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1 Introduction

The environmental fate of a compound is, *inter alia*, determined by transformation and distribution processes which, in turn, are strongly dependant on the specific environmental conditions. In general, there are three major approaches for experimental environmental fate studies. Field tests allow for the clarification of a substances behaviour under realistic conditions, whereas laboratory simulation (e.g. OECD guideline 308, 2002) and screening tests (e.g. OECD guideline 301, 1992) display only a certain detail of the entire scenario. Since field tests are quite costly, time consuming and deliver very complex data, their realisation is usually limited to a small number of cases. In this light, laboratory tests are the normally applied tools for the investigation of individual environmental processes, providing high comparability and reproducibility due to standardised test conditions (Löffler et al. 2004). Numerous laboratory test systems have been established allowing for the investigation of a chemical's fate under a variation of relevant environmental conditions in terrestrial, aquatic and other scenarios (Brodsky et al. 1997, Hill et al. 1994, Freitag et al. 1982, Freitag et al. 1985). Respective standardised test procedures are an important part of the risk assessment of environmental relevant chemicals. Tests according to OECD guidelines 301 C and D represent standardised laboratory screening tests, conducted under aerobic conditions, in which the test system is inoculated with microorganisms derived from domestic sewage, activated sludge or secondary effluent. The biodegradation of high concentrations of the test substance is measured by non-specific parameters like dissolved organic carbon (DOC), biochemical oxygen demand (BOD) or CO₂ production.

In order to collect data that apply to more realistic conditions a new water-sediment system was developed. To ensure a high degree of comparability of experimental data from water-only and water-sediment studies a test design close to the OECD Guideline 301 C (MITI I) was chosen.

In the following, the experimental work between May 2006 and April 2007 is reported, including the following main aspects:

- Experimental biodegradation kinetics for a set of three test compounds in water and sediment are presented.
- Search was performed in order to identify factors, which may influence the results of the biodegradation studies in the water-sediment system (confounding factors).

2 Material and Methods

Material and methods, including the test design, description of microbial inoculum, sediment, overlying water and the validation of the experimental procedures were described in Deliverables 2.3.1 (Meller et al. 2005) and 2.3.2 (Junker et al. 2006). For detailed information, please refer to these documents.

2.1 Confounding factors

Biodegradation of chemicals is influenced by many factors depending on the compound itself as well as on biological and physicochemical conditions. On the one hand the test system became more complex by the integration of the sediment compartment. This is because bioavailability, transformation processes and degradation could be affected by sediment-related parameters like sorption, sequestration and cross-coupling. On the other side, such factors can be used to investigate the differences between the water-only and the water-sediment test system and thus can provide additional important information on the environmental fate of a compound.

For this purpose, the intention was to identify and investigate the most important confounding factors for the newly developed water-sediment system by using a particularly suitable test substance. With regard to this objective, the "ideal compound" should combine the following properties:

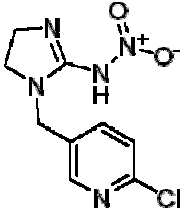
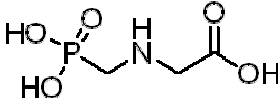
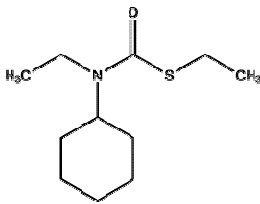
- biologically degradable
- sorption to sediment
- water soluble (approx. 75-100 mg/L)
- nonvolatile
- nontoxic for microorganisms

Since it was not possible to identify a compound combining all these properties, three test substances were chosen, which seemed to be suitable for different reasons (see 2.2).

2.2 Test substances

The physicochemical parameters of the test substances are shown in Tab. 1. For imidacloprid, glyphosate and cycloate, stock solutions were prepared by dissolving the test item in test medium. Aniline was used as reference substance. Imidacloprid and glyphosate were tested at 100 mg/L whereas cycloate was tested at 75 mg/L due to its lower water solubility.

Tab. 1: Physicochemical parameters of the test substances used in the studies with the water-only and the water-sediment system

	Imidacloprid	Glyphosate	Cycloate
CAS-No.:	105827-78-9	1071-83-6	1134-23-2
Empirical formula:	C ₉ H ₁₀ ClN ₅ O ₂	C ₃ H ₈ NO ₅ P	C ₁₁ H ₂₁ NOS
Structural formula:			
Molecular weight [g/mol]:	255.66	169.08	215.37
Water solubility [mg/L]:	514 (20°C) ^[2]	11,600 (25°C) ^[1]	75 (20°C)
Log P_{ow}:	3.7 ^[2]	-3.5 ^[1]	3.88 ^[4]
K_{oc}:	132 - 310 ^[2]	-	12941 ^[3]
Vapour pressure [mm Hg]:	1.0*10 ⁻⁷ ^[2]	7.5*10 ⁻⁸ ^[1]	1.6*10 ⁻⁵
Henry constant [atm·m²/mol]:	6.5*10 ⁻¹¹ ^[2]	< 1.44*10 ⁻¹² ^[1]	6.12*10 ⁻³ Pa m ³ /mol
Soil adsorption coefficient K_d [g/m³]:	0.956 - 4.18 ^[2]	61 ^[1]	-
Hydrolysis (DT₅₀):	> 30 d ^[2]	> 35 d ^[1]	30 d ^[3]
Photolysis (DT₅₀):	< 1 h ^[2]	stable to light	219 d (water, pH7, 20°C) ^[5]
ThOD_(NH4) [mg/L]:	81.4	56.8	183.9

^[1] Schuette (1998); ^[2] Fossen (2006); ^[3] www.pesticideinfo.org; ^[4] according to MSDS for cycloate, which was provided together with the test item; ^[5] California Environmental Protection Agency (1995)

Imidacloprid is a systemic insecticide that enters the target pest via ingestion or direct contact. Its low Henry's law constant indicates that it has low volatility from water. The high water solubility and low K_{oc} for imidacloprid indicates a low tendency for adsorption to soil particles.

Since breakdown in surface waters is primarily due to aqueous photolysis, all test vessels were covered by aluminium foil. It was chosen as test substance since NoMiracle partners from Pillar 3 were planning to study imidacloprid as well.

Glyphosate is a non-selective systemic herbicide used to control many annual and perennial grasses and broadleaf weeds plus many tree and brush species. It was chosen because of its high water solubility and the fact that in water, glyphosate is strongly adsorbed to suspended organic and mineral matter. Furthermore, glyphosate is broken down primarily by microorganisms and its half-life in pond water ranges from 12 days to 10 weeks. NoMiracle partners from Pillar 3 were planning to study glyphosate as well.

Cycloate is a selective herbicide for pre-plant use only. It is used to control many broadleaf weeds, annual grasses, and nutsedge in fields for sugar beets, table beets, and spinach. It was chosen as test substance because of its relatively high log P_{ow} and the good water solubility. Furthermore, biodegradation experiments according to OECD 301 E showed nearly 100% biodegradation within 28 days (California Environmental Protection Agency, 1995). However, the Henry constant of $6.12 \cdot 10^{-3} \text{ Pa m}^3/\text{mol}$ indicates volatility.

To ensure an optimal comparability of the results, each compound was tested using the water-only and the new water-sediment test system in parallel. Furthermore, two different approaches were used for application of the test substance for the water-sediment systems in order to investigate the influence of the sediment compartment:

- a) the aqueous stock solution was applied directly to the water phase
- b) the aqueous stock solution was mixed into the sediment.

In the latter case (b) the same amount of test substance was mixed into the sediment as applied directly to the water phase.

3 Results and Discussion

3.1 Identification of confounding factors

The identification of relevant confounding factors has not been completed yet, because it depends on the properties of the test substance, which physicochemical parameters are particularly suitable.

There are two main categories which are supposed to influence the rate of biodegradation (Lyman et al. 1990):

- 1) factors that influence the availability and concentration of the compound to be degraded or that affect size and activity of the microbial population
- 2) factors that directly influence the degradation rate itself.

As shown in Tab. 2, all of the factors can be classified as substrate-related, organism-related or environment-related.

Table 2: Factors which may affect biodegradation (Lyman et al. 1990)

Substrate-related	Organism-related	Environment-related
<ul style="list-style-type: none"> ▪ Physicochemical parameters ▪ Concentration 	<ul style="list-style-type: none"> ▪ Species composition of population ▪ Population density (inoculum concentration) ▪ Previous history ▪ Interspecies interactions ▪ Intraspecies interaction ▪ Enzymatic make-up and activity 	<ul style="list-style-type: none"> ▪ Temperature ▪ pH ▪ Oxygen availability ▪ Nutrients ▪ Illumination ▪ Sediment organic matter ▪ Ammonium

3.1.1 Substrate-related factors

There are many correlations between the physicochemical properties of a compound and its biodegradability. Water insoluble substances are supposed to have a lower potential for biodegradation than compounds with a high water solubility because an insoluble compound may not reach the reaction site in the microbial cell, for example (Lyman et al. 1990). Another

factor which may affect biodegradation is the BOD/COD-ratio whereby compounds with a ratio less than 0.01 are classified as relatively undegradable (e.g. chloroform), between 0.01 and 0.1 as moderately degradable (e.g. acetonitrile) and greater than 0.1 as relatively degradable (e.g. aniline) (Lyman et al. 1990). Furthermore, structure-activity relationships between hydrolysis and microbial degradation have been reported (Paris et al. 1982).

With regard to the investigations with the water-sediment system, these substance-related factors will not be changed because all experiments will be performed using the same test compound. However, another substance-related factor is the test item concentration. Biodegradation may be limited if the test item concentration is too low due to lack of sufficient stimulus to initiate enzymatic responses, whereas too high concentrations may be toxic or inhibitory. Thus, investigation of different test item concentration may be one possible attempt.

3.1.2 Organism-related factors

The species composition as well as inoculum concentration, previous history of inoculum, inter- and intraspecific interactions and the ability of the bacteria to degrade the compound are important factors which may influence biodegradation. In order to minimise these organism-related factors, the inoculum used in the experiments is derived from microbial populations from sewage treatment plants, rivers and lakes of different sizes in rural, urban and industrial areas to ensure the presence of a great variety of degrading organisms. Therefore, these organism-related factors will not be investigated within the biodegradation experiments.

3.1.3 Environment-related factors

The main focus of the investigations will be on environment-related factors because they are comparatively easy to modify within the experiments.

Temperature: According to their optimum temperature, microorganisms are classified as psychrophiles (< 20°C), mesophiles (20°C - 42°C) and thermophiles (> 42°C) (Schlegel 1992). In general, biodegradation increases with increasing temperature within these temperature ranges. The ideal temperature for wastewater treatment processes is between 20°C and 35°C. Furthermore, some other environmental parameters may be affected by different temperatures.

Adsorption, for example, is an exothermic process and usually decreases with increasing temperature. Thus, considering adsorption to sediment particles, variations in temperature may result in different biodegradation rates using the water-sediment system.

pH: The growth optimum of most degrading microorganisms is between pH 6 and 8 (Lyman et al. 1990). In addition, microbial oxidation is most rapid within this range, whereas fungi prefer slightly acidic conditions. Hambrick et al. (1980) showed that mineralisation rates increased with increasing pH between 5.0 and 8.0.

Oxygen: In aerobic environments oxygen availability often limits the growth of bacteria in closed laboratory systems. However, as reported in Deliverable D.2.3.2 (Junker et al. 2006), the end of the exponential phase of degradation in experiments using the water-sediment system was not caused by an oxygen deficiency but by a lack of DOC in the water phase. Although anaerobic conditions in deeper sediment layers can not be excluded, the experiments will be performed following ready biodegradability tests (OECD 1992) under aerobic conditions in which a high concentration of the test substance is used and the biodegradation rate is measured by non-specific parameters like DOC, BOD and CO₂. Therefore, the oxygen content will not be used to investigate factors affecting the biodegradation rate.

Nutrients: Nutrients are required for metabolic reactions in addition to the substrate for the induction of enzymes necessary for degradation. However, the test substance should be the only source of carbon used in the experiments apart from the mineral medium and the peat contained in the artificial sediment and therefore no other carbon sources will be added to the test system.

Illumination: All experiments are performed in the dark in order to prevent higher degradation rates due to photodegradation. In addition, when testing photosensitive compounds, the test vessels will be covered by aluminium foil.

Sediment organic carbon / adsorption: Some compounds may be physically trapped in pores or may be adsorbed onto sediment particles, suspended solids or organic matter and therefore will significantly reduce the amount of test substance available for biodegradation. Thus, porewater can act as a pathway for chemicals that bind to the sediment (Lapota et al. 2000). In addition, Hinga (2003) found that degradation rates of the same classes of aromatic hydrocarbons in

sediments correlate with sediment total organic carbon. Since sediment grain size often correlates with sediment TOC it seems likely that grain size parameters would exhibit relationships with degradation rate. Thus, variations in sediment composition or the use of natural sediments with high and low organic carbon content may be approaches to investigate important confounding factors.

Ammonium: The principle of the manometric measurement is based on the fact that under the consumption of oxygen the carbon of organic compounds is oxidised to CO₂, the formed CO₂ is trapped and the pressure in the closed system decreases. Therefore, additional oxygen consumption by nitrifying processes related to the organic material of the artificial sediment or related to the biodegradation of nitrogen-containing compounds is prevented by using Allylthiourea (ATU). Hence, an influence of ammonia on the biodegradation rate can be excluded.

In conclusion, the confounding factors which will be investigated most likely are test substance concentration, temperature, pH and sediment composition. However, it depends on the properties of the test substance, which parameters will be particularly suitable.

3.2 Biodegradation test with imidacloprid in water and sediment

In order to check if imidacloprid has a negative effect on microorganisms at 100 mg/L, two test vessels with aniline and imidacloprid were investigated for the water-sediment system. The results show that imidacloprid did not affect the biodegradation of aniline. Whereas the degradation curves of the reference substance aniline for the water-only and for the water-sediment system show the characteristic progression, indicating that the used inoculum was in good condition, imidacloprid was not degraded, neither in the water-only system nor in the water-sediment system. Therefore, no differences in biodegradation could be observed with regard to the application method (via the water phase and via the sediment) in the water-sediment system. This corresponds to the biodegradability predicted by BIOWIN, where imidacloprid is classified as not readily biodegradable with an ultimate half-life of months (Dimitrov and Mekenyan 2006). However, a BOD of 31.9% was predicted by CATABOL (Dimitrov and Mekenyan 2006). The low predictability of biodegradation could be explained by a low predictability of the observed metabolic fate of this compound.

The degradation curves of imidacloprid and the reference substance aniline are presented in Fig. 1.

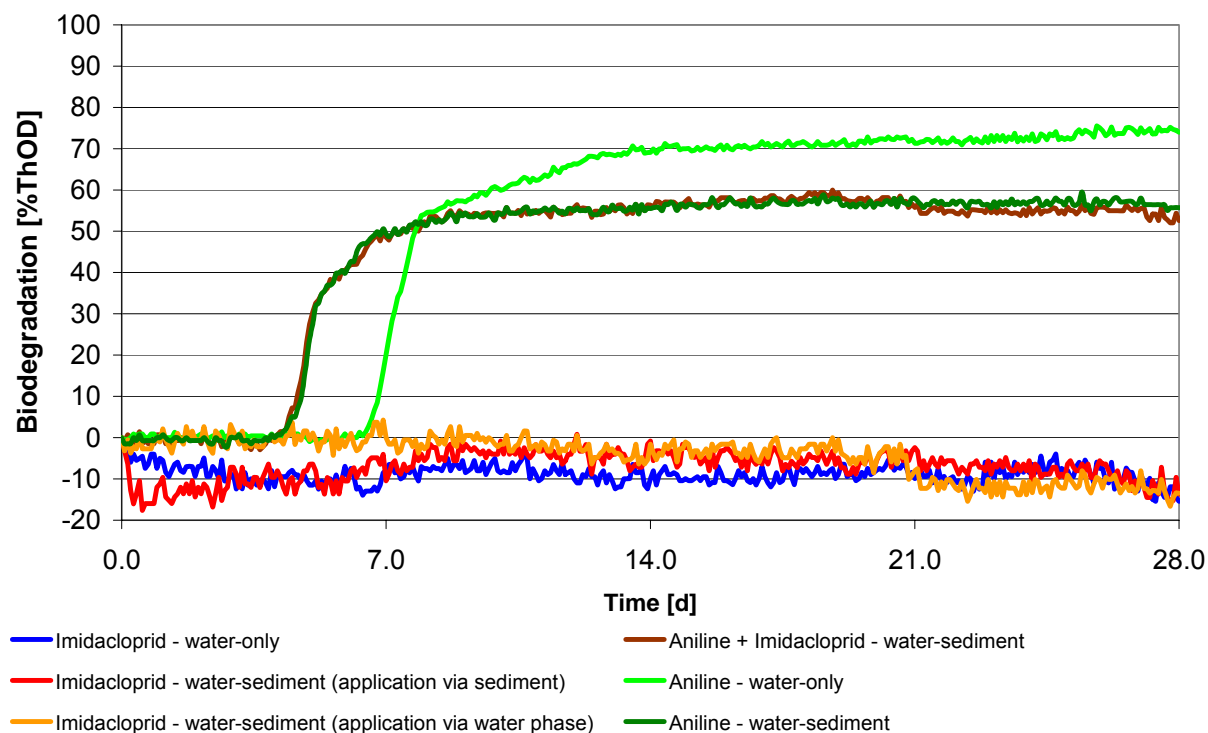


Fig. 1: Biodegradation of imidacloprid (mean; n = 4) and aniline (mean; n = 2) in the water-only system (mean; n = 4) and in the new water-sediment system

The biodegradation [%ThOD] at different time points during the course of the test is presented in Tab. 2.

Tab. 2: Degradation rates at the different time points during the test with imidacloprid

Substance	Biodegradation [% ThOD]			
	8.0 d ^[1]	14 d	21 d	28 d
Aniline – water only	54.1	69.1	71.3	74.0
Aniline – water sediment	51.4	55.5	58.0	55.7
Aniline + imidacloprid	52.3	56.3	55.8	52.6
Imidacloprid – water-only	0.0	0.0	0.0	0.0
Imidacloprid – water-sediment (application via water phase)	0.0	0.0	0.0	0.0
Imidacloprid – water-sediment (application via sediment)	0.0	0.0	0.0	0.0

^[1] corresponding to the end of the exponential degradation phase for aniline

3.3 Biodegradation test with glyphosate in water and sediment

The degradation curves of glyphosate and the reference substance aniline are presented in Fig. 2.

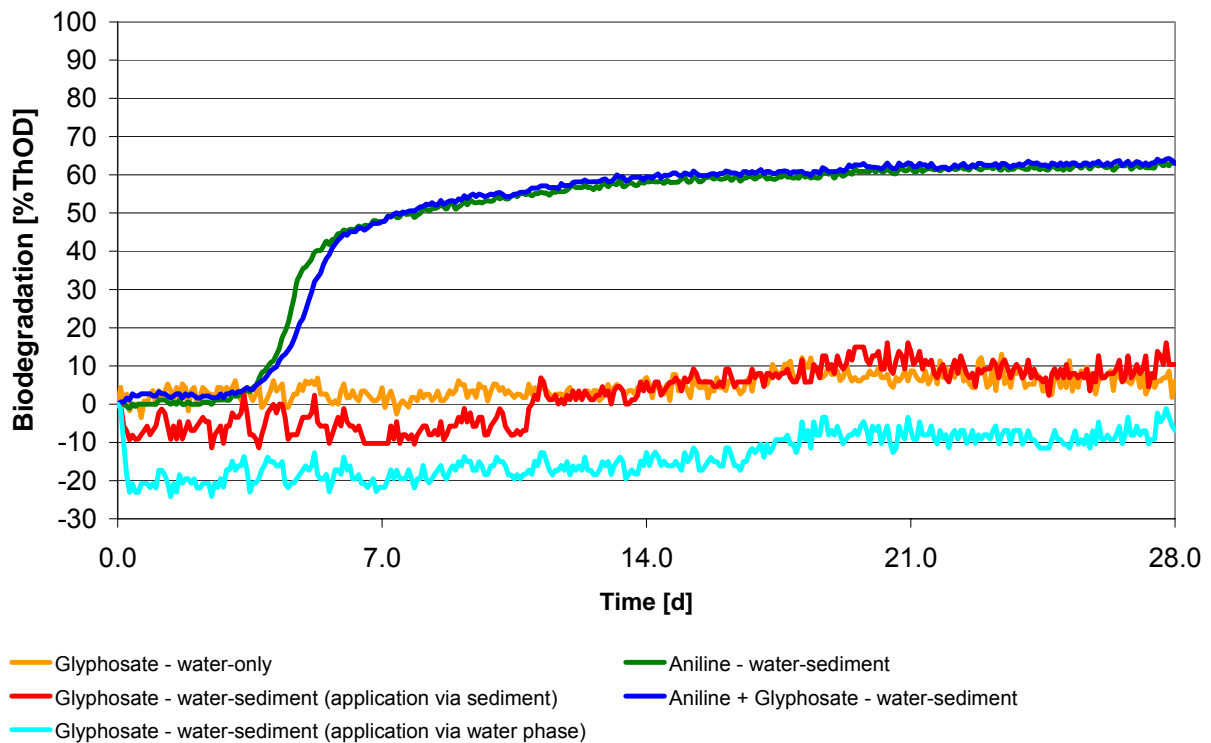


Fig. 2: Biodegradation of glyphosate (mean; $n = 4$) and aniline in the new water-sediment system

In order to check if glyphosate has a negative effect on microorganisms at 100 mg/L, two test vessels with aniline and glyphosate were investigated for the water-sediment system. The results show that glyphosate did not affect the biodegradation of aniline. Whereas the degradation curves of the reference substance aniline for the water-sediment system show the characteristic progression, indicating that the used inoculum was in good condition, glyphosate was not degraded, neither in the water-only system nor in the water-sediment system with degradation rates $\leq 10\%$ after 28 days. The differences in biodegradation with regard to the application method (via the water phase and via the sediment) in the water-sediment system are supposed to be within the normal variation limit. This does not completely correspond to the biodegradability predicted by BIOWIN, where glyphosate is classified as readily biodegradable

by the MITI linear model and not readily biodegradable by the MITI nonlinear model with an ultimate half-life of weeks (Dimitrov and Mekenyan 2006). Furthermore, a BOD of 73.4% was predicted by CATABOL with an ultimate half-life of 14 days (Dimitrov and Mekenyan 2006). The comparison between observed and simulated biodegradation pathways by CATABOL resulted in a predictability of observed biodegradation pathways of 33.3%.

The biodegradation [%ThOD] at different time points during the course of the test is presented in Tab. 4.

Tab. 4: Degradation rates at the different time points during the test with glyphosate

Substance	Biodegradation [% ThOD]			
	6.0 d ⁽¹⁾	14 d	21 d	28 d
Aniline – water sediment	45.6	58.2	62.1	62.4
Aniline + glyphosate	44.5	59.5	63.0	63.0
Glyphosate – water-only	5.3	7.0	8.7	6.1
Glyphosate – water-sediment (application via water phase)	0.0	0.0	0.0	0.0
Glyphosate – water-sediment (application via sediment)	0.0	5.8	13.8	10.3

⁽¹⁾ corresponding to the end of the exponential degradation phase for aniline

3.4 Biodegradation test with cycloate in water and sediment

In order to check if cycloate has a negative effect on microorganisms at 75 mg/L, two test vessels with aniline and cycloate were investigated for the water-sediment system. The results show that cycloate did not affect the biodegradation of aniline. Whereas the degradation curves of the reference substance aniline for the water-sediment system show the characteristic progression, indicating that the used inoculum was in good condition, cycloate was not degraded, neither in the water-only system nor in the water-sediment system. Since the principle of the measurements is based on manometric changes (negative pressure) in the closed system, the data for application of cycloate directly to the water phase of the water-sediment system could not be evaluated since there was an increase in pressure in two out of three replicates. This could be explained by volatilisation of cycloate.

The degradation curves of cycloate and the reference substance aniline are presented in Fig. 3.

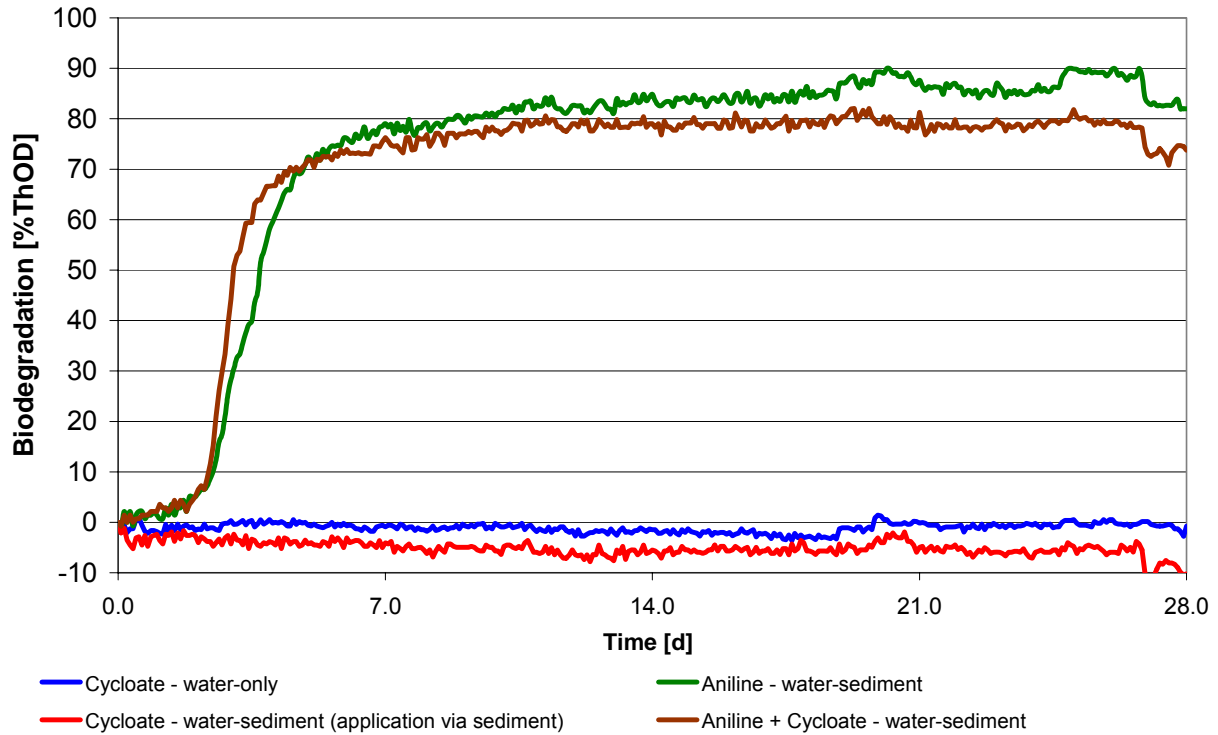


Fig. 3: Biodegradation of cycloate in the water-only system (mean; n = 4) and in the new water-sediment system (mean; n = 3) and biodegradation of aniline in the water-sediment system (mean; n = 2)

In order to check if cycloate has a negative effect on microorganisms at 75 mg/L, two test vessels with aniline and cycloate were investigated for the water-sediment system. The results show that cycloate did not affect the biodegradation of aniline. Whereas the degradation curves of the reference substance aniline for the water-sediment system show the characteristic progression, indicating that the used inoculum was in good condition, cycloate was not degraded, neither in the water-only system nor in the water-sediment system. Since the principle of the measurements is based on manometric changes (negative pressure) in the closed system, the data for application of cycloate directly to the water phase of the water-sediment system could not be evaluated since there was an increase in pressure in two out of three replicates. This could be explained by volatilisation of cycloate.

The biodegradation [%ThOD] at different time points during the course of the test is presented in Tab. 4.

Tab. 4: Degradation rates at the different time points during the test with cycloate

Substance	Biodegradation [% ThOD]				
	4.5 d ^[1]	7 d	14 d	21 d	28 d
Aniline – water sediment	49.4	59.3	63.6	65.7	62.6
Aniline + cycloate	52.8	57.2	59.3	61.0	55.3
Cycloate – water-only	0.0	0.0	0.0	0.0	0.0
Cycloate – water-sediment (application via water phase)	0.0	0.0	0.0	0.0	0.0
Cycloate – water-sediment (application via sediment)	0.0	0.0	0.0	0.0	0.0

^[1] corresponding to the end of the exponential degradation phase for aniline

4 Conclusion and Outlook

Experimental biodegradation tests for a set of three test compounds (imidacloprid, glyphosate, cycloate) in water and sediment were performed. Unfortunately, none of the tested compounds proved to be suitable for the investigation of factors which may influence biodegradation in the water-sediment system (confounding factors) because none of them were degraded within the test period of 28 days. Therefore, only a preselection of potentially suitable parameters could be made. The reference substance aniline was nearly completely degraded, indicating that the used inoculum was in good condition. The results do not completely correspond to the biodegradability predicted by Dimitrov and Mekenyan (2006) using BIOWIN models and CATABOL.

Within the next period (May 07 - April 08), biodegradation experiments will be continued for further selected substances. To ensure an optimal comparability of the results, each compound is tested using the water-only approach and the new water-sediment test system in parallel. In doing so, existing differences between the test systems will be identified in order to continually improve the comparability of experimental biodegradation data obtained for the different compartments. In this context, the main focus will be on the identification of a test substance which is suitable to investigate the most important confounding factors. If possible, these parameters will be investigated using a factorial design approach. Besides the experimental part, a systematic search for additional degradation data from literature and competent authorities will be performed for the aquatic compartment.

The results of both, biodegradation experiments and literature search, will provide data which are needed by other partners in WP 2.3 to build a database for biodegradation parameters of organic compounds in water and sediment. Thus, an analysis of degradation parameters across chemicals and media will be possible and improved quantitative structure-property relationships (QSPRs) can be developed.

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