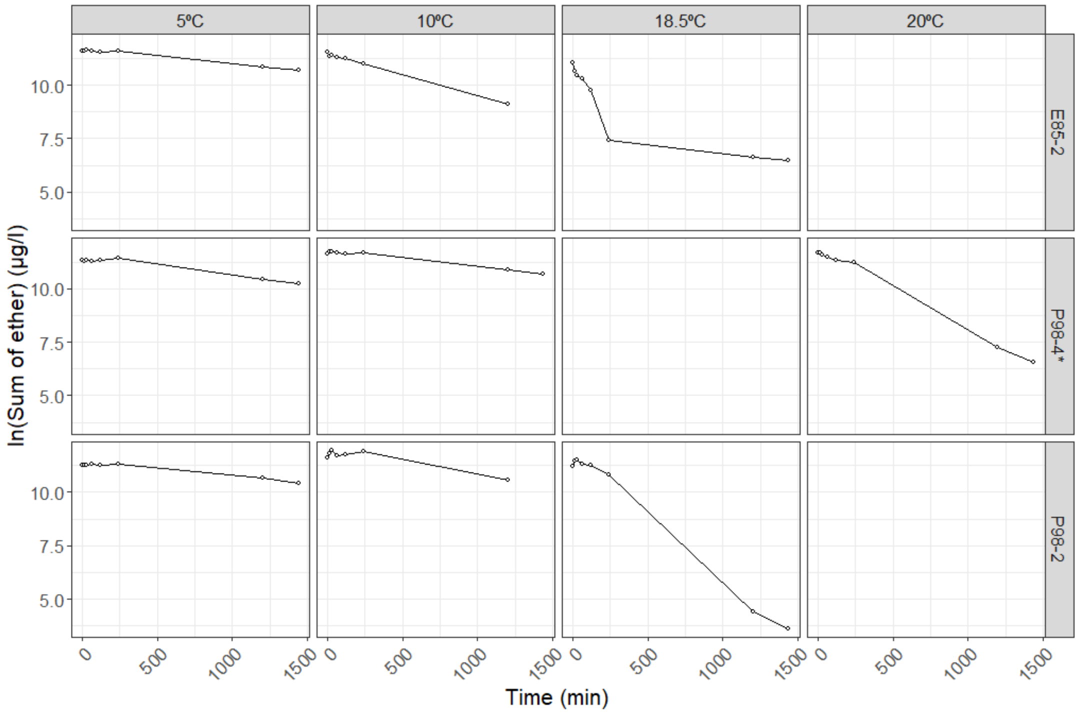

Figure 19. Plots of the sum of ether on a logarithmic scale vs time for the aeration test. Plots are separated by the temperature of the test and by fuel.

The practical implications from these bench-scale studies are that for fuel spills during the cold period, with water temperatures below 5 °C, ether concentration over time is almost entirely reduced by dilution.

Robust modelling of ether concentration achieved

From the results of the aeration test, a linear regression model was constructed to explain the evaporation of ether. The equation below reasonably estimates the evaporation rate of the sum of ethers.

$$\ln(sum\ ether) = \beta_0 + \beta_1 Time + \beta_2 Temp + \beta_3 Ethanol + \beta_4 Time * Temp + \beta_5 Time * Ethanol + \beta_6 Temp * Ethanol + \beta_7 Temp * Ethanol * Time$$

Where $\beta_0 = 11.15, \beta_1 = 1.64e-3, \beta_2 = 5.45e-2, \beta_3 = 1.12e-2, \beta_4 = -3.75e-4, \beta_5 = -1.95e-5, \beta_6 = 1.61e-3, \beta_7 = 2.82e-6$.

The model has an adjusted R-squared value of 0.84, indicating that the chosen variables can explain much of the variance in the total ether concentration. It is thus possible to model the evaporation of ethers in a water mixture. It should, however, be emphasised that this model describes the system created in the laboratory well. Applying the results to real-world situations may significantly alter the results. However, an attempt to apply it in practice is presented in Appendix 1.

6 Materials and method

6.1 Preparation of test solution

500 ml of tap water and 50 ml of each fuel were used to mix fuel with water. Water and fuel were added to a 1 L Duran bottle with Teflon lined screw cap. The mixing was performed with a Rotier ra20 from Gerhardt that rotated about 20 revolutions per minute (rpm) for about 15 hours. After mixing, the mixture was moved to a separator funnel, allowing it to settle for about 4 hours. The aqueous phase with fuel (water accommodated fraction, WAF) was collected in a new Duran bottle and stored in a fridge before sample preparation.

6.2 Chemical analysis

6.2.1 Water extracts quantification

This section describes the analytical procedure used to measure the concentrations of selected fuel hydrocarbons in the WAF. Liquid-liquid extraction (LLE) of the WAF was performed inside a pyrolysed glass tube (25 mL) for each sample. 4 mL of each WAF sample was extracted with 1 mL of dichloromethane (DCM). Pyrolysed glassware was used for the extraction to avoid external contamination. 250 ng of surrogate internal standard (IS) (toluene-d_8 & hexadecane-d_{34}) were added before LLE, followed by 1 min vortex mixing. Extraction using an organic solvent, already spiked with internal standard, was performed assuming equilibrium between the two phases. The organic phase (1 mL) was transferred to precleaned Eppendorf glass tubes, and 200 mg of Sodium Sulphate (Na_2SO_4) was added to remove any remaining water residues, followed by mixing using a vortex mixer for 1 min. The tubes were centrifugated at 14,000 rpm for 10 minutes to recover the DCM without Na_2SO_4. 250 ng of volumetric IS (benzene-d_6) was added to facilitate the method’s recovery calculation before the injection.

6.2.2 Fuel product quantification

This section describes the quantitative analysis method employed to measure the content of selected hydrocarbon compounds in the selected fuels. Each fuel sample was diluted 400 times using a two-step dilution to 1 mL of DCM to avoid overloading the analytical instrumentation. A previously pyrolysed glass vial (1.5 mL) and 250 ng of each IS were added (toluene-d_8. hexadecane-d_{34} and benzene-d_6). The analyses were conducted using gas chromatography coupled to mass spectrometry GC/MS system (Agilent 5977B GC/MSD; Agilent Technologies. Inc. Santa Clara. CA. USA) in electron impact ionisation mode (EI).

An analytical separation column, developed explicitly for excellent resolution of petroleum process products, including paraffins, isoparaffins, aromatics, naphthalenes, and olefins (PIANO), was used (HP-PONA column; length 50 m; inner diameter 0.2 mm; stationary phase thickness 0.5 µm). The column was configured for high-resolution performance analysis of petroleum process products, including paraffins, isoparaffins, aromatics, naphthalenes, and olefins (PIANO). The instrument was equipped with an auto-injector (Agilent 7683B). The injection was in pulsed splitless mode, while the injection volume was 1 µL. Integration was made with MassHunter software version B.04.00 for quantitative analysis (Agilent Technologies. Inc. 2008). Quantification of 17 selected PAHs (Table S3), including those recommended by the Environmental Protection Agency (EPA) (Keith, 2015) which are frequently found in environmental monitoring samples, was also performed using high performance liquid chromatography with fluorescence detection (HPLC-FL) and diode array detection (DAD) (Swit et al. 2022). A mixture of fluorinated PAHs was used as surrogate IS andcalibration standards of different concentrations (0.1 – 1 500 µg/L) in acetonitrile. The reference method utilized was according to the Swedish Standard SS-EN 17503:2022 (SIS, 2022).

6.2.2.1 Quantified substances

The quantified substances can be divided into groups, such as ethers, BTEX-compounds and aromatics, aliphatic compounds, and esters. Groups are specified in Appendix 2. Common occurring ethers are methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE) and tertamyl-methyl ether (TAME).

Due to the focus on predicting odour properties from concentrations, the best resolution was needed for the low molecular weight substances, such as BTEX and ethers, since these are expected to contribute most to odour (WHO, 2005; WHO, 2008). The BTEX group of compounds consists of benzene, toluene, ethylbenzene and xylene (m-, o-, and p-xylene). Other aromatic compounds common in fuels are 3-ethyl-toluene, propyl- and trimethyl- benzene. The aliphatic compounds studied in this project have ranged from hexane (C_6) to tetracosane (C_{24}). Shorter alkanes may interfere with the solvent peak in the GC chromatographic separation due to extractions from water and dilution of the fuels performed using DCM. Cyclohexane and n-hexane were challenging to evaluate due to the small amounts of impurities in the DCM used for dilution and extraction.

6.3 Odour threshold test

Tests for determining odour thresholds were performed following the SS-EN 1622:2006 European standard, using the paired test (10.2.2) from the full method (10.3.2). The threshold odour number (TON) used in the standard and this report is defined as the dilution ratio beyond which the sample has no perceptible odour.

Test panels for odour assessment were assembled by the four drinking water producers (DWP) participating in the project, which consisted of 3-11 personnel from production, laboratory, and management. In short, every panel member was given a dilution series with dilutions of the WAF. Each dilution series consisted of five different dilutions of the WAF. Each panel member noted for which bottle (or for none) an odour could be sensed, compared to reference water.

The assessments were performed on two separate occasions, with the DWPs evaluating the fuel types according to Table 4.

Table 4. Fuel types that were sent to each DWP. First round (X). Second round (Y). Third round (Z).

Fuel	DWP 1	DWP 2	DWP 3	DWP 4
HVO1	(Y)		X	X (Y)
D-SE1	X (Y)		(Y)	X
D-SE2		X (Y)		X (Y)
D-SE3		X (Y)	(Y)	X
E85-1	X (Y)		X (Y)	
P98-1	X	(Y)	X	(Y)
P95-1	X (Y)	X (Y)		
P95-2		X	X (Y)	(Y)
P98-2			Z	Z
MGO-2		Z	Z	
D-EU1	Z	Z		
D-EU2	Z			Z

The different dilution series were prepared at IVL and sent to the DWP. Per SS-EN 1622:2006, the dilutions prepared followed a geometric series. Dilution factors were set to 2 in the first and 2.5 in the second and third rounds. A factor of 2.5 in the second round was chosen to increase the difference in concentration between two successive dilutions.

Not all results from the odour tests could be used. In most cases, at least one of the panel members noticed a deviating smell in the weakest dilution or did not notice any odour throughout the whole dilution series. In those cases, the definition of odour threshold in SS-EN 1622:2006 could not be applied since it requires the geometric mean of the results from all panel members. Instead, the odour threshold was defined as the dilution at which 50% of the panel members noticed an odour, which sometimes is a more practical indicator used in odour threshold determinations (Alberta Health, 2020; DEPA, 2002; Department of Environmental Protection, West Australia, 2002). This definition meant that more data could be used.

Regarding the method used, it cannot be ruled out that there is some bias in the odour test results since the odour testing procedure could not be entirely randomised regarding smelling reference water and samples. The panel members knew which bottle was the reference (randomisation would put demands on the procedure that could not be practically met). If there is such a bias, it would most likely mean that a panel noticed the odour too early.

6.3.1 Statistics

Data on odour thresholds and concentrations in WAF were analysed using a linear mixed model. The odour threshold number (TON) was the response variable. WAF concentrations were explanatory variables and fuel type was a random effect. Using fuel type as a random effect compensates for the fact that observations from the same fuel type are correlated and that fuel types with more than one observation are not dominant in the model estimation. In mixed-effects terminology, explanatory variables are commonly referred to as fixed effects.

The dataset has more variables (chemicals) than observations (results from odour tests). Since linear models can only be fitted when the number of observations is larger than the number of variables, choosing a model that included many chemical compounds as explanatory variables was impossible. Thus, a smaller selection of chemical compounds had to be made. For this purpose, correlations between each chemical and the odour threshold and each chemical with every other chemical were investigated. Furthermore, the variable selection technique Lasso regression (Least Absolute Shrinkage and Selection Operator) was used. Lasso regression is an automated method used for variable selection. It reduces the coefficient estimates of non-important variables to zero. Results from the Lasso regression were used as a guide for constructing the linear predictor in the mixed model. In all statistical models, the upper bound concentration was used if the concentration was below the detection limit of the chemical analysis.

6.4 Ecotoxicity to freshwater organisms

6.4.1 Preparation of test solution

The ecotoxicity was evaluated for seven fuels. Water/fuel solutions (WAF) were prepared for these tests, separate from those used for odour threshold tests. 100 mg fuel was used per litre of growth medium. The mixture was stirred overnight using a magnetic stirrer at sufficient speed to create a vortex on the surface, after which the solution was allowed to settle for 4 hours.

6.4.2 Alga

Growth-inhibiting effects on alga were evaluated using the OECD 201 test.

The test organism was a laboratory culture of the green alga *Pseudokirchneriella subcapitata*. A qualification test using potassium dichromate ($K_2Cr_2O_7$) was performed on 1 July 2021 to ensure the test organism's quality. E_rC_{50} (the concentration where reproduction for 50 % of the test organisms is affected) was between 0.9 and 1.5 mg/L, confirming that the culture was suitable as a test organism according to OECD TG No. 201. The growth medium composition during the test was as specified in Appendix 3 in OECD TG No. 201.

A new extraction of the test concentration (loading rate) was prepared as described in OECD No. 23. The test item was weighed using a small beaker and then rinsed into a glass bottle with 2000 mL of growth medium with minimal headspace and closed using parafoil. The test concentration was stirred overnight at a speed that created a small vortex at the surface. After settling for 4 hours, the water phase was collected from the bottle and subsequently transferred to test glass vessels. The process was performed in darkness at room temperature (20-22 °C).

The highest test concentration (loading rate) was 100 mg/L, and the subsequent concentrations were made by dilution.

The nutrient concentration in the growth medium was the same in the control and the test solution. Six replicates were used for the control, while the test solutions were tested in triplicates. Test bottles were 250 ml glass E-flasks with 100 ml solution, incubated on a shaking table with approximately 100 µE m-2 s-1 continuous illumination at a temperature of 21-24±2 °C. The pH in all solutions was measured at the start of the test and again in all replicates after 72 hours. The incubation period was 72 hours, with sampling at 24. 48. and 72 hours. The biomass in the samples was measured by counting the number of cells/mL using an optical microscope.

At the start of the test, approximately 100 mL of the highest test concentrations of the test items and the control, without substrate, were fixated with sulphuric acid (H_2SO_4) for future reference analysis regarding total organic carbon (TOC) content. Another E-flask containing the highest test concentration of the test items and the control was incubated on the same shaking table as the control and the test solution. These flasks were not inoculated with algae but were used for reference analysis, showing the test item’s integrity after 72 hours.

Reference analysis was made to confirm that the test concentration was stable during the test. Samples from the highest test concentrations and the control (growth medium) were collected at 0 h and 72 h. fixated with sulphuric acid and stored at +4°C until analysis regarding the TOC content.

Cell count is one of the proposed surrogate parameters for biomass according to the OECD TG No. 201. The average specific growth rate (µ) for a particular period is calculated as the logarithmic increase in the biomass from the equation for every single vessel of the control and test concentrations:

$$\mu = \frac{\ln cells/mL_{72h} - \ln cells/mL_{0h}}{3\ (time\ in\ days)}$$

An average growth rate and variance estimates are calculated for the control and test concentrations. The specific growth rate for each test concentration is then related to the average growth rate of the control:

$$\%\ I_r = \frac{\mu_c - \mu_r}{\mu_c \times 100}$$

where:

$\% I_r$ = per cent inhibition in average specific growth rate;
μ_c = mean value for average specific growth rate (μ) in the control group;
μ_r = average specific growth rate (μ) for the test concentration replicate.

The No Observed Effect Concentration (NOEC), the highest concentration with no statistical difference from the control ($p > 0.05$), was determined using an unpaired t-test.

6.4.3 Bacteria

Toxicity to bacteria was evaluated using the standard ISO 11348. Microtox.

The method shows the inhibition of the luminescence emitted by the marine bacterium *Vibrio fischeri* (NRRL B-11177).

6.4.4 Crustaceans

Crustacean reproduction tests were performed according to the standard EPA/600/4-89/001.

Daphnia magna was kept in 1.5 L glass beakers. Cultivation was performed in stabilised water (aquarium water), partially renewed every week. The animals used in the experiment were single-grown in the “EPA moderately hard” medium. According to the standard, the animals were fed with 0.25 ml YTC (Yeast, Trout and Cerophyll feeding stuff) once a day. The cultivation occurred in subdued lighting with a light period of 16 hours per day. The temperature was 25.0 ± 0.5 °C during both cultivation and trials. The test animals had a maximum age difference of 16 h at the start, plucked from 30 single-grown mother animals.

The test was conducted in 5 sample concentrations, and blank samples were placed in a dilution series. Each concentration contained 10 animals and double-blank samples. The 50 ml styrene beakers consisted of 15 ml of solution and one animal in each beaker. New solutions were prepared thrice weekly, while a new stock solution was prepared weekly. Beakers were tempered for about 6 hours before the crayfish were moved. The test animals were added across the dilution series, meaning sibling fry was spread to a maximum between the experimental concentrations. The test material was stored at +1 °C until the start of the trial. pH did not require any adjustment since it was stable (7.1) during exposure. Oxygen saturation was within the limits of the standard in all concentrations.

The experiment was aborted after 17 days when 80% of the animals in the control released their third fry litter. According to the standard, more than 60% of the animals in the blank must release their third litter before the experiment is considered complete. The mortality rate in the blank was 0% (must not exceed 20%). The average frying in the blank was acceptable, with 16.4 cubs. The method accepts average frying of 15 (C *double*) cubs in blank samples.

The statistical calculation of toxicity was performed using a computer-based calculation program developed by the USEPA for this type of test called BOOTSTRAP. The results have been calculated using the statistical measures EC_{10} and EC_{50} (Effect Concentration. where EC_{50} equals halved frying compared to the blank) and a 95% confidence interval.

6.4.5 Degradation

Degradation tests were performed following the standard SSEN-ISO 7827.

By inoculating WAF samples with wastewater sludge, degradation of fuel constituents was estimated from the change in dissolved organic carbon (DOC) content during a 28-day degradation period.

A test volume of 1 000 ml in 2 L flasks was used for six different samples: test material (FT1 o FT2) as a duplicate, blank (FB), control (FC), inhibition control (FI) and abiotic control (FS) in a single set. The glass flasks were stirred with magnets throughout the test period. DOC was measured on day 0. 7. 14. 21 and 28.

6.4.6 Respiration test

Following ISO 8192, the test was executed to evaluate the inhibitory effects of WAF samples on the respiration rate of activated sludge microorganisms. The procedure began by collecting activated sludge from a municipal wastewater treatment plant. The sludge was then concentrated by sedimentation and centrifugation.

The WAF was used in 0.5, 5.0 and 50% dilutions. Oxygen consumption by the sludge was continuously monitored using a respirometer to measure the microbial respiration rate. The test duration was set as per the standard’s guidelines.

6.5 Aeration test

The procedure commenced with preparing water-accommodated fractions (WAF) mixtures utilising three distinct fuel types: P98, P98 with ethanol addition, and E85, which represent ethanol concentrations of 5%, 45%, and 85%, respectively. Three (3) replicate samples were prepared for each ethanol concentration, culminating in nine (9) WAF mixtures.

A water bath, shown in Figure 20, regulated the sample temperature after the WAF preparation. The water bath temperature was adjusted to one of the desired set points: 5°C, 10°C, 18.5°C or 20°C, corresponding to the experimental requirements. The prepared WAF mixture was then dispensed into a beaker. Subsequently, the beaker containing the WAF mixture was submerged in the temperature-controlled water bath.

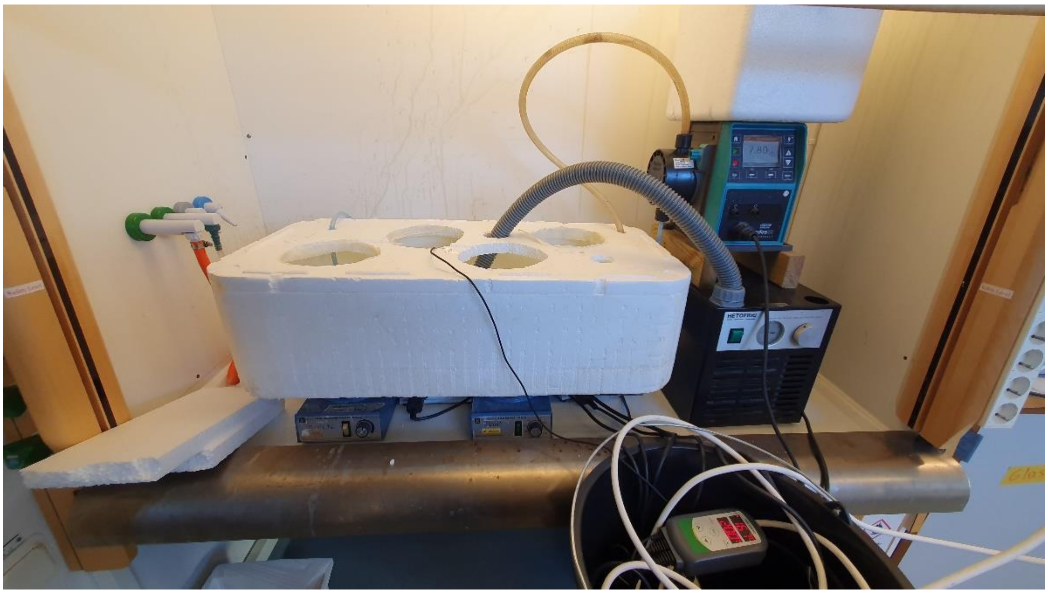

Figure 20. The water bath which has a cooling apparatus controlled by a temperature sensor.

A magnetic stirrer was placed into the beaker with the WAF mixture to facilitate homogenisation in the next phase. The beaker was then sealed with aluminium foil to prevent evaporation, with the magnetic stirrer activated at a speed sufficient to induce a vortex in the liquid without incorporating air into the mixture. A 20-minute equilibration period was allowed to ensure a uniform temperature distribution and constituents within the mixture. Upon the completion of the equilibration period, the aluminium foil was removed. The temperature within the beaker was then measured to confirm the maintenance of the desired temperature setting.

The initial sample, the 0-sample, was collected immediately after the equilibration phase. Sampling was conducted at a depth of approximately 3 cm below the surface and 3 cm from the edge of the beaker to avoid wall effects and ensure a representative sample. Following the initial sample, the beaker was left uncovered, and additional samples were extracted at specific time intervals: 15, 30, 60, 120, 240, 1200, and 1440 minutes. This approach aimed to capture the dynamic changes in the WAF mixture over time and provide a comprehensive profile of the mixture’s constituents under controlled experimental conditions.

6.5.1 Statistics

The design of the aeration test is a so-called full factorial design (Montgomery, 2021) in which the test is replicated for every combination of the variables in the study. Here, three fuels were tested at X temperatures and Y time points. Hence, 3*X*Y=Z samples were collected and analysed for chemical composition.

The resulting data was then analysed statistically by the following linear regression model:

$$\ln(C) = \beta_0 + \beta_1 Time + \beta_2 Temp + \beta_3 Ethanol + \beta_4 Time * Temp + \beta_5 Time * Ethanol + \beta_6 Temp * Ethanol + \beta_7 Temp * Ethanol * Time + Remainder$$

where $\ln(C)$ is the natural logarithm of the concentration of the sum of ether in the water. The natural logarithm was applied since the concentration decline is expected to be exponential with time. The logarithm will then linearise data, making it suitable to model with a linear model. Also, the parameter estimates are then interpreted as percentage changes in C (ether concentration?) for a one unit increase in either *Time*, *Temp* or *Ethanol*, which is convenient.

All interactions were included, represented by the variables β_4, β_5, β_6 and β_7. The motivation for including interactions is that the relation between C and one variable can be moderated based on the magnitude of another variable.

The estimated model then gives an equation for predicting the concentration of a compound in a WAF based on the elapsed time since the aeration began, the water temperature and the ethanol concentration of the fuel used.

7 Literature study

7.1 Fuels on the Swedish market

Recent data from the Drivkraft Sverige/Infostat (web, 2023-11-21) show the volumes of fuel in Sweden delivered from the fuel companies to service stations (Figure 21). Diesel has emerged as the predominant fuel in terms of delivery since 2010. No publicly available information has been found on the proportions of 98-octane and 95-octane petrol volumes.

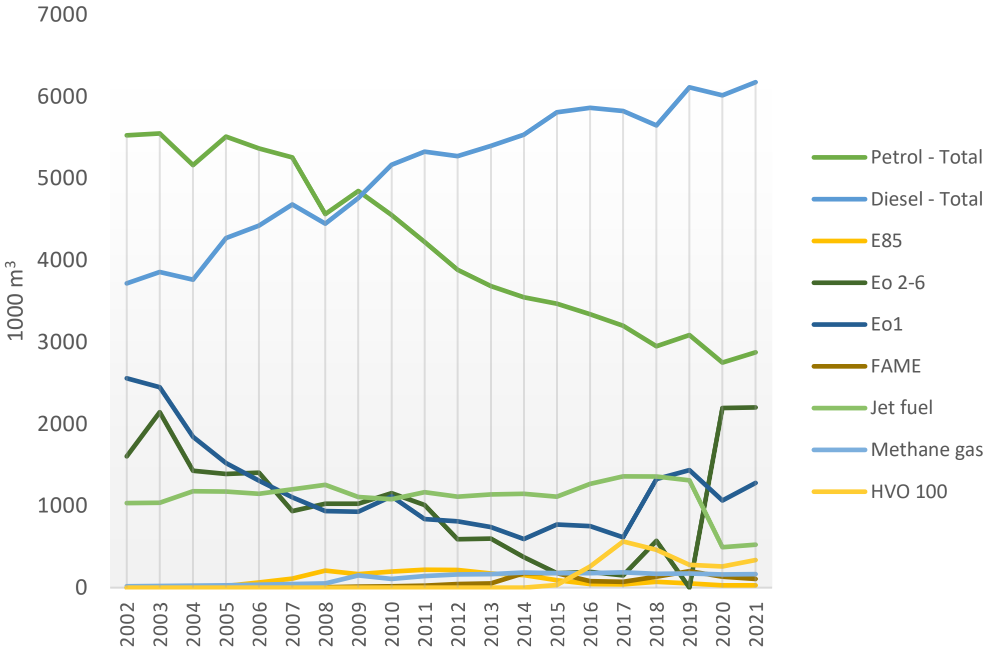

Figure 21. Volumes of different fuels, sold on the Swedish market. From Drivkraft Sverige/Infostat.

7.1.1 Regulated substances in Swedish fuels

The Swedish Fuel Act (SFS 2011:319) has regulated some substances in fuels since 2011, as shown in Table 5.

Table 5. Limits of components in Environment Class 1 diesel and petrol fuels, according to the Swedish Fuel Act (SFS 2011:319).

Substance	MK1 petrol (vol %)	MK1 diesel (vol %)
Olefines, max	13.0	Not regulated
Aromatic hydrocarbons, max	35.0	5
Benzene, max	1.0	Not regulated
Cyclohexane, max	-	Not regulated
n-hexane, max	-	Not regulated
Ethers with more than 5 carbons (MTBE, ETBE), max	22.0	Not regulated
Fatty acid methyl esters, max	Not regulated	7.0
Polycyclic aromatic hydrocarbons, max	Not regulated	Not detectable

7.1.2 Fuel Standards Across Europe

The focus of European standards for fuels is to ensure compatibility across the continent. Individual countries can make minor adjustments to these standards to align with national legislation. These standards are subject to updates approximately every five years, though this frequency may vary based on emerging needs. However, it is consistently observed that these standards do not emphasise environmental considerations; instead, the primary goal is to produce effective and safe fuels for engines. Environmental regulations are left to the discretion of individual countries and legislative bodies.

7.1.3 Diesel fuel

European diesel fuel adheres to the EN590 standard, which sets various requirements, including a cap on poly-aromatic hydrocarbons (PAHs), limiting them to 7% of the fuel. The diesel fuel specification in Sweden is even more stringent for MK1 diesel, following the EN590 standard and the Swedish standard SS155435. This Swedish standard enforces stricter limits on aromatics content, with a maximum of 5% allowed for total aromatic hydrocarbons and a specific exclusion of PAHs to address environmental and health concerns, as seen in Table 5.

Additionally, the performance of diesel in cold weather is a paramount consideration. This is assessed by measuring the Cold Filter Plugging Point (CFPP), the temperature at which the fuel waxes to the extent that it can block a standardised filter, and the Cloud Point, which is the temperature at which wax crystals start to form at the bottom of the container. These characteristics ensure that diesel vehicles can operate effectively in colder climates without fuel-related issues. The CFPP standards in Sweden are adapted to seasonal and regional climatic variations to ensure reliability and functionality.

7.1.4 Petrol

The European petrol standard EN228 is also supplemented in Sweden with additional requirements for MK1 petrol, as per SFS 2011:319, §4. These requirements include reducing permissible olefin content from 18% to 13% and heightened mandates for additives. An oxygen content of 2.7% limits the maximum allowable content of MTBE/ETBE. This restriction means that with a 10% ethanol blend, there is less room to add MTBE/ETBE than lower ethanol blends, such as the 5% found in 98-octane fuels.

It is noteworthy that MTBE (Methyl Tertiary-Butyl Ether) was phased out in Japan in 2001 and the USA and Canada in 2006 due to concerns over water contamination risks. Despite these concerns, MTBE is still utilised in Europe. Conversely, ETBE (Ethyl Tertiary-Butyl Ether) is primarily used in Europe and Japan and is driven by legislative biofuel mandates. ETBE is favoured in regions with stringent biofuel regulations because it is derived from ethanol. This renewable resource helps meet the sustainability criteria outlined in such policies.

7.1.5 Ethanol fuel E85

European E85 complies with the standard EN15293, which specifies a list of potential denaturing agents known not to affect engines adversely. The decision on approved denaturing agents is left to the individual countries. According to regulation HSLF-FS 2022:63, fuel containing more than 70% m/m alcohol must be denatured with either 25g/kg MTBE + 5g/kg isobutanol or 25g/kg ETBE + 5g/kg isobutanol. The octane numbers RON > 104 and MON > 88 are attainable with correct blending. However, these levels are generally considered indicative, as they might be beyond the measurement capability of standard methods.

7.1.6 Hydraulic oils

Hydraulic oil is essential in a hydraulic system to transfer energy, as well as lubrication, antiwear, cleaning and rust prevention of components. The physicochemical properties – including oxidation stability, incompressibility and anti-foaming properties, shear stability, seal adaptability, anti-emulsification and hydrolysis stability, anti-rust property and filterability - are all critical parameters in the oil's performance characteristics(1). These properties are obtained through additives to the base oil type.

According to the base oil type, hydraulic oils can be distinguished into mineral, synthetic and biological-based (bio-based):

- Mineral oils consist of a base oil often obtained from the distillation of crude petroleum oil.
- Synthetic oils are based on synthetic esters produced through chemical reactions between alcohol and fatty acid, or polyalphaolefins (PAO) and polyalkylene glycols (PAG).
- Bio-based oils are mostly synthetic with fatty acids based on vegetable oils of plant and, to a small extent, animal origin(2).

Hydraulic oils used in Sweden must always meet the Swedish standard SS-155434's environmental requirements, stipulating that hydraulic oil must be biodegradable and minimise water toxicity. RISE Research Institutes of Sweden has published the "SP list" since 1998, including hydraulic fluids that meet the environmental requirements according to Swedish standards. This list is updated at most every calendar month[1].

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[1] https://www.ri.se/en/what-we-do/services/hydraulic-fluids-which-meet-environmental-requirements-in-ss-15-54-34

7.2 Potential online sensors

Standard monitoring programs for oil detection - i.e., the concentration of dispersed and dissolved oil constituents – require laboratory techniques, e.g., infrared absorption, gravimetric techniques, gas chromatography and fluorometry. Coupled with laboratory data, sensors with high time resolution allow for fast detection and monitoring of oil spills. *In situ* sensors use one of the following techniques: ultraviolet (UV), focused ultrasonic acoustics, fibre optic sensing, image analysis, light scattering and photoacoustic. UV fluorescence is one of the most common field measurement techniques, and polyaromatic hydrocarbons (PAH:s) are key in oil detection using *in situ* fluorometric techniques.

7.2.1 Principle technologies

Table 6 lists the suitability and the limit of detection (LOD) ranges of commercially available *in situ* sensors based on UV-visible and fluorescence spectroscopy to measure BTEX, ethers, PAH:s, and TOC. TOC is a non-specific but inclusive parameter for total organic carbon monitoring, useful in detecting the presence of many organic contaminants, including petroleum products (Assman et al., 2017).

Table 6. Suitability and limit of detection (LOD) range of some commercially available in situ sensors based on UV-visible and fluorescence spectroscopic techniques to measure benzene, toluene, ethylbenzene and xylene (BTEX), ethers, polyaromatic hydrocarbons (PAHs) and total organic carbon (TOC).

Technique	BTEX	ethers	PAHs	TOC
UV-visible spectroscopy	active LOD: 0.05 mg/L benzene in drinking water (100 mm path length) (Broeke et al., 2005)	inactive	active	active LOD: 0.3 - 1 mg/L (10 mm path length)
Fluorescence spectroscopy	active LOD: 10 µg/L	inactive	active LOD: 0.1 -0.5 µg/L	active (Carstea et al., 2020)
Ion Mobility Spectrometer		active LOD: 10-100 mg/L (Borsdorf H. et al., 2005; de Lacy Costello B.P.J., et al., 2005)		

7.2.2 Background NOM concentrations in Swedish surface waters

Figure 22 shows box and whisker plots of TOC concentration data from the Swedish environmental monitoring retrieved from the Miljödata-MVM database as examples of TOC background values in Swedish surface water sources (SLU, 2023). Data were collected since 1996 from different monitoring stations of Lake Mälaren, the third largest lake in Sweden.

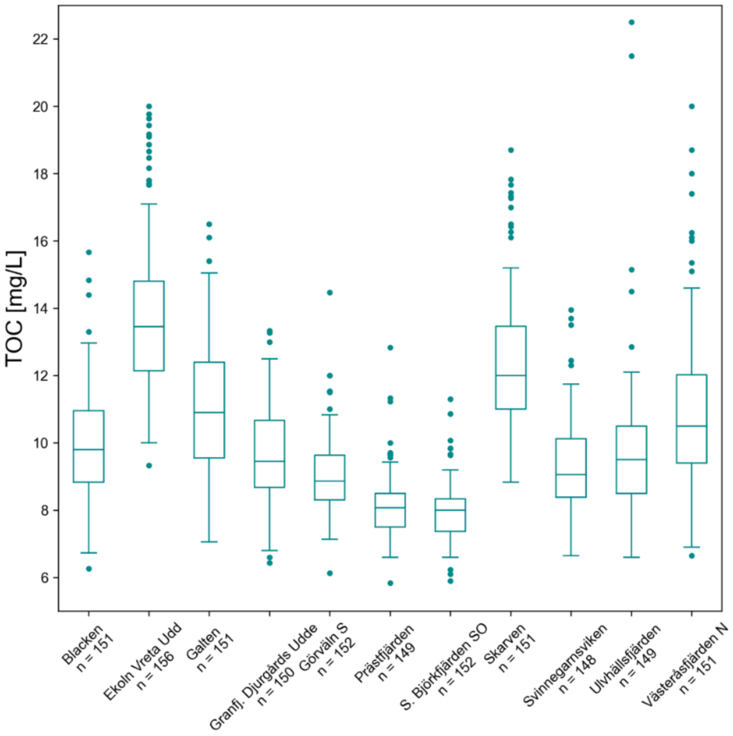

Figure 22. Box and whisker plot of concentrations (mg/L) of n total organic carbon (TOC) data from the Swedish environmental monitoring retrieved from the Miljödata-MVM database. The values are average concentrations of field replicates, if available.

7.2.3 Applicability of sensors for oil detection

This study has shown that ether is the most important substance to monitor from a drinking water perspective. MTBE is, however, invisible in UV/Vis and fluorescence spectroscopy (Broeke 2005). TOC analysers cannot distinguish between natural organics (e.g., algae, decomposed plant matter) and hydrocarbons from fuels. Therefore, the odour threshold of 4 µg/L of ether will be difficult to detect given the natural background levels of TOC in surface waters. Sensors detecting PAHs might be useful, given that there are PAHs in the fuel, which is not valid for MK1 diesel. Ion mobility spectrometry (IMS) can detect MTBE, with reported sensitivities from low mg/L to 100 mg/L (Borsdorf H. et al., 2005; de Lacy Costello B.P.J., et al., 2005). Given the odour threshold found by this project of 1.4-4 µg/L for ether in a WAF, the reported sensitivities are insufficient.

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Appendix 1 - Implementation of experimental results on a spill

Introduction

This section outlines a straightforward model and an accompanying Microsoft Excel tool, both developed using data from this project. The model explicitly addresses a query relevant to drinking water producers:

"At what distance must a petrol spill containing ether occur to prevent its taste or smell from being detectable at my raw water intake?"

Specifically designed to evaluate the risk of fuel spills near raw water intakes, the model integrates factors such as horizontal advection, water velocity, and spreading angle and data on ether's evaporation into the atmosphere. A vital feature of the model is incorporating a critical odour threshold for ether, determined to be 1-4 µg/l through odour tests. This threshold is essential for assessing when a spill's dispersion plume will reach a raw water intake and the distance required for the ether concentration to fall below this level. This appendix details the model's structure, the data it relies on, and preliminary results from two different scenarios.

Method description

The model is based on calculations and experiments that quantify the proportion of ether that dissolves in water at 20°C (see section 5.2). We assume a fuel spill of X m^3 (Vol_{Fuel}) over a circular water surface with an initial radius (r_0). The user can select three fuels: E85, Petrol 95 Octance and Petrol 98 Octane. From this selection, the concentration of ether in the fuel (C_{Ether}) in g/L, water accommodated fraction (WAF, WAF_{Ether}) of ether in µg/L and percentage ethanol ($Vol\%_{Ethanol}$) is obtained.

The model generously assumes that all WAF available ether is mixed into the water down to the depth of the raw water intake (Z). The ether is diluted in the initial amount of water, giving an ether starting concentration (c_0) in aqueous solution in µg/L (Eq. 1).

$$c_0 = \frac{Vol_{Fuel} \times c_{ether} \times 1000 \times Sol_{ether}}{\pi \times Z \times {r_0}^2} \qquad \textit{Equation 1}$$

Where:
Sol_{ether} is the ether percentage in an aqueous solution (c_{ether}/ WAF_{ether}).

The expansion of the plume is calculated in an iterative procedure, given a time step (dt) that the user defines. A conceptual model is shown in Figure 1 and Figure 2 below.

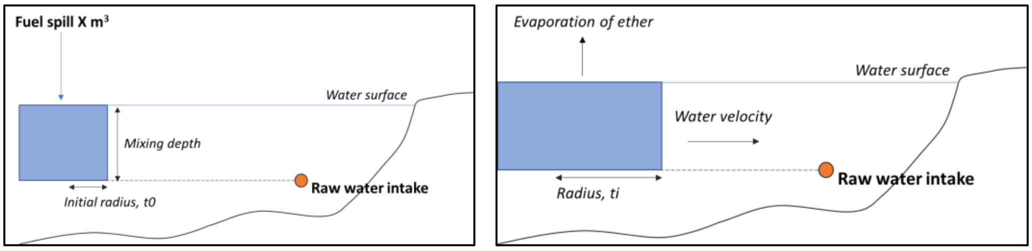


Figure 1 Conceptual model. To the left: fuel spill in an initial radius at time t0. The ether in the fuel is assumed to mix in the water column corresponding to a water depth of the raw water intake (worst case). To the right: the plume is expanding in the water velocity direction. The radius is increasing in each time step. A part of the ether in the water evaporates in each time step.

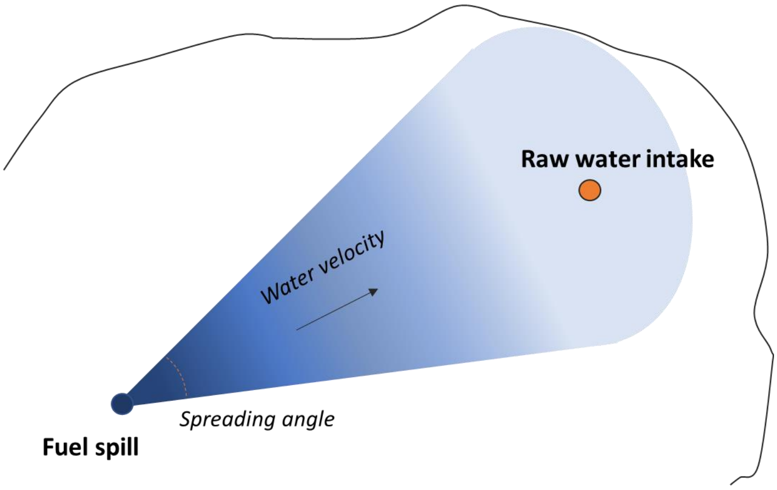


Figure 2 Conceptual model. The plume, viewed from above. The concentration of ether is decreasing due to being diluted in a larger volume of water, and a part of the ether evaporates in each time-step.

To estimate the spreading rate of the plume, assumptions about surface water velocity are required. It is important to note that our model focuses on horizontal advection and only considers fuel transport. The user can select the water velocity (v) in the direction of the raw water intake and specify a spreading angle (θ) for the plume. This angle affects how fast ether is diluted in the plume - a narrower angle results in higher concentrations at longer distances and vice versa. The water volume in each time step (i) is calculated by Eq. 2.

$$V_i = \left(V_i - V_i \times \frac{\theta}{360}\right) + Z \times \pi \times {r_i}^2 \times \frac{\theta}{360} \qquad \textit{Equation 2}$$

The concentration of ether in aqueous solution in time step i (c_i) in µg/L is then given by Eq. 3.

$$c_i = m_{ether,i} \times V_i \times 1000 \qquad \textit{Equation 3}$$

Where:
$m_{ether,\,i}$ is the mass of ether in the aqueous solution in each time step.

In addition, the model considers ether evaporation to the atmosphere in each time step, which is dependent on time, temperature (T), and ethanol concentration in the water at each time step ($c_{ethanol}$). The evaporation rate is based on data collected from the evaporation tests carried out within the framework of the project, where we analysed the ether evaporation over time in relation to temperature and ethanol concentration. The ether evaporation is described in chapter **5.5** of the report.

Thus, ether concentration is corrected by subtracting the evaporated ether (g/dt) from M_{Ether} in each time step to account for evaporation.

The odour limit of ether is assumed to be 4 µg/L, which is used as a threshold value in the model (note that the user is free to assign any number as a threshold value in the model). The model generates results in terms of:

- The time it takes for the plume to reach the odour threshold value.
- The radius at which an ether concentration is reached below the odour threshold value.

Results

Two hypothetical cases of spills are shown here to demonstrate the model. The underlying assumptions are shown in Table 1. Each spill is modelled for two different temperatures differing regarding the water velocity.

Table 1. Assumptions were made for the two cases, the results of which are displayed in subsequent figures.

Parameter	Case 1	Case 2
Spill volume	14 m^3	14 m^3
Fuel type	98-octane petrol	98-octane petrol
Initial radius, r_0	15 m	15 m
Depth to raw water intake	20 m	20 m
Water velocity	0.5 cm/s	2 cm/s
Spreading angle	45°	45°
Water temperature	20°C	20°C
Evaporation	With/without	With/without

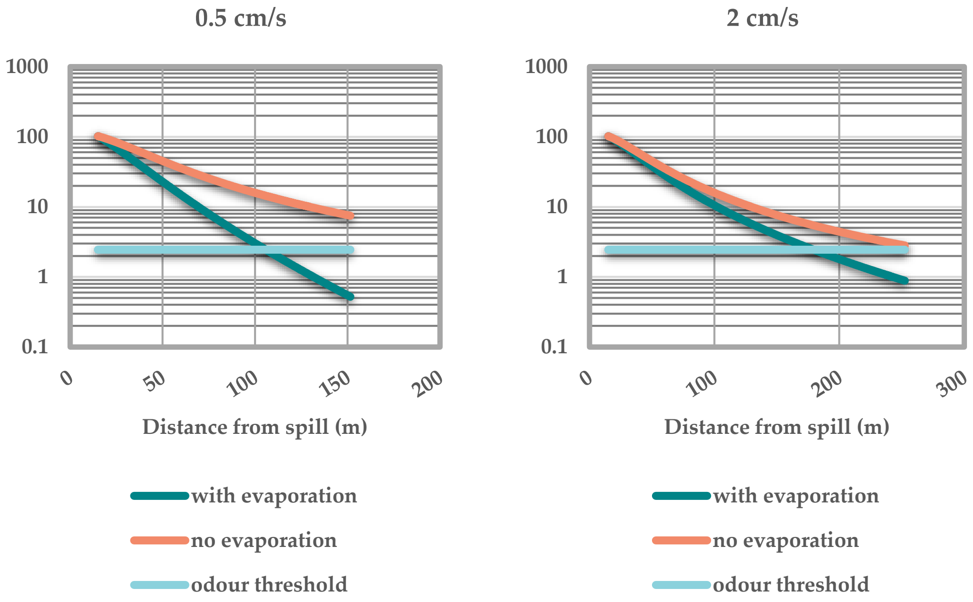


Figure 3 Concentration of ether as a function of the distance from the spill in meters.

A key figure for the model is the distance at which the concentration is below the odour threshold. Case 1 (with evaporation) corresponds to a radius of about 250 m in 5°C water and 110 m in 20°C water. For case 2 (with evaporation), where water velocity was increased, water temperature was less influential. The ether concentration was below the odour threshold at 180 and 260 m, respectively, in 20 and 5°C water.

Simplifications and assumptions

Water dispersion models are vital in studying how contaminants move and settle in aquatic environments. Typically, these models integrate various factors to accurately simulate substance dispersion in water bodies. Key factors include:

- Turbulence and vertical mixing
- Sedimentation and upwelling
- Thermocline and halocline effects
- Large-scale circulation

However, our model simplifies this approach by focusing mainly on horizontal advection—the movement of water in a horizontal direction—and the volatilisation or evaporation of substances. This simplification allows us to address scenarios where horizontal movement is the predominant factor in transporting contaminants within the water body.

While this streamlined approach is practical in specific contexts, it’s essential to understand its limitations. Our model does not account for vertical movements such as upwelling or sedimentation, nor does it consider vertical diffusion. Therefore, its applicability is best suited to situations where horizontal advection is the key driver of dispersion, and other factors like vertical processes and large-scale circulation have minimal influence on the movement of pollutants.

Appendix 2 – Chemical data

Table A1. List of chemicals included in the GC/MS analysis and their characteristic ions

Chemical name	**Quantifier; Qualifier ions**	**SPIMFAB classification**
MTBE	73.1; 57.1	ethers
1-hexene	56.1; 84.1	aliphates >C5-C8
n-hexane (C6H14)	57.1; 86	aliphates >C5-C8
Ethyl tert-butyl ether (ETBE)	59.1; 87.1	ethers
Benzene	78.1; 77.1	BTEX
Cyclohexane	56.1; 69.1. 84.1	aliphates >C5-C8
tert-Amyl methyl ether (TAME)	73.1; 87.1	ethers
1-heptene	56.1; 98.1	aliphates >C5-C8
Heptane	71.1; 100.1	aliphates >C5-C8
Methylcyclohexane	83.1; 98.1	aliphates >C5-C8
Toluene	91.1; 92.1	BTEX
1-octene	83.1; 112.1	aliphates >C5-C8
Octane	85.1; 114.1	aliphates >C5-C8
Ethylcyclohexane	83.1; 112.1	aliphates >C5-C8
Ethylbenzene	91.1; 106.1	BTEX
1-nonene	69.1; 126.1	aliphates >C8-C10
Nonane	85.1; 128.1	aliphates >C8-C10
Propylcyclohexane	83.1; 126.1	aliphates >C8-C10
Propylbenzene	91.1; 120.1	aromates >C8-C10
1-decene	70.1; 140.1	aliphates >C8-C10
Decane	71.1; 142.2	aliphates >C8-C10
Butylcyclohexane	83.1; 140.1	aliphates >C8-C10
Butylbenzene	91.1; 134.1	aromates >C8-C10
1-undecene	70.1; 126.1	aliphates >C10-C12
Pentylcyclohexane	83.1; 82.1. 154.1	aliphates >C10-C12
Pentylbenzene	91.1; 148.1	aromates >C10-C16
1-dodecene	69.1; 83.1. 168.1	aliphates >C10-C12

Chemical name	**Quantifier; Qualifier ions**	**SPIMFAB classification**
2,3-dimethylpentane	56.1; 71.1. 100.1	aliphates >C8-C10
p-Xylene	91.1; 106.1	BTEX
Cumene	105.1; 120.1	aromates >C8-C10
Trimethylbenzene	105.1	aromates >C8-C10
3-Ethyltoluene	120.1	aromates >C8-C10
Isobutylbenzene	91.1; 134.1	aromates >C8-C10
Decaline	138.1; 96.1	aliphates >C10-C12
1,2,4,5-tetramethylbenzene	119.1; 134.1	aromates >C10-C16
Benzene-d_6	84.1	BTEX
Toluene-d_8	98.1; 100.1	BTEX
Octane (C_8H_{18})	85.1; 114.1	aliphates >C5-C8
Nonane (C_9H_{20})	85.1; 128.1	aliphates >C8-C10
Decane ($C_{10}H_{22}$)	71.1; 142.2	aliphates >C8-C10
Undecane ($C_{11}H_{24}$)	71.1; 156.2	aliphates >C10-C12
Dodecane ($C_{12}H_{26}$)	57.1; 71.1. 170.2	aliphates >C10-C12
Tridecane ($C_{13}H_{28}$)	57.1; 71.1. 184.2	aliphates >C12-C16
Tetradecane ($C_{14}H_{30}$)	57.1; 71.1. 198.2	aliphates >C12-C16
Pentadecane ($C_{15}H_{32}$)	57.1; 71.1. 212.2	aliphates >C12-C16
Hexadecane ($C_{16}H_{34}$)	57.1; 71.1. 226.3	aliphates >C12-C16
Hexadecane-d_{34} ($C_{16}D_{34}$)	66.1; 50.1	aliphates >C12-C16
Heptadecane ($C_{17}H_{36}$)	57.1; 71.1. 240.3	aliphates >C16-C35
Octadecane ($C_{18}H_{38}$)	57.1; 71.1. 254.3	aliphates >C16-C35
Nonadecane ($C_{19}H_{40}$)	57.1; 71.1. 268.4	aliphates >C16-C35
Eicosan ($C_{20}H_{42}$)	57.1; 71.1. 282.3	aliphates >C16-C35
Oleic acid. methyl ester	55.1; 74. 264.3	ester
Heneicosane ($C_{21}H_{44}$)	57.1; 71.1. 296.3	aliphates >C16-C35
Docosane ($C_{22}H_{46}$)	57.1; 71.1. 85.1	aliphates >C16-C35
Tricosane ($C_{23}H_{48}$)	57.1; 71.1. 85.1	aliphates >C16-C35
Tetracosane ($C_{24}H_{50}$)	57.1; 71.1. 338.4	aliphates >C16-C35

PAH substance	Quantifier; Qualifier ions	Class
Naphthalene		PAH-L
Acenaphthene		PAH-L
Acenaphthylene		PAH-L
Fluorene		PAH-M
Phenanthrene		PAH-M
Anthracene		PAH-M
Fluoranthene		PAH-M
Pyrene		PAH-M
Benzo(a)anthracene		PAH-H
Chrysene		PAH-H
Benzo(b)fluoranthene		PAH-H
Benzo(k)fluoranthene		PAH-H
Benzo(a)pyrene		PAH-H
Dibenzo(a,h)anthracene		PAH-H
Benzo(ghi)perylene		PAH-H
Indeno(1,2,3-cd)pyrene		PAH-H
Benzo(j)fluoranthene		PAH-H

Table A2. Concentrations (g/L) of substances in fuel samples.

(g/L)	E85-2	E85-3	E85-4	D-SE4	D-SE5	D-SE6	D-SE7	HVO2	HVO3	HVO4	MGO-1	MGO-2	D-EU1	D-EU2	EO1-1	EO1-2
MTBE	7.12	8.59	7.66	0.20	0.20	0.22	0.19	0.17	0.18	0.20	0.15	0.17	0.05	0.05	0.03	0.03
Ethyl tert-butyl ether (ETBE)	0.01	0.01	0.00	<0.0001	<0.0001	<0.0001	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.01	0.02	0.00	0.00
tert-Amyl methyl ether (TAME)	0.00	<0.0001	<0.0001	<0.0001	0.00	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00
Benzene	1.43	1.33	0.91	0.01	0.01	0.01	0.01	0.00	<0.001	0.00	0.04	0.07	0.05	0.03	0.01	0.01
Toluene	9.59	12.07	7.88	0.06	0.08	0.06	0.12	0.01	0.00	0.04	0.26	0.40	0.59	0.31	0.11	0.09
Ethylbenzene	2.47	3.06	1.87	0.04	0.05	0.05	0.04	0.00	0.00	0.01	0.22	0.23	0.44	0.14	0.26	0.14
p-Xylene	6.28	7.97	4.80	0.12	0.14	0.16	0.13	0.01	0.00	0.03	0.65	0.65	0.39	0.16	0.16	0.10
Propylbenzene	0.83	0.90	0.57	0.04	0.05	0.07	0.04	0.00	0.00	0.00	0.22	0.23	0.49	0.17	0.38	0.24
Butylbenzene	0.13	0.14	0.10	0.05	0.06	0.07	0.05	0.00	0.00	0.01	0.22	0.23	0.49	0.20	0.43	0.25
Cumene	0.37	0.51	0.32	0.03	0.03	0.05	0.03	<0.001	<0.001	0.00	0.15	0.10	0.33	0.11	0.18	0.16
Trimethylbenzene	2.69	3.30	2.04	0.14	0.14	0.19	0.13	0.00	0.00	0.02	0.68	0.78	2.26	0.82	1.58	1.11
3-Ethyltoluene	4.36	6.21	3.95	0.42	0.41	0.49	0.34	0.02	0.03	0.06	1.57	2.08	5.29	2.63	3.51	2.15
Isobutylbenzene	0.07	0.06	0.05	0.02	0.02	0.03	0.02	0.00	0.00	0.00	0.08	0.06	0.15	0.07	0.13	0.07
Pentylbenzene	0.01	0.02	0.01	0.06	0.08	0.09	0.06	0.00	0.00	0.00	0.29	0.44	0.21	0.10	0.40	0.11
1,2,4,5-tetramethylbenzene	0.40	0.60	0.39	0.17	0.16	0.21	0.11	0.02	0.03	0.03	0.30	0.80	1.70	0.60	0.64	0.37
Oleic acid, methyl ester	0.03	0.02	0.28	5.38	4.28	5.44	5.52	0.01	0.61	0.25	0.74	0.68	15.67	8.46	0.43	0.74
1-hexene	1.41	1.46	0.98	0.10	0.11	0.06	0.13	0.22	0.25	0.24	0.09	0.10	7.30	4.61	4.88	2.79
n-hexane	1.41	1.45	0.98	0.10	0.11	0.06	0.13	0.22	0.25	0.24	0.08	0.11	<0.00327	<0.00327	<0.00327	<0.00327
Cyclohexane	0.80	0.72	0.54	<0.0024	<0.0024	<0.0024	<0.0024	<0.0024	<0.0024	<0.0024	<0.002413	<0.002413	<0.002413	<0.002413	<0.002413	<0.002413
1-heptene	0.04	3.64	0.73	0.00	0.00	<0.001	0.00	<0.001	<0.001	0.02	0.05	0.09	0.00	0.06	<0.001	0.00
Heptane	1.78	1.44	1.18	0.07	0.09	<0.003	0.12	0.22	0.26	0.27	0.13	0.22	0.53	0.11	0.10	0.02
Methylcyclohexane	0.76	1.03	0.68	0.25	0.35	0.38	0.26	0.04	0.04	0.05	0.51	0.64	1.17	0.34	0.42	0.12
1-octene	0.06	0.03	0.05	0.03	0.04	0.04	0.03	0.00	0.01	0.01	0.05	0.08	0.11	0.04	0.06	0.02
Ethylcyclohexane	0.09	0.16	0.10	0.39	0.38	0.55	0.28	0.02	0.03	0.05	0.51	0.40	0.86	0.32	0.47	0.32
Octane	1.35	1.08	1.04	0.44	0.59	0.36	0.70	0.54	0.73	1.00	0.94	0.84	2.38	0.52	0.59	0.47
1-nonene	0.01	0.05	0.01	0.04	0.06	0.08	0.04	0.01	0.01	0.01	0.08	0.04	0.19	0.05	0.09	0.14
Propylcyclohexane	0.02	0.04	0.02	0.74	0.56	1.06	0.51	0.02	0.02	0.06	0.55	0.44	1.12	0.54	1.11	0.67
1-decene	0.00	0.01	0.00	0.09	0.02	0.13	0.05	0.00	0.01	0.01	0.06	0.07	0.17	0.07	0.21	0.12
Butylcyclohexane	0.01	0.01	0.01	1.20	1.08	1.57	1.00	0.01	0.02	0.07	0.63	0.50	1.39	0.67	1.14	0.73
2,3-dimethylpentane	1.43	1.49	0.89	0.03	0.03	<0.0001	0.04	0.07	0.08	0.06	0.02	0.05	0.04	0.02	0.02	0.00

(g/L)	**E85-2**	**E85-3**	**E85-4**	**D-SE4**	**D-SE5**	**D-SE6**	**D-SE7**	**HVO2**	**HVO3**	**HVO4**	**MGO-1**	**MGO-2**	**D-EU1**	**D-EU2**	**EO1-1**	**EO1-2**
Nonane	0.49	0.49	0.43	1.78	2.11	2.12	2.32	0.63	0.88	1.83	2.48	1.78	8.17	2.13	3.80	4.96
Decane	0.12	0.13	0.15	4.45	4.66	5.48	5.16	0.93	1.14	2.16	3.50	3.30	16.98	5.47	8.29	7.16
1-undecene	0.00	0.01	0.00	0.07	0.07	0.09	0.06	0.00	0.00	0.01	0.05	0.05	0.14	0.06	0.12	0.07
Pentylcyclohexane	0.00	0.00	0.01	1.26	1.17	1.70	1.02	0.01	0.02	0.09	0.55	0.50	0.95	0.66	0.86	0.53
1-dodecene	0.00	0.00	0.01	0.87	0.99	1.17	0.76	0.02	0.04	0.10	0.54	0.61	0.09	0.05	0.06	<0.0001
Decaline	0.01	0.01	0.03	4.12	4.20	5.27	3.10	0.03	0.05	0.24	1.00	1.97	3.64	1.80	3.62	1.16
Undecane	0.02	0.07	0.10	6.17	6.92	8.42	6.51	0.91	1.20	1.99	3.50	4.32	18.07	6.15	7.93	7.51
Dodecane	0.01	0.02	0.10	8.27	9.12	10.65	7.96	0.98	1.25	2.26	4.01	4.49	14.31	6.84	7.13	6.79
Tridecane	0.01	0.01	0.12	9.41	10.12	12.04	8.57	0.74	0.96	1.97	4.42	5.26	10.26	7.08	7.95	7.27
Tetradecane	0.01	0.01	0.09	6.12	6.33	7.93	5.67	0.99	2.02	1.41	3.22	3.71	10.90	7.45	6.23	5.65
Pentadecane	0.01	0.01	0.08	4.91	5.83	7.40	5.93	3.14	5.74	5.06	3.44	4.57	11.30	6.57	5.80	5.96
Hexadecane	0.01	0.01	0.07	5.30	4.80	6.03	4.79	5.85	5.29	8.02	3.04	4.36	9.18	5.88	4.68	5.20
Heptadecane	0.01	0.00	0.08	3.08	2.89	5.07	3.11	2.08	2.21	3.33	3.96	4.49	8.75	5.33	4.40	5.75
Octadecane	0.01	0.00	0.05	4.28	4.14	3.76	4.85	5.61	6.30	7.86	3.31	3.29	8.55	4.87	4.07	5.79
Nonadecane	0.00	0.00	0.01	1.74	1.49	2.23	1.59	0.09	0.14	0.33	3.33	3.33	8.11	5.45	4.93	7.37
Eicosane	0.00	0.00	0.01	1.05	1.09	1.25	1.13	0.10	0.13	0.28	4.28	2.86	7.44	4.80	4.82	6.78
Heneicosane	0.00	0.00	0.00	0.47	0.59	0.56	0.58	0.04	0.05	0.06	3.82	2.03	5.83	3.56	3.69	5.87
Docosane	0.00	0.00	0.00	0.26	0.40	0.37	0.42	0.00	0.01	0.08	2.29	1.29	6.29	4.16	4.52	6.82
Tricosane	<0.0001	<0.0001	<0.0001	0.11	0.22	0.20	0.26	0.01	0.01	0.05	1.81	0.86	5.59	3.60	4.24	6.12
Tetracosane	<0.0001	<0.0001	0.00	0.03	0.06	0.06	0.08	0.01	0.01	0.02	1.45	0.61	4.33	2.73	3.69	4.21

(g/L)	P95-1	D-SE2	D-SE3	HVO1	D-SE1	P98-1	E85-1	P95-2	P98-2	P98-3	P98-4	P95-3	P95-4	P95-5	P95-6
MTBE	0.056	0.043	<0.002	0.0043	<0.002	12.74	6.59	0.105	10.32	4.75	9.42	0.20	0.21	0.19	0.23
Ethyl tert-butyl ether (ETBE)	0.06	0.52	0.002	0.43	0.01	2.83	0.43	0.15	2.85	16.54	0.00	0.01	0.00	0.00	0.17
tert-Amyl methyl ether (TAME)	0.00038	<0.000001	<0.000001	<0.000001	<0.000001	0.50	0.022	0.045	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Benzene	2.76	0.01	0.0027	0.01	<0.001	2.98	0.38	3.07	3.34	4.66	2.28	4.35	4.16	4.50	6.26
Toluene	29.50	0.10	0.02	0.11	0.01	33.53	6.45	27.10	32.25	39.12	28.62	33.61	33.73	33.64	44.08
Ethylbenzene	5.94	0.01	0.005	0.003	0.01	8.47	1.19	6.32	8.64	9.87	4.87	8.93	8.77	9.04	11.17
p-Xylene	17.87	0.11	0.08	0.07	0.08	21.65	3.77	16.85	20.07	23.93	9.47	21.91	20.44	21.44	27.03
Propylbenzene	1.85	0.03	0.03	0.01	0.03	2.59	0.37	1.92	2.52	3.11	1.33	2.70	2.81	2.82	3.21
Butylbenzene	0.23	0.03	0.03	0.01	0.04	0.42	0.05	0.32	0.36	0.43	0.13	0.45	0.19	0.48	0.45
Cumene	0.829	0.019	0.022	0.003	0.025	1.47	0.17	1.23	1.50	1.64	0.75	1.68	1.55	1.57	1.58
Trimethylbenzene	10.26	0.15	0.14	0.05	0.16	14.64	2.19	11.39	8.09	10.30	5.72	9.54	9.36	9.19	11.63
3-Ethyltoluene	12.37	0.25	0.31	0.10	0.34	17.82	2.84	13.99	15.82	19.36	10.64	18.01	17.88	16.97	22.00
Isobutylbenzene	0.15	0.02	0.03	0.003	0.03	0.27	0.03	0.18	0.16	0.24	0.07	0.24	0.19	0.23	0.25
Pentylbenzene	0.02	0.03	0.04	0.003	0.05	0.02	0.0020	0.0197	0.05	0.05	0.01	0.06	0.09	0.05	0.05
1,2,4,5-tetramethylbenzene	2.82	0.22	0.26	0.10	0.26	4.05	0.61	3.12	1.76	1.84	0.93	1.92	2.11	1.81	1.95
Oleic acid, methyl ester	0.002	38.18	18.97	0.17	32.62	0.003	0.01	0.001	0.00	0.04	0.00	0.02	0.01	0.02	0.03
1-hexene	0.32	<0.0009	<0.0009	<0.0009	<0.0009	1.19	<0.0009	1.12	2.85	3.68	<0.0009	4.61	5.02	6.40	7.60
n-hexane	3.55	<0.0033	<0.0033	<0.0033	<0.0033	4.16	<0.0033	7.21	2.74	3.65	<0.0033	4.58	4.95	6.34	7.60
Cyclohexane	5.32	<0.0024	<0.0024	<0.0024	<0.0024	3.75	0.08	4.78	1.12	4.73	<0.0024	4.22	3.46	3.90	2.29
1-heptene	12.04	0.02	0.0037	<0.001	<0.001	30.51	1.18	15.86	17.24	3.90	17.44	2.37	1.96	5.62	1.17
Heptane	5.20	0.09	0.05	0.31	<0.00016	5.60	0.80	5.50	4.22	6.80	4.53	4.92	4.02	6.89	8.91
Methylcyclohexane	2.25	0.33	0.39	0.05	0.35	1.39	0.27	1.71	1.57	3.09	0.91	1.27	3.14	3.10	4.73
1-octene	0.04	0.04	0.04	0.01	0.05	0.11	0.02	0.10	0.10	0.06	0.04	0.19	0.18	0.25	0.18
Ethylcyclohexane	0.19	0.35	0.41	0.03	0.49	0.11	0.01	0.15	0.22	0.27	0.05	0.34	0.35	0.36	0.80
Octane	2.81	0.59	0.53	1.09	0.33	2.49	0.52	3.02	2.18	3.73	1.26	3.41	2.68	4.64	6.81
1-nonene	0.02	0.05	0.06	0.01	0.06	0.07	0.01	0.12	0.14	0.07	0.02	0.12	0.04	0.17	0.15
Propylcyclohexane	0.05	0.58	0.65	0.05	0.83	0.03	0.005	0.04	0.05	0.05	0.01	0.08	0.09	0.07	0.26
1-decene	0.01	0.07	0.08	0.01	0.14	0.01	0.002	0.02	0.02	0.02	0.00	0.01	0.04	0.02	0.02
Butylcyclohexane	0.02	0.88	1.09	0.07	1.36	0.02	0.002	0.02	0.03	0.02	0.01	0.02	0.03	0.02	0.04
2,3-dimethylpentane	3.92	0.04	0.02	0.12	<0.00020	4.31	0.39	3.76	3.86	4.83	1.53	4.51	4.49	5.01	5.09

(g/L)	P95-1	D-SE2	D-SE3	HVO1	D-SE1	P98-1	E85-1	P95-2	P98-2	P98-3	P98-4	P95-3	P95-4	P95-5	P95-6
Nonane	1.04	1.76	1.63	1.91	1.77	0.67	0.16	1.03	0.63	1.07	0.25	1.36	0.97	1.67	2.97
Decane	0.27	4.26	4.32	2.65	6.05	0.21	0.05	0.35	0.24	0.29	0.07	0.39	0.43	0.49	0.83
1-undecene	0.03	0.05	0.05	0.02	0.11	0.07	0.0207	0.0393	0.05	0.00	0.03	0.02	0.02	0.03	0.01
Pentylcyclohexane	0.00	0.87	1.04	0.05	1.49	0.00	0.0004	0.0022	0.01	0.01	0.00	0.01	0.01	0.01	0.01
1-dodecene	0.001	0.36	0.47	0.01	0.54	0.0016	0.0002	0.0002	0.00	0.01	0.00	0.01	0.02	0.01	0.00
Decaline	0.01	3.09	3.93	0.15	4.86	0.01	0.00	0.01	0.02	0.06	0.01	0.04	0.03	0.04	0.04
Undecane	0.09	7.03	7.40	2.48	10.54	0.17	0.01	0.13	0.24	0.30	0.13	0.17	0.27	0.17	0.21
Dodecane	0.01	8.66	8.91	2.38	12.46	0.03	0.01	0.02	0.05	0.22	0.03	0.08	0.11	0.10	0.11
Tridecane	0.01	9.23	9.38	2.15	12.76	0.02	0.004	0.01	0.01	0.09	0.00	0.02	0.02	0.03	0.05
Tetradecane	0.0022	7.93	8.69	2.97	11.36	0.01	0.002	0.004	0.00	0.02	<0.0004	0.01	0.00	0.01	0.02
Pentadecane	0.0030	7.30	6.70	7.39	9.05	0.004	0.002	0.003	0.00	0.02	0.00	0.01	0.00	0.01	0.02
Hexadecane	0.0021	6.03	5.04	14.27	5.36	0.0050	0.0010	0.0014	0.00	0.01	0.00	0.01	0.00	0.01	0.02
Heptadecane	0.0025	4.66	4.64	9.46	4.31	0.0012	0.0007	0.0013	0.00	0.01	0.00	0.01	0.00	0.00	0.01
Octadecane	0.0023	5.38	4.20	16.49	2.96	0.0012	0.0005	0.0007	0.00	0.01	0.00	0.00	0.00	0.00	0.01
Nonadecane	0.0003	2.03	1.93	0.42	2.20	0.0004	<0.0002	0.0005	0.00	0.00	<0.0002	0.00	0.00	0.00	0.00
Eicosane	0.0003	1.40	1.37	0.38	1.44	0.0003	0.0002	0.0003	0.00	0.00	<0.0001	0.00	0.00	0.00	0.00
Heneicosane	0.00011	0.85	0.87	0.05	0.97	0.0004	<0.0001	0.0001	<0.0001	0.00	<0.0001	0.00	<0.0001	0.00	0.00
Docosane	0.0002	0.46	0.47	0.17	0.54	0.0001	<0.0001	0.0002	<0.0001	0.00	<0.0001	0.00	0.00	0.00	0.00
Tricosane	<0.0001	0.14	0.13	0.04	0.21	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Tetracosane	<0.0001	0.03	0.03	0.03	0.06	<0.0001	<0.00000026	0.00016	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table A3. PAH concentrations in eight fuel samples.

(g/L)	**P98-4**	**P95-4**	**MGO-1**	**MGO-2**	**D-EU1**	**D-EU2**	**EO1-1**	**EO1-2**
Naphthalene	7.9	14	5	1.3	1.1	0.8	1.1	2.4
Acenaphthene	0.65	1.2	0.26	0.42	0.13	0.2	0.11	0.35
Acenaphthylene	0.039	0.11	<0.039	<0.038	<0.0370	<0.038	<0.037	<0.038
Fluorene	0.1	0.14	1.5	0.49	0.14	0.5	0.2	1.7
Phenanthrene	0.094	0.14	2.3	1.5	0.2	1.1	0.42	4.4
Anthracene	0.042	0.064	0.019	0.024	0.0038	0.019	0.012	0.077
Fluoranthene	0.019	0.027	0.17	0.12	0.023	0.084	0.03	0.37
Pyrene	0.034	0.06	1	1.5	0.26	0.58	0.55	1.5
Benzo(a)anthracene	0.044	0.11	2.6	3.5	0.73	1.6	0.6	3.9
Chrysene	<0.0020	0.27	<0.0023	<0.0023	<0.0022	<0.0023	<0.0022	<0.0023
Benzo(b)fluoranthene	<0.0026	0.015	0.017	0.036	0.0065	0.014	0.01	0.0056
Benzo(k)fluoranthene	<0.0015	0.056	0.0045	0.0055	<0.0016	0.0019	<0.0017	<0.0017
Benzo(a)pyrene	0.0067	0.031	0.0071	0.0063	<0.0021	0.0022	<0.0021	0.0032
Dibenzo(a,h)anthracene	<0.0019	0.039	0.0072	0.0086	<0.0021	0.0023	<0.0021	<0.0021
Benzo(ghi)perylene	0.0057	0.014	0.0064	<0.0042	<0.0041	<0.0042	<0.0041	<0.0042
Indeno(1,2,3-cd)pyrene	<0.0074	0.0092	<0.0086	<0.0085	<0.0082	<0.0084	<0.0083	<0.0084
Benzo(j)fluoranthene	<0.0037	0.0094	0.023	0.018	<0.0041	0.0098	<0.0041	0.021

Table A4. Concentrations (µg/L) of substances in water/fuel mixtures (WAF).

(µg/L)	D-SE1	D-SE2	D-SE3	HVO1	P98-1	P98-2	P95-1	P95-2	P95-3	E85-1	E85-2	EO1-1	EO1-2	D-EU1	MGO-2
MTBE	517.0	1581.9	58.7	828.0	80571.9	81595.7	2143.8	3858.7	3390.7	95557.9	59664.1	10.6	24.2	217.0	4.7
Ethyl tert-butyl ether (ETBE)	1.3	143.1	0.8	113.1	22426.4	26027.7	5.3	23.3	211.2	1004.0	798.5	0.3	0.9	11.7	<0.2
tert-Amyl methyl ether (TAME)	<0.1	0.1	<0.1	0.7	82.8	37.6	0.8	5.4	0.4	14.3	4.0	<0.2	2.8	<0.2	<0.2
Benzene	9.2	33.4	12.8	27.0	6502.5	7769.9	7577.6	8347.0	9425.0	8310.7	1863.9	21.6	25.2	157.0	357.6
Toluene	37.2	122.2	39.1	76.2	19718.5	25790.7	21746.2	20915.3	22937.3	31770.5	6985.2	114.3	125.0	607.3	684.3
Ethylbenzene	10.7	14.8	6.9	4.4	1742.5	1983.6	1559.2	1805.9	1740.8	3010.6	544.3	29.7	47.7	112.3	87.0
p-Xylene	35.3	50.1	22.8	14.6	4368.0		4549.7	4689.0		9472.4		27.8	28.2	90.4	68.4
Propylbenzene	6.7	7.4	2.6	0.7	167.0	119.2	133.8	173.5	85.9	477.4	33.5	50.4	18.7	32.5	21.6
Butylbenzene	7.0	7.2	1.4	0.5	8.3	1.9	5.0	10.0	3.2	42.9	1.9	13.6	5.3	11.2	5.3
Cumene	6.1	5.9	2.8	0.4	104.0		77.3	126.6		231.6		32.4	17.8	30.4	15.9
Trimethylbenzene	41.2	41.6	16.8	5.7	1090.3	325196.9	885.7	1223.6	295281.6	3052.8	150299.1	244.1	100.5	177.3	116.9
3-Ethyltoluene	89.5	84.4	40.1	13.2	1618.6	374895.7	1279.0	1780.0	319302.8	4151.3	161050.4	828.0	216.0	476.4	331.7
Isobutylbenzene	5.7	6.6	1.2	0.2	5.8	100.5	4.0	6.0	67.5	30.7	601.5	8.2	2.1	3.9	1.9
Pentylbenzene	10.2	10.2	3.1	0.3	0.7	4.3	0.6	1.2	8.8	1.8	0.2	16.2	8.1	25.0	17.3
1,2,4,5-tetramethylbenzene	69.1	64.5	21.3	11.3	215.8	76971.9	129.2	205.0	79572.2	731.7	65199.9	30.3	12.6	59.8	47.1
Oleic acid, methyl ester	6959.7	9438.6	582.6	59.8	2.3		1.7	115.9		12.7		3.2	0.7	511.1	5.5
1-hexene	<0.1	<0.1	<0.1	<0.1	87.2		24.3	48.5		195.2					
n-hexane	4076.5	1861.1	32.2	4066.2	1226.9		1564.0	500.8		1137.1					
Cyclohexane	<100	<100	<100	<100	<100		<100	<100		1224.9					
1-heptene	6.5	7.2	5.0	4.6	326.0		125.8	110.4		1157.9		<1.5	<1.5	<1.5	<1.5
Heptane	<0.1	<0.1	<0.1	<0.1	32.5	87.7	5.8	9.9	18.9	641.5	11.3	<8	<8	<8	<8
Methylcyclohexane	48.1	41.3	13.1	1.5	19.8	49.8	44.4	21.9	15.1	273.6	1.4	4.0	1.8	20.6	20.3
1-octene	21.8	15.9	3.9	0.5	1.2		2.1	1.5		28.8		<0.4	<0.4	0.6	0.4
Ethylcyclohexane	67.3	60.7	12.9	2.0	1.1	2.8	2.1	1.3	0.6	20.4	0.1	1.9	2.1	8.6	4.3
Octane	51.1	92.0	16.9	51.4	22.8	15.1	24.2	19.6	2.5	489.1	0.9	1.1	1.4	14.6	3.2
1-nonene	11.3	8.2	1.5	0.4	1.1		0.3	1.3		6.5		0.3	0.3	0.6	<0.2
Propylcyclohexane	129.8	123.9	22.0	2.3	0.3	0.2	0.4	0.4	0.3	3.5	0.1	7.9	2.2	9.4	3.0
1-decene	23.1	13.8	2.5	0.6	0.1		<0.2	<0.2		0.8		0.5	<0.2	0.9	<0.2
Butylcyclohexane	248.4	226.7	42.0	5.7	0.3	5.6	0.2	0.2	7.0	1.8	0.0	6.4	1.9	12.4	2.8
2,3-dimethylpentane	<0.1	<0.1	<0.1	<0.1	26.8		26.5	13.2		406.9		<4.5	<4.5	<4.5	<4.5

(µg/L)	D-SE1	D-SE2	D-SE3	HVO1	P98-1	P98-2	P95-1	P95-2	P95-3	E85-1	E85-2	EO1-1	EO1-2	D-EU1	MGO-2
Nonane	324.9	329.2	58.1	129.4	6.5		8.3	8.5		162.7		20.1	12.9	61.1	9.0
Decane	1207.4	920.5	169.2	232.4	5.9		4.4	6.2		46.7		68.9	20.8	157.7	15.6
1-undecene	65.2	2.9	8.2	0.7	0.2		0.1	1.6		1.7		1.3	0.2	1.7	0.3
Pentylcyclohexane	317.5	250.2	46.3	4.8	0.0	0.2	0.0	0.1	0.5	0.4	0.0	2.5	1.4	8.7	2.9
1-dodecene	208.5	246.8	43.8	4.1	0.2		<0.2	0.2		0.4		0.7	<0.2	2.6	0.7
Decaline	886.6	813.0	161.0	12.3	0.2	19.4	0.2	0.2	11.8	3.4	4.9	17.7	3.8	36.0	14.0
Undecane	2361.3	1576.9	292.8	240.9	0.7		0.4	29.2		20.6		34.8	20.7	175.8	23.4
Dodecane	2395.4	1768.4	391.3	220.9	4.0		1.0	3.9		8.3		21.0	19.9	145.7	26.7
Tridecane	2581.4	2027.7	402.0	194.6	2.4		1.6	2.5		4.6		15.7	19.6	143.9	28.6
Tetradecane	2656.6	2105.9	341.7	289.8	10.3		8.0	9.8		11.4		14.9	22.4	148.4	32.6
Pentadecane	2192.3	2056.3	314.1	914.6	11.9		15.0	8.4		6.5		16.1	23.4	154.4	42.6
Hexadecane	1413.6	1721.5	254.6	2190.0	36.4		35.1	33.0		33.2		15.3	21.9	142.4	40.3
Heptadecane	881.5	1461.4	202.0	1152.8	2.3		2.2	4.0		3.8		13.8	22.5	126.8	35.8
Octadecane	570.2	1247.0	153.6	2091.5	9.0		13.2	15.2		15.2		10.9	22.5	122.8	26.9
Nonadecane	393.9	417.1	60.1	33.8	3.0		4.7	3.0		2.5		12.2	24.4	82.4	27.2
Eicosane	251.8	279.1	42.7	30.8	0.5		0.7	0.8		1.2		8.9	17.4	68.5	16.6
Heneicosane	143.1	145.3	24.5	<0.1	<0.1		<0.1	<0.1		<0.1		5.5	9.3	36.9	8.3
Docosane	88.1	91.8	12.9	13.4	0.7		0.8	0.6		0.5		5.4	8.9	33.4	6.2
Tricosane	32.6	23.7	2.4	2.8	0.0		<0.1	<0.1		<0.1		4.1	6.8	25.2	3.9
Tetracosane	8.2	3.7	0.5	1.2	0.3		0.1	0.2		0.2		3.4	6.0	18.8	4.2

Table A5. Concentrations (µg/L) of substances in water/fuel mixtures (WAF) during evaporation experiments.

Fuel sample	Temp [°C]	Time [min]	MTBE [µg/l]	ETBE [µg/l]	Benzene [µg/l]	tert-Amyl methyl ether [µg/l]	Heptane [µg/l]	Methyl cyclohexane [µg/l]	Toluene [µg/l]	Octane [µg/l]	Ethylcyclo-hexane [µg/l]	Ethyl-benzene [µg/l]	Propyl-benzene [µg/l]
P98-2	18,5	0	31071,0	41916,8	1749,8	20,8	74,0	6,8	7588,2	13,5	0,9	421,2	37,1
P98-2	18,5	15	39332,2	56814,4	6806,5	30,3	181,2	48,0	22604,6	38,2	3,7	1353,4	114,4
P98-2	18,5	30	40223,8	57943,2	7284,1	28,2	209,8	59,2	23885,5	43,7	4,3	1422,5	122,5
P98-2	18,5	60	34905,1	47697,1	2562,9	19,1	114,2	19,1	10714,9	25,1	2,0	618,0	59,7
P98-2	18,5	120	31856,9	43932,3	3078,8	15,2	168,8	34,0	12085,7	40,3	3,5	694,3	68,6
P98-2	18,5	240	21850,9	26734,0	900,3	6,2	119,8	15,3	4062,7	32,9	2,1	240,7	26,8
P98-2	18,5	1200	66,6	16,8	2,8	0,1	58,3	0,7	10,4	0,4	0,2	3,4	0,1
P98-2	18,5	1440	24,9	12,4	3,1	0,2	130,7	1,6	6,0	0,3	0,3	3,2	0,2
E85-2	18,5	0	62130,5	1449,6	9327,8	1,8	95,2	40,4	27283,5	10,7	2,4	1572,3	91,6
E85-2	18,5	15	41629,9	1249,8	2910,5	1,0	77,0	15,2	9979,2	7,9	1,3	525,6	33,1
E85-2	18,5	30	33485,9	1482,1	143,9	0,4	78,7	2,4	665,5	0,9	0,2	41,9	2,8
E85-2	18,5	60	28247,1	1375,9	159,6	0,7	79,2	2,1	658,1	3,4	0,3	39,7	2,7
E85-2	18,5	120	16120,0	1284,4	11,6	0,7	75,9	1,4	48,2	2,0	0,2	5,3	0,2
E85-2	18,5	240	518,1	1177,7	4,3	0,6	76,7	1,2	14,5	0,9	0,1	3,5	0,1
E85-2	18,5	1200	67,3	695,1	2,5	0,3	75,7	0,9	7,1	0,5	0,1	3,0	0,1
E85-2	18,5	1440	19,8	640,2	2,2	0,0	129,8	1,7	2,0	0,6	0,1	2,5	0,0
P98-2	10	0	33796,1	73524,5	844,7	7,0	55,1	1,9	4197,4	1,1	0,3	205,1	14,9
P98-2	10	15	40624,2	94332,4	2459,9	8,2	51,2	3,5	9058,2	0,9	0,5	502,2	31,7
P98-2	10	30	43768,6	110315,6	6871,5	11,4	66,1	22,0	20916,6	5,5	0,9	995,3	63,1
P98-2	10	60	36705,6	82704,8	1384,5	7,2	59,9	3,0	5369,7	0,8	0,3	261,1	17,9
P98-2	10	120	39973,4	85579,5	1528,3	7,2	59,7	3,6	6462,6	1,6	0,4	343,8	20,3

Fuel sample	Temp [°C]	Time [min]	MTBE [µg/l]	ETBE [µg/l]	Benzene [µg/l]	tert-Amyl methyl ether [µg/l]	Heptane [µg/l]	Methyl cyclohexane [µg/l]	Toluene [µg/l]	Octane [µg/l]	Ethylcyclo-hexane [µg/l]	Ethyl-benzene [µg/l]	Propyl-benzene [µg/l]
P98-2	10	240	46767,0	102587,2	1831,7	7,8	62,8	5,8	6565,1	2,2	0,3	321,6	25,3
P98-2	10	1200	19077,0	19245,6	8,0	1,2	66,9	1,0	33,0	1,9	0,3	6,1	0,2
E85-2	10	0	101892,7	2417,7	11842,8	6,5	103,3	45,0	33320,3	14,1	2,2	2329,2	174,2
E85-2	10	15	81002,4	1918,3	419,6	2,8	66,8	1,9	2302,9	0,7	0,2	160,3	10,9
E85-2	10	30	87461,1	2245,7	3392,9	3,6	69,3	9,7	11666,5	6,0	0,7	808,0	57,8
E85-2	10	60	79488,1	1838,5	2324,5	2,9	70,8	7,3	8230,9	6,4	0,6	488,6	38,9
E85-2	10	120	74161,8	1714,4	811,0	2,5	59,7	2,4	3102,8	2,4	0,3	182,8	13,3
E85-2	10	240	57428,3	1617,0	57,9	1,3	56,3	1,3	239,5	0,6	0,2	16,8	0,9
E85-2	10	1200	7810,8	1113,9	1,4	0,3	58,4	0,9	3,4	0,4	0,1	3,1	0,0
P98-4*	10	0	113812,8	58,5	12691,4	39,3	0,0	21,8	42012,6	7,6	1,1	2426,5	141,3
P98-4*	10	15	121691,9	53,1	13065,0	40,6	0,0	23,0	43117,8	7,9	1,1	2477,2	141,0
P98-4*	10	30	121981,4	46,3	8370,1	36,2	0,0	11,3	29913,0	4,8	0,8	1644,9	96,5
P98-4*	10	60	119607,9	45,8	9778,4	35,6	0,0	17,4	33964,4	6,4	0,9	1929,9	119,6
P98-4*	10	120	112208,9	36,5	4520,9	29,4	0,0	5,9	17014,2	3,3	0,6	910,7	55,9
P98-4*	10	240	115171,2	34,6	4484,6	28,0	0,0	8,8	16998,3	4,2	0,7	834,9	50,0
P98-4*	10	1200	52978,7	4,6	24,5	4,6	0,0	-0,8	132,6	0,5	0,3	8,5	0,6
P98-4*	10	1440	44464,0	3,9	10,3	3,2	0,0	-0,9	60,0	0,5	0,3	4,2	0,3
P98-4*	20	0	119022,5	57,5	11302,4	45,4	28,4	14,7	39358,8	6,6	0,9	2256,9	129,8
P98-4*	20	15	118471,3	53,7	11151,0	42,2	33,7	17,4	38036,8	7,4	0,9	2081,3	115,6
P98-4*	20	30	105637,3	37,9	1412,9	29,4	0,0	0,4	7810,7	1,2	0,3	449,1	31,2
P98-4*	20	60	95674,8	32,7	1326,4	24,6	0,0	1,4	7712,6	2,7	0,5	502,1	46,3
P98-4*	20	120	82613,6	20,9	2041,6	17,0	2,1	2,6	8421,1	1,8	0,4	442,4	27,2
P98-4*	20	240	76457,7	19,7	2659,2	14,8	0,0	1,5	11056,8	2,6	0,5	569,2	36,9

Fuel sample	Temp [°C]	Time [min]	MTBE [µg/l]	ETBE [µg/l]	Benzene [µg/l]	tert-Amyl methyl ether [µg/l]	Heptane [µg/l]	Methyl cyclohexane [µg/l]	Toluene [µg/l]	Octane [µg/l]	Ethylcyclo-hexane [µg/l]	Ethyl-benzene [µg/l]	Propyl-benzene [µg/l]
P98-4*	20	1200	1381,2	0,8	5,5	0,0	0,0	0,0	22,2	0,6	0,3	1,7	0,1
P98-4*	20	1440	688,8	0,6	3,6	0,0	0,0	0,0	14,2	0,7	0,3	1,2	0,1
P98-2	5	0	78344,1	63,1	12458,3	47,4	24,3	15,1	32802,7	6,1	1,2	2083,5	106,2
P98-2	5	15	77083,8	55,6	10033,4	41,2	15,2	11,9	27172,3	4,8	1,0	1405,0	70,6
P98-2	5	30	77527,5	51,9	8157,4	38,9	12,0	7,5	25884,7	3,9	0,9	1443,1	77,0
P98-2	5	60	80305,0	50,3	8174,0	37,9	14,7	9,7	26290,1	4,5	0,9	1438,2	77,8
P98-2	5	120	77677,3	43,8	5872,0	33,4	7,6	6,8	20020,5	3,6	0,8	1044,2	57,2
P98-2	5	240	82756,0	47,4	4250,3	36,5	2,3	3,3	14800,7	2,4	0,7	737,7	41,0
P98-2	5	1200	43128,2	8,3	30,8	7,1	0,0	0,0	152,0	1,2	0,6	9,1	0,5
P98-2	5	1440	32654,2	4,7	6,1	3,8	0,0	0,0	31,3	1,3	0,7	2,1	0,1
P98-4*	5	0	81625,9	55,1	12080,9	41,9	26,5	15,8	35788,1	7,3	1,2	2034,8	103,6
P98-4*	5	15	80708,9	51,5	10444,1	39,0	26,0	15,8	33766,9	8,1	1,2	2021,3	114,4
P98-4*	5	30	82784,0	46,8	8489,7	35,7	17,0	11,4	29613,9	6,8	1,1	1710,1	98,6
P98-4*	5	60	79136,1	42,3	6559,6	32,9	12,4	9,0	23640,8	6,0	1,1	1322,2	76,4
P98-4*	5	120	83049,8	37,9	4158,1	29,5	9,4	5,5	15648,3	4,4	0,9	802,9	47,8
P98-4*	5	240	93446,2	39,0	2438,3	32,2	7,9	2,1	10042,6	3,0	0,8	482,1	27,8
P98-4*	5	1200	33621,0	3,4	3,9	2,4	3,1	0,0	17,4	1,3	0,7	1,4	0,1
P98-4*	5	1440	28419,0	2,8	3,6	1,5	1,3	0,0	14,3	1,3	0,7	1,2	0,1
E85-2	5	0	108742,9	1725,9	12572,2	2,7	31,4	34,7	37917,3	9,3	2,4	2187,9	121,8
E85-2	5	15	108971,5	1697,0	9605,0	2,5	24,7	24,4	29996,5	7,9	1,9	1680,7	94,5
E85-2	5	30	109357,8	1667,6	7715,8	2,4	17,8	17,6	24826,7	6,9	1,6	1343,9	76,0
E85-2	5	60	105417,1	1600,3	4938,0	2,1	9,2	9,4	17334,4	5,6	1,2	922,3	52,6
E85-2	5	120	102215,2	1619,0	1772,3	1,9	5,1	2,3	7342,3	2,2	0,7	388,0	22,0

Fuel sample	Temp [°C]	Time [min]	MTBE [µg/l]	ETBE [µg/l]	Benzene [µg/l]	tert-Amyl methyl ether [µg/l]	Heptane [µg/l]	Methyl cyclohexane [µg/l]	Toluene [µg/l]	Octane [µg/l]	Ethylcyclo-hexane [µg/l]	Ethyl-benzene [µg/l]	Propyl-benzene [µg/l]
E85-2	5	240	105615,6	1680,1	679,7	2,0	2,1	1,0	3072,1	0,7	0,5	162,9	8,7
E85-2	5	1200	50062,3	1417,0	3,3	0,4	1,6	0,2	12,1	0,9	0,4	1,1	0,2
E85-2	5	1440	43451,9	1397,7	3,8	0,3	1,4	0,6	13,0	0,9	0,5	0,9	0,2

Appendix 3 – Ecotoxicological data

GROWTH INHIBITION TEST USING *PSEUDOKIRCHNERIELLA SUBCAPITATA*

subjected to the water-accomodated fraction of two different samples: D2 and D1

Report E21-042
Härslöv February 2022

ALS
TOXICON AB

Contents

Appendixes

1: Protocol for “Freshwater Algae and Cyanobacteria, Growth Inhibition Test”

2: Report from analysis of TOC. ALS report ST2134887

SUMMARY

During the winter 2021/2022, a study on the test items “D2” and “D1” was performed by Toxicon AB, Härslöv, Sweden. The study included one method for ecotoxicological testing.

The method used was:

- OECD Guidelines for the Testing of Chemicals No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011).

The test was performed on the water-accommodated fraction (WAF) of the test items. The method for WAF is outlined in OECD series on testing and assessment no. 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

In the method OECD TG No. 201, the No Observed Effect Concentration (NOEC) value is defined as the highest tested concentration not statistically different from the control (unpaired t-test $p>0,05$).

Results from the algae test is presented in table 1.

Table 1. Nominal NOEC-value and E_rC-values (mg/L) for *Pseudokirchneriella subcapitata* determined after 72 hours of exposure to the loading rate (100 mg/L) of the test items D2 and D1.

72 h			
Test item	**NOEC mg/L**	**E_rC_{10} mg/L**	**E_rC_{50} mg/L**
D2	**100**	**> 100**	**> 100**
D1	**100**	**> 100**	**> 100**

Introduction

Samples of the products “D2” and “D1” were received at Toxicon AB on November 2nd, 2021 for environmental assessment. The tests were performed according to OECDs Guidelines for testing of chemicals.

Test laboratory:

Toxicon AB
Rosenhällsvägen 29
SE-261 92 HÄRSLÖV
Sweden
Telephone: +46 418 70 700

Study director: Anders Walstad ……………………………… Date: 2022-02-09 ……………………..
Anders Walstad

Performance:

Anders Walstad
Susanne Fritz

Sponsor:

IVL Svenska Miljöinstitutet AB
Box 210 60
SE-100 31 STOCKHOLM
SWEDEN

Sponsors reference: Johan Strandberg

Test articles:

Sponsors sample code	Toxicon sample code
D2	E21-042-1
D1	E21-042-2

Test methods

The methods used were:

- OECD Guidelines for the Testing of Chemicals No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011). Toxicon SOP T003.
- OECD Series on testing and assessment No. 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

Freshwater Alga and Cyanobacteria, Growth Inhibition Test – OECD 201

The method used was OECD Guidelines for the Testing of Chemicals No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011). Toxicon SOP T003.

The purpose of the test is to determine the effects of a substance on the growth of a freshwater microalgae. Exponentially growing cultures of algae are exposed to the water-accommodated fraction (WAF) of the test items over a period of 72 hours. Despite of the relatively brief test duration, effects over several generations can be assessed. The response variable is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations (loading rates) of the test substances. The response is evaluated as the function of the exposure concentration in comparison to the average growth of an unexposed control culture. Growth, and growth inhibition, are quantified from measurements of the algal biomass (dry weight per volume) as a function of time. Cell counts (cells/ml) are used as a surrogate parameter to dry weight (OECD TG No. 201). The test endpoint is inhibition of growth, which is expressed as the logarithmic increase in biomass (average specific growth rate) during the period of exposure.

Test organism

The test organism is a laboratory culture of the green alga *Pseudokirchneriella subcapitata*. The inoculum is taken from Toxicon's culture of *P. subcapitata* (NIVA CHL 1), which originates from NIVA, Oslo, Norway. A qualification test using potassium dichromate was performed on the 1st of July, 2021 in order to assure the quality of the test organism. E_rC_{50} was found to be between 0,9 and 1,5 mg/L, which confirmed that the culture was suitable as a test organism according to OECD TG No. 201.

Growth medium (dilution solution)

The composition of the growth medium was as specified in Annex 3 in OECD TG No. 201.
Recipe for the growth medium is presented in table 2.
The growth medium had a pH of 8,1±0,2.

Table 2. Preparation of the growth medium. The substances were added to reversed osmosis water, <10 µS/cm.

Single substance	µg/L	Single substance	µg/L
NH_4Cl	15000	H_3BO_3	185
$MgCl_2$ x 6 H_2O	12000	$MnCl_2$ x 4 H_2O	415
$CaCl_2$ x 2 H_2O	18000	$ZnCl_2$	3
$MgSO_4$ x 7 H_2O	15000	$CoCl_2$ x 6 H_2O	1,5
KH_2PO_4	1600	$CuCl_2$ x 2 H_2O	0,01
$FeCl_3$ x 6 H_2O	64	Na_2MoO_4 x 2 H_2O	7
Na_2EDTA x 2 H_2O	100	$NaHCO_3$	50000

Preparation of test solution

Fresh extraction of the test concentration (loading rate) was prepared as described in OECD No. 23. The test item was weighed in using a small beaker and then rinsed into a glass bottle with 2000 millilitres of growth medium with minimal head space and closed using para foil (table 2). The test concentration was stirred over night at a speed that created a small vortex at the surface. After being allowed to settle for 4 hours, the water phase was collected from the bottle and subsequently transferred to test glass vessels. The process was performed in darkness at room temperature (20-22 °C).

The highest test concentration (loading rate) was 100 mg/L and the subsequent concentrations were made using dilution.

Ecotoxicological testing

The nutrient concentration in the growth medium was the same in the control as in the test solution. Six replicates each were used for the control while the test solutions were tested in triplicates. Test bottles were 250 ml glass E-flasks with 100 ml solution, incubated on a shaking table with approximately 100 µE m-2 s-1 continuous illumination at a temperature of 21-24±2 °C. The pH in all solutions

was measured at the test start, and again in all replicates after 72 hours. The incubation period was 72 hours with sampling at 24, 48, and 72 hours. As a measurement of the biomass in the samples, the number of cells/mL was counted using an optical microscope. At the start of the test, approximately 100 mL of the highest test concentrations of the test items and the control were fixated with sulphuric acid for future reference analysis regarding the content of total organic carbon. Another E-flask containing the highest test concentration of the test items and the control were incubated on the same shaking table as the control and the test solution. These flasks were not inoculated with algae but were used for reference analysis showing the integrity of the test item after 72 hours.

Reference analysis

Reference analysis was made in order to certify that the test concentration was stable during the test. Samples from the highest test concentrations and from the control (growth medium) were collected at 0 h and 72 h and fixated with sulphuric acid and stored in +4°C until analysis regarding the content of total organic carbon.

Calculations

Cell count is one of the proposed surrogate parameters for biomass according to the OECD TG No. 201. The average specific growth rate (µ) for a specific period is calculated as the logarithmic increase in the biomass from the equation for each single vessel of the control and test concentrations:

$\mu = (\ln \text{cells/ml 72 hrs} - \ln \text{cells/ml 0 hrs})/3$ (time in days).

For the control and test concentrations, an average growth rate along with variance estimates is calculated. The specific growth rate for each test concentration is then related to the average growth rate of the control:

$\% I_r = (\mu_c - \mu_r)/\mu_c * 100$

where:

$\% I_r$ = percent inhibition in average specific growth rate;
μ_c = mean value for average specific growth rate (μ) in the control group;
μ_r = average specific growth rate (μ) for the test concentration replicate.

The No Observed Effect Concentration (NOEC), the highest concentration with no statistical difference to the control ($p > 0,05$), is determined using unpaired t-test.

Results

Freshwater Alga and Cyanobacteria, Growth Inhibition Test – OECD 201

The criteria for the validity of the test according to OECD TG No. 201 were fulfilled:

- The biomass in the control culture increased by a factor of more than 16 within 72 hours.
- The mean coefficient of variation of average specific growth rates in the control culture did not exceed 35%.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 7%.

Calculated response variables for the control group and the highest test concentration (loading rate) (mean ± coefficient variation) are presented in table 3. The growth of the algae in the control and in the test concentration during the 72 hours of exposure is presented as cells/mL from the cell counting (figure 1 and 2). NOEC and the E_rC-values are presented in table 3.

There was no statistical difference (unpaired t-test, $p > 0,05$) in the variable average specific growth rate between the control and the highest test concentration (loading rate) of 100 mg/L, and therefore the NOEC-value is determined to be 100 mg/L.
There was a significant difference between the control and the concentrations 12,5 and 25 mg/L of the test sample D2, but since it was a stimulating effect it is not regarded a toxic effect in this case.

Primary data are presented in appendix 1.

Table 3. Calculated response variables for the control and the loading rate (mean ± coefficient of variation) after 72 hours of exposure to the highest test concentrations of the test items D2 and D1.

	72 h			
Test item Loading rate 100 mg/L	**Specific growth rate (µ) Mean ± CV (%)**	**NOEC mg/L**	**E_rC_{10} mg/L**	**E_rC_{50} mg/L**
Control	1,52 ± 2,97	-	-	-
D2	1,45 ± 4,65	100	> 100	> 100
D1	1,58 ± 2,21	100	> 100	> 100

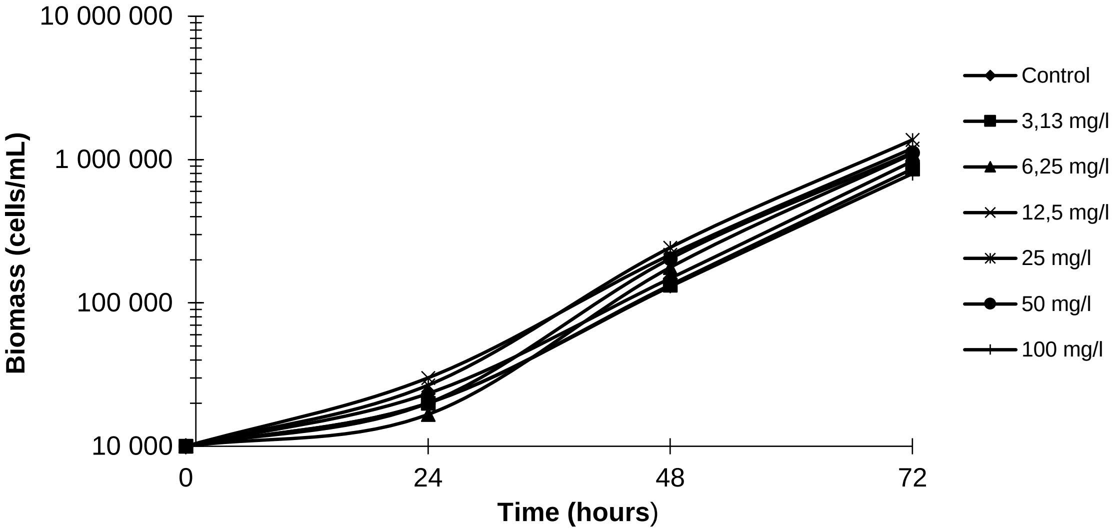


Figure 1. The biomass (cells/mL) in the control and in the test concentrations (loading rate) after 0, 24, 48 and 72 hours of exposure to the test item D2.

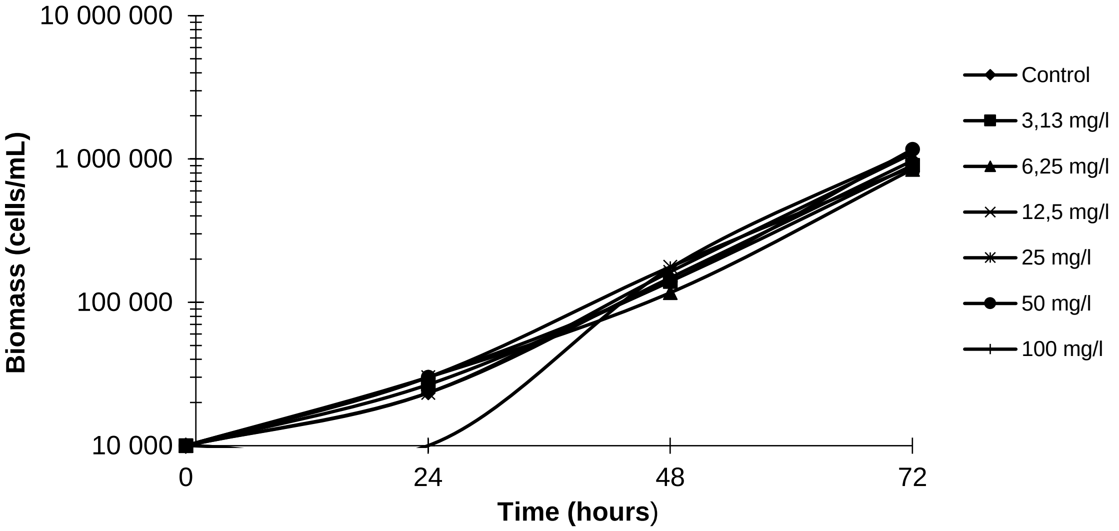


Figure 2. The biomass (cells/mL) in the control and in the test concentrations (loading rate) after 0, 24, 48 and 72 hours of exposure to the test item D1.

Reference analysis

Even if there was a distinct smell from the recovered WAFs, the results from reference analysis indicate that very little of the test samples are in fact present in the WAF. These low levels make it difficult to say something about the stability of the test items D2 and D1. Results from the reference analysis are presented in table 4 and appendix 2.

Table 4. Results from the analysis of total organic carbon at the beginning (0 h) and the end (72 h) of the exposure.

Test Sample Loading rate 100 mg/L	**TOC (mg/L)**	
	0 h	**72 h**
Control	**0,77**	**1,16**
D2	**0,64**	**< 0,50**
D1	**< 0,50**	**< 0,50**

References

1. OECD Guideline for the testing of chemicals No. 201 “Freshwater Alga and Cyanobacteria, Growth Inhibition Test”. Adopted 2011.

2. OECD series on testing and assessment No. 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

Appendix 1

Protocol for “Freshwater Algae and Cyanobacteria, Growth Inhibition Test”

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**	Dokumentnr: **PROT 033-3**
Godkänd av: Thomas Olsson Datum: **2010-02-05**	Gäller från: **2010-02-01** Sid av: **1(1)**

Datum: 21-11-22 till 21-11-26
Projekt nr: E21-042
Laboratorium: Toxicon AB
Testartikel: D2 (E21-042-1)
Försöksledare: Anders Walstad
Utförande: Anders Walstad, Susanne Fritz

Testart: *Pseudokirchneriella subcapitata*
Klon: NIVA CHL1

Konc	pH		Celler/ml (CPML)				(µ) tillväxt-	% inhibering
mg/l	0	72	0 (h)	24 (h)	48 (h)	72 (h)	hastighet	
kontroll 1	7,9	7,6	10000	30000	150000	1210000	1,60	
kontroll 2		7,5	10000	30000	190000	950000	1,52	
kontroll 3		7,6	10000	20000	120000	960000	1,52	
kontroll 4		7,7	10000	20000	150000	790000	1,46	
kontroll 5		7,8	10000	20000	160000	980000	1,53	
kontroll 6		7,9	10000	20000	120000	950000	1,52	
medel	**7,9**	**7,7**	**10000**	**23333**	**148333**	**973333**	**1,52**	
3,13	7,9	7,9	10000	20000	110000	880000	1,49	2,04
		8,0	10000	20000	140000	850000	1,48	2,79
		8,0	10000	20000	150000	850000	1,48	2,79
medel	**7,9**	**8,0**	**10000**	**20000**	**133333**	**860000**	**1,48**	**2,54**
6,25	7,9	8,1	10000	10000	170000	1160000	1,58	-4,01
		8,1	10000	10000	200000	1060000	1,55	-2,04
		8,1	10000	30000	160000	1070000	1,56	-2,24
medel	**7,9**	**8,1**	**10000**	**16667**	**176667**	**1096667**	**1,57**	**-2,76**
12,5	7,9	8,2	10000	50000	270000	1200000	1,60	-4,75
		8,2	10000	30000	210000	1290000	1,62	-6,33
		8,2	10000	10000	170000	1100000	1,57	-2,85
medel	**7,9**	**8,2**	**10000**	**30000**	**216667**	**1196667**	**1,59**	**-4,64**
25	7,9	8,3	10000	40000	240000	1400000	1,65	-8,12
		8,3	10000	30000	240000	1610000	1,69	-11,18
		8,3	10000	10000	250000	1110000	1,57	-3,04
medel	**7,9**	**8,3**	**10000**	**26667**	**243333**	**1373333**	**1,64**	**-7,45**
50	7,9	8,3	10000	20000	160000	1040000	1,55	-1,62
		8,3	10000	30000	200000	1210000	1,60	-4,93
		8,2	10000	10000	250000	1110000	1,57	-3,04
medel	**7,9**	**8,2**	**10000**	**20000**	**203333**	**1120000**	**1,57**	**-3,20**
100	7,9	8,2	10000	10000	110000	640000	1,39	9,00
		8,2	10000	20000	150000	790000	1,46	4,40
		8,2	10000	30000	130000	960000	1,52	0,13
medel	**7,9**	**8,2**	**10000**	**20000**	**130000**	**796667**	**1,45**	**4,51**
Sign	AWA	AWA	AWA	AWA	AWA	AWA	SF	SF

	Grafiskt	Probit
EC (I) 10:	> 100	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	100	

Övriga observationer:

Signatur: A WA ....

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**	Dokumentnr: **PROT 033-3**

Godkänd av: Thomas Olsson	Datum: **2010-02-05**	Gäller från: **2010-02-01**	Sid av: **1(1)**

Datum: 21-11-22 till 21-11-26
Laboratorium: Toxicon AB
Försöksledare: Anders Walstad
Utförande: Anders Walstad, Susanne Fritz

Projekt nr: E21-042
Testartikel: D1 (E21-042-2)

Testart: *Pseudokirchneriella subcapitata*
Klon: NIVA CHL1

Konc mg/l	pH		Celler/ml (CPML)				(µ) tillväxt-hastighet	% inhibering
	0	72	0 (h)	24 (h)	48 (h)	72 (h)		
kontroll 1	7,9	7,6	10000	30000	150000	1210000	1,60	
kontroll 2		7,5	10000	30000	190000	950000	1,52	
kontroll 3		7,6	10000	20000	120000	960000	1,52	
kontroll 4		7,7	10000	20000	150000	790000	1,46	
kontroll 5		7,8	10000	20000	160000	980000	1,53	
kontroll 6		7,9	10000	20000	120000	950000	1,52	
medel	**7,9**	**7,7**	**10000**	**23333**	**148333**	**973333**	**1,52**	
3,13	7,9	8,2	10000	60000	140000	910000	1,50	1,30
		8,2	10000	10000	130000	870000	1,49	2,29
		8,2	10000	10000	150000	930000	1,51	0,83
medel	**7,9**	**8,2**	**10000**	**26667**	**140000**	**903333**	**1,50**	**1,47**
6,25	7,9	8,2	10000	30000	130000	830000	1,47	3,32
		8,2	10000	20000	110000	830000	1,47	3,32
		8,2	10000	40000	110000	880000	1,49	2,04
medel	**7,9**	**8,2**	**10000**	**30000**	**116667**	**846667**	**1,48**	**2,89**
12,5	7,9	8,2	10000	30000	190000	920000	1,51	1,06
		8,2	10000	20000	160000	880000	1,49	2,04
		8,2	10000	40000	180000	840000	1,48	3,05
medel	**7,9**	**8,2**	**10000**	**30000**	**176667**	**880000**	**1,49**	**2,05**
25	7,9	8,3	10000	50000	220000	1130000	1,58	-3,44
		8,3	10000	10000	150000	1220000	1,60	-5,11
		8,3	10000	10000	120000	930000	1,51	0,83
medel	**7,9**	**8,3**	**10000**	**23333**	**163333**	**1093333**	**1,56**	**-2,57**
50	7,9	8,3	10000	30000	130000	1000000	1,54	-0,76
		8,3	10000	30000	170000	1290000	1,62	-6,33
		8,4	10000	30000	120000	1210000	1,60	-4,93
medel	**7,9**	**8,3**	**10000**	**30000**	**140000**	**1166667**	**1,58**	**-4,01**
100	7,9	8,3	10000	10000	210000	1040000	1,55	-1,62
		8,3	10000	10000	190000	1270000	1,61	-5,99
		8,3	10000	10000	120000	1090000	1,56	-2,65
medel	**7,9**	**8,3**	**10000**	**10000**	**173333**	**1133333**	**1,58**	**-3,42**
Sign	AWA	AWA	AWA	SF	SF	AWA	SF	SF

	Grafiskt	Probit
EC (I) 10:	> 100	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	100	

Övriga observationer:

Signatur: A WA

Appendix 2

Report from analysis of TOC. ALS report ST2134887

Analyscertifikat

Ordernummer	: **ST2134887**	Sida	: 1 av 3
Kund	: **Toxicon AB**	Projekt	: ----
Kontaktperson	: Anders Walstad	Beställningsnummer	: ----
Adress	: Sverige	Provtagare	: ----
		Provtagningspunkt	: ----
		Ankomstdatum, prover	: 2021-12-01 12:20
E-post	: anders.walstad@alsglobal.com	Analys påbörjad	: 2021-12-03
Telefon	: ----	Utfärdad	: 2021-12-13 16:42
C-O-C-nummer (eller Orderblankett-nummer)	: ----	Antal ankomna prover	: 6
Offertnummer	: ST2020SE-TOX-AB0001 (OF200580)	Antal analyserade prover	: 6

Generell kommentar

Denna rapport får endast återges i sin helhet, om inte utfärdande laboratorium i förväg skriftligen godkänt annat. Laboratoriet tar inget ansvar för information i denna rapport som har lämnats av kunden, eller resultat som kan ha påverkats av sådan information. Beträffande laboratoriets ansvar i samband med uppdrag, se vår webbplats www.alsglobal.se

Signatur	*Position*
Niels-Kristian Terkildsen	Laboratoriechef

Analysresultat

Matris: **VATTEN**

Provbeteckning	**E21-042-Blank T0**
Laboratoriets provnummer	*ST2134887-001*
Provtagningsdatum / tid	*ej specificerad*

Parameter	**Resultat**	MU	*Enhet*	*LOR*	*Analyspaket*	*Metod*	*Utf.*
Övrigt							
TOC	0.77	± 0.15	mg/L	0.50	TOC	W-TOC-IR	PR

Matris: **VATTEN**

Provbeteckning	**E21-042-Blank T72**
Laboratoriets provnummer	*ST2134887-002*
Provtagningsdatum / tid	*ej specificerad*

Parameter	**Resultat**	MU	*Enhet*	*LOR*	*Analyspaket*	*Metod*	*Utf.*
Övrigt							
TOC	1.16	± 0.23	mg/L	0.50	TOC	W-TOC-IR	PR

Matris: **VATTEN**

Provbeteckning	**E21-042-1 T0**
Laboratoriets provnummer	*ST2134887-003*
Provtagningsdatum / tid	*ej specificerad*

Parameter	**Resultat**	MU	*Enhet*	*LOR*	*Analyspaket*	*Metod*	*Utf.*
Övrigt							
TOC	0.64	± 0.13	mg/L	0.50	TOC	W-TOC-IR	PR

Matris: **VATTEN**

Provbeteckning	**E21-042-1 T72**
Laboratoriets provnummer	*ST2134887-004*
Provtagningsdatum / tid	*ej specificerad*

Parameter	**Resultat**	MU	*Enhet*	*LOR*	*Analyspaket*	*Metod*	*Utf.*
Övrigt							
TOC	<0.50	----	mg/L	0.50	TOC	W-TOC-IR	PR

Matris: **VATTEN**

Provbeteckning	**E21-042-2 T0**
Laboratoriets provnummer	*ST2134887-005*
Provtagningsdatum / tid	*ej specificerad*

Parameter	**Resultat**	MU	*Enhet*	*LOR*	*Analyspaket*	*Metod*	*Utf.*
Övrigt							
TOC	<0.50	----	mg/L	0.50	TOC	W-TOC-IR	PR

Matris: **VATTEN**

Provbeteckning	**E21-042-2 T72**
Laboratoriets provnummer	*ST2134887-006*
Provtagningsdatum / tid	*ej specificerad*

Parameter	**Resultat**	MU	*Enhet*	*LOR*	*Analyspaket*	*Metod*	*Utf.*
Övrigt							
TOC	<0.50	----	mg/L	0.50	TOC	W-TOC-IR	PR

Metodsammanfattningar

Analysmetoder	*Metod*
W-TOC-IR	Bestämning av TOC med IR detektion enligt metod baserad på CSN EN 1484, CSN EN 16192 och SM 5310.

Nyckel: **LOR** = Den rapporteringsgräns (LOR) som anges är standard för respektive parameter i metoden. Rapporteringsgränsen kan påverkas vid t.ex. spädning p.g.a. matrisstörningar, begränsad provmängd eller låg torrsubstanshalt.

MU = Mätosäkerhet

***** = Asterisk efter resultatet visar på ej ackrediterat test, gäller både egna lab och underleverantör

Mätosäkerhet:

Mätosäkerheten anges som en utvidgad osäkerhet (enligt definitionen i "Evaluation of measurement data- Guide to the expression of uncertainty in measurement", JCGM 100:2008 Corrected version 2010) beräknad med täckningsfaktor lika med 2 vilket ger en konfidensnivå på ungefär 95%.
Mätosäkerhet anges endast för detekterade ämnen med halter över rapporteringsgränsen.

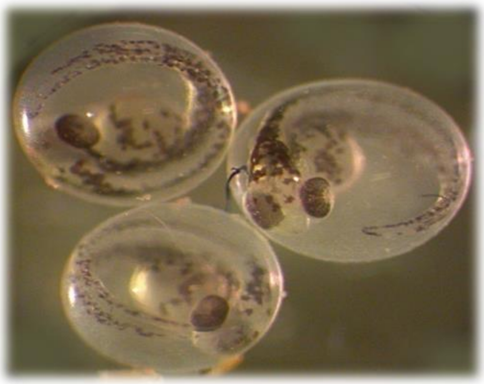
Mätosäkerhet från underleverantör anges oftast som en utvidgad osäkerhet beräknad med täckningsfaktor 2. För ytterligare information kontakta laboratoriet.

Utförande laboratorium (teknisk enhet inom ALS Scandinavia eller anlitat laboratorium (underleverantör)).

	Utf.
PR	*Analys utförd av* ALS Czech Republic s.r.o Prag, Na Harfe 336/9 Prag Tjeckien 190 00 Ackrediterad av: CAI Ackrediteringsnummer: 1163

GROWTH INHIBITION TEST USING *PSEUDOKIRCHNERIELLA SUBCAPITATA*

subjected to the water-accomodated fraction of five different samples: E85-2, P95-3, P98-2, D-EU1 and E01-1



Report E23-025.2

Härslöv October 2023

Contents

Appendices

1: Protocol for “Freshwater Algae and Cyanobacteria, Growth Inhibition Test”
2: Report from analysis of TOC, Anox Kaldnes report 2023-249

Summary

This report is a revised version of report E23-025. Report E23-025.2 replaces report E23-025 in its entirety.

During the autumn 2023, a study on the test items “E85-2”, “P95-3”, “P98-2”, “D-EU1” and “E01-1” was performed by Toxicon AB, Härslöv, Sweden. The study included one method for ecotoxicological testing.

The method used was:
- OECD Guidelines for the Testing of Chemicals No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011).

The test was performed on the water-accommodated fraction (WAF) of the test items. The method for WAF is outlined in OECD series on testing and assessment no. 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

In the method OECD TG No. 201, the No Observed Effect Concentration (NOEC) value is defined as the highest tested concentration not statistically different from the control (unpaired t-test *p*>0,05).

Results from the algae test is presented in Table 1.

Table 1. Nominal NOEC-value and E_rC-values (mg/L) for *Pseudokirchneriella subcapitata* determined after 72 hours of exposure to a WAF from the loading rate (100 mg/L) of the respective test items E85-2, P95-3, P98-2, D-EU1 and E01-1.

Test item	**NOEC mg/L**	**E_rC_{10} mg/L**	**E_rC_{50} mg/L**
E85-2	50	> 100	> 100
P95-3	100	> 100	> 100
P98-2	50	86.5	> 100
D-EU1	100	> 100	> 100
E01-1	50	67.2	> 100

Introduction

Samples of the products “E85-2”, “P95-3”, “P98-2”, “D-EU1” and “E01-1” were received at Toxicon AB on August 16th, 2023 for environmental assessment. The tests were performed according to OECDs Guidelines for testing of chemicals.

Test laboratory:

Toxicon AB
Rosenhällsvägen 29
SE-261 92 HÄRSLÖV
Sweden
Telephone: +46 418 70 700

Study director: Anders Walstad ………………………… Date: 2023-10-31 ………………………..
Anders Walstad

Performance:

Anders Walstad (Toxicon AB)
Susanne Fritz (Toxicon AB)
Carolina Cammernäs (AnoxKaldnes, Veolia Water Techn. AB)

Sponsor:

IVL Svenska Miljöinstitutet AB
Box 210 60
SE-100 31 STOCKHOLM
SWEDEN

Sponsors reference: Johan Strandberg

Test articles:

Sponsors sample code	Toxicon sample code
E85-2	E23-025-1
P95-3	E23-025-2
P98-2	E23-025-3
D-EU1	E23-025-4
E01-1	E23-025-5

Test methods

The methods used were:

- OECD Guidelines for the Testing of Chemicals No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2011). Toxicon SOP T003.
- OECD Series on testing and assessment No. 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

Freshwater Alga and Cyanobacteria, Growth Inhibition Test – OECD 201

The purpose of the test is to determine the effects of a substance on the growth of a freshwater microalgae. Exponentially growing cultures of algae are exposed to the water-accommodated fraction (WAF) of the test items over a period of 72 hours. Despite of the relatively brief test duration, effects over several generations can be assessed. The response variable is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations (loading rates) of the test substances. The response is evaluated as the function of the exposure concentration in comparison to the average growth of an unexposed control culture. Growth, and growth inhibition, are quantified from measurements of the algal biomass (dry weight per volume) as a function of time. Cell counts (cells/ml) are used as a surrogate parameter to dry weight (OECD TG No. 201). The test endpoint is inhibition of growth, which is expressed as the logarithmic increase in biomass (average specific growth rate) during the period of exposure.

Test organism

The test organism is a laboratory culture of the green alga *Pseudokirchneriella subcapitata*. The inoculum is taken from Toxicon's culture of *P. subcapitata* (NIVA CHL 1), which originates from NIVA, Oslo, Norway. A qualification test using potassium dichromate was performed on June 19th, 2023 in order to assure the quality of the test organism. E_rC_{50} was found to be 1.5 mg/L which is in the range 0.9 – 1.5 mg/L confirming that the culture was suitable as a test organism according to OECD TG No. 201.

Growth medium (dilution solution)

The composition of the growth medium was as specified in Annex 3 in OECD TG No. 201. Recipe for the growth medium is presented in Table 2. The growth medium had a pH of 8.1±0.2.

Table 2. Preparation of the growth medium. The substances were added to reversed osmosis water, <10 µS/cm.

Single substance	µg/L	Single substance	µg/L
NH_4Cl	15000	H_3BO_3	185
$MgCl_2$ x 6 H_2O	12000	$MnCl_2$ x 4 H_2O	415
$CaCl_2$ x 2 H_2O	18000	$ZnCl_2$	3
$MgSO_4$ x 7 H_2O	15000	$CoCl_2$ x 6 H_2O	1,5
KH_2PO_4	1600	$CuCl_2$ x 2 H_2O	0.01
$FeCl_3$ x 6 H_2O	64	Na_2MoO_4 x 2 H_2O	7
Na_2EDTA x 2 H_2O	100	$NaHCO_3$	50000

Preparation of test solution

Fresh extraction of the test concentration (loading rate) was prepared as described in OECD No. 23. Using a small beaker, the test items were weighed and then rinsed into glass bottles with 4000 millilitres of growth medium. The bottles had 20-25 % head space and were closed using Teflon lined caps (Table 2). The test concentration was stirred over night at a speed that created a small vortex at the surface. After being allowed to settle for 1 hour, the water phase was collected from the bottle and subsequently transferred to test glass vessels. The process was performed at room temperature (20-22 °C).

The highest test concentration (loading rate) was 100 mg/L and the subsequent concentrations were prepared using dilution.

Ecotoxicological testing

The nutrient concentration in the growth medium was the same in the control as in the test solution. Six replicates each were used for the control while the test solutions were tested in triplicates. Test bottles were 250 ml glass E-flasks with 100 ml solution, incubated on a shaking table with approximately 100 µE m-2 s-1 continuous illumination at a temperature of 21-24±2 °C. The pH in all solutions was measured at the test start, and again in all replicates at the completion of the test. The incubation period was 72 hours with sampling at 24, 48, and 72 hours. As a measurement of the biomass in the samples, the number of cells/mL was counted using an optical microscope.

Reference analysis

At the start of the test, approximately 100 mL of the highest test concentrations of the test items and the control were placed in refrigerator for future reference analysis regarding the content of total organic carbon (TOC). Another E-flask containing the highest test concentration of the test items and the control were incubated on the same shaking table as the control and the test solution. These flasks were not inoculated with algae but were used for reference analysis showing the integrity of the test item after 72 hours.

The reference analysis was performed by AnoxKaldnes (Veolia Water Technologies AB).

Calculations

Cell count is one of the proposed surrogate parameters for biomass according to the OECD TG No. 201. The average specific growth rate (µ) for a specific period is calculated as the logarithmic increase in the biomass from the equation for each single vessel of the control and test concentrations:

$$\mu = \frac{\ln N_L - \ln N_0}{t_L - t_0}$$

where:

t_0	is the time of teststart (days)
t_L	is the time of exposure at the end of the test (days)
N_0	is the initial, nominal, concentration of cells
N_L	is the nominal cell concentration at t_L

For the control and test concentrations, an average growth rate along with variance estimates is calculated. The specific growth rate for each test concentration is then related to the average growth rate of the control:

$I_{\mu i} = [(\mu_c - \mu_i)/\ \mu_c] * 100$

where:
$I_{\mu i}$ = percent inhibition of specific growth rate in replicate *i*;
μ_c = mean value for average specific growth rate (μ) in the control group;
μ_r = average specific growth rate (μ) for the test concentration replicate.

The No Observed Effect Concentration (NOEC), the highest concentration with no statistical difference to the control (p >0,05), is determined using unpaired t-test.

Results

Freshwater Alga and Cyanobacteria, Growth Inhibition Test – OECD 201

The criteria for the validity of the test according to OECD TG No. 201 were fulfilled:

- The biomass in the control culture increased by a factor of more than 16 within 72 hours.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 7%.
- The pH did not change with more than 1.5 units during the test.

Calculated response variables for the control group and the highest test concentration (loading rate) (mean ± coefficient variation) are presented in Table 3. The growth of the algae in the control and in the test concentration during the 72 hours of exposure is presented as cells/mL from the cell counting (Figures 1-5). NOEC and the E_rC-values are presented in Table 3.

There was no statistical difference (unpaired t-test, p>0,05) in the variable average specific growth rate between the control and the highest test concentration (loading rate) of 100 mg/L in the test items P95-3 and D-EU1 and therefore the NOEC-value was determined to be 100 mg/L for those test items.

The highest test concentrations of the test items E85-2, P98-2 and E01-1 did display a significant difference ($p<0.05$) from their respective control, and therefore the NOEC for those test items were 50 mg/L. Any significant difference between a control and a test item that is the result of a stimulating effect has not been considered a toxic effect in this study.

Primary data are presented in Appendix 1.

Table 3. Calculated response variables for the control and the loading rate (mean ± coefficient of variation) after 72 hours of exposure to the highest test concentrations of the test items E85-2, P95-3, P98-2, D-EU1 and E01-1. Control was the control for sample E85-2 and P95-3 while Control 2 was used as control for samples P98-2 and D-EU1. Control 3 was used for E01-1.

Test item Loading rate 100 mg/L	Specific growth rate (µ) Mean ± CV (%)	NOEC mg/L	E_rC_{10} mg/L	E_rC_{50} mg/L
Control	1.51 ± 4.11	-	-	-
E85-2	1.37 ± 3.63	50	> 100	> 100
P95-3	1.41 ± 6.07	100	> 100	> 100
Control 2	1.58 ± 4.89	-	-	-
P98-2	1.38 ± 4.63	50	86.5	> 100
D-EU1	1.48 ± 1.05	100	> 100	> 100
Control 3	1.44 ± 2.42	-	-	-
E01-1	1.16 ± 7.42	50	67.2	> 100

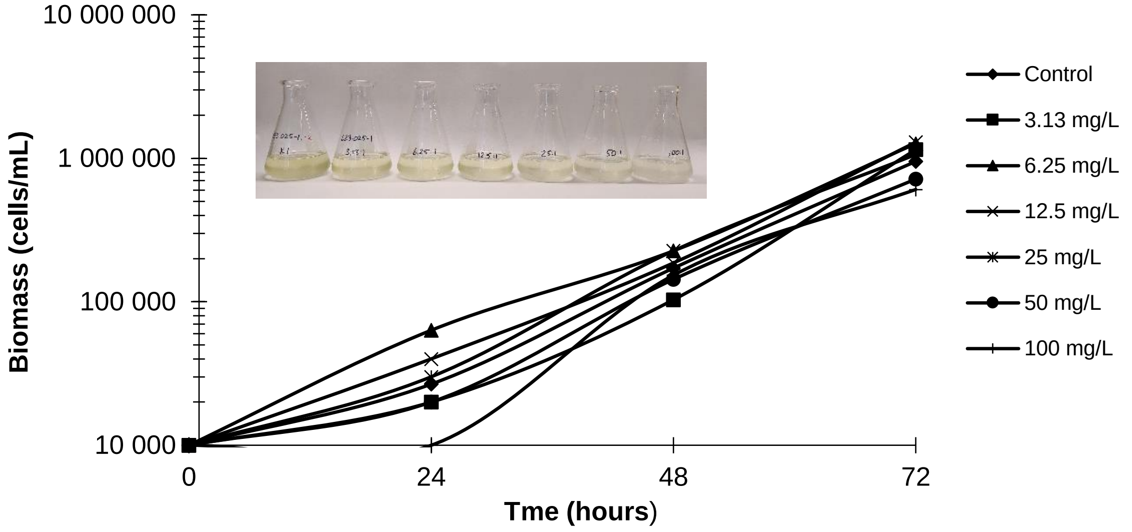


Figure 1. The biomass (cells/mL) in the control and in the test concentrations (loading rate, WAF) after 0, 24, 48 and 72 hours of exposure to the test item E85-2.

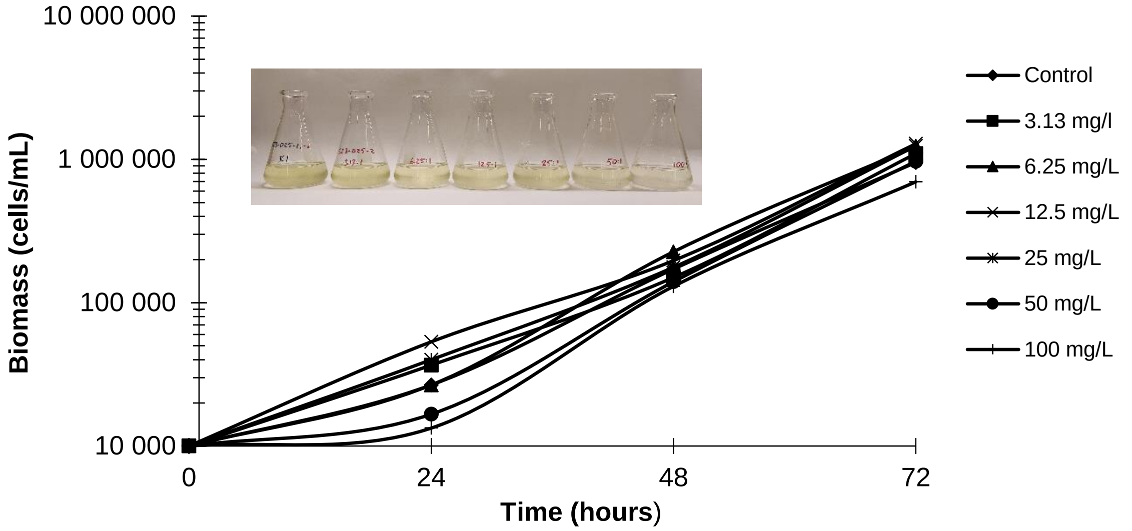


Figure 2. The biomass (cells/mL) in the control and in the test concentrations (loading rate, WAF) after 0, 24, 48 and 72 hours of exposure to the test item P95-3.

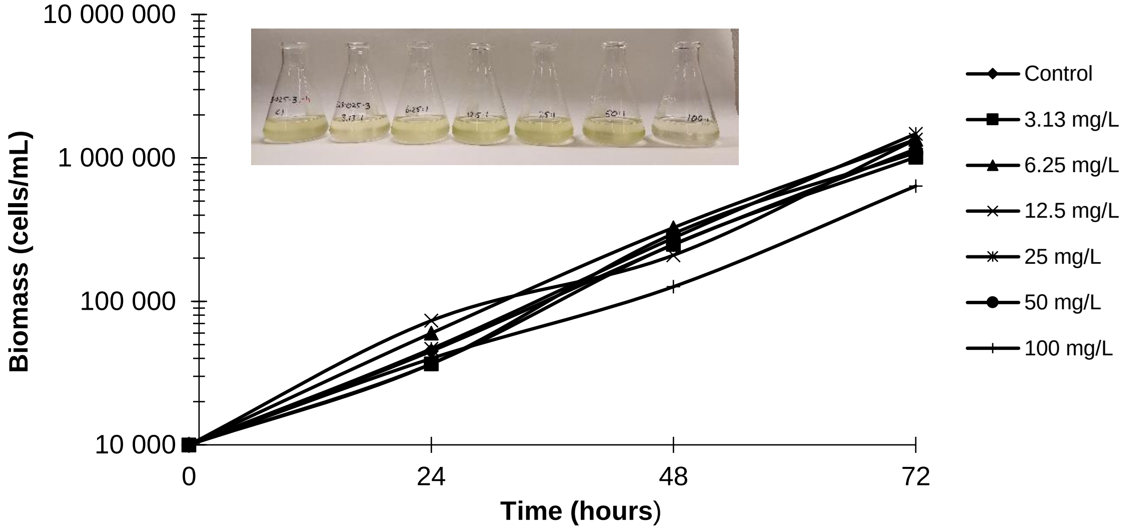


Figure 3. The biomass (cells/mL) in the control and in the test concentrations (loading rate, WAF) after 0, 24, 48 and 72 hours of exposure to the test item P98-2.

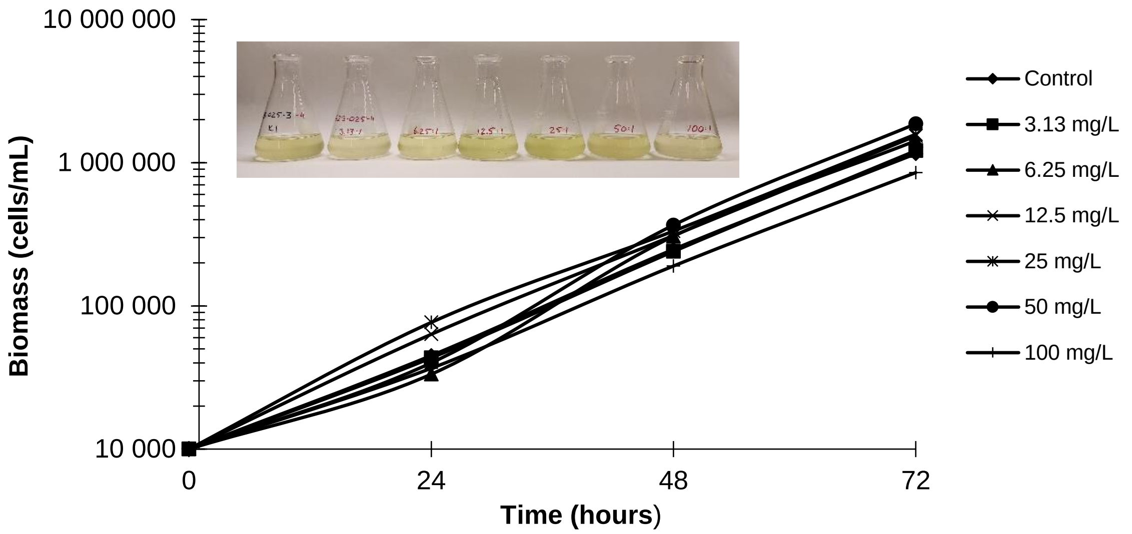


Figure 4. The biomass (cells/mL) in the control and in the test concentrations (loading rate, WAF) after 0, 24, 48 and 72 hours of exposure to the test item D-EU1.

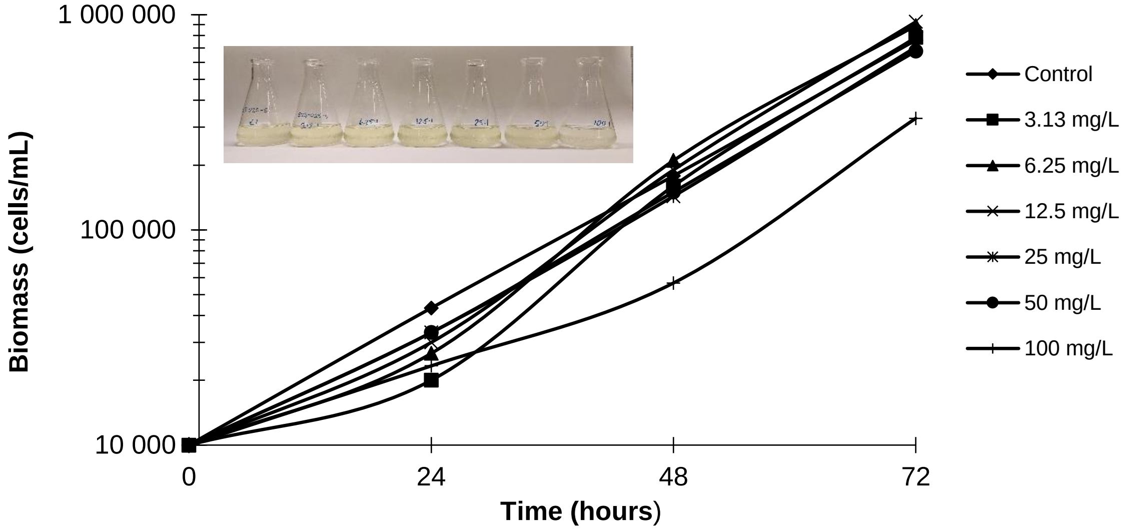


Figure 5. The biomass (cells/mL) in the control and in the test concentrations (loading rate, WAF) after 0, 24, 48 and 72 hours of exposure to the test item E01-1.

Reference analysis

The results from the analysis of TOC indicate that the WAFs from E85-2, P95-3 and P98-2 are stable during the 72 hours of exposure to the algae. The low concentrations of TOC in D-EU1 and E01-1 makes it difficult to draw any conclusions regarding the stability of these test items. Results from the reference analysis are presented in Table 4 and Appendix 2.

Table 4. Results from the analysis of total organic carbon at the beginning (0 h) and the end (72 h) of the exposure. Control was the control from sample E85-2 and P95-3 while Control 2 was used as control with samples P98-2, D-EU1 and E01-1.

Test Sample Loading rate 100 mg/L	**TOC (mg/L)**	
	0 h	**72 h**
Control	< 1.0	< 1.0
E85-2	49	48
P95-3	6.9	6.6
P98-2	4.2	3.8
D-EU1	< 1.0	< 1.0
E01-1	1.2	< 1.0
Control 2	< 1.0	< 1.0

References

1. OECD Guideline for the testing of chemicals No. 201 “Freshwater Alga and Cyanobacteria, Growth Inhibition Test”. Adopted 2011.

2. OECD series on testing and assessment No. 23 (Guidance document on aquatic toxicity testing of difficult substances and mixtures).

Appendix 1

Protocol for “Freshwater Algae and Cyanobacteria, Growth Inhibition Test”

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**		Dokumentnr: **PROT 033-3**
Godkänd av: Thomas Olsson	Datum: **2010-02-05**	Gäller från: **2010-02-01** Sid av: **1(1)**

Datum: 230912 till: 230915
Laboratorium: Toxicon AB
Försöksledare: Anders Walstad
Utförande: Anders Walstad, Susanne Fritz

Projekt nr: E23-025
Testartikel: E85-2
(E23-025-1)

Testart: *Pseudokirchneriella subcapitata*
Klon: NIVA CHL1

Konc mg/l	pH		Celler/ml (CPML)				(µ) tillväxthastighet	% inhibering
	0	72	0 (h)	24 (h)	48 (h)	72 (h)		
kontroll 1	7,87	7,50	10000	10000	140000	1220000	1,60	
kontroll 2		7,39	10000	40000	210000	920000	1,51	
kontroll 3		7,23	10000	20000	180000	1040000	1,55	
kontroll 4		7,24	10000	60000	150000	750000	1,44	
kontroll 5		7,21	10000	10000	250000	1010000	1,54	
kontroll 6		7,23	10000	20000	100000	770000	1,45	
medel	**7,9**	**7,3**	**10000**	**26667**	**171667**	**951667**	**1,51**	
3,13	7,82	7,24	10000	10000	110000	1160000	1,58	-4,68
		7,28	10000	30000	60000	1040000	1,55	-2,27
		7,45	10000	20000	140000	1250000	1,61	-6,32
medel	**7,8**	**7,3**	**10000**	**20000**	**103333**	**1150000**	**1,58**	**-4,43**
6,25	7,82	7,48	10000	100000	240000	1320000	1,63	-7,52
		7,46	10000	30000	220000	1140000	1,58	-4,30
		7,57	10000	60000	220000	1340000	1,63	-7,86
medel	**7,8**	**7,5**	**10000**	**63333**	**226667**	**1266667**	**1,61**	**-6,56**
12,5	7,83	7,52	10000	50000	150000	1390000	1,64	-8,66
		7,50	10000	40000	190000	1070000	1,56	-2,90
		7,52	10000	30000	220000	1430000	1,65	-9,29
medel	**7,8**	**7,5**	**10000**	**40000**	**186667**	**1296667**	**1,62**	**-6,95**
25	7,84	7,45	10000	10000	200000	1080000	1,56	-3,11
		7,42	10000	30000	240000	950000	1,52	-0,28
		7,39	10000	50000	240000	1160000	1,58	-4,68
medel	**7,8**	**7,4**	**10000**	**30000**	**226667**	**1063333**	**1,55**	**-2,69**
50	7,96	7,17	10000	10000	120000	780000	1,45	4,06
		7,16	10000	10000	180000	720000	1,43	5,82
		7,16	10000	40000	130000	650000	1,39	8,08
medel	**8,0**	**7,2**	**10000**	**20000**	**143333**	**716667**	**1,42**	**5,99**
100	8,07	6,96	10000	10000	120000	520000	1,32	12,99
		6,94	10000	10000	210000	600000	1,36	9,84
		6,87	10000	10000	130000	700000	1,42	6,44
medel	**8,1**	**6,9**	**10000**	**10000**	**153333**	**606667**	**1,37**	**9,76**
Sign	AWA	AWA	AWA	SF	SF	SF	SF	SF

	Grafiskt	Probit
EC (I) 10:	> 100	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	50,0	

Övriga observationer:

Signatur: A WA

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**	Dokumentnr: **PROT 033-3**
Godkänd av: Thomas Olsson Datum: **2010-02-05**	Gäller från: **2010-02-01** Sid av: **1(1)**

Datum: 230912 till: 230915
Laboratorium: Toxicon AB
Försöksledare: Anders Walstad
Utförande: Anders Walstad, Susanne Fritz

Projekt nr: E23-025
Testartikel: P95-3
(E23-025-2)

Testart: *Pseudokirchneriella subcapitata*
Klon: NIVA CHL1

Konc mg/l	pH		Celler/ml (CPML)				(µ) tillväxt-hastighet	% inhibering
	0	72	0 (h)	24 (h)	48 (h)	72 (h)		
kontroll 1	7,87	7,50	10000	10000	140000	1220000	1,60	
kontroll 2		7,39	10000	40000	210000	920000	1,51	
kontroll 3		7,23	10000	20000	180000	1040000	1,55	
kontroll 4		7,24	10000	60000	150000	750000	1,44	
kontroll 5		7,21	10000	10000	250000	1010000	1,54	
kontroll 6		7,23	10000	20000	100000	770000	1,45	
medel	**7,9**	**7,3**	**10000**	**26667**	**171667**	**951667**	**1,51**	
3,13	7,86	7,21	10000	40000	150000	1320000	1,63	-7,52
		7,53	10000	10000	100000	920000	1,51	0,43
		7,31	10000	60000	200000	1050000	1,55	-2,49
medel	**7,9**	**7,4**	**10000**	**36667**	**150000**	**1096667**	**1,56**	**-3,19**
6,25	7,72	7,39	10000	20000	240000	1250000	1,61	-6,32
		7,52	10000	30000	210000	1320000	1,63	-7,52
		7,61	10000	30000	230000	1130000	1,58	-4,10
medel	**7,7**	**7,5**	**10000**	**26667**	**226667**	**1233333**	**1,60**	**-5,98**
12,5	7,66	7,55	10000	60000	320000	1260000	1,61	-6,50
		7,52	10000	40000	150000	1010000	1,54	-1,63
		7,62	10000	60000	120000	1580000	1,69	-11,48
medel	**7,7**	**7,6**	**10000**	**53333**	**196667**	**1283333**	**1,61**	**-6,54**
25	7,73	7,66	10000	20000	230000	1170000	1,59	-4,87
		7,61	10000	40000	150000	1400000	1,65	-8,82
		7,61	10000	60000	150000	1150000	1,58	-4,49
medel	**7,7**	**7,6**	**10000**	**40000**	**176667**	**1240000**	**1,61**	**-6,06**
50	7,85	7,60	10000	30000	100000	900000	1,50	0,91
		7,51	10000	10000	160000	910000	1,50	0,67
		7,54	10000	10000	160000	1100000	1,57	-3,51
medel	**7,9**	**7,6**	**10000**	**16667**	**140000**	**970000**	**1,52**	**-0,64**
100	7,93	7,32	10000	20000	130000	620000	1,38	9,12
		7,26	10000	10000	140000	560000	1,34	11,36
		7,31	10000	10000	120000	910000	1,50	0,67
medel	**7,9**	**7,3**	**10000**	**13333**	**130000**	**696667**	**1,41**	**7,05**
Sign	AWA	AWA	AWA	SF	SF	SF	SF	SF

	Grafiskt	Probit
EC (I) 10:	> 100	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	100	

Övriga observationer:

Signatur: A WA ....

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**	Dokumentnr: **PROT 033-3**

Godkänd av: Thomas Olsson	Datum: **2010-02-05**	Gäller från: **2010-02-01**	Sid av: **1(1)**

Datum: 230919 till: 230922
Laboratorium: Toxicon AB
Försöksledare: Anders Walstad
Utförande: Anders Walstad, Susanne Fritz

Projekt nr: E23-025
Testartikel: P98-2
(E23-025-3)

Testart: *Pseudokirchneriella subcapitata*
Klon: NIVA CHL1

Konc	pH		Celler/ml (CPML)				(µ) tillväxt-hastighet	% inhibering
mg/l	0	72	0 (h)	24 (h)	48 (h)	72 (h)		
kontroll 1	7,75	7,26	10000	60000	390000	1330000	1,63	
kontroll 2		7,68	10000	50000	310000	1310000	1,63	
kontroll 3		7,78	10000	30000	220000	1140000	1,58	
kontroll 4		7,64	10000	50000	190000	1360000	1,64	
kontroll 5		7,40	10000	70000	220000	1050000	1,55	
kontroll 6		7,67	10000	10000	160000	740000	1,43	
medel	**7,8**	**7,6**	**10000**	**45000**	**248333**	**1155000**	**1,58**	
3,13	7,11	7,62	10000	50000	300000	870000	1,49	5,56
		7,59	10000	50000	180000	1180000	1,59	-0,89
		7,60	10000	10000	270000	990000	1,53	2,83
medel	**7,1**	**7,6**	**10000**	**36667**	**250000**	**1013333**	**1,54**	**2,50**
6,25	7,13	7,66	10000	70000	350000	1320000	1,63	-3,26
		7,69	10000	30000	340000	1510000	1,67	-6,10
		7,84	10000	80000	290000	1210000	1,60	-1,42
medel	**7,1**	**7,7**	**10000**	**60000**	**326667**	**1346667**	**1,63**	**-3,59**
12,5	7,12	7,90	10000	60000	180000	1190000	1,59	-1,07
		7,89	10000	70000	220000	1390000	1,64	-4,35
		7,93	10000	90000	230000	1450000	1,66	-5,24
medel	**7,1**	**7,9**	**10000**	**73333**	**210000**	**1343333**	**1,63**	**-3,55**
25	7,43	7,96	10000	30000	320000	1600000	1,69	-7,33
		7,96	10000	20000	230000	1270000	1,61	-2,44
		7,96	10000	90000	280000	1550000	1,68	-6,65
medel	**7,4**	**8,0**	**10000**	**46667**	**276667**	**1473333**	**1,66**	**-5,47**
50	7,64	7,91	10000	40000	260000	1150000	1,58	-0,34
		7,93	10000	30000	320000	1010000	1,54	2,40
		7,82	10000	40000	310000	1110000	1,57	0,41
medel	**7,6**	**7,9**	**10000**	**36667**	**296667**	**1090000**	**1,56**	**0,82**
100	7,73	7,63	10000	10000	110000	540000	1,33	15,64
		7,67	10000	50000	130000	590000	1,36	13,77
		7,44	10000	60000	140000	780000	1,45	7,87
medel	**7,7**	**7,6**	**10000**	**40000**	**126667**	**636667**	**1,38**	**12,43**
Sign	AWA	AWA	AWA	SF	SF	SF	SF	SF

	Grafiskt	Probit
EC (I) 10:	86,5	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	50,0	

Övriga observationer:

Signatur: AWA

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**	Dokumentnr: **PROT 033-3**
Godkänd av: Thomas Olsson Datum: **2010-02-05**	Gäller från: **2010-02-01** Sid av: **1(1)**

Datum: 230919 till: 230922

Laboratorium: Toxicon AB

Försöksledare: Anders Walstad

Utförande: Anders Walstad, Susanne Fritz

Projekt nr: E23-025

Testartikel: D-EU1

(E23-025-4)

Testart: *Pseudokirchneriella subcapitata*

Klon: NIVA CHL1

Konc	pH		Celler/ml (CPML)				(µ) tillväxt-	% inhibering
mg/l	0	72	0 (h)	24 (h)	48 (h)	72 (h)	hastighet	
kontroll 1	7,75	7,26	10000	60000	390000	1330000	1,63	
kontroll 2		7,68	10000	50000	310000	1310000	1,63	
kontroll 3		7,78	10000	30000	220000	1140000	1,58	
kontroll 4		7,64	10000	50000	190000	1360000	1,64	
kontroll 5		7,40	10000	70000	220000	1050000	1,55	
kontroll 6		7,67	10000	10000	160000	740000	1,43	
medel	**7,8**	**7,6**	**10000**	**45000**	**248333**	**1155000**	**1,58**	
3,13	7,22	7,43	10000	60000	250000	1400000	1,65	-4,50
		7,45	10000	20000	180000	1060000	1,55	1,38
		7,42	10000	50000	290000	1180000	1,59	-0,89
medel	**7,2**	**7,4**	**10000**	**43333**	**240000**	**1213333**	**1,60**	**-1,34**
6,25	7,16	7,37	10000	40000	370000	1440000	1,66	-5,10
		7,58	10000	40000	230000	1300000	1,62	-2,94
		7,72	10000	20000	320000	1530000	1,68	-6,38
medel	**7,2**	**7,6**	**10000**	**33333**	**306667**	**1423333**	**1,65**	**-4,80**
12,5	7,32	7,80	10000	20000	230000	1310000	1,63	-3,10
		7,99	10000	60000	340000	1610000	1,69	-7,46
		8,17	10000	110000	360000	1700000	1,71	-8,61
medel	**7,3**	**8,0**	**10000**	**63333**	**310000**	**1540000**	**1,68**	**-6,39**
25	7,41	8,05	10000	90000	350000	1870000	1,74	-10,62
		8,08	10000	90000	340000	1560000	1,68	-6,79
		8,18	10000	50000	310000	1310000	1,63	-3,10
medel	**7,4**	**8,1**	**10000**	**76667**	**333333**	**1580000**	**1,68**	**-6,84**
50	7,55	8,12	10000	20000	380000	1830000	1,74	-10,17
		8,04	10000	50000	390000	1770000	1,73	-9,46
		8,02	10000	50000	330000	2010000	1,77	-12,15
medel	**7,6**	**8,1**	**10000**	**40000**	**366667**	**1870000**	**1,74**	**-10,59**
100	7,54	8,02	10000	10000	200000	830000	1,47	6,55
		7,53	10000	80000	150000	900000	1,50	4,84
		7,42	10000	20000	220000	830000	1,47	6,55
medel	**7,5**	**7,7**	**10000**	**36667**	**190000**	**853333**	**1,48**	**5,98**
Sign	AWA	AWA	SF	SF	SF	SF	SF	SF

	Grafiskt	Probit
EC (I) 10:	> 100	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	100	

Övriga observationer:

Signatur: A WA

Titel: **Protokoll för tillväxthämning hos mikroalger enligt OECD TG 201, SS-EN ISO 8692, EU Dir 92/69/EEC**		Dokumentnr: **PROT 033-3**	
Godkänd av: Thomas Olsson	Datum: **2010-02-05**	Gäller från: **2010-02-01**	Sid av: **1(1)**

Datum: 230919 till: 230922

Laboratorium: Toxicon AB

Försöksledare: Anders Walstad

Utförande: Anders Walstad, Susanne Fritz

Projekt nr: E23-025

Testartikel: E01-1

(E23-025-5)

Testart: *Pseudokirchneriella subcapitata*

Klon: NIVA CHL1

Konc mg/l	pH		Celler/ml (CPML)				(µ) tillväxthastighet	% inhibering
	0	72	0 (h)	24 (h)	48 (h)	72 (h)		
kontroll 1	7,72	7,63	10000	60000	230000	820000	1,47	
kontroll 2		7,79	10000	20000	200000	690000	1,41	
kontroll 3		7,81	10000	20000	210000	670000	1,40	
kontroll 4		7,66	10000	70000	130000	720000	1,43	
kontroll 5		7,68	10000	60000	180000	800000	1,46	
kontroll 6		7,68	10000	30000	120000	870000	1,49	
medel	**7,7**	**7,7**	**10000**	**43333**	**178333**	**761667**	**1,44**	
3,13	7,24	7,77	10000	20000	170000	780000	1,45	-0,66
		7,64	10000	30000	150000	760000	1,44	-0,05
		7,66	10000	10000	160000	810000	1,46	-1,53
medel	**7,2**	**7,7**	**10000**	**20000**	**160000**	**783333**	**1,45**	**-0,75**
6,25	7,32	7,72	10000	30000	150000	960000	1,52	-5,45
		7,74	10000	20000	240000	730000	1,43	0,88
		7,76	10000	30000	240000	980000	1,53	-5,93
medel	**7,3**	**7,7**	**10000**	**26667**	**210000**	**890000**	**1,49**	**-3,50**
12,5	7,39	7,77	10000	50000	260000	1080000	1,56	-8,17
		7,71	10000	20000	170000	850000	1,48	-2,64
		7,62	10000	20000	140000	850000	1,48	-2,64
medel	**7,4**	**7,7**	**10000**	**30000**	**190000**	**926667**	**1,51**	**-4,48**
25	7,55	7,64	10000	40000	160000	730000	1,43	0,88
		7,54	10000	30000	140000	740000	1,43	0,56
		7,53	10000	30000	130000	650000	1,39	3,56
medel	**7,6**	**7,6**	**10000**	**33333**	**143333**	**706667**	**1,42**	**1,66**
50	7,63	7,66	10000	30000	110000	790000	1,46	-0,95
		7,62	10000	60000	170000	620000	1,38	4,65
		7,64	10000	10000	170000	620000	1,38	4,65
medel	**7,6**	**7,6**	**10000**	**33333**	**150000**	**676667**	**1,40**	**2,78**
100	7,78	7,71	10000	40000	70000	380000	1,21	15,96
		7,57	10000	10000	50000	370000	1,20	16,58
		7,51	10000	20000	50000	240000	1,06	26,58
medel	**7,8**	**7,6**	**10000**	**23333**	**56667**	**330000**	**1,16**	**19,70**
Sign	AWA	AWA	AWA	SF	SF	SF	SF	SF

	Grafiskt	Probit
EC (I) 10:	67,2	
EC (I) 50:	> 100	
EC (I) 90:		
NOEC:	50,0	

Övriga observationer:

Signatur: A.W.A.

Appendix 2

Report from analysis of TOC. AnoxKaldnes report 2023-249

VEOLIA

SWEDAC ACKREDITERING
Ackred. nr. 2031
Provning
ISO/IEC 17025

RAPPORT
utfärdat av ackrediterat laboratorium
REPORT issued by an Accredited Laboratory

AnoxKaldnes Analyslaboratoriet
Analysrapport

Uppdragsgivare

Toxicon AB
Att: Anders Walstad
Rosenhällsvägen 29
261 92 HÄRSLÖV

Provuppgifter

Datum:	2023.10.11
Uppdragsnr:	2023-249
Ank.datum:	2023.10.05
Ank. Temp.:	Fryst

Analysresultat

2023-249-1 **E23-025-0, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

2023-249-2 **E23-025-0 ,72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

2023-249-3 **E23-025-1, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	49	mg/l	± 8%	231010

2023-249-4 **E23-025-1, 72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	48	mg/l	± 8%	231010

2023-249-5 **E23-025-2, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	6,9	mg/l	± 21%	231010

2023-249-6 **E23-025-2, 72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	6,6	mg/l	± 21%	231010

2023-249-7 **E23-025-3, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	4,2	mg/l	± 21%	231010

2023-249-8 **E23-025-3, 72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	3,8	mg/l	± 21%	231010

Den angivna mätosäkerheten är beräknad med täckningsfaktor k=2, vilket ger en konfidensnivå på ca 95%.
Denna rapport får endast återges i sin helhet, om inte utförande laboratorium i förväg skriftligen godkänt annat.

Om provtagningsdatum ej är angivet förutsätter laboratoriet att provtagning skett inom föreskriven tid.

Resultaten gäller för provet såsom det har mottagits.

AnoxKaldnes Analyslaboratoriet
Analysrapport

Uppdragsnr: 2023-249

2023-249-9 **E23-025-4, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

2023-249-10 **E23-025-4, 72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

2023-249-11 **E23-025-5, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	1,2	mg/l	± 21%	231010

2023-249-12 **E23-025-5, 72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

2023-249-13 **E23-025-0-2, 0h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

2023-249-14 **E23-025-0-2, 72h**

Parameter	Metod	Resultat	Enhet	Mätosäkerhet	Analysdat.
Icke flyktigt totalt organiskt kol (NVOC-TOC)	SS-EN ISO 20236:2021	<1,0	mg/l		231010

DocuSigned by:
62AA59B5D9B34E1...

Carolina Cammernäs
Stf teknisk ledare

Den angivna mätosäkerheten är beräknad med täckningsfaktor k=2, vilket ger en konfidensnivå på ca 95%.
Denna rapport får endast återges i sin helhet, om inte utförande laboratorium i förväg skriftligen godkänt annat.

Om provtagningsdatum ej är angivet förutsätter laboratoriet att provtagning skett inom föreskriven tid.

Resultaten gäller för provet såsom det har mottagits.

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(EPA/600/4-89/001)

Uppdragsgivare

IVLSvenska Miljöinstitutet AB
Johan Strandberg
Box 210 60
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **Evolution diesel.**	Startdatum: 211213	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 5.6, 11.2, 22.5, 45 o 90.0.	Spädmedium: "EPA moderately hard" 80-100 mg/l ($CaCO_3$) och Konduktivitet 31 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	7,1	31,2	17	**11** (8,3 - 13)	**21** (19 - 22)	6 11 22 45 90	- 21 21 68 100 100

BILAGA

1) Dos-respons diagram   2) Rådata; yngelsättning   3) Rådata; Konduktivitet, pH och syre.

Kommentarer

Provet har toxiska egenskaper i testsystemet.

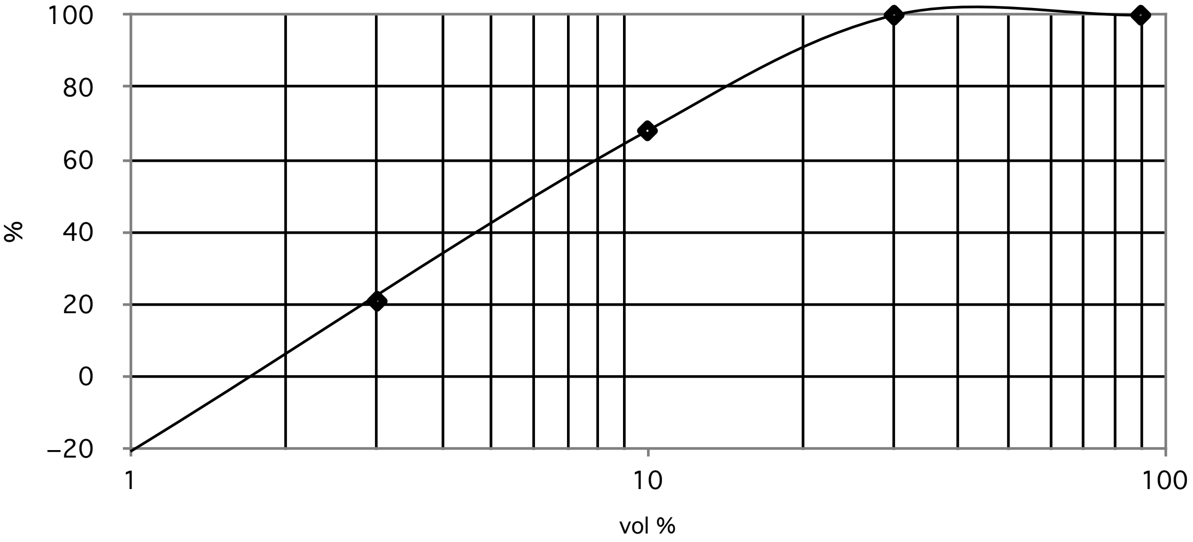
2021-01-17

GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

KRÄREPIVL220117A

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika prov-konc och blank (0). d = död (kan bero på skada vid överflyttning till nytt provkärl).*

Prov	Konc (vol%)	Yngelsättning 1	2	3	4	5	6	7	8	9	10	Summa	yngel/ Hona
	0	20	16	13	13	15	19	19	16	16	18	165	16,5
1	5,6	18	22	19	19	22	21	22	23	20	14	200	20,0
	11	19	+	11	16	21	15	20	+	18	11	131	13,1
	22	9	4	+	11	10	+	+	10	+	9	53	5,3
	45	+	+	+	+	+	+	+	+	+	+	0	0
	90	+	+	+	+	+	+	+	+	+	+	0	0

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc	Kond		pH		O2 (%)	
	(vol%)	Före	efter	före	efter	före	Efter
0 ref	0	31,2	31,9	7,1	7,3	> 90	> 90
1	5,6	26,5	31,0	7,2	7,2	> 90	> 90
	11	-	-	-	-	-	-
	22	26,7	30,7	7,2	7,2	> 90	> 90
	45	-	-	”	-	-	-
	90	28,7	31,2	7,2	7,4	> 90	> 90

* gäller koncentrerat prov.

Metodbeskrivning

Kräftdjuret Dapnia *magna* kulturhålls i 1,5 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som delvis förnyas varje vecka.

De i försöket använda djuren har singelodlats i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5°C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur och dubbla blankprov. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Nya lösningar bereds 3 gånger per vecka. Ny stamlösning har beretts varje vecka. Bägare tempereras under ca 6 h innan kräftorna flyttas över. Djuren matas en gång per dygn. Testbägarna i försöksbacken ställs i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids maximalt mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 16 h. De har plockats ur en population om 30 singelodlade moderdjur.

Testmaterialet har förvarats kylt (+1°C) till försöksstart. pH, Konduktivitet och syrenivå redovisas i rådatatabell 3. pH har inte krävt någon justering (7,1). pH och är stabilt under exponeringsperioden. Syremättnad är god i samtliga koncentrationer.

Försöket har avbrutits efter 17 dygn då 80% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull innan försöket ska anses klart.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 16,4. Metoden accepterar en genomsnittlig yngelsättning om 15 (*C dubbla*) ungar i blankprov.

Den statistiska beräkningen av toxiciteten har utförts med hjälp av ett datorbaserat beräkningsprogram som EPA tagit fram för den här typen av tester och kallas för BOOTSTRAP. Resultaten har beräknats med det statistiska måttet som EC-10 och EC50-värden (Effect Concentration, EC50 = halverad yngelsättning jämfört med blank) och 95% konfidensintervall med hjälp av programvaran. Hämningen vid varje testkoncentration har beräknats för varje provkonc och dosrespons-diagram har ritats.

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(EPA/600/4-89/001)

Uppdragsgivare

IVLSvenska Miljöinstitutet AB
Johan Strandberg
Box 210 60
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **HVO**	Startdatum: 211213	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 5.6, 11.2, 22.5, 45 o 90.0.	Spädmedium: ”EPA moderately hard” 80-100 mg/l ($CaCO_3$) och Konduktivitet 31 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	7,6	30,6	17	**10** (7,9 - 16)	**82** (73 - 90)	6 11 22 45 90	- 4,5 12 21 40 54

BILAGA

1) Dos-respons diagram 2) Rådata; yngelsättning 3) Rådata; Konduktivitet, pH och syre.

Kommentarer

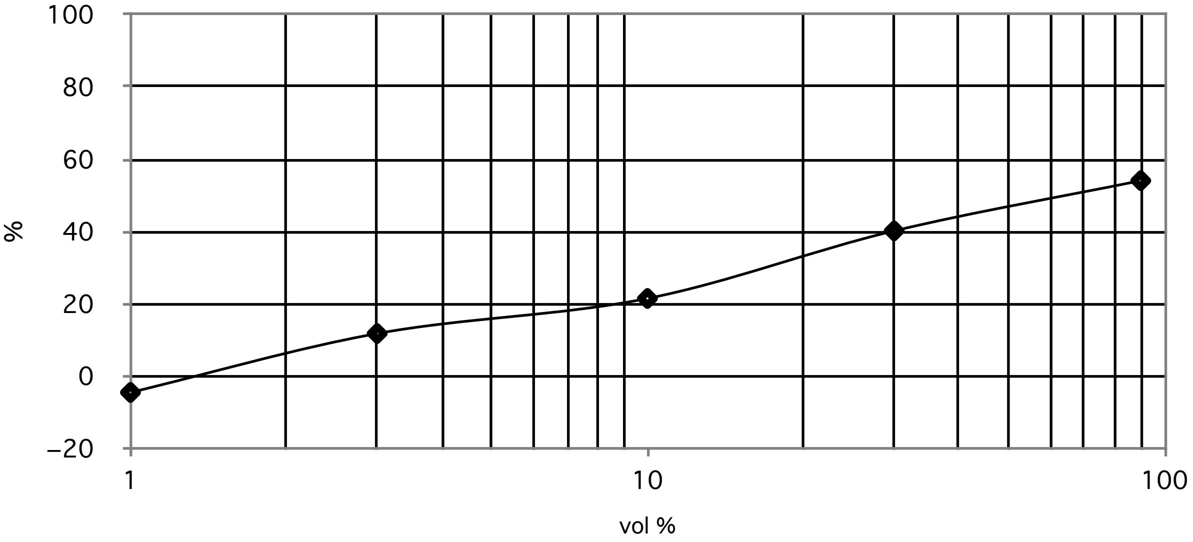
Provet har toxiska egenskaper i testsystemet.

2021-01-17

GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika provkonc och blank (0). + = död.*

Prov	Konc (vol%)	Yngelsättning 1	2	3	4	5	6	7	8	9	10	Summa	yngel/ Hona
	0	15	13	14	16	19	18	17	17	19	18	166	16,6
1	5,6	12	17	20	14	15	18	19	21	18	19	173	17,3
	11	15	13	14	16	20	6	16	25	8	13	146	14,6
	22	15	13	7	10	17	16	11	17	12	12	130	13,0
	45	12	12	14	11	8	13	+	17	12	+	99	9,9
	90	13	11	+	13	16	+	6	3	6	8	76	7,6

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc	Kond		pH		O2 (%)	
	(vol%)	Före	efter	före	efter	före	Efter
0 ref	0	31,2	31,9	7,1	7,3	> 90	> 90
1	5,6	29,6	28,7	7,2	7,3	> 90	> 90
	11	-	-	-	-	-	-
	22	27,9	31,2	7,2	7,5	> 90	> 90
	45	-	-	”	-	-	-
	90	27,6	29,9	7,3	7,6	> 90	> 90

* gäller koncentrerat prov.

Metodbeskrivning

Kräftdjuret Dapnia *magna* kulturhålls i 1,5 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som delvis förnyas varje vecka.

De i försöket använda djuren har singelodlats i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5^{o}C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur och dubbla blankprov. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Nya lösningar bereds 3 gånger per vecka. Ny stamlösning har beretts varje vecka. Bägare tempereras under ca 6 h innan kräftorna flyttas över. Djuren matas en gång per dygn. Testbägarna i försöksbacken ställs i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids maximalt mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 16 h. De har plockats ur en population om 30 singelodlade moderdjur.

Testmaterialet har förvarats kylt (+1^{o}C) till försöksstart. pH, Konduktivitet och syrenivå redovisas i rådatatabell 3. pH har inte krävt någon justering (7,1). pH och är stabilt under exponeringsperioden. Syremättnad är god i samtliga koncentrationer.

Försöket har avbrutits efter 17 dygn då 80% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull innan försöket ska anses klart.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 16,4. Metoden accepterar en genomsnittlig yngelsättning om 15 (*C dubbla*) ungar i blankprov.

Den statistiska beräkningen av toxiciteten har utförts med hjälp av ett datorbaserat beräkningsprogram som EPA tagit fram för den här typen av tester och kallas för BOOTSTRAP. Resultaten har beräknats med det statistiska måttet som EC-10 och EC50-värden (Effect Concentration, EC50 = halverad yngelsättning jämfört med blank) och 95% konfidensintervall med hjälp av programvaran. Hämningen vid varje testkoncentration har beräknats för varje provkonc och dosrespons-diagram har ritats.

MICROTOX

¨ISO11348-3¨

Uppdragsgivare

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JOHAN STRANDBERG
Box 21060
100 31 STOCKHOLM

Prov och provberedning

Provbeteckning 1) Jämviktad EVO-diesel ursprung 2) ” ” dag 0 3) ” ” dag 28	**Saltkorrektion** 2%	**Bakteriebatch** 10131020A (Aqua Science)
Spädserie (vol%) (1) 0, 11, 22, 45 o 90 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 65 %. Zinksulfat 2,2 mg/l 71 %. Kaliumdikromat 18,7 mg/l 25 %.

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 90 vol% (%)
1	1	7,7	0,1 - 20	5 15 30	> 90 > 90 > 90	> 90 > 90 > 90	- 4,1 - 18 - 20
2	1	7,6	0,1 – 20	5 15 30	> 90 > 90 > 90	> 90 > 90 > 90	- 9,3 - 13 - 11
3	1	7,6	0,1 - 20	5 15 30	> 90 > 90 > 90	> 90 > 90 > 90	3,2 - 11 - 19

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 o 2 har analyserats den 1 och prov 3 den 29 december.

2021-12-29

GS EKOTOX

Göran Svenstam
EKOTOXIKOLOG

MICROTOX

¨ISO11348-3¨

Uppdragsgivare

IVL Svenska Miljöinstitutet AB
JOHAN STRANDBERG
Box 21060
100 31 STOCKHOLM

Prov och provberedning

Provbeteckning 1) Jämviktad HVO-diesel ursprung 2) ” ” dag 0 3) ” ” dag 28	**Saltkorrektion** 2%	**Bakteriebatch** 10131020A (Aqua Science)
Spädserie (vol%) (1) 0, 11, 22, 45 o 90 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 65 %. Zinksulfat 2,2 mg/l 71 %. Kaliumdikromat 18,7 mg/l 25 %.

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 90 vol% *(%)*
1	1	7,7	0,1 - 20	5 15 30	> 90 > 90 > 90	> 90 > 90 > 90	*- 6,8* *- 12* *- 9,4*
2	1	7,6	0,1 – 20	5 15 30	> 90 > 90 > 90	> 90 > 90 > 90	*- 14* *- 11* *- 6,3*
3	1	7,6	0,1 – 20	5 15 30	> 90 > 90 > 90	> 90 > 90 > 90	*- 2,6* *- 13* *- 15*

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 o 2 har analyserats den 1 och prov 3 den 29 december.

2021-12-29

GS EKOTOX

Göran Svenstam
EKOTOXIKOLOG

MICIVL211229B

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

IVL Svenska Miljöinstitutet AB
Johan Strandberg
Box 21060
100 31 STOCKHOLM

PROV

(1) Evolution diesel.

Provet har analyserats med start den 30 november. Provkoncentrationen har varit maximal med 98,6 vol %. pH har vid start varit 7,6.

RESULTAT (mg/l)

Flaska		**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)
FT1	DOC	< 2,0	-	-	-	< 2,0	**0**
FT2	DOC	< 2,0	-	-	-	< 2,0	**0**
	TOC	3,5	-	-	-	3,6	**0**
FI	”	< 2,0 *	< 2,0	-	-	< 2,0	**0 ***
FS	”	< 2,0	-	-	-	< 2,0	**0**
FB	DOC	< 2,0	-	-	-	< 2,0	**-**
	TOC	5,1	-	-	-	2,6	**-**
FC	DOC	41	< 2,0	-	-	-	**> 95**

FT	testmaterial (kolv 1 o 2)	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inocculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2021-01-18

GS Ekotox

INOCULUM

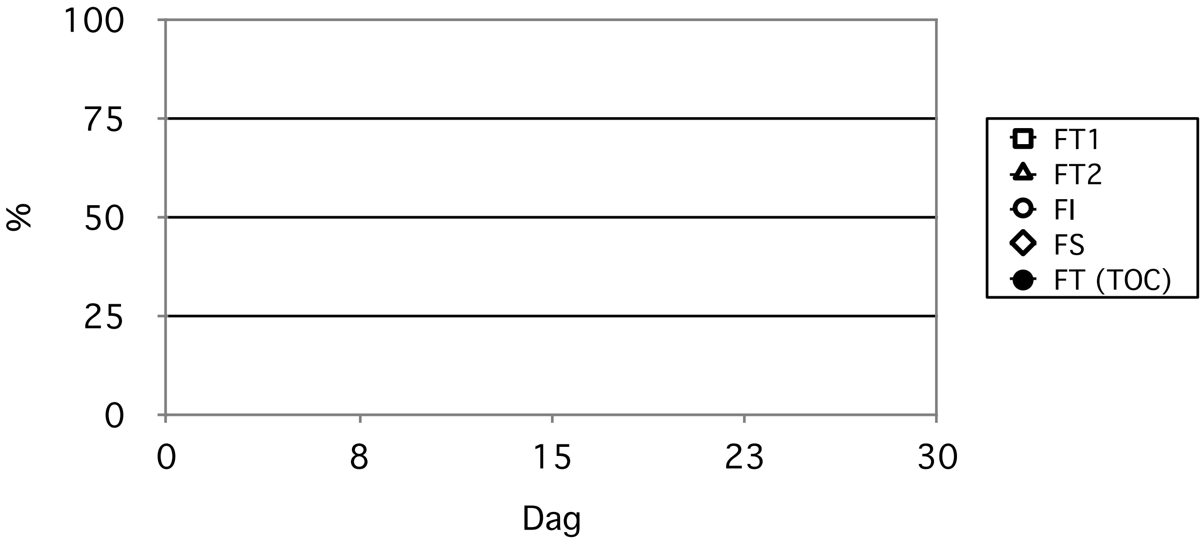
Det aktivta slammet kommer från luftningsbassängen vid Bromma reningsverk, Nockebyanläggningen. Det har tillväxt under en vecka på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var god (41 till < 2 mg/l) under de första sju dygnen (FC). Inhibitionskontrollen (FI) visar inte några toxiska egenskaper hos testmaterialet..

UTRUSTNING

Testvolym 1 000 ml i e-kolvar. Testmaterial (FT1 o FT2) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.5±0.5°C. TOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen. Testmaterialets låga löslighet i vatten medför att DOC-halten understiger 2,0 mg/l. Det är för lågt för att kunna redovisas som nedbrytningskurvor i diagrammet.



KOMMENTAR

- Testmaterialet har låg löslighet i vatten och DOC-halten är lägre än vad analysmetoden klarar av. Rekommenderad halt är 10 - 40 mg/l. Lösligheten av testmaterialet understiger 2 mg/l. TOC-halten visar ingen förändring med 3,5 mg/l vid start och 3,5 mg/l vid avslut.
- Inhibitionskontrollen (FI); visar att halten faller från 41 (extern kolkälla) till < 2 mg/l under den första veckan varför inte någon märkbar toxicitet kan föreligga. Halterna försvinner under analysgränsen redan första veckan.
- FS-kolven; dvs den sterila kontrollen, kan inte visa någon förändring då halterna är under analysgränsen båda vid start och avslut.
- pH har varit stabilt med pH 7,6 - 7,7 vid start och avslut. Syrenivån har varit god under hela perioden.

METOD

Genom att följa förändringen av DOC-halten (Dissolved Organic Carbon) i 6 reaktionskolvar kan reduktionen av biologiskt betingad nedbrytbarhet beskrivas för den 28 dygn långa nedbrytningsperioden. Samtliga kolvar rörs om med magneter under hela testperioden. Reaktionskolvarna utgörs av 6 st 2 000 ml E-kolvar med 1 000 ml reaktionslösning i varje kolv. Dubbel uppsättning av testmaterialet (FT1 och FT2). En kolv för inhibitionskontroll (FI), abiotisk kontroll (FS), blankprov (FB) och kontroll av ympens aktivitet (FC).

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

IVL Svenska Miljöinstitutet AB
JOHAN STRANDBERG
Box 21060
100 31 STOCKHOLM

PROV

(1) HVO

Provet har analyserats med start den 30 november. Provkoncentrationen har varit maximal med 98,6 vol %. pH har vid start varit 7,6.

RESULTAT (mg/l)

Flaska		**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)
FT1	DOC	< 2,0	-	-	-	< 2,0	**0**
FT2	DOC	< 2,0	-	-	-	< 2,0	**0**
	TOC	5,2	-	-	-	3,0	**0**
FI	”	< 2,0 *	< 2,0	-	-	< 2,0	**0 ***
FS	”	< 2,0	-	-	-	< 2,0	**0**
FB	DOC	< 2,0	-	-	-	< 2,0	**-**
	TOC	5,1	-	-	-	2,6	**-**
FC	DOC	41	< 2,0	-	-	-	**> 95**

FT	testmaterial (kolv 1 o 2)	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inocculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2021-01-18

GS Ekotox

INOCULUM

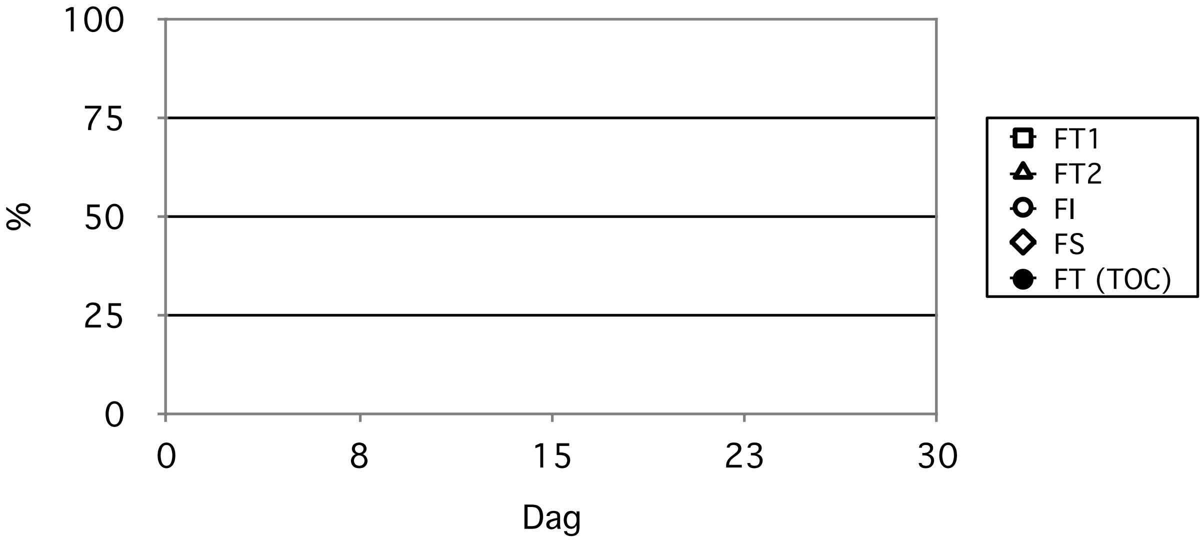
Det aktivta slammet kommer från luftningsbassängen vid Bromma reningsverk, Nockebyanläggningen. Det har tillväxt under en vecka på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var god (41 till < 2 mg/l) under de första sju dygnen (FC). Inhibitionskontrollen (FI) visar inte några toxiska egenskaper hos testmaterialet.

UTRUSTNING

Testvolym 1 000 ml i e-kolvar. Testmaterial (FT1 o FT2) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.5±0.5°C. TOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen. Testmaterialets låga löslighet i vatten medför DOC-halten understiger 2,0 mg/l. Det är för lågt för att kunna redovisas som nedbrytningskurvor i diagrammet.



KOMMENTAR

- Testmaterialet har låg löslighet i vatten och DOC-halten är lägre än vad analysmetoden klarar av. Rekommenderad halt är 10 - 40 mg/l. Lösligheten av testmaterialet understiger 2 mg/l. TOC-halten visar ingen förändring med 3,5 mg/l vid start och 3,5 mg/l vid avslut.
- Inhibitionskontrollen (FI); visar att halten faller från 41 (extern kolkälla) till < 2 mg/l under den första veckan varför inte någon märkbar toxicitet kan föreligga. Halterna försvinner under analysgränsen redan första veckan.
- FS-kolven; dvs den sterila kontrollen, kan inte visa någon förändring då halterna är under analysgränsen båda vid start och avslut.
- pH har varit stabilt med pH 7,6 - 7,7 vid start och avslut. Syrenivån har varit god under hela perioden.

METOD

Genom att följa förändringen av DOC-halten (Dissolved Organic Carbon) i 6 reaktionskolvar kan reduktionen av biologiskt betingad nedbrytbarhet beskrivas för den 28 dygn långa nedbrytningsperioden. Samtliga kolvar rörs om med magneter under hela testperioden. Reaktionskolvarna utgörs av 6 st 2 000 ml E-kolvar med 1 000 ml reaktionslösning i varje kolv. Dubbel uppsättning av testmaterialet (FT1 och FT2). En kolv för inhibitionskontroll (FI), abiotisk kontroll (FS), blankprov (FB) och kontroll av ympens aktivitet (FC).

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(ITM rapport 20)

Uppdragsgivare

IVL Svenska Miljöinst AB
Johan Strandberg
Box 21060
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **B95-3**	Startdatum: 231109	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 1.5, 3.1, 6.2, 12.5 o 25.	Spädmedium: "EPA moderately hard" 80-100 mg/l ($CaCO_3$). Konduktivitet 30 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	8,0	2,6	14	**3,3**	**5,7**	1,6 3,1 6,2 12,5 25,0	- 15 - 2 64 100 100

BILAGA

1) Dos-respons diagram 2) Rådata; yngelsättning 3) Rådata; Konduktivitet, pH och syre.

Kommentarer

Provet visar toxiska egenskaper i testsystemet. Provet verkar stimulerande upp till 3 vol% då kräftdjurets yngelsättning ökar i förhållande till blankprovets. Vid 25 vol% var yngelsättningen 0 och toxiciteten därmed 100%.

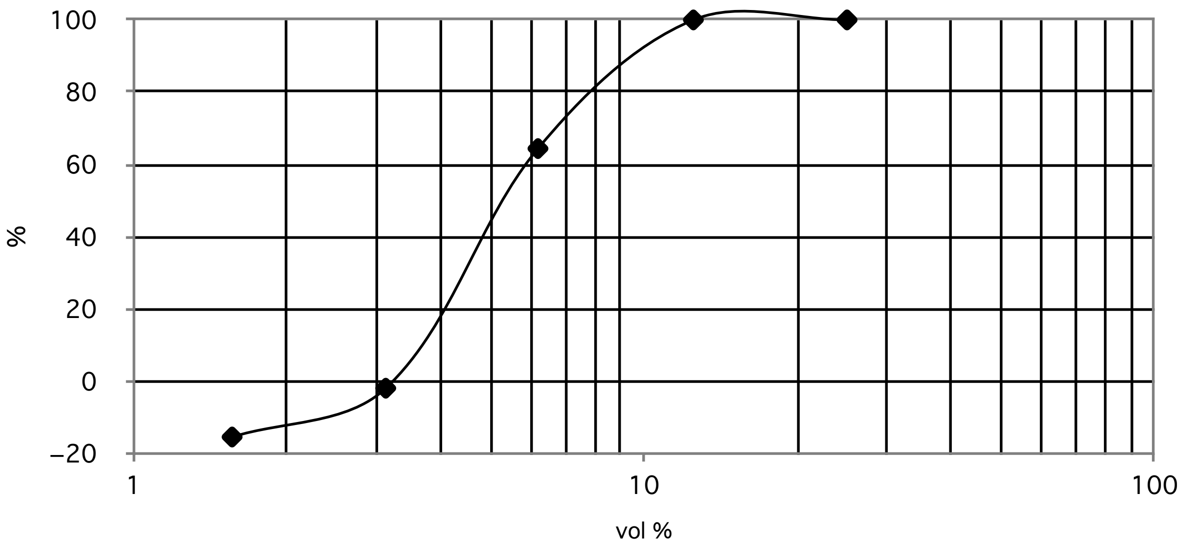
2023-12-08

GS Ekotox

Göran Svenstam
Ekotoxikolog

REPRODUKTIONSHÄMNING BILAGA

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika prov-konc och blank (0). d = död (kan bero på skada vid överflyttning till nytt provkärl).*

Prov Konc	Yngelsättning										Summa	yngel/
(vol%)	5	6	7	8	9	10	11	12	13	14		Hona
0	23	18	20	23	16	21	22	25	18	22	208	20,8
1,6	27	22	28	20	21	25	26	26	18	27	240	24,0
3,1	22	25	21	17	24	19	20	24	19	21	212	21,2
6,2	14	0	0	3	9	0	12	16	20	0	74	7,4
12	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc	Kond (mS/m)		pH		O2 (% av mättnad)	
	(vol%)	Före	efter	före	efter	Före	Efter
0	0	26,9	32,5	8,3	7,5	> 90	> 90
1	1,6	2,6 *	28	8,0 *	7,5	> 90	> 90
	3,1	”					
	6,2	”	27	”	7,6	> 90	> 90
	12,5	”	21				
	25	”	16	”	7,6	> 90	> 90

* gäller 100 vol % prov.

Metodbeskrivning

Kräftdjuret *Daphnia magna* kulturhålls i 1 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som byts med 100% varje vecka.

De i försöket använda djuren singelodlas i två generationer i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5°C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur med lika många djur i blankprovet. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Tre gånger per vecka bereds nya lösningar som tempereras under 6 h och foder tillsätts innan kräftorna flyttas över. Istället för att slumpa ut testbägarna i försöksbacken ställs de i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 6 h. De har plockats ur en population om 50 singelodlade moderdjur.

Testmaterialet har förvarats nerkylt till försöksstart. pH, Konduktivitet och syrenivå redovisas i rådatatabell 3. pH har varit bra i samtliga provkoncentrationen. Provet har ett gott syreinnehåll vid både start och avslut i samtliga koncentrationer. Ett lågt saltinnehåll gör att högre provkoncentrationer får mycket låg konduktivitet. Inom redovisat koncentrations-intervall bör det dock inte vara några problem eftersom yngelsättningen är 0 redan vid 12,5 vol % då konduktiviteten var 21 mS/m.

Försöket har avbrutits efter 14 dygn då 100% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull för att försöket ska avbrytas.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 20,8. Det är något bättre än stipulerade 15,0 (gäller *Ceriodaphnia dubia*).

Toxiciteten har bestämts genom linjär regression.

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(ITM rapport 20)

Uppdragsgivare

IVL Svenska Miljöinst AB
JOHAN STRANDBERG
Box 21060
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **B98-2**	Startdatum: 231109	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 1.6, 3.1, 6.2, 12.5 o 25.	Spädmedium: ”EPA moderately hard” 80-100 mg/l ($CaCO_3$). Konduktivitet 30 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	6,6	0,5	14	**5,6**	**8,0**	1,6 3,1 6,2 12,5 25,0	3,8 6,7 18 100 100

BILAGA

1) Dos-respons diagram   2) Rådata; yngelsättning   3) Rådata; Konduktivitet, pH och syre.

Kommentarer

Provet visar toxiska egenskaper i testsystemet. Provet har toxiska egenskaper. Vid 12 vol% var yngelsättningen 0 och toxiciteten därmed 100%.

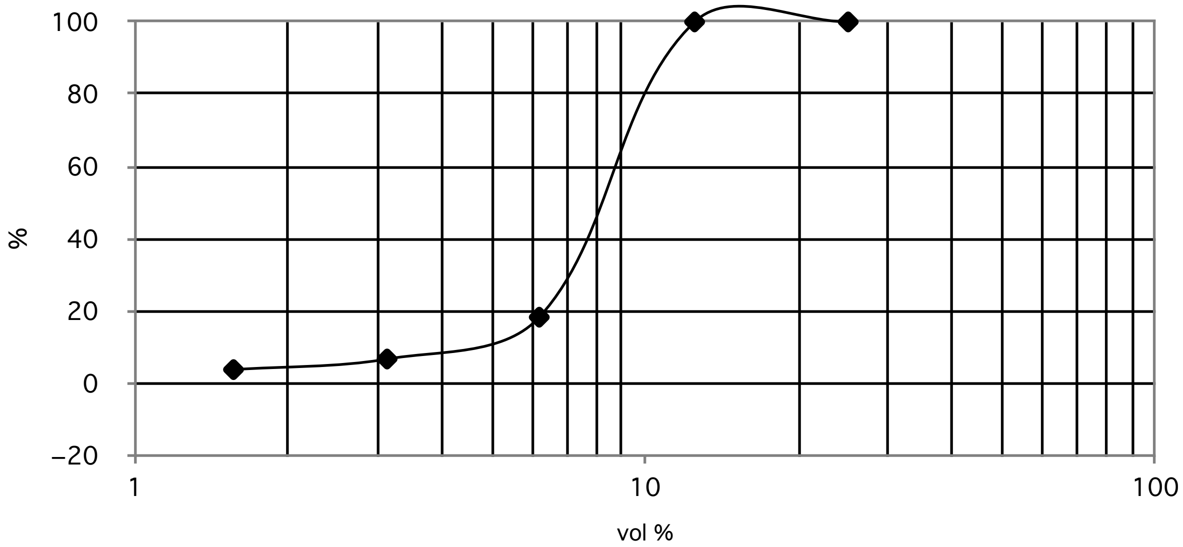
2023-12-08

GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

REPRODUKTIONSHÄMNING BILAGA

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika prov-konc och blank (0). d = död (kan bero på skada vid överflyttning till nytt provkärl).*

Prov Konc	Yngelsättning										Summa	yngel/
(vol%)	5	6	7	8	9	10	11	12	13	14		Hona
0	23	18	20	23	16	21	22	25	18	22	208	20,8
1,6	22	19	18	20	25	21	17	19	22	17	200	20,0
3,1	16	13	20	26	17	26	21	22	23	10	194	19,4
6,2	19	21	18	13	17	14	17	16	14	21	170	170
12	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc	Kond (mS/m)		pH		O2 (% av mättnad)	
	(vol%)	Före	efter	före	efter	Före	Efter
0	0	26,9	32,5	8,3	7,5	> 90	> 90
1	1,6	0,5 *	28	8,0 *	7,5	> 90	> 90
	3,1	”					
	6,2	”	27	”	7,5	> 90	> 90
	12,5	”	22				
	25	”	16	”	8,0	> 90	> 90

* gäller 100 vol % prov.

Metodbeskrivning

Kräftdjuret *Daphnia magna* kulturhålls i 1 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som byts med 100% varje vecka.

De i försöket använda djuren singelodlas i två generationer i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5^{o}C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur med lika många djur i blankprovet. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Tre gånger per vecka bereds nya lösningar som tempereras under 6 h och foder tillsätts innan kräftorna flyttas över. Istället för att slumpa ut testbägarna i försöksbacken ställs de i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 6 h. De har plockats ur en population om 50 singelodlade moderdjur.

Testmaterialet har förvarats nerkylt till försöksstart. pH, Konduktivitet och syrenivå redovisas i tabell 3. pH har varit inom godkända intervall i samtliga provkoncentrationen. Provet har ett gott syreinnehåll vid både start och avslut i samtliga koncentrationer. Ett lågt saltinnehåll gör att högre provkoncentrationer får mycket låg konduktivitet. Inom redovisat koncentrations-intervall bör det dock inte vara några problem eftersom yngelsättningen är 0 redan vid 12,5 vol % då konduktiviteten var 22 mS/m.

Försöket har avbrutits efter 14 dygn då 100% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull för att försöket ska avbrytas.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 20,8. Det är något bättre än stipulerade 15,0 (gäller *Ceriodaphnia dubia*).

Toxiciteten har bestämts genom linjär regression.

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(ITM rapport 20)

Uppdragsgivare

IVL Svenska Miljöinst AB
JOHAN STRANDBERG
Box 21060
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **E85-2**	Startdatum: 231109	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 1.6, 3.1, 6.2, 12.5 o 25.	Spädmedium: ”EPA moderately hard” 80-100 mg/l ($CaCO_3$). Konduktivitet 30 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	6,5	0,4	14	**5,4**	**10**	3,1 6,2 12,5 25,0 50,0	1,9 17 64 100 100

BILAGA

1) Dos-respons diagram   2) Rådata; yngelsättning   3) Rådata; Konduktivitet, pH och syre.

Kommentarer

Provet visar toxiska egenskaper i testsystemet. Provet har toxiska egenskaper. Vid 25 vol% var yngelsättningen 0 och toxiciteten därmed 100%.

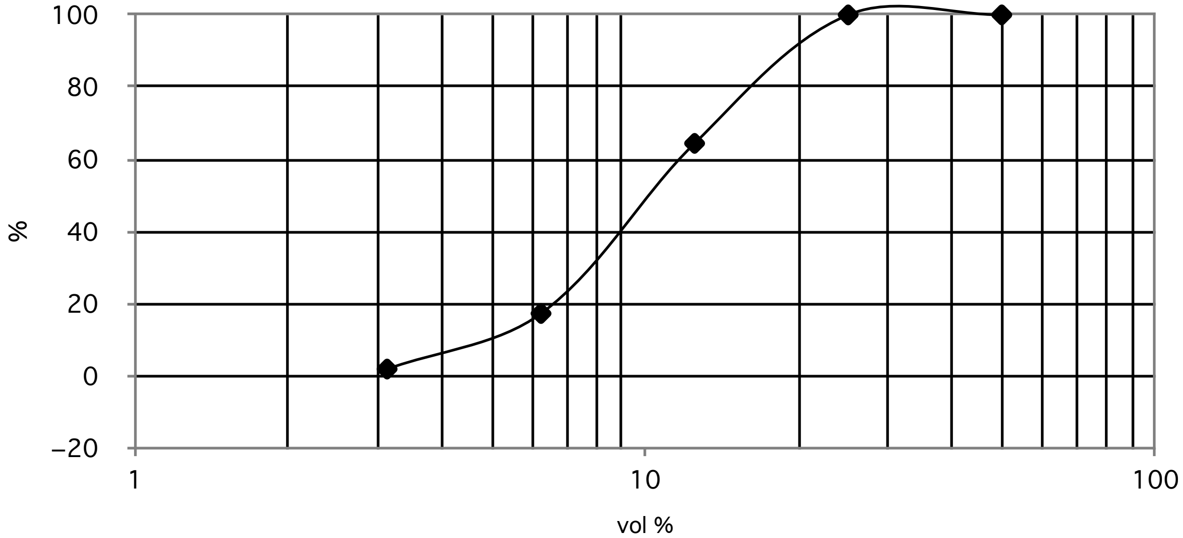
2023-12-08

GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

REPRODUKTIONSHÄMNING BILAGA

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika prov-konc och blank (0). d = död (kan bero på skada vid överflyttning till nytt provkärl).*

Prov Konc (vol%)	Yngelsättning										Summa	yngel/Hona
	5	6	7	8	9	10	11	12	13	14		
0	23	18	20	23	16	21	22	25	18	22	208	20,8
3,1	22	17	24	23	14	19	18	21	24	22	204	20,4
6,2	21	17	14	21	20	19	15	9	16	20	172	17,2
12	2	11	14	5	1	7	12	7	8	7	74	7,4
25	0	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0	0

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc (vol%)	Kond (mS/m)		pH		O2 (% av mättnad)	
		Före	efter	före	efter	Före	Efter
0	0	26,9	32,5	8,3	7,5	> 90	> 90
1	3,1	0,4 *	30	6,5 *	7,5	> 90	> 90
	6,2	”					
	12,5	”	27	”	7,9	> 90	> 90
	25	”	16				
	50	”	3,7	”	8,3	> 90	> 90

* gäller 100 vol % prov.

Metodbeskrivning

Kräftdjuret *Daphnia magna* kulturhålls i 1 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som byts med 100% varje vecka.

De i försöket använda djuren singelodlas i två generationer i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5^{o}C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur med lika många djur i blankprovet. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Tre gånger per vecka bereds nya lösningar som tempereras under 6 h och foder tillsätts innan kräftorna flyttas över. Istället för att slumpa ut testbägarna i försöksbacken ställs de i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 6 h. De har plockats ur en population om 50 singelodlade moderdjur.

Testmaterialet har förvarats nerkylt till försöksstart. pH, Konduktivitet och syrenivå redovisas i tabell 3. pH har varit inom godkända intervall i samtliga provkoncentrationen. Provet har ett gott syreinnehåll vid både start och avslut i samtliga koncentrationer. Ett lågt saltinnehåll gör att högre provkoncentrationer får mycket låg konduktivitet. Inom redovisat koncentrations-intervall bör det dock inte vara några problem eftersom yngelsättningen är 0 redan vid 25 vol % då konduktiviteten var 16 mS/m.

Försöket har avbrutits efter 14 dygn då 100% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull för att försöket ska avbrytas.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 20,8. Det är något bättre än stipulerade 15,0 (gäller *Ceriodaphnia dubia*).

Toxiciteten har bestämts genom linjär regression.

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(ITM rapport 20)

Uppdragsgivare

IVL Svenska Miljöinst AB
JOHAN STRANDBERG
Box 21060
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **D-EU1**	Startdatum: 231109	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 0,4, 0,8,1.6, 3.1 o 6.2.	Spädmedium: ”EPA moderately hard” 80-100 mg/l ($CaCO_3$). Konduktivitet 30 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	6,7	0,8	14	**0,6**	**1,0**	0,4 0,8 1,6 3,1 6,2	- 13 31 93 100 100

BILAGA

1) Dos-respons diagram   2) Rådata; yngelsättning   3) Rådata; Konduktivitet, pH och syre.

Kommentarer

Provet visar toxiska egenskaper i testsystemet. Provet har toxiska egenskaper. Vid 6 vol% var yngelsättningen 0 och toxiciteten därmed 100%.

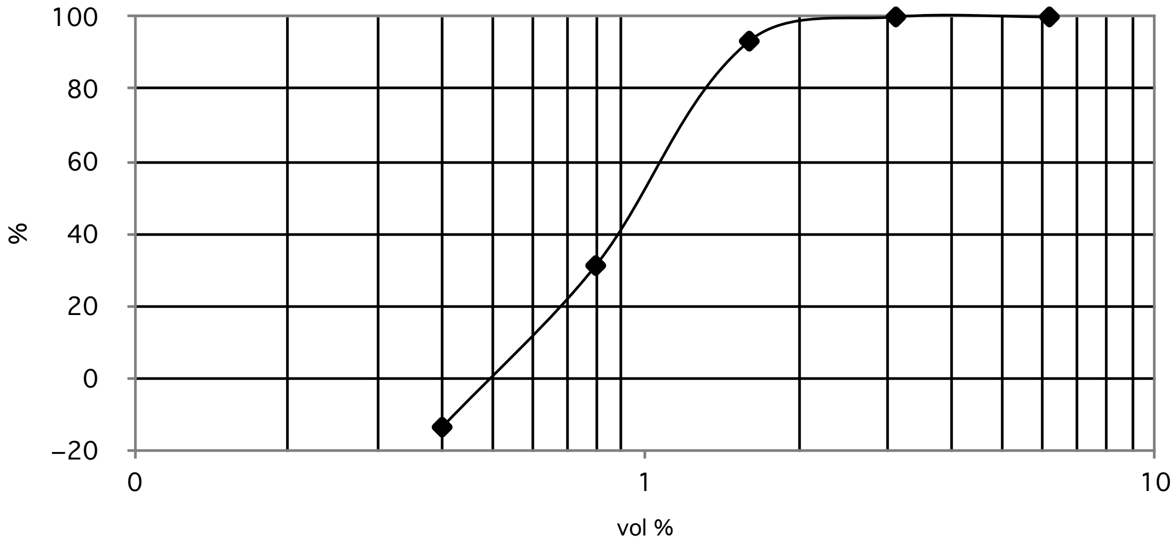
2023-12-08

GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

REPRODUKTIONSHÄMNING BILAGA

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika prov-konc och blank (0). d = död (kan bero på skada vid överflyttning till nytt provkärl).*

Prov Konc (vol%)	Yngelsättning 5	6	7	8	9	10	11	12	13	14	Summa	yngel/ Hona
0	23	18	20	23	16	21	22	25	18	22	208	20,8
0,4	26	28	23	17	25	26	23	18	24	26	236	23,6
0,8	18	13	15	18	15	19	12	13	10	10	143	14,3
1,6	0	0	2	3	0	8	0	0	1	0	14	1,4
3,1	0	0	0	0	0	0	0	0	0	0	0	0
6,2	0	0	0	0	0	0	0	0	0	0	0	0

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc (vol%)	Kond (mS/m) Före	efter	pH före	efter	O2 (% av mättnad) Före	Efter
0	0	26,9	32	8,3	7,5	> 90	> 90
1	0,4	0,8 *	31	6,7 *	7,5	> 90	> 90
	0,8	”					
	1,6	”	29	”	7,5	> 90	> 90
	3,1	”					
	6,2	”	27	”	7,5	> 90	> 90

* gäller 100 vol % prov.

Metodbeskrivning

Kräftdjuret *Daphnia magna* kulturhålls i 1 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som byts med 100% varje vecka.

De i försöket använda djuren singelodlas i två generationer i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5°C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur med lika många djur i blankprovet. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Tre gånger per vecka bereds nya lösningar som tempereras under 6 h och foder tillsätts innan kräftorna flyttas över. Istället för att slumpa ut testbägarna i försöksbacken ställs de i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 6 h. De har plockats ur en population om 50 singelodlade moderdjur.

Testmaterialet har förvarats nerkylt till försöksstart. pH, Konduktivitet och syrenivå redovisas i tabell 3. pH har varit inom godkända intervall i samtliga provkoncentrationen. Provet har ett gott syreinnehåll vid både start och avslut i samtliga koncentrationer. Mycket utspädda lösningar ger inget problem med saltinnehållet.

Försöket har avbrutits efter 14 dygn då 100% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull för att försöket ska avbrytas.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 20,8. Det är något bättre än stipulerade 15,0 (gäller *Ceriodaphnia dubia*).

Toxiciteten har bestämts genom linjär regression.

REPRODUKTIONSFÖRSÖK, KRÄFTDJUR

(ITM rapport 20)

Uppdragsgivare

IVL Svenska Miljöinst AB
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Box 21060
100 31 STOCKHOLM

PROV OCH PROVHANTERING

Provbeteckning: (1) **EO1-1**	Startdatum: 231109	Kräftdjur: *Daphnia magna*
Spädserie: (vol%) (1) 0, 1.5, 3.1, 6.2, 12,5 o 25.	Spädmedium: ”EPA moderately hard” 80-100 mg/l ($CaCO_3$). Konduktivitet 30 mS/m.	

RESULTAT (95% konf.int)

Prov	Späd.	pH	Kond. (mS/m)	Exp.tid (d)	**EC10** (vol%)	**EC50** (vol%)	Konc (vol%)	Hämning (%)
1	1	7,6	4,7	8	**4,2**	**6,9**	1,5 3,1 6,2 12,5 25	- 4,3 - 19 41 97 100

BILAGA

1) Dos-respons diagram 2) Rådata; yngelsättning 3) Rådata; Konduktivitet, pH och syre.

Kommentarer

Provet visar toxiska egenskaper i testsystemet. Provet har stimulerande egenskaper upp till 6 vol% då kräftdjurets yngelsättning ökar i förhållande till blankprovets. Vid 25 vol% var yngelsättningen 0 och toxiciteten därmed 100%.

2023-12-01

GS Ekotox

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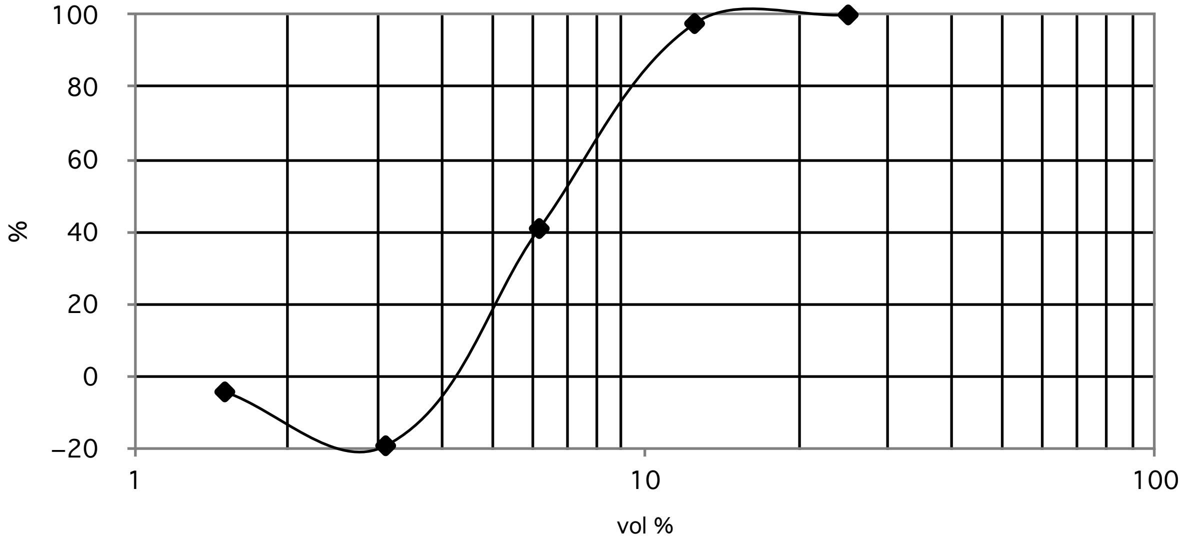
Ekotoxikolog
KRÄrepIVL231201e

REPRODUKTIONSHÄMNING BILAGA

1. *Dosrespons kurva, hämning (%) av yngelsättning vid varje provkonc (vol%).*



2. *Rådata; Yngelsättning hos varje i de olika prov-konc och blank (0). d = död (kan bero på skada vid överflyttning till nytt provkärl).*

Prov Konc	Yngelsättning										Summa	yngel/
(vol%)	5	6	7	8	9	10	11	12	13	14		Hona
0	23	18	20	23	16	21	22	25	18	22	208	20,8
1,5	18	19	24	28	27	22	18	19	22	20	217	21,7
3,1	25	20	33	27	15	26	27	28	25	22	248	24,8
6,2	15	11	12	15	14	7	10	12	16	11	123	12,3
12,5	0	0	0	2	0	0	3	0	0	0	5	0,5
25,0	0	0	0	0	0	0	0	0	0	0	0	0,0

3. *Rådata; Konduktivitet (mS/m), pH och syrehalt (mg/l) före och efter exponeringstiden.*

Prov	Konc	Kond (mS/m)		pH		O2 (% av mättnad)	
	(vol%)	Före	efter	före	efter	Före	Efter
0	0	26,9	32,5	8,3	7,5	> 90	> 90
1	1,5	4,7 *	29	7,6 *	7,5	> 90	> 90
	3,1	”					
	6,2	”	28	”	7,8	> 90	> 90
	12,5	”	22				
	25,0	”	16	”	8,2	> 90	> 90

* gäller 100 vol % prov.

Metodbeskrivning

Kräftdjuret *Daphnia magna* kulturhålls i 1 l glasbägare. Odlingen görs i stabiliserat vatten (akvarievatten) som byts med 100% varje vecka.

De i försöket använda djuren singelodlas i två generationer i det använda spädmediet EPA moderately hard. Djuren fodras med 0,25 ml YTC (Yeast, Trout och Cerophyll foderblandning enligt metodbeskrivningen). Odlingen sker i dämpad belysning med en ljusperiod om 16 h per dygn. Temperatur var 25,0 +/- 0,5°C vid både odling och försök.

Testet har genomförts i 5 provkoncentrationer och blankprov. Varje koncentration innnehåller 10 djur med lika många djur i blankprovet. Bägarna utgörs av 50 ml i styren med 15 ml lösning och ett djur i varje bägare. Tre gånger per vecka bereds nya lösningar som tempereras under 6 h och foder tillsätts innan kräftorna flyttas över. Istället för att slumpa ut testbägarna i försöksbacken ställs de i raka spädserier. Testdjuren tillsätts sedan tvärs spädserien. Det innebär att syskonyngel sprids mellan försökskoncentrationerna. Testdjuren har vid start en åldersskillnad på maximalt 6 h. De har plockats ur en population om 50 singelodlade moderdjur.

Testmaterialet har förvarats nerkylt till försöksstart. pH, Konduktivitet och syrenivå redovisas i rådatatabell 3. pH har varit något högt i den högsta koncentrationen. Men det bör inte påverka resultatet. Även blankprovet har något förhöjt pH-värde vid avslut. Provet har ett gott syreinnehåll vid både start och avslut i samtliga koncentrationer. Lite högre konduktivitet ger inget problem med saltinnehållet.

Försöket har avbrutits efter 14 dygn då 100% av djuren i kontrollen släppt sin 3:e yngelkull. Enligt metodbeskrivningen måste mer än 60% av djuren i blanken släppa sin tredje kull för att försöket ska avbrytas.

Dödligheten i blanken har varit 0% (får inte överstiga 20%). Den genomsnittliga yngelsättningen i blanken har varit godkänd med 20,8. Det är något bättre än stipulerade 15,0 (gäller *Ceriodaphnia dubia*).

Toxiciteten har bestämts genom linjär regression och grafisk avläsning.

MICROTOX

¨ISO11348-3¨

Uppdragsgivare

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Prov och provberedning

Provbeteckning 1) Jämviktad **B95-3**, ursprung 2) ” ” dag 0 3) ” ” dag 28	**Saltkorrektion** 2%	**Bakteriebatch** 22KY01301 (Modern Water)
Spädserie (vol%) (1) 0, 0.4, 2.0, 10 o 50 (duplikat). (2) 0, 0.56, 1.1, 2.2, 4.5 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 60 %. Zinksulfat 2,2 mg/l 65 %. Kaliumdikromat 18,7 mg/l 32 %

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 50 o 4,5 vol% (%)
1	1	8,6 - 7,0	0,0 - 20	5 15 30	0,67 (0,57 - 0,79) 0,84 (0,63 - 1,1) 0,72 (0,46 - 1,1)	2,5 (2,2 - 2,8) 3,1 (2,4 - 3,9) 2,5 (2,1 - 3,0)	100 (50v%) 100 (”) 100 (”)
2	2	7,8	0,1 – 20	5 15 30	3,9 (2,7 - 5,7) 6,7 (4,7 - 9,4) 6,1 (5,8 - 6,3)	29 (3,2 - 134) 17 (8,3 - 35) 48 (41 - 56)	21 (4,5v%) 12 (”) 17 (”)
3	2	7,6	0,6 - 20	5 15 30	> 4.5 > 4.5 > 4.5	> 4.5 > 4.5 > 4.5	- 21 (4,5v%) - 21 (”) - 7,8 (”)

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 har analyserats den 4 oktober, prov 2 den 12 oktober och prov 3 den 23 november.

2023-11-27

GS EKOTOX

Göran Svenstam
EKOTOXIKOLOG

MICROTOX

¨ISO11348-3¨

Uppdragsgivare

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Prov och provberedning

Provbeteckning	**Saltkorrektion**	**Bakteriebatch**
1) Jämviktad **B98-2**, ursprung 2) ” ” dag 0 3) ” ” dag 28	2%	22KY01301 (Modern Water)
Spädserie (vol%) (1) 0, 0.4, 2.0, 10 o 50 (duplikat). (2) 0, 0.56, 1.1, 2.2, 4.5 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 60 %. Zinksulfat 2,2 mg/l 65 %. Kaliumdikromat 18,7 mg/l 32 %

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 50 o 4,5 vol% (%)
1	1	6,6	0,0 - 20	5 15 30	0,33 (0,30 - 0,37) 0,54 (0,48 - 0,61) 0,53 (0,44 - 0,65)	1,9 (1,8 - 1,9) 2,5 (2,3 - 2,7) 2,3 (2,0 - 2,6)	95 (50v%) 95 (”) 97 (”)
2	2	7,8	0,1 – 20	5 15 30	0,39 (0,24 - 0,64) 0,59 (0,40 - 0,86) 0,29 (0,18 - 0,47)	2,5 (2,5 - 2,5) 3,8 (3,6 - 4,1) 2,5 (2,5 - 2,5)	59 (4,5v%) 53 (”) 59 (”)
3	2	7,7	0,0 - 20	5 15 30	> 4.5 > 4.5 > 4.5	> 4.5 > 4.5 > 4.5	- 15 (4,5v%) - 6,9 (”) 12 (”)

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 har analyserats den 4 oktober, prov 2 den 12 oktober och prov 3 den 27 november.

2023-11-27

GS EKOTOX

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Prov och provberedning

Provbeteckning 1) Jämviktad **E85-2**, ursprung 2) ” ” dag 0 3) ” ” dag 28	**Saltkorrektion** 2%	**Bakteriebatch** 22KY01301 (Modern Water)
Spädserie (vol%) (1) 0, 0.4, 2.0, 10 o 50 (duplikat). (2) 0, 0.56, 1.1, 2.2, 4.5 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 60 %. Zinksulfat 2,2 mg/l 65 %. Kaliumdikromat 18,7 mg/l 32 %

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 50 o 4,5 vol% (%)
1	1	6,5	0,0 - 20	5 15 30	0,33 (0,30 - 0,37) 0,54 (0,48 - 0,61) 0,53 (0,44 - 0,65)	1,9 (1,8 - 1,9) 2,5 (2,3 - 2,7) 2,3 (2,0 - 2,6)	100 100 100
2	2	7,7	0,1 – 20	5 15 30	3,9 (2,7 - 5,7) 6,7 (4,7 - 9,4) 6,1 (5,8 - 6,3)	29 (3,2 - 134) 17 (8,3 - 35) 48 (41 - 56)	95 95 97
3	2	7,4	0,2 - 20	5 15 30	4.5 4.5 4.5	> 4.5 > 4.5 > 4.5	5,6 (4,5v%) 8,7 (”) 24 (”)

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 har analyserats den 4 oktober, prov 2 den 12 oktober och prov 3 den 27 november.

2023-11-27

GS EKOTOX

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Uppdragsgivare

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Prov och provberedning

Provbeteckning 1) Jämviktad **D-EU1**, ursprung 2) ” ” dag 0 3) ” ” dag 28	**Saltkorrektion** 2%	**Bakteriebatch** 22KY01301 (Modern Water)
Spädserie (vol%) (1) 0, 0.4, 2.0, 10 o 50 (duplikat). (2) 0, 0.56, 1.1, 2.2, 4.5 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 60 %. Zinksulfat 2,2 mg/l 65 %. Kaliumdikromat 18,7 mg/l 32 %

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 50 o 4,5 vol% *(%)*
1	1	6,7	0,0 - 20	5 15 30	4,6 (3,2 - 6,5) 3,9 (3,6 - 4,1) 2,5 (2,2 - 2,9)	14 (12 - 15) 15 (14 - 16) 14 (14 - 15)	*76 (50v%)* *79 (”)* *87 (”)*
2	2	7,7	0,1 – 20	5 15 30	> 4,5 > 4,5 > 4,5	> 4,5 > 4,5 > 4,5	*- 11 (4,5v%)* *- 20 (”)* *- 11 (”)*
3	2	7,2	0,1 - 20	5 15 30	> 4.5 > 4.5 > 4.5	> 4.5 > 4.5 > 4.5	*- 22 (4,5v%)* *- 19 (”)* *- 9,7 (”)*

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 har analyserats den 4 oktober, prov 2 den 12 oktober och prov 3 den 27 november.

2023-11-27

GS Ekotox

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MICROTOX

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Uppdragsgivare

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Prov och provberedning

Provbeteckning 1) Jämviktad **EO1-1**, ursprung 2) ” ” dag 0 3) ” ” dag 28	**Saltkorrektion** 2%	**Bakteriebatch** 22KY01301 (Modern Water)
Spädserie (vol%) (1) 0, 0.4, 2.0, 10 o 50 (duplikat). (2) 0, 0.56, 1.1, 2.2, 4.5 (duplikat).	**Ljuskorrektion** Nej	**Referens**, hämning (30min) 3,5 Diklorfenol 3,4 mg/l 60 %. Zinksulfat 2,2 mg/l 65 %. Kaliumdikromat 18,7 mg/l 32 %

Resultat vol% (95% konf int)

Prov	Späds.	PH-justering	Salinitet-Justering	Tid (min)	EC20	EC50	Inhib vid 50 o 4,5 vol% *(%)*
1	1	9,2 - 7,0	0,0 - 20	5 15 30	3,7 (3,2 - 4,5) 4,0 (3,2 - 4,9) 3,5 (2,4 - 5,3)	11 (10 - 13) 12 (10 - 15) 14 (12 - 17)	*85 (50v%)* *85 (”)* *83 (”)*
2	2	7,7	0,1 – 20	5 15 30	> 4,5 > 4,5 > 4,5	> 4,5 > 4,5 > 4,5	*- 3,3 (4,5v%)* *- 6,5 (”)* *- 2,1 (”)*
3	2	7,7	0,1 - 20	5 15 30	> 4.5 > 4.5 > 4.5	> 4.5 > 4.5 > 4.5	*- 4,6 (4,5v%)* *- 8,4 (”)* *- 3,3 (”)*

Kommentarer

Beräkningar har gjorts med ¨Microtox data capture and reporting program version 7.80¨. Prov 1 har analyserats den 4 oktober, prov 2 den 12 oktober och prov 3 den 27 november.

2023-11-27

GS Ekotox

Göran Svenstam
Ekotoxikolog

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

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PROV

1 EO1-1

Provet har analyserats med start den 11 oktober. Provkoncentrationen av jämviktad prov var 5,0 vol%, med hänsyn till utfall i analys av respirationshämning. pH i reaktionslösningen var 7,9 vid start. Vid avslut var pH 7,2. Toxiska egenskaper som stör nedbrytningsprocessen kan inte noteras. Mänden organiskt material som löser sig i vattenfasen är mycket begränsad. Den beräknade reduktionsgraden begränsas av TOC-analysens känslighet. Nedbrytningsgraden kan därför vara betydligt högre än den som analysen redovisar.

RESULTAT (DOC mg/l, där inte annat anges)

Prov	**Flaska**	**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)	
								Medel
1	**FT1**	2,9	4,1	2,1	2,6	< 2	**> 31**	**> 31**
	FT2	-	2,3	2,0	2,1	< 2	**> 31**	
	FT (TOC)	3,7	-	-	-	< 2	**> 46**	
	FI	2,9 *	2,4	2,6	2,8	< 2	**> 31 ***	
	FS	2,9	-	-	-	< 2	**> 31**	
	FB	< 2	< 2	< 2	< 2	> 2	**-**	
	FB (TOC)	2,6	-	-	-	2,2	**-**	
	FC	46	2,6	-	-	-	**> 95**	

FT	testmaterial	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inoculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2023-12-01
GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

INOCULUM

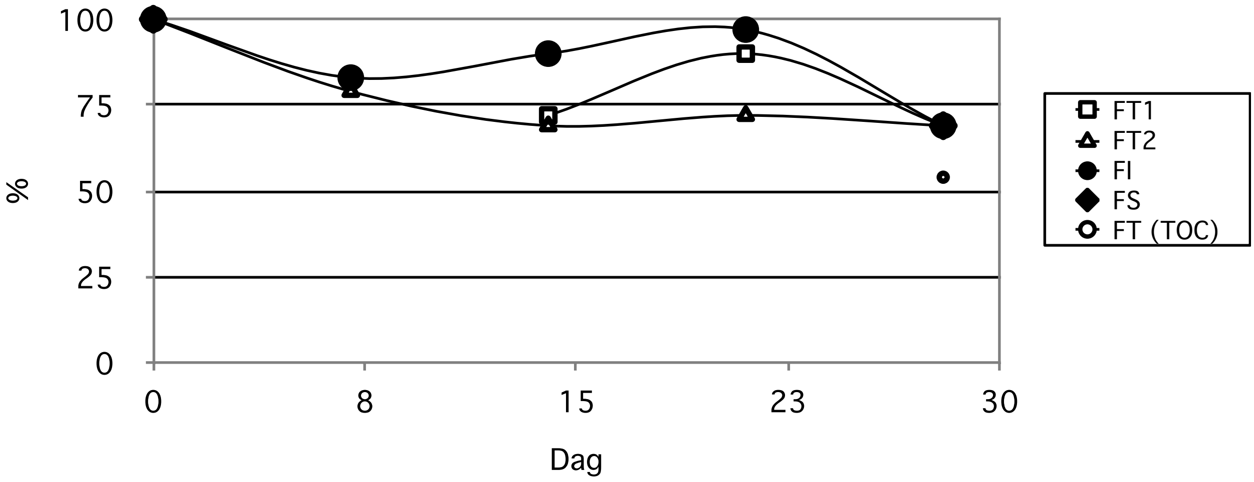
Försedimenterat avloppsvatten från Bromma reningsverk. Den har tillväxt under flera dygn på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var hög, NaAc (46 till 2,6 mg/l på under 7 dygn (FB). Testmaterialets kontroll av toxicitet (FI) visar att ingen toxicitet påverkar nedbrytningsförloppet.

UTRUSTNING

Testvolym 1000 ml i e-kolvar. Testmaterial (FT) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.5±0.5^{o}C. DOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen.



KOMMENTAR

Lösligheten av organiskt material är väldigt låg. En 5 %-ig lösning innehåller 3,7 mg/l som TOC och 2,9 mg/l som DOC. Den låga lösligheten gör att avståndet till analysgränsen (2,0 mg/l) blir begränsad både avseende TOC och DOC. Den genomsnittliga (FT1 och FT2) nedbrytningsgraden beräknas till 31 % för DOC resp 46 % för TOC.

Reduktionen är helt beroende av nedbrytning eftersom den abiotiska kontrollen (FS) visar samma utfall i jämförelse med testkolvarna.

Inhibitionskontrollen (FI) visar att testmaterialet inte har toxiska egenskaper mot nedbrytningsprocessen. Tillsatsen av extern kolkälla, genom referenskemikalien (Natriumacetat) kan inte påverka inte reduktionsgraden eftersom analysgränsen är den begränsande faktorn. Ytterligare tillsats av kol kan eventuellt leda till ytterligare ökad nedbrytningsgrad.

Syrehalten har varit god under perioden. pH har drivit något under perioden. Vid start var pH 7,9 och vid avslut 7,2. pH bör ligga inom intervallet 6,0 – 8,0 för att säkerställa att inte nedbrytningen påverkas negativt. Förändringen ligger inom godkännt intervall och bedöms inte ha någon påverkan på nedbrytningsförloppet.

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

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PROV

1 B95-3

Provet har analyserats med start den 11 oktober. Provkoncentrationen av jämviktad prov var 5,0 vol%, med hänsyn till utfall i analys av respirationshämning. pH i reaktionslösningen var 8,0 vid start. Vid avslut uppmättes 7,6. Toxiska egenskaper som stör nedbrytningsprocessen kan inte noteras.

RESULTAT (DOC mg/l, där inte annat anges)

Prov	**Flaska**	**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)	Medel
1	**FT1**	180	7,1	4,2	5,5	3,8	**98**	**98**
	FT2	-	4,1	4,2	4,2	2,9	**98**	
	FT (TOC)	200	-	-	-	5,1	**97**	
	FI	180 *	8,1	6,4	6,3	3,7	**98 ***	
	FS	180	-	-	-	160	**11**	
	FB	< 2	< 2	< 2	< 2	< 2	**-**	
	FB (TOC)	2,6	-	-	-	3,4	**-**	
	FC	46	2,6	-	-	-	**> 95**	

FT	testmaterial	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inoculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2023-12-04
GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

INOCULUM

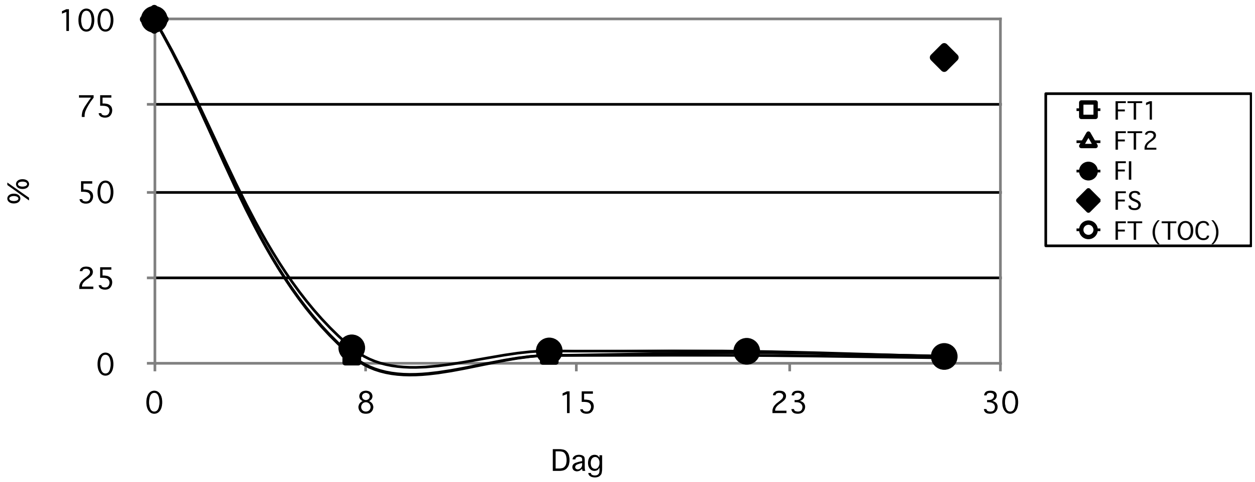
Försedimenterat avloppsvatten från Bromma reningsverk. Den har tillväxt under flera dygn på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var hög, NaAc (46 till 2,6 mg/l på under 7 dygn (FB). Testmaterialets kontroll av toxicitet (FI) visar att ingen toxicitet påverkar nedbrytningsförloppet.

UTRUSTNING

Testvolym 1000 ml i e-kolvar. Testmaterial (FT) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.2±0.5°C. DOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen.



KOMMENTAR

Försöket visar att provet organiska innehåll har en hög grad av nedbrytning, både avseende DOC och TOC. Den genomsnittliga (FT1 och FT2) nedbrytningsgraden beräknas till 98 % för DOC resp 97 % för TOC.

Inhibitionskontrollen (FI) visar att testmaterialet inte har toxiska egenskaper mot nedbrytningsprocessen. Tillsatsen av extern kolkälla, genom referenskemikalien (Natriumacetat) har samma grad av nedbrytning. Ökad tillgång på lättillgängligt kol ser därför inte ut att öka nedbrytningsgraden.

Den abiotiska kontrollen (FS) reducerar DOC-innehållet med 11 % under nedbrytningsperioden. Det utgörs normalt av komponenter med högre ångtryck som avgår till atmosfären under nedbrytningsperioden. Den fraktionen kan inte anses vara ej nedbrytbar utan helt enkelt för flyktig för att finnas kvar i reaktionslösningen för att här reduceras.

Syrehalten har varit god under perioden. pH har däremot drivit något under perioden. Vid start var pH 8,0 och vid avslut 7,6. pH bör ligga inom intervallet 6,0 – 8,0 för att säkerställa att inte nedbrytningen påverkas negativt. pH har således varit klart inom intervallet under perioden.

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

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PROV

1   P98-2

Provet har analyserats med start den 11 oktober. Provkoncentrationen av jämviktad prov var 5,0 vol%, med hänsyn till utfall i analys av respirationshämning. pH i reaktionslösningen var 7,6 vid start. Vid avslut var pH 7,7. Toxiska egenskaper som stör nedbrytningsprocessen kan inte noteras.

RESULTAT (DOC mg/l, där inte annat anges)

Prov	**Flaska**	**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)	Medel
1	**FT1**	91	5,8	6,1	3,2	2,6	**97**	**98**
	FT2	-	6,9	4,4	3,1	2,1	**98**	
	FT (TOC)	97	-	-	-	5,5	**94**	
	FI	91 *	6,7	4,8	4,1	> 2,0	**> 98 ***	
	FS	90	-	-	-	82	**9,9**	
	FB	< 2	< 2	< 2	< 2	< 2,0	**-**	
	FB (TOC)	2,6	-	-	-	2,2	**-**	
	FC	46	2,6	-	-	-	**> 95**	

FT	testmaterial	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inoculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2023-12-04
GS Ekotox

Göran Svenstam
Ekotoxikolog

INOCULUM

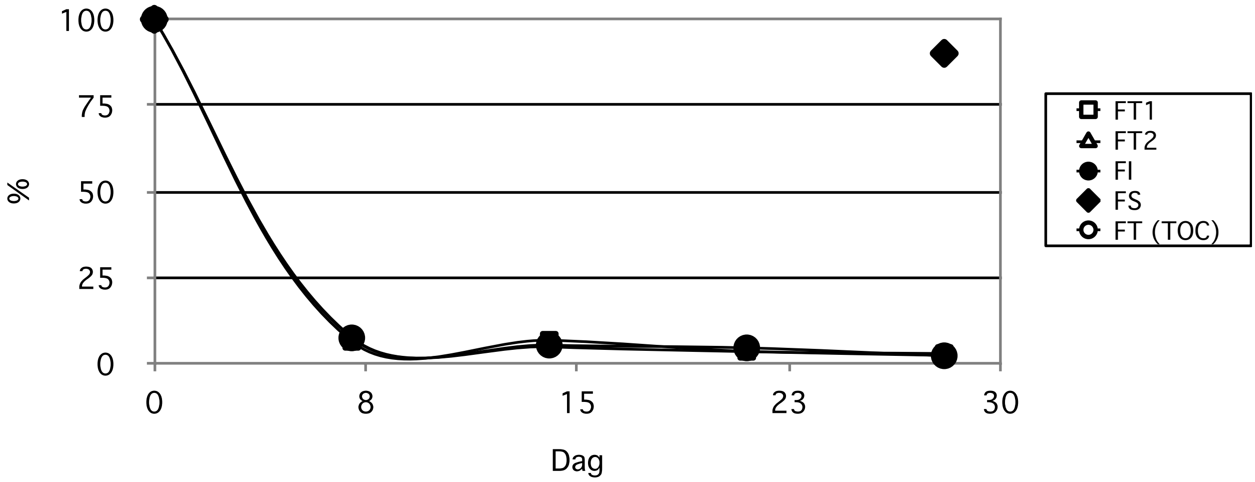
Försedimenterat avloppsvatten från Bromma reningsverk. Den har tillväxt under flera dygn på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var hög, NaAc (46 till 2,6 mg/l på under 7 dygn (FB). Testmaterialets kontroll av toxicitet (FI) visar att ingen toxicitet påverkar nedbrytningsförloppet.

UTRUSTNING

Testvolym 1000 ml i e-kolvar. Testmaterial (FT) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.5±0.5°C. DOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen.



KOMMENTAR

Försöket visar att provet organiska innehåll har en hög grad av nedbrytning, både avseende DOC och TOC. Den genomsnittliga (FT1 och FT2) nedbrytningsgraden beräknas till 98 % för DOC resp 94 % för TOC.

Inhibitionskontrollen (FI) visar att testmaterialet inte har toxiska egenskaper mot nedbrytningsprocessen. Tillsatsen av extern kolkälla, genom referenskemikalien (Natriumacetat) har samma grad av nedbrytning. Ökad tillgång på lättillgängligt kol ser därför inte ut att öka nedbrytningsgraden.

Den abiotiska kontrollen (FS) reducerar DOC-innehållet med 10 % under nedbrytningsperioden. Det utgörs normalt av komponenter med högre ångtryck som avgår till atmosfären under nedbrytningsperioden. Den fraktionen kan inte anses vara ej nedbrytbar utan helt enkelt för flyktig för att finnas kvar i reaktionslösningen för att här reduceras.

Syrehalten har varit god under perioden. pH har däremot drivit något under perioden. Vid start var pH 8,0 och vid avslut 7,7. pH bör ligga inom intervallet 6,0 – 8,0 för att säkerställa att inte nedbrytningen påverkas negativt. pH har således varit klart inom intervallet under perioden.

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

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PROV

1 E85-2

Provet har analyserats med start den 11 oktober. Provkoncentrationen av jämviktad prov var 5,0 vol%, med hänsyn till utfall i analys av respirationshämning. pH i reaktionslösningen var 7,9 vid start. Vid avslut var pH 7,4. Toxiska egenskaper som stör nedbrytningsprocessen kan inte noteras.

RESULTAT (DOC mg/l, där inte annat anges)

Prov	**Flaska**	**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)	Medel
1	**FT1**	1 300	260	30	10	12	**99**	**99**
	FT2	-	570	16	16	16	**99**	
	FT (TOC)	1 400	-	-	-	34	**98**	
	FI	1 300 *	440	25	11	9,7	**99 ***	
	FS	1 300	-	-	-	1 100	**15**	
	FB	< 2	< 2	< 2	< 2	< 2,0	**-**	
	FB (TOC)	2,6	-	-	-	2,2	**-**	
	FC	46	2,6	-	-	-	**> 95**	

FT	testmaterial	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inoculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2023-12-04
GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

INOCULUM

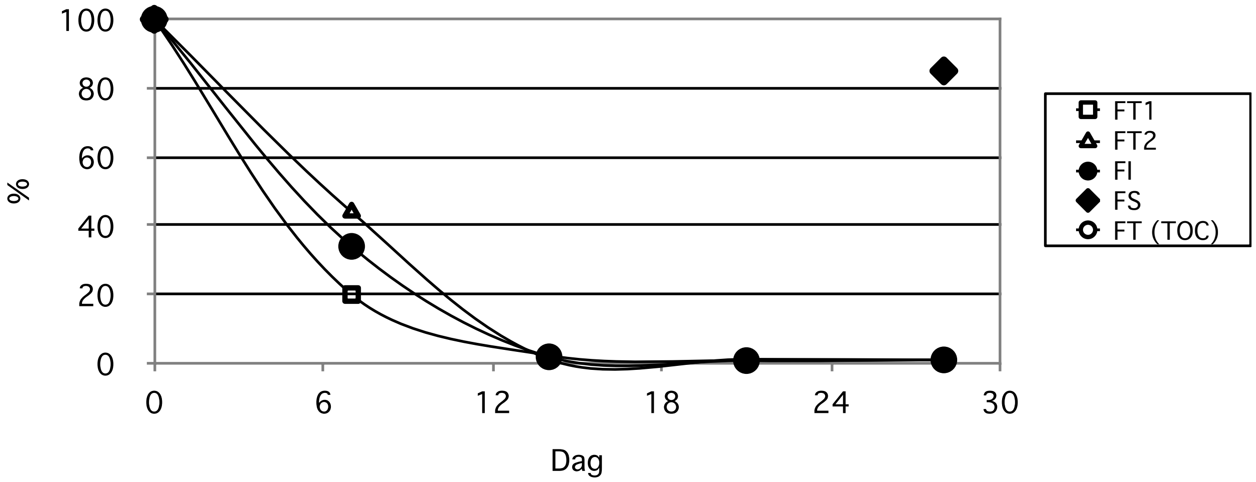
Försedimenterat avloppsvatten från Bromma reningsverk. Den har tillväxt under flera dygn på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var hög, NaAc (46 till 2,6 mg/l på under 7 dygn (FB). Testmaterialets kontroll av toxicitet (FI) visar att ingen toxicitet påverkar nedbrytningsförloppet.

UTRUSTNING

Testvolym 1000 ml i e-kolvar. Testmaterial (FT) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.5 ± 0.5^{o}C. DOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen.



KOMMENTAR

Försöket visar att provet organiska innehåll har en hög grad av nedbrytning, både avseende DOC och TOC. Den genomsnittliga (FT1 och FT2) nedbrytningsgraden beräknas till 99 % för DOC resp 98 % för TOC.

Inhibitionskontrollen (FI) visar att testmaterialet inte har toxiska egenskaper mot nedbrytningsprocessen. Tillsatsen av extern kolkälla, genom referenskemikalien (Natriumacetat) har samma grad av nedbrytning. Ökad tillgång på lättillgängligt kol ser därför inte ut att öka nedbrytningsgraden.

Den abiotiska kontrollen (FS) reducerar DOC-innehållet med 15 % under nedbrytningsperioden. Det utgörs normalt av komponenter med högre ångtryck som avgår till atmosfären under nedbrytningsperioden. Det går inte att utasluta att fraktionen är nedbrytbar. Den är bara för flyktig för att finnas kvar i reaktionslösningen för att där evetuellt reduceras.

Syrehalten har varit god under perioden. pH har däremot drivit något under perioden. Vid start var pH 8,0 och vid avslut 7,4. pH bör ligga inom intervallet 6,0 – 8,0 för att säkerställa att inte nedbrytningen påverkas negativt. pH har således varit klart inom intervallet under perioden.

NEDBRYTBARHET

(SSEN-ISO7827)

Uppdragsgivare

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Box 21060
100 31 STOCKHOLM

PROV

1 EO1-1

Provet har analyserats med start den 11 oktober. Provkoncentrationen av jämviktad prov var 5,0 vol%, med hänsyn till utfall i analys av respirationshämning. pH i reaktionslösningen var 7,9 vid start. Vid avslut var pH 7,7. Toxiska egenskaper som stör nedbrytningsprocessen kan inte noteras. Mänden organiskt material som löser sig i vattenfasen är mycket begränsad. Den beräknade reduktionsgraden begränsas av TOC-analysens känslighet. Nedbrytningsgraden kan därför vara betydligt högre än den som analysen redovisar.

RESULTAT (DOC mg/l, där inte annat anges)

Prov	**Flaska**	**Dag 0**	**7**	**14**	**21**	**28**	Reduktion (%)	Medel
1	**FT1**	< 2	< 2	< 2	< 2	< 2,0	**-**	**31**
	FT2	-	2,0	2,1	< 2	< 2,0	**-**	
	FT (TOC)	3,7	-	-	-	< 2,0	**> 46**	
	FI	*	2,4	2,6	< 2,0	< 2,0	**- ***	
	FS	< 2,0	-	-	-	< 2,0	**-**	
	FB	< 2	< 2	< 2	< 2	< 2,0	**-**	
	FB (TOC)	2,6	-	-	-	2,2	**-**	
	FC	46	2,6	-	-	-	**> 95**	

FT	testmaterial	FI	testmaterial; inhibitionskontroll
FS	” ; abiotisk kontroll	FB	blank
FC	kontroll	*	med avs. på testmaterialet

Bilagor

Beskrivning 1) Inoculum 2) Utrustning 3) Nedbrytningsdiagram 4) Kommentar 5) Metod

2023-12-04
GS Ekotox

Göran Svenstam
EKOTOXIKOLOG

INOCULUM

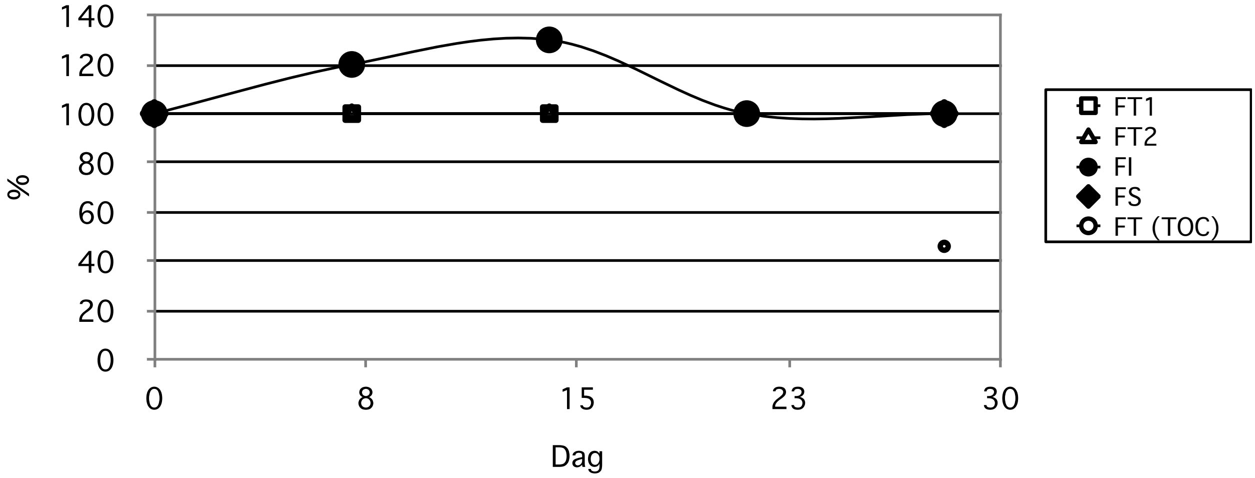
Försedimenterat avloppsvatten från Bromma reningsverk. Den har tillväxt under flera dygn på syntetiskt avloppsvatten. Slamkoncentrationen har varit 25 mgSS/l. Slammets nedbrytande förmåga var hög, NaAc (46 till 2,6 mg/l på under 7 dygn (FB). Testmaterialets kontroll av toxicitet (FI) visar att ingen toxicitet påverkar nedbrytningsförloppet.

UTRUSTNING

Testvolym 1000 ml i e-kolvar. Testmaterial (FT) som duplikat, Blank (FB) och Kontroll (FC), Inhibitionskontroll (FI) och Abiotisk kontroll (FS) i enkel uppsättning. Temperaturen har varit inom intervallet 21.5±0.5°C. DOC-analyser har utförts på instrument med katalytisk förbränning och IR-detektion.

DIAGRAM

Diagram 1. Relativ andel (%) organiskt material (DOC) i förhållande till startkoncentrationen.



KOMMENTAR

Testsubstansen, det med vatten jämviktade oljeprovet, löser endast i begränsad omfattning i vattenlösningen. Halten är redan i försökets start lägre än analysgränsen i TOC/DOC-analysen. Av den anledningen går det inte att verifiera i vilken grad det orgaiska innehållet är nedbrytbart. Halten vid avslut är fortfarande < 2,0mg/l.

Inhibitionskontrollen (FI) visar att testmaterialet inte har toxiska egenskaper mot nedbrytningsprocessen.

Den abiotiska kontrollen (FS), där inga nedbrytande mikroorganismer tillsats, visar ingen abiotisk reduktion eftersom halterna är under analysgränsen både vid start och avslut.

Syrehalten har varit god under perioden. pH har däremot drivit under perioden. Vid start var pH 7,9 och vid avslut 7,7. pH bör ligga inom intervallet 6,0 – 8,0 för att säkerställa att inte nedbrytningen påverkas negativt. pH har således varit klart inom intervallet under perioden.

RESPIRATIONSHÄMNING

(ISO 8192 "Met A")

Uppdragsgivare

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PROV

(1) B95-3

Försöken har genomförts den 5 mars. Spädserien har varit 0.5, 5.0 och 50.0 vol %. Samma provkoncentrationer, men utan ymptillsats, har utgjort den fyskikalisk kemisk kontrollen. pH var vid start 8,0. Efter exponeringsperioden var pH 7,8 i högsta provkoncentrationen.

INOCULUM

Aktivt slam har uttagits den 4 oktober. De har koncentrerats genom sedimentering och centrifugering. Slamkoncentrationen i försöket var 25 mgSS/l. Respiration har beräknats som mgO2/gSS*h. Konditionstest (EC50) mot 3,5 Diklorfenol (DCP) 7,9 mg/l. Metoden redovisar resultat från ringtest där EC50 återfinns i intervallet 5 – 30 mg/l.

RESULTAT (vol%)

Prov	Beräkning	EC20	EC50	Hämning vid 50 vol% *(%)*
1	grafisk linjär regression	8,0 6,3	22 23	*67*

Bilagor

1) Beräkningar 2) Dosrespons diagram 3) Rådata i diagramform 4) Kommentarer 5) Kort metodbeskrivning.

2023-10-09

GS Ekotox AB

Göran Svenstam
EKOTOXIKOLOG

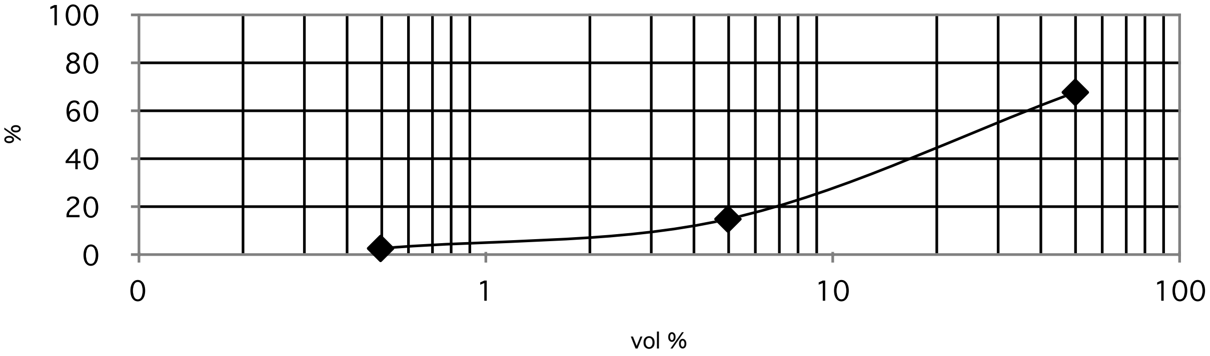
1 Beräkningar

Beräknad respirationshastighet och hämning med utgångspunkt från uppmätta syrehalter i reaktionslösningarna för de olika aktiva provkoncentrationerna. Hämningen har beräknats med justering för den abiotiska kontrollen (fyskemkontroll) om så varit nödvändigt.

Testmaterial (vol %)	**Respiration** (mgO2/gSS*h)	**Hämning** (%)
Blank I **II**	34,5 34,4 34,45	-
DCP 5,0 mg/l	23,2	**32,6**
DCP 20 mg/l	5,4	**84,4**
1 0,5 fyskemkontroll	33,2 0,04	**2,5**
1 5,0 fyskemkontroll	27,5 0,19	**14,7**
1 50 fyskemkontroll	1,3 - 0,18	**67,5**

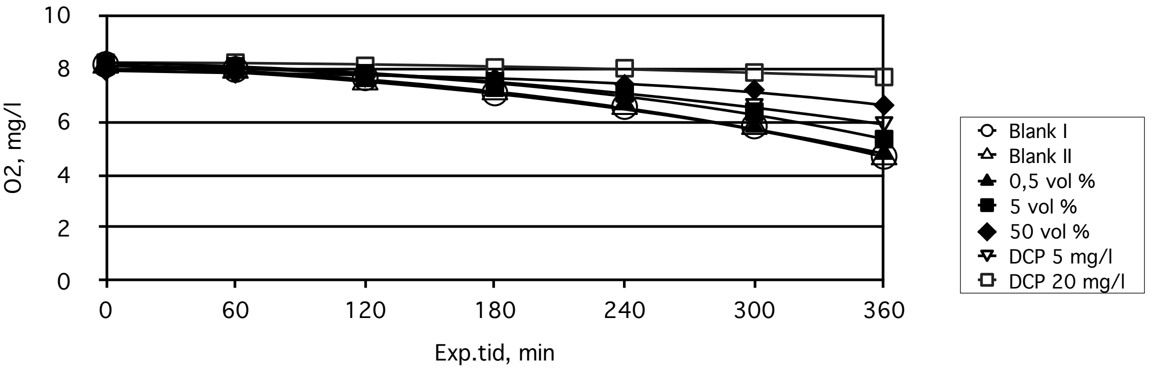
2 Dosrespons diagram.

Dosrespons kurva. Hämning av respiration i delproven.



3 Rådata i diagramform

Respirationen visad som uppmätt syrehalt i reaktionslösningarna. Blankprov (duplikat) och de 5 provkoncentrationer samt referens (3,5 DCP). Tid (min) och syrehalt (mg/l).



4 Kommentarer

Ett aktivt slam förbrukar syre när det bryter ner organiskt material. Om testmaterialet är toxiskt hämmas slammets aktivitet och syreförbrukningen går ned. Genom att mäta syrehalten och bestämma hastigheten på syreförbrukningen mellan de olika provkoncentrationerna kan man rita in hämningsvärdena i ett sk dosrespons-diagram. Är hastigheten lägre jämfört med blankproven så är testmaterialet därmed hämmande och att betrakta som en toxisk respons.

Det aktuella provet ses en påtaglig toxicitet i testsystemet inom det använda spädintervallet 0,5 - 50 vol %. Påverkan från den fyskem kontrollen är begränsad men har räknats in vid beräkning av hämning vid varje provkoncentration.

Hämningen från testmaterialet är betydande. En 20 %-ig hämning inställer sig vid 8 vol % och 50 % vid 22 vol %.

5 Kort metodbeskrivning

Blank, spädserien av testmaterialet och referenssubstansen sätts till 300 ml vinklerflaskor och ympas med ”respirerande aktivt slam” och syntetiskt avloppsvatten. Samma spädserie av testmaterialet sätts också i vinklerflaskor utan ymptillsats (fyskem kontroll) men med syntetiskt avloppsvatten. Vinklerflaskorna mäts avseende syreinnehåll vid upprepade tillfällen, dock inte längre än att syrenivån överstiger 2,0 mg/l. De abiotiska kontrollerna avläses endast vid start och avslutning av försöket.

RESPIRATIONSHÄMNING

(ISO 8192 "Met A")

Uppdragsgivare

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PROV

(1) B98-2

Försöken har genomförts den 5 mars. Spädserien har varit 0.5, 5.0 och 50.0 vol %. Samma provkoncentrationer, men utan ymptillsats, har utgjort den fyskikalisk kemisk kontrollen. pH var vid start 8,0. Efter exponeringsperioden var pH 7,8 i högsta provkoncentrationen.

INOCULUM

Aktivt slam har uttagits den 4 oktober. De har koncentrerats genom sedimentering och centrifugering. Slamkoncentrationen i försöket var 25 mgSS/l. Respiration har beräknats som mgO2/gSS*h. Konditionstest (EC50) mot 3,5 Diklorfenol (DCP) 7,9 mg/l. Metoden redovisar resultat från ringtest där EC50 återfinns i intervallet 5 – 30 mg/l.

RESULTAT (vol%)

Prov	Beräkning	**EC20**	**EC50**	Hämning vid 50 vol% *(%)*
1	grafisk linjär regression	4,1 3,6	13 12	*87*

Bilagor

1) Beräkningar 2) Dosrespons diagram 3) Rådata i diagramform 4) Kommentarer
5) Kort metodbeskrivning.

2023-10-09

GS Ekotox AB

Göran Svenstam
EKOTOXIKOLOG

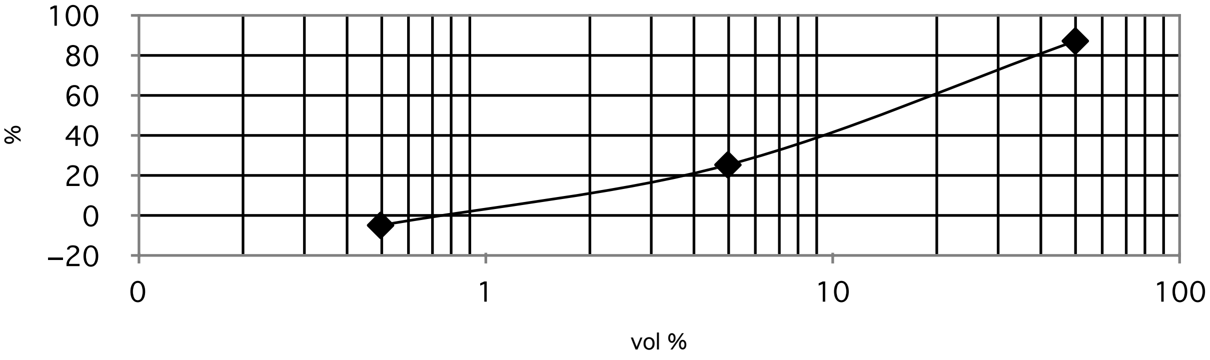
1 Beräkningar

Beräknad respirationshastighet och hämning med utgångspunkt från uppmätta syrehalter i reaktionslösningarna för de olika aktiva provkoncentrationerna. Hämningen har beräknats med justering för den abiotiska kontrollen (fyskemkontroll) om så varit nödvändigt.

Testmaterial (vol %)	**Respiration** (mgO2/gSS*h)	**Hämning** (%)
Blank I **II**	34,5 34,4 34,45	-
DCP 5,0 mg/l	23,2	**32,6**
DCP 20 mg/l	5,4	**84,4**
1 0,5 fyskemkontroll	36,2 0,00	**- 5,1**
1 5,0 fyskemkontroll	27,1 - 0,13	**25,1**
1 50 fyskemkontroll	0,67 - 0,22	**86,9**

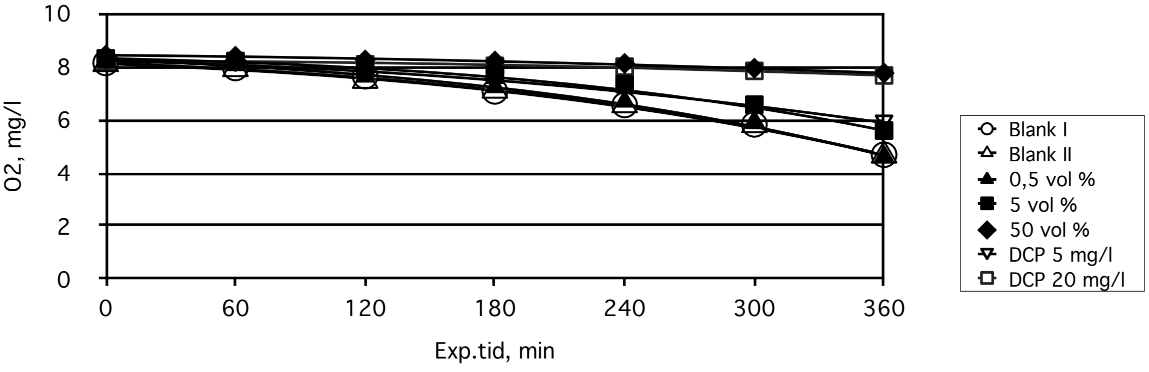
2 Dosrespons diagram.

Dosrespons kurva. Hämning av respiration i delproven.



3 Rådata i diagramform

Respirationen visad som uppmätt syrehalt i reaktionslösningarna. Blankprov (duplikat) och de 5 provkoncentrationer samt referens (3,5 DCP). Tid (min) och syrehalt (mg/l).



4 Kommentarer

Ett aktivt slam förbrukar syre när det bryter ner organiskt material. Om testmaterialet är toxiskt hämmas slammets aktivitet och syreförbrukningen går ned. Genom att mäta syrehalten och bestämma hastigheten på syreförbrukningen mellan de olika provkoncentrationerna kan man rita in hämningsvärdena i ett sk dosrespons-diagram. Är hastigheten lägre jämfört med blankproven så är testmaterialet därmed hämmande och att betrakta som en toxisk respons.

Det aktuella provet ses en påtaglig toxicitet i testsystemet inom det använda spädintervallet 0,5 - 50 vol %. Påverkan från den fyskem kontrollen är begränsad men har räknats in vid beräkning av hämning vid varje provkoncentration.

Hämningen från testmaterialet är betydande. En 20 %-ig hämning inställer sig vid 4 vol % och 50 % vid 13 vol %.

5 Kort metodbeskrivning

Blank, spädserien av testmaterialet och referenssubstansen sätts till 300 ml vinklerflaskor och ympas med ”respirerande aktivt slam” och syntetiskt avloppsvatten. Samma spädserie av testmaterialet sätts också i vinklerflaskor utan ymptillsats (fyskem kontroll) men med syntetiskt avloppsvatten. Vinklerflaskorna mäts avseende syreinnehåll vid upprepade tillfällen, dock inte längre än att syrenivån överstiger 2,0 mg/l. De abiotiska kontrollerna avläses endast vid start och avslutning av försöket.

RESPIRATIONSHÄMNING

(ISO 8192 "Met A")

Uppdragsgivare

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100 31 STOCKHOLM

PROV

(1) E85-2

Försöken har genomförts den 5 mars. Spädserien har varit 0.5, 5.0 och 50.0 vol %. Samma provkoncentrationer, men utan ymptillsats, har utgjort den fyskikalisk kemisk kontrollen. pH var vid start 8,0. Efter exponeringsperioden var pH 7,9 i högsta provkoncentrationen.

INOCULUM

Aktivt slam har uttagits den 4 oktober. De har koncentrerats genom sedimentering och centrifugering. Slamkoncentrationen i försöket var 25 mgSS/l. Respiration har beräknats som mgO2/gSS*h. Konditionstest (EC50) mot 3,5 Diklorfenol (DCP) 7,9 mg/l. Metoden redovisar resultat från ringtest där EC50 återfinns i intervallet 5 – 30 mg/l.

RESULTAT (vol%)

Prov	Beräkning	**EC20**	**EC50**	Hämning vid 50 vol% *(%)*
1	grafisk linjär regression	4,2 4,6	18 17	*74*

Bilagor

1) Beräkningar 2) Dosrespons diagram 3) Rådata i diagramform 4) Kommentarer 5) Kort metodbeskrivning.

2023-10-09

GS Ekotox AB

Göran Svenstam
EKOTOXIKOLOG

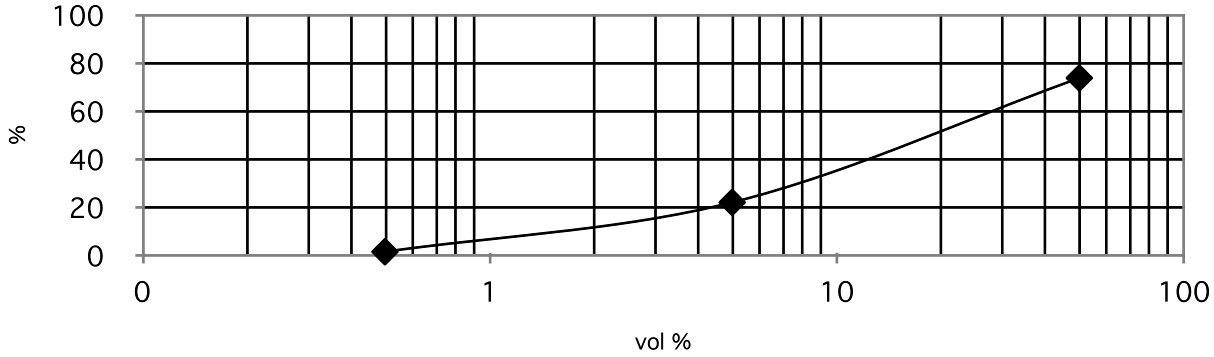
1 Beräkningar

Beräknad respirationshastighet och hämning med utgångspunkt från uppmätta syrehalter i reaktionslösningarna för de olika aktiva provkoncentrationerna. Hämningen har beräknats med justering för den abiotiska kontrollen (fyskemkontroll) om så varit nödvändigt.

Testmaterial (vol %)	**Respiration** (mgO2/gSS*h)	**Hämning** (%)
Blank I **II**	47,9 49,5 48,7	**-**
DCP 5,0 mg/l	23,2	**32,6**
DCP 20 mg/l	5,4	**84,4**
1 0,5 fyskemkontroll	43,5 0,45	**1,4**
1 5,0 fyskemkontroll	35,2 0,28	**22,0**
1 50 fyskemkontroll	10,2 0,26	**73,7**

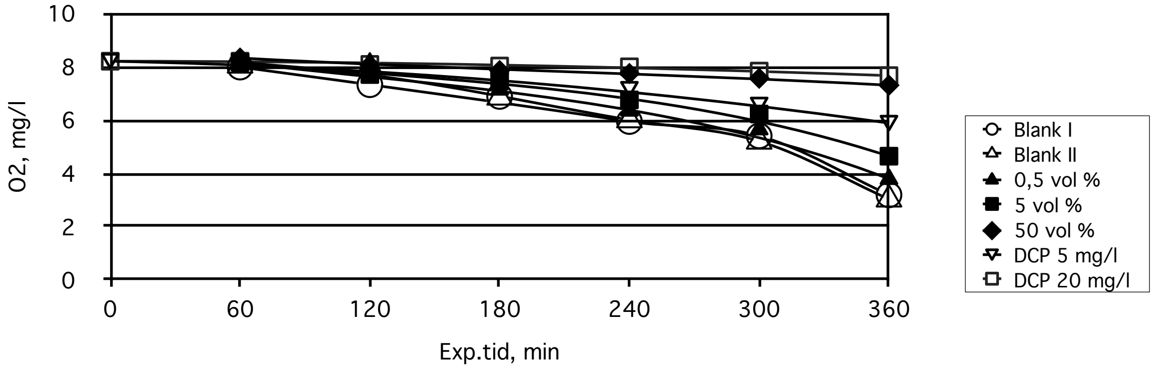
2 Dosrespons diagram.

Dosrespons kurva. Hämning av respiration i delproven.



3 Rådata i diagramform

Respirationen visad som uppmätt syrehalt i reaktionslösningarna. Blankprov (duplikat) och de 5 provkoncentrationer samt referens (3,5 DCP). Tid (min) och syrehalt (mg/l).



4 Kommentarer

Ett aktivt slam förbrukar syre när det bryter ner organiskt material. Om testmaterialet är toxiskt hämmas slammets aktivitet och syreförbrukningen går ned. Genom att mäta syrehalten och bestämma hastigheten på syreförbrukningen mellan de olika provkoncentrationerna kan man rita in hämningsvärdena i ett sk dosrespons-diagram. Är hastigheten lägre jämfört med blankproven så är testmaterialet därmed hämmande och att betrakta som en toxisk respons.

Det aktuella provet ses en påtaglig toxicitet i testsystemet inom det använda spädintervallet 0,5 - 50 vol %. Påverkan från den fyskem kontrollen är begränsad men har räknats in vid beräkning av hämning vid varje provkoncentration.

Hämningen från testmaterialet är betydande. En 20 %-ig hämning inställer sig vid 4 vol % och 50 % vid 17 vol %.

5 Kort metodbeskrivning

Blank, spädserien av testmaterialet och referenssubstansen sätts till 300 ml vinklerflaskor och ympas med ”respirerande aktivt slam” och syntetiskt avloppsvatten. Samma spädserie av testmaterialet sätts också i vinklerflaskor utan ymptillsats (fyskem kontroll) men med syntetiskt avloppsvatten. Vinklerflaskorna mäts avseende syreinnehåll vid upprepade tillfällen, dock inte längre än att syrenivån överstiger 2,0 mg/l. De abiotiska kontrollerna avläses endast vid start och avslutning av försöket.

RESPIRATIONSHÄMNING

(ISO 8192 "Met A")

Uppdragsgivare

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Box 21060
100 31 STOCKHOLM

PROV

(1) D-EU1

Försöken har genomförts den 5 mars. Spädserien har varit 0.5, 5.0 och 50.0 vol %. Samma provkoncentrationer, men utan ymptillsats, har utgjort den fysikalisk kemisk kontrollen. pH var vid start 8,0. Efter exponeringsperioden var pH 7,9 i högsta provkoncentrationen.

INOCULUM

Aktivt slam har uttagits den 4 oktober. De har koncentrerats genom sedimentering och centrifugering. Slamkoncentrationen i försöket var 25 mgSS/l. Respiration har beräknats som mgO2/gSS*h. Konditionstest (EC50) mot 3,5 Diklorfenol (DCP) 7,9 mg/l. Metoden redovisar resultat från ringtest där EC50 återfinns i intervallet 5 – 30 mg/l.

RESULTAT (vol%)

Prov	Beräkning	**EC20**	**EC50**	Hämning vid 50 vol% *(%)*
1	grafisk linjär regression	12 13	55 55	*48*

Bilagor

1) Beräkningar 2) Dosrespons diagram 3) Rådata i diagramform 4) Kommentarer 5) Kort metodbeskrivning.

2023-10-09

GS Ekotox AB

Göran Svenstam
EKOTOXIKOLOG

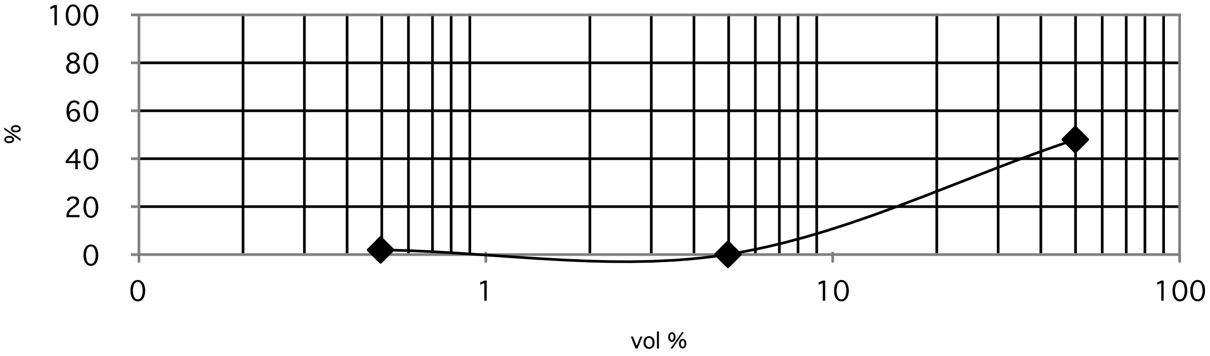
1 Beräkningar

Beräknad respirationshastighet och hämning med utgångspunkt från uppmätta syrehalter i reaktionslösningarna för de olika aktiva provkoncentrationerna. Hämningen har beräknats med justering för den abiotiska kontrollen (fyskemkontroll) om så varit nödvändigt.

Testmaterial (vol %)	**Respiration** (mgO2/gSS*h)	**Hämning** (%)
Blank I **II**	47,9 49,5 48,7	-
DCP 5,0 mg/l	23,2	**32,6**
DCP 20 mg/l	5,4	**84,4**
1 **0,5** fyskemkontroll	44,8 0,30	**1,8**
1 **5,0** fyskemkontroll	45,7 0,30	**0,0**
1 **50** fyskemkontroll	23,4 0,20	**47,8**

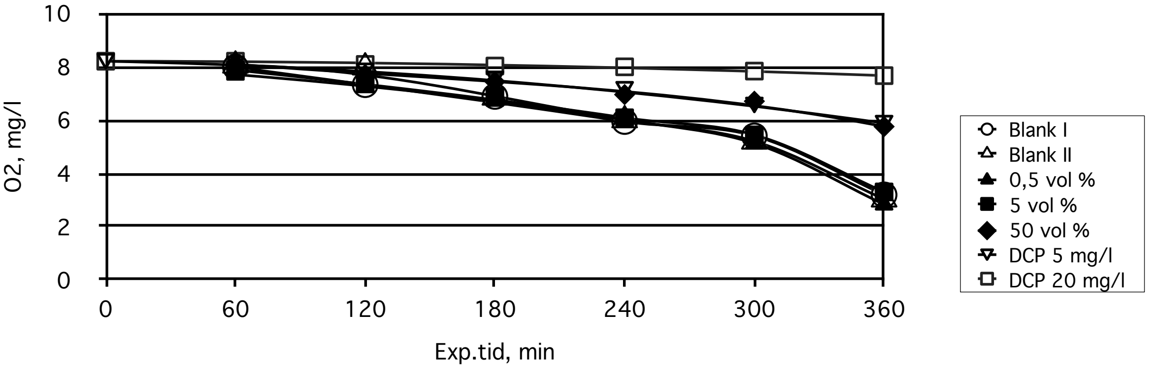
2 Dosrespons diagram.

Dosrespons kurva. Hämning av respiration i delproven.



3 Rådata i diagramform

Respirationen visad som uppmätt syrehalt i reaktionslösningarna. Blankprov (duplikat) och de 5 provkoncentrationer samt referens (3,5 DCP). Tid (min) och syrehalt (mg/l).



4 Kommentarer

Ett aktivt slam förbrukar syre när det bryter ner organiskt material. Om testmaterialet är toxiskt hämmas slammets aktivitet och syreförbrukningen går ned. Genom att mäta syrehalten och bestämma hastigheten på syreförbrukningen mellan de olika provkoncentrationerna kan man rita in hämningsvärdena i ett sk dosrespons-diagram. Är hastigheten lägre jämfört med blankproven så är testmaterialet därmed hämmande och att betrakta som en toxisk respons.

Det aktuella provet ses en påtaglig toxicitet i testsystemet inom det använda spädintervallet 0,5 - 50 vol %. Påverkan från den fyskem kontrollen är begränsad men har räknats in vid beräkning av hämning vid varje provkoncentration.

Hämningen från testmaterialet är betydande. En 20 %-ig hämning inställer sig vid 12 vol % och 50 % vid 55 vol %.

5 Kort metodbeskrivning

Blank, spädserien av testmaterialet och referenssubstansen sätts till 300 ml vinklerflaskor och ympas med ”respirerande aktivt slam” och syntetiskt avloppsvatten. Samma spädserie av testmaterialet sätts också i vinklerflaskor utan ymptillsats (fyskem kontroll) men med syntetiskt avloppsvatten. Vinklerflaskorna mäts avseende syreinnehåll vid upprepade tillfällen, dock inte längre än att syrenivån överstiger 2,0 mg/l. De abiotiska kontrollerna avläses endast vid start och avslutning av försöket.

RESPIRATIONSHÄMNING

(ISO 8192 "Met A")

Uppdragsgivare

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Box 21060
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PROV

(1)   EO1-1

Försöken har genomförts den 26 oktober. Spädserien har varit 0.5, 5.0 och 50.0 vol %. Samma provkoncentrationer, men utan ymptillsats, har utgjort abiotisk kontroll. pH var vid start 7,9. Efter exponeringsperioden var pH 8,0 i den högsta provkoncentrationen.

INOCULUM

Aktivt slam har uttagits den 26 oktober. De har koncentrerats genom sedimentering och centrifugering. Slamkoncentrationen i försöket var 25 mgSS/l. Respiration har beräknats som mgO2/gSS*h. Konditionstest (EC50) mot 3,5 Diklorfenol (DCP) 7,9 mg/l. Det är en normal känslighet för metoden.

RESULTAT (vol%)

Prov	Beräkning	**EC20**	**EC50**	Hämning vid 50 vol% *(%)*
1	grafisk linjär regression	> 50 > 50	> 50 > 50	*−2,2*

Bilagor

1) Beräkningar   2) Dosrespons diagram   3) Rådata i diagramform   4) Kommentarer   5) Kort metodbeskrivning.

2023-10-26

GS Ekotox AB

Göran Svenstam
EKOTOXIKOLOG

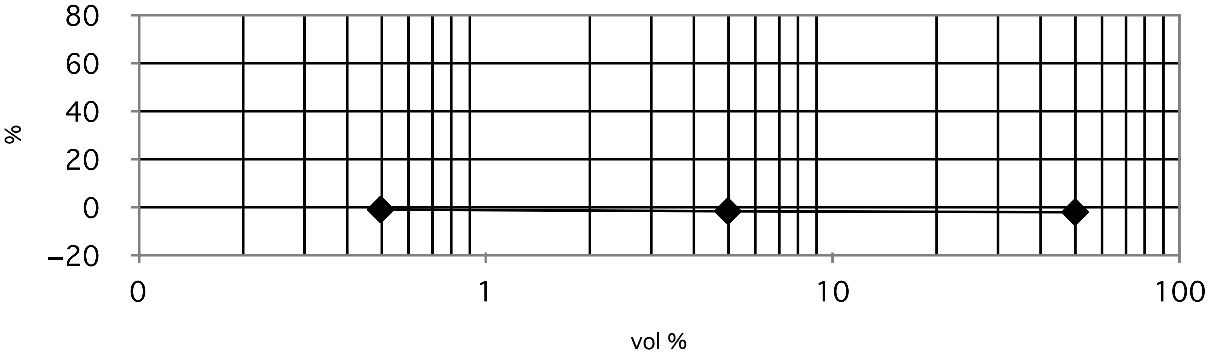
1 Beräkningar

Beräknad respirationshastighet och hämning med utgångspunkt från uppmätta syrehalter i reaktionslösningarna för de olika aktiva provkoncentrationerna. Hämningen har beräknats med justering för den abiotiska kontrollen (fyskemkontroll) om så varit nödvändigt.

Testmaterial (vol %)	**Respiration** (mgO2/gSS*h)	**Hämning** (%)
Blank I **II**	44,3 44,5 44,4	**-**
DCP 5,0 mg/l	28,6	**35,5**
DCP 20 mg/l	8,9	**80,0**
1 **0,5** fyskemkontroll	44,9 0,32	**- 1,1**
1 **5,0** fyskemkontroll	45,2 0,32	**- 1,8**
1 **50** fyskemkontroll	45,4 0,32	**- 2,2**

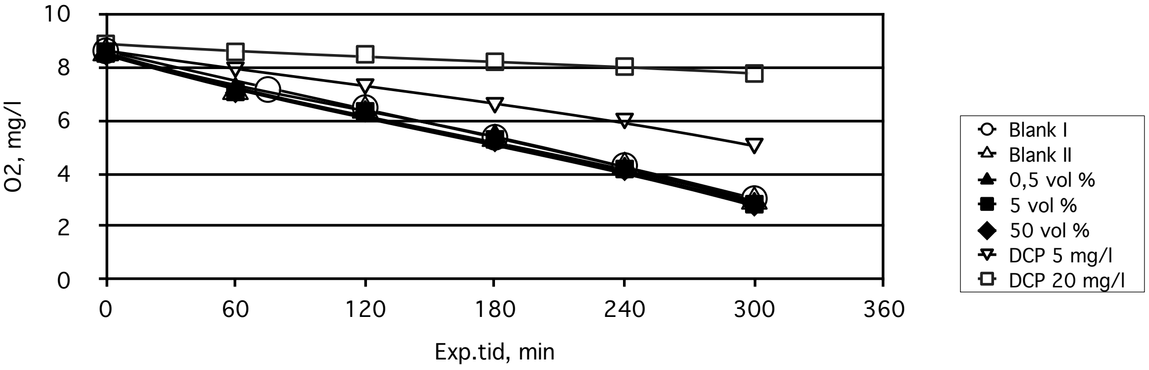
2 Dosrespons diagram.

Dosrespons kurva. Hämning av respiration i delproven.



3 Rådata i diagramform

Respirationen visad som uppmätt syrehalt i reaktionslösningarna. Blankprov (duplikat) och de 5 provkoncentrationer samt referens (3,5 DCP). Tid (min) och syrehalt (mg/l).



4 Kommentarer

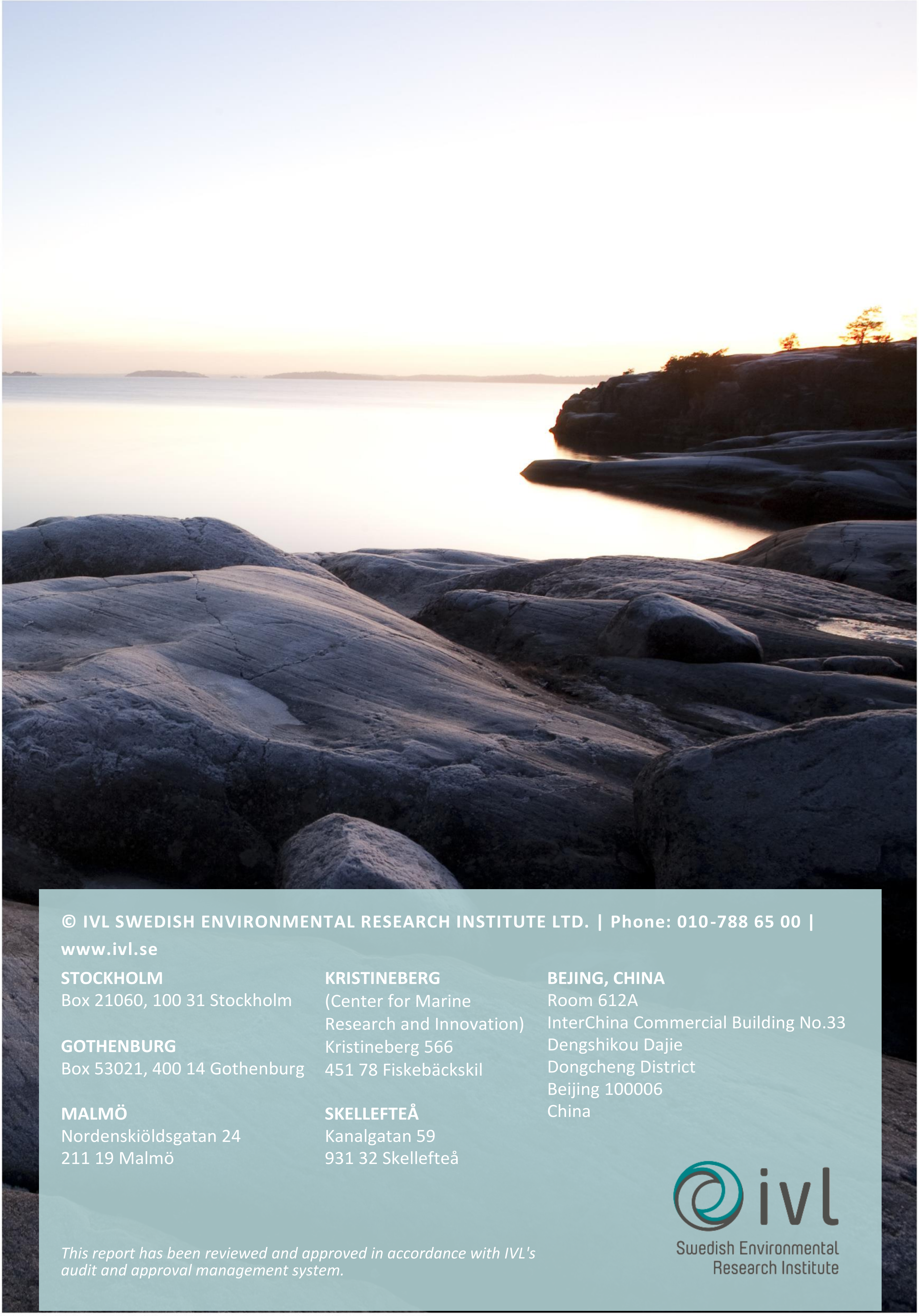
Ett aktivt slam förbrukar syre när det bryter ner organiskt material. Om testmaterialet är toxiskt hämmas slammets aktivitet och syreförbrukningen går därför ner. Genom att med jämna mellanrum registrera syrehalten går det att bestämma vilken hastighet syret förbrukas. Hastigheten omräknas i hämningsvärden utifrån blankprovens syreförbrukning och avsätts i ett sk dosrespons-diagram. Är hastigheten lägre jämfört med blankproven så är testmaterialet därmed hämmande och att betrakta som en toxisk respons.

Det aktuella provet ses ingen toxicitet i testsystemet inom det använda spädintervallet 0,5 - 50 vol %. Påverkan från den abiotiska kontrollen är mycket begränsad men har räknats in vid beräkning av hämning vid varje provkoncentration.

Hämningen från testmaterialet är negativ vid samtliga provkoncentrationer. Negativ hämning innebär att provet har stimulerande egenskaper på det respirerande ”aktiva slammet”.

5 Kort metodbeskrivning

Blank, spädserien av testmaterialet och referenssubstansen sätts till 300 ml vinklerflaskor och ympas med respirerande ”aktivt slam” och syntetiskt avloppsvatten. Samma spädserie av testmaterialet sätts också i vinklerflaskor utan ymptillsats (fyskem kontroll) men med syntetiskt avloppsvatten. Vinklerflaskorna mäts avseende syreinnehåll vid upprepade tillfällen, dock inte längre än att syrenivån överstiger 2,0 mg/l. De abiotiska kontrollerna avläses endast vid start och avslutning av försöket.



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This report has been reviewed and approved in accordance with IVL's audit and approval management system.

Swedish Environmental
Research Institute