TEXTE
78/2022

Refinement of the P assessment of ionisable substances: Distribution and degradation of anionic, neutral and cationic organic chemicals in water-sediment systems

German Environment Agency

Umwelt Bundesamt

TEXTE 78/2022

Ressortforschungsplan of the Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection

Project No. (FKZ) 3715 65 415 2

Report No. FB000178/ENG

Refinement of the P assessment of ionisable substances: Distribution and degradation of anionic, neutral and cationic organic chemicals in water-sediment systems

by

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On behalf of the German Environment Agency

Imprint

Publisher
Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
Tel: +49 340-2103-0
Fax: +49 340-2103-2285
buergerservice@uba.de
Internet: www.umweltbundesamt.de

/umweltbundesamt.de
/umweltbundesamt

Report performed by:
Institut für Umweltforschung, RWTH Aachen University
Worringerweg 1
52074 Aachen

Report completed in:
August 2019

Edited by:
Section IV 2.3 Chemicals
Dr. Juliane Ackermann, Ulrich Jöhncke

Publication as pdf:
http://www.umweltbundesamt.de/publikationen

ISSN 1862-4804

Dessau-Roßlau, July 2022

Abstract

The identification of persistent (P) bioaccumulative (B) and toxic (T) substances under the EU regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) was developed to assess neutral organic compounds. Under environmental conditions, however, organic chemicals can be ionised leading to a different environmental behaviour. The objective of the project was to investigate the behaviour of anionic and cationic organic chemicals compared to neutral organic chemicals in sediment and surface water in order to refine the P assessment under REACH. Three radiolabelled model substances were used with 4-*n*-dodecylbenzene sulfonic acid sodium salt (DS^-), 4-*n*-dodecylphenol (DP) and 4-*n*-dodecylbenzyltrimethyl ammonium chloride (DA^+) representing the anionic, non-ionic and cationic compound, respectively. Simulation studies according to OECD 308 (*Aerobic and Anaerobic Transformation in Aquatic Sediment Systems*) and OECD 309 (*Aerobic Mineralization in Surface Water - Simulation Biodegradation Test)* were performed.

The objective of the simulation study following OECD guideline 308 was to investigate the behaviour of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in a water-sediment system over an incubation period of 120 days. The sediment was extracted sequentially using different solvents (aqueous $CaCl_2$ solution, methanol and acetonitrile). The sediment incubated with ^{14}C-DA^+ was additionally extracted under Soxhlet conditions. After 120 days of incubation, mineralisation of ^{14}C-DS^- and ^{14}C-DP accounted for 68 % and 63 % AR, respectively. The cationic compound ^{14}C-DA^+ was mineralised to a lesser extent (6 % AR). The direct bioavailability of the test substances, based on their mineralisation and portions in the $CaCl_2$ fraction, decreased as follows: ^{14}C-DS^- > ^{14}C-DP > ^{14}C-DA^+. NER formation was highest for ^{14}C-DA^+ (33 % AR), followed by ^{14}C-DS^- (19 % AR) and ^{14}C-DP (14 % AR) after 120 days of incubation. Half-lives (DT_{50}) of the test substances for the overall water-sediment system and for the sediment ($DT_{50,sed}$) and water phase ($DT_{50,w}$) separately were calculated. For the overall test system, half-lives decreased as follows: ^{14}C-DA^+ (162 days) > ^{14}C-DS^- (22 days) and ^{14}C-DP (14 days). $DT_{50,sed}$ of ^{14}C-DA^+, ^{14}C-DP and ^{14}C-DS^- amounted 267 days, 24 days and 122 days, respectively. $DT_{50,w}$ accounted for < 1 day for all test substances.

In order to investigate the behaviour of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in surface water, simulation studies following OECD 309 was performed. Preliminary surface water tests were performed as pelagic tests (surface water only) and as suspended sediment tests (surface water with addition of suspended sediment) using ^{14}C-DP and ^{14}C-DS^-. No significant difference between the biodegradation ^{14}C-DP and ^{14}C-DS^- was observed between both test systems: For ^{14}C-DS^-, mineralisation accounted for up to 51 % in the pelagic test and up to 56 % in the suspended sediment test at the end of the test (after 60 days). For ^{14}C-DP, mineralisation was up to 56 % in the pelagic test and up to 60 % in the suspended sediment test.

The main surface water test was run as a suspended sediment test at a concentration of 10 µg/L and 100 µg/L. Additionally, abiotic degradation of the model substances was examined under sterile conditions using autoclaved surface water treated with sodium azide and gamma irradiated sediment. After an incubation time of 62 days (10 µg/L) and 60 days (100 µg/L) under non-sterile conditions, mineralisation accounted for 75 % AR (^{14}C-DS^-) and 69 % AR (^{14}C-DP), and 63 % AR (^{14}C-DS^-) and 58 % AR (^{14}C-DP), respectively. Mineralisation of ^{14}C-DA^+ was 7 % AR at both test concentrations. Under sterile conditions, mineralisation of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ was neglectable (< 0.1 % AR). After 60 days, highest NER formation was observed for ^{14}C-DP (21 % AR) followed by ^{14}C-DA^+ (14 % AR) and ^{14}C-DS^- (9 % AR). NER formation of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ under sterile conditions was considerably lower (0.1 %, 0.6 % and 5.5 % AR, respectively). Half-lives of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ decreased as follows: DA^+ (26 days at 10

µg/L and 13 days at 100 µg/L) > DP (2 days at 10 µg/L and 1 day at 100 µg/L) > DS^- (1 day at both test concentrations).

Using three differently charged model substances with high structural similarity, we showed that a positive charge has a negative impact on the degradation of organic chemicals in both a water-sediment system and surface water, compared to no charge. A negative charge, however, affects the degradation of a chemical positively. Consequently, a compound with a positive charge exhibits higher half-lives resulting in a higher persistence compared to neutral or negatively charged compounds. With respect to the refinement of the P assessment, we recommend using the degradation half-life of the total water-sediment system of OECD 308 as an indicator for persistence of both ionic and neutral organic chemicals. We propose performing the simulation study following OECD 309 as a pelagic test since its degradation capacity was nearly identical compared to the suspended sediment test and this test design minimises potential NER formation.

Kurzbeschreibung

Die Identifizierung persistenter (P), bioakkumulierender (B) und toxischer(T) Stoffe unter der EU-Chemikalienverordnung REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) wurde für die Bewertung neutraler, organischer Chemikalien entwickelt. Jedoch können organische Chemikalien unter Umweltbedingungen ionisiert vorliegen, was eine Veränderung ihres Abbauverhaltens in der Umwelt bewirkt. Ziel dieses Projekts war die Untersuchung der Verteilung und des Abbaus anionischer und kationischer organischer Chemikalien im Vergleich zu nicht-ionischen Chemikalien in Sediment und Oberflächenwasser. Mit Hilfe der gewonnenen Daten soll das Bewertungskonzept hinsichtlich der Persistenz ionischer und ionisierbarer Stoffe verbessert werden. Als Modellsubstanzen dienten drei radioaktiv markierte organische Chemikalien: 4-*n*-Dodecylbenzolsulfonsäure Natriumsalz (DS^-, anionisch), 4-*n*-Dodecylphenol (DP, neutral) und 4-*n*-Dodecylbenzyltrimethylammoniumchlorid (DA^+, kationisch). Mit diesen Substanzen wurden Simulationsstudien nach den OECD Richtlinien 308 (*Aerobic and Anaerobic Transformation in Aquatic Sediment Systems*) und 309 (*Aerobic Mineralization in Surface Water - Simulation Biodegradation Test*) durchgeführt.

Ziel der Simulationsstudie nach OECD 308 war, das Verhaltens von ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in einem Wasser-Sediment-System über eine Inkubationszeit von 120 Tagen zu untersuchen. Das Sediment wurde sequenziell mit unterschiedlichen Lösungsmitteln (wässrige $CaCl_2$-Lösung, Methanol und Acetonitril) extrahiert. Für ^{14}C-DA^+ wurde zusätzlich eine Soxhlet-Extraktion mit Methanol durchgeführt. Nach einer Inkubationszeit von 120 Tagen wurden von ^{14}C-DS^- und ^{14}C-DP 68 % bzw. 63 % der applizierten Radioaktivität (AR) mineralisiert. Der mineralisierte Anteil von ^{14}C-DA^+ betrug 6 % AR. Anhand der Mineralisationsraten und den Anteilen der Modellsubstanzen im $CaCl_2$-Extrakt wurde die direkte Bioverfügbarkeit ermittelt. Diese nahm wie folgt ab: ^{14}C-DS^- > ^{14}C-DP > ^{14}C-DA^+. Die höchste Menge an nicht-extrahierbaren Rückständen (NER) nach 120 Tagen wurde für ^{14}C-DA^+ beobachtet (33 % AR), gefolgt von ^{14}C-DS^- (19 % AR) und ^{14}C-DP (14 % AR). Die Berechnung der Halbwertszeit (DT_{50}) erfolgte sowohl für das Gesamtsystem als auch für das Sediment ($DT_{50,sed}$) und die Wasserphase getrennt ($DT_{50,w}$). Im Gesamtsystem nahm die DT_{50} wie folgt ab: ^{14}C-DA^+ (162 Tage) >^{14}C-DS^- (22 Tage) > ^{14}C-DP (14 Tage). $DT_{50,sed}$ betrug für ^{14}C-DA^+ 267 Tage, für ^{14}C-DP 24 Tage und für ^{14}C-DS^- 22 Tage. Deutlich geringere Halbwertszeiten (< 1 Tag) aller Modellsubstanzen wurden für die Wasserphase ermittelt.

Das Verhalten der Modellsubstanzen in Oberflächenwasser wurde anhand von Simulationsstudien nach OECD 309 untersucht. Zur Untersuchung des Abbaus von ^{14}C-DS^- und ^{14}C-DP wurden zunächst Vorversuche als pelagische Tests (nur Oberflächenwasser) und

suspendierte Sedimenttests (Oberflächenwasser mit Zugabe von suspendiertem Sediment) durchgeführt. Hinsichtlich des biologischen Abbaus der Testsubstanzen konnte kein wesentlicher Unterschied zwischen beiden Testdesigns festgestellt werden: Im pelagischen Test betrug die Mineralisation von ^{14}C-DS$^-$ bis zu 51 % AR, im suspendierten Sedimenttest wurden an Versuchsende (nach 60 Tagen) bis zu 56 % AR mineralisiert. Die mineralisierten Anteile von ^{14}C-DP betrugen im pelagischen Test bis zu 56 % AR und im suspendierten Sedimenttest bis zu 60 % AR nach 60 Tagen.

Hauptversuche zum Abbau von ^{14}C-DS$^-$, ^{14}C-DP und ^{14}C-DA$^+$ wurden als suspendierter Sedimenttestunter Verwendung verschiedener Testkonzentrationen (10 µg/l und 100 µg/l) durchgeführt. Zusätzlich wurde der abiotische Abbau der Modellsubstanzen unter sterilen Bedingungen untersucht. Unter nicht-sterilen Bedingungen wurden nach 62 Tagen (10 µg/l) bzw. 60 Tagen (100 µg/l) 75 % AR (^{14}C-DS$^-$) und 69 % AR (^{14}C-DP) bzw. 63 % AR (^{14}C-DS$^-$) und 58 % AR (^{14}C-DP) mineralisiert. Die Mineralisation von ^{14}C-DA$^+$ lag bei beiden Testkonzentrationen bei 7 % AR. Unter sterilen Bedingungen war die Mineralisation vernachlässigbar (< 0,1 % AR). Nach 60 Tagen wurden die höchsten Mengen an NER für ^{14}C-DP beobachtet (21 % AR), gefolgt von ^{14}C-DA$^+$ (14 % AR) und ^{14}C-DS$^-$ (9 % AR). Unter sterilen Bedingungen wurden deutlich geringere NER Mengen von ^{14}C-DS$^-$, ^{14}C-DP und ^{14}C-DA$^+$ gebildet (0,1 %, 0,6 % und 5,5 % AR). Die Halbwertszeiten nahmen bei einer Testkonzentration von 10 µg/l wie folgt ab: ^{14}C-DA$^+$ (26 Tage) > ^{14}C-DP (2 Tage) > ^{14}C-DS$^-$ (1 Tag). Bei einer Testkonzentration von 100 µg/l betrug die DT_{50} von ^{14}C-DA$^+$ 13 Tage, von ^{14}C-DP und ^{14}C-DS$^-$ jeweils 1 Tag.

Unter Verwendung unterschiedlich geladener Modellsubstanzen, die ansonsten eine hohe strukturelle Ähnlichkeit besitzen, konnte gezeigt werden, dass eine positive Ladung im Vergleich zu ungeladenen Substanzen einen negativen Einfluss auf den Abbau organischer Chemikalien im Wasser-Sediment-System und Oberflächenwasser hat. Im Vergleich zu ungeladenen Stoffen beeinflusst eine negative Ladung den Abbau jedoch positiv. Aufgrund der geringeren Abbaubarkeit weisen positiv geladene Substanzen höhere Halbwertszeiten auf als negativ geladene oder neutrale Substanzen und sind deutlich persistenter als diese. Im Hinblick auf die Persistenzbewertung ionischer und ionisierbarer Stoffe empfehlen wir die Verwendung der DT_{50} des gesamten Wasser-Sediment-Systems als Bewertungsgrundlage sowohl für ionische als auch für neutrale organische Chemikalien. Des Weiteren sollte der pelagische Test nach OECD 309 als Standardtest Anwendung finden, da er eine ähnlich hohe Abbauleistung wie der Test unter Zugabe von suspendiertem Sediment aufweist, jedoch das Potential zur Bildung von NER begrenzt.

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List of abbreviations

AR	Applied Radioactivity
DA^+	4-*n*- Dodecylbenzyltrimethyl ammonium chloride
DP	4-*n*-Dodecylphenol
DS^-	4-*n*-Dodecylbenzene sulfonic acid sodium salt
DT_{50}	half-life
DTAC	Dodecyltrimethyl ammonium chloride
Dw	dry weight
ER	Extractable residues
kGy	kilo Gray
Log K_{ow}	Logarithm of the octanol/water partition coefficient
min	minutes
NER	Non-extractable residues
NP	Nonylphenol
OECD	Organisation for Economic Co-operation and Development
OTAC	Octadecyltrimethyl ammonium chloride
P	Persistence
PBT	Persistent, bioaccumulative and toxic substances
RA	Radioactivity
Radio-HPLC	Radio-High Performance Liquid Chromatography
Radio-TLC	Radio-Thin Layer Chromatography

Summary

The concept for identifying persistent (P), bioaccumulative (B) and toxic (T) and very toxic and very bioaccumulative (vPvB) substances under the EU regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals; No. 1907/2006) was developed for the assessment of neutral organic compounds. The criteria for the identification of PBT and vPvB substances are set in Annex XIII of REACH and based on non-ionic and hydrophobic reference substances. Under environmental conditions and depending on pH, organic compounds can be ionised which influences their physico-chemical properties and their environmental behaviour. Therefore, there is a need to examine whether a refinement of the persistence (P) assessment of ionic and ionisable compounds is necessary.

In the present study, the influence of a chemical charge on the behaviour and degradation of organic compounds in a water-sediment system and in surface water was investigated in order to refine the P assessment under REACH. Three ^{14}C-labelled model substances were used with 4-*n*-dodecylbenzene sulfonic acid sodium salt (^{14}C-DS$^-$), 4-*n*-dodecylphenol (^{14}C-DP) and 4-*n*-dodecylbenzyltrimethyl ammonium chloride (^{14}C-DA$^+$) representing the anionic, non-ionic and cationic model substance, respectively. Using ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$, simulation studies following OECD guideline 308 (*Aerobic and Anaerobic Transformation in Aquatic Sediment Systems*) and OECD guideline 309 (*Aerobic Mineralization in Surface Water - Simulation Biodegradation Test*) were performed. Natural sediment and surface water were collected from a rainwater retention basin in Aachen, Germany.

The objective of the simulation study following OECD guideline 308 was to investigate the behaviour of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in a water-sediment system after 1, 7, 14, 30, 60 and 120 days of incubation. Each assay contained 100 g sediment (dry weight) and 300 mL surface water. The flasks were sealed with a CO_2 trap containing soda lime pellets in order to quantify mineralisation of the test substances. At each sampling time, the water phase was removed, and the sediment was sequentially extracted using different solvents (aqueous $CaCl_2$ solution, methanol and acetonitrile) in order to determine extractable residues (ER). For ^{14}C-DA$^+$, an additional 24-hour Soxhlet extraction with methanol was performed after the sequential solvent extraction. The extracted sediment was subjected to combustion analysis in order to quantify the formation of non-extractable residues (NER). Soda lime pellets were dissolved in hydrochloric acid (25 %) and the released $^{14}CO_2$ measured by LSC to quantify mineralisation. The distribution of the applied radioactivity (AR) among the water phase, mineralised, extractable and non-extractable residues was determined. ER were further analysed for the parent compounds and metabolites using radio-TLC and radio-HPLC. After 120 days of incubation, mineralisation of ^{14}C-DS$^-$ and ^{14}C-DP accounted for 68 % and 63 % AR, respectively. The cationic compound ^{14}C-DA$^+$ was mineralised to a lesser extent (6 % AR after 120 days). The direct bioavailability of the test substances, based on their mineralisation and portions in the $CaCl_2$ fraction, was as follows: ^{14}C-DS$^-$ > ^{14}C-DP > ^{14}C-DA$^+$. After 120 days of incubation, highest NER formation was observed for ^{14}C-DA$^+$ (33 % AR) followed by ^{14}C-DS$^-$ (19 % AR) and ^{14}C-DP (14 % AR). A rapid degradation of ^{14}C-DS$^-$ and ^{14}C-DP into mainly more polar transformation products was observed for both the water phase and sediment-derived extracts. Metabolites of ^{14}C-DA$^+$ were mainly found in the water phase, whereas the major ^{14}C-fraction in the sediment-derived extracts (in particular in Soxhlet extracts) consisted of the parent compound. Half-lives (DT_{50}) of the test substances in the overall water-sediment system decreased as follows: ^{14}C-DA$^+$ (162 days) > ^{14}C-DS$^-$ (22 days) > ^{14}C-DP (14 days). When the half-lives were calculated for the sediment ($DT_{50,sed}$) and water phase ($DT_{50,w}$) separately, $DT_{50,sed}$ of ^{14}C-DA$^+$, ^{14}C-DP and ^{14}C-DS$^-$ were 267 days, 24 days and 22 days, respectively. Considerably lower half-lives of all test substances were obtained for the water phase (< 1 day).

In order to investigate the behaviour of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water, simulation studies following OECD 309 were performed. Preliminary surface water tests were performed as pelagic tests (surface water only) and as suspended sediment tests (surface water with addition of suspended sediment) using ^{14}C-DP and ^{14}C-DS$^-$. Each assay contained 150 mL surface water (pelagic test) or 150 mL surface water with 0.15 g sediment (dry weight) (suspended sediment test). The assays were sealed with a CO_2 trap with soda lime pellets to quantify mineralisation of ^{14}C-DP and ^{14}C-DS$^-$. The assays were incubated for 1, 3, 7, 14, 28 and 60 days. As proposed by OECD 309, sampling was performed by withdrawing sub-samples of the water phases (10 mL) and replacing the soda lime at each sampling time. At the end of the 60-day incubation period, the sediment was extracted using methanol and acetonitrile. The extracted sediment was dried and combusted in order to quantify NER formation. For ^{14}C-DS$^-$, mineralisation accounted for up to 51 % in the pelagic test and up to 56 % in the suspended sediment test at the end of the test (after 60 days). For ^{14}C-DP, mineralisation was up to 56 % in the pelagic test and up to 60 % in the suspended sediment test. In the suspended sediment test, NER formation accounted for up to 3.4 % AR (^{14}C-DP) and 0.5 % AR (^{14}C-DS$^-$). In general, low recoveries (up to 80 %) were obtained using the sampling technique of withdrawing sub-samples. Therefore, multiple flasks were used in the main experiments and whole flasks were harvested at each sample interval. The main experiments following OECD 309 were performed as a suspended sediment test at two different test concentrations (10 µg/L and 100 µg/L). Each assay contained 100 mL surface water, 0.1 g sediment (dry weight) and was sealed with a CO_2 trap with soda lime pellets to quantify mineralisation. The assays were incubated for 7, 14, 30 and 62 days (10 µg/L) and 1, 7, 14, 30 and 60 days (100 µg/L). At each sampling time, the water phase was separated from the sediment particles by filtration and the sediment particles were sequentially extracted using methanol and acetonitrile. The water phase and the pooled sediment extract were analysed using radio-TLC and radio-HPLC. The extracted sediment was combusted for NER quantification. For quantification of mineralised portions, soda lime pellets were dissolved in hydrochloric acid (25 %) and the released $^{14}CO_2$ measured by LSC. Additionally, the suspended sediment test was performed under sterile conditions in order to investigate abiotic degradation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$. Sterile conditions were obtained by using autoclaved surface water treated with sodium azide and gamma irradiated sediment. The test was run at a test concentration of 10 µg/L over an incubation time of 30 days. After 62 days of incubation under non-sterile conditions (10 µg/L), mineralisation of ^{14}C-DS$^-$ and ^{14}C-DP accounted for 75 % AR and 69 % AR, respectively. After 60 days of incubation under non-sterile conditions (100 µg/L), mineralisation was 63 % AR (^{14}C-DS$^-$) and 58 % AR (^{14}C-DP). For ^{14}C-DA$^+$, mineralisation accounted for 7 % AR at both test concentrations. Under sterile conditions, mineralisation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ was neglectable (< 0.1 % AR). For both test concentrations, the highest amounts of radioactivity were extracted from the sediment incubated with ^{14}C-DA$^+$ (18 % AR), followed by ^{14}C-DP (3 % AR) and ^{14}C-DS$^-$ (2 % AR). At the end of the 60-day incubation period, highest NER formation was observed for ^{14}C-DP (21 % AR) followed by ^{14}C-DA$^+$ (14 % AR) and ^{14}C-DS$^-$ (9 % AR). NER formation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ under sterile conditions accounted for 0.1 %, 0.6 % and 5.5 % AR, respectively. Besides the parent compounds, more polar and less polar metabolites were detected. At a test concentration of 10 µg/L, half-lives of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ decreased as follows: ^{14}C-DA$^+$ (26 days) > ^{14}C-DP (2 days) > ^{14}C-DS$^-$ (1 day). At a concentration of 100 µg/L, DT_{50} decreased in the same order: ^{14}C-DA$^+$ (13 days) > ^{14}C-DP and ^{14}C-DS$^-$ (1 day). Under sterile test conditions, ^{14}C-DS$^-$ and ^{14}C-DA$^+$ were found unchanged in the water phase, whereas ^{14}C-DP and transformation products were detected in some samples.

The results of the simulation study following OECD 308 showed that a stronger sorption of the positively charged compound to sediment particles together with a higher non-extractable fraction result in a higher persistence of the cationic compound compared to the non-ionic and

anionic compound. This can be confirmed by the results of the suspended sediment tests according to OECD 309, although the formation of NER for the uncharged model substance was highest in these tests. In general, the results of the present study showed that the degradation of the three model substances ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in a water-sediment system and in surface water with suspend sediments was influenced by their chemical charge. A positive charge leads to a reduced degradation of the substance in both test systems compared to a negative and no charge.

According to REACH annex XIII, a substance is regarded as persistent/very persistent if the P/vP criterion in a single environmental compartment is fulfilled. The results of our water-sediment study, however, showed that separately determined half-lives of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ for the sediment and water column are less reliable than the half-lives calculated for the overall water-sediment system. We therefore recommend using the half-life for the total water-sediment system (OECD 308) for the P assessment of both ionic and non-ionic organic chemicals. Simulation studies following OECD 309 leave too much scope for the experimental setup which has been shown to affect the outcome of the study. In preliminary tests using both the pelagic and suspended sediment version, we showed that the degradation capacity of the pelagic test was nearly identical compared to the suspended sediment test. In main experiments using the suspended sediment test, appreciable quantities of NER were formed despite the addition of only low amounts of sediment. We therefore propose the use of the pelagic test in the context of P assessment since this test design minimises potential NER formation.

Zusammenfassung

Das Bewertungskonzept zur Identifizierung persistenter (P), bioakkumulierender (B) und toxischer Stoffe sowie Stoffe mit sehr persistenten und sehr bioakkumulierenden Eigenschaften (vPVB) unter der europäischen Chemikalienverordnung REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) wurde für neutrale organische Stoffe entwickelt. Anhand der in Annex XIII der REACH-Verordnung definierten Kriterien erfolgt die Identifizierung von PBT- bzw. vPvB-Stoffen. Diese Kriterien wurden jedoch basierend auf nicht-ionischen, hydrophoben Referenzchemikalien erstellt. In der Umwelt und in Abhängigkeit des pH-Wertes können organische Chemikalien jedoch ionisiert vorliegen, wodurch deren physiko-chemischen Eigenschaften und somit ihr Umweltverhalten beeinflusst werden. Daher ist eine Überprüfung des derzeit gültigen Konzepts hinsichtlich der Persistenzbewertung ionischer und ionisierbarer Stoffe notwendig. In der vorliegenden Arbeit soll der Einfluss einer chemischen Ladung auf das Schicksal ionisierbarer organischer Chemikalien in der Umwelt untersucht werden. Als Modellsubstanzen dienten 4-n-Dodecylbenzolsulfonsäure Natriumsalz (DS^-, anionisch), 4-n-Dodecylphenol (DP, neutral) und 4-n-Dodecylbenzyltrimethylammoniumchlorid (DA^+, kationisch). Mit diesen Substanzen wurden Simulationsstudien in aquatischen Sedimentsystemen in Anlehnung an die OECD Richtlinie 308 (Aerobic and Anaerobic Transformation in Aquatic Sediment Systems) und in Oberflächenwasser mit suspendiertem Sediment in Anlehnung an OECD 309 (Aerobic Mineralization in Surface Water - Simulation Biodegradation Test) durchgeführt. Das verwendete Sediment und Oberflächenwasser wurden aus einem Regenrückhaltebecken in Aachen (Deutschland) entnommen.

Ziel der Simulationsstudie nach OECD 308 war die Untersuchung des Verhaltens von ^{14}C-DS^-, ^{14}C-DP und ^{14}C-DA^+ in einem Wasser-Sediment-System nach einer Inkubationszeit von 1, 7, 14, 30, 60 und 120 Tagen. Jeder Versuchsansatz bestand aus 100 g Sediment (Trockengewicht) und 300 ml Wasser. Zur Bestimmung der mineralisierten Anteile der Testsubstanzen wurden die Ansätze mit einer mit Natronkalk befüllten CO2-Falle verschlossen. An jedem Aufarbeitungstag wurde die Wasserphase abgenommen und das Sediment mit unterschiedlichen Lösemitteln (wässrige CaCl2-Lösung, Methanol und Acetonitril) sequentiell extrahiert, um den Anteil der extrahierbaren Rückstände (ER) zu bestimmen. Das Sediment, welches mit ^{14}C-DA^+ inkubiert wurde, wurde zusätzlich einer 24-stündigen Soxhlet-Extraktion mit Methanol unterzogen. Zur Bestimmung der nicht-extrahierbaren Rückstände (NER) wurde eine Verbrennungsanlayse des extrahierten Sediments durchgeführt. Der Natronkalk wurde in 25 %-iger Salzsäure aufgelöst und die Menge an freigesetztem $^{14}CO_2$ mittels LSC bestimmt. Die Verteilung der applizierten Radioaktivität (AR) auf die Wasserphase, mineralisierte, extrahierbare und nicht-extrahierbare Anteile wurde ermittelt. Die Wasserphasen und extrahierbaren Anteile wurden mittels Radio-DC und Radio-HPLC auf die Ausgangssubstanzen und Transformationsprodukte untersucht. Nach einer Inkubationszeit von 120 Tagen betrug die Mineralisation von ^{14}C-DS^- und ^{14}C-DP 68 % bzw. 63 % AR. ^{14}C-DA^+ wurde in geringerem Maße mineralisiert (6 % AR). Basierend auf der Mineralisationsrate und dem Anteil der Ausgangssubstanzen im CaCl2-Extrakt, nahm die direkte Bioverfügbarkeit wie folgt ab: ^{14}C-DS^- > ^{14}C-DP > ^{14}C-DA^+. Nach 120 Tagen betrug die Menge an NER für ^{14}C-DA^+ 33 % AR, gefolgt von ^{14}C-DS^- (19 % AR) und ^{14}C-DP (14 % AR). Die Analyse der Wasserphase und extrahierbaren Anteile ergab, dass DS- und DP sowohl im Wasser als auch im Sediment zu hauptsächlich polaren Transformationsprodukten abgebaut wurden. Im Falle von ^{14}C-DA^+ konnten Metabolite hauptsächlich in der Wasserphase nachgewiesen werden, wohingegen im Sediment der Großteil der Radioaktivität als Ausgangssubstanz vorlag. Die Halbwertszeiten (DT_{50}) der Modellsubstanzen nahmen im gesamten Wasser-Sediment-System wie folgt ab: ^{14}C-DA^+ (162 Tage) > ^{14}C-DS^- (22 Tage) > ^{14}C-DP (14 Tage). Des Weiteren wurden die Halbwertszeiten für das Sediment ($DT_{50,sed}$) und die Wasserphase ($DT_{50,w}$) getrennt

bestimmt. $DT_{50,sed}$ von ^{14}C-DA^+, ^{14}C-DP und ^{14}C-DS^- lag bei 267 Tagen, 24 Tagen bzw. 22 Tagen. $DT_{50,w}$ war für alle Testsubstanzen deutlich niedriger (< 1 Tag).

Um den Abbau der Modellsubstanzen in Oberflächenwasser zu untersuchen, wurden Simulationsstudien nach OECD 309 durchgeführt. Zur Untersuchung des Abbaus von ^{14}C-DS^- und ^{14}C-DP wurden zunächst Vorversuche als pelagische Tests (nur Oberflächenwasser) und suspendierte Sedimenttests (Oberflächenwasser mit Zugabe von suspendiertem Sediment) durchgeführt. Jeder Versuchsansatz bestand dabei aus 150 ml Oberflächenwasser (pelagischer Test) oder 150 ml Oberflächenwasser mit 0,15 g Sediment (Trockengewicht) (suspendierter Sedimenttest). Die Ansätze wurden mit einer mit Natronkalk befüllten CO_2-Falle verschlossen und 1, 3, 7, 14, 28 und 60 Tage inkubiert. An jedem Aufarbeitungszeitpunkt wurden 10 ml-Aliquots aus der Wasserphase entnommen und die CO_2-Falle mit frischem Natronkalk befüllt. Am Ende der 60-tägigen Inkubationszeit wurde zusätzlich das Sedimentmit Methanol und Acetonitril extrahiert und anschließend zur NER-Bestimmung verbrannt. Die mineralisierten Anteile von ^{14}C-DS^- betrugen im pelagischen Test bis zu 51 % AR und im suspendierten Sedimenttest bis zu 56 % AR. Für ^{14}C-DP betrug die Mineralisation im pelagischen Test bis zu 56 % AR und im suspendierten Sedimenttest bis zu 60 % AR. Im suspendierten Sedimenttest lag an Testende die Menge an NER bei 0,5 % AR (^{14}C-DS^-) und 3,4 % AR (^{14}C-DP). Aufgrund niedriger Wiederfindungsraten (bis zu 80 %) wurde in den Hauptversuchen auf die Entnahme von Teilproben verzichtet und stattdessen an jedem Messzeitpunkt der gesamte Ansatz aufgearbeitet. Die Hauptversuche wurden als suspendierter Sedimenttest unter Verwendung unterschiedlicher Testkonzentrationen (10 µg/l und 100 µg/l) durchgeführt. Jeder Versuchsansatz enthielt 100 ml Oberflächenwasser und 0,1 g Sediment (Trockengewicht) und wurde mit einer CO_2-Falle versehen. Die Ansätze wurden 7, 14, 30 und 60 Tage (10 µg/l) und 1, 7, 14, 30 und 60 Tage (100 µg/l) inkubiert. An jedem Aufarbeitungstag wurde die Wasserphase von den Sedimentpartikeln durch Filtration getrennt und das Sediment wurde sequentiell mit Methanol und Acetonitril extrahiert. Die Wasserphase und der vereinigte Sedimentextrakt wurden mittels Radio-DC und Radio-HPLC analysiert. Das extrahierte Sediment wurde verbrannt, um den Anteil der NER zu bestimmen. Zur Bestimmung der Mineralisation wurde der Natronkalk in 25 %-iger Salzsäure gelöst und das freigesetzte $^{14}CO_2$ mittels LSC gemessen. Zusätzlich wurde der suspendierte Sedimenttest unter sterilen Bedingungen durchgeführt, um den abiotischen Abbau von ^{14}C-DS^-, ^{14}C-DP und ^{14}C-DA^+ zu untersuchen. Hierzu wurde das Oberflächenwasser zunächst autoklaviert und mit Natriumazid versetzt und das Sediment gamma-bestrahlt. Die Ansätze wurden bei einer Testkonzentration von 10 µg/l über einen Zeitraum von 30 Tagen inkubiert. Bei einer Konzentration von 10 µg/l wurden am Testende 75 % AR (^{14}C-DS^-) bzw. 69 % (^{14}C-DP) mineralisiert. Bei der höheren Testkonzentration betrugen diese Anteile 63 % AR (^{14}C-DS^-) bzw. 58 % AR (DP). Die Mineralisation von ^{14}C-DA^+ lag bei beiden Konzentrationen bei 7 % AR. Unter sterilen Bedingungen war die Mineralisation von ^{14}C-DS^-, ^{14}C-DP und ^{14}C-DA^+ vernachlässigbar (< 0,1 % AR). Generell wurden am Testende die höchsten Mengen an Radioaktivität von dem mit 14C-DA+ inkubierten Sediment extrahiert (18 % AR), gefolgt von ^{14}C-DP (3 % AR) und ^{14}C-DS^- (2 % AR). Nach einer Inkubationszeit von 60 Tagen wurde die höchste Menge an NER für ^{14}C-DP beobachtet (21 % AR), gefolgt von ^{14}C-DA^+ (14 % AR) und ^{14}C-DS^- (9 % AR). Unter sterilen Bedingungen lag die Menge an NER von 14C-DS^-, ^{14}C-DP und ^{14}C-DA^+ jeweils bei 0,1 %, 0,6 % und 5,5 % AR. Neben den Ausgangssubstanzen wurden polarere und unpolarere Metaboliten in der Wasserphase und im Sedimentextrakt nachgewiesen. ^{14}C-DS^- und ^{14}C-DA^+ wurden unter sterilen Bedingungen kaum metabolisiert, wohingegen ^{14}C-DP und zwei Transformationsprodukte nachgewiesen wurden. Die Halbwertszeiten nahmen bei einer Konzentration von 10 µg/l bzw. 100 µg/l wie folgt ab: ^{14}C-DA^+ (26 Tage) > ^{14}C-DP (2 Tage) > ^{14}C-DS^- (1 Tag) bzw. ^{14}C-DA^+ (13 Tage) > ^{14}C-DP und ^{14}C-DS^- (jeweils 1 Tag).

Die Ergebnisse aus den Simulationsstudien nach OECD 308 zeigen, dass positiv geladene Substanzen stärker an Sedimentpartikel binden als negativ oder ungeladene Substanzen und folglich höhere Mengen an NER bilden. Somit trägt eine positive Ladung zu einer erhöhten Persistenz einer organischen Chemikalie im Wasser-Sediment-System bei. Anhand der Ergebnisse der suspendierten Sedimenttests nach OECD 309 lässt sich dies bestätigen, obwohl in diesen Tests die Bildung von NER für die ungeladene Modellsubstanz am höchsten war. Generell zeigen die Ergebnisse der vorliegenden Arbeit, dass das Verhalten und der Abbau der Modellsubstanzen DS^-, DP und DA^+ sowohl im Wasser-Sediment-System als auch in Oberflächenwasser von deren Ladung beeinflusst wird. Eine positive Ladung führte in beiden Testsystemen zu einem geringeren Abbau, wohingegen eine negative Ladung einen positiven Effekt auf die Abbaubarkeit hat im Vergleich zu ungeladenen Stoffen.

Nach Annex XIII der REACH-Verordnung gilt eine Substanz als persistent/sehr persistent, wenn das P/vP Kriterium in einem einzelnen Umweltkompartiment erfüllt ist. Jedoch zeigten unsere Ergebnisse der Wasser-Sediment-Studie, dass die separat ermittelten Halbwertszeiten von DS^-, DP und DA^+ für das Sediment und die Wasserphase weniger aussagekräftig sind als jene, die für das Gesamtsystem (OECD 308) bestimmt wurden. Daher empfehlen wir, die Persistenzbewertung von sowohl ionischen als auch nicht-ionischen organischen Chemikalien basierend auf den Halbwertszeiten des Gesamtsystems durchzuführen. Wir konnten zeigen, dass die OECD Richtlinie 309 ein hohes Maß an experimentellem Freiraum lässt, wodurch unterschiedliche Ergebnisse erzielt werden können. Anhand von Vorversuchen, die sowohl als pelagischer als auch als suspendierter Sedimenttest durchgeführt wurden, konnten wir zeigen, dass der pelagische Test eine ähnlich hohe Abbauleistung aufweist wie der suspendierte Sedimenttest. In Hauptversuchen mit suspendiertem Sediment wurden trotz Zugabe geringer Sedimentmengen deutliche Mengen an NER gebildet. Basierend auf diesen Ergebnissen empfehlen wir daher die Verwendung des pelagischen Tests im Rahmen der Persistenzbewertung, auch in Hinblick auf die geringe Bildung von NER in diesem Testsystem.

1 Introduction

1.1 Aim of the project

Little is known about the degradation of ionic organic substances in water and sediments and how their chemical charge influences their biodegradability. The identification of persistent (P) bioaccumulative (B) and toxic (T) substances under the EU regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) was developed to assess neutral organic compounds. However, nearly 50 % of the chemicals pre-registered at the European Chemicals Agency (ECHA) are ionised under environmental conditions (Franco *et al.*, 2010). Since the charge of chemicals strongly influences their properties and environmental behaviour, the currently valid concept under the REACH regulation probably does not allow a sufficient assessment of ionic or ionisable substances. The objective of the project is to examine whether a refinement of the P assessment of ionic and ionisable substances under REACH is necessary. For this purpose, simulation studies following OECD guidelines 308 (*Aerobic and Anaerobic Transformation in Aquatic Sediment Systems*) and 309 (*Aerobic Mineralisation in Surface Water - Simulation Biodegradation Test*) are conducted.

Special attention should be paid to the choice of model substances, as the compounds must meet the following requirements:

a) structural identity of hydrophobic part

b) polar head carries positive, negative or no charge to simulate ionisation under environmental pH

c) environmental relevance (wide range of commercial and consumer use)

1.2 Key questions

The behaviour and degradation of differently charged organic compounds in sediment and surface water were investigated with regard to the following key questions:

- Is there an influence of charge on the degradation of these compounds in sediment and surface water?
- Does the presence of suspended sediments influence the degradation of organic compounds in surface water?

Scientific hypotheses to test were:

- Charged organic compounds have a higher affinity to sediment particles due to interactions with the sediment matrix than uncharged compounds.
- There is a difference between positively and negatively charged compounds in terms of their affinity to binding to sediment particles.
- The degradation of organic compounds in surface water increases in the presence of suspended sediments.

1.3 Choice of model substances

All three chosen test substances are surfactants. To examine the influence of a negative charge on the behaviour of a compound 4-n-dodecylbenzenesulfonic acid sodium salt (abbreviated below as "DS$^-$") was used (Figure 1). DS$^-$ belongs to the group of linear alkylbenzene sulfonates (LAS$^-$). With a pK_a of -1.84 (Table 1), DS$^-$ is a strong acid and exists as anionic compound under environmental pH (Sixt, 1998). LAS- are widely used for the production of detergents and household cleaners (de Wolf and Feijtel, 1998).

Figure 1: Molecular structure of DS$^-$.

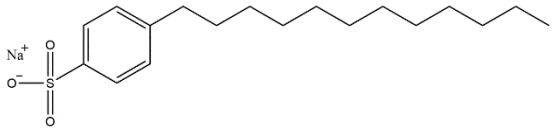


Table 1: Physiochemical properties of DS$^-$.

4-*n*-Dodecylbenzenesulfonic acid sodium salt	
CAS No.	25155-30-0
Molecular weight	348.48 g/mol
Water solubility	300 mg/L
Vapour pressure	$3*10^{-13}$ Pa
Log K_{OW}	1.96
pK_a	- 1.84

4-*n*-Dodecylphenol (abbreviated below as "DP") represented the non-ionic test compound belonging to the group of alkylphenols (Figure 2). Long-chain alkyl phenols are widely used as precursors during the synthesis of lubricant additives, intermediates for the production of fuel system cleaners and as a component in ink resins (Product Safety Summary, SI Group). Physicochemical properties of DP are given in Table 2 .

Figure 2: Molecular structure of DP.

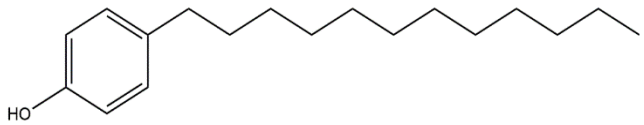


Table 2: Physiochemical properties of DP.

4-*n*-Dodecylphenol	
CAS No.	104-43-8
Molecular weight	262.44 g/mol
Solubility in water (bulk, C_{12} fraction)	2.1 mg/L (25 °C), 0.031 mg/L (25 °C)
Density (20 °C)	0.94 g/cm^3
Vapour pressure	$9.19*10^{-13}$ Pa

4-*n*-Dodecylphenol	
Log K_{OW}	6.58 (7.14 [Brooke et al. 2007])

4-*n*-Dodecylbenzyltrimethyl ammonium chloride (DA^+) was used as a cationic model substance (Figure 3). Quaternary ammonium compounds ($QACs^+$) are primarily applied in fabric softeners and antiseptic agents in laundry detergents (Boethling, 1984; Ying, 2006). Limited data on physical properties of DA^+ are available (Table 3).

Figure 3: Molecular structure of DA^+.

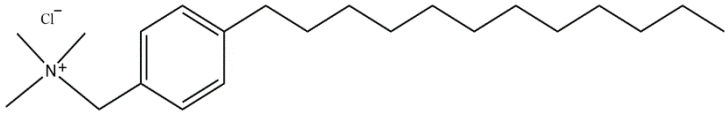


Table 3: Physiochemical properties of DA^+.

4-*n*-Dodecylbenzyltrimethyl ammonium chloride	
CAS No.	19014-05-2
Molecular weight	354.02 g/mol
Log K_{OW}	3.48

All substances are ^{14}C- radiolabelled in the aromatic ring (uniform) and purchased from the following suppliers (Table 4).

Table 4: Purchase of radiochemicals and information on specific activity and chemical purity.

Compound	Supplier	Information
4-*n*-Dodecylbenzenesulfonic acid [phenyl ring-^{14}C(U)] sodium salt	ARC – American Radiolabeled Chemicals, Inc., St. Louis, USA	Specific activity: 140 mCi/mmol Chemical purity > 99%
4-*n*-Dodecylphenol [ring-^{14}C(U)]	ARC – American Radiolabeled Chemicals, Inc., St. Louis, USA	Specific activity: 77 mCi/mmol Chemical purity > 99%
4-*n*-Dodecylbenzyltrimethylammonium chloride [ring-^{14}C(U)]	BlyChem LTD, Billingham, England	Specific activity: 89 mCi/mmol Chemical purity > 98 %

The corresponding unlabelled substances were also supplied from the respective companies. Stock solutions of DS^- were prepared using ethanol:water (7:3, v/v), DP and DA^+ were dissolved in ethanol (absolute).

1.4 Ionic Substances

Ionic substances are either positively charged (cation), negatively charged (anion) or carry both a negative and a positive charge (amphoteric compounds). Ionisation of a chemical is directly dependent on its electronic structure (Calvet, 1989). Some compounds, such as quaternary ammonium compounds ($QACs^+$, e.g. paraquat always occur in cationic form in soils and sediments. In contrast, ionisation of weak bases with pK_a values between 3 and 8 depends on the pH of the liquid phase of the respective environmental compartment (Calvet 1989).

The pK_a value of a compound is defined as the negative base-10 logarithm of the acid dissociation constant (K_a). K_a is a measure for the strength of an acid in solution; the lower the

pK_a value, the stronger the acid. A compound such as 4-*n*-dodecyl phenol is not expected to dissociate under environmental pH conditions due to its pK_a value higher than 9.9 (Brooke and Agency, 2007). Therefore, 4-*n*-dodecyl phenol was chosen as a non-ionisable compound in this project.

1.5 Degradation of Ionic Substances in the Environment

For the behaviour and fate of substances in the environment, the ability of microorganisms to metabolise and degrade these compounds is essential. Linear alkylbenzene sulfonates (LAS^-) can be degraded by certain white rot fungi, whereby degradation takes place primarily at the alkyl chain (Yadav *et al.*, 2001). However, biotransformation of LAS^- can also happen at the aromatic ring (Gledhill, 1975). Quaternary ammonium compounds ($QACs^+$) tend to be more persistent in the environment (Li and Brownawell, 2010), yet several bacterial species such as *Pseudomonas* are capable of degrading QACs under aerobic conditions (Tezel and Pavlostathis, 2015).

In general, the mineralisation is a suitable tool for assessing the biodegradation of organic compounds. Mineralisation is the complete degradation of a compound into carbon dioxide by microorganisms. The evolving amount of CO_2 thus provides information on the biodegradability of the respective compound. Another helpful tool for the P assessment of organic compounds is its half-life (DT_{50}). According to the European Regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), DT_{50} describes the time after which the concentration of the compound was reduced by half due to degradation. Half-lives are also used in other regulations in order to assess a chemical's persistence, for example Plant Protection Ordinance (EC No 1107/2009) and Drug Ordinance (2001/83/EC). It is, however, difficult to distinguish between whether the reduction of the initial concentration is due to degradation or dissipation processes. Another problem arising in the context of determining half-lives is the formation of non-extractable residues (NER, see chapter 1.7) and if NER can be regarded as degradation. In case the parent compound has become non-extractable, NER are not considered as degradation under REACH (ECHA, 2017). The criteria for the P assessment of chemicals according to the REACH regulation are given in Table 5. A substance fulfils the P criterion if its half-live in fresh or marine water is higher than 40 or 60 days, respectively. For fresh or estuarine sediment or soil, the threshold value is 120 days or for marine sediment 180 days. A substance is identified as very persistent (vP) in case its degradation half-life in marine or fresh water is higher than 60 days. The threshold for sediment or soil is 180 days.

Table 5: Criteria for the P assessment of chemicals under REACH.

Environmental compartment	Degradation half-life
Fresh or estuarine water	> 40 days
Marine water	> 60 days
Fresh or estuarine sediment	> 120 days
Marine sediment	> 180 days
Soil	> 120 days

Source: COMMISSION REGULATION (EU) No 253/2011.

In a simulation study, a half-life of 2 days for LAS^- was reported (Knaebel *et al.*, 1994), Waters *et al.* (1989) determined half-lives between 7 and 22 days. In studies on the degradation of LAS^- in waste and ground water higher persistence of LAS^- and LAS-derived residues was observed (Field *et al.*, 1992). It was assumed that differences in the microbial community and

biogeochemical conditions within the water layer had adverse effects on the degradability of LAS. Mesocosm studies using nonylphenol (NP), one of the most important compounds in the group of alkylphenols, reveal half-lives of 28 to 104 days (Maguire, 1999). Additionally, sunlight photodegradation plays an important role in the degradation process of NP (Neamţu and Frimmel, 2006; Li *et al.*, 2013).

The identification of substances having PBT or vPvB properties was based on selected reference chemicals with non-ionic and hydrophobic properties (Matthies *et al.*, 2016). Since estimated 33 % of the chemicals on the REACH pre-registration list were ionisable at pH 7 (Franco *et al.*, 2010), the currently used approach based on neutral chemicals is unsuitable for ionic and ionisable substances (Grisoni *et al.*, 2015).

1.6 The Influence of Sorption on the Degradation of Ionic Substances

Sorption is defined as a physical or chemical process by which a substance can either be attached to the surface of another substance (adsorption) or enters the volume of another material (absorption). The process of releasing a substance from or through a surface is desorption.

Sorption intensity is influenced by soil and sediment characteristics such as pH, soil texture and organic matter content (Gevao *et al.*, 2000). Substance properties (molecular size and structure, chemical charge) also affect sorption processes in the environment (Senesi, 1992): Non-charged molecules can be attached to non-polar surfaces by hydrogen bonds or hydrophobic interactions, whereas charged molecules bind to soil particles via ion exchange. Soil particles with a negatively charged surface bind cations (cation exchanger), anion exchangers are positively charged and bind anions. Due to negatively charged surfaces of clay minerals and soil organic matter in soils and sediments, cation exchange is the predominant type of binding (Gevao *et al.*, 2000). With increasing sorption of chemicals to soil particles its degradability decreases (Haigh, 1996). It was shown that the charge of $QACs^+$ facilitates their binding to sediment particles and impeded their degradability (Li and Brownawell, 2010). In contrast to that, negatively charged substances are more rapidly degradable due to their reduced affinity to the mainly negatively charged surface of soil organic matter (SOM) (Kah and Brown, 2007).

In addition to chemical charge, the spatial structure of a compound effects its sorption behaviour and hence its degradability. Branched 4-NP showed lower sorption to SOM than its linear form (Düring *et al.*, 2002). The size of branched molecules prevented them from diffusing into soil aggregates and interacting with soil components.

1.7 Non-extractable residues (NER)

According to the IUPAC definition, bound or non-extractable residues (NER) are defined as ‘chemical species originating from pesticides, used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues’ (Roberts, 1984). This definition was slightly modified by adding the condition that ‘the extraction method must not substantially change the compounds themselves or the structure of the matrix’ (Führ and Ophoff, 1998). As far as is currently known, altering the matrix cannot be excluded by using more vigorous extraction methods such as Soxhlet extraction or Accelerated Solvent Extraction (ASE). Furthermore, biogenic residues are not considered by the above-mentioned definitions (Schäffer *et al.*, 2018). NER can be classified into three different types (Kästner *et al.*, 2014; Schäffer *et al.*, 2018) : Xenobiotic residues strongly adsorbed or entrapped within the soil organic matter (sequestered NER, type I), covalently bound residues (type II) and biogenic NER (type III) which are derived from biotic degradation. Due to the conversion of

carbon from the compounds into microbial biomass and biomolecules such as amino acids, type III NER do not pose any risk. Type I NER pose a higher risk as they can be released from soils or sediments over time (Gevao *et al.*, 2001). Therefore, knowledge about the formation of NER of chemicals in soils and sediments is important for assessing their risk in the environment, since their persistence is influenced to a high extent by the fraction of NER.

2 Preliminary Tests

2.1 Analysis of the Sediment/Sediment Characteristics

Sediment and associated surface water were obtained from a rainwater retention basin near the Institute for Environmental Research of Aachen University (50°46`53.5``N 6°02`30.9``E). Data on the sediment are summarized in Table 6.

Table 6: Properties of the sediment and concentration of various substances.

Sediment properties	Measured values
pH value	7.4
Conductivity	120 µS/cm
Total Organic Carbon (TOC)	3.36 mg/L
Chloride	<10 mg/L
Sulfate	< 20 mg/L
Lead	< 7 mg/L
Cadmium	< 0.5 mg/L
Mercury	< 0.2 mg/L
PAHs (sum)	2.1 mg/kg dw
PCBs (sum)	< 0.015 mg/kg dw

Source: Chemical analysis of the sediment by GEOTAIX Umwelttechnologie GmbH, date of analysis: June 2015.

Prior to use, the sediment was wet sieved with a 2 mm sieve. The surface water was filtrated by suction filtration (8µm- filter paper) in order to remove larger particles and small animals. Sediment and water were stored at 5 °C in the dark. The water content of the sediment ranged between 26 % and 31.2 %. To classify the soil texture of the sediment, a particle fractionation was conducted (for details see 3rd progress report). The sediment consists of 2.5 % sand, 21.4 % clay and 76.1 % silt and was classified as silt loam. Organic matter (OM) and organic carbon (C_{org}) content was determined by loss on ignition (Heiri *et al.*, 2001). OM accounted for 10.01 ± 0.16 %, C_{org} was 5.80 ± 0.09 %. According to OECD 308, the sediment can be characterised by a high organic carbon content.

2.2 Establishment of Extraction Methods

Prior to the preliminary experiments suitable methods for extracting the different test substances were established and refined by recovery tests. The test concentrations were selected following suggestions in OECD 307. For analytical reasons and based on the concentration of LAS^- in soils that have not received sewage sludge (Jensen, 1999), a test concentration of 1 mg/kg was chosen. This concentration was applied to each test assay with 1 mg/kg corresponding to 0.02 mg/20g sediment (dry weight). The amounts of sediment and test substances are shown in Table 7. Each test substance was applied separately to the sediment in a centrifuge beaker.

Table 7: Amounts of the test substances per centrifuge beaker. Each beaker contained 62 g sediment (wet weight). The recovery test was performed using duplicates of each test substance.

Test substance	Amount of test substance
^{14}C-DS$^-$ DS$^-$ (unlabelled)	0.6 µg (0.0083 MBq) 19.4 µg
^{14}C-DP DP (unlabelled)	0.8 µg (0.0083 MBq) 19.2 µg
^{14}C-DA$^+$ DA$^+$ (unlabelled)	1.6 µg (0.017 MBq) 18.4 µg

After application of the test substance the sediment was immediately extracted using four different solvents: aqueous calcium chloride solution (0.01 mol/L), methanol, a methanol:water mixture (1:1) and acetonitrile. The calcium chloride solution was used to extract the bioavailable portion. The different organic solvents were used to extract the potentially bioavailable fraction and different metabolites. After addition of the solvent, the samples were shaken for 15 minutes (170 rpm) and centrifuged (2400 x g). The volume of the supernatant was determined and the radioactivity in this extract was measured. This step was performed three times for each solvent except for the extraction with calcium chloride solution. After extraction the sediment was dried and combusted to determine the amount of NER.

Using this extraction scheme, 101.8 % and 97.5 % of the initially applied amount of ^{14}C-DS$^-$ and ^{14}C-DP were recovered, respectively. Only 69.2 % of the applied amount of ^{14}C-DA$^+$ were recovered. In order to achieve a better recovery for ^{14}C-DA$^+$, an additional 24-hour Soxhlet-extraction with methanol was performed subsequent to the sequential solvent extraction. Using this extraction scheme, the recovery of ^{14}C-DA$^+$ accounted for 98.8 %. This extraction scheme was applied to the experiments following OECD 308 (water-sediment study).

2.3 Distribution and fate of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in a water-sediment system following OECD 308

2.3.1 Experimental Setup

At the time when the preliminary water-sediment study was conducted, the cationic model substance was not provided yet. The test was run using only ^{14}C-DP and ^{14}C-DS$^-$.

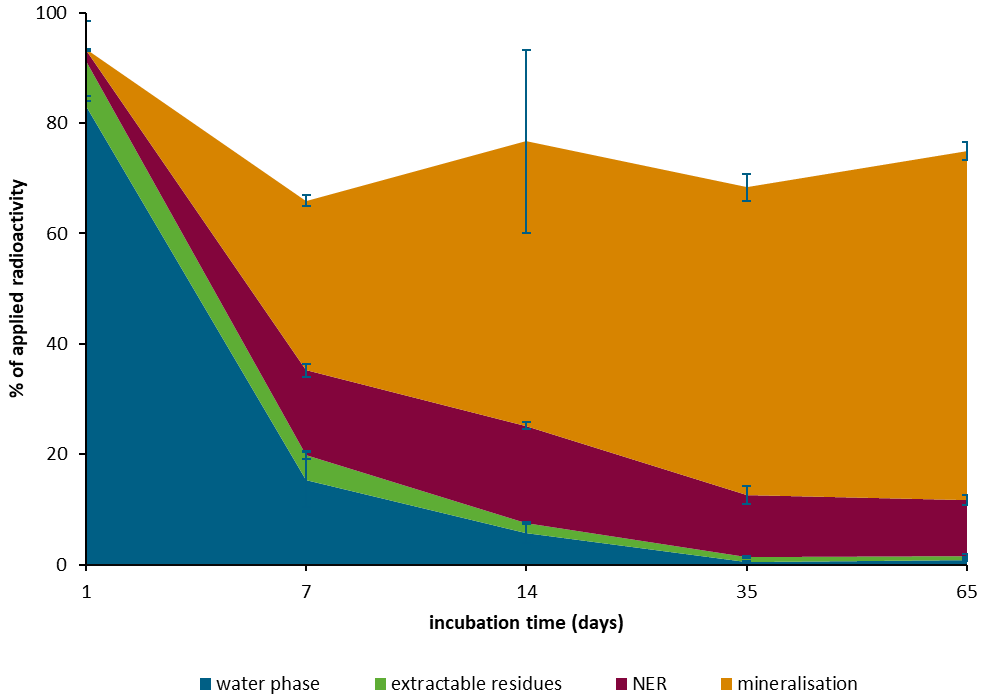
After manual homogenization of the sediment, 160 g sediment (wet weight, ca. 50 g dry weight) were put in 500 mL flasks and 300 mL water were added. The flasks were sealed with a CO_2 trap containing soda lime pellets in order to quantify mineralisation of the test substances. The CO_2 trap contained glass wool impregnated with paraffin in order to absorb possible volatile organic residues formed during degradation of the test substances. All samples were stored on an orbital shaker (50 rpm) at 20 ± 2 °C in the dark. Incubation time was 4 hours and 7, 14, 35 and 65 days. The test was performed using duplicates. For analytical reasons and based on the concentration of LAS$^-$ in soils that have not received sewage sludge (Jensen, 1999), a test concentration of 1 mg/kg was chosen resulting in a test concentration of 0.05 mg/50 g sediment (dry weight) for each test substance. The applied amount of ^{14}C-labelled DS$^-$ was 1.2 µg (0.017 MBq) and 48.8 µg unlabelled DS$^-$; 1.5 µg ^{14}C-DP (0.017 MBq) and 48.5 µg unlabelled DP. The test substances were applied to the water phase by means of a Hamilton syringe.

After incubation, the water layer was removed, and duplicate 1 mL samples were measured by liquid scintillation counting (LSC) to determine the radioactivity in the water phase. The sediment was extracted according to the extraction scheme (see chapter 2.2). A combustion analysis was performed in order to quantify the amount of NER. Soda lime pellets were dissolved in hydrochloric acid (25 %), the released $^{14}CO_2$ was trapped in scintillation cocktail and measured by LSC. The glass wool was extracted using 10 mL *n*-hexane. The amount of radioactivity in the *n*-hexane extract was measured by LSC.

2.3.2 Results

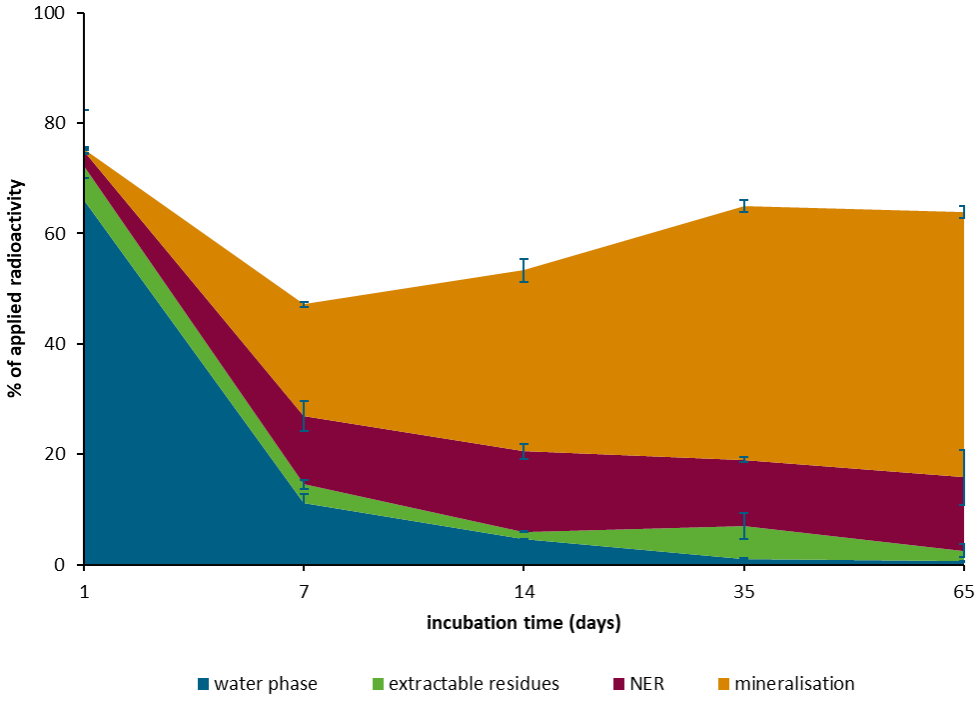
The distribution of the applied radioactivity (AR) of ^{14}C-DS$^-$ and ^{14}C-DP in the water-sediment system over the 65-day incubation period is shown in Figure 4 and Figure 5, respectively. The recovery of ^{14}C-DS$^-$ and ^{14}C-DP ranged between 76.0 % - 98.4 % and 49.7 % - 76.1 %, respectively. Both substances rapidly disappeared from the water phase. After 14 days, only 5.8 % AR (^{14}C-DS$^-$) and 4.6 % AR (^{14}C-DP) were found in the water phase. With increasing incubation time, less radioactivity was extractable. After 65 days, only 0.9 % and 1.8 % AR of ^{14}C-DS$^-$ and ^{14}C-DP could be extracted, respectively. The formation of NER of ^{14}C-DS$^-$ and ^{14}C-DP increased during the first 14 days of incubation and decreased to 10.0 % AR (^{14}C-DS$^-$) and 13.33 % AR (^{14}C-DP) at the end of the incubation period. Both test substances were rapidly mineralised. After 65 days, 63.3 % of the initially applied amount of ^{14}C-DS$^-$ were converted into $^{14}CO_2$. Mineralisation of ^{14}C-DP was slightly lower (48.2 % AR after 65 days). No volatile products of ^{14}C-DS$^-$ and ^{14}C-DP were formed during incubation of the assays.

Figure 4: Distribution of the AR (%) of ^{14}C-DS$^-$ among water phase, extractable, non-extractable and mineralised residues in the water-sediment system over the 65-day incubation period. Data points represent mean values of two replicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

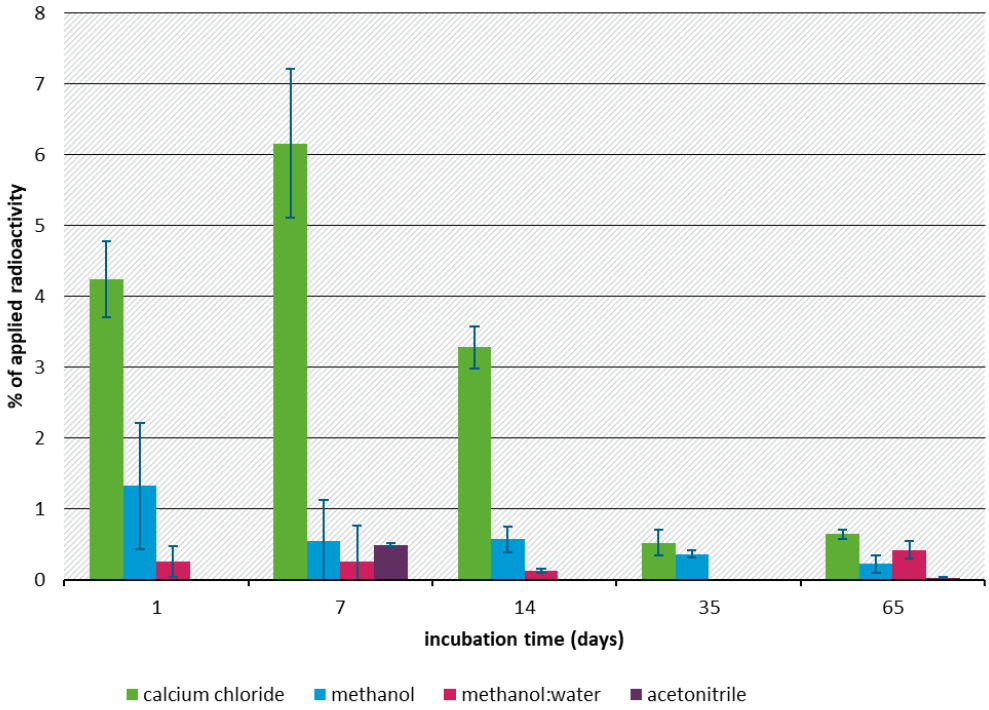
Figure 5: Distribution of the AR (%) of ^{14}C-DP among water phase, extractable, non-extractable and mineralised residues in the water-sediment system over the 65-day incubation period. Data points represent mean values of two replicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

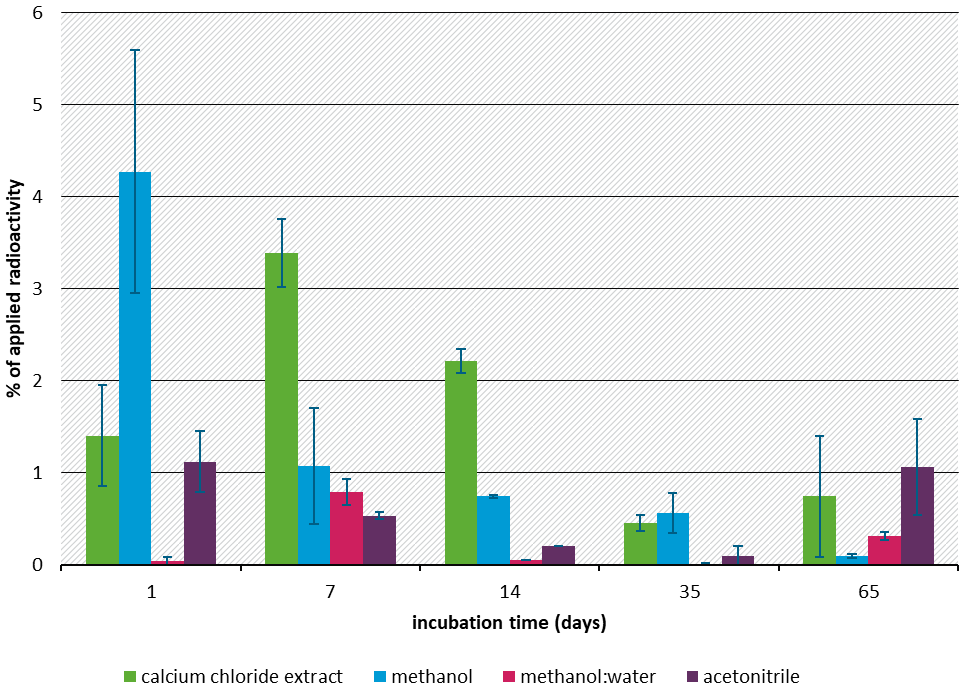
The proportion of radioactivity within the extractable fraction of ^{14}C-DS$^-$ and ^{14}C-DP is shown in Figure 6 and Figure 7. The highest amounts of ^{14}C-DS$^-$ residues were extracted using aqueous calcium chloride solution. The portion decreased from 4.2 % AR (day 1) to 0.6 % AR (day 65). Using organic solvents, below 1.5 % AR were extractable over time. The highest amounts of ^{14}C-DP residues were found in the methanol extract at day 1 (4.3 % AR) and in the calcium chloride extracts at day 7 and 14. In general, only small quantities of ^{14}C-DS$^-$ and ^{14}C-DP were extractable over time. This may be due to the texture of the sediment. After addition of fresh solvent to the sediment in the beaker, mechanical agitation using the orbital shaker was not sufficient for dissolving the sediment from the bottom. Therefore, manual stirring was performed prior to placing the samples on the orbital shaker in the main experiments.

Figure 6: Distribution of ^{14}C-DS^{-} between the different sediment extracts over the 65-day incubation time. The extraction was performed using aqueous calcium chloride solution (0.01 mol/L), methanol, methanol:water (1:1) and acetonitrile successively. Data points represent mean values of two replicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 7: Distribution of ^{14}C-DP between the different sediment extracts over the 65-day incubation time. The extraction was performed using aqueous calcium chloride solution (0.01 mol/L), methanol, methanol:water (1:1) and acetonitrile successively. Data points represent mean values of two replicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

2.4 Distribution and fate of DS$^-$, DP and DA$^+$ in surface water following OECD 309

2.4.1 Experimental Setup

The preliminary surface water test was also performed using ^{14}C-DP and ^{14}C-DS$^-$. The test was conducted with surface water only (pelagic test) and with addition of suspended sediment (1 g/L dry weight, suspended sediment test). Each 500 mL flask contained 150 mL natural surface water (pelagic test). In the suspended sediment test, 0.45 g sediment (wet weight) were added to 150 mL surface water resulting in a sediment concentration of 1 g/L (dry weight). The flasks were sealed with a CO_2 trap containing soda lime pellets and glass wool impregnated with paraffin. Paraffin was used to absorb possible volatile organic products formed during degradation of the test substances. Test concentrations were 10 µg/L and 100 µg/L in order to imitate environmental conditions. For the test at a concentration of 10 µg/L, each flask contained 150 mL surface water and 1.5 µg ^{14}C-DS$^-$ or ^{14}C-DP. For the test at a higher concentration, 1.1 µg ^{14}C-DS$^-$ and 13.9 µg unlabelled DS$^-$ or 1.6 µg ^{14}C-DP and 13.5 µg unlabelled DP were applied to a flask containing 150 mL surface water.

In addition to the non-sterile assays, sterile controls were used for examining possible abiotic degradation. The test water and sediment were autoclaved (126 °C, 30 min). A DMSO-reducation-assay for quantification of the microbial activity in the sediment (for details see 3rd progress report) showed that autoclaving was not sufficient (DMSO-reduction rate 125 ng/(g dw * h)). Therefore, the sediment was gamma irradiated (30 kGy, ^{60}Co) in order to obtain

sterile conditions in the main experiments. The flasks were incubated at 20 °C in the dark on an orbital shaker (50 rpm). Incubation time was 1, 3, 7, 14, 28 and 60 days. The test was performed in duplicates. At each sampling time 10 mL of the water phase were taken from both the non-sterile and sterile assays. The amount of radioactivity in the water phase was determined by LSC. After 60 days, the whole flasks were harvested. The water samples were used for radiochemical analysis (radio-Thin-Layer Chromatography, radio-TLC). Solvent system for DS^- was chloroform: methanol: water: formic acid (50:50:3:1; v/v/v/v). For DP, a mixture of *n*-hexane: diethyl ether: acetic acid (20:80:1; v/v/v) was used. Unlabelled DS^- and DP served as reference substances. The soda lime was dissolved in hydrochloric acid and the amount of $^{14}CO_2$ was measured. For determination of volatile organic compounds, the glass wool was extracted using 10 mL *n*-hexane. The amount of radioactivity in the *n*-hexane extract was measured. The sediment was separated from the water phase by centrifugation (3900 x g, 10 minutes). After addition of 50 mL methanol, the samples were shaken for 15 minutes (170 rpm) and centrifuged (2400 x g). The supernatant was collected and its radioactivity was measured. This step was performed using methanol:water (1:1, 50 mL) and acetonitrile (50 mL). After extraction, the sediment was combusted to quantify NER formation.

2.4.2 Results

In general, the recovery was not sufficient in this test series. Ideally, the mass balance should range between 90 % and 110 % (OECD 309). In the pelagic test, the recovery of ^{14}C-DS^- was mostly below 80 % (Table 8). Similar results were obtained when the test was performed using suspended sediment (Table 9). At the end of the suspended sediment test, the sediment was collected and extracted using methanol, methanol:water (1:1) and acetonitrile. The extracts were pooled (sediment extract, 'SE'). In both test systems (pelagic and suspended sediment) at different concentrations, the formation of volatile products was negligible (below 0.1 % after 60 days).

Table 8: Preliminary surface water test: Distribution of applied radioactivity (AR, in %) of ^{14}C-DS^- in the pelagic version of the surface water test (OECD 309). Test concentrations of 10 µg/L and 100 µg/L.

incubation time [days]	WP (10 µg/L)	$^{14}CO_2$ (10 µg/L)	recovery (10 µg/L)	WP (100 µg/L)	$^{14}CO_2$ (100 µg/L)	recovery (100 µg/L)
1	79.65 ± 3.32	0.3	79.95	76.15 ± 0.40	0.04	76.19
3	72.77 ± 0.40	8.08	80.85	60.60 ± 5.90	13.01	73.61
7	49.05 ± 4.63	18.53	67.59	33.58 ± 1.29	17.94	51.52
14	45.47 ± 3.11	30.18	75.65	31.41 ± 1.52	40.05	71.46
28	39.41 ± 3.48	37.51	76.92	25.82 ± 0.27	46.99	72.80
60	35.43 ± 2.77	44.27	82.70	24.47 ± 0.09	51.37	84.52

Table 9: Preliminary surface water test: Distribution of applied radioactivity (AR, in %) of ^{14}C-DS^- in the suspended sediment version of the surface water test (OECD 309). Test concentrations of 10 µg/L and 100 µg/L.

incubation time [days]	WP (10 µg/L)	$^{14}CO_2$ (10 µg/L)	recovery (10 µg/L)	WP (100 µg/L)	$^{14}CO_2$ (100 µg/L)	recovery (100 µg/L)
1	70.12 ± 1.85	0.21	70.33	65.22 ± 1.89	0.09	65.32

incubation time [days]	WP (10 µg/L)	$^{14}CO_2$ (10 µg/L)	recovery (10 µg/L)	WP (100 µg/L)	$^{14}CO_2$ (100 µg/L)	recovery (100 µg/L)
3	59.53 ± 3.69	8.42	67.95	48.27 ± 12.80	8.28	56.56
7	33.07 ± 0.82	24.11	57.18	26.84 ± 3.24	14.81	41.65
14	32.85 ± 0.40	36.06	68.91	26.30 ± 1.89	44.67	70.97
28	27.40 ± 0.49	41.34	68.75	20.86 ± 0.79	47.31	68.16
60	31.70 ± 0.63	49.36	82.49*	19.52 ± 2.08	56.07	77.13**

*including 1.07% SE and 0.35% NER; **including 1.05% SE and 0.49 % NER

In the pelagic test, the recovery of ^{14}C-DP was slightly lower compared to ^{14}C-DS$^-$ (Table 10). At the end of the test period in the suspended sediment test, the recovery increased to an acceptable range (Table 11).

Table 10: Preliminary surface water test: Distribution of applied radioactivity (AR, in %) of ^{14}C-DP in the pelagic version of the surface water test (OECD 309). Test concentrations of 10 µg/L and 100 µg/L.

incubation time [days]	WP (10 µg/L)	$^{14}CO_2$ (10 µg/L)	recovery (10 µg/L)	WP (100 µg/L)	$^{14}CO_2$ (100 µg/L)	recovery (100 µg/L)
1	68.47 ± 20.49	0.04	68.51	50.97 ± 6.38	0.07	51.04
3	62.71 ± 6.80	2.69	65.40	45.12 ± 1.72	8.10	53.22
7	54.30 ± 13.33	10.86	65.16	32.19 ± 0.19	16.67	48.86
14	52.87 ± 15.75	18.01	70.88	30.52 ± 1.86	52.89	83.41
28	46.88 ± 14.74	22.32	69.20	24.32 ± 0.12	54.85	79.17
60	55.27 ± 13.61	26.22	81.49	32.89 ± 4.31	56.38	89.28

Table 11: Preliminary surface water test: Distribution of applied radioactivity (AR) of ^{14}C-DP in the suspended sediment version of the surface water test (OECD 309). Test concentrations of 10 µg/L and 100 µg/L.

incubation time [days]	WP (10 µg/L)	$^{14}CO_2$ (10 µg/L)	recovery (10 µg/L)	WP (100 µg/L)	$^{14}CO_2$ (100 µg/L)	recovery (100 µg/L)
1	74.56 ± 3.78	0.42	74.98	42.47 ± 0.76	0.14	42.62
3	55.52 ± 2.07	7.68	63.20	41.37 ± 0.85	9.68	51.05
7	35.14 ± 1.42	22.40	57.55	27.39 ± 3.87	26.71	54.09
14	40.74 ± 1.28	35.21	75.94	27.25 ± 3.34	44.05	71.30
28	35.79 ± 0.32	41.04	76.83	23.69 ± 1.08	49.55	73.24
60	45.46 ± 4.71	51.06	101.87*	22.30 ± 0.57	60.32	88.77**

*including 2.94 % SE and 2.41 % NER; ** including 2.74 % SE and 3.41 % NER

Low recovery rates could be due to the sampling technique. At each sampling time, only an aliquot of 10 mL was taken out of the 150 mL water sample. During sediment extraction, centrifugation was used in order to separate the extract from sediment particles. This procedure might also have led to losses of radioactivity, since a precise separation of solvent and particles was not possible. Therefore, the main experiments were performed by using multiple flasks and harvesting whole flasks at each sample interval. Instead of centrifugation, the sediment was separated from the water phase by filtration. The sediment/solvent mixture was also filtrated rather than centrifuged. After extraction, the cellulose filters with the extracted sediment were combusted for NER quantification.

A radio-TLC analysis was performed using the water phases of ^{14}C-DS$^-$ and ^{14}C-DP after 1 day and 60 days of incubation (pelagic test at a concentration of 10 µg/L). After 1 day, only a small portion of ^{14}C-DS$^-$ was detected in the water sample. Two more polar and one less polar transformation products were detected. The sample of day 60 contained one less polar transformation product but no parent substance. The water phase of ^{14}C-DP at day 1 contained small amounts of ^{14}C-DP, three more polar transformation products and unidentifiable start activity. The sample of day 60 contained a high portion of start activity, two more polar transformation products and ^{14}C-DP.

The R_f values of the unlabelled reference substances were calculated on the basis of their visible spot on the plate. Hence inaccuracies occurred and led to fluctuating R_f values. Therefore, radiolabelled reference substances were used in the main experiments. There was no significant difference between the radio-TLC results of the pelagic test and the suspended sediment test.

The mineralisation of the sterile controls was very low (below 1 % after 60 days for both substances; except DP pelagic, 10 µg/L with 8 %), although the DMSO-reduction assay revealed microbial activity in the sediment (125 ng/(g dw * h)). The main experiments were conducted using autoclaved water (126 °C, 30 min) with the addition of sodium azide (10-20 g/L) and gamma irradiated sediment (30 kGy, ^{60}Co) to ensure sterile conditions.

In order to investigate the biodegradation through sediment-associated microorganisms in surface water, the main experiments were carried out as suspended sediment tests. The amendment of sediment particles additionally allows the quantification of NER.

3 Main experiment: Distribution and fate of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in a water-sediment system (OECD 308)

3.1 Experimental Setup

The water-sediment study was performed according to OECD guideline 308 using ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$. The test design was identical to the setup in the preliminary test. Incubation time was 1, 7, 14, 30, 60 and 120 days. Triplicates were used for each sampling time. The test concentration of 1 mg/kg was based on the concentration of LAS$^-$ in soils that have not received sewage sludge (Jensen, 1999). The applied amounts of ^{14}C-labelled and unlabelled DS$^-$, DP and DA$^+$ are summarised in Table 12.

Table 12: Applied amounts of radiolabelled and non-labelled DS$^-$, DP and DA$^+$ in the water-sediment study. Concentrations were based on the application rate and amount of sediment per flask, resulting in a test concentration of 0.1 mg/100 g sediment (dry weight).

Test substance	Concentration	Amount of test substance
^{14}C-DS$^-$ DS$^-$ (unlabelled)	0.1 mg / 100 g sediment (dw)	16.8 µg (0. 3 MBq) 83.2 µg
^{14}C-DP DP (unlabelled)	0.1 mg / 100 g sediment (dw)	30.0 µg (0.3 MBq) 70.0 µg
^{14}C-DA$^+$ DA$^+$ (unlabelled)	0.1 mg / 100 g sediment (dw)	26.9 µg (0.2 MBq) 73.1 µg

The samples were treated as described in chapter 2.3.1. With increasing incubation time less radioactivity was extractable using methanol:water (1:1). Therefore, methanol extracts were pooled with methanol:water extracts of the respective test substance. At the end of the test, the methanol:water extraction step was skipped. For ^{14}C-DA$^+$, a 24-hour Soxhlet-extraction was performed subsequent to the sequential solvent extraction, since the sequential solvent extraction was not exhaustive for this test substance (see chapter 2.2).

3.2 Radiochemical analysis

In order to quantify the parent substances and to detect transformation products, radio-TLC of the extracts was performed. If available, 250 Bq of each extract were applied to the TLC plate. Solvent system for DS$^-$ was chloroform: methanol: water: formic acid (50:50:3:1; v/v/v/v), for DP *n*-hexane: diethyl ether: acetic acid (20:80:1; v/v/v) and for DA$^+$ methanol with addition of sodium bromide (100 mL methanol + 5.15 g NaBr). Radiolabelled DS$^-$, DP and DA$^+$ served as reference substances. For each substance, the corresponding molecule without the 12 carbon-tail served as unlabelled reference substance (benzenesulfonic acid sodium salt for DS$^-$, phenol for DP and benzyltrimethylammonium chloride for DA$^+$, respectively). Based on the radio-TLC results, selected samples were analysed using radio-HPLC. Composition of the mobile phase was adjusted to the different test substances. For DS$^-$ and DP, the mobile phase consisted of water + 0.1 % acetic acid (eluent A) and acetonitrile + 0.1 % acetic acid (eluent B). The mobile phase of DA$^+$ was water + 0.1 % trifluoroacetic acid (eluent A) and acetonitrile + 0.1 % trifluoroacetic acid (eluent B). The gradient elution programme was aligned with the respective test substance (Table 13, Table 14 and Table 15).

Table 13: Radio-HPLC gradient elution programme for DS^-. The mobile phase was composed of water + 0.1 % acetic acid (Eluent A) and acetonitrile + 0.1 % acetic acid (Eluent B). HPLC was run using a Phenomenex Synergi 4 µm Polar-RP 80A column.

Time [min]	A [%]	B [%]
0.00	70	30
5.00	70	30
20.00	0	100
25.00	0	100
35.00	70	30

Table 14: Radio-HPLC gradient elution programme for DP. The mobile phase was composed of water + 0.1 % acetic acid (Eluent A) and acetonitrile + 0.1 % acetic acid (Eluent B). HPLC was run using a Phenomenex Synergi 4 µm Polar-RP 80A column.

Time [min]	A [%]	B [%]
0.00	70	30
5.00	70	30
15.00	0	100
25.00	0	100
35.00	70	30

Table 15: Radio-HPLC gradient elution programme for DA^+. The mobile phase was composed of water + 0.1 % trifluoroacetic acid (Eluent A) and acetonitrile + 0.1 % trifluoroacetic acid (Eluent B). HPLC was run using a Phenomenex Synergi 4 µm Polar-RP 80A column.

Time [min]	A [%]	B [%]
0.00	70	30
5.00	70	30
15.00	0	100
25.00	0	100
35.00	70	30

3.3 Determination of degradation half-life (DT_{50})

Degradation half-lives of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in the water-sediment system (incubation temperature 20 ± 2 °C) were calculated using the software CAKE (Computer Assisted Kinetic Evaluation, Tessella Altran Group, Version 3.3). CAKE provides four different kinetic models to describe the change in substance concentration with time: Single First-Order (SFO), First-Order Multi-Compartment (FOMC), Double First-Order in Parallel (DFOP) and the bi-phasic Hockey-Stick model (HS). Calculations were carried out following a guidance document (Boesten *et al.*, 2005) and performed using all four kinetic models. The DT_{50} values were chosen according to the model which provided the best fit of the data. Calculations were based on the portions of ^{14}C-

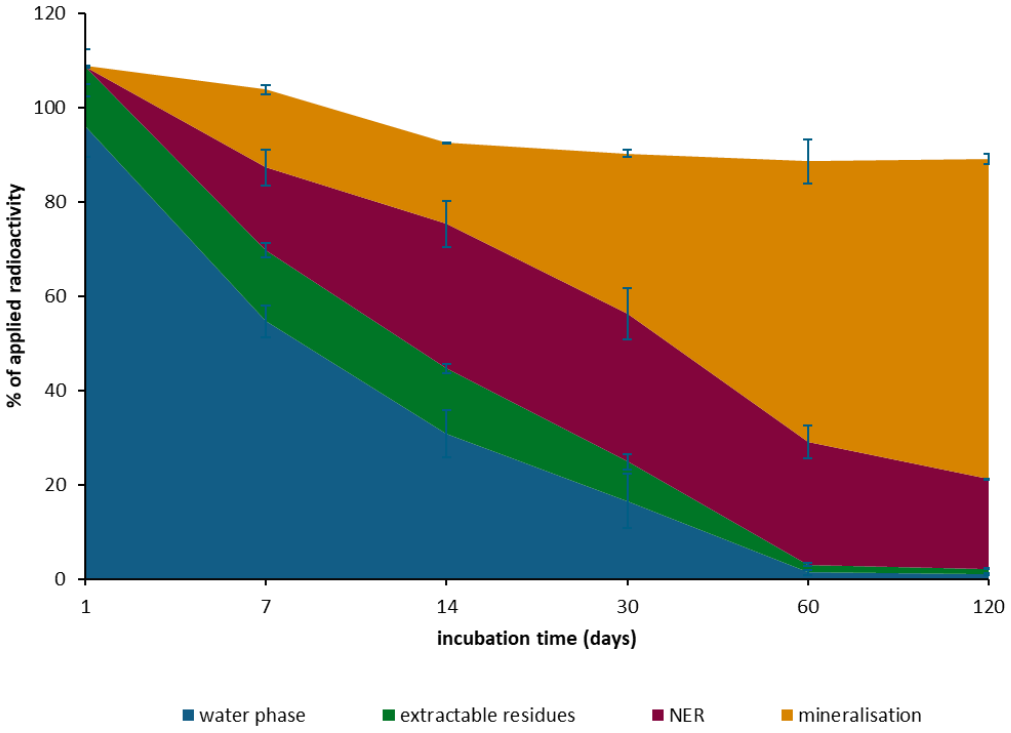
DS^-, ^{14}C-DP and ^{14}C-DA^+ in the water phases and extractable fractions determined by radio-TLC analysis. It was assumed that the mineralised, volatile and non-extractable fraction did not contain the parent substances. Calculations were run for the overall system and for the water and sediment phase separately. Since 12 °C represents the average EU outdoor temperature, this temperature should be reflected in simulation studies (European Commission, 2003). Therefore, extrapolation of calculated degradation half-lives to 12 °C was conducted following Beulke *et al.* (2005) and European Food Safety Authority (2008).

3.4 Results

3.4.1 Distribution of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in the water-sediment system

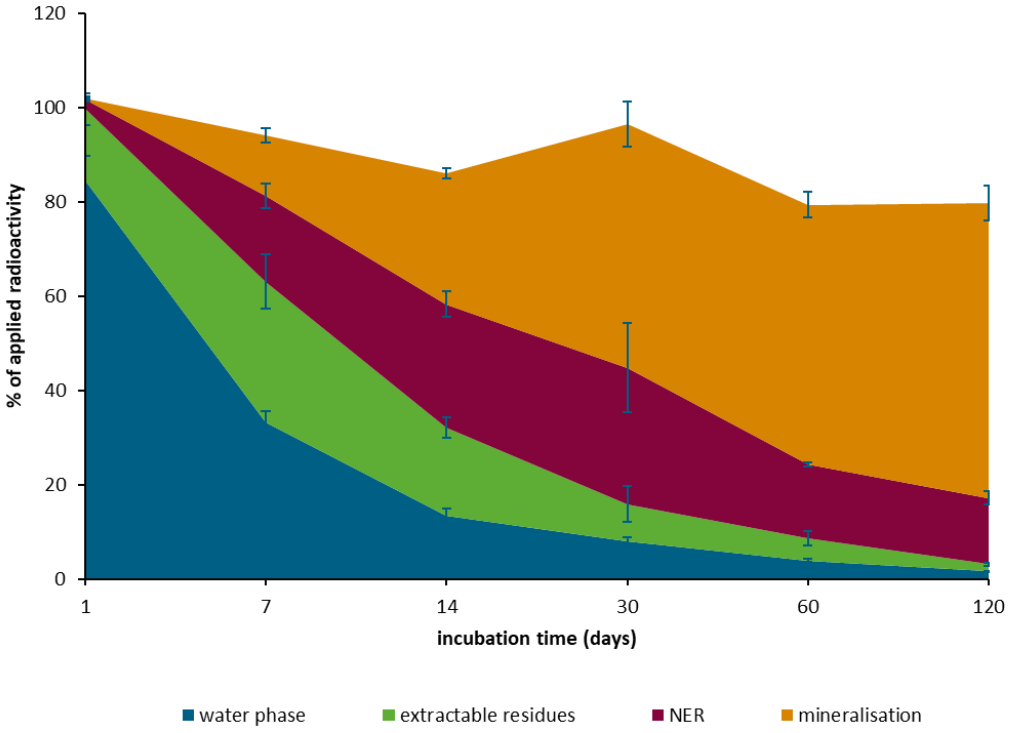
The distribution of the applied radioactivity (AR) of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in the water-sediment system over the 120-day incubation period is shown in Figure 8, Figure 9 and Figure 10, respectively. The recovery of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ ranged between 88.6 % - 108.9 %, 79.4 % - 101.8 % and 76.3 % - 89.5 %, respectively (appendix A.1).^{14}C-DS^- and ^{14}C-DP rapidly disappeared from the water phase. After 60 days, 1.3 % AR (^{14}C-DS^-) and 3.9 % AR (^{14}C-DP) were detected in the water phase. Residues of ^{14}C-DA^+ in the water phase decreased more continuously (19 % AR at day 60). With increasing incubation time, less ^{14}C-DS^- and ^{14}C-DP residues were extractable (below 2 % after 120 days of incubation). The extractable fraction of ^{14}C-DA^+ increased to 24 % during the first 30 days of incubation and decreased slightly in the further course of the experiment (19 % AR at day 120). Highest NER formation was observed for ^{14}C-DA^+ with 38 % AR after 60 days of incubation followed by ^{14}C-DS^- (31 % AR at day 30) and ^{14}C-DP (28 % AR at day 30). The formation of NER of all test substances decreased slightly after reaching its maximum. Mineralisation of ^{14}C-DS^- and ^{14}C-DP accounted for 68 % AR and 63 % AR after 120 days, respectively. Low amounts of ^{14}C-DA^+ were mineralised (6 % AR after 120 days).

Figure 8: Distribution of the AR (%) of ^{14}C-DS$^-$ among water phase, extractable, non-extractable and mineralised residues in the water-sediment system over the 120-day incubation period. Data points represent mean values of triplicates with standard deviation.



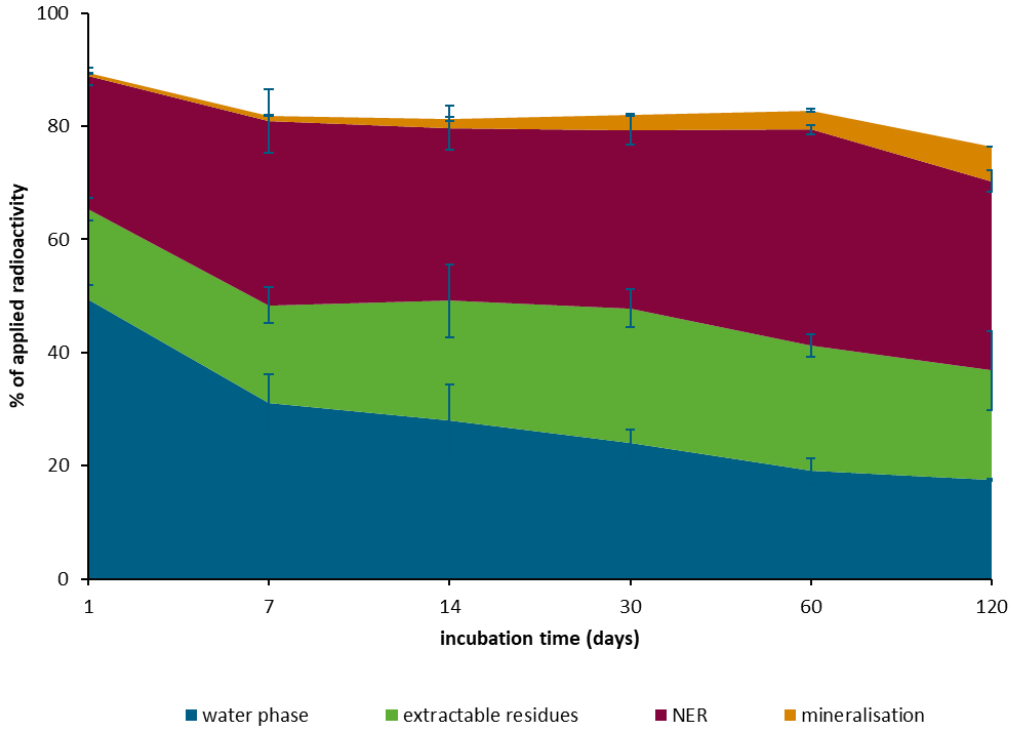
Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 9: Distribution of the AR (%) of ^{14}C-DP among water phase, extractable, non-extractable and mineralised residues in the water-sediment system over the 120-day incubation period. Data points represent mean values of triplicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

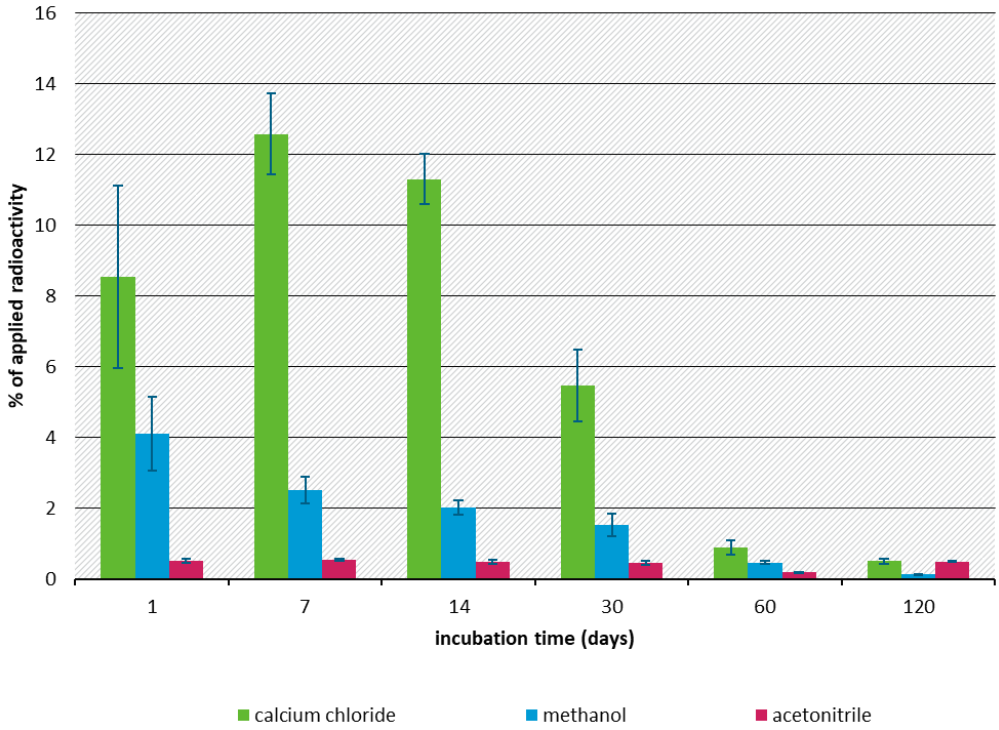
Figure 10: Distribution of the AR (%) of ^{14}C-DA^{+} among water phase, extractable, non-extractable and mineralised residues in the water-sediment system over the 120-day incubation period. Data points represent mean values of triplicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

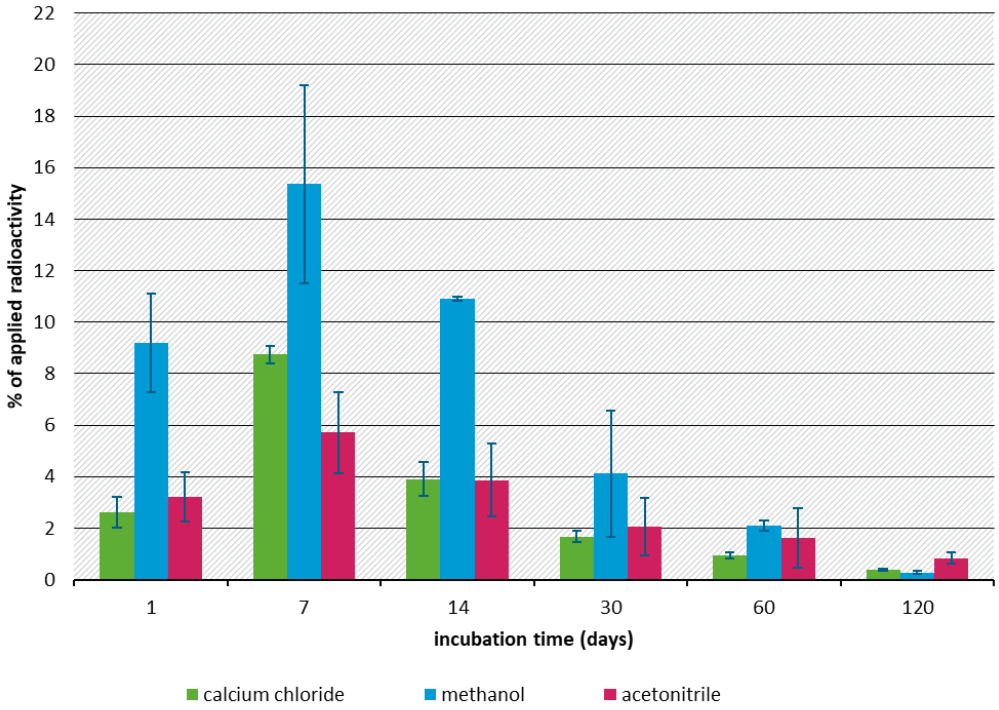
The extractable fractions of ^{14}C-DS^{-}, ^{14}C-DP and ^{14}C-DA^{+} contained less than 30 % of the applied radioactivity (Figure 11, Figure 12, Figure 13 and appendix A.2). After a slight increase during the first 7 days of incubation, the extractability of ^{14}C-DS^{-} and ^{14}C-DP decreased over time, whereas the extractability of ^{14}C-DA^{+} increased during the first 30 days of incubation followed by a marginal decrease until the end of the incubation period. For ^{14}C-DS^{-}, the calcium chloride extracts contained the highest amounts of radioactivity compared to the methanol and acetonitrile extracts. For ^{14}C-DP, the highest amounts of radioactivity were generally extracted using methanol. The major ^{14}C fractions of ^{14}C-DA^{+} were found in the Soxhlet extracts.

Figure 11: Distribution of AR of ^{14}C-DS^{-} between the different sediment extracts over the 120-day incubation time. Data points represent mean values of triplicates with standard deviation.



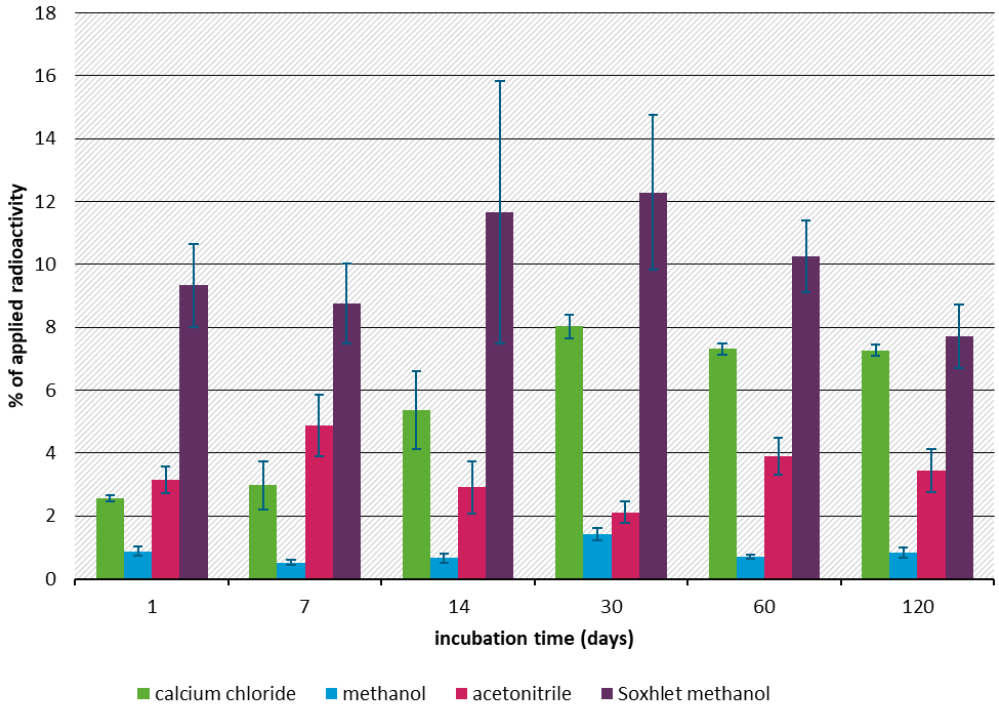
Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 12: Distribution of AR of ^{14}C-DP between the different sediment extracts over the 120-day incubation time. Data points represent mean values of triplicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 13: Distribution of AR of ^{14}C-DA$^+$ between the different sediment extracts over the 120-day incubation time. Data points represent mean values of triplicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

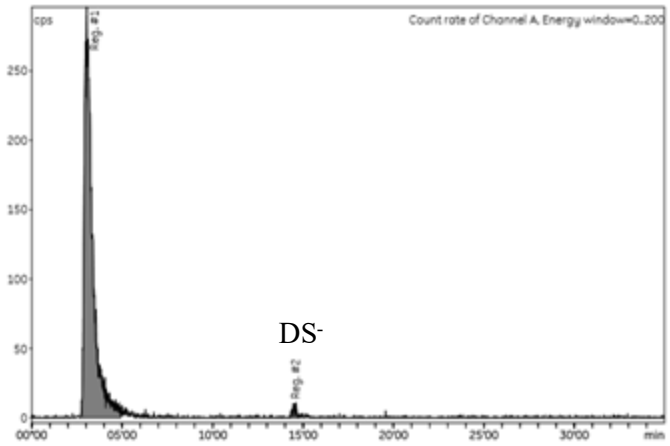
3.4.2 Radiochemical analysis (radio-TLC and radio-HPLC)

For ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$, the water phases, calcium chloride extracts, acetonitrile extract (and Soxhlet-extract for ^{14}C-DA$^+$ only) were analysed using radio-TLC (appendix A.3). In case the radioactivity in an extract was too low, replicates were pooled. Based on the results of the radio-TLC analyses, selected samples of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ were analysed using radio-HPLC (appendix A.4).

Using radio-TLC, small amounts of ^{14}C-DS$^-$ could be detected in the water phases at day 60. In the samples of day 1, 7, 14, 30 and 120, only polar transformation products were found. Additionally, a less polar transformation product was detected in the water phase at day 7. The calcium chloride extracts of day 1, 7 and 30 contained small amounts of the parent substance DS$^-$. In all samples, polar transformation products were detected. After an incubation time of 7 days, the calcium chloride extract contained also a less polar transformation product. DS$^-$ was detected in the methanol extracts during the first 30 days of incubation. In all samples, polar transformation products were found. In addition, a less polar metabolite was detected in the methanol extracts after 7, 14 and 60 days of incubation. Due to low amounts of radioactivity in the acetonitrile extracts, only the samples of day 30 and 60 could be analysed. In both extracts, only a less polar transformation product was detected. In general, the highest amounts of ^{14}C-DS$^-$ could be extracted from the sediment using aqueous calcium chloride solution. All samples of ^{14}C-DS$^-$ contained small amounts of radioactivity which remained at the starting spot of the TLC plate ('start activity', R_f value ~0.00).

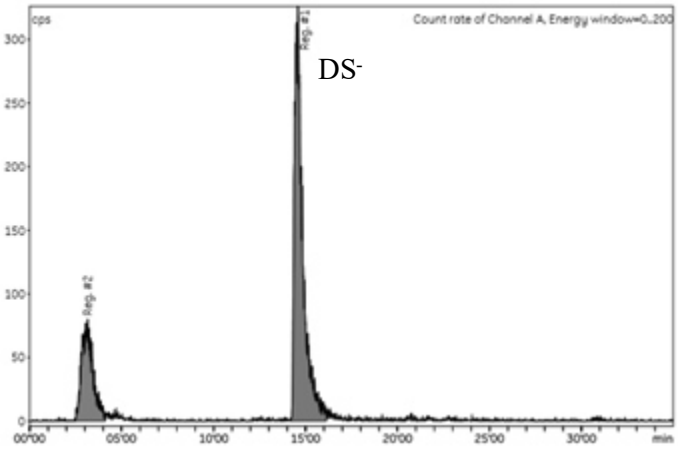
Figure 14 shows the radio-HPLC chromatogram of the sample DS- water phase day 1. Only small amounts of the parent substance were detected in the water phase (1.3 % AR). The major ^{14}C fraction was found as a polar transformation product (RT= 3 min) containing 94.7 % AR.

Figure 14: Radio-HPLC chromatogram of the water phase of DS- after 1 day of incubation. Based on co-chromatography, the right signal (reg. #2) was identified as DS- (retention time: 14.3 minutes). A polar transformation product eluted after 3 minutes (reg. #1).



The methanol extract of DS- after 1 day of incubation contained DS- (3.1 % AR) and a polar transformation product (RT = 3 min) which contained 1.0 % AR (Figure 15).

Figure 15: Radio-HPLC chromatogram of the methanol extract of DS- after 1 day of incubation. Based on co-chromatography, the right signal (reg. #1) was identified as DS- (retention time: 14.3 minutes). A polar transformation product eluted after 3 minutes (reg. #2).

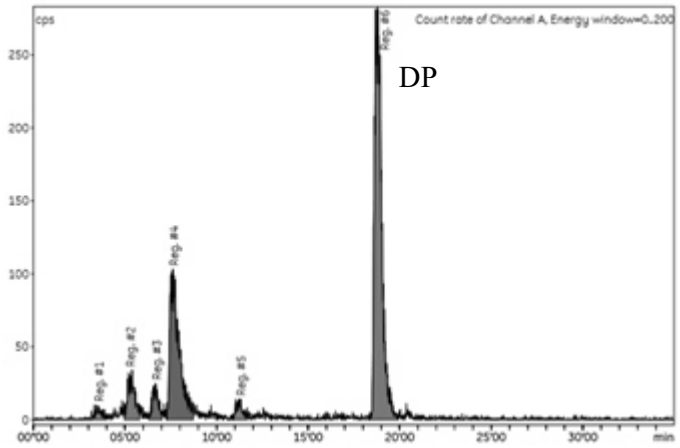


The radio-TLC analysis of the water phases of ^{14}C-DP showed that the parent substance was rapidly transformed into metabolites. After 1 day of incubation, ^{14}C-DP, three more polar and a less polar transformation product were detected. Small amounts of ^{14}C-DP were found after 14, 60 and 120 days of incubation. The water phase of day 7 contained only a polar transformation product. All radio-TLC chromatograms contained high amounts of start activity. Therefore, these samples were analysed using radio-HPLC. The start activity was separated into more polar metabolites (appendix A.4: Figure 36). In all calcium chloride extracts, ^{14}C-DP was detected in low amounts (below 1.0 % AR). Additionally, start activity and four polar transformation products were detected. The calcium chloride extract of day 1 contained also a less polar

transformation product. ^{14}C-DP was detected in all methanol extracts over time. In addition, start activity, three polar and a less polar transformation product were found. In all acetonitrile extracts, ^{14}C-DP was detected. Additionally, start activity, two polar and two less polar transformation products were found. In general, the highest amounts of ^{14}C-DP could be extracted from the sediment using methanol.

Figure 16 shows the radio-HPLC chromatogram of the sample DP water phase day 1. The portion of DP accounted for 49.8 % AR. A metabolite with a retention time of 7.4 min (reg. #4) contained 22.7 % AR, whereas the other transformation products contained less than 6 % AR.

Figure 16: Radio-HPLC chromatogram of the water phase of DP after 1 day of incubation. Based on co-chromatography, the right signal (reg. #6) was identified as DP (ret. time: 18.5 minutes). Five polar transformation products (regions #1 to #5) were found.

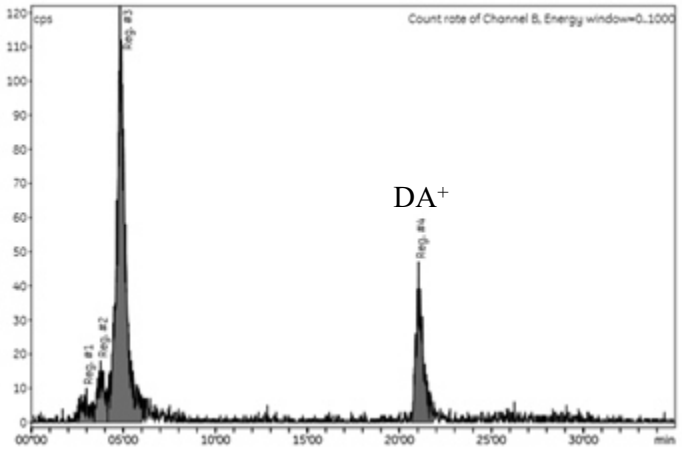


The methanol extract of DP after 1 day of incubation contained only the parent substance with a portion of 9.2 % AR.

^{14}C-DA^{+}, start activity and a polar transformation product were detected in all water phases using radio-TLC. During the first 14 days of incubation, two further transformation products were found in the water phases. The parent substance could not be detected in the calcium chloride extract. Four polar transformation products and start activity were found. The methanol extract contained small amounts of ^{14}C-DA^{+}, three polar transformation products and start activity. In the acetonitrile extract, small amounts of ^{14}C-DA^{+}, three polar transformation products, a less polar product and start activity were detected. The Soxhlet extract at day 1 contained two polar transformation products and start activity. After an incubation time of longer than 1 day, ^{14}C-DA^{+}, start activity, a polar and a less polar transformation product could be detected. The highest amounts of the parent substance were extracted under Soxhlet conditions.

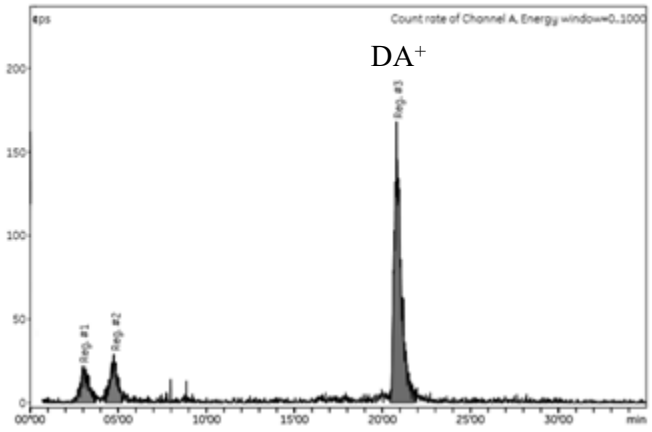
Figure 17 shows the radio-HPLC chromatogram of the sample DA^{+} water phase day 1. The portion of DA^{+} in the water phase accounted for 10.1 % AR. A transformation product with a RT of 4.5 minutes (reg. #3) contained 33.7 % AR. Regions #1 and #2 contained 2.0 % and 3.5% AR, respectively.

Figure 17: Radio-HPLC chromatogram of the water phase of DA^+ after 1 day of incubation. Based on co-chromatography, the right signal (reg. #4) was identified as DA^+ (RT: 20.4 minutes). Three polar transformation products (regions #1 to #3) were found.



The methanol extract of the sediment after 7 days of incubation contained DA^+ (reg. #3, < 1.0 % AR) and two polar transformation products (regions #1 and #2) with portions below 1.0 % AR (Figure 18).

Figure 18: Radio-HPLC chromatogram of the methanol extract of DA^+ after 7 days of incubation. Based on co-chromatography, the right signal (reg. #3) was identified as DA^+ (ret. time: 20.4 minutes). Two polar transformation products (regions #1 and #2) were found.



3.4.3 Determination of degradation half-lives (DT_{50})

The degradation of ^{14}C-DS^-, ^{14}C-DA^+ and ^{14}C-DP in the water-sediment system was characterised by a fast initial decrease followed by a slower decline. Thus, degradation was best described using the bi-phasic kinetic model DFOP with degradation rate constants k_1 and k_2 (Table 16). Since low values for k_1 were most likely caused by a transfer of the model substances from the water phase to the sediment rather than by degradation, k_2 was chosen to compare half-lives of the model substances in the overall water-sediment system.

In the overall water-sediment system, the highest half-live was found for DA^+ (162 days), followed by DS^- (22.1 days) and DP (14.1 days) $DT_{50,water}$ of DA^+, DP and DS^- was considerably lower (< 1 d), whereas higher half-lives were determined for the sediment (267 days, 24.2 days and 22 days, respectively).

Table 16: Degradation half-lives of DS^-, DP and DA^+ in the overall water-sediment system ($DT_{50,system}$), in the water column ($DT_{50,water}$) and sediment ($DT_{50,sed}$) at 20 °C. Calculations were performed using CAKE (version 3.3).

Test substance	$DT_{50,system}$	$DT_{50,water}$	$DT_{50,sed}$
DS^-	22.1 d	0.1 d	22 d
DP	14.1 d	0.3 d	24.2 d
DA^+	162 d	0.2 d	267 d

Table 17 shows the degradation half-lives of the test substances after extrapolation to 12 °C.

Table 17: Degradation half-lives of DS^-, DP and DA^+ in the overall water-sediment system ($DT_{50,system}$), in the water column ($DT_{50,water}$) and sediment ($DT_{50,sed}$) after extrapolation to 12 °C. Calculations were performed using CAKE (version 3.3).

Test substance	$DT_{50,system}$	$DT_{50,water}$	$DT_{50,sed}$
DS-	47 d	0.2 d	47 d
DP	30 d	0.6 d	51 d
DA+	346 d	0.4 d	570 d

4 Main Experiment: Distribution and fate of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water (OECD 309)

4.1 Experimental Setup

The surface water test was performed according to OECD guideline 309 using ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$. For the suspended sediment test, 500 mL flasks were filled with 100 mL natural surface water and 0.33g sediment (wet weight). The flasks were sealed with a CO_2 trap containing soda lime pellets. In the preliminary test, no volatile organic products were formed. Therefore, no paraffin-impregnated glass wool was used. Test concentrations were 10 µg/L and 100 µg/L in order to represent environmental conditions (OECD 309). For the test at a concentration of 10 µg/L, 1µg ^{14}C-DS$^-$, ^{14}C-DP or ^{14}C-DA$^+$ (0.015 MBq, 0.011 MBq or 0.010 MBq, respectively) were applied to the assays. For the test with a concentration of 100 µg/L, 10 µg of ^{14}C-DS$^-$, ^{14}C-DP or ^{14}C-DA$^+$ (0.15 MBq, 0.11 MBq or 0.10 MBq, respectively) were applied to the assays. The test substances were applied to the water phase by means of a Hamilton syringe.

The flasks were incubated at 20 °C in darkness on an orbital shaker (50 rpm). Incubation times were 7, 14, 30 and 62 days (test concentration of 10 µg /L) and 1, 7, 14, 30 and 60 days (test concentration of 100 µg/L). The experiments were performed in duplicates (10 µg/L) and triplicates (100 µg/L). At each sampling time, the water phase was separated from sediment particles by filtration prior to measuring ^{14}C levels in the water phase. The radioactivity in the water phase was determined by measuring duplicate 1 mL aliquots using LSC. The sediment was transferred to 250 mL centrifuge beakers, 50 mL methanol were added and the samples were shaken for 15 minutes on an orbital shaker (170 rpm). The methanol extract was separated from sediment particles by filtration and collected. 50 mL acetonitrile were added to the sediment and filter in the centrifuge beaker and shaken again. After filtration, methanol and acetonitrile extracts were combined (hereafter referred to as sediment extract). Duplicate 1 mL samples were taken from the sediment extract and measured using LSC. Afterwards, the extracted sediment particles and the filter paper were combusted to determine the amount of NER. The soda lime was dissolved in hydrochloric acid (25 %) and the amount of $^{14}CO_2$ was measured using LSC.

Additionally, abiotic degradation of the test substances was examined by using gamma irradiated sediment (30 kGy, ^{60}Co) and autoclaved water (126 °C, 30 minutes) with addition of sodium azide (10 g/L). The test under sterile conditions was performed at a concentration of 10 µg/L and with addition of 0.1 g gamma irradiated sediment (dry weight). The flasks were incubated at 20 °C in darkness on an orbital shaker (50 rpm). Incubation times were 7, 14, and 30 days. At each time point, the sediment was separated from the water phase by centrifugation (3900 x g, 10 minutes). After addition of 50 mL methanol, the samples were shaken for 15 minutes (170 rpm) and centrifuged (2400 x g). The supernatant was collected and its radioactivity was measured. This extraction step was repeated using acetonitrile (50 mL). After extraction, the sediment was combusted to determine the amount of NER. Since this experiment was performed prior to the surface water tests under non-sterile conditions, the previous extraction method (see chapter 2.4.1) was used. The soda lime was dissolved in hydrochloric acid (25 %) and the released $^{14}CO_2$ was measured by LSC to quantify mineralisation.

4.2 Radiochemical analysis

Radio-TLC analyses were conducted with water phases and sediment extracts. Solvent systems for DS$^-$, DP and DA$^+$ were identical to the ones described in chapter 3.2. Unlabelled DS$^-$, DP and

DA^+ served as reference substances in the tests with a concentration of 10 µg/L. Due to poor visibility of the unlabelled compounds under UV light, radiolabelled DS^-, DP and DA^+ were used for the test at 100 µg/L. Based on the results of radio-TLC analyses, selected samples were analysed using radio-HPLC. Radio-HPLC analysis of the samples at a test concentration of 10 µg/L was performed using a different HPLC-system. Thus, a slightly different gradient elution programme was used (Table 18 and Table 19).

Table 18: Radio-HPLC gradient elution programme for DS- and DA+. For DS-, mobile phase was composed of water + 0.1 % acetic acid (Eluent A) and acetonitrile + 0.1 % acetic acid (Eluent B). For DA+, 0.1 % trifluoroacetic acid was added instead of acetic acid. HPLC was run using a Phenomenex Synergi 4 µm Polar-RP 80A column.

Time [min]	A [%]	B [%]
0.00	95	5
5.00	95	5
35.00	0	100
40.00	0	100
45.00	95	5

Table 19: Radio-HPLC gradient elution programme for DP. The mobile phase was composed of water + 0.1 % acetic acid (Eluent A) and acetonitrile + 0.1 % acetic acid (Eluent B). HPLC was run using a Phenomenex Synergi 4 µm Polar-RP 80A column.

Time [min]	A [%]	B [%]
0.00	95	5
5.00	95	5
25.00	0	100
40.00	0	100
45.00	95	5

The gradient elution programme for the test at 100 µg/L was identical to the one used for the water-sediment study (see Table 13, Table 14 and Table 15).

4.3 Determination of degradation half-life (DT_{50})

Degradation half-lives of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in the surface water test were calculated using the software CAKE (Computer Assisted Kinetic Evaluation, Tessella Altran Group, Version 3.3). Calculations were carried out following the guidance document of FOCUS (2006), performed using all four kinetic models and based on the portions of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ in the extractable fraction determined by radio-TLC analysis. The DT_{50} values were chosen according to the model which provided the best fit of the data. The mineralised, volatile and non-extractable fraction was disregarded since these fractions were not expected to contain the parent substances. In addition, extrapolation of calculated degradation half-lives to 12 °C was conducted.

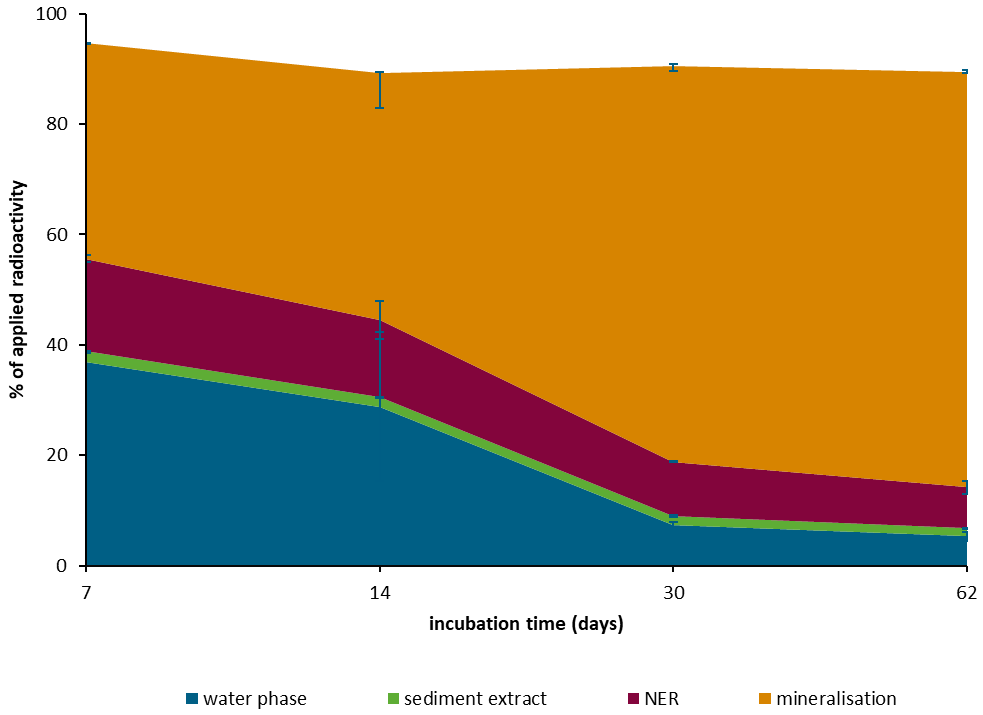
4.4 Results

4.4.1 Distribution of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water with addition of suspended sediment

The distribution of the applied radioactivity (AR) of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water with suspended sediment at a concentration of 10 µg/L is shown in Figure 19, Figure 20 and Figure 21, respectively. The recovery of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ ranged between 89.2 % - 94.7 %, 87.5 % - 94.2 % and 93.4 % - 99.0 %, respectively (appendix A.5). High $^{14}CO_2$ evolution was observed for ^{14}C-DS$^-$ and ^{14}C-DP with portions of 40 % AR after an incubation time of 7 days. At the end of the test, 75 % (^{14}C-DS$^-$) and 69 % (^{14}C-DP) AR were mineralised. Mineralisation of ^{14}C-DA$^+$ accounted for 6.7 % AR after 62 days of incubation. For ^{14}C-DS$^-$ and ^{14}C-DP, the amount of radioactivity in the water phase, sediment extract and the non-extractable fraction decreased with increasing incubation time. For ^{14}C-DA$^+$, ^{14}C levels in the water phase increased from 36.9 % AR (day 7) to 62.3 % AR (day 62), whereas the sediment extract contained between 40.5 % AR (day 7) and 17.9 % AR (day 62). The formation of NER decreased from 19.5 % AR (day 7) to 9.4 % AR (day 14) and then remained constant.

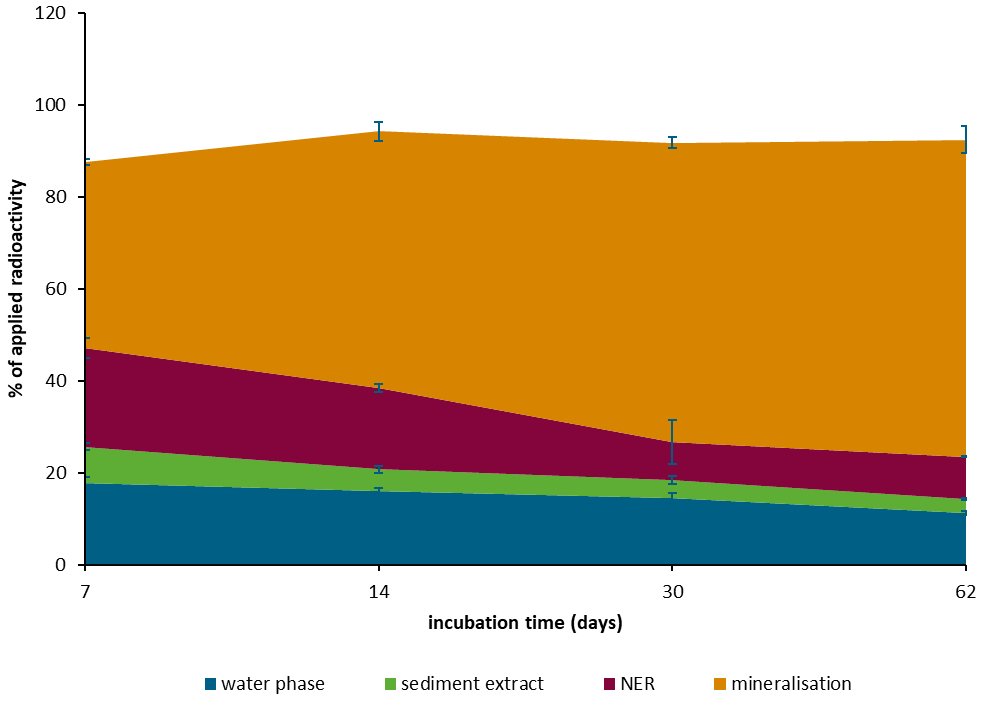
Under sterile conditions, mineralisation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ was below 0.1 % AR after 30 days of incubation (appendix A.6). The non-extractable fraction of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ accounted for 0.1 %, 0.6 % and 5.5 % AR, respectively.

Figure 19: Distribution of the AR (%) of ^{14}C-DS$^-$ among water phase, sediment extract, non-extractable and mineralised residues in the surface water test over the 62-day incubation period at a test concentration of 10 µg/L. Data points represent mean values of two replicates with standard deviation.



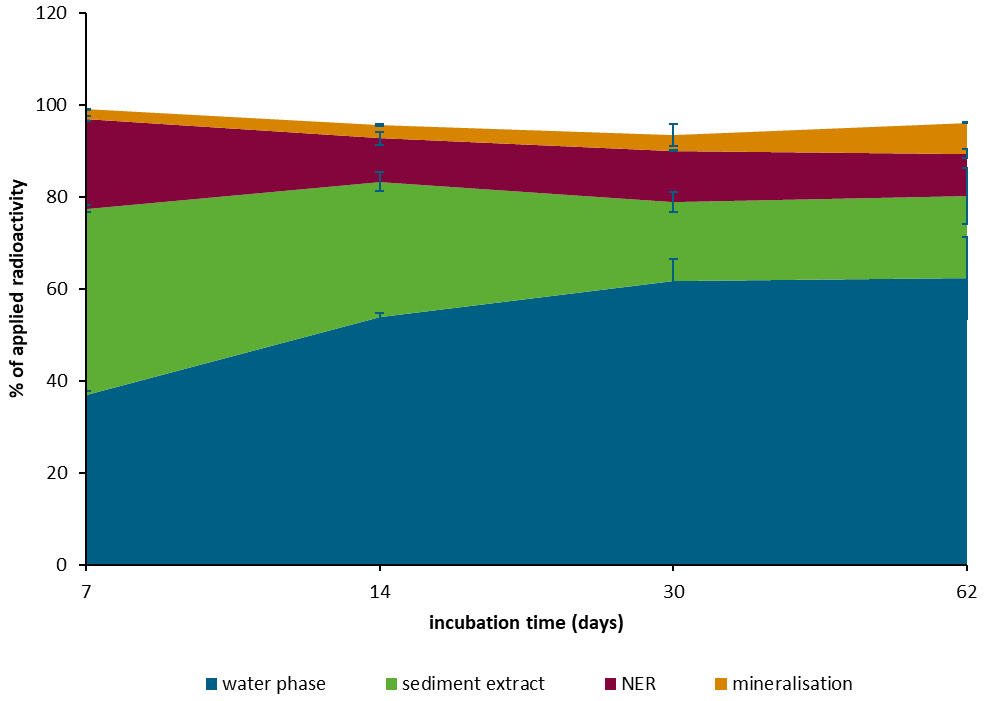
Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 20: Distribution of the AR (%) of ^{14}C-DP among water phase, sediment extract, non-extractable and mineralised residues in the surface water test over the 62-day incubation period at a test concentration of 10 µg/L. Data points represent mean values of two replicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

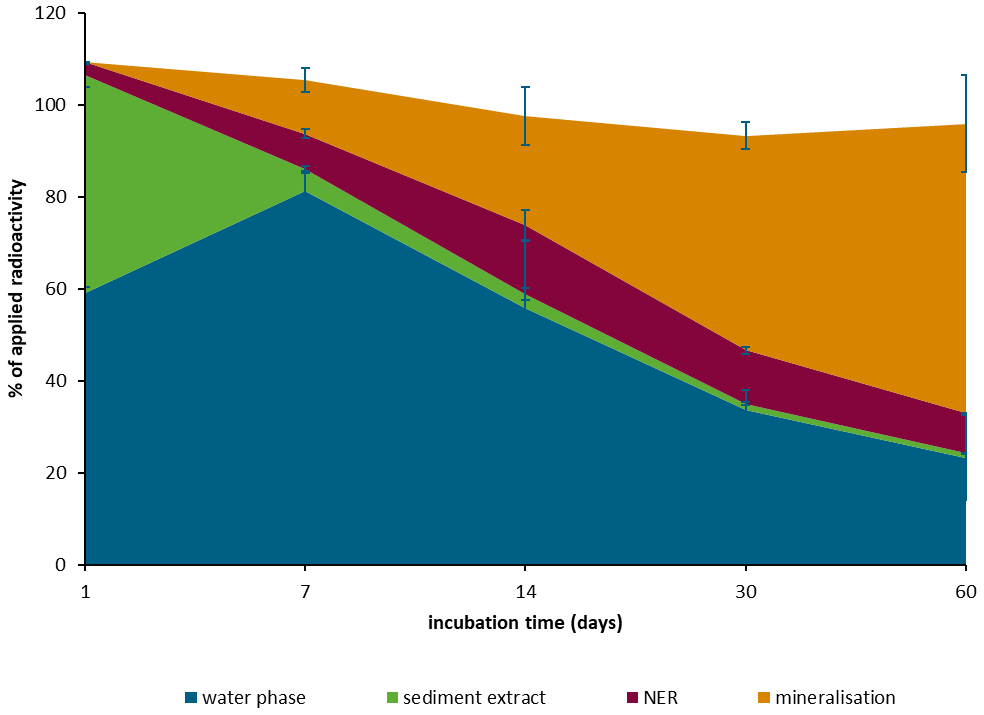
Figure 21: Distribution of the AR (%) of ^{14}C-DA^{+} among water phase, sediment extract, non-extractable and mineralised residues in the surface water test over the 62-day incubation period at a test concentration of 10 µg/L. Data points represent mean values of two replicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

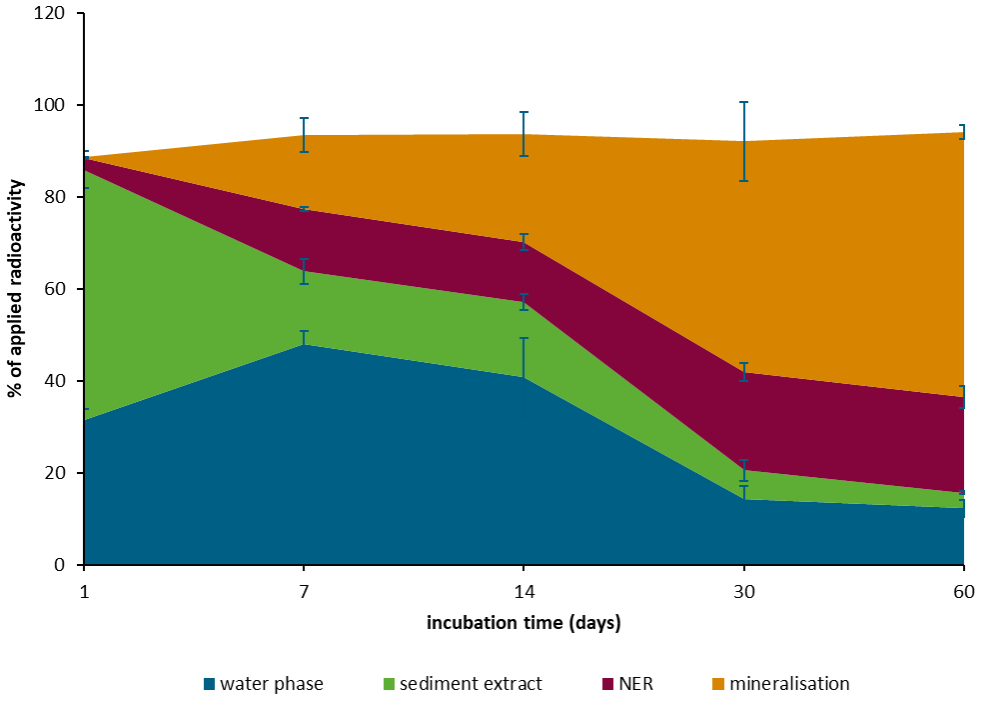
The distribution of the applied radioactivity of ^{14}C-DS^{-}, ^{14}C-DP and ^{14}C-DA^{+} in the test system at a concentration of 100 µg/L are shown in Figure 22, Figure 23 and Figure 24, respectively. The recovery of ^{14}C-DS^{-}, ^{14}C-DP and ^{14}C-DA^{+} ranged between 93.3 % - 109.2 %, 88.5 % - 94.0 % and 90.7 % - 95.5 %, respectively (appendix A.5). After 7 days of incubation, 11.7 % AR (^{14}C-DS^{-}) and 16.2 % AR (^{14}C-DP) were mineralised to $^{14}CO_2$. After 60 days of incubation, these portions increased to 63.0 % AR (^{14}C-DS^{-}) and 57.3 % AR (^{14}C-DP). Mineralisation of ^{14}C-DA^{+} accounted for 7.5 % after 60 days of incubation. The portion of ^{14}C-DS^{-} in the water phase decreased from 59.0 % AR (day 1) to 23.3 % AR (day 60), whereas the portion of ^{14}C-DP increased from 31.4 % AR (day 1) to 48.1 % AR after 7 days of incubation and decreased to 12.2 % AR after 60 days. The portion of ^{14}C-DA^{+} in the water phase increased from 26.0 % AR (day 1) to 57.8 % AR (day 60). The extractability of all test substances decreased over time. NER formation of ^{14}C-DS^{-} increased during the first 14 days of incubation to 15.0 % AR and decreased to 8.7 % AR at day 60. For ^{14}C-DP, NER formation increased over time. For ^{14}C-DA^{+}, NER formation increased during the first 7 days of incubation to 25.7 % AR and decreased to 14.0 % AR at day 60.

Figure 22: Distribution of the AR (%) of ^{14}C-DS^{-} among water phase, sediment extract, non-extractable and mineralised residues in the surface water test over the 60-day incubation period at a test concentration of 100 µg/L. Data points represent mean values of triplicates with standard deviation.



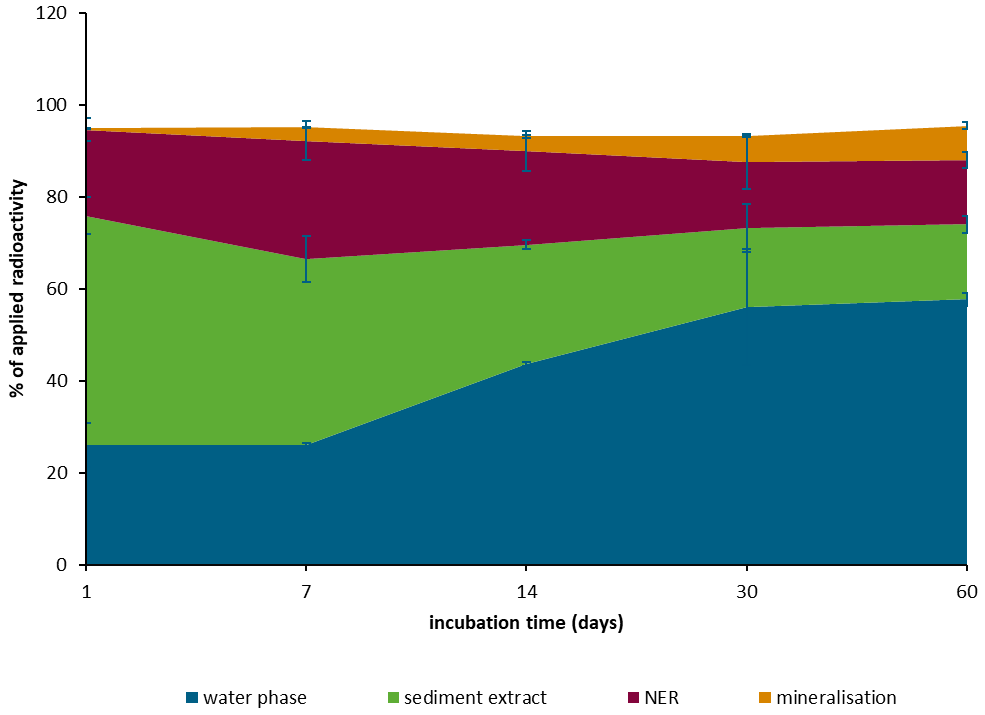
Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 23: Distribution of the AR (%) of ^{14}C-DP among water phase, sediment extract, non-extractable and mineralised residues in the surface water test over the 60-day incubation period at a test concentration of 100 µg/L. Data points represent mean values of triplicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

Figure 24: Distribution of the AR (%) of ^{14}C-DA^{+} among water phase, sediment extract, non-extractable and mineralised residues in the surface water test over the 60-day incubation period at a test concentration of 100 µg/L. Data points represent mean values of triplicates with standard deviation.



Source: own illustration, Institute for Environmental Research, RWTH Aachen University

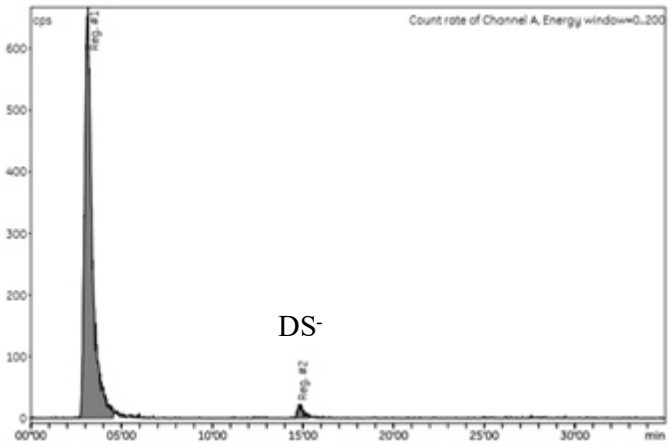
4.4.2 Radiochemical analysis (radio-TLC and radio-HPLC)

For ^{14}C-DS^{-}, ^{14}C-DP and ^{14}C-DA^{+}, the water phases and sediment extracts were analysed for the parent substances and possible transformation products (appendix A.7). Based on the results of the radio-TLC analyses, selected samples of ^{14}C-DS^{-}, ^{14}C-DP and ^{14}C-DA^{+} were analysed using radio-HPLC.

At a test concentration of 10 µg/L, start activity, five polar and two less polar transformation products of DS^{-} were found in the water phase. The parent substance could not be detected in the water phase. The sediment extract contained start activity, four polar and two less polar transformation products. Small amounts of ^{14}C-DS^{-} (below 1.0 % AR) were also detected in the sediment extract after 7 days of incubation. At a test concentration of 100 µg/L, DS^{-} and start activity were detected in all water samples. Additionally, some samples contained two polar and a less polar transformation product. In all sediment extracts, ^{14}C-DS^{-} was detected. In addition, start activity, three polar and three less polar transformation products could be detected.

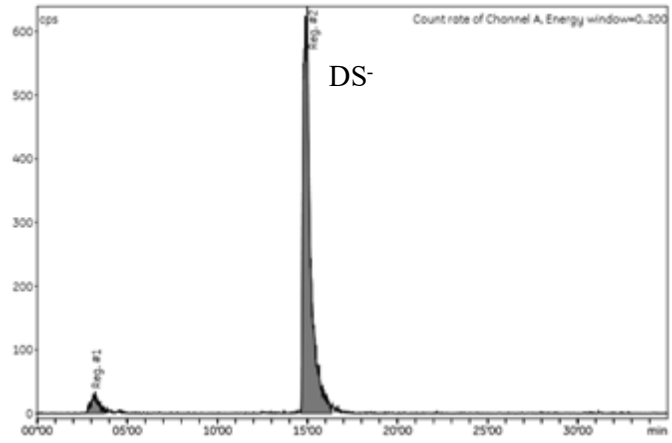
Figure 25 shows the radio-HPLC chromatogram of the sample DS^{-} water phase day 1. Only small amounts of the parent substance were detected in the water phase (reg. #2, 1.3 % AR). A polar transformation product was detected (reg. #1) containing about 57.7 % AR.

Figure 25: Radio-HPLC chromatogram of the water phase of DS^- after 1 day of incubation. Test concentration 100 µg/L. Based on co-chromatography, the right signal (reg. #2) was identified as DS^- (RTe: 14.5 minutes). A polar transformation product eluted after 3 minutes (reg. #1).



The sediment extract of ^{14}C-DS^- after 1 day of incubation contained higher amounts of DS^- (reg. #2, 45.2 % AR) and a polar transformation product (reg. #1) which contained 2.2 % AR (Figure 26).

Figure 26: Radio-HPLC chromatogram of the sediment extract of DS^- after 1 day of incubation. Test concentration 100 µg/L. Based on co-chromatography, the right signal (reg. #2) was identified as DS^- (RT: 14.5 minutes). A polar transformation product eluted after 3 minutes (reg. #1).

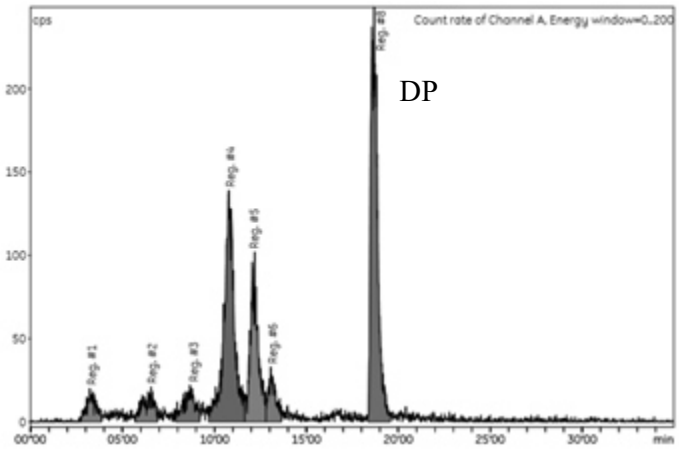


For ^{14}C-DP and at a test concentration of 10 µg/L, ^{14}C-DP and start activity were detected in all water samples. Additionally, the sample of day 7 contained a less polar transformation product. The sediment extracts contained ^{14}C-DP, start activity, four polar metabolites and a less polar transformation product. At a test concentration of 100 µg/L, ^{14}C-DP was detected in the water phases of day 1, 7, 14 and 30. Start activity was found in all water samples. Additionally, some samples contained three polar and a less polar transformation product. In the sediment extracts of day 1, 7, 14 and 30, ^{14}C-DP was detected. In addition, start activity, three polar and a less polar transformation products were found.

Figure 27 shows the radio-HPLC chromatogram of the sample DP water phase day 1 (test concentration 100 µg/L). The portion of ^{14}C-DP (reg. #8) accounted for 11.5 % AR. Two polar

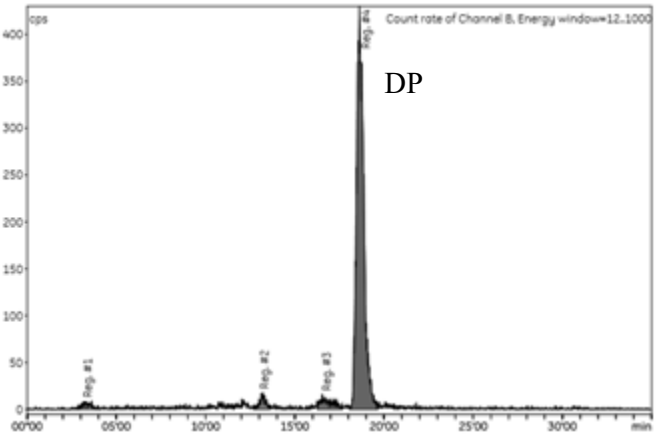
transformation products (region #4 and #5) contained 9.5 % and 5.0 % AR, respectively. Further metabolites were detected in small amounts (below 1.5 % AR).

Figure 27: Radio-HPLC chromatogram of the water phase of DP after 1 day of incubation. Test concentration 100 µg/L. Based on co-chromatography, the right signal (reg. #8) was identified as DP (RT: 18.4 minutes). Six polar transformation products (regions #1 - #6) were detected.



The sediment extract of ^{14}C-DP after 1 day of incubation contained higher amounts of DP (reg. #4, 49.8 % AR) and three polar transformation products (reg. #1- #3) which contained below 2.0 % AR (Figure 28).

Figure 28: Radio-HPLC chromatogram of the sediment extract of DP after 1 day of incubation. Test concentration 100 µg/L. Based on co-chromatography, region #4 was identified as DP (RT: 18.4 minutes). Three polar transformation were found (reg. #1 - #3).

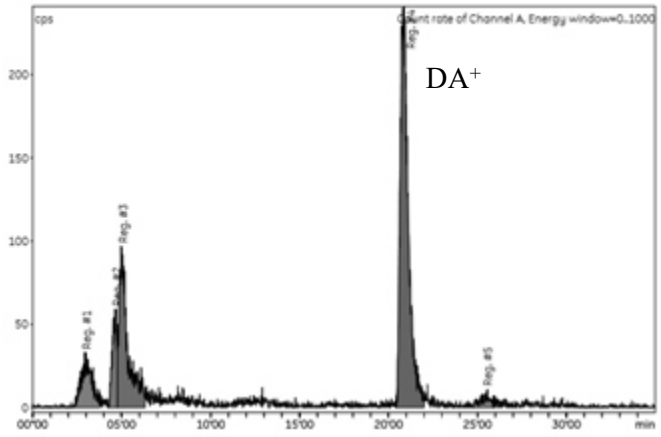


For ^{14}C-DA$^+$ and at a test concentration of 10 µg/L, ^{14}C-DA$^+$, start activity and two polar transformation products were detected in the water phases by radio-TLC. The sediment extracts contained ^{14}C-DA$^+$, start activity, a polar and a less polar transformation product. At a test concentration of 100 µg/L, DA+, start activity, four polar and two less polar transformation products were found in the water phases. The sediment extracts contained ^{14}C-DA$^+$ and start activity. In addition, a polar transformation product was detected in the sediment extracts after 30 and 60 days.

Figure 29 shows the radio-HPLC-chromatogram of the sample DA$^+$ water phase day 1 (test concentration 100 µg/L). The portion of ^{14}C-DA$^+$ (reg. #4) accounted for 14.9 % AR. Three polar

transformation products were found with portions below 7.0 % AR. A less polar transformation product (reg. #5) was detected with a portion of < 1.0 % AR.

Figure 29: Radio-HPLC chromatogram of the water phase of DA^+ after 1 day of incubation. Test concentration 100 µg/L. Based on co-chromatography, region #4 was identified as DA^+ (RT: 20.4 minutes). Three polar transformation products (regions #1 - #3) and a less polar transformation product (region #5) were detected.



In the sediment extract of ^{14}C-DA^+ after 1 day of incubation, only the parent substance was detected with a portion of 25.9 % AR.

The formation of transformation products was less pronounced under sterile conditions (appendix A.9). Using radio-TLC, ^{14}C-DS^- and start activity were detected in the water phases after an incubation time of 7, 14 and 30 days. In the sediment extracts, ^{14}C-DS^-, start activity and a less polar transformation product were found. ^{14}C-DP, start activity, a polar and a non-polar metabolite were detected in the water phases and sediment extracts. DA^+ and start activity were detected in the water phases. The sediment extracts contained ^{14}C-DA^+, start activity and a less polar transformation product. Using radio-HPLC, only ^{14}C-DS^- could be detected in the water phase and sediment extract after 30 days of incubation. ^{14}C-DP and three polar metabolites were found in the water phase at day 30. No transformation product was detected in the sediment extract. For ^{14}C-DA^+, only the parent substance was detected in the water phase and sediment extract after 30 days of incubation.

4.4.3 Determination of degradation half-lives (DT_{50})

The degradation of the test substances in the surface water test was best described using the Single First-Order (SFO) kinetic (Table 20). At both test concentrations, the highest half-lives were found for DA^+ followed by DP and DS^-.

Table 20: Degradation half-lives of DS^-, DP and DA^+ in the surface water test at a concentration of 10 µg/L ($DT_{50,10\ µg/L}$) and at a concentration of 100 µg/L ($DT_{50,100\ µg/L}$) at 20 °C. Calculations were performed using CAKE (version 3.3).

Test substance	$DT_{50,10µg/L}$	$DT_{50,100\ µg/L}$
DS^-	1.4 d	1.0 d
DP	1.5 d	1.2 d
DA^+	26.4 d	13.4 d

Table 21 shows the degradation half-lives of the test substances after extrapolation to 12 °C.

Table 21: Degradation half-lives of DS^-, DP and DA^+ in the surface water test at a concentration of 10 µg/L ($DT_{50,10\ µg/L}$) and at a concentration of 100 µg/L ($DT_{50,100\ µg/L}$) after extrapolation to 12 °C. Calculations were performed using CAKE (version 3.3).

Test substance	$DT_{50,10µg/L}$	$DT_{50,100\ µg/L}$
DS^-	3 d	2 d
DP	3 d	3 d
DA^+	56 d	45 d

5 Discussion

5.1 Distribution and fate of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in a water-sediment system

In the water-sediment study, ^{14}C-DS$^-$ and ^{14}C-DP were rapidly degraded in the test system, whereas ^{14}C-DA$^+$ was degraded considerably slower. The extractability of ^{14}C-DS$^-$ and ^{14}C-DP from the sediment decreased throughout the incubation period. The amount of ^{14}C-DA$^+$-derived residues in the extractable fraction increased within the first 30 days of incubation and then slightly decreased over time. Based on the mineralisation of the test substances and their portions in the $CaCl_2$ extracts, the direct bioavailability decreased as follows: ^{14}C-DS$^-$ > ^{14}C-DP > ^{14}C-DA$^+$. NER formation of ^{14}C-DA$^+$ was higher compared to ^{14}C-DS$^-$ and ^{14}C-DP. The degradation half-life of ^{14}C-DA$^+$ at incubation temperature (20 °C) was 162 days, whereas DT_{50} of ^{14}C-DS$^-$ and ^{14}C-DP were much lower (22 days and 14 days, respectively).

5.1.1 Ultimate degradation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in the water-sediment system

The ultimate degradation of a chemical is determined by the mineralised fraction, which represents the inorganic degradation product of the parent compound (CO_2). For ^{14}C-DS$^-$ and ^{14}C-DP, mineralisation represented the major degradation pathway in the water-sediment system with $^{14}CO_2$ evolution of ^{14}C-DS$^-$ being slightly higher at the end of the experiment compared to ^{14}C-DP. Higher ultimate biodegradability of anionic surfactants compared to non-ionic surfactants was observed by Gledhill (1975). Haudin *et al.* (2013) reported that mineralisation of ^{14}C-labelled sodium linear dodecylbenzene sulfonate (^{14}C-LAS$^-$) in soil accounted for 20 - 32 % after 140 days, whereas 4-*n*-NP was mineralised more slowly (4 - 7 %). Lashermes *et al.* (2010) investigated the mineralisation of 4-*n*-NP and LAS$^-$ in compost. He found that LAS$^-$ was mineralised to larger extents than the neutral compound NP. In another study using compost, mineralisation was also the major route of dissipation of LAS$^-$ and 4-*n*-NP with LAS$^-$ being mineralised to a higher extent than 4-*n*-NP (Lashermes *et al.*, 2010). Although ultimate degradation of LAS$^-$ is rapid and more pronounced than the one of NP (Jiménez *et al.*, 1991; Shan *et al.*, 2011), several studies showed that 4-*n*-NP was also mineralised to large extents in soil and seawater (Ekelund *et al.*, 1993; Topp and Starratt, 2000; Hesselsøe *et al.*, 2001).

Contrary to the behaviour of the anionic and neutral model substances, ultimate biodegradation of ^{14}C-DA$^+$ was very low. The evolution of low portions of $^{14}CO_2$ indicated that the aromatic part of the molecule was degraded considerably more slowly. Knaebel *et al.* (1994) examined the mineralisation of anionic, cationic and non-ionic surfactants in soil and found that ^{14}C-LAS$^-$ were rapidly mineralised to high extents (50-70 % after 60 days) followed by dodecyl linear alcohol ethoxylate (LAE) with 30 – 55 % after 60 days. Lower $^{14}CO_2$ evolutions (5 - 20 % after 60 days) were reported for stearyl trimethylammonium chloride (STAC$^+$). Another study by Knaebel *et al.* (1996) dealing with the degradation of differently charged ^{14}C-labelled compounds in different types of soil constituents showed that dodecyltrimethyl ammonium chloride (DTAC$^+$) was mineralised to much lower extents than LAS$^-$ and LAE. In a study by Claßen (2019), the influence of an ionic functional group on the degradation of organic compounds in soil was investigated. Using ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$, highest mineralisation was observed for ^{14}C-DS$^-$ (65 % AR), followed by ^{14}C-DP (44 % AR) and ^{14}C-DA$^+$ (38 % AR) after an incubation time of 124 days.

The results of the present study showed that the mineralisation of an organic chemical is influenced by its charge. A positive charge leads to a considerably lower ultimate degradation

compared to no charge, whereas a negative charge leads to a slightly higher one compared to no charge.

5.1.2 Determination of extractable and non-extractable fractions of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+

The sediment incubated with ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ was subjected to a sequential extraction using different solvents. The sequential extraction method is used to determine the binding strength between a chemical and soil or sediment and its bioavailability for organisms living in the matrix (Schaeffer *et al.*, 2018). The extractable fraction can be divided into the easily bioavailable fraction, potentially bioaccessible fraction and slowly desorbable fraction. The first extraction step was performed using a 0.01 M $CaCl_2$ solution in order to mimic the molarity and ionic strength of the soil pore water (Houba *et al.*, 2000; Peijnenburg *et al.*, 2007). Subsequently, the sediment was extracted with organic solvents and solvent/water mixtures (methanol, methanol:water and acetonitrile) to determine the portion of the desorbable, potentially bioaccessible fraction. For ^{14}C-DA^+, this extraction method was not exhaustive. According to (Schaeffer *et al.*, 2018), sequential extractions should be performed until less than 5 % of the radioactivity released from the first extraction step is obtained. Therefore, the sequential solvent extraction was followed by a more vigorous Soxhlet extraction in order to determine the slowly desorbable fraction.

In general, only low amounts of radioactivity were found in the directly bioavailable fraction of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ ($CaCl_2$ extract). The portions decreased with increasing incubation time indicating that sorption of the test substances to the sediment increased with aging. Aging refers to the increased contact time between a chemical and soil or sediment, which can lead to a more strongly association of a chemical with soil or sediment components over time (Gevao *et al.*, 2000). At the end of the 120-day incubation period, the highest portion of ^{14}C-residues in the $CaCl_2$ extract was found for ^{14}C-DA^+. These results are in accordance with the findings of Claßen (2019). In their study, the portion of radioactivity in the $CaCl_2$ extract of ^{14}C-DA^+ after 124 days was significantly higher compared to ^{14}C-DS^- and ^{14}C-DP. Higher amounts of ^{14}C-DP were found in the potentially bioaccessible fraction obtained by solvent extraction compared to ^{14}C-DS^- and ^{14}C-DA^+. Strongest affinity to the sediment matrix was observed for the cationic compound DA^+ with the highest portions of ^{14}C found in the slowly desorbable fraction obtained by Soxhlet extraction. Contrary to this observation, Claßen (2019) reported that the highest amount of radioactivity was extracted from soil using methanol.

NER formation of the test substances decreased as follows: ^{14}C-DA^+ > ^{14}C-DS^- > ^{14}C-DP. In contrast to moderate NER formation of ^{14}C-DS^- in the present study (maximum 31 % at day 30), Haudin *et al.* (2013) reported that between 60 % and 79 % of ^{14}C-LAS were non-extractable from soil-compost mixtures after 14 days. However, the amount of organic carbon in soil containing compost is much higher than in native soil or sediments (Kästner *et al.*, 1999). LAS^- adsorption mechanisms on soil or sediment underlie interactions between the negatively charged sulfonate group with polar groups (e.g. hydroxyl and phenol groups) of soil humus or hydroxyl groups on the surface of minerals and oxides (Laha and Luthy, 1992; Ou *et al.*, 1996). Furthermore, the negative charge of ^{14}C-DS^- can lead to a repulsion of the molecule from the mainly negatively charged surfaces of clay minerals and organic matter components (Wauchope *et al.*, 2002). ^{14}C-DP showed the lowest NER formation in the water-sediment system. In contrast, Barriuso *et al.* (2008) stated that the presence of a chemical reactive group leads to an increased formation of NER. For NP, for example, NER formation was the major degradation pathway in soil (Shan *et al.*, 2011; Liu *et al.*, 2014). These findings suggest that the degradation pathway of alkyl phenols differs depending on which simulation study, testing on sediment

(OECD 308) or soil (OECD 307), is chosen. In a study on the formation, classification and identification of NER of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in soil, highest amounts of NER were observed for ^{14}C-DP followed by ^{14}C-DA$^+$ and ^{14}C-DS$^-$ (Claßen 2019). In the present study, highest NER formation was observed for ^{14}C-DA$^+$ (maximum 38 % at day 60). Due to negatively charged surfaces of clay minerals and soil organic matter, cation exchange is the predominant type of binding leading to an increased sorption of positively charged compounds in soils and sediments (Mordaunt *et al.*, 2005; Kah and Brown, 2006; Cycoń *et al.*, 2012). The present findings are in accordance with previous studies which corroborated strong sorption and reduced bioavailability of QACs$^+$ in sediments (Ismail *et al.*, 2010; Li and Brownawell, 2010; Sarkar *et al.*, 2010).

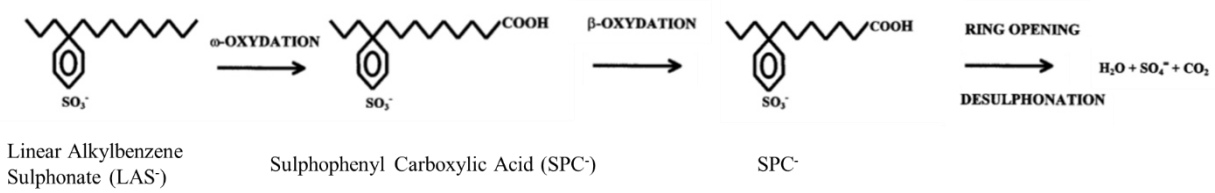
Regarding the influence of a chemical charge on the extractability and NER formation of organic chemicals, it can be concluded that a positive charge increases the binding affinityof a compound to sediment organic matter leading to a reduced extractability and increased NER formation compared to non-charged compounds. Furthermore, our results showed that also a negative charge has a positive impact on NER formation, albeit this impact is less pronounced compared to a positive charge.

5.1.3 Transformation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$

Using radio-TLC, transformation products with lower R_f values compared to ^{14}C-DS$^-$ (R_f 0.79) were found in the water samples, indicating that these metabolites were more polar than ^{14}C-DS$^-$. The easily bioavailable fraction obtained by $CaCl_2$ extraction contained only small amounts of the parent substance DS$^-$ (below 1 % AR) which indicates a reduced direct bioavailability of the substance for organisms living in the sediment. However, high $^{14}CO_2$ evolution implies that the test substance was easily bioavailable for microbial mineralisation. In the potentially bioavailable fraction (extraction using organic solvents), the parent substance and more polar metabolites were detected. For ^{14}C-DP, a rapid transformation was observed in the water phase. However, radio-TLC chromatograms contained high amounts of start activity. Radio-HPLC analyses revealed that this radioactivity was mainly composed of more polar transformation products. In the easily bioavailable fraction ($CaCl_2$), ^{14}C-DP was detected in low amounts (below 0.1 % AR). As mentioned for ^{14}C-DS$^-$, high mineralisation of ^{14}C-DP implies that this test substance was easily bioavailable for microorganisms in the test system. Only low amounts (1.8 % AR at day 7) of ^{14}C-DP were found in the potentially bioavailable fraction. ^{14}C-DA$^+$ and a polar transformation product were detected in all water phases using radio-TLC. No parent substance could be detected in the directly bioavailable fraction. In the potentially bioavailable fraction, ^{14}C-DA$^+$, four polar transformation products and a less polar metabolite were detected. Subsequent to the sequential solvent extraction, a 24 hour Soxhlet extraction with methanol was performed. The Soxhlet extracts contained ^{14}C-DA$^+$, two more polar and a less polar transformation product. When comparing the portions of ^{14}C-DA$^+$ in the different extracts, the highest amount of the parent substance was extracted under Soxhlet conditions (1.4 % AR at day 30). Thus, a direct bioavailability of ^{14}C-DA$^+$ for organisms in the sediment is unlikely.

A previous study showed that LAS$^-$ isomers were readily degradable when the terminal methyl group was positioned as far away from the sulphophenyl group as possible (Bayona *et al.*, 1986). This was the case for the molecule used in the present study. Perales *et al.* (1999) described the LAS$^-$ biodegradation pathway in river water as a three-stage process. Initially, LAS$^-$ are degraded into sulphophenyl carboxylic acid (SCP) by ω-oxidation. After shortening of the alkyl chain several times (β-oxidation), the sulphonate group is removed (desulphonation) and the aromatic ring is cleaved. Ultimate degradation products are water, carbon dioxide and sulphate (Figure 30).

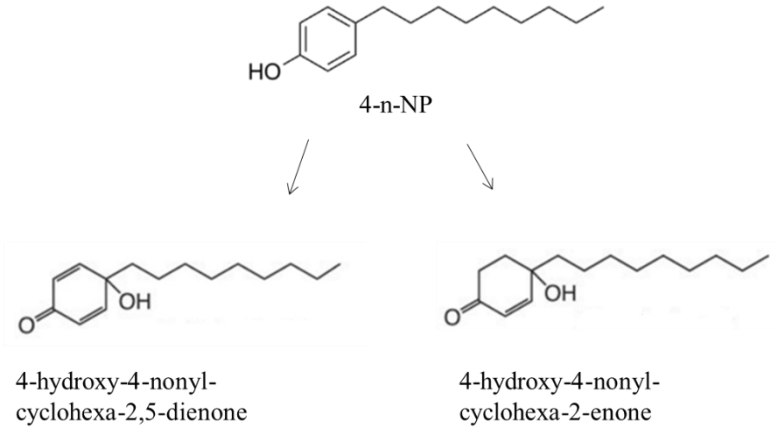
Figure 30: Biodegradation pathway of LAS$^-$.



Adapted from Perales et al. (1999).

Under aerobic conditions, NP was found to be biodegradable (Corti *et al.*, 1995; Hesselsøe *et al.*, 2001; Soares *et al.*, 2006). Ultimate degradation products are water and carbon dioxide (Ahel *et al.*, 1994). Gabriel *et al.* (2005) studied the degradation pathway of 4-*n*-NP and identified two more polar transformation products originating from 4-*n*-NP (Figure 31).

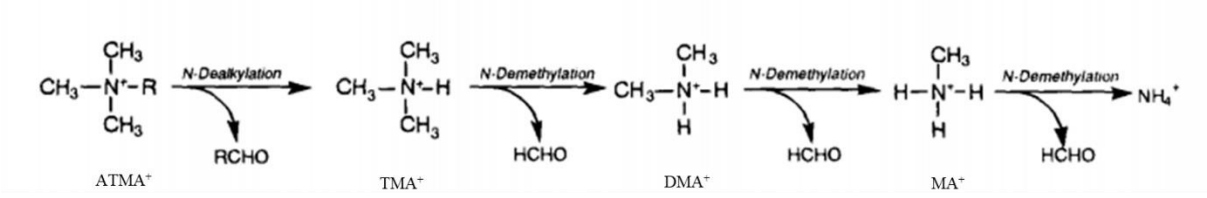
Figure 31: Degradation pathway of 4-n-NP by *Sphingomonas xenophaga* in minimal medium.



Adapted from Gabriel et al. (2005).

Besides biodegradation, sunlight photodegradation (photolysis) plays an important role in the degradation process of NP (Maguire, 1999; Neamţu and Frimmel, 2006; Liu *et al.*, 2014). Nishiyama *et al.* (1995) investigated the degradation pathway of alkyltrimethl ammonium salts (ATMA$^+$) in activated sludge. ATMA$^+$ were degraded to trimethylamine (TMA$^+$) by N-dealkylation. In a second and third step, TMA$^+$ was degraded to dimethylamine (DMA$^+$) and methylamine (MA$^+$) by N-demethylation which is rarely detected (Figure 32).

Figure 32: Possible degradation pathway of alkyltrimethylammonium salts (ATMA$^+$) by activated sludge.

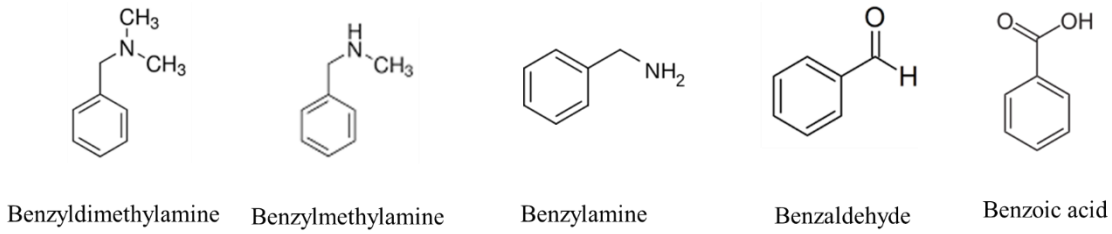


Adapted from Nishiyama et al. (1995).

This pathway was also observed for the degradation of dodecyltrimethyl ammonium bromide (DTAB$^+$) by two bacteria strains of *Psdeudomonas fluorescens* (Nishiyama and Nishihara, 2002).

In a study on the biodegradation of benzyldimethylalkylammonium chloride (BAC^+), Patrauchan and Oriel (2003) showed that during BAC^+ biodegradation, benzyldimethylamine, benzylmethylamine, benzylamine, benzaldehyde and benzoic acid were formed (Figure 33).

Figure 33: Possible transformation products of benzyldimethylalkylammonium chloride (BAC^+).



Benzyldimethylamine Benzylmethylamine Benzylamine Benzaldehyde Benzoic acid

Adapted from Patrauchan and Oriel (2003).

In the present study, we determined the direct bioavailability of organic chemicals based on their levels of mineralisation and portions in the $CaCl_2$ extract. A chemical carrying a positive charge shows a reduced bioavailability compared to uncharged chemicals, while a negative charge increases the bioavailability compared to no charge.

5.1.4 Degradation half-lives of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$

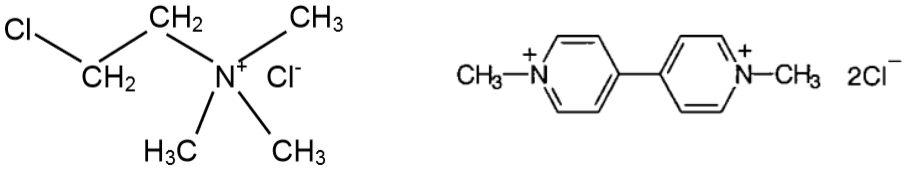
Degradation half-lives of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in the overall water-sediment system decreased as follows: ^{14}C-DA$^+$ (162 days) > ^{14}C-DS$^-$ (22 days) > ^{14}C-DP (14 days). This tendency could also be observed by Claßen (2019) where DT_{50} of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in soil was as follows: ^{14}C-DA$^+$ (62 days) > ^{14}C-DS$^-$ (18 days) > ^{14}C-DP (10 days). When the half-lives were calculated for the sediment ($DT_{50,sed}$) and water phase ($DT_{50,water}$) separately, large deviations could be observed for ^{14}C-DA$^+$ ($DT_{50,sed}$: 267 days, $DT_{50,water}$: 0.2 days), ^{14}C-DS$^-$ ($DT_{50,sed}$: 22 days, $DT_{50,water}$: 0.1 days) and ^{14}C-DP ($DT_{50,sed}$: 24 days, $DT_{50,water}$: 0.3 day) and

In a study on biodegradation of ^{14}C-LAS$^-$ in soil, degradation half-lives ranged from 2 to 20 days (Nielsen *et al.*, 1997). Holt *et al.* (1989) determined the fate of LAS$^-$ in sludge amended soils and observed rapid removal of LAS from the soils with calculated half-lives ranging from 7 to 22 days. Küchler and Schnaak (1997) reported LAS$^-$ half-lives between 3 days in laboratory studies and 7 days in field trial. Ward and Larson (1989) used ^{14}C-labelled LAS$^-$ homologs containing 10 to 14 carbon atoms in the alkyl chain to examine their biodegradation in sludge-amended agricultural soil. The calculated half-lives ranged between 18 and 26 days. For linear 4-NP, calculated half-life in soil was 1.4 days (Shan *et al.*, 2011). In a study on the degradation of 4-NP in soil following the addition of biosolids, DT_{50} values were between 12 and 25 days (Langdon *et al.*, 2011). Chang *et al.* (2007) investigated the biodegradation of NP in soil and reported degradation half-lives of NP between 5.6 and 12.3 days. Similar half-lives of NP in soil were calculated by Topp and Starratt (2000) with DT_{50} from 4.5 days to 16.7 days. In another study, DT_{50} of 4-*n*-NP in soil was found to be 4 days (Dubroca *et al.*, 2005). Games *et al.* (1982) reported that octadecyltrimethylammonium chloride ($OTAC^+$) was rapidly degraded in laboratory studies using activated sludge with half-lives of methyl ^{14}C-labelled and alkyl ^{14}C-labelled $OTAC^+$ of 28 hours and 40 hours, respectively. For the cation diethylesterdimethylammonium chloride, degradation half-life in sediment was 1.1 days (Giolando *et al.*, 1995). In their study, river water and sediment were taken downstream from a municipal sewage treatment plant. Short degradation half-lives of these $QACs^+$ can most likely be attributed to the adaption of microorganisms to degrade these compounds as was observed by Takenaka *et al.* (2007). The sediment used in the present study was taken from a stormwater retention pond without

sewage discharge. Thus, it can be considered as pristine sediment without adaption of microorganisms resulting in a higher degradation half-life of ^{14}C-DA$^+$. Higher half-lives of the cationic model substance can also be caused by its toxicity towards microorganisms, since QACs$^+$ are known to have toxic effects on aquatic organisms (Garcıa *et al.*, 2001; Roberts and Costello, 2003). However, Claßen (2019) showed that the microbial activity in soil was not inhibited by ^{14}C-DA$^+$ over an incubation time of 124 days.

High DT_{50} values could be found for other chemicals carrying a positive charge, for example the quaternary ammonium salt chlormequat chloride (CC$^+$, (Cycoń *et al.*, 2012) and paraquat (Amondham *et al.*, 2006) (Figure 34). For CC$^+$, a degradation half-life of 71 days for a silt loam soil was calculated. The half-life of paraquat in a tropical soil was 46 days.

Figure 34: Molecular structures of cationic chemicals. Left: Chlormequat chloride (CC$^+$), also known as chlorocholine chloride. Right: Paraquat



Chlormequat chloride (CC$^+$) Paraquat

Adapted from Cycoń et al. (2012) and Bromilow (2004).

Our results showed that half-lives are influenced by a chemical charge. A positive charge leads to an increase of DT_{50} values, while a negative charge leads to lower half-lives compared to no charge.

5.2 Distribution and fate of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water

In general, the results of the degradation studies in surface water at concentrations of 10 µg/L and 100 µg/L were similar to those obtained by the water-sediment study. High amounts of ^{14}C-DS$^-$ and ^{14}C-DP were mineralised, whereas the evolution of ^{14}C-DA$^+$ derived carbon dioxide was very low over the 60-day incubation period. Reduced degradation of the positively charged compound was also reflected by degradation half-lives (at 20 °C): DT_{50} of ^{14}C-DA$^+$ was 26 days (10 µg/L) and 13 days (100 µg/L), whereas DT_{50} of ^{14}C-DS$^-$ and ^{14}C-DP were much lower (< 2 days). At a test concentration of 10 µg/L, highest NER formation could be observed for ^{14}C-DP. At 100 µg/L, NER formation of ^{14}C-DA$^+$ was higher compared to ^{14}C-DS$^-$ and ^{14}C-DP during the first 14 days of incubation. Thereafter, highest amounts of NER were observed for ^{14}C-DP (21 % AR at day 60) followed by ^{14}C-DA$^+$ (14 % AR) and ^{14}C-DS$^-$ (9 % AR). Under sterile test conditions, mineralisation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ was below 0.1 % AR. Highest amounts of NER were observed for ^{14}C-DA$^+$ (6 % AR). For ^{14}C-DS$^-$ and ^{14}C-DP, NER formation was below 1 % AR. Radiochemical analyses of the water phases and sediment extracts showed that in some cases transformation products were detected besides the parent substances.

5.2.1 Ultimate degradation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water

At both test concentrations, ^{14}C-DS$^-$ and ^{14}C-DP were rapidly mineralised to large extents, whereas $^{14}CO_2$ evolution of ^{14}C-DA$^+$ was very low. Remarkably, in both versions of the surface water test, the mineralisation of all test substances after 60 days (62 days) of incubation was higher than the amount mineralised at the same incubation time in the water-sediment system.

Especially in the case of ^{14}C-DA$^+$, $^{14}CO_2$ evolution was observed being twice as high as in the water-sediment study. Generally low mineralisation rates of the cationic test substance ^{14}C-DA$^+$ can be explained by reduced bioavailability, since strong adsorption of QACs$^+$ to sediment particles leads to recalcitrance to biodegradation (Zhang *et al.*, 2015). Due to the addition of low amounts of suspended sediment in the surface water test, only a small portion of ^{14}C residues sorbed to the sediment particles on the bottom of the flask. The major portion of radioactivity was found in the water phase and became accessible for microbial degradation which was reflected by an increased mineralisation (7.5 % AR after 60 days compared to 3.4 % AR after the respective time in the water-sediment system). In general, mineralisation of all three test substances in the surface water test was higher compared to the water-sediment study although only 0.3 g sediment were added to the test flasks. It can be assumed, that higher mineralisation rates are a result of the increased contact zone between microorganisms on the surface of the suspended particles and the surrounding water.

When the test was performed under sterile conditions, $^{14}CO_2$ evolution was very low indicating that abiotic degradation took place only to a limited extent. Claßen (2019) stated that mineralisation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in gamma irradiated soil was negligible. In a study on the effects of a microbial community on the biodegradation of anionic, non-ionic and cationic surfactants in soil, Knaebel and Vestal (1992) reported that abiotic controls showed no mineralisation of LAS$^-$, dodecyl linear alcohol ethoxylate (DAE) and dodecyltrimethyl ammonium chloride (DTA$^+$). Mineralisation of ^{14}C-4-*n*-NP accounted for less than 0.5 % in sterilized soil (Shan *et al.*, 2011).

In surface water with suspended sediments, a negative charge has a positive impact on a chemical's ultimate degradation, whereas a positive charge results in a reduced degradability compared to no charge. However, levels of mineralisation of all test substances were slightly higher in the surface water test compared to the water-sediment system indicating that the addition of suspended sediments enhanced biodegradability.

5.2.2 Determination of extractable and non-extractable fractions of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$

The suspended sediment particles were extracted using methanol and acetonitrile prior to combustion for NER quantification. At both test concentrations, the highest amounts of radioactivity were extracted from the sediment incubated with ^{14}C-DA$^+$. However, NER formation of ^{14}C-DA$^+$ was not substantially higher compared to ^{14}C-DS$^-$ and ^{14}C-DP. At the end of the 60-day incubation period, highest NER formation was observed for ^{14}C-DP (21 % AR, test concentration 100 µg/L) followed by ^{14}C-DA$^+$ (14 % AR) and ^{14}C-DS$^-$ (9 % AR). These results are in accordance with Claßen (2019) who reported that highest NER formation was observed for the non-ionic test substance ^{14}C-DP, followed by ^{14}C-DA$^+$ and ^{14}C-DS$^-$. Although only very low amounts of sediment were used in the surface water test following OECD 309, notable amounts of NER were formed for all test substances. Increased NER formation in the suspended sediment test can be traced back to the enlarged surface of suspended particles which increased the sorption capacity of the sediment (Shrestha *et al.*, 2016). Under sterile conditions, NER formation decreased as follows: ^{14}C-DA$^+$ > ^{14}C-DP > ^{14}C-DS$^-$. These results are in accordance with Claßen (2019), who found that considerable amounts of NER of ^{14}C-DA$^+$ (16 % AR) were formed after 14 days of incubation in sterile soil. NER formation of ^{14}C-DP and ^{14}C-DS$^-$ accounted for 3 % and 1.5 %, respectively.

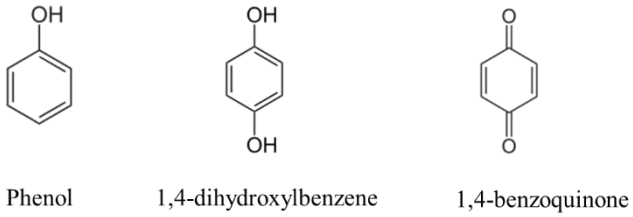
In absence of microorganisms, a positive charge enhances the formation of NER in surface waters, whereas under non-sterile conditions NER formation is more pronounced for uncharged compounds.

5.2.3 Transformation of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$

Using radio-TLC, only low amounts of the parent substances were detected in the water phases and sediment extracts. The major ^{14}C-fractions were found as more polar metabolites and a less polar transformation product. Using radio-HPLC, a major transformation product of ^{14}C-DS$^-$ was detected in both water phases and sediment extracts and coincides with the analytical results of the water-sediment study. Thus, degradation of ^{14}C-DS$^-$ in the surface water test follows the same pathway as in the water-sediment study. For ^{14}C-DP, the formation of several more polar metabolites within a short time of incubation was observed indicating the shortening of the alkyl chain by microbial degradation. Additionally, one less polar transformation product was detected in the sediment extract. During biodegradation of alkyl phenols (e.g. 4-NP), the formation of mainly polar metabolites and occasionally less polar transformation products could be observed (Bokern *et al.*, 1998; Shan *et al.*, 2010).

Under sterile conditions, transformation products of ^{14}C-DP were detected in the water phase and sediment extract. These metabolites may be the result of abiotic degradation of ^{14}C-DP as some of them could not be detected in the corresponding sample under non-sterile conditions. Abiotic degradation processes include hydrolysis, photolysis or oxidation/reduction (ECHA, 2017). As mentioned before, photochemical degradation (photolysis) of alkylphenols is an important abiotic degradation pathway. Neamţu and Frimmel (2006) investigated the photochemical degradation of NP in river water in presence and absence of natural organic matter and identified phenol, 1,4-dihydroxylbenzene and 1,4-benzoquinone as intermediate products (Figure 35).

Figure 35: Structures of intermediate products during photochemical degradation of nonylphenol.



In our study, the formation of a more polar and a less polar transformation product of ^{14}C-DP was observed in both the water phase and sediment extract which may include one of the above-mentioned abiotic degradation products. In sorption tests using ^{14}C-DP, the test substance was abiotically degraded with the formation of a more polar transformation product (Claßen, 2019).

For ^{14}C-DA$^+$, radiochemical analyses showed that the ^{14}C residues in the water phase consisted mainly of polar metabolites whereas in almost all sediment extracts only the parent substance was detected. These results are largely identical to those obtained in the water-sediment study and indicate the same degradation pathway in sediments and water bodies. Similar to the degradation pathway described by Nishiyama *et al.* (1995), Takenaka *et al.* (2007) identified five transformation products during the biodegradation of the cation n-dodecyltrimethyl ammonium chloride, among them n-dodecyldimethyl amine, trimethyl amine and dimethyl amine. Khan *et al.* (2015) investigated the degradation of ^{14}C-labelled dodecylbenzyldimethyl ammonium chloride (DTA$^+$) and identified benzylmethyl amine and dodecyldimethyl amine as transformation products.

5.2.4 Degradation half-lives of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$

Degradation half-lives of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water decreased as follows: ^{14}C-DA$^+$ (10 µg/L: 26 days and 100 µg/L: 13 days) > ^{14}C-DP (10 µg/L: 2 days and 100 µg/L: 1 day) > ^{14}C-DS$^-$ (10 µg/L: 1 day and 100 µg/L: 1 day). In contrast to the water-sediment study, DT_{50} of the neutral test substance ^{14}C-DP was slightly higher compared to the anionic compound ^{14}C-DS$^-$ which correlates positively with the increased formation of ^{14}C-DP derived NER in the surface water test. However, these results corroborate that a positive charge has a stronger impact on a chemical's half-life than a negative or no charge. In a simulation study with soil, similar short half-lives of ^{14}C-LAS$^-$ were reported by Knaebel *et al.* (1990) (1.1 – 3.6 days). For the investigation of the degradation of NP in seawater with the addition of small amounts of sediment, Ekelund *et al.* (1993) used a low concentration of NP similar to the one used in our study (11 µg/L) and found a half-life of 35 days. However, it should be noted that their study was performed at 11 °C instead of 20 °C in the present study. When the present half-lives of ^{14}C-DP were extrapolated to 12 °C, DT_{50} values were still lower (3.2 days at 10 µg/L and 9.6 days at 100 µg/L) than the half-life reported by Ekelund *et al.* (1993).

Although half-lives of ^{14}C-DA$^+$ in the surface water test were lower than in the sediment-water study, a positive charge seems to have a negative impact on the degradation of a chemical in the aquatic environment compared to no charge. In contrast, a negative charge enhances the degradation resulting in lower half-lives compared to no charge.

6 Recommendations for refinement of the P assessment of ionisable compounds

The assessment of the persistence (P) of a substance in the environment is based on its degradation half-life. According to annex XIII of the REACH regulation, substances with a degradation half-life of ≥ than 40 days in fresh water, ≥ than 120 days in sediment or ≥ than 120 days in soil fulfil the P criterion and are considered as being persistent in the environment. A substance fulfils the criterion of being very persistent (vP) when its degradation half-life is ≥ than 60 days in fresh water or ≥ than 180 days in soil or sediment. According to REACH annex XIII, however, a substance is regarded as P if the persistence criterion in a single environmental compartment is fulfilled. For the model substances used in this project (DS^-, DP and DA^+), half-lives for both the sediment and water column separately as well as for the overall water-sediment system were determined and compared to the half-lives in soil following OECD guideline 307 (*Aerobic and Anaerobic Transformation in Soil*) (Table 22).

Table 22: DT_{50} of ^{14}C-DP, ^{14}C-DS^- and ^{14}C-DA^+ in soil (OECD 307), in a water-sediment system (OECD 308, divided into sediment, water column and the overall system) and in surface water with suspended sediments (OECD 309) at two different test concentrations (10 µg/L and 100 µg/L) after extrapolation to 12 °C.

Test substance	soil (OECD 307)*	sediment (OECD 308)	water column (OECD 308)	overall system (OECD 308)	surface water 10 µg/L (OECD 309)	surface water 100 µg/L (OECD 309)
DS^-	21 d	47 d	< 1 d	47 d	3 d	2 d
DP	39 d	51 d	< 1 d	30 d	3 d	3 d
DA^+	132 d	570 d	< 1 d	346 d	56 d	45 d

* data originate from Claßen (2019)

In the water-sediment system, a comparison between half-lives of the model substances determined for sediment, water and the entire system showed that the DT_{50} for the water column was lowest for all three substances. Our results indicate that the degradation of a substance is overestimated in case only its DT_{50} in the water column is considered. Consequently, this substance would not fulfil the P criterion under REACH. Low half-lives in water are, however, caused by dissipation due to partitioning to the sediment rather than by degradation of a substance. For ^{14}C-DP and ^{14}C-DA^+, the half-lives determined for the sediment (51 days and 570 days, respectively) were higher compared to the half-lives calculated for the entire system (30 days and 346 days, respectively). Since the water-sediment system of OECD 308 does not allow a clear differentiation between the compartments sediment and water, half-lives for the entire system are more reliable.

Based on our findings, we therefore recommend using the degradation half-life of the entire system of OECD 308 as an indicator for persistence of both ionic and non-ionic organic chemicals. A significant difference between $DT_{50,sed}$ and $DT_{50,water}$ was observed for the cationic test substance ^{14}C-DA^+. This difference can be traced back to the increased sorption of ^{14}C-DA^+ to the negatively charged surfaces of the sediment.

The OECD guideline 309 aims at the assessment of biodegradation of a substance in aerobic surface water. This guideline provides scope for the experimental setup and test concentrations. The test can be performed by using surface water only ("pelagic test") or as a "suspended

sediment test" with the addition of sediment (0.01 to 1 g/L) in order to simulate a water body with resuspended sediment particles. The use of at least two different test concentrations differing by a factor of 5 to 10 with a maximum concentration of 100 µg/L is recommended. During incubation, sampling can be performed by withdrawal of sub-samples from each replicate or by harvest of whole flasks at each sampling time.

In this project, we performed a preliminary surface water test in both versions (pelagic and suspended sediment) using two different test concentrations of ^{14}C-DP and ^{14}C-DS$^-$ (10 µg/L and 100 µg/L). At each sampling time, sub-samples were withdrawn (10 mL aliquots). At the end of the incubation period, the whole flasks were harvested. Using this sampling technique, the recovery of ^{14}C-DP was not sufficient in both the pelagic and suspended sediment test (up to 79 % after 28 days of incubation). The highest recoveries were obtained when the whole flasks were harvested at the end of the 60-day incubation period (up to 102 %). For ^{14}C-DS$^-$, similar results were obtained with recoveries in both test systems up to 77 % after 28 days of incubation. The highest recoveries were obtained when the whole flasks were harvested at the end of the 60-day incubation period (up to 85 %). Thus, the preliminary tests did not fulfil the validity criteria proposed by the guideline (90-110 %). We conclude that the withdrawal of sub-samples instead of harvesting the whole flask does not provide a reliable method in order to obtain valid results for the assessment of biodegradation in surface water. Therefore, the other sampling option recommended by OECD 309 was chosen for the main experiments resulting in better recoveries. For the anionic test substance ^{14}C-DS$^-$, no significant difference regarding the mineralisation was observed between both test versions and concentrations. For ^{14}C-DP, a considerably lower mineralisation was only observed in the pelagic test at a low concentration. In the suspended sediment test, the amount of NER could only be determined at the end of the 60-day incubation period when whole flasks were harvested, and the sediment particles were extracted and combusted. NER formation was very low with ^{14}C-DP forming slightly higher amounts of NER than ^{14}C-DS$^-$.

The pelagic version of the surface water test is recommended for the P assessment under REACH, since the addition of sediment in a suspended sediment test can enhance the biodegradation of a substance and the presence of sediment particles increases the potential for NER formation (ECHA, 2017). For ^{14}C-DS$^-$, mineralisation accounted for up to 51 % in the pelagic test and up to 56 % in the suspended sediment test. For ^{14}C-DP, mineralisation was up to 56 % in the pelagic test and up to 60 % in the suspended sediment test. Thus, we substantiate the assumption regarding the enhanced biodegradation due to the addition of sediment, albeit biodegradation was only marginally higher when suspended sediment was added.

In the suspended sediment test, the amount of NER could only be determined at the end of the 60-day incubation period. NER formation was very low with ^{14}C-DP forming slightly higher amounts of NER than ^{14}C-DS$^-$. Despite the addition of low amounts of sediment (1 g/L) in the main experiments performed as suspended sediment test, moderate NER formation of the model substances could be observed. In the water-sediment system, amounts of NER accounted for 26 % AR (^{14}C-DS$^-$), 16 % AR (^{14}C-DP) and 38 % AR (^{14}C-DA$^+$) after 60 days of incubation. In the surface water test with suspended sediment, NER formation accounted for 7 % AR for ^{14}C-DS$^-$, 9 % AR for ^{14}C-DP and 9% ^{14}C-DA$^+$ after 62 days of incubation (10 µg/L) and 9 % AR (^{14}C-DS$^-$), 21 % AR (^{14}C-DP) and 14 % AR (^{14}C-DA$^+$) after 60 days of incubation (100 µg/L). These results substantiate the aforementioned assumptions of potential NER formation in suspended sediment tests. This applies in particular for the non-ionic test substance ^{14}C-DP, as its formation of NER was higher in the suspended sediment test (100 µg/L) compared to the water-sediment study. In contrast, NER formation of the ionic test substances was less pronounced in the suspended sediment test compared to the water-sediment system.

Moreover, we also showed that the choice of the test concentration has no major impact on the biodegradability of the model substances. At a high concentration, for example, the cationic test substance ^{14}C-DA$^+$ has a DT_{50} of 45 days and thus fulfils the P criterion. At a lower concentration, ^{14}C-DA$^+$ also fulfils the P-criterion with a half-life of 56 days. In contrast, half-lives of the non-ionic and anionic test substances were almost the same at both test concentrations. It is therefore recommended to perform the surface water test following OECD 309 using lower test concentrations (1 µg/L to 10 µg/L) instead of 100 µg/L since these concentrations seem to better reflect biodegradation in the environment. The suspended sediment test (OECD 309) closely resembles the water-sediment system of OECD 308 in terms of the obtained results as was observed by Shrestha *et al.* (2016). With regard to the choice of the test design (pelagic test or suspended sediment test), we therefore propose the use of the pelagic test since its degradation capacity was nearly identical compared to the suspended sediment test. Additionally, the pelagic test should be preferred since only low amounts of NER were formed in this test design.

The determination of NER is recommended in simulation studies following OCD 309 (ECHA, 2017) with an amount of suspended matter of 15 mg/L in order to represent the level of suspended solids in EU surface waters (European Communities, 2011). According to ECHA (2017), the test water in the pelagic test contains also suspended matter onto which the test substances can adsorb and form NER. However, quantification and characterisation of these NER with regard to sequestered, covalently bound and biogenic NER is difficult due to very low amounts of suspended matter in the pelagic test and suspended sediment in the suspended sediment test.

When the half-lives of ^{14}C-DP, ^{14}C-DS$^-$ and ^{14}C-DA$^+$ in soil following OECD guideline 307 were determined, the lowest DT_{50} was calculated for ^{14}C-DP (21 days) followed by ^{14}C-DS$^-$ (39 days) and ^{14}C-DA$^+$ (132 days). In the simulation studies following OECD 308 and 309, the lowest half-life was determined for ^{14}C-DP (OECD 308: 14 days) and ^{14}C-DS$^-$ (OECD 309: 1 day. These findings suggest that not only for ionic substances a refinement is required, but also for neutral organic chemicals. This refinement should include the testing of different environmental compartments for the P assessment, since the outcome strongly depends on the simulation study. Furthermore, the OECD guideline 309 leaves too much experimental freedom which also affects the outcome of the study as reported by Shrestha *et al.* (2016). Thus, this guideline requires further refinement for the P assessment of ionic and non-ionic organic compounds.

7 Conclusion

The present study aimed at investigating the influence of a chemical charge on the degradation of organic chemicals in the aquatic environment in order to refine the P assessment under REACH.

Using three differently charged model substances with high structural similarity, simulation studies following OECD guidelines 308 and 309 were performed. Based on the obtained data, we showed that a positive charge has a negative impact on the ultimate degradation of an organic chemical, whereas a negative charge facilitates it. The binding affinity of a compound to the sediment matrix increases when the compound carries a positive charge leading to a reduced extractability compared to uncharged and negatively charged compound. Furthermore, a positive charge leads to reduced bioavailability for organisms living in the sediment compared to uncharged chemicals, while a negative charge increases the bioavailability compared to no charge. In OECD 308, NER formation was enhanced by a positive charge. A negative charge has a positive impact on NER formation, albeit this impact is less pronounced compared to the positive charge. In OECD 309, NER formation is more pronounced for uncharged compounds. In both simulation studies, a positive charge leads to considerably higher half-lives, while a negative charge leads to lower half-lives compared to no charge. In OECD 308, however, the half-lives calculated for the sediment phase and the water phase separately showed significant deviations from the half-lives calculated for the entire test system as was especially the case for the positively charged test substance.

With respect to the refinement of the P assessment, we recommend using the degradation half-life in the entire system of OECD 308 as an indicator for persistence of both ionic and non-ionic organic chemicals. Based on the comparison of the half-lives of the model substances in the water-sediment system with their half-lives in soil (OECD 307), we suggest that simulation studies for all three environmental compartments (soil, sediment and surface water) should be performed in order to generate reliable data on the degradation of ionic substances.

The surface water test following OECD 309 can either be performed by using surface water only (“pelagic test”) or as a “suspended sediment test” with the addition of sediment in order to simulate a water body with resuspended sediment particles. According to REACH, the pelagic version of the surface water test is recommended for the P assessment. Based on our findings, we suggest performing the pelagic test since its degradation capacity was nearly identical compared to the suspended sediment test and this test design minimises potential NER formation.

8 List of references

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A Appendix

A.1 Distribution of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in the water-sediment system

Table 23: Distribution of the applied radioactivity (in %) of ^{14}C-DS$^-$ in the water-sediment system. The radioactivity in the water phase, extractable residues (ER), non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 120-day incubation time are shown. Data points represent mean values of triplicates with standard deviation.

day	water phase	ER	NER	$^{14}CO_2$	recovery
1	95.95 ± 6.38	12.64 ± 3.62	0.05 ± 0.01	0.22 ± 0.05	108.86 ± 8.77
7	54.65 ± 3.36	15.09 ± 1.52	17.47 ± 3.87	16.53 ± 0.96	103.74 ± 1.97
14	30.78 ± 4.97	13.81 ± 0.97	30.72 ± 4.9	17.17 ± 0.1	92.48 ± 1.34
30	16.52 ± 5.74	8.35 ± 1.63	31.31 ± 5.45	34.06 ± 0.81	91.07 ± 6.69
60	1.32 ± 0.45	1.69 ± 0.30	26.01 ± 3.39	59.56 ± 4.65	88.58 ± 6.88
120	0.97 ± 0.28	1.12 ± 0.09	19.11 ± 0.11	67.84 ± 1.04	89.23 ± 0.6

Table 24: Distribution of the applied radioactivity (in %) of ^{14}C-DP in the water-sediment system. The radioactivity in the water phase, extractable residues (ER), non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 120-day incubation time are shown. Data points represent mean values of triplicates with standard deviation.

day	water phase	ER	NER	$^{14}CO_2$	recovery
1	84.57 ± 5.14	15.03 ± 3.46	2.13 ± 0.59	0.09 ± 0.02	101.83 ± 2.52
7	33.26 ± 2.40	29.80 ± 5.76	18.12 ± 2.64	12.94 ± 1.52	94.11 ± 2.98
14	13.48 ± 1.46	18.66 ± 2.13	26.07 ± 2.68	27.77 ± 1.13	85.98 ± 1.84
30	8.02 ± 0.75	7.84 ± 3.79	28.90 ± 9.51	51.72 ± 4.72	96.48 ± 8.10
60	3.91 ± 0.27	4.64 ± 1.50	15.75 ± 0.45	55.07 ± 2.62	79.37 ± 1.45
120	1.6 ± 0.06	1.49 ± 0.32	14.07 ± 1.35	62.54 ± 3.79	79.69 ± 3.29

Table 25: Distribution of the applied radioactivity (in %) of ^{14}C-DA$^+$ in the water-sediment system. The radioactivity in the water phase, extractable residues (ER), non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 120-day incubation time are shown. Data points represent mean values of triplicates with standard deviation.

day	water phase	ER	NER	$^{14}CO_2$	recovery
1	49.40 ± 2.54	15.92 ± 2.03	23.48 ± 1.60	0.65 ± 0.01	89.45 ± 0.92
7	31.11 ± 5.04	17.27 ± 3.09	32.57 ± 5.66	0.94 ± 0.06	81.90 ± 1.73
14	28.08 ± 6.3	21.04 ± 6.44	30.57 ± 3.92	1.57 ± 0.32	81.26 ± 1.21

day	water phase	ER	NER	$^{14}CO_2$	recovery
30	23.96 ± 2.45	23.86 ± 3.39	31.53 ± 2.67	2.62 ± 0.19	81.97 ± 2.5
60	19.03 ± 2.33	22.15 ± 2.00	38.24 ± 1.94	3.36 ± 0.26	82.78 ± 2.95
120	17.54 ± 0.21	19.28 ± 7.02	33.48 ± 1.94	6.04 ± 0	76.34 ± 7.02

A.2 Distribution of ^{14}C-DS^-, ^{14}C-DP and ^{14}C-DA^+ within the extractable residue fraction of the sediment

Table 26: Distribution of the applied radioactivity (in %) of ^{14}C-DS^- between the different sediment extracts over the 120-day incubation time. The extraction was performed using aqueous calcium chloride solution (0.01 mol/L), methanol, and acetonitrile successively. For ^{14}C-DA^+, a Soxhlet extraction using methanol was performed afterwards. Data points represent mean values of triplicates with standard deviation.

day	calcium chloride	methanol	acetonitrile
1	8.54 ± 2.58	4.11 ± 1.04	0.51 ± 0.05
7	12.58 ± 1.14	2.51 ± 0.39	0.54 ± 0.04
14	11.30 ± 0.72	2.02 ± 0.20	0.48 ± 0.06
30	5.46 ± 1.01	1.53 ± 0.32	0.45 ± 0.06
60	0.90 ± 0.20	0.45 ± 0.04	0.18 ± 0.02
120	0.50 ± 0.07	0.13 ± 0.00	0.49 ± 0.01

Table 27: Distribution of the applied radioactivity (in %) of ^{14}C-DP between the different sediment extracts over the 120-day incubation time. The extraction was performed using aqueous calcium chloride solution (0.01 mol/L), methanol, and acetonitrile successively. Data points represent mean values of triplicates with standard deviation.

day	calcium chloride	methanol	acetonitrile
1	2.63 ± 0.59	9.19 ± 1.91	3.22 ± 0.96
7	8.74 ± 0.34	15.36 ± 3.84	5.70 ± 1.58
14	3.91 ± 0.64	10.90 ± 0.08	3.85 ± 1.41
30	1.67 ± 0.24	4.44 ± 2.45	2.06 ± 1.11
60	0.93 ± 0.12	2.09 ± 0.21	1.62 ± 1.17
120	0.38 ± 0.03	0.28 ± 0.06	0.83 ± 0.22

Table 28: Distribution of the applied radioactivity (in %) of ^{14}C-DA$^+$ between the different sediment extracts over the 120-day incubation time. The extraction was performed using aqueous calcium chloride solution (0.01 mol/L), methanol, and acetonitrile successively. A Soxhlet extraction using methanol was performed afterwards. Data points represent mean values of triplicates with standard deviation.

day	calcium chloride	methanol	acetonitrile	Soxhlet methanol
1	2.56 ± 0.11	0.88 ± 0.15	3.15 ± 0.43	9.33 ± 1.34
7	2.97 ± 0.76	0.52 ± 0.08	4.86 ± 0.98	8.76 ± 1.27
14	5.38 ± 1.24	0.66 ± 0.15	2.91 ± 0.83	11.66 ± 4.19
30	8.03 ± 0.37	1.42 ± 0.21	2.12 ± 0.35	12.29 ± 2.47
60	7.31 ± 0.19	0.70 ± 0.07	3.89 ± 0.59	10.26 ± 1.15
120	7.27 ± 0.19	0.84 ± 0.17	3.44 ± 0.69	7.72 ± 1.02

A.3 Radio-TLC analysis of the water phases, calcium chloride extracts, methanol extracts and acetonitrile extracts (and Soxhlet extracts) of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ over the 120-day incubation period in the water-sediment system

Table 29: Radio-TLC analysis of water phases (WP) of ^{14}C-DS$^-$ in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS$^-$ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

WP	^{14}C-DS$^-$ (0.797)	M1 (0.113)	M2 (0.226)	M3 (0.305)	M4 (0.377)	M5 (0.479)	M6 (0.550)	M7 (0.640)	M8 (0.679)	M9 (0.805)	SA
day 1	-	-	-	-	-	-	-	92.55	-	-	3.4
day 7	-	-	-	-	-	-	-	51.77	-	1.90	-
day 14	-	-	-	2.24	-	-	26.67	-	-	-	2.63
day 30	-	-	-	-	5.27	-	-	11.50	-	-	≤ 1.00
day 60	≤ 1.00	-	≤ 1.00	-	-	-	-	≤ 1.00	-	-	≤ 1.00
day 120	-	≤ 1.00	-	≤ 1.00	-	-	-	≤ 1.00	-	-	≤ 1.00

Table 30: Radio-TLC analysis of the, calcium chloride ($CaCl_2$) extracts of ^{14}C-DS^- in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS^- as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

$CaCl_2$	^{14}C-DS^- (0.797)	M1 (0.113)	M2 (0.226)	M3 (0.305)	M4 (0.377)	M5 (0.479)	M6 (0.550)	M7 (0.640)	M8 (0.679)	M9 (0.805)	SA
day 1	2.93	-	-	-	-	1.17	-	3.16	4.22	-	≤ 1.00
day 7	2.15	-	-	-	-	-	3.97	-	-	6.12	≤ 1.00
day 14	-	-	-	1.5	-	2.48	5.66	-	-	-	≤ 1.00
day 30	≤ 1.00	-	-	-	-	2.20	2.01	-	-	-	≤ 1.00
day 60	-	≤ 1.00	≤ 1.00	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00	-	≤ 1.00
day 120	-	≤ 1.00	≤ 1.00			≤ 1.00	-	-	≤ 1.00	-	≤ 1.00

Table 31: Radio-TLC analysis of the methanol (MeOH) extracts of ^{14}C-DS^- in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS^- as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

MeOH	^{14}C-DS^- (0.797)	M1 (0.113)	M2 (0.226)	M3 (0.305)	M4 (0.377)	M5 (0.479)	M6 (0.550)	M7 (0.640)	M8 (0.679)	M9 (0.805)	SA
day 1	2.43	-	-	-	-	-	-	1.40	-	-	≤ 1.00
day 7	≤ 1.00	-	-	-	-	-	-	-	1.75	0.15	≤ 1.00
day 14	≤ 1.00	-	-	-	≤ 1.00	-	-	≤ 1.00	1.26	0.26	≤ 1.00
day 30	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00	≤ 1.00	-	-	≤ 1.00
day 60	-	-	≤ 1.00	-	-	≤ 1.00	≤ 1.00	-	≤ 1.00	≤ 1.00	≤ 1.00
day 120	-	≤ 1.00	-	-	-	≤ 1.00	-	-	≤ 1.00		≤ 1.00

Table 32: Radio-TLC analysis of the acetonitrile (ACN) extracts of ^{14}C-DS$^-$ in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS$^-$ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

ACN	^{14}C-DS$^-$ (0.797)	M1 (0.113)	M2 (0.226)	M3 (0.305)	M4 (0.377)	M5 (0.479)	M6 (0.550)	M7 (0.640)	M8 (0.679)	M9 (0.805)	SA
day 30	-	-	-	-	-	-	-	-	-	≤ 1.00	≤ 1.00
day 60	-	-	-	-	-	-	-	-	-	≤ 1.00	≤ 1.00

Table 33: Radio-TLC analysis of the water phases (WP) of ^{14}C-DP in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

WP	^{14}C-DP (0.520)	M1 (0.118)	M2 (0.169)	M3 (0.221)	M4 (0. 305)	M5 (0.433)	M7 (0.650)	M8 (0.679)	M9 (0.805)	SA
day 1	14.48	13.75	25.38	-	11.84	-	11.00	-	-	9.31
day 7	-	25.62	-	-	-	-	-	-	-	7.38
day 14	≤ 1.00	≤ 1.00	-	-	-	-	-	-	-	12.69
day 30	-	-	-	-	-	-	-	-	-	8.02
day 60	-	-	-	-	-	-	-	-	-	3.91
day 120	≤ 1.00	-	-	-	-	-	-	-	-	1.51

Table 34: Radio-TLC analysis of the calcium chloride ($CaCl_2$) extracts of ^{14}C-DP in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

$CaCl_2$	^{14}C-DP (0.520)	M1 (0.118)	M2 (0.169)	M3 (0.221)	M4 (0. 305)	M5 (0.433)	M7 (0.650)	M8 (0.679)	M9 (0.805)	SA
day 1	≤ 1.00	≤ 1.00	≤ 1.00	-	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00
day 7	≤ 1.00	-	5.76	-	-	-	-	-	-	2.92
day 14	≤ 1.00	-	1.49	1.07	-	-	-	-	-	1.19
day 30	≤ 1.00	≤ 1.00	≤ 1.00	-	-	-	-	-	-	≤ 1.00
day 60	≤ 1.00	≤ 1.00	-	≤ 1.00	-	-	-	-	-	≤ 1.00
day 120	≤ 1.00	-	-	-	-	-	-	-	-	≤ 1.00

Table 35: Radio-TLC analysis of the methanol (MeOH) extracts of ^{14}C-DP in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

MeOH	^{14}C-DP (0.520)	M1 (0.118)	M2 (0.169)	M3 (0.221)	M4 (0.305)	M5 (0.433)	M7 (0.650)	M8 (0.679)	M9 (0.805)	SA
day 1	8.92	-	-	-	-	-	-	-	-	≤ 1.00
day 7	11.78	-	2.29	-	-	-	-	-	-	≤ 1.00
day 14	8.90	-	≤ 1.00	-	-	-	-	-	-	1.19
day 30	2.70	-	-	-	-	-	-	≤ 1.00	-	≤ 1.00
day 60	≤ 1.00	-	-	≤ 1.00	-	≤ 1.00	-	≤ 1.00	-	≤ 1.00
day 120	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00	-	-	-	≤ 1.00

Table 36: Radio-TLC analysis of the acetonitrile (ACN) extracts of ^{14}C-DP in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

ACN	^{14}C-DP (0.520)	M1 (0.118)	M2 (0.169)	M3 (0.221)	M4 (0. 305)	M5 (0.433)	M7 (0.650)	M8 (0.679)	M9 (0.805)	SA
day 1	3.11	-	-	-	-	-	-	-	-	≤ 1.00
day 7	5.42	-	-	-	-		-	-	-	≤ 1.00
day 14	3.37	-	-	≤ 1.00	-	≤ 1.00	-	-	-	≤ 1.00
day 30	≤ 1.00	-	-	-	-	-	-	≤ 1.00	-	≤ 1.00
day 60	≤ 1.00	-	-	≤ 1.00	-	≤ 1.00	-	≤ 1.00	-	≤ 1.00
day 120	≤ 1.00	-	-	≤ 1.00	-	≤ 1.00	≤ 1.00	-	-	≤ 1.00

Table 37: Radio-TLC analysis of the water phases (WP) of ^{14}C-DA^{+} in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA+ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

WP	^{14}C-DA^{+} (0.550)	M1 (0.113)	M2 (0.169)	M3 (0.256)	M4 (0.367)	M5 (0.474)	M6 (0.690)	SA
day 1	4.94	24.74	-	18.60	-	-	-	2.41
day 7	1.25	12.12	-	14.94	≤ 1.00	-	-	≤ 1.00
day 14	≤ 1.00	26.25	-	≤ 1.00	-	-	-	≤ 1.00
day 30	≤ 1.00	21.08	-	-	-	-	-	1.67
day 60	≤ 1.00	17.87	-	-	-	-	-	≤ 1.00
day 120	≤ 1.00	16.48	-	-	-	-	-	≤ 1.00

Table 38: Radio-TLC analysis of the calcium chloride ($CaCl_2$) extracts of ^{14}C-DA^+ in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA^+ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

$CaCl_2$	^{14}C-DA^+ (0.550)	M1 (0.113)	M2 (0.169)	M3 (0.256)	M4 (0.367)	M5 (0.474)	M6 (0.690)	SA
day 1	-	2.38	-	-	-	-	-	≤ 1.00
day 7	-	1.74	-	1.13	-	≤ 1.00	-	≤ 1.00
day 14	-	5.02	-	-	-	≤ 1.00	-	≤ 1.00
day 30	-	7.60	-	-	-	-	-	≤ 1.00
day 60	-	6.98	-	-		-	-	≤ 1.00
day 120	-	5.78	-	-	≤ 1.00	≤ 1.00	-	≤ 1.00

Table 39: Radio-TLC analysis of the methanol (MeOH) extracts of ^{14}C-DA^+ in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA^+ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

MeOH	^{14}C-DA^+ (0.550)	M1 (0.113)	M2 (0.169)	M3 (0.256)	M4 (0.367)	M5 (0.474)	M6 (0.690)	SA
day 1	≤ 1.00	-	-	-	-	-	-	≤ 1.00
day 7	≤ 1.00	≤ 1.00	-	-	≤ 1.00	-	-	≤ 1.00
day 14	-	≤ 1.00	-	-	≤ 1.00	-	-	≤ 1.00
day 30	≤ 1.00	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00
day 60	≤ 1.00	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00
day 120	≤ 1.00	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00

Table 40: Radio-TLC analysis of the acetonitrile (ACN) extracts of ^{14}C-DA^+ in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA^+ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

ACN	^{14}C-DA^+ (0.550)	M1 (0.113)	M2 (0.169)	M3 (0.256)	M4 (0.367)	M5 (0.474)	M6 (0.690)	SA
day 1	≤ 0.1	-	-	-	-	-	-	≤ 0.1

ACN	^{14}C-DA^{+} (0.550)	M1 (0.113)	M2 (0.169)	M3 (0.256)	M4 (0.367)	M5 (0.474)	M6 (0.690)	SA
day 7	4.51	-	-	-	-	-	≤ 1.00	≤ 1.00
day 14	2.54	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00
day 30	1.12	-	-	≤ 1.00	-	≤ 1.00	-	≤ 1.00
day 60	2.89	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00
day 120	1.93	-	-	-	-	1.51	-	-

Table 41: Radio-TLC analysis of the Soxhlet methanol extracts of ^{14}C-DA^{+} in the water-sediment system after an incubation time of 1, 7, 14, 30, 60 and 120 days. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA^{+} as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

Soxhlet MeOH	^{14}C-DA^{+} (0.550)	M1 (0.113)	M2 (0.169)	M3 (0.256)	M4 (0.367)	M5 (0.474)	M6 (0.690)	SA
day 1	-	4.50	-	-	3.75	-	-	≤ 1.00
day 7	8.68	-	-	-	-	-	-	≤ 1.00
day 14	9.69	-	-	-	-	2.83	-	≤ 1.00
day 30	11.71	-	-	-	-	-	≤ 1.00	≤ 1.00
day 60	8.67	-	-	-	-	1.27	-	≤ 1.00
day 120	5.82	-	-	-	-	1.68	≤ 1.00	≤ 1.00

A.4 Radio-HPLC analysis of selected samples of water phases, calcium chloride extracts, methanol extracts and acetonitrile extracts (and Soxhlet extracts) of ^{14}C-DS^{-}, ^{14}C-DP and ^{14}C-DA^{+} over the 120-day incubation period in the water-sediment system

Table 42: : Radio-HPLC analysis of selected samples of water phases (WP), calcium chloride ($CaCl_2$), methanol (MeOH) and acetonitrile (ACN) extracts of ^{14}C-DS^{-} in the water-sediment system. The portions of the parent substance and metabolites are shown as % AR Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS- as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DS^{-} (14.34)	M1 (3.03)	M2 (3.14)	M3 (4.59)	M4 (5.31)	M5 (20.46)	M6 (21.42)	M7 (22.55)	M8 (23.43)
WP day 1	1.30	94.65	-	-	-	-	-	-	-
CaCl2 day 1	-	-	7.82	≤ 1.00	-	-	-	-	-
MeOH	3.10	1.01	-	-	-	-	-	-	-

sample	^{14}C-DS^{-} (14.34)	M1 (3.03)	M2 (3.14)	M3 (4.59)	M4 (5.31)	M5 (20.46)	M6 (21.42)	M7 (22.55)	M8 (23.43)
day 1									
WP day 7	-	54.65	-	-	-	-	-	-	-
MeOH day 7	≤ 1.00	1.84	-	-	-	-	-	-	-
$CaCl_2$ day 14	-	-	10.05	-	1.25	-	-	-	-
$CaCl_2$ day 30	-	2.16	2.38	≤ 1.00	-	-	-	-	-
MeOH day 30	≤ 1.00	≤ 1.00	-	≤ 1.00	-	≤ 1.00	≤ 1.00	≤ 1.00	≤ 1.00
ACN day 30	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00	-	≤ 1.00	-
WP day 60	-	1.11	-	≤ 1.00	-	-	-	-	-
WP day 120	-	≤ 1.00	-	≤ 1.00	-	-	-	-	-
MeOH day 120	≤ 1.00	≤ 1.00	-	-	-	≤ 1.00	-	-	-

Table 43: Radio-HPLC analysis of selected samples of water phases (WP), calcium chloride (CaCl2), methanol (MeOH) and acetonitrile (ACN) extracts of ^{14}C-DP in the water-sediment system. Retention times are shown in brackets. The portions of the parent substance and metabolites are shown as % AR The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DP (18.41)	M1 (3.30)	M2 (5.13)	M3 (6.39)	M4 (7.35)	M5 (9.14)	M6 (10.43)	M7 (11.15)	M8 (12.20)	M9 (20.19)
WP day 1	49.81	1.29	5.36	3.43	22.67	-	-	2.00	-	-
ACN day 1	3.22	-	-	-	-	-	-	-	-	-
WP day 7	≤ 1.00	2.52	-	1.92	-	≤ 1.00	-	18.37	9.59	-
MeOH day 7	12.16	≤ 1.00	-	-	-	-	-	1.52	≤ 1.00	≤ 1.00
WP day 14	≤ 1.00	3.40	≤ 1.00	2.12	-	1.51	1.93	≤ 1.00	1.43	-
CaCl2 day 14	≤ 1.00	≤ 1.00	≤ 1.00	≤ 1.00	-	-	1.60	-	1.35	-

sample	^{14}C-DP (18.41)	M1 (3.30)	M2 (5.13)	M3 (6.39)	M4 (7.35)	M5 (9.14)	M6 (10.43)	M7 (11.15)	M8 (12.20)	M9 (20.19)
WP day 30	-	6.16	≤ 1.00	-	-	-	-	-	-	-
ACN day 30	≤ 1.00	≤ 1.00	≤ 1.00	-	-	-	≤ 1.00	-	-	≤ 1.00
WP day 60	≤ 1.00	1.56	1.14	-	-	-	-	-	-	-
WP day 120	≤ 1.00	≤ 1.00	≤ 1.00	-	-	-	-	≤ 1.00	≤ 1.00	≤ 1.00
$CaCl_2$ day 120	≤ 1.00	≤ 1.00	≤ 1.00	-	-	-	-	-	-	-

Figure 36: Radio-TLC and radio-HPLC analysis of the sample 'DP water phase day 60'. The start activity (SA) was separated into DP and several more polar metabolites when the sample was injected into the HPLC system.

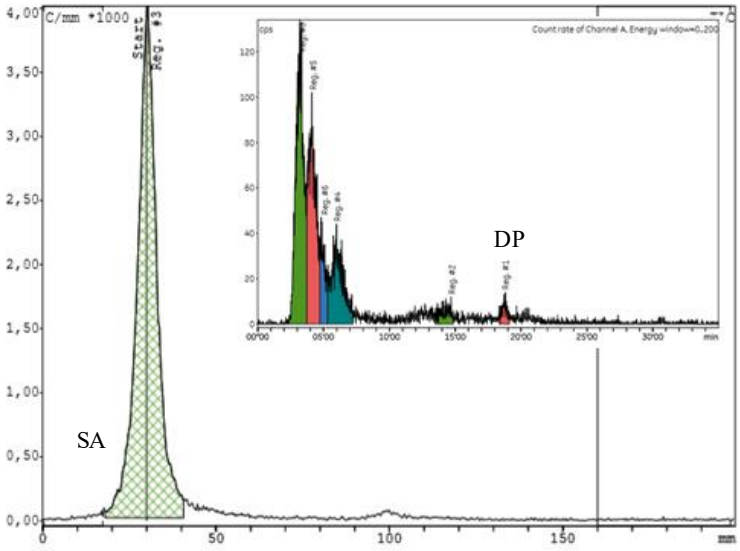

Table 44: Radio-HPLC analysis of selected samples of water phases (WP), calcium chloride ($CaCl_2$), methanol (MeOH), acetonitrile (ACN) and Soxhlet methanol extracts of ^{14}C-DA$^+$ in the water-sediment system. The portions of the parent substance and metabolites are shown as % AR Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA+ as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DA$^+$ (20.41)	M1 (3.04)	M2 (3.48)	M3 (4.00)	M4 (4.51)	M5 (5.26)	M6 (6.00)	M8 (12.20)	M9 (16.31)	M10 (17.17)	M11 (23.32)
WP day 1	10.09	2.01	3.50	-	33.71	-	-	-	-	-	-
$CaCl_2$ day 1	-	-	-	≤ 0.1	-	-	≤ 0.1	-	-	-	-
WP day 7	1.39	4.27	-	7.86	-	17.59	-	-	-	-	-
MeOH day 7	≤ 1.00	≤ 1.00	-	-	≤ 1.00	-	-	-	-	-	-
ACN day 7	3.90	-	-	-	-	-	-	-	-	-	≤ 1.00
MeOH day 14	≤ 1.00	≤ 1.00	-	-	≤ 1.00	-	-	-	≤ 1.00	-	≤ 1.00
Soxhlet day 14	10.63	-	-	-	-	-	-	-	≤ 1.00	≤ 1.00	-
WP day 30	-	3.78	-	-	19.80	-	-	-	-	-	-
MeOH day 30	≤ 1.00	≤ 1.00	-	-	≤ 1.00	-	-	-	-	-	-
Soxhlet day 30	11.61	-	-	-	-	-	-	≤ 1.00	-	≤ 1.00	-
WP day 60	-	3.05	6.49	-	-	-	9.48	-	-	-	-

A.5 Distribution of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water with addition of suspended sediment

Table 45: Distribution of the applied radioactivity (in %) of ^{14}C-DS$^-$ in surface water with suspended sediment at a test concentration of 10 µg/L. The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation (14CO2) and recovery over the 62-day incubation time are shown. Data points represent mean values of two replicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
7	36.90 ± 1.69	1.88 ± 0.07	16.85 ± 0.61	39.09 ± 0.14	94.71 ± 1.01
14	28.78 ± 13.52	1.72 ± 0.12	13.98 ± 3.40	44.74 ± 6.27	89.22 ± 10.77

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
30	7.42 ± 0.52	1.59 ± 0.23	9.80 ± 0.14	71.73 ± 0.92	90.54 ± 1.08
62	5.30 ± 0.81	1.55 ± 0.04	7.32 ± 1.21	75.29 ± 0.23	89.46 ± 0.21

Table 46: Distribution of the applied radioactivity (in %) of ^{14}C-DP in surface water with suspended sediment at a test concentration of 10 µg/L. The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 62-day incubation time are shown. Data points represent mean values of two replicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
7	17.65 ± 1.37	8.00 ± 0.77	21.46 ± 2.11	40.40 ± 0.60	87.51 ± 0.92
14	15.98 ± 0.68	4.69 ± 0.70	17.70 ± 0.93	55.87 ± 2.08	94.24 ± 2.52
30	14.52 ± 1.12	3.88 ± 0.85	8.23 ± 4.87	65.09 ± 1.16	91.72 ± 4.06
62	11.26 ± 0.48	3.01 ± 0.21	9.17 ± 0.14	68.96 ± 2.99	92.40 ± 3.54

Table 47: Distribution of the applied radioactivity (in %) of ^{14}C-DA$^+$ in surface water with suspended sediment at a test concentration of 10 µg/L. The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 62-day incubation time are shown. Data points represent mean values of two replicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
7	36.90 ± 0.76	40.52 ± 0.73	19.51 ± 0.53	2.05 ± 0.09	98.97 ± 0.64
14	53.73 ± 0.98	29.54 ± 2.01	9.41 ± 1.37	2.80 ± 0.22	95.48 ± 0.11
30	61.65 ± 4.79	17.20 ± 2.20	11.13 ± 0.10	3.42 ± 2.36	93.41 ± 5.05
62	62.34 ± 8.90	17.87 ± 6.07	9.15 ± 1.05	6.71 ± 0.13	96.08 ± 1.65

Table 48: Distribution of the applied radioactivity (in %) of ^{14}C-DS$^-$ in surface water with suspended sediment at a test concentration of 100 µg/L. The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation (14CO2) and recovery over the 60-day incubation time are shown. Data points represent mean values of triplicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
1	58.98 ± 1.47	47.39 ± 2.56	2.80 ± 0.09	0.04 ± 0.03	109.21 ± 1.14
7	81.33 ± 3.73	4.78 ± 0.53	7.58 ± 0.93	11.71 ± 2.60	105.40 ± 0.89
14	55.70 ± 14.71	3.11 ± 1.34	14.97 ± 3.22	23.79 ± 6.27	97.57 ± 6.46
30	33.52 ± 4.53	1.47 ± 0.27	11.55 ± 0.73	46.74 ± 2.86	93.28 ± 1.79
60	23.27 ± 9.33	0.88 ± 0.18	8.74 ± 0.14	62.93 ± 10.52	95.82 ± 1.62

Table 49: Distribution of the applied radioactivity (in %) of ^{14}C-DP in surface water with suspended sediment at a test concentration of 100 µg/L. The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 60-day incubation time are shown. Data points represent mean values of triplicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
1	31.43 ± 2.35	54.45 ± 4.05	2.56 ± 0.20	0.09 ± 0.03	88.53 ± 2.10
7	48.05 ± 2.69	15.75 ± 2.73	13.45 ± 0.39	16.18 ± 3.63	93.42 ± 4.20
14	40.70 ± 8.67	16.42 ± 1.70	13.01 ± 1.75	23.49 ± 4.85	93.63 ± 1.85
30	14.26 ± 2.85	6.19 ± 2.28	21.47 ± 2.00	50.12 ± 8.55	92.04 ± 1.72
60	12.19 ± 1.91	3.42 ± 0.27	20.85 ±2.41	57.53 ± 1.49	94.00 ± 2.09

Table 50: Distribution of the applied radioactivity (in %) of ^{14}C-DA$^+$ in surface water with suspended sediment at a test concentration of 100 µg/L. The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation ($^{14}CO_2$) and recovery over the 60-day incubation time are shown. Data points represent mean values of triplicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
1	26.04 ± 4.74	49.83 ± 4.02	18.72 ± 2.47	0.23 ± 0.06	94.83 ± 1.78
7	25.91 ± 0.51	40.63 ± 5.00	25.66 ± 4.32	2.86 ± 0.18	95.06 ± 1.90
14	43.59 ± 0.47	25.93 ± 0.99	20.40 ± 4.41	3.19 ± 0.29	90.71 ± 2.01
30	56.05 ± 12.63	17.13 ± 5.28	14.40 ± 5.98	5.60 ± 0.30	93.18 ± 2.29
60	57.72 ± 1.40	16.24 ± 1.81	14.02 ± 1.81	7.47 ± 0.72	95.46 ± 0.62

A.6 Distribution of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in surface water with addition of suspended sediment under sterile conditions (test concentration 10 µg/L)

Table 51: Distribution of the applied radioactivity (in %) of ^{14}C-DS$^-$ in surface water with suspended sediment under sterile conditions (test concentration of 10 µg/L). The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation (14CO2) and recovery over the 30-day incubation time are shown. Data points represent mean values of two replicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
7	20.77 ± 8.14	13.23 ± 0.30	0.17 ± 0.03	0.01 ± 0.00	34.18 ± 7.80
14	14.38 ± 7.16	59.36 ± 0.58	0.20 ± 0.01	0.04 ±0.05	73.97 ± 7.84
30	18.66 ± 0.41	49.99 ±7.13	0.11 ± 0.04	0.01 ± 0.02	68.77 ± 7.30

Table 52: Distribution of the applied radioactivity (in %) of ^{14}C-DP in surface water with suspended sediment under sterile conditions (test concentration of 10 µg/L). The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation (14CO2) and recovery over the 30-day incubation time are shown. Data points represent mean values of two replicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
7	12.54 ± 8.77	18.26 ± 12.05	1.26 ± 0.85	0.02 ± 0.01	32.07 ± 22.38
14	6.86 ± 5.08	57.49 ± 5.16	0.75 ± 0.02	0.02 ± 0.00	65.13 ± 0.05
30	19.77 ± 0.30	52.12 ± 8.11	0.63 ± 0.20	0.06 ± 0.03	72.58 ± 8.41

Table 53: Distribution of the applied radioactivity (in %) of ^{14}C-DA$^+$ in surface water with suspended sediment under sterile conditions (test concentration of 10 µg/L). The radioactivity in the water phase, sediment extract, non-extractable residues (NER), mineralisation (14CO2) and recovery over the 30-day incubation time are shown. Data points represent mean values of two replicates with standard deviation.

day	water phase	sediment extract	NER	$^{14}CO_2$	recovery
7	28.37 ± 1.76	29.60 ±1.47	2.82 ± 1.38	0.01 ±0.01	60.80 ±1.61
14	19.48 ± 5.82	43.83 ± 2.99	4.12 ± 1.38	0.00 ± 0.00	67.49 ± 10.86
30	20.76 ± 2.62	31.03 ± 1.49	5.54 ± 4.73	0.00 ± 0.00	60.68 ± 10.08

A.7 Radio-TLC analysis of the water phases and sediment extracts of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in the surface water test with suspended sediment

Table 54: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DS$^-$ in the surface water test after an incubation time of 7, 14, 30 and 62 days. Test concentration 10 µg/L. The portions of the parent substance and metabolites are shown as % AR. Rf values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS- as reference substance. Data points represent mean values of two replicates. M = metabolite. SA = start activity. - = not detected.

sample	^{14}C-DS$^-$ (0.699)	M1 (0.187)	M2 (0.279)	M3 (0.367)	M4 (0.479)	M5 (0.592)	M6 (0.754)	M7 (0.859)	SA
WP day 7	-	-	-	32.47	-	2.95	-	-	1.30
WP day 14	-	1.74	-	-	16.82	5.00	-	1.91	3.30
WP day 30	-	≤ 1.00	≤ 1.00	1.48	≤ 1.00	1.48	≤ 1.00	≤ 1.00	2.02
WP day 62	-	-	-	-	-	3.77	≤ 1.00	-	≤ 1.00
SE	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00	≤ 1.00	≤ 1.00	≤ 1.00

sample	^{14}C-DS$^-$ (0.699)	M1 (0.187)	M2 (0.279)	M3 (0.367)	M4 (0.479)	M5 (0.592)	M6 (0.754)	M7 (0.859)	SA
day 7									
SE day 14	-	-	≤ 1.00	-	≤ 1.00	≤ 1.00	≤ 1.00	≤ 1.00	≤ 1.00
SE day 30	-	-	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00	≤ 1.00
SE day 62	-	≤ 1.00	≤ 1.00	-	-	≤ 1.00	≤ 1.00	-	≤ 1.00

Table 55: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DS$^-$ in the surface water test after an incubation time of 1, 7, 14, 30 and 60 days. Test concentration 100 µg/L. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS$^-$ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

sample	^{14}C-DS$^-$ (0.808)	M4 (0.479)	M5 (0.592)	M6 (0.674)	M7 (0.754)	M8 (0.859)	M9 (0.926)	M10 (0.987)	SA
WP day 7	-	-	-	32.47	-	2.95	-	-	1.30
WP day 14	-	1.74	-	-	16.82	5.00	-	1.91	3.30
WP day 1	14.75	-	-	44.34	-	-	-	-	0.29
WP day 7	10.57	-	-	69.13	-	-	-	-	0.65
WP day 14	1.01	-	52.91	-	-	-	-	-	1.40
WP day 30	3.34	-	-	22.34	-	0.84	-	-	6.71
WP day 60	1.16	-	13.97	-	-	-	-	-	8.25
SE day 1	42.65	-	-	4.75	-	-	-	-	-
SE day 7	≤ 1.00	-	≤ 1.00	2.72	-	-	-	-	≤ 1.00
SE day 14	≤ 1.00	≤ 1.00	≤ 1.00	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00
SE day 30	≤ 1.00	-	≤ 1.00	≤ 1.00	-	-	≤ 1.00	≤ 1.00	≤ 1.00
SE day 60	≤ 1.00	-	≤ 1.00	-	-	-	-	-	≤ 1.00

Table 56: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DP in the surface water test after an incubation time of 7, 14, 30 and 62 days. Test concentration 10 µg/L. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Data points represent mean values of two replicates. M = metabolite. SA = start activity. - = not detected.

sample	^{14}C-DP (0.532)	M1 (0.187)	M2 (0.209)	M3 (0.386)	M4 (0.414)	M5 (0.686)	SA
WP day 7	≤ 1.00	7.76	-	-	-	-	9.35
WP day 14	1.00	-	-	-	-	-	14.83
WP day 30	≤ 1.00	-	-	-	-	-	13.64
WP day 62	≤ 1.00	-	-	-	-	-	11.01
SE day 7	3.49	≤ 1.00	-	-	≤ 1.00	≤ 1.00	3.25
SE day 14	≤ 1.00	-	≤ 1.00	≤ 1.00	-	-	2.56
SE day 30	≤ 1.00	-	≤ 1.00	≤ 1.00	-	-	≤ 1.00
SE day 62	≤ 1.00	≤ 1.00	-	≤ 1.00	-	-	≤ 1.00

Table 57: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DP in the surface water test after an incubation time of 1, 7, 14, 30 and 60 days. Test concentration 100 µg/L. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

sample	^{14}C-DP (0.550)	M1 (0.047)	M2 (0.156)	M3 (0.254)	M4 (0.328)	M5 (0.695)	SA
WP day 1	7.86	-	15.71	-	1.60	1.34	3.79
WP day 7	2.31	-	35.07	-	≤ 1.00	≤ 1.00	10.32
WP day 14	3.51	-	28.33	-	-	-	8.13
WP day 30	≤ 1.00	3.93	2.88	-	-	-	7.15
WP day 60	-	-	≤ 1.00	-	-	-	11.48
SE day 1	43.67	-	3.27	-	1.80	2.66	2.71
SE day 7	11.17	-	2.22	≤ 1.00	-	-	2.10
SE day 14	11.45	-	2.19	1.40	-	-	1.46
SE day 30	3.23	-	-	≤ 1.00	≤ 1.00	-	2.59
SE day 60	-	-	≤ 1.00	-	-	-	3.22

Table 58: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DA$^+$ in the surface water test after an incubation time of 7, 14, 30 and 62 days. Test concentration 10 µg/L. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA$^+$ as reference substance. Data points represent mean values of two replicates. M = metabolite. SA = start activity. - = not detected.

sample	^{14}C-DA$^+$ (0.590)	M1 (0.123)	M2 (0.261)	M3 (0.864)	SA
WP day 7	22.87	12.55	-	-	1.19
WP day 14	33.31	17.74	-	-	2.01
WP day 30	23.73	33.28	-	-	4.62
WP day 62	11.53	43.63	3.67	-	3.37
SE day 7	37.56	2.02	-	≤ 1.00	1.00
SE day 14	28.94	-	-	-	≤ 1.00
SE day 30	16.63	-	-	-	≤ 1.00
SE day 62	13.82	2.52	-	-	≤ 1.00

Table 59: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DA$^+$ in the surface water test after an incubation time of 1, 7, 14, 30 and 60 days. Test concentration 100 µg/L. The portions of the parent substance and metabolites are shown as % AR. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA$^+$ as reference substance. Data points represent mean values of triplicates. M = metabolite. SA = start activity. - = not detected.

sample	^{14}C-DA$^+$ (0.550)	M1 (0.095)	M1 (0.151)	M2 (0.251)	M3 (0.338)	M4 (0.779)	M6 (0.923)	SA
WP day 1	13.79	-	8.33	-	-	1.29	-	2.05
WP day 7	33.38	5.71	5.94	-	≤ 1.00	-	-	2.08
WP day 14	15.55	-	-	16.70	-	-	10.32	1.31
WP day 30	11.40	39.80	-	-	-	-	-	4.48
WP day 60	1.33	-	47.63	-	1.91	-	-	6.34
SE day 1	48.83	-	-	-	-	-	-	1.00
SE day 7	37.98	-	-	-	-	-	-	2.63
SE day 14	25.41	-	-	-	-	-	-	≤ 1.00
SE day 30	13.25	3.04	-	-	-	-	-	≤ 1.00
SE day 60	12.99	-	2.71	-	-	-	-	≤ 1.00

A.8 Radio-HPLC analysis of selected samples of water phases and sediment extracts of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in the surface water test

Table 60: Radio-HPLC analysis of selected samples of water phases (WP) of ^{14}C-DS$^-$ in the surface water test. Test concentration 10 µg/L. The portions of the parent substance and metabolites are shown as % AR. Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS$^-$ as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DS$^-$ (25.78)	M1 (4.40)	M2 (7.07)
WP day 7	-	35.96	≤ 1.00
WP day 14	-	27.11	1.67

Table 61: Radio-HPLC analysis of selected samples of water phases (WP) and sediment extracts (SE) of ^{14}C-DS$^-$ in the surface water test. Test concentration 100 µg/L. The portions of the parent substance and metabolites are shown as % AR. Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DS$^-$ as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DS$^-$ (14.35)	M1 (3.13)	M2 (4.51)	M3 (13.32)	M4 (17.13)	M5 (22.26)
WP day 1	1.29	57.69	-	-	-	-
WP day 7	≤ 1.00	67.49	-	1.98	11.87	-
WP day 14	-	55.70	-	-	-	-
WP day 30	≤ 1.00	29.87	2.74	-	-	-
SE day 1	45.22	2.17	-	-	-	-
SE day 7	3.77	≤ 1.00	-	-	-	≤ 1.00
SE day 14	2.35	≤ 1.00	≤ 1.00	≤ 1.00	-	-
SE day 60	≤ 1.00	≤ 1.00	-	≤ 1.00	-	≤ 1.00

Table 62: Radio-HPLC analysis of selected samples of water phases (WP) and sediment extracts (SE) of 14C-DP in the surface water test. Test concentration 10 µg/L. The portions of the parent substance and metabolites are shown as % AR. Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. Due to low 14C levels in the extracts, samples were pooled. M = metabolite. - = not detected.

sample	^{14}C-DP (28.40)	M1 (4.48)	M2 (15.93)	M3 (20.37)	M4 (31.15)
WP day 7	≤ 1.00	2.75	3.97	10.27	-
WP day 14-62	-	10.67	-	-	-
SE day 7-62	3.70	-	-	-	1.20

Table 63: Radio-HPLC analysis of selected samples of water phases (WP) and sediment extracts (SE) of ^{14}C-DP in the surface water test. Test concentration 100 µg/L. The portions of the parent substance and metabolites are shown as % AR. Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DP as reference substance. M = metabolite. - = not detected

sample	^{14}C-DP (18.41)	M1 (3.15)	M2 (4.35)	M3 (6.31)	M4 (7.06)	M5 (8.47)	M6 (10.47)	M7 (12.04)	M8 (13.05)	M9 (20.17)
WP day 1	11.47	1.15	-	1.31	-	1.50	9.46	5.04	1.49	-
WP day 7	≤ 1.00	2.42	1.14	2.45	1.38	4.80	24.38	10.62	-	-
WP day 14	≤ 1.00	3.57	-	2.36	-	6.94	17.81	9.45	-	-
WP day 30	0.10	9.30	1.09	-	-	-	1.82	1.37	-	-
WP day 60	≤ 0.1	7.36	1.53	-	-	≤ 1.00	≤ 1.00	≤ 1.00	-	-
SE day 1	49.80	0.94	-	-	-	-	-	-	1.42	-
SE day 7	10.16	1.15	-	-	-	1.17	≤ 1.00	1.06	≤ 1.00	-
SE day 60	1.53	≤ 1.00	-	-	-	-	-	-	-	1.39

Table 64: Radio-HPLC analysis of selected samples of water phases (WP) and sediment extracts (SE) of ^{14}C-DA$^+$ in the surface water test. Test concentration 10 µg/L. The portions of the parent substance and metabolites are shown as % AR. Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA$^+$ as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DA$^+$ (31.50)	M1 (3.82)	M2 (13.63)	M3 (16.53)	M4 (36.95)
WP day 7	22.00	≤ 1.00	4.04	3.33	1.56
WP day 14	38.64	≤ 1.00	7.45	6.74	-
WP day 62	11.77	7.46	10.71	32.40	-
SE day 7	40.52	-	-	-	-
SE day 14	29.54	-	-	-	-

Table 65: Radio-HPLC analysis of selected samples of water phases (WP) and sediment extracts (SE) of ^{14}C- DA$^+$ in the surface water test. Test concentration 100 µg/L. The portions of the parent substance and metabolites are shown as % AR. Retention times are shown in brackets. The identification of the parent substance was based on co-chromatography using radiolabelled DA$^+$ as reference substance. M = metabolite. - = not detected.

sample	^{14}C-DA$^+$ (20.41)	M1 (1.08)	M2 (2.57)	M3 (3.05)	M4 (4.18)	M5 (4.41)	M6 (5.00)	M7 (12.04)	M8 (22.10)	M9 (25.39)
WP day 1	14.87	-	2.37	-	-	1.89	6.33	-	-	≤ 1.00
WP day 7	10.13	-	-	2.36	4.05	7.58	-	-	≤ 1.00	≤ 1.00
WP day 14	9.04	1.67	-	9.26	-	23.03	-	-	-	≤ 1.00
WP day 30	5.91	-	-	14.07	-	36.07	-	-	-	-
WP day 60	-	-	-	16.53	-	41.19	-	-	-	-
SE day 14	25.93	-	-	-	-	-	-	-	-	-
SE day 60	10.94	-	-	1.60	-	2.32	-	-	-	1.38

A.9 Radio-TLC analysis of water phases and sediment extracts of ^{14}C-DS$^-$, ^{14}C-DP and ^{14}C-DA$^+$ in the surface water test under sterile conditions (test concentration 10 µg/L)

Table 66: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DS$^-$ in the surface water test after an incubation time of 7, 14 and 30 days under sterile conditions. Test concentration 10 µg/L. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using unlabelled DS$^-$, DP and DA$^+$ as reference substances. M = metabolite. SA = start activity. x = detected. - = not detected.

sample	DS$^-$ (0.650)	M1 (0.810)	SA
WP day 7	x	-	x
WP day 14	x	-	x
WP day 30	x	-	x
SE day 7	x	x	x
SE day 14	x	x	x
SE day 30	x	-	x

Table 67: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DP in the surface water test after an incubation time of 7, 14 and 30 days under sterile conditions. Test concentration 10 µg/L. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using unlabelled DS$^-$, DP and DA$^+$ as reference substances. M = metabolite. SA = start activity. x = detected. - = not detected.

sample	DP (0.500)	M1 (0.202)	M2 (0.617)	SA

sample	DP (0.500)	M1 (0.202)	M2 (0.617)	SA
WP day 7	x	x	x	x
WP day 14	x	-	-	x
WP day 30	x	-	-	x
SE day 7	x	x	x	x
SE day 14	x	x	x	x
SE day 30	x	-	-	x

Table 68: Radio-TLC analysis of water phases (WP) and sediment extracts (SE) of ^{14}C-DA^{+} in the surface water test after an incubation time of 7, 14 and 30 days under sterile conditions. Test concentration 10 µg/L. R_f values are shown in brackets. The identification of the parent substance was based on co-chromatography using unlabelled DS^{-}, DP and DA^{+} as reference substances. M = metabolite. SA = start activity. x = detected. - = not detected.

sample	DA^{+} (0.500)	M1 (0.762)	SA
WP day 7	x	-	x
WP day 14	x	-	x
WP day 30	x	-	x
SE day 7	x	x	x
SE day 14	x	x	x
SE day 30	x	x	x