Forschungszentrum Jülich GmbH

Institut für Chemie und Dynamik der Geosphäre: Agrosphäre

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# The intrinsic microbial degradation potential of shallow aquifers with respect to crop protection agents

I n a u g u r a l  D i s s e r t a t i o n

zur

Erlangung des Grades

Doktor der Agrarwissenschaften

(Dr. agr.)

der

Hohen Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität

zu Bonn

Vorgelegt am 31.01.2010

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Tag der mündlichen Prüfung: 26 März 2010

Erscheinungsjahr: 2010

**ABSTRACT**

Active substances in crop protection agents (CPA) are assessed by a community procedure, and a comprehensive dossier including data and tests is part of the application. The studies must be performed according to specified standards and by certified testing facilities.

Recently, it is discussed, whether it is necessary to extend these tests to aquifers to investigate the environmental behaviour of CPA in the groundwater. This was the main motivation to develop a *dynamic artificial aquifer incubation system* (DAISY) at the lab-scale for standard use (Good Laboratory Practice, GLP). DAISY is operated fully automated via online control (Figure 19; page 125) at different redox conditions (Figure 12; page 48), and it is possible to simulate different groundwater flow rates (Figure 11; page 47). This is important, as I showed that dynamic flow conditions generally increase the degradation kinetics of the model compound benzoic acid (BA), an effect that can also be assumed for the degradation of other aromatic substances (3.1.3). A test system is now available that enables to study the intrinsic microbial degradation potential of shallow aquifers at realistic low concentrations of ≤ 10 µg $l^{-1}$.

As part of the work I conducted, I compiled important information on the design of future groundwater studies. BA was used to investigate processes relevant to degradation within the scope of developing a static and dynamic test system. It was shown that simple physical parameters, such as the dynamic solubility of carbon dioxide ($CO_2$) in water, were not taken into account in groundwater studies in the past (12). This led, for example, to an incorrect estimation of the existing degradation potential in the existing BA studies (Figure 15; page 56). It was also determined that continuous absorption of $CO_2$, by e.g. sodium hydroxide that is widely used for the absorption of mineralized $^{14}C$-labelled test substances (53;140;141), interferes considerably with the chemical groundwater equilibrium. The reason for this is the unspecific absorption of $CO_2$, its supply from the groundwater bicarbonates, and the resulting enrichment of $OH^-$ ions (2.2.4). This ultimately gives rise to a strong increase in the pH value (3.1.2), and future degradation studies should therefore completely dispense without continuous absorption of $CO_2$.

The test systems developed within the scope of this work (2.5) were also used to investigate the current intrinsic degradation potential of three shallow aquifers for Imidacloprid (IMI), Isoproturon (IPU) and Diketonitrile (DKN). In various long-term incubation studies, a general degradation potential was assessed under oxygen-, iron-, and sulfur-reducing conditions and at a concentration of 0.1, 1, and 10 µg $l^{-1}$. It was revealed, however, that the lower mineralization rates led to accumulation of metabolites becoming the most important parameter for estimating the degradation potential at a concentration of ≤ 10 µg $l^{-1}$. The combined application of test substances had e.g. an effect on the degradation of individual substances here (3.2.2). Furthermore, I demonstrated the degradation of IPU under anaerobic conditions for the first time (3.2.1).

Not all of the findings from the BA acid studies (3.1.4) could be taken into account when setting up and conducting the long-term studies (> 360 days). Moreover, the test systems were only developed in the final stages of this work and led to a patent application for the dynamic test system, as well as a contract for another GLP study from the same sponsor. The test systems and methods developed enable a realistic estimate to be made of the intrinsic microbial degradation potential of aquifers near the surface in the laboratory under GLP conditions. Further studies will be conducted to estimate the hazard potential associated with different test substances and to transfer the results to the field scale.

The intrinsic degradation potential of the aerobic shallow aquifers was analysed in enrichment cultures, for which a variety of carbon (C) sources were available (2.4). The microorganisms metabolised IPU as both the only C-source as well as co-metabolically. This was accompanied by an increase in the microbial biomass. Furthermore, using fingerprint techniques (DGGE) a strong displacement of the microbial population was confirmed (3.3). The experimental approach (Figure 39; page 141) has good potential to isolate future bacterial strains with a specific degradation potential for individual CPA.

**ZUSAMMENFASSUNG**

Innerhalb der Europäischen Union werden die Wirkstoffe von Pflanzenschutzmitteln (PSM) in einem Gemeinschaftsverfahren bewertet, zu dem ein umfangreiches Dossier mit Daten und Tests erstellt wird. Die Studien müssen dabei nach vorgegebenen Normen von zertifizierten Versuchseinrichtungen durchgeführt werden.

Aktuell wird diskutiert, ob die Notwendigkeit besteht, das bestehende Dossier auch auf Grundwasserleiter auszudehnen, um das Umweltverhalten von PSM im Grundwasser zu untersuchen. Ein essentieller Meilenstein ist hierbei die Entwicklung eines dynamischen artifiziellen Testsystems, um Grundwasserstudien standardisiert unter der „Guten Laborpraxis“ (GLP) im Labormassstab durchführen zu können. Dies war die wesentliche Motivation für die Entwicklung des dynamischen Testsystems DAISY, das einen vollautomatischen Remotebetrieb (Figure 19; page 125) unter verschiedenen Redoxbedingungen (Figure 12; page 48), bei der gleichzeitigen Simulation verschiedener Grundwasserflusssraten (Figure 11; page 47), ermöglicht. Der Simulation verschiedener Flussraten kommt eine besondere Bedeuting zu, da ich im Rahmen der Arbeit zeigen konnte, dass dynamische Flussbedingungen die Abbaukinetik der Modellsubstanz Benzoesäure (BA) grundsätzlich erhöhen, ein Effekt der auch für den Abbau anderer aromatischer Substanzen angenommen werden kann (3.1.3). Ein Testsystem ist jetzt verfügbar, welches es ermöglicht, dass intrinsische mikrobielle Abbaupotential oberflächenaher Grundwasserleiter bzgl. PSM bei realistischen geringen Konzentrationen von ≤ 10 µg $l^{-1}$ zu untersuchen.

Darüber hinaus wurden wesentliche Erkenntnisse erarbeitet, die das Design zukünftiger Grundwasserstudien betreffen. Stellvertretend für viele aromatische Moleküle diente BA als Modellsubstanz, um die den Abbau betreffenden relevanten Prozesse im Rahmen der Entwicklung eines statischen und dynamischen Testsystems zu untersuchen. Es zeigte sich, dass einfache physikalische Parameter, wie z.B. die Löslichkeit von Kohlenstoffdioxid ($CO_2$) in Wasser, in Grundwasserstudien bislang keine Berücksichtigung fanden (12). Dieses führte in den vorliegenden BA-studien zu einer falschen Einschätzung des vorhandenen Abbaupotenzials (Figure 15; page 56). Es wurde ebenfalls festgestellt, dass die kontinuierliche Absorption von $CO_2$, durch z.B. Natronlauge, die in Abbaustudien häufig für die Absorption von mineralisiertem $^{14}C$-markiertem Kohlenstoff eingesetzt wird (53;140;141), einen starken Effekt auf das chemische Grundwassergleichgewicht zur Folge hat. Begründet ist dies durch die unspezifische Absorption des $CO_2$, dessen Nachlieferung aus dem Bikarbonat des Grundwassers und der daraus resultierenden Anreicherung von $OH^-$ Ionen (2.2.4). Diese führt letztendlich zu einem starken Anstieg des pH Wertes (3.1.2) und zukünftige Abbaustudien sollten deshalb vollständig auf die kontinuierliche Absorption von $CO_2$ verzichten.

Die im Rahmen dieser Arbeit entwickelten Testsysteme wurden ferner dazu eingesetzt, das aktuelle intrinsische Abbaupotenzial dreier oberflächennaher Grundwasserleiter gegenüber Imidacloprid (IMI), Isoproturon (IPU) und Diketonitrile (DKN) zu untersuchen. In verschiedenen Langzeitstudien konnte ich ein generelles Potenzial unter sauerstoff-, eisen- und schwefelreduzierenden Bedingungen und bei einer Konzentration von 0.1, 1, und 10 µg $l^{-1}$ nachweisen. Es zeigte sich jedoch, dass, bedingt durch die geringen Mineralisationsraten, es zu einer Anreicherung der verschiedenen Abbauzwischenprodukte kam. Diese waren somit die wichtigere Kenngröße, um das Abbaupotenzial bei einer Konzentration ≤ 10 µg $l^{-1}$ abzuschätzen. In diesem Zusammenhang hatte die gemeinsame Applikation der Testsubstanzen oftmals einen positiven Effekt auf den Abbau einzelner Substanzen (3.2.2). Des Weiteren wurde für IPU erstmals ein Abbau unter anaeroben Bedingungen nachgewiesen werden (3.2.1).

Nicht alle Erkenntnisse der BA-studien konnten für das Setup und die Aufarbeitung der Langzeitstudien (> 360 Versuchstage) Berücksichtigung finden (3.1.4). Darüber hinaus wurde die Entwicklung der Testsysteme erst mit dem Ende der Arbeit abgeschlossen, die für das dynamische Testsystem in einer Patentanmeldung, sowie in einer weiteren GLP Auftragstudie durch den Sponsor resultierte. Die entwickelten Testsysteme und Methoden ermöglichen eine realistische Abschätzung des intrinsischen mikrobiellen Abbaupotenzials oberflächennaher Grundwasserleiter im Labor unter GLP Bedingungen. Weitere Studien sollten folgen, um eine Abschätzung des Gefährdungspotenzials verschiedener Testsubstanzen zu ermitteln und eine Übertragung der Ergebnisse auf die Feldskala zu ermöglichen.

Desweiteren wurde das intrinsische Abbaupotenzial des aeroben oberflächenahen Grundwasserleiters in mikrobiellen Anreicherungskulturen untersucht, denen verschiedene Kohlenstoffquellen (C-quellen) zur Verfügung gestellt wurden (2.4). Die Mikroorganismen waren in der Lage, IPU sowohl als alleinige C-quelle, als auch co-metabolisch zu verstoffwechseln, einhergehend mit einer Zunahme der mikrobiellen Biomasse. Darüber hinaus konnte ich mittels einer Fingerprint-technik (DGGE) eine starke Verschiebung der mikrobiellen Population nachweisen (3.3). Der Versuchsansatz hat großes Potenzial, um zukünftig Bakterienstämme zu isolieren, die ein spezifisches Abbaupotenzial gegenüber einzelnen PSM besitzen.

**DANKSAGUNG**

INTRAC war ein Kooperationsprojekt zwischen der BayerCorpScience AG und dem Forschungszentrum Jülich und ich möchte dem Institutsleiter der Agrosphäre, Herrn Prof. Dr. Harry Vereecken, ganz herzlich für die Möglichkeit danken, die Arbeit an seinem Institut durchzuführen. Darüber hinaus hat Herr PD. Dr. Peter Burauel wohl maßgeblich dazu beigetragen, dass ich den Weg nach Jülich gefunden habe und ich letztendlich hier - wenn jetzt auch in ganz anderer Funktion - meine Heimat gefunden habe. Peter auch Dir herzlichen Dank für Dein Engagement. Auch wenn es jetzt etwas länger gedauert hat, ist es ist Dir sicherlich ein Trost, dass Deine Abteilung auch eine exzellente Schule für Wissenschaftsmanager ist. Erwähnen möchte ich auch die finanzielle Unterstützung der BayerCropScience AG, ohne die diese Arbeit nicht möglich gewesen wäre. Ich danke den Kollegen Herrn Dr. Fritz, Herrn Dr. Stork, Herrn Prof. Dr. Babczinski und Herrn Sneikus vom Institut für Metabolismusforschung und Umweltverhalten, die mit ihrer Expertise zum Gelingen dieser Arbeit beigetragen haben. Bei mir entstand der Wunsch sich zu bedanken im Wesentlichen aus dem Gefühl heraus endlich fertig zu sein, und ich möchte hier vor allem Wolfgang Tappe aus der Agrosphäre danken. Danken für die zahllosen Diskussion, seine guten Ideen - die das Projekt ganz entscheidend mitgeprägt haben – und für seinen großen persönlichen Einsatz in Sachen INTRAC. Hier möchte ich mich auch bei Joost Groeneweg, Thomas Muckenheim und Sirgit Kummer bedanken, die mich - jeder auf seine Art und Weise - bei den mikrobiologischen Fragestellungen unterstützt haben. Radioagronomie und die damit zusammenhängende Expertise findet man in der Agrosphäre am Besten bei Werner Mittelstaedt, Ulrike Langen, Martina Krause and Stefan Köppchen. Allen einen ganz herzlichen Dank für Ihre Hilfe, die mich in die Tiefen von LSC, HPLC und LS-MS geführt haben. Die Zusammenarbeit mit diesem Team hat mir immer besonders viel Spaß gemacht, und es freut mich ganz besonders das DAISY bei Euch eine Zukunft gefunden hat. Im Bezug auf DAISY und die entwickelte Kontrolleinheit ist Ferdinand Engels, Norman Hermes and vor allem Ansgar Weuthen zu danken, die die Automatisierung von Seiten der Elektrotechnik umgesetzt haben. Ein Wort möchte ich noch zu den allgemeinen Arbeitsbedingungen im Forschungszentrum Jülich verlieren. Ich habe es immer als Privileg empfunden, in Jülich wissenschaftlich arbeiten zu dürfen. Ich kann deshalb nur jedem empfehlen, sich selbst einmal einen Eindruck von den exzellenten Möglichkeiten im Stetternicher Forst zu verschaffen. So habe ich hier unter anderem einen Holländer und Belgier angetroffen, die mit mir immer wieder während der Mittagpause um die Wette laufen wollten. Auch das gehört dazu und deshalb vielen Dank an Ros und Jan. Zum Schluss möchte ich noch allen Personen aus meinem privaten und kollegialen Umfeld danken, die mich in dieser Sache unterstützt haben. Danke Steffi, dass Du mich immer wieder bestärkt hast und mir den Rücken frei gehalten hast. Du hast ganz entscheidend dazu beigetragen, dass die Arbeit jetzt doch noch fertig geworden ist.

**CONTENT**

## Abbreviations and Symbols

| | |
|---|---|
| ACN | Acetonitrile |
| AR | Applied radioactivity |
| AI | Active ingredient |
| BA | Benzoic acid |
| CPA | Crop protection agent |
| cpm | Counts per minute |
| DAISY | Dynamic aquifer incubation system |
| DGGE | Denaturing gradient gel electrophoresis |
| DOC | Dissolved organic carbon |
| DKN | Diketonitrile |
| DM | Dry matter |
| DNA | Deoxyribonucleic acid |
| DT50 | Half-life time |
| GLP | Good laboratory practice |
| HCl | Hydrochloride acid |
| HPLC | High performance liquid chromatography |
| IMI | Imidacloprid |
| IPU | Isoproturon |
| K | Krauthausen |
| LC | Liquid chromatography |
| LSC | Liquid scintillation counting |
| LUFA | Landwirtschaftliche Untersuchungs- und Forschungsanstalt |
| M | Microcosm |
| MB | Mulder-Beilen |
| MBq | Mega Becquerel |
| mbs | Meter below surface |
| NA | Nature A |
| n.a. | not analyzed |
| n.c. | not calculated |
| n.d. | not detectable |
| NRW | North Rhine Westphalia |
| RA | Radioactivity |
| rpm | Revolutions per minute |
| SD | Standard deviation |
| TLC | Thin layer chromatography |
| UV | Ultraviolet |

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# 1 INTRODUCTION

Pollution of water bodies such as lakes, rivers, and groundwater is a major environmental problem. No access to clean water causes worldwide deaths and diseases (1;2) of about 14000 people daily (1). Humans depend on water, and the protection of water resources is becoming more and more a grand challenge for a sustainable life. Up to date, agricultural production accounts for the usage of 70% of the freshwater resources worldwide. Surface water and groundwater have often been studied and managed as separate resources, although they are a continuum (3). Aquifers that provide sustainable fresh groundwater to urban areas and for agricultural irrigation are typically close to the ground surface and depend on recharge by fresh water. This recharge is typically from rivers or meteoric water (precipitation) that percolates into the aquifer through overlying soils and unsaturated subsoils (4). For this reason, the water quality of shallow aquifers particularly depends on the filter and buffering capacity of the overlaying unsaturated matrix. Due to intensive agricultural practice that mostly comes along with intensive pest control, the filter and buffering capacity of soils can be overstrained and as a consequence thereof, contaminations of groundwater might occur (5).

Modern plant protection means guaranteeing sustainable plant production and simultaneously safeguarding the legitimate interests of consumer and environmental protection. The term plant protection comprises many preventative non-chemical measures and is much more than the use of chemical pesticides. Despite this, chemical pesticides are still one of the cornerstones of modern agriculture, and in Germany domestic sales rose e.g. from 29,769 t in 1994 to 40,744 t in 2007. The European Union has set drinking water standards of 0.1 µg $l^{-1}$ for an individual pesticide, and 0.5 µg $l^{-1}$ for the total number of pesticides and related substances to protect humans for any toxicological side effects of these substances (6). Application modes and physical-chemical behaviour should generally prevent the appearance of these crop protection (CPA) agents in e.g. groundwater reservoirs. However, groundwater contaminations occur (7), and there is only sparse knowledge about the intrinsic microbial degradation potential of aquifers (8). It is therefore even more surprising that aquifers near the surface have rarely been studied in terms of their degradation potential. One of the main reasons for this is the lack of an adequate study design permitting laboratory investigations under realistic conditions and a subsequent transfer of results to the field scale.

Near-natural aquifers are more or less black boxes with respect to e.g. treshold levels of low concentrated contaminants for the induction of potentially proceeding biological degradation processes, or even more with respect to their degradation kinetics.

In degradation studies, the application of radiolabeled test substances has been proven to be of exceptional value, especially when dealing with extremely low concentrations. But even if radiolabeled test substances are used, it is a special challenge to determine the metabolisation of CPA at concentrations in the range of ≤ 10 µg $l^{-1}$, as e.g. specific labeling intensities for organic molecules are limited due to the risk of radiolysis. The distribution of the applied radioactivity between different compartments (e.g. water, aquifer material, gas-phase) accounts for an additional challenge concerning the detection limits of the analytical methods applied (e.g. liquid scintillation counting, radio-HPLC).

**In this work**, the intrinsic microbial degradation potential of three near natural shallow aquifers was studied with respect to Isoproturon (IPU), Imidacloprid (IMI), and Diketonitrile (DKN). The studies were accomplished under simulated aquifer conditions at the lab-scale with $^{14}C$-labeled test compounds. In order to mimic the specific natural conditions of the respective aquifers at the lab-scale, a static and a so-called “dynamic” artifical aquifer system were developed. The test systems enabled a long term incubation (> 360 days), the use of $^{14}C$ radiolabeled test substances, and the recovery of mass balances. Both systems were checked on the basis of mass balances, determined in $^{14}C$ benzoic acid (BA) test series. BA is easily degraded by microorganisms (9-11), and can be used as a model compound for the degradation of aromatic molecules in aquifers (12). Prior to the experiments, the groundwater and aquifer materials were sampled and handled with special techniques that allowed for keeping their particular chemical and physical properties as close as possible to their natural conditions (2.2). An elaborate sampling device (sonic drill technique) prevented aquifer material from being contaminated with microorganisms from above soil layers.

## 1.1 BACKGROUND

The fate of CPA in topsoils is regularly studied at lab-, lysimeter-, or field-scale (15), and existing data sets focus on concentration ranges of about 100 µg $l^{-1}$ and higher. These results are non-transferable to metabolic rates of diffuse concentration loads ≤ 10 µg $l^{-1}$ in near-natural aquifers, although the physiological capacities of subsurface microorganisms and their potential to degrade xenobiotics seem to be the same, as those found in microorganisms from agricultural soils (14). Compared to topsoils, near-natural aquifers are characterized by e.g. a different availability of in- and organic nutrients, variable redox conditions, and minor changes in temperature (13;15-18). The dissolved organic carbon content (DOC) of the test aquifer Krauthausen (2.2.1) is e.g. just about 1.1 mg $l^{-1}$, which is roughly the equilibrium concentration between DOC input rate, typically in forms of recalcitrant humic substances, and microbial DOC degradation rate.

The pH is of special importance, which can determine the environmental behaviour of a xenobiotic, as e.g. described for sulfadiazine (19). The presence of aerobic, facultative aerobic, and anaerobic bacteria in aerobic aquifers suggests the existence of anaerobic microhabitats. This is additionally an important parameter to determine the microbiology of a site, which is governing the degradation pathway of a CPA (17). Reduced redox conditions favour e.g. the dehalogenation of chlorinated compounds (20), but on the other hand many CPA contain aromatic structures, which are more easily cleaved under aerobic than under anaerobic conditions (21). Nevertheless, the measurement of the redox potential (Eh) is complex, due to the relationship between Eh measured in bulk soil, and the concentration of $NO_3^-$, $Mn^{2+}$, $Fe^{2+}$, and $[SO_4]^{2-}$ in the soil solution (22-24).

Aquifers have a broad array of microbial metabolic capabilities that is comparable to agricultural soils, which is related to the complexity, and heterogeneity of the aquifer matrix itself (16). Microbial populations consist predominantly of bacteria, which have a central function in the biotic degradation of many CPA. They can live immobilised to sediment surfaces, or suspended in the groundwater, being at least mobile by the lateral movement of the groundwater (17). The population size is decreasing with increasing depth (17;25), and the horizontal distribution is also heterogeneous and differs greatly, e.g. as a function of soil and sediment type (14).

Subsurface bacteria appear to be rather inactive, but seem to be well adapted to the stringent oligotrophic conditions of their environment (26;27). The absence of a direct correlation between plate count numbers and degradation activity (culture media are selective for metabolic types of organisms, and some bacteria are even not able to produce colonies) illustrates that it is necessary to differentiate between metabolic active and inactive microorganisms (17). The mineralization of CPA generally requires multiple enzymatic steps (e.g. removal of side-chains, followed by ring cleavage), including reactions like e.g. hydroxylation, N-dealkylation, decarboxylation, dechlorination, beta-oxidation, ester-hydrolysis, or sulfoxidation (21;28). Several factors influence the time for enzyme induction (lag period), and synthesis prior to microbial degradation (29). Enzymatic modifications usually initiate the biodegradation of non-aromatic molecule structures (28), and several enzymes can degrade different structures, as described for the organophosphates, the phenoxyalkanoic acids, and a large group of related compounds including acetanilide, carbamates and phenylureas (30).

The concentration of the inducing CPA can enhance or accelerate biodegradation (29) in response to agricultural application rates at high frequencies (31). The microbial adaptation and cross-adaptation can lead to shortened lag phases, as well as enhanced degradation rates, as observed in pure cultures, aquatic environments, soils, and subsurface aquifers (29;32;33).

For example, the previous exposure to the herbicide EPTC (S-ethyl dipropyl (thiocarbamate)) resulted in the microbial adaptation, as evidenced by increased degradation and mineralization of EPTC (34). Shorter and more reproducible lag periods may reflect the time required for growth of a small population of bacteria, already having a specific degradation potential. On the other hand, extended and variable lag periods seem to indicate that microorganisms are actually not capable to degrade the respective CPA. The time might be required to evolve the ability to degrade the CPA through mutation, genetic exchange, or selection (33). The author further reported that specific p-nitrophenol (PNP) degrading bacteria did not respond to the addition of PNP at low concentrations. The exposition of a small population of microorganisms to a low substrate concentration, under less than ideal conditions, did not lead to the expected enzyme induction and activation under near natural aquifer conditions. The threshold level, which is the minimum concentration necessary to produce a measurable physiological effect, was not reached.

The presence of inhibitors can also delay the induction of biodegradation by toxicity of the inhibitor itself, or as a competitive substrate for the specific microbes under consideration (29). On the other hand, if CPA are present in only very low concentrations, the addition of organic nutrients can also promote microbial degradation (35). However, the presence of a CPA at threshold levels (≤ 1 µg $l^{-1}$) must not necessarily lead to microbial metabolism, or co-metabolism (18). Instead, the amount of molecules in these habitats might slow their degradation by failing to induce, or activate enzymes, necessary for their degradation (36;37). If the concentration of the substance is additionally low, there may not be an increase in the total number of the heterotrophic bacteria, whereas the number of specific degraders may increase (38). Very low concentrations might also cause an increase in degradation rates, but without detectable increases in population size (36;37). Degradation kinetics can also change, as e.g. found for MCPP [(+/-)-2-(4-chloro-2-methylphenoxy) propanoic acid; mecoprop] and 2,4-D [2,4-dichlorophenoxyacetic acid]. Non-growth kinetics were typically found below 10 µg $l^{-1}$, whereas growth-linked kinetics with accelerating degradation rates were measured at higher concentrations (18;39). The authors suggested the existence of specific threshold concentrations to which microorganisms decompose a single substrate under steady state conditions. This is in accordance with calculated threshold concentrations of Schmidt et al. (40), who suggested a growth-linked degradation of approximately 0.2 µg $l^{-1}$ for many organic chemicals in an aquatic environment. In such cases, biotransformation is supposed to occur by co-metabolism as secondary substrate, constant and independently of the test substance concentration (41;42).

The mineralization of xenobiotics, either by microbial individuals or by linked consortia, depends on several factors, and it is speculated that four major variables regulate the rate of degradation in soils (17;43;44):

1.) The molecular structure of the CPA.
2.) The quantities and distribution of “reactive sites” in the ecosystem that can degrade the CPA (e.g. ions, microbial cells), as well as the accessibility at these sites.
3.) The degradability of CPA by living cells, as well as their activity.
4.) The fractions of the soil, like e.g. the mineral or organic matter content.

Factors regulating the variables are:

1.) The total amount of CPA.
2.) The test site itself.
3.) The available electron acceptors (e.g. oxygen).
4.) The ambient temperature.
5.) The water availability.
6.) The pH.

A guideline from the Netherlands recommends the use of $^{14}C$-labelled test compounds for aquifer degradation studies under different redox conditions at the lab-scale, using water-saturated batch (static), or column (dynamic) systems (45). Depending on the specific scientific question, each approach has its advantages and biases (15). The design of a batch system is simple, and studies can include a large number of replicates (12;46;47). However, batch systems can reflect only a limited part of the biotic and abiotic complexity, as they e.g. are not able to consider the probable impact of groundwater flow on degradation kinetics. The design of a column system is more complex, including a source reservoir, matrix space, waste container, and peristaltic pump. Breakthrough curves of CPA are determined by in- and effluent measurements that enables additionally a metabolite screening, but the number of treatments and applications is limited (48-53). However, aquifer studies should generally reflect natural aquifer conditions, in particular to the concentration of test subsances (≤ 10 µg $l^{-1}$) and flow conditions (38;53;54).

**In this work**, test systems were developed to assess the microbial degradation activity at static, as well as at dynamic flow conditions at the lab-scale. Liquid scintillation counting (LSC) was used to quantify the radioactivity, whereas high-performance liquid chromatography (HPLC) was used to identify active ingredients, metabolites, and their relative distribution.

The physiology of bacteria was additionally studied in small bioreactors, referred to as Retentostat, operated with complete biomass retention using internal cross-flow filtration (55). A cumulative selective enrichment of aquifer bacteria took place, due to the complete retention of the bacteria inside the bioreactor at continuous supply of different organic media (3.3). Growth conditions were reflecting to some extend the natural growth conditions concerning substrate availability inside the respective aquifer. The method ensured a continuous accumulation of the bacterial biomass at lowest growth rates to access the microbial aquifer degradation potential.

## 1.2 OBJECTIVES

**Artificial aquifer incubation systems at the lab-scale**: The first objective of the thesis was to develop a static, as well as a dynamic artificial aquifer test system at the lab-scale for standard use (Good Laboratory Practice, GLP). Both systems should be airtight, operable under aerobic and anaerobic conditions, and mimic natural aquifer conditions. The application range of the test systems was dedicated to near-natural aquifers, as at higher concentration (≥ 100 µg $l^{-1}$) clogging problems (due to microbial growth), or oxygen depletion (due to higher organic carbon turnover rates) were most likely.

**Aquifer degradation studies and their comparability**: The second objective was to establish standard methods for aquifer degradation studies concerning setup, and analytics to improve the comparability between the static and dynamic study design, as well as between different experimental approaches.

**The study of the current aquifer degradation activity**: The third objective was to study the intrinsic microbial degradation activity of shallow aquifers with respect to Isoproturon (IPU), Imidacloprid (IMI), and Diketonitrile (DKN) at the lab-scale under static and dynamic flow, as well as under oxygen, iron- and sulphur-reducing conditions at a concentration of 0.1, 1 and 10 µg $l^{-1}$. Results should represent the current microbial degradation activity of the selected aquifers Krauthausen, Mulder-Beilen, and Nature A at natural aquifer conditions.

**The study of the aquifer degradation potential**: Besides these three objectives, the microbial degradation potential was additionally studied under optimized growth conditions to characterize the degradation potential of the indigenous aquifer microorganisms. These experiments were not part of the thesis, and are illustrated only briefly.

# 2 MATERIAL AND METHODS

For the degradation studies, a lab facility, including an atmospheric chamber, was installed in the lysimeter basement of the institute (http://www.fz-juelich.de) to match natural aquifer conditions with respect to light (dark), and temperature throughout long-term operation. During the studies, the basement temperature was synchronized to the temperature amplitude in 1.5 mbs of the test field Merzenhausen (Forschungszentrum Jülich), as below this depth, temperature fluctuations are only marginal (56). The atmospheric chamber (Figure 11; page 48) was filled with forming gas (95/5 ⇔ $N_2/H_2$) to control iron- and sulphur reducing conditions during incubation (> 450 days). Besides the specifications of the sponsor, the degradation studies were set up in accordance to a guideline for the transformation of xenobiotics in aquifers by means of incubation studies, issued in the Netherlands (45).

## 2.1 TEST SUBSTANCES

The following chapter will summarize some key facts about the selected test substances, which were provided by the sponsor (BayerCropScience).

### 2.1.1 BENZOIC ACID

Test substance was [$^{14}$C-UL] benzoic acid, and [phenyl-UL-$^{14}$C] benzoic acid.

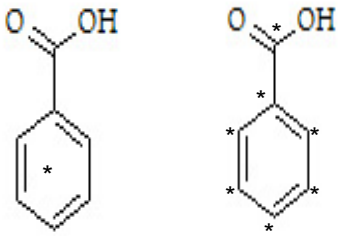



**Figure 1: Benzoic acid: Chemical structure and label position***

| | |
|---|---|
| Nomenclature: | Common name: Benzoic acid (9) |
| Other names: | Benzene carboxylic acid, phenyl carboxylic acid |
| Molecular weight: | 122.12 g $mol^{-1}$ |
| Molecular formula: | $C_7H_6O_2$ |
| Form: | White, needle-like crystals |
| Melting point: | 122°C |
| Boiling point: | 249°C |

| | |
|---|---|
| Solubility: | In water: 2.9 g $l^{-1}$ (20 °C); 69 g $l^{-1}$ (95 °C) |
| Stability: | Stable under common conditions of use, and storage |
| Vapour pressure: | 0.11 to 0.53 Pa (20°C) |
| log Kow: | 1.9 |
| Specific radioactivity: | 120-mCi $mmol^{-1}$ |
| Radiochemical purity: | > 99% |

Benzoic acid (BA) is mainly known as a food preservative, but it occurs naturally as well in many plants and animals. Well established derivatives are salicylic acid, 2-O-acetylsalicyclic acid, also known as aspirin, and as food additive E210 as pure compound, and E211, E212, or E213 as salts (11). BA is easily degradable by microorganisms, and it is taken up either by passive diffusion, or via highly specific permeases (57). The degradation pathway is supposed to be via aerobic, as well as anaerobic microbial mineralization (58). The aerobic metabolism is characterized by an extensive demand of molecular oxygen, essential for the hydroxylation, and activation of the aromatic ring structure, leading to a small number of different aromatic intermediates (59;60). The most common is benzoyl-CoA, a central intermediate in the degradation pathway of a large number of aromatic substrates (61). The anaerobic degradation starts with the activation of benzoic acid to benzoyl-CoA by a benzoate-CoA ligase (62). The subsequent ring reduction to a non-aromatic compound can differ in several ways (60). The volatilization and adsorption potential to sediment or soil particles is low (9), and mobility studies of $^{14}C$-labeled BA in different soils showed that BA is moderately mobile. The mobility increases with pH increase, whereas it decreases with anion exchange capacity increase (11;63;64). A study with an alkaline soil showed a mineralization up to 63% within 10 weeks, initial concentration 2 mg 100 $g^{-1}$ (65). At aerobic conditions, the calculated $DT_{50}$ in groundwater was 41 hrs, initial concentration from 1 to 100 µg $l^{-1}$ (66). Described half-life times ranged from 7.2 to 76.8 hrs in a sandy soil, and the mineralization did not vary in any systematic way with depth. Mineralization kinetics in the saturated and overlying vadose zone were quite similar and included no lag period. The author concluded that most of the microbes are already adapted to the utilization of aromatic substrates, as a high proportion of humic substances include aromatic moieties (67). BA was also included as a model compound in a chalk aerobic aquifer study, and was mineralized up to 60% within 144 days in all depths (12). Several microorganisms are isolated which are able to degrade BA. Among others, they include fungal species such as *Rhodotorula glutinis*, other yeast-like fungi (68), the mould *Penicillium frequentans* (69), and bacteria such as *Alcaligenes denitrificans* (57), *Rhodopseudomonas palustris*, and several strains of denitrifying *pseudomonads* (58;60;61;65).

## 2.1.2 ISOPROTURON

Test substance is [phenyl-UL-$^{14}$C] Isoproturon (70).

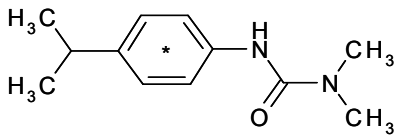



**Figure 2: Isoproturon: Chemical structure and label position***

| | |
|---|---|
| Nomenclature: | Common name: Isoproturon |
| Chemical name (IUPAC): | 3 -(4-isopropylphenyl)-1,1-dimethylurea |
| Molecular weight: | 206.3 g $mol^{-1}$ |
| Molecular formula: | $C_{12}H_{18}N_2O$ |
| Form: | Colourless crystals |
| Melting point: | 158°C; (technical 153-156 °C) |
| Vapour pressure: | 3.15 x $10^{-3}$ mPa (20 °C); 8.1 x $10^{-3}$ mPa (25 °C) |
| log Kow: | 2.5 (20 °C) |
| Henry: | 1.46 x $10^{-5}$ Pa $m^3$ $mol^{-1}$ |
| Density: | 1.2 (20 °C) |
| Solubility: | In water: 65 mg $l^{-1}$ (22 °C) |
| Stability: | Very stable to light, acids, and alkalis |
| Specific radioactivity | 12.3 MBq $mg^{-1}$ |
| Radiochemical purity | > 99% |

The non-polar phenylurea herbicide Isoproturon (IPU) is used for pre- and post emergence control of annual grasses and broad-leaved weeds in wheat, rye, and barley crops (70). It is widely used, and frequently detected in ground- and surface water, exceeding the drinking water limit of the European Commission (5). This is probably due to its moderate persistence, as well as relatively low adsorption to soil particles (70). The persistence for at least 3 years after treatment, as well as a leaching potential was e.g. shown in a field study from the UK (71). Microbial degradation is the significant mechanism for the dissipation of Isoproturon from soil (72-74), although photochemical, as well as chemical processes can be involved (75;76). The mineralization of the phenyl-moiety is slow, and its extent is often limited. It achieves about 5 to 25% within 2 and 3 months, often attended by the formation of large fractions of bound residues (71;77).

The mineralization in soil includes the metabolic pathway of N-demethylation to N-(4-isopropylphenyl)-N-methylurea (MDIPU), and hydroxylation to 3-(4-(2-hydroxyisopropyl)-phenyl)-1.1-dimethylurea (74;78;79), which are subsequently degraded to several other metabolites, including DDIPU, and 4IA (80;81). The mineralization potential decreases with increasing depth, and is either absent or slow in groundwater and aquifers (12;80;82-84). Johnsen et al. (82) observed a small but significant sorption together with a partial mineralization of IPU in a chalk aquifer, leading to the accumulation of MDIPU. Kristensen et al. (12) reported about the vertical mineralization potential of IPU in a chalk aquifer (0.15 to 4.45 mbs). They measured a mineralization up to 37% in the plough layer, and up to 6% in the chalk, attended by lag periods of at least 40 days within 258 days. This is in accordance to a study with sandy aquifer material (0.06 to 7.68 mbs) from Denmark (52), where the mineralization was greatest in the plough layer (upper 0.30 m), and reached about 14% within 267 days. IPU was recalcitrant, and showed a small time-depending sorption in a column study with sandy, gravely aquifer material within 140 days. IPU was not mineralized in batch study with a sandy aquifer profile (0 to 7.7 mbs) from Denmark within 270 days, as well as in a batch study with in-situ groundwater and aquifer material, whether incubated at aerobic or anaerobic conditions (85). Larsen et al. (86) measured that IPU was not anaerobically mineralized in the presence of nitrate with sandy aquifer material within 312 days, and Larson and Aamand (83) failed to detect any mineralization with denitrifying, sulphate reducing or methanogenic aquifer material within 312 days at 10°C. They assumed that the cleavage of the aromatic ring, according to current knowledge, is an oxygen demanding step. Several studies failed to isolate IPU degrading microorganisms in liquid cultures (75;80). This could be related to the insufficient exposure of the used soils towards IPU (81;87), as a mixed bacterial culture was isolated from a field regularly treated with IPU for over 10 years, having the ability to mineralize IPU (31). The high pH of the field (> 7) possibly favoured specific bacteria, suggesting an in-situ adaption upon highly repeated application to the metabolism of IPU (31;72;81;88). Sorensen et al. (89) isolated the bacteria strain SRS2 from the mixed culture that mineralized about 50% of IPU by successive N-demethylation, cleavage of the urea side chain and mineralization of the phenyl structure. They identified SRS2 as a *Sphingomonas sp*., a microorganism that belongs to the alpha-subdivision of the *proteobacteria*. SRS2 is unable to grow on rich media, suggesting the adaptation to an oligotrophic environment. If the strain was cultured together with strain SRS1, isolated from the same field and unable to degrade IPU or its known metabolites, the mineralization of IPU was significantly enhanced. The authors concluded that SRS2 might be auxotrophic, and required components supplied by synergistic interactions between the two strains (90).

The narrow pH optimum of SRS2 (7 to 7.5) might be responsible for the spatial variation of degradation rates across the field (91;92). The aging of IPU, MDIPU and 4IA in an agricultural soil study for 131 days implied that the partial degradation might lead to a reduced mineralization by SRS2, due to sorption (93). These suggestions are in accordance with results of Walker et al. (84), who reported a significant negative linear relationship between $DT_{50}$ values (from 6 to 30 days), and soil pH in an agricultural soil previously treated regularly with IPU for over 8 years.

### 2.1.3 IMIDACLOPRID

Test substance is [methylene-$^{14}$C] Imidacloprid (70).

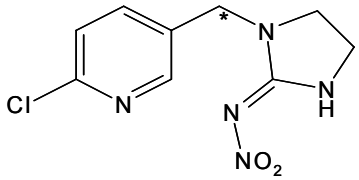



**Figure 3: Imidacloprid: Chemical structure and label position***

| | |
|---|---|
| Nomenclature: | Common name: Imidacloprid |
| Chemical name (IUPAC): | 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine |
| Molecular weight: | 255.7 g $mol^{-1}$ |
| Molecular formula: | $C_9H_{10}ClN_5O_2$ |
| Melting point: | 144°C |
| Vapour pressure: | 4 x $10^{-7}$ mPa (20°C); 9 x $10^{-7}$ mPa (25°C) |
| log $K_{ow}$: | 0.57 (21°C) |
| Solubility: | In water: 0.61 g $l^{-1}$ (20°C) |
| Stability: | Stable to hydrolysis at pH 5 - 11 |
| Specific radioactivity: | 4.11 MBq $mg^{-1}$ |
| Radiochemical purity: | > 99% |

Imidacloprid (IMI) is a polar, systemic, chloro-nicotinyl insecticide used in soil, seed, and foliar for the control of sucking insects, including rice hoppers, aphids, thrips, whiteflies, termites, turf insects, soil insects, and some beetles (94). It is affecting the transmission of stimuli in the insect nervous system by the blockage in a type of neuronal pathways (nicotinergic). These are more abundant in insects than in warm-blooded animals, making the chemical more toxic to insects than to warm-blooded animals.

The blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insects paralysis, and eventually death (95). The main elimination pathway is due to microbial degradation, and half-life times ($DT_{50}$) depend on e.g. the soil type, the use of organic fertilizers, and the presence or absence of ground cover (96-102). The absence of any detectable metabolite accounting for more than 10% of applied amounts indicates that the primary attack of the parent molecule determines the aerobic degradation kinetic (main metabolites in soil include Imidacloprid-urea (1-(6-chloro-3-pyridinylmethyl)-2-imidazolidinone), 6 chloro-nicotinyl acid, and 6-hydroxynicotinic acid). First steps of the anaerobic degradation are promoted, leading to the formation of Desnitro Imidacloprid, and no new degradation products, not already known from aerobic degradation, are formed (103-105). Lee et. al. (99) studied the microbial degradation of IMI in a field lysimeter, and postulated the accumulation of intermediate anaerobic degradation products. They measured an increase in $^{14}CO_2$ evolution, when the redox conditions switched from anaerobic to aerobic. $DT_{50}$ of different field studies ranged between 74 and 174 days, and the presence of a ground cover shortened $DT_{50}$ (48 days) by a factor of three. The degradation via photolysis resulted in a $DT_{50}$ of 39 days (103;106). A desorption hysteresis is reported for IMI, and for its metabolites that is increasing with incubation time (107-110). Low application rates show highest sorption (111;112), affected by smectite, as well as organic carbon content (109;110;113;114). The physical-chemical properties may indicate a mobility of IMI, but column-, field-, and lysimeter studies did not confirm a leaching potential into deeper soil layers (115;116). Only Jenkins (117) suggested a leaching potential in sensitive soils, such as porous or gravelly soils, depending on irrigation practices and preferential flow.

### 2.1.4 DIKETONITRILE

Test substance is [phenyl-UL-$^{14}$C] Diketonitrile (70).

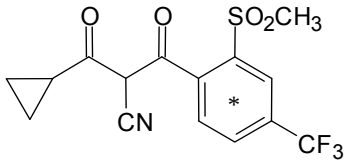



**Figure 4: Diketonitrile: Chemical structure and label position***

| | |
|---|---|
| Nomenclature: | Common name: Diketonitrile<br>(Main metabolite and active ingredient of the herbicide Isoxaflutole) |
| Chemical name (IUPAC): | 2-cyano-3-cyclopropyl-1-(2-methanesulfonyl-4-trifluoromethyl-phenyl) propane-1.3-dione |

| | |
|---|---|
| Molecular weight: | 206.3 g $mol^{-1}$ |
| Molecular formula: | $C_{12}H_{18}N_2O$ |
| log Kow: | 0.4 |
| Solubility: | In water: 326 mg $l^{-1}$ |
| Specific radioactivity | 4.3 MBq $mg^{-1}$ |
| Radiochemical purity | > 98% |

Diketonitrile (DKN) is the main metabolite, and active ingredient of the pre-emergence herbicide Isoxaflutole (5-cyclopropyl-1.2-oxazol-4-yl α,α,α-trifluoro-2-mesyl-p-tolyl ketone). Isoxaflutole (ISO) belongs to the isoxazole class of herbicides, and is applied at low doses (between 75 and 150 g $ha^{-1}$) for grass and broadleaf control in corn (maize) and sugar cane (118). DKN is a potent inhibitor of the primary molecular target 4-hydroxyphenylpyruvate dioxygenase (HPPD). The inhibition affects the carotenoid biosynthesis, resulting in bleaching of newly developed leaves, followed by growth suppression and necrosis prior to the plant death (119;120). The reduction of the carotenoid and chlorophyll content is associated with an indirect in vivo inhibition of the phytoene desaturase by the depletion of its essential co-factor plastoquinone, caused by the inhibition of HPPD (119;121).

In soil, water, and plants, ISO converts fast to the more mobile DKN that hydrolysis to 2-methanesulonyl-4-trifluoromethyl benzoic acid, a herbicidal inactive benzoic acid derivative (122). A grass treatment did not promote the hydrolysis in a lysimeter study, but significantly influenced the transport of DKN through enhanced evapotranspiration (123). The author assessed a poor correlation between the conversion of DKN and the microbial biomass, suggesting a mainly abiotic process. A hypothetical pathway for the chemical cleavage may involve an oxidative mechanism and the possible involvement of fungal oxidases (124). In contrary, Taylor-Lovell et al. (125) reported about a reduced conversion in abiotic controls and concluded that the dissipation of DKN is primarily a biological process. In agreement, Beltran et al. (126;127) concluded that the isomerisation of ISO is a chemical process, whereas the subsequent conversion of DKN is biologically mediated (128;129). They assessed a strong correlation between conversion and the degree of susceptibility, being most rapid in tolerant corn and slowest in susceptible *Abutilon theophrasti*. DKN laboratory half-life times ($DT_{50}$) range between 20 and 30 days (122;130). Mitra et al. (131-133) increased the reversible sorption of DKN by the amendment of organic matter. They concluded a positive sorption-desorption hysteresis, influenced primary by the organic matter and secondly by the clay content. DKN has a higher mobility in soil compared to ISO, and strong precipitation events seem to enhance the leaching potential (125) that decreases, as the organic matter- and clay content increases (133).

### 2.1.5 APPLICATION

Isoproturon (IPU), Imidacloprid (IMI), and Diketonitrile (DKN) were provided as powders, which were diluted in distilled water, whereas benzoic acid (BA) was solved in ethanol. To avoid the addition of extra organic carbon to the oligotrophic aquifer environment, the ethanol was evaporated by $N_{2,}$ and BA was resolved in double distilled water. Stock and application solutions of all test substances were directly prepared before application, and radioactivity and radiochemical purity were checked by LSC, and HPLC. It was impossible to install an analytical balance in the lysimeter hall, and the test substances were directly applied into the groundwater by a pipette (METTLER TOLEDO, Rainin, EDP3), as well as into a vial (PACKARD) to check the applied radioactivity by LSC.

## 2.2 AQUIFERS AND AQUIFER MATERIALS

Aquifer materials and groundwater were sampled from the water-saturated zone by the use of special sampling techniques to avoid the affection of microorganisms originating from topsoil or other layers. The aerobic aquifer material was sampled by a sonic drill technique (Figure 6; page 35), whereas the groundwater was collected from an already installed well by a peristaltic pump (Figure 21; page 126). Anaerobic aquifers were sampled by J. Smelt from the Alterra Institute Wageningen (134), who developed a special method to sample anaerobic aquifer material and groundwater from the water saturated zone (Figures 23, 24; pages 127, 128). For all aquifer materials, temperature (10°C), moisture content (water saturated), and lighting conditions (dark) were maintained during sampling, transport, storage, and long-term operation. Groundwater and aquifer materials were analyzed by LUFA NRW, BayerCropScience, and Forschungszentrum Jülich to determine the physical and chemical characteristics (Tables 10 - 13; pages 78 - 81).

### 2.2.1 AEROBIC TEST SITE KRAUTHAUSEN

**Krauthausen**: Near the city of Jülich, province North Rhine-Westphalia
Coordinates: N latitude 52 ° 38’ 025’’; E longitude 25° 30’ 079’’
Contact: Forschungszentrum Jülich GmbH, ICG-4, Leo Brand Strasse, D-52425 Jülich

As a part of the geological so-called “Zwischenscholle”, the gravel, sandy aquifer “Krauthausen” is located in the southern part of the Lower Rhine region Germany, approximately 10 km northwest of the city of Düren, and 7 km southeast from the Forschungszentrum Jülich between the river Rur, and the opencast Hambach.

The test site (Figure 5; page 34) is about 200 × 70 m in size, and has a permanent grass cover (fallow land), influenced by agricultural activity of the surrounding area (production of sugar beets, winter wheat, and barley). Flood plain deposits at the top, and a clay layer at the bottom, originated from the branched Rur river system, limit the first aquifer, which has a thickness of about 9 m (Figure 20; page 125).

**Figure 5: Aerobic test site Krauthausen**

The average annual precipitation in this area is about 690 mm, and the groundwater table varies between 1 and 3 mbs throughout the year. Mean groundwater velocity is about 2 m per day, but geo-electric and seismic tomography showed locations with high and low groundwater flow velocities (135;136). The groundwater has a high bicarbonate content, and the pH is about 6.8 (Table 9; page 77). Intensive agricultural land use of the region causes high concentrations of nitrate. Content of dissolved oxygen increases up to 7.7 mg $l^{-1}$, and Eh values vary between 250 mV and 300 mV. Clay and silt content of the aquifer varies between 0.5 and 7.5%, and the mean total porosity is 26% ± 7%. Mean cationic exchange capacity of aquifer sediments is 0.44 meq 100 $g^{-1}$, and mean specific surface is 0.7 $m^2$ $g^{-1}$ (137).

The aquifer material was sampled from the water-saturated zone from 4 to 6.5 meter below surface (mbs) by the use of a sonic drill module (Figure 6; page 35). The device generated vibrations (200 Hz) that caused the fluidization of the surrounding sediment (1 to 5 mm), which reduced the friction at the head of a special sampler, referred to as aqua lock sample system (Figure 25; page 128). For the groundwater, a plastic tube was implemented into an observation well of the test field (Figure 21; page 126), and a Teflon® tube was released inside.

Afterwards, the groundwater was sampled by a peristaltic pump (6 l $h^{-1}$) that was finally stored in sterilized bottles (SCHOTT, 10 l).

**Figure 6: Aerobic aquifer material sampling by Axiom & Eijkelkamp**

### 2.2.2 ANAEROBIC TEST SITE MULDER-BEILEN AND NATURE A

**Mulder-Beilen**: Near the village of Beilen in the province Drenthe (NL)
Coordinates: N latitude 52 ° 51' 6.393''; E longitude 6° 28' 57.410'' (Bessel)
Contact: Mr. J.H. Mulder, De Musels 3, 9411 VN Beilen

The agricultural field above the aquifer **Mulder-Beilen** is situated in the "valley" of a small water stream, and has always been used as a pasture. The sandy aquifer is directly in contact with the peat topsoil (70 to 80 cm), and might sometimes include a thin layer of silt at 1.3 to 1.5 mbs (Figure 7; page 35). The groundwater table varies between 0.7 and 1.3 mbs, and Eh values, measured by platinum electrode, as well as flow-through cell, vary between 228 mV and 245 mV, and 39 mV and 95 mV, respectively. A quantitative iron/sulphur test confirmed iron-reducing conditions (Table 9; page 77). Pesticide use (mecoprop, MCPA, or glyphosate) was minimal, and restricted on spots to control undesired plant species. Groundwater analysis by the sponsor showed no IMI, and IPU concentrations > 50 ng $l^{-1}$.

**Figure 7: Anaerobic test site Mulder-Beilen**

**Nature A**: Near village of Slootdorp, province N-Holland (NL)
Coordinates: N latitude 52° 51' 25,284''; E longitude 4° 44' 44,944'' (Bessel)
Contact: Hoogheemraadschap (NL) Noorderkwartier, Gorslaan 60; 1441 RG Purmerend

This aquifer **Nature A** does not underlie an agricultural field, but a strip of natural land (about 60 m wide), grown with trees and wild plants (Figure 8; page 36). The test site is situated on a slope between two agricultural areas with different surface levels that are used as grassland alternated with flower-bulb growing (Figure 22; page 127). At the sampling spot, a clay layer exists under the loamy and humic topsoil (about 30 cm), which reaches to about 1.8 mbs with a higher clay content at greater depth, and a very dark colour (reduced and with some organic matter). Apparently, the clay layer acts as a barrier for the (upper) groundwater flow from the area with the higher surface level. The fine to middle fine sand layer beneath (with some shells) appeared strongly reduced, and Eh values, measured by platinum electrode, as well as flow-through cell, vary between 173 mV and 187 mV, and -86 mV and 4 mV, respectively (Table 9; page 77). A quantitative iron/sulphur test also confirmed sulphur reducing conditions, and groundwater analysis by the sponsor showed no IMI and IPU concentrations > 50 ng $l^{-1}$.

**Figure 8: Anaerobic test site Nature A**

**Aquifer materials and groundwater were sampled** by digging a ditch until the groundwater level was reached. Afterwards, a hole, which was stabilized by a casing tube, was further deepened by a special bailer (Figure 24; page 128). Aquifer materials were collected from the specific strata by a special core sampling tube, while $N_2$ was injected into the hole to prevent oxygen contact. Aquifer materials were stored in polyethylene containers, which were flushed by argon gas, again to minimize oxygen contact (Figure 9; page 37). A peristaltic pump was finally used to sample the groundwater that were then stored in sterilized flasks (2.5 l).

**Figure 9: Anaerobic aquifer material sampling by J. Smelt**

### 2.2.3 DRY MATTER CONTENT

The dry matter content (%) was calculated by the following equation:

$$\text{Dry matter content [\%]} = \frac{\text{(initial weight + tare weight - gross weight)}}{\text{(gross weight - tare weight)}} * 100$$

**Table 1: Dry matter content – calculation**

Petri dishes (3 x) were scaled (tare weight), and wet aquifer material (50 g) was weighed in (initial weight) to determine the dry matter content (DM) for each charge of aquifer material. Samples were dried to constant weight (105°C, 12 hrs), and gross weights were determined after the samples were cooled down in an exsiccator.

### 2.2.4 CARBON DIOXIDE IN AQUEOUS SOLUTIONS AND MODIFICATION OF GROUNDWATER

The solubility of $CO_2$ in aqueous solutions depends upon several aspects (138), as:

1.) The pressure of $CO_2$ in equilibrium with the solution - the solubility increases with the pressure.
2.) The temperature – the solubility decreases with increasing temperature.
3.) The pH – the solubility increases with increasing pH (133;139).

In aqueous solution, $CO_2$ exists in the following different species:

1.) $CO_2$ (g) $\Leftrightarrow$ $CO_2$ (l)
2.) $CO_2$ (l) + $H_2O$ $\Leftrightarrow$ $H_2CO_3$
3.) $H_2CO_3$+ $H_2O$ $\Leftrightarrow$ $H_3O^+$ + $HCO_3^-$ $pK_{a1}$ (25°C) = 6.37
4.) $HCO_3^-$ + $H_2O$ $\Leftrightarrow$ $H_3O^+$ + $CO_3^{2-}$ $pK_{a2}$ (25°C) = 10.25

First, it simply dissolves (1), related to a chemical equilibrium with carbonic acid (2). At equilibrium, most of the $CO_2$ remains as solvated molecular $CO_2$, and only a small fraction (0.2 to 1%) is converted into carbonic acid ($H_2CO_3$) that dissociates in two steps (3/4). Bicarbonate ($HCO_3^-$), and carbonate ($CO_3^{2-}$) are dominating at a pH > 8.3, $CO_3^{2-}$ at a pH > 12.3, and $CO_2$ at a pH < 4.3 (Figure 42; page 144). All these carbonate anions can interact with the cationic present in the groundwater to form insoluble carbonates (e.g. limestone ($CaCO_3$), or manganese carbonate ($MgCO_3$) (142).
The sampling of aquifer materials and groundwater from deeper layers (2.2) was affected by an atmospheric pressure change for $CO_2$ that resulted in a minor solubility of $CO_2$, which finally increased the pH of the collected groundwater (Table 14; page 81). This “natural” increase was stronger for the aquifer Krauthausen, probably due to the lower sampling depth, and could not be prevented, as the studies were accomplished at the normal atmospheric pressure.
Sodium hydroxide and soda lime are strong sinks for $CO_2$, and both are regularly used in degradation studies with $^{14}C$-labelled test compounds to absorb mineralized $^{14}CO_2$ from the gas phase (6;12;13;17;53;140;141). However, the benzoic acid (BA) studies had shown that the continuous absorption of $CO_2$ by the use of $CO_2$ absorber traps resulted in a displacement of the groundwater chemical equilibrium (Table 15; page 81).

The increase of the groundwater pH was correlated with an increase of the hydroxide ion concentration, which was related to the transformation of bicarbonate that was caused by the diffusion potential of the $CO_2$ sinks, strongly depending on incubation time (2.2.4). The groundwater samples were modified to stabilize the pH as follows: A magnetic stirrer was used to warm up the acidified (HCL, 6 M) groundwater (50 to 60°C) in order to support the degassing of $CO_2$ by $N_2$ (aquifer Mulder-Beilen and Nature A), or synthetic air (aquifer Krauthausen). After 12 hrs, groundwater samples were cooled down to about 10°C, and sodium hydroxide (1 M) was added to adjust the pH to about 7. $Na_2HPO_4$ and $KH_2PO_4$, (each 1 mM) were finally added to re-buffer the groundwater to pH 6.8.

## 2.3 DETERMINATION OF RADIOACTIVITY

The following chapter provides information about the detection of the applied radioactivity by liquid scintillation counting (LSC), and radio high performance liquid chromatography (HPLC). Both methods were used to accomplish quantitative (LSC) and qualitative (HPLC) analysis.

### 2.3.1 LIQUID SCINTILLATION COUNTING

$^{14}C$ measurements were accomplished by a liquid scintillation counter (CANBERRA, TRI-CARBTM 2500TR) with quench correction by means of sample-specific calibration series, and external standardization. For the measurement, the sample solutions were mixed with a scintillation cocktail, typically composed of an aromatic organic solvent, a scintillation substance, and suitable solubilizers. The detection limit of the liquid scintillation counter (LSC) is defined as the sense of a discrimination limit of the smallest quantifiable amount of radioactivity, differentiated from a zero value in a statistically significant manner. Based on the double zero value, this amount was 0.4 Bq for all measured LSC samples. Specific background values were determined by measuring blank samples that matched the study samples as close as possible in terms of cocktail, vial type, volume, and sample composition and standards of known $^{14}C$ radioactivity were additionally measured for internal standardization.

#### 2.3.1.1 $^{14}CO_2$ GAS PHASE AND DISSOLVED $^{14}CO_2$

Aqueous sodium hydroxide (NaOH) solution (1.5 ml, molarity (M) 0.5) was filled into test tubes (5 ml) to absorb **$^{14}CO_2$ from the gas phase** of the static, as well as dynamic test systems after the following equation:

$$2NaOH + CO_2 + H_2O \Leftrightarrow Na_2CO_3 + 2H_2O$$

The test tubes were processed as follows: The NaOH solution was transferred into LSC vials (PACKARD), and the tubes were twice rinsed by distilled water (5 ml) to trap $^{14}C$ residuals. The water additionally enhanced the solubility of carbonates, as only single phases are measurable by LSC. Afterwards, INSTA-Gel plus (10 ml) was added to the samples, and $^{14}CO_2$ gas phase was measured by LSC.

**Dissolved $^{14}CO_2$ from the groundwater and the extracts** was measured after the same principle, following the separation of dissolved organic $^{14}C$. Liquid samples were carefully filled into flasks (SCHOTT, Duran) that were immediately closed airtight. Temperature and atmospheric pressure were kept constant, and sample disturbance was minimized to avoid losses of dissolved $CO_2$. Afterwards, a syringe was used to acidify the samples (HCL, 37%) via a septum, and ultra sonic waves (ROTH, Elmasonic S 15, 3 x 5 min) were used to release the dissolved $^{14}CO_2$, which was then trapped in aqueous NaOH solution. The procedure ensured to measure even the smallest amounts, as the dissolved $^{14}CO_2$ was trapped from the entire sample volume.

The **absorption kinetic** of the NaOH traps was determined by a sodium bicarbonate ($NaH^{14}CO_3$) study (0.5 - 11 hrs) to fix the required absorption time after the release of the dissolved $^{14}CO_2$. As more than 50% of the released $^{14}CO_2$ was absorbed from the gas phase within 1.5 hrs, and about 99% within 11 hrs (Figure 27; page 129) the absorption time was fixed to 12 hrs. The total **absorption capacity** of the NaOH traps was additionally calculated on molari masses, and the maximum applied quantities of Isoproturon ($C_{12}H_{18}N_2O$), Imidacloprid ($C_9H_{10}ClN_5O_2$), and Diketonitrile ($C_{15}H_{12}F_3NO_4S$), which were all $^{14}C$ ring-labeled at only one atom (2.1). For the calculation, it was assumed that 100% of the relative carbon content of IPU (70%), IMI (42%), and DKN (50%) were converted. Results confirmed that the quantity, and molarity of the NaOH trap was sufficient to aborb 100% of the relative carbon contents, even if the test substances were applied in a mixture at a concentration of 10 µg $l^{-1}$ ($7.5 \times 10^{-3}$ Mol sodium hydroxide ⇔ approximately $1.53 \times 10^{-9}$ Mol $CO_2$ Isoproturon, $7.39 \times 10^{-10}$ Mol $CO_2$ Imidacloprid, and $6.26 \times 10^{-10}$ Mol $CO_2$ Diketonitrile).

At the beginning of my thesis, two additional methods were developed to separate dissolved organic $^{14}C$ and dissolved $^{14}CO_2$. **Method A:** Quantities of dissolved organic $^{14}C$ and dissolved $^{14}CO_2$, as well as dissolved organic $^{14}C$ (after acidification with HCL, 37%, and outgassing with $N_2$ for 45 min) were measured to calculate dissolved $^{14}CO_2$, as the difference of the two measurements. However, if the metabolic rates are very low, this method can not be recommended. LSC samples had a strong quench, and acidified blank samples had higher backgrounds than alkaline blank samples. Unfortunately, this effect was not linear, and it was not possible to establish a calibration curve.

**Method B:** Aliquots (20 ml) of the liquid samples were filled into vials (PACKARD), and placed into a customized separation device (Figure 43; page 144). Afterwards, the samples were acidified (HCL, 37%), and outgassed with $N_2$ (45 min) to trap the dissolved $^{14}CO_2$ in two vials, connected in-line, and filled with 15 ml OXYSOLVE scintillator (ZINSSER). Aliquots (3 x 5 ml) of the remaining liquids were finally mixed with INSTA-Gel plus (10 ml), and all samples were measured by LSC. This method had the advantage to process up to 20 samples on each run, but the couplings had to be replaced rapidly because they were corroded by the volatile scintillator solution.

#### 2.3.1.2 DISSOLVED ORGANIC $^{14}C$

Groundwater aliquots (20 ml) of the dynamic test system (2.5.2) were sampled, and stored in flasks (SCHOTT, 500 ml). After the separation of dissolved $^{14}CO_2$ (2.3.1.1), aliquots of the remaining groundwater (3 x 5 ml) were mixed with INSTA-Gel plus (10 ml) to quantify the dissolved organic $^{14}C$ by LSC. If the radioactivity was below the detection limit of LSC, the liquid samples were evaporated by the means of a Syncore® Analyst (BUECHI), which allowed for a smooth concentration of the samle volume down to pre-defined residual volumes of 0.3, 1.0, or 3.0 ml, respectively.

#### 2.3.1.3 EXTRACTABLE ORGANIC $^{14}C$

Aquifer materials were extracted three times with methanol (70 ml) to differentiate between the extractable, and non-extractable organic $^{14}C$. Acetonitrile and ethanol, which are generally used in degradation studies to extract solid sample material, did both not fit, as the sample composition (extract plus scintillator; INSTA Gel plus, Ultima Gold) influenced the LSC measurement. LSC quench and count per minute (CPM) rate were affected by phase separation, precipitation, and ionic strength of the sample itself. Aquifer materials were tranferred into centrifuge tubes (BECKMANN, 250 ml), and NaOH (0.5 M, 1 ml) was added (pH > 8.5) to exclude losses of dissolved $^{14}CO_2$. The samples were then placed for 30 min on a vertical shaker (BÜEHLER & OTTO, SM-30), centrifuged for 10 min at 14.000 rpm (BECKMANN, J2-21), and filtrated (fluted filter, typ 101, 240 mm). The aquifer materials were extracted twice again, and extracts were merged in flasks (SCHOTT, 500 ml). Afterwards, extractable organic $^{14}C$, and dissolved $^{14}CO_2$ were separated (2.3.1.1), and aliquots (3 x 5 ml) of the remaining extract were finally mixed with INSTA-Gel plus (10 ml) to measure the amount of extractable organic $^{14}C$ by LSC.

#### 2.3.1.4 NON-EXTRACTABLE ORGANIC $^{14}C$

Quantitative $^{14}C$ radioactivity measurements by LSC are based on liquid samples. Therefore, the non-extractable organic $^{14}C$ fraction was combusted, and trapped as $^{14}CO_2$ in scintillator solution by the use of an automated biological oxidizer (ZINSSER, Robox 192). Extracted aquifer materials were dried to constant weight (12 hrs, 105°C), and homogenized (15 min, 400 rpm) by the use of a vibratory disc mill (RETSCH, PM 400).

Aliquots (3 x 3 g) were then combusted, and the non-extractable organic $^{14}C$ was trapped as $^{14}CO_2$ in 15 ml OXYSOLVE scintillator (ZINSSER). Several samples ranged below the detection limit of the LSC, and it was not possible to establish a mass balance. This was due to the low applied radioactivity, organic carbon detection limit of the oxidizer (300 mg per single probe), as well as maximum sample size of the oxidizer (3 g). Specific background values were determined by blank samples for each run and aquifer, and standards of known $^{14}C$ radioactivity were additionally combusted for internal standardization.

### 2.3.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Radio high-performance liquid chromatography (HPLC) was used to identify active ingredients, metabolites, and their relative distribution. However, analysis were only accomplished, if the radioactivity of the samples exceeded the detection limit of HPLC (about 10 Bq per sample). Liquid samples were separated in a solid phase column under high pressure (MZ Analysentechnik Mainz, PefectSIL Target ODS-3, 3 µm, 150 * 4.6 mm) with the maximum HPLC injection volume (200 µl). The polarity of the mobile phase was gradually modified by means of a gradient program (Table 26; page 87), where the aqueous solution became increasingly non-polar after the addition of acetonitrile (flow 1 ml $min^{-1}$, 0.1 wt% aqueous $H_3PO_4$). Substances with a large number of polar OH groups, in this case the metabolites, were first routed to the detector after chromatographic separation. The detection was then performed by a radioactivity detector (BERTHOLD, type LB506C), equipped with a solid scintillator (yttrium glass), and the radioactivity of the injected sample was quantified by the area under a peak. Qualitative information concerning sample composition (relative distribution, integration % of all) was finally determined by a HPLC analysis program (WINFLOW™ Version 1.3, Radiochromatography Software*)*. If the radioactivity was below the detection limit of HPLC, the liquid samples were concentrated by freeze-drying. Sample solutions (20 ml) were filled into a vial (PACKARD), coated by cellulose cloths, and stored in a freezer (-18°C). The frozen samples were dried in a freeze-drying unit (STERIS, Lyovac GT2) to constant weight, and the radioactivity was resolved in 1 ml acetonitrile (ACN).

Precipitation, probably carbonates, remained undissolved, but were transferred, together with the acetonitrile, into an Eppendorf vial (2 ml). Samples were then centrifuged (5 min, 12000 rpm, HETTICH, Mikro Rapid), and the supernatants were used to rinse the vials (PACKARD) to trap $^{14}C$ residuals. Afterwards, the liquid samples were measured by HPLC, whereas the remaining precipitations were diluted in distilled water (5 ml), mixed with 10 ml INSTA-Gel plus (PACKARD), and measured by LSC to determine $^{14}C$ residuals.

## 2.4 THE INTRINSIC MICROBIAL DEGRADATION POTENTIAL - ENRICHMENT OF MICROBIAL AQUIFER COMMUNITIES IN BIOREACTORS

The following chapter provides information about the enrichment of microbial communities in small bioreactors (Figure 39; page 141). This method ensured a continuous separation and feedback of the bacterial biomass and high cell densities at lowest growth rates in long term continuous cultivations (143).

### 2.4.1 ENRICHMENT IN RETENTOSTATS

Microbial communities of the aquifer Krauthausen (2.2.1) were enriched according to Tappe et al. (55). Three Retentostats were filled with groundwater (300 ml), derived from observation well 69 of the test field Krauthausen (Figure 21; page 126). The microbial communities were enriched by a continuous supply of a specific media (144) that included peptone, yeast, and the selected CPA as organic carbon sources (Table 2; page 43).

| | Retentostat 1 (R1) | R2 | R3 |
|---|---|---|---|
| Peptone [mg $l^{-1}$] | 25 | 25 | - |
| Yeast [mg $l^{-1}$] | 25 | 25 | - |
| Isoproturon [mg $l^{-1}$] | - | 0.5 | 10 |
| Imidacloprid [mg $l^{-1}$] | - | 0.5 | 10 |
| Diketonitrile [mg $l^{-1}$] | - | 0.5 | 10 |

**Table 2: Enrichment cultures: Organic media components (10 l)**

Anorganic media components (Table 17; page 82) were added to double distilled water, and placed for 12 hrs on a stirrer to dissolve all components. Afterwards, the organic media components were added, and the water was filtrated (0.2 µm) to sterilize the media.

The media were supplied at a flow rate of approximately 50 ml $h^{-1}$, and aliquots of R1 (2 ml), R2 (2 ml), and R3 (5 x 2ml) were sampled after 4, 10, 50, 71, and 77 days of enrichment. Microbial populations were characterized by bacterial cell number (2.4.5), and denaturing gradient gel electrophoresis (DGGE) of PCR products (2.4.3). Starting with incubation day 50, the in- and outflow test substance concentrations of R2 and R3 were measured by HPLC (2.4.4).

### 2.4.2 DNA EXTRACTION, PURIFICATION AND POLYMERASE CHAIN REACTION

The total community DNA was extracted from sterile filtrated groundwater (2 l) of the test aquifer Krauthausen (2.2.1), and from aliquots of the three enrichment cultures (5 x 2 ml) using Fast DNA kit (Q-BIOgene, BIO 101 Systems). DNA was purified using Wizard®DNA Clean-Up System (PROMEGA), extraction and purification followed the instructions of the manufacturer. 16S rDNA from nucleotide 968 to nucleotide 1401 was amplified using the eubacterial universal primer set U-968-GC, and L-1401. Polymerase chain reaction (PCR) was performed with a total volume of 50 µl in 0.2 ml reaction tubes, using the following profile: Denaturation for 10 min at 95°C, 35 cycles consisting of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and elongation at 72°C for 1 min. PCR was finished by a final elongation step at 72°C for 10 min, and aliquots (5 µl) of each amplification reaction were then analyzed on 1.2% w/v agarose gels, cast and run in 1x TAE buffer. Gels were finally stained with ethidium bromide, and documented using PDQuest (BIO-RAD).

### 2.4.3 DENATURING GRADIENT GEL ELECTROPHORESIS

Denaturing gradient gel electrophoresis (DGGE) of PCR products (145) was performed using a DCode system (BIO-RAD). The PCR products encounted increasingly higher concentrations of chemical denaturant as they migrated through a polyacrylamide (6%) gel with gradients between 30% and 70% denaturants (urea-formamide). Gels were prepared, and polymerized to a sheet of PAG film (AMERSHAM Biosciences, GelBond) for being stabilized during the subsequent staining steps. Upon reaching a threshold denaturant concentration, the weaker melting domains of the double-stranded PCR product begun to denature at which time migration slows dramatically. Differing sequences of DNA (from different bacteria) denatured at different denaturant concentrations resulting in a pattern of bands. Each band is theoretically representing a different bacterial strain present in the community. Gels were running in 1x TAE buffer for 16 hrs at a constant voltage (100 V), and temperature (60°C). After electrophoresis, the gels were silver stained, and scanned (AGFA, DUOScan f 40).

### 2.4.4 HIGH PERFORMANCE LIQUID CHROMOTOGRAPHY

The concentation of non-radiolabeled test substances was measured in the in- and outflow of R2 and R3 by HPLC. The active ingredients were determined by a UV detector (GYNKOTHEK, UVD 160) at a wavelength of 270 nm for Imidacloprid (IMI), 251 nm for Isoproturon (IPU), and 300 nm for Diketonitrile (DKN).

### 2.4.5 BACTERIAL CELL NUMBER

The bacterial cell number of the enrichment cultures, as well as their distribution was measured using an automated particle counter (BECKMANN Coulter, Multisizer II). The particle concentration was calculated after the following equation:

$$\text{Particle/ ml} = \left( \frac{\text{buffer volume [ml] + sample volume [ml]}}{\text{sample volume [ml]}} \right) \times \left( \frac{\text{particle counts}}{\text{measured volume [µl]}} \times 1000 \right)$$

**Table 3: Bacterial cell number: Particle concentration (particles ml$^{-1}$)**

## 2.5 DEVELOPMENT OF A STATIC AND DYNAMIC TEST SYSTEM FOR AQUIFER DEGRADATION STUDIES

The following chapter provides information about the development, setup, and testing of a static, as well as a dynamic artifical aquifer incubation system at the lab-scale, referred to as Microcosm (2.5.1) and DAISY (2.5.2). Both systems were used to study the intrinsic microbial degradation potential of the selected aquifers (2.2) with respect to Isoproturon (IPU), Imidacloprid (IMI), and Diketonitrile (DKN). The comparability between the static and dynamic approach was additionally characterized with the main intention to identify the relevant parameters with respect to aquifer degradation studies. In the following studies, the headspace and the groundater of the dynamic test system was sub-sampled in regular time intervals to measure the amount of $^{14}CO_2$ gas phase, dissolved organic $^{14}C$, and dissolved $^{14}CO_2$ groundwater. At time of final processing, the amount of extractable organic $^{14}C$, as well as non-extractable organic $^{14}C$ was additionally measured to establish a mass balance. This was in contrast to the static system, where a mass balance was determined at each sampling point.

### 2.5.1 THE STATIC INCUBATION SYSTEM

Specifications of the static Microcosm incubation test system were as follows: Simple design, easy to handle in- and outside of atmospheric chambers in order to run an ample of replicates. The sponsor additionally decided to set up the incubations with the highest achievable ratio of aquifer material to groundwater to allow for a water table of about 2 cm above sediment, depending of the grain size of the selected aquifer. A glass flask (SCHOTT, 250 ml) was selected as Microcosm that was placed on a precision balance (METTLER TOLEDO) to fill in the wet aquifer material by a spatula, excluding stones > 0.5 cm, and to add the groundwater by the use of a pipette (METTLER TOLEDO, Rainin, E3). After application (2.1.5), the Microcosms were closed airtight until processing with a special screw cap (Figure 10; page 46) that was equipped with an absorber trap for $^{14}CO_2$ (2.3.1.1). Setup, sample processing, and analytical methods were checked on the basis of mass balances, determined in two $^{14}C$ benzoic acid (BA) studies using first Milli-Q water, and subsequently methanol as eluant. Both studies did not distinguish between dissolved $^{14}CO_2$ and dissolved organic $^{14}C$, since it was not known at this time that a large portion of $^{14}CO_2$ remained dissolved in the groundwater (3.1.4).

The **Microcosm incubation study - M1** and **Microcosm incubation study - M2**. were set up with aquifer material (100 g, DM), as well as groundwater (40 ml) from the aquifer Krauthausen (2.2.1), and [UL-$^{14}C$]-benzoic acid (2.1.1) was applied at a concentration of 4.5 µg $l^{-1}$. After 12 hrs of incubation, the microcosms were processed as described in chapter (2.3).

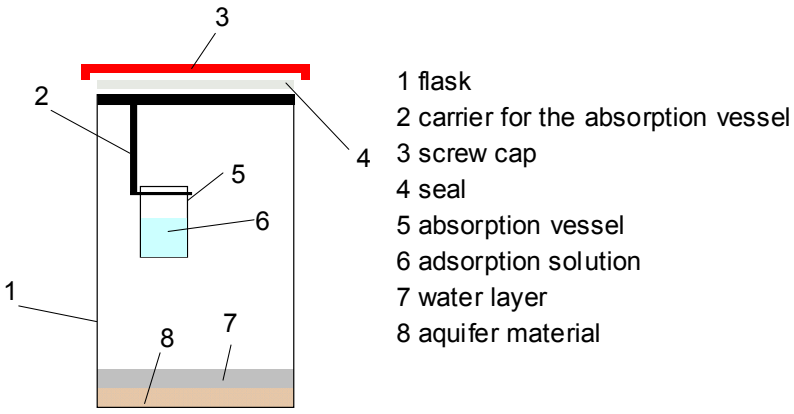



**Figure 10: The Microcosm incubation test system: Technical draft**

## 2.5.2 THE DYNAMIC INCUBATION SYSTEM

Specifications of the dynamic incubation system (DAISY) were as follows: Simple and technical reliable, operable in- and outside of atmospheric chambers to accomplish long-term degradation studies ≥ 360 days. Airtight to establish mass balances by the use of $^{14}C$ radiolabeld test substances (concentration ≤ 10 µg $l^{-1}$), and monitored by an automated online control unit (Figure 19; page 125), which can be used to adjust different flow velocities of at least 20 replicates. With respect to the Microcosm (2.5.1), the dynamic test systems were set up with a different aquifer material (150 g, DM) to groundwater (450 ml) ratio to enable dynamic flow conditions, as well as the sub-sampling of the test system. Two flasks (A, B), a glass column (C), and peristaltic pump (D) were connected in line (A, C, B, D, A) by Teflon®-PVA, and stainless steel tubes (Figure 11; page 47).

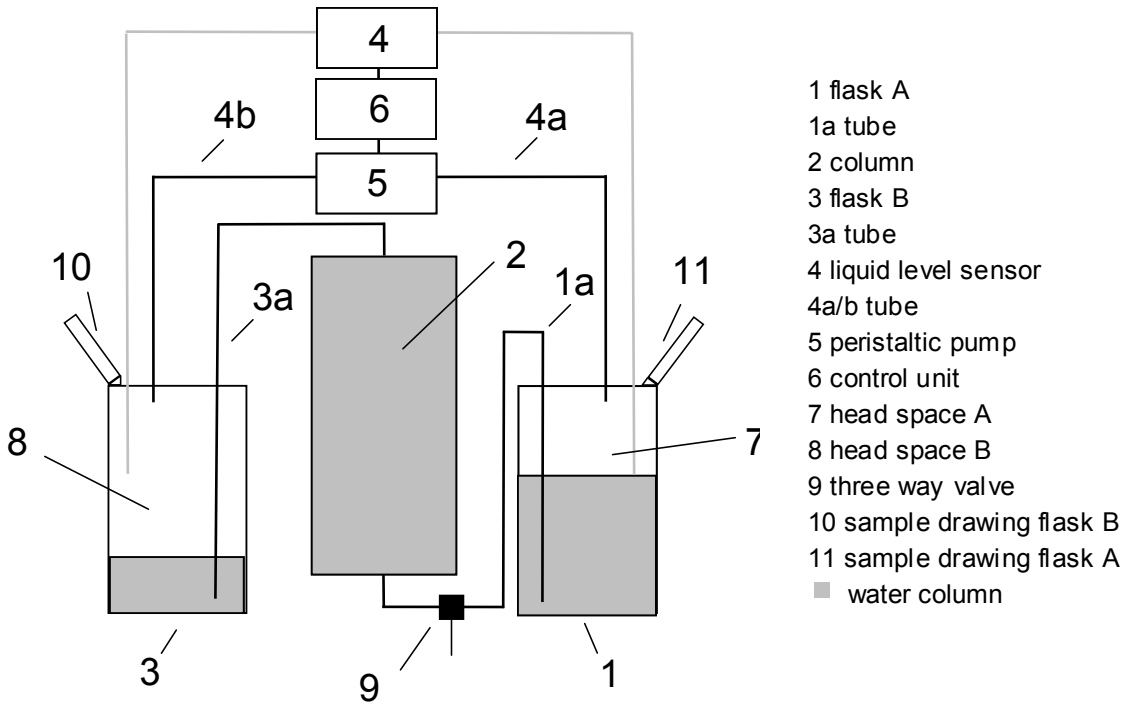



**Figure 11: The DAISY incubation test system: Technical draft**

The test systems were then closed airtight by screw caps, equipped with an absorber trap for $^{14}CO_2$, and a liquid level sensor  The test systems were filled with groundwater (flasks), and aquifer material (column) to establish a constant water column between flask A, column, and flask B. After the the test systems were closed airtight, the groundwater flow was achieved by generating an atmospheric pressure difference between the two headspaces of the flasks (shifting of the constant water column, due to high- and low pressure). The adjustable, bi-directional, continuous, and automated flow is comparable to the principle of a beam balance.

For DAISY, the weights were substituted through high- and low pressure, realised by a switchable peristaltic pump, operated in online mode by the control unit that monitored the water level inside the two flasks (Figure 19; page 125). The test systems were placed on a precision balance (METTLER TOLEDO) to fill in the aquifer material by a spatula, excluding stones > 0.5 cm, and to fill in the groundwater by the use of a pipette (EPPENDORF, Easypet). Afterwards, the test substances were applied (2.1.5), and the test systems were closed until sub-sampling, or final processing (Figure 12; page 48). Prior to the automated version, the flow was first achieved by gravitation, and alternate lifting of flask A and B. The flow volume was adjusted by the difference in height between flask A and B.

**Figure 12: The DAISY incubation test system: Design and experimental setup inside an atmospheric chamber**

Like for the Microcosm, the DAISY was checked on the basis of mass balances, determined in three $^{14}C$ benzoic acid (BA) studies:

1.) The long-term DAISY incubation study - D1.
2.) The mid-term DAISY incubation study - D2.
3.) The short-term DAISY incubation study - D3.

The automation was not performed at the start of the BA studies, and the flasks were lifted from Monday to Friday twice a day. Contrary to the mid- and short-term incubation test series, the first long-term incubation study did additionally not distinguish between dissolved $^{14}CO_2$ and dissolved organic $^{14}C$. It was not known at this time that a large portion of the $^{14}CO_2$ remained dissolved in the groundwater (2.2.4).

The **long-term DAISY incubation study - D1** was set up in triple repetition with aquifer material (150 g, DM) and groundwater (450 ml, unmodified) from the test aquifer Krauthausen (2.2.1). [UL-$^{14}C$]-benzoic acid (2.1.1) was applied at a concentration of 0.1 and 10 µg $l^{-1}$ and sampling was scheduled after 1, 3, 7, 11, 15, 21, 35, 56, and 84 days of incubation. The processing was accomplished as described in chapter (2.3).

The **mid-term DAISY incubation study - D2** was set up in fourfold repetition with aquifer material (100 g, DM) and groundwater (300 ml, unmodified). [UL-$^{14}C$]-benzoic acid was applied only at a concentration of 10 µg $l^{-1}$, and the sampling was scheduled after 1, 4, 6, 8, 11, and 15 days of incubation. Processing was accomplished as in **D1**, but dissolved $^{14}CO_2$ was fixed by the addition of sodium hydroxide, as well as dissolved $^{14}CO_2$ and dissolved organic $^{14}C$ were separated by method A (2.3.1.1).

The **short-term DAISY incubation study - D3** was set up and processed like **D2**, with the following exceptions: The sampling was scheduled after 3, 6, 8, 24, 32, 48, 56, 120, 144, and 168 hrs of incubation, [Phenyl-UL-$^{14}C$]-benzoic (2.1.1) was used, and the dissolved $^{14}CO_2$ was trapped in $^{14}CO_2$ absorber traps (2.3.1.1).

### 2.5.3 COMPARABILITY OF THE STATIC AND DYNAMIC TEST SYSTEM

It was scheduled to compare degradation rates of the static and dynamic test system to study the effect of groundwater flow on degradation kinetics. **However, the specifications of the static and dynamic test system (2.5.1/ 2.5.2) were different in more than one factor, and it was not possible to compare the degradation rates between the two test systems.**

As a consequence, it was necessary to implement a third test system that was filled with the same quantity of aquifer material and groundwater like the dynamic test system to match the absolute amount of the test substance, as well as the number of microorganisms. For this “new” test system, referred to as Column test system, a self-made glass column was used that was closed airtight until processing by a special screw cap, equipped with an absorber trap for $^{14}CO_2$ (2.3.1.1). Degradation rates were then compared again on the basis of mass balances, determined in one $^{14}C$ benzoic acid (BA) test series. In contrast to all other experiments, this study was accomplished at 25°C to study the estimated small, but specific differences at enhanced microbial metabolic rates more precisely.

The **comparability incubation study - C1** included samplings in triple (Microcosm and Column test system), or twofold repetition (DAISY) after 9, 24, 33, 48 hrs of incubation. The test systems were filled with aquifer material (50 g, DM), as well as groundwater (Microcosm 20 ml; Column/ DAISY 350 ml) from the aquifer Krauthausen (2.2.1), and [Phenyl-UL-$^{14}$C]-benzoic acid (2.1.1) was applied at a concentration of 1 µg $l^{-1}$. After the application, aquifer material and groundwater were mixed within the Microcosm and Column test system once to ensure the uniform distribution of test substance at the beginning of the study. As the groundwater flow rate was adjusted to about 30 cm $h^{-1}$, this was not necessary for DAISY. All incubations were processed as described in chapter (2.3.1), including the separation of dissolved $^{14}CO_2$ and dissolved organic $^{14}$C.

## 2.6 THE ACTUAL MICROBIAL DEGRADATION ACTIVITY

The actual microbial degradation activity of the aquifer Krauthausen (2.2.1), Mulder-Beilen, and Nature A (2.2.2) was studied with respect to the selected test substances Isoproturon (2.1.2), Imidacloprid (2.1.3), and Diketonitrile (2.1.4). It was scheduled to accomplish long-term degradation studies using the static Microcosm (> 450 days), as well as dynamic DAISY (> 360 days) test system to study the effect of groundwater flow on the degradation kinetics. Four incubation studies were accomplished:

1.) The microcosm incubation study - M3.
2.) The microcosm incubation study - M4.
3.) The DAISY incubation study - D4.
4.) The DAISY incubation study - D5.

The incubation studies were started after about ½ year of experimental work, and it was not possible to consider all findings of the benzoic acid studies, which were developed meanwhile (3.1.4). Therefore, the first **Microcosm incubation study - M3** and the first **DAISY incubation study - D4** were accomplished with unmodified groundwater, which caused an increase of the pH (3.1.2). The second **Microcosm incubation study - M4** was set up with unmodified groundwater, as well as modified groundwater to “bridge” the results between **M3** and **M4**. This was in contrast to the second **DAISY incubation study - D5** that was accomplished only with modified groundwater, as the findings of the “bridging” were meaningful for both systems. **M4** and **D5** were additionally and contrary to **M3** and **D4** set up one week before the application of test substances to measure redox, pH, and oxygen, as well as to adjust the flow volume of the groundwater (> ½ of the total groundwater volume) and the velocity (ml $min^{-1}$) for DAISY.

The first **Microcosm incubation study - M3** scheduled samplings after 0, 225, and 450, as well as after 0, 90, 180, 270, 360, 450 days of incubation (Table 22; page 85). A total number of 186 Microcosms were filled with aquifer material (100 g, DM), and unmodified groundwater (40 ml) from the aquifer Krauthausen, Mulder-Beilen, and Nature A (2.2). The effect of temperature on degradation kinetics was studied by including samples that were incubated at 20°C, and biotic degradation was additionally checked by sterilized samples. The test substances were applied as single substances, as well as in a mixture (1:1:1) at a concentration of 0.1, 1, and 10 µg $l^{-1}$ (Table 21; page 85). Due to the number of replicates, the application was done on three consecutive days. The samples were processed as described in chapter (2.3) with the exception that the differentation of dissolved organic $^{14}C$ and dissolved $^{14}CO_2$ started with incubation day 180.

The second **Microcosm incubation study - M4** scheduled samplings after 0, 180, 330, as well as after 0, 90, 180, 270, 330 days of incubation (Table 24; page 86). A total number of 132 Microcosms were filled with aquifer material (100 g, DM), as well as groundwater (Nature A 65 ml; Krauthausen and Mulder-Beilen 40 ml) from the three selected test aquifers (2.2). The study included variants with modified (2.2.4), as well as unmodified groundwater (Table 23; page 86) to study the effect of the groundwater treatment. Afterwards, the three selected test substances were applied, and the incubations were processed as described for **M3**.

The first **DAISY incubation study - D4** scheduled a sub-sampling interval of 45 days of incubation (Table 25; page 87). A total number of 22 DAISY´s were filled with aquifer material (150 g, DM), and unmodified groundwater (450 ml) from the three selected test aquifers (2.2). Isoproturon, Imidacloprid and Diketonitrile were applied as single substances, as well as in a mixture (1:1:1) at a concentration of 0.1, 1 and 10 µg $l^{-1}$. All incubations were processed as described in chapter (2.3), including the differentation of dissolved organic $^{14}C$ and dissolved $^{14}CO_2$.

The second **DAISY incubation study - D5** started with the same variants (Table 25; page 87) and specifications as **D4**, but instead of using modified groundwater (buffer, 1 mM), and applying the test substances after nine days of stable operation at a constant pH.

## 2.7 SUMMARIZED TEST STUDY DESIGN

### Development of a static and a dynamic test system for aquifer degradation studies

| Test Study | Test substance | Test aquifer | Flow conditions | Incubation days | Groundwater | Dissolved $^{14}CO_2$ | pH |
|---|---|---|---|---|---|---|---|
| Microcosm - M1 | BA | K | static | ½ | unmodified | not determined | increasing |
| Microcosm - M2 | BA | K | static | ½ | unmodified | not determined | increasing |
| C1 | BA | K | static<br>dynamic | 2 | unmodified | not determined | increasing |
| DAISY -D1 | BA | K | dynamic | 84 | unmodified | not determined | increasing |
| DAISY -D2 | BA | K | dynamic | 15 | unmodified | not determined | increasing |
| DAISY -D3 | BA | K | dynamic | 7 | unmodified | not determined | increasing |

### The microbial degradation activity

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Microcosm - M3 | IPU<br>IMI<br>DKN | K<br>MB<br>NA | static | 450 | unmodified | not determined | increasing |
| Microcosm - M4 | IPU<br>IMI<br>DKN | K<br>MB<br>NA | static | 330 | modified<br>unmodified | measured | stable<br>increasing |
| DAISY -D4 | IPU<br>IMI<br>DKN | K<br>MB<br>NA | dynamic | 330 | unmodified | not determined | increasing |
| DAISY -D5 | IPU<br>IMI<br>DKN | K<br>MB<br>NA | dynamic | 300 | modified | measured | stable |

**Table 4: Summarized test study design (BA = Benzoic acid, K = Krauthausen, MB = Mulder-Beilen, NA = Nature A, IPU = Isoproturon, IMI = Imidacloprid, DKN = Diketonitrile)**

# 3 RESULTS AND DISCUSSION

## 3.1 DEVELOPMENT OF A STATIC AND DYNAMIC TEST SYSTEM FOR AQUIFER DEGRADATION STUDIES

As benzoic acid was mineralized within a few hours, it was possible to test the two developed test systems (2.5) based on the sum of the radioactivity detected in the respective fractions of the different studies (2.5.1; 2.5.2). DAISY was introduced as a dynamic test system that enables the determination of mass balances by the use of $^{14}C$ radiolabeled test substances, recommended for aquifer degradation studies at a concentration ≤ 10 µg $l^{-1}$ (45).

### 3.1.1 THE STATIC INCUBATION SYSTEM

The radioactive balance of the first **Microcosm incubation study - M1** was based on the sum of the radioactivity detected in the following fractions:

1.) Mineralized $^{14}CO_2$ gas phase.
2.) Extractable organic $^{14}C$.
3.) Non-extractable organic $^{14}C$.

The radioactive balance of the study was 89.3%±1.6% (Table 18; page 82) of the applied radioactivity (AR). This confirmed that benzoic acid (BA) was an appropriate model compound to check the static and dynamic test systems on the basis of mass balances (12) at a concentration range ≤ 10 µg $l^{-1}$. BA was additionally suitable to assess the microbiol activity of the selected aquifers, as between 4.5 and 7.1% of AR were mineralized ($^{14}CO_2$ gas phase) within 12 hrs of incubation. The extractable organic $^{14}C$ fraction ranged between 48.3 and 51.6%, whereas the non-extractable organic $^{14}C$ was between 31.8 and 35.1%. The standard deviations of LSC measurements were acceptable (< 2.0%), but about 11.6% of AR were not recovered. Therefore, the eluant Milli-Q water was replaced by methanol to reliably stop the microbial degradation during processing.
The radioactive balance of the **second Microcosm study - M2** was detected in the same fractions as for **M1**, and 99.0%±2.5% (Table 19; page 83) of the applied radioactivity (AR) was recovered. The change of the eluant improved the recovery of the applied radioactivity, and setup, processing, and analytics were adapted for the dynamic incubation system (DAISY).

### 3.1.2 THE DYNAMIC INCUBATION SYSTEM

The radioactive balance of the first **long-term DAISY incubation study - D1** was based on the sum of the radioactivity detected in the following fractions:

1.) Mineralized $^{14}CO_2$ gas phase.
2.) Organic $^{14}C$ groundwater (including extractable organic $^{14}C$).
3.) Non-extractable organic $^{14}C$.

Only one system of the triplicate incubation at 0.1 µg $l^{-1}$ could be processed (one system failed due to leakage, and one due to sodium hydroxide contamination), and the radioactive balance was 95.7% of the applied radioactivity (AR). The mineralization ($^{14}CO_2$ gas phase) increased during incubation from 8.2% to 63.4%, whereas the organic $^{14}C$ in the groundwater decreased from 78.9 to 10.1%, and the non-extractable organic $^{14}C$ fraction was 22.3% at final processing. The radioactive balance of the 10 µg $l^{-1}$ concentration was 95.9%±0.2%, and the mineralization ($^{14}CO_2$ gas phase) increased from 4.1%±0.3% to 48.1%±0.7%, whereas the organic $^{14}C$ in the groundwater decreased from 71.2%±0.5% to 26.7%±0.5%, and the non-extractable organic $^{14}C$ fraction was 21.1%±0.9% at final processing (Table 20; page 84, Figure 13; page 54).

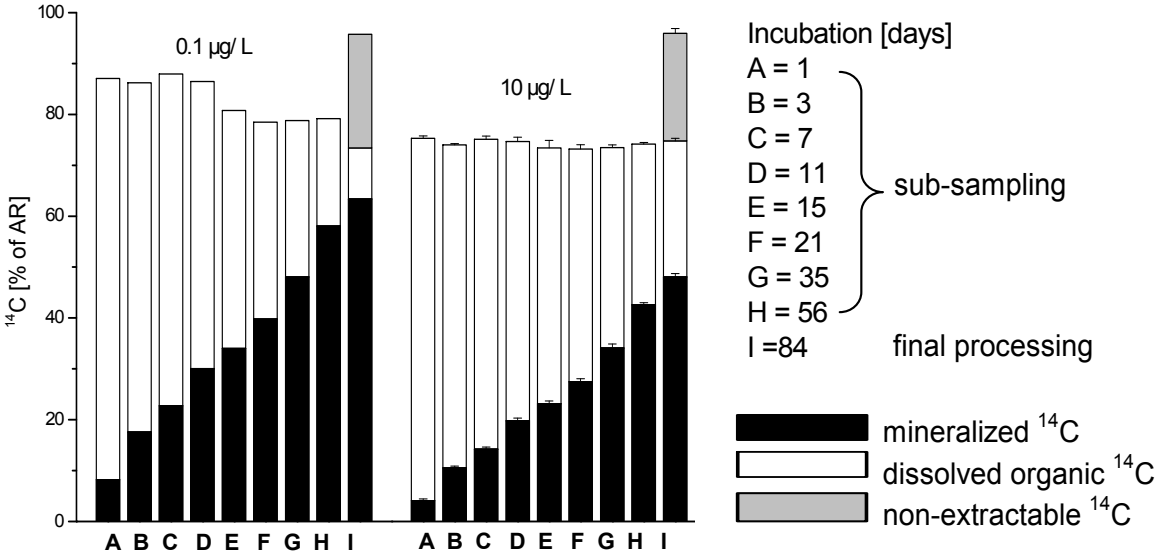



**Figure 13: The long-term DAISY incubation study – D1: Distribution of applied radioactivity [%]**

The mass balances and standard deviations were acceptable, and the study design was unaltered. However, the test substance concentrations varied with respect to the fraction of organic $^{14}C$ in the groundwater, as well as $^{14}C$ in the gas phase (Figure 13; page 54).

If the incubation time increased, the relative ratio decreased for $^{14}C$ in the gas phase, whereas it increased for organic $^{14}C$ in the groundwater (Table 20; page 84). The groundwater samples were acidified, and measured by LSC to study this effect in detail. Results showed that a huge amount of mineralized $^{14}CO_2$ still remained as dissolved $^{14}CO_2$ in the groundwater. The dissolved $^{14}CO_2$ was slowly, but steadily released to the gas phase during the incubation time, probably due to the low diffusion kinetic in the groundwater (68). The benzoic acid degradation kinetic was consequently highly underestimated, and methods were considered to measure the dissolved $^{14}CO_2$ fraction.

The radioactive balance of the second **mid-term DAISY incubation study - D2** was based on the sum of the radioactivity detected in the following fractions:

1.) Mineralized $^{14}CO_2$ gas phase.
2.) Dissolved $^{14}CO_2$ groundwater and extract.
3.) Organic $^{14}C$ groundwater and extract.
4.) Non-extractable organic $^{14}C$.

The radioactive balance was 97.5%±11.3%, and standard deviations were increased as compared to the previous study. Higher standard deviations were especially measured for the combustion, where the basic extraction (pH > 8.5) enhanced the quench of the LSC, and the radioactivity of the solid samples additionally ranged close to the detection limit of LSC. The mineralization ($^{14}CO_2$: gas phase, groundwater, extract) increased up to **43.4%±3.2% within 4 days** of incubation (Figure 14; page 56). This confirmed the extreme underestimation of the BA degradation kinetic, as for **D1** the mineralization, in this case only the $^{14}CO_2$ gas phase, was just **42% after 56 days** of incubation (Figure 15; page 56). The maximum turnover was already reached before incubation day 4, and the sampling intervals were shortened for the following study **D3**.

The setup and processing of the third **short-term DAISY incubation study - D3** was unaltered, and the radioactive balance was based on the sum of the radioactivity detected in the same fractions as for **D2**. The radioactive balance was 110.3%±13.9% for the 0.1 µg $l^{-1}$ concentration, and 83.7±0.37% for the 10 µg $l^{-1}$ concentration. The larger deviation from full mass balance was most likely a result of the frequent, and short sampling intervals at high metabolic rates with respect to the kinetic of the sodium hydroxide traps (2.3.1.1). However, the mass balances were in the range for degradation studies with $^{14}C$-radiolabeled test substances (140), but should be improved once again for the following studies.

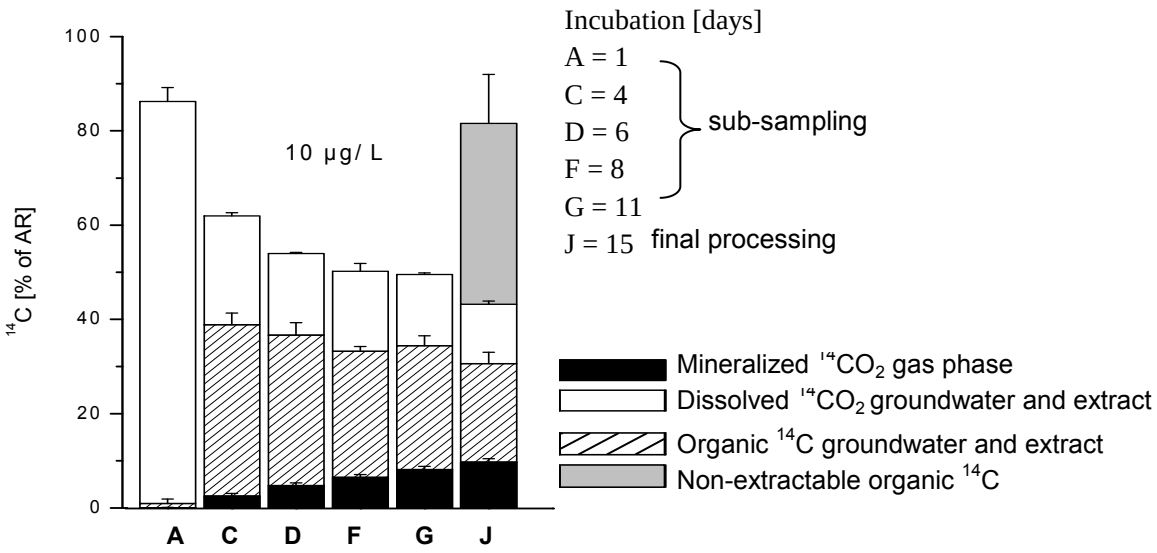



**Figure 14: The mid-term DAISY incubation study - D2: Distribution of applied radioactivity [%]**

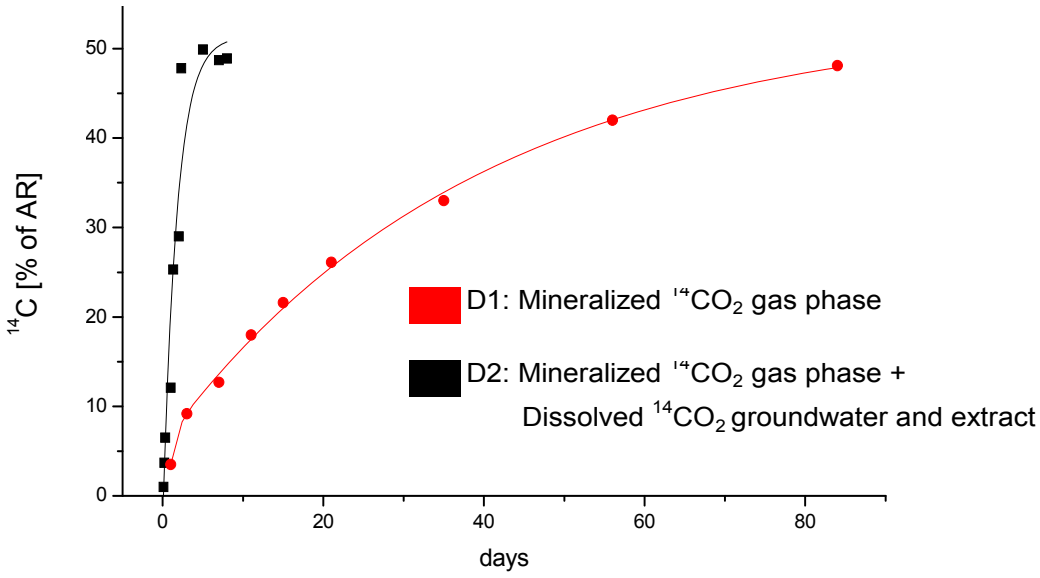



**Figure 15: The DAISY incubation test system and the amount of dissolved $^{14}CO_2$: Comparison of incubation studies D1/ D2**

The mineralization of [Phenyl-UL-$^{14}$C]-benzoic acid confirmed the cleavage of the aromatic ring structure that was already assumed in the studies with [UL-$^{14}$C]-benzoic acid (3.2.1). For the 10 µg $l^{-1}$ concentration, the mineralization reached 12.9% within 24 hrs, 29.7% within 48 hrs of incubation, and a maximum of about 50% after 56 hrs.

The degradation was generally faster, and about 6% higher compared to the 0.1 µg $l^{-1}$ concentration. The extractable organic $^{14}C$ fraction was below 5% for both concentrations, whereas the non-extractable organic $^{14}C$ was about twice for the 0.1 µg $l^{-1}$ concentration (57.9%±4.4%). However, the samples of the 0.1 µg $l^{-1}$ concentration were measured close to the detection limit of LSC, influenced by specific background values.

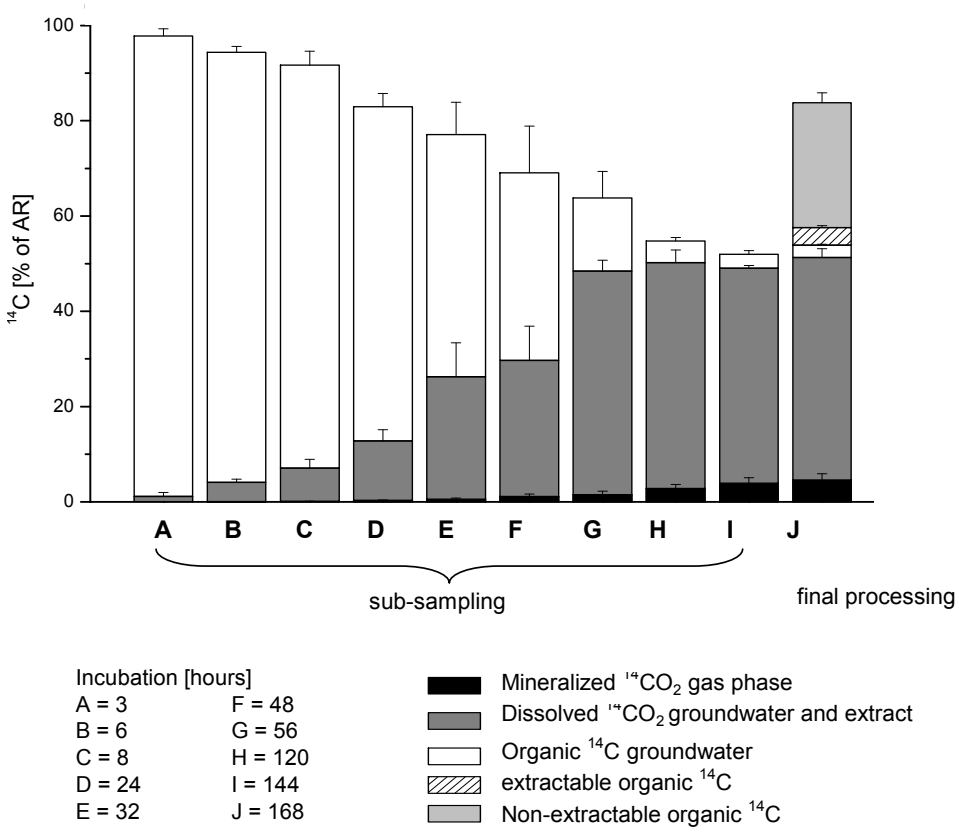



**Figure 16: The short-term DAISY incubation study - D3: Distribution of applied radioactivity [%]**

### 3.1.3 COMPARABILITY OF THE STATIC AND DYNAMIC TEST SYSTEM

The degradation rates of the Microcosm, DAISY, and Column test system were compared in the **comparison study - C1** that was set up and processed as the previous study **D3**. The mass balances were determined at each sampling point for the two static systems, and radioactive balances ranged between 86.5±4.9 and 93.8%±1.7 for the Column test system, as well as between 81.9±0.8 and 95.9%±10.7 for the Microcosm. The headspace and the groundwater of the dynamic system was sub-sampled, and mass balance was 94.3%±2.1 at final processing.

The study was accomplished at 25°C, which generally increased the BA degradation kinetic as compared to the studies at 10°C (2.5.2). After 48 hours, the mineralization (=$^{14}CO_2$ gas phase + groundwater + extract) was 53.3% for the DAISY, 46.1% for the static column, and 33.4% for the Microcosm (Figure 17; page 59). At the start of the thesis, it was scheduled to compare the degradation rates of the Microcosm and DAISY test system (1.2) to study the influence of groundwater flow on degradation kinetics. Based on the results of this study, this was obviously impossible to accomplish, as the study design was different in more than one factor. The specifications of the static and dynamic test system (2.5.1, 2.5.2) did not take into account the aquifer material (CPA "sink") to groundwater ratio (CPA "source"). Both systems were set up with the same concentration (µg $l^{-1}$), but with a different absolute amount of test substance (µg), groundwater (ml), and aquifer material (g). It was also not possible to compare the two test systems based on metabolic rates (µg) per aquifer material (g), as Microcosm and DAISY were set up with a different ratio of aquifer material and groundwater. In the studies concerning the microbial degradation activity, these ratios differed for example, by a factor of 7.5 (Table 16; page 82).

**DAISY** and the **Column test system** had the same aquifer packing to equalize contact surfaces, and both test systems were filled with the same amount of aquifer material and groundwater to match the relative and absolute amount of test substance. After the application and uniform distribution of test substances, this experimental setup enabled to study the effect of water flow on degradation kinetics, as both systems differed in only one factor, the groundwater flow. Results showed that the mineralization was about twice that for the DAISY, and dynamic flow conditions enhanced the BA degradation kinetic. Within 9 hrs of incubation, total mineralization reached **46.6% for DAISY**, whereas only **24.2%** were mineralized for the **Column test system**. The degradation-limiting factor was almost certainly the availability of test substance, as 57.0% (static column), and 24.6% (DAISY) of the applied radioactivity (AR) remained dissolved in the groundwater. The aquifer microorganisms were probably mainly immobile, and attached to the solid aquifer matrix. Furthermore, as about 20% of AR (DAISY and SC) was not extractable, and BA is known to have a low adsorption potential (9), it was assumed that the non-extractable organic $^{14}C$ fraction was mainly incorporated into the microbial biomass, and not bound to sediment particles. After 48 hrs, the $^{14}CO_2$ gas phase fraction was 5.1% for the Column test system, and 24.3% for DAISY. Although the two systems had a different headspace volume, and two traps were used within the dynamic system, the dynamic flow conditions enhanced the diffusion of dissolved $^{14}CO_2$ into the gas phase, most probably due to the movement of the groundwater.

The study was accomplished with aquifer material that was stored at +4°C for about two years. Results showed that the microbial degradation potential of the aquifer with respect to BA was unaltered, and comparable to those, determined in the studies immediately after sampling. The storage time and storage conditions probably had no negative effect on the general microbial degradation potential of all selected aquifers.

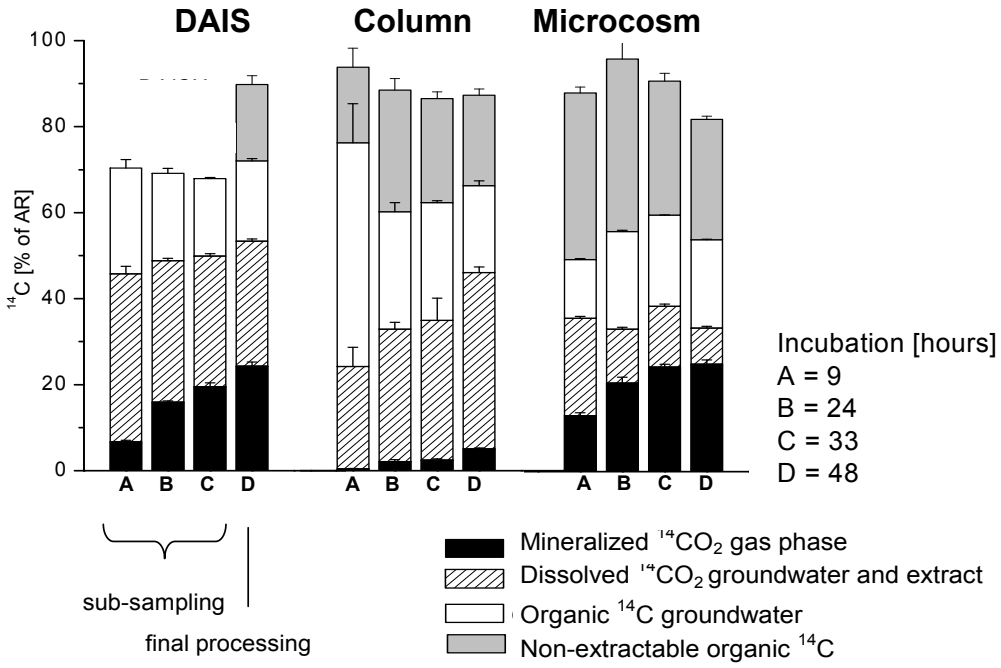



**Figure 17: The comparison study – C1: Distribution of applied radioactivity [%]**

### 3.1.4 CONCLUSIONS

Sparse knowledge was available with respect to the degradation of CPA in near-natural aquifers, and even no data were availabe about specific test systems at the lab-scale for aquifer degradation studies. However, If the degradation of CPA is studied in aquifers, the study design must take into account the special physical and microbiological settings of a water-saturated matrix. The BA studies clarified that e.g. the amount of dissolved $^{14}CO_2$, which is not yet considered in other studies (12), or the pH, which is governing the degradation pathway of a xenobiotic (19), are of particular importance. At a concentration of ≤ 10 µg $l^{-1}$, analytics are additionally a special challenge. On the one hand to detect mineralization and metabolisation of CPA at treshold levels, on the other hand to characterize microorganisms with respect to population shift, growth, or maintenance.

The **comparability study C1** confirmed that the dynamic flow conditions enhanced the degradation kinetic of BA, which can also be accepted for Imidacloprid, Isoproturon, and Diketonitrile. At static flow conditions, the relevant transport mechanism was molecular diffusion, which led to the transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion. This was in contrast to DAISY, where the transport was dominated by the given groundwater flow.

Based on the results of the BA studies, and with respect to a guideline from the Netherlands (45), the following specifications were derived to study the microbial degradation potential of a shallow aquifer with respect to CPA.

1.) Degradation rates of the Microcosm and DAISY test system are only comparable, if the incubations are set up with the same specifications, in particluar with respect to the concentration (µg $l^{-1}$), absolute amount of test substance (µg), and groundwater (ml) to aquifer material (g) ratio.
2.) Degradation studies in water-saturated matrices should be set up with regard to the groundwater chemistry, in particular to the solubility of $CO_2$ in aqueous solutions, which is variable and strongly influences the pH (2.2.4).
3.) Degradation studies in water-saturated matrices have to consider the amount of dissolved $^{14}CO_2$, otherwise degradation kinetics are highly underestimated (2.5.3). As many water-saturated aquifer, as well as topsoil degradation studies did not consider the dissolved $CO_2$ fraction, the degradation rates of several CPA should be confirmed.
4.) The Microcosm and DAISY test systems were developed to accomplish degradation studies with near-natural aquifer material. Due to the concentration (≤ 10 µg $l^{-1}$) and organic carbon content (≤ 0.5%), the accumulation of $CO_2$ within the two test systems was in the non-toxic range.
5.) Degradation studies are regulary set up with sodium hydroxide or soda lime to trap mineralized $CO_2$ (53;141). This leads to a pH shift, due to a displacement of the groundwater equilibrium (Table 15; page 81). As the pH can govern the pathway of CPA (19), the degradation rates of several studies should be confirmed.
6.) Degradation studies should be set up <u>without</u> sodium hydroxide or soda lime traps. In this case, $CO_2$ gas phase could be measured by flushing the gas phase of the test system at each sampling intervall, whereas accumulated dissolved $CO_2$ should be separated and measured as already described (2.3.1.1).

7.) Degradation studies should use analytical methods as e.g. FT-ICR-MS or LC-MS to study the mineralization and metabolisation of CPA in detail. At a concentration range ≤ 10 µg $l^{-1}$, LSC and Radio-HPLC are only of restricted use, as the specific detection limits are reached to several metabolites (due to e.g. limited specific radioactivity, or distribution of applied radioactivity).

## 3.2 THE MICROBIAL DEGRADATION ACTIVITY

The studies with respect to the microbial degradation activity were already set up when the comparability study C1 (2.5.3) was accomplished, and it was not possible to change the set of settings to consider the results that were developed meanwhile. Therefore, the degradation rates of Microcosm and DAISY were not comparable, and it was not possible to study the effect of groundwater flow on degradation kinetics of Imidacloprid, Isoproturon, and Diketonitrile. This strongly limited the informative value of the microbial degradation activity studies.

On the other hand, standard methods for aquifer degradation studies were established concerning setup and analytics (3.1.4), and DAISY was introduced as a new artifical aquifer test system at the lab-scale to study the microbial degradation activity with respect to CPA. The groundwater was modified for the long-term incubation studies, which was only a compromise to suppress the increase in the pH value by continious carbon dioxide trapping. Further studies should be set up without sodium hydroxide traps, and $^{14}CO_2$ could be measured after flushing the headspace of the respective test system. Liquid scintillation counting was used to provide quantitative information with respect to the mineralization [% of AR] of selected CPA. Although the detection limits of LSC were reached at a concentration of 0.1 µg $l^{-1}$, future studies should still use this method to determine the radiolabeled test substances in the respective fractions. This is important to access the mineralization of CPA at reasonable diffuse concentration. As the mineralisation in aquifers is generally low, the metabolisation is of special importance. Methods as FT-ICR-MS should be used to study the metabolisation of test substances at concentrations ≤ 10 µg $l^{-1}$ in more detail. Radio-HPLC is only of limited use, as the specific detection limits are reached.

### 3.2.1 THE STATIC INCUBATION SYSTEM

The first **Microcosm study - M3** was set up with unmodified groundwater, and sodium hydroxide was used to trap $^{14}CO_2$ from the gas phase. This had a deep impact on the chemical groundwater equilibrium (Table 15; page 81), and increased the pH. The pH was measured in the thin water layer (about 0.5 cm) above the aquifer material, and it was impossible to determine a general trend.

pH-Values ranged between 6.9 and 8.7 for Krauthausen, ≈80% of all values ranged between 8.0 and 8.7, between 4.6 and 8.6 for Mulder-Beilen, ≈40% of all values ranged between 7.0 and 7.5, and between 7.0 and 9.0 for Nature A, ≈60% of all values ranged between 8.5 and 9.0 (Tables 75 - 87; pages 115 - 124). The dissolved $^{14}CO_2$ and dissolved organic $^{14}C$ fractions were separated starting with incubation day 180, and the liquid samples were not analyzed by radio-HPLC. It was also impossible to determine a mass balance for the 0.1 µg $l^{-1}$ variants, as the radioactivity of the liquid and solid samples was often below the detection limit of the LSC. **M3** suffered from the methodological weaknesses that considerably limit the validity that can be drawn from the results. Therfore, only general statements could be derived from **M3**, especially with respect to the scheduled "bridging" with the following second **Microcosm study – M4**.

**M4** was set up with water-saturated aquifer material, and modified groundwater. For the aquifer Nature A, the microcosms had to be filled with a total groundwater volume of 65 ml to buffer the bicarbonate of the groundwater that was bound to the aquifer material (the molarity of the buffer was limited by the groundwater chemistry). This was in contrast to Krauthausen and Mulder-Beilen, where the specifications of the sponsor had to be fulfilled and 40 ml groundwater were added. Unfortunately, the BA results showed that this limited the comparability between the respective aquifers, due to a different aquifer material to groundwater ratio (3.1.3).

The radioactive balance of **Microcosm incubation study - M3** was based on the sum of the radioactivity detected in the following fractions:

1.) $^{14}CO_2$ gas phase.
2.) Dissolved $^{14}CO_2$, (1+2 = mineralization).
3.) Dissolved organic $^{14}C$ groundwater.
4.) Extractable organic $^{14}C$ (3 + 4 = extraction).
5.) Non-extractable organic $^{14}C$ (= combustion).

A mineralization potential with respect to **IPU** was assessed in all aquifers and it generally increased, when the redox potential decreased (Krauthausen < Mulder-Beilen < Nature A). This was a new result, since up till now the mineralization of IPU at reducing redox conditions was not described in the literature. Most authors supposed that the cleavage of the aromatic ring is an oxygen demanding step (83;85;86). The adsorption potential was correlated with the type of sediment, being strongest for the aquifer Nature A, followed Mulder-Beilen and Krauthausen (Tables 75, 78, 79, 82, 83, 86, 87; pages 115, 118, 118, 121, 121, 124, 124).

**IMI** was mineralized in all selected test aquifers and at all concentrations. However, a strong variation (> 10% for Mulder-Beilen; > 7% for Krauthausen) was measured between all values (Tables 76, 78, 80, 82, 84, 87; pages 116, 118, 119, 121, 122, 124), and it was impossible to detect a general trend. The adsorption potential was low for the aquifers Krauthausen and Mulder-Beilen (10 µg $l^{-1}$), and higher for Nature A. This was probably due to the specific physical and chemical characteristics of the respective sediments (Tables 10, 11, 12; pages 78, 79, 80), as it is known that the adsorption of IMI is affected by e.g. the smectite-, as well as organic carbon content (109;112-114).

The mineralization potential of **DKN** decreased with increasing concentration of test substance, being greatest for the aquifer Nature A, followed by Krauthausen and Mulder-Beilen (Tables 77, 82, 85, 86, 87; pages 117, 121, 123, 124, 124). This was in contrast to the adsorption potential, which was highest for the aquifer Nature A at 0.1 µg $l^{-1}$. Mulder-Beilen and Krauthausen had only a low adsorption potential, and the non-extractable organic $^{14}C$ fraction was < 6.5% of AR after 450 days of incubation.

| Aquifer | Concentration [µg l⁻¹] | Incubation [days] | Mineralization [%] | Extraction [%] | Combustion [%] |
|---|---|---|---|---|---|
| K | 0.1 | 450 | 4.56 | 80.21 | n.d. |
| | 1 | 450 | 4.53 | 79.17 | 11.16 |
| | 10 | 270 | 8.77 | - | - |
| | 10 | 450 | 2.06 | 77.84 | 7.33 |
| MB | 0.1 | 450 | 12.93 | 37.83 | n.d. |
| | 1 | 450 | 13.48 | 40.11 | 36.32 |
| | 10 | 450 | 10.28 | 45.26 | 32.04 |
| NA | 0.1 | 450 | 14.28 | 7.63 | n.d. |
| | 1 | 450 | 15.44 | 9.29 | 71.13 |
| | 10 | 360 | 16.08 | - | - |
| | 10 | 450 | 5.39 | 4.60 | 72.48 |

**Table 5: The microcosm incubation study - M3: Selected values Isoproturon [% of AR] of tables 75, 78, 79, 82, 83, 86, 87 (pages 115, 118, 118, 121, 121, 124, 124)**

**IPU, IMI, and DKN** were also applied in a mixture (1:1:1), and the adsorption-, as well as the mineralization potential of the selected aquifers was comparable to the results of the single substances (Nature A > Mulder-Beilen > Krauthausen). If the test substances were incubated at 20°C, the mineralization was generally enhanced (Table 86; page 124). The test substances were not significantly mineralized in the biological control samples, which confirmed the biotic degradation of IMI, IPU, and DKN (Table 87; page 124).

However, it was not possible to determine specific degradation rates of the active ingredients, as the radioactivity of the liquid samples was below the detection limit of HPLC.

| Aquifer | Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] |
|---|---|---|---|---|---|
| K | 0.1 | 450 | 11.9 | 84,82 | - |
| | 1 | 450 | 0.48 | 88.70 | 6.11 |
| | 10 | 450 | 0.18 | 90.51 | 2.77 |
| MB | 0.1 | 450 | 5.54 | 78.68 | - |
| | 1 | 450 | 0.96 | 89.76 | 2.69 |
| | 10 | 360 | 0.29 | 90.56 | 1.85 |
| | 10 | 450 | 0.46 | 76.42 | 3.14 |
| NA | 0.1 | 450 | 21.15 | 12.59 | |
| | 1 | 450 | 11.38 | 18.78 | 56.12 |
| | | 450 | 8.91 | 42.10 | 37.88 |

**Table 6: The microcosm incubation study - M3: Selected values Diketonitrile [% of AR] of tables 77, 82, 85, 86, 87 (pages 117, 121, 123, 124, 124)**

The second **Microcosm incubation study - M4** was processed, and evaluated in the same manner as **M3**. A mass balance was not determined for the 0.1 µg$l^{-1}$ concentration because the radioactivity of the $^{14}CO_2$ gas phase and non-extractable organic $^{14}C$ fraction was below the detection limit of LSC and HPLC. The recovery of all other incubations was acceptable and ranged between 89 and 103% of the applied radioactivity (Tables 42, 45, 48, 51, 53, 56, 59, 62, 64, 67, 73; pages 101, 102, 104, 105, 106, 107, 108, 110, 111, 112, 114).

The study included unmodified and modified groundwater samples (K, MB, NA, mixture, 0.1 and 10 µg $l^{-1}$) to confirm that the "re-buffering" of the groundwater had no effect on the degradation of selected CPA. However, on the one hand it was difficult to measure the pH in the supernatants (about 0.5 cm), and on the other hand the pH of the unmodified samples was additionally increased by the continuous $CO_2$ absorption. This strongly limited the comparability of the incubations, and future studies should be set up without the use of sodium hydroxide.

If the CPA were applied as single substances, the pH of the unmodified samples was increased by the continuous absorption of $CO_2$, whereas the pH of the modified samples fluctuated more or less around the adjusted value. A general upward trend of the unmodified groundwater pH was measured, if the selected test substances were applied in a mixture. However, the pH of the modified samples was also slighlty increased, which was unexpected and only detected in the mixtures.

The distribution of the applied radioactivity (AR) was similar between the unmodified and modified incubations for all fractions, aquifers, and concentrations (Tables 51, 62, 73; pages 105, 110, 114). This indicated that the “re-buffering” of the groundwater had no significant effect on the aquifer degradation activity. Due to the fluctuating pH values, it was impossible to make a clear statement, which showed that a revision of the test system design was necessary. Future studies should be set up without continuous $^{14}CO_2$ absorption, as e.g. already mentioned in the conclusions of the BA studies (3.1.4), to avoid the displacement of the chemical groundwater equilibrium and finally the increase of pH (Table 15; page 81).

The mineralization [% of AR] of **Isoproturon (IPU)** in the **aerobic** aquifer **Krauthausen** (modified groundwater) was nearly constant for the 0.1 µg $l^{-1}$ concentration after 90 days of incubation (Table 42; page 101), which was in contrast to the 10 µg $l^{-1}$ concentration. HPLC analysis showed that the metabolization of the active ingredient [AI] was low and stopped after 170 days of incubation (Figure 35; page 137, table 44; page 102). Metabolites of IPU were further degraded and finally mineralized up to 1.7% (0.0725 µg $kg^{-1}$) within 310 days of incubation (Table 42; page 101). This is in accordance with results from a chalk aquifer, where the mineralization of the phenyl-moiety was low and its extend was limited (71;77). However, if IPU was applied in a mixture with modified, as well as unmodified groundwater, the metabolization of the parent compound was about 20% higher, probably due to the “interaction” with IMI and DKN (Table 51; page 105). The aquifer Krauthausen had only a low adsorption potential with respect to IPU, applied as single substance and in a mixture, probably due to the low organic carbon content and sediment properties (Table 10; page 78). After 310 days of incubation, more than 85% of the applied radioactivity were extractable, and only < 5% were not extractable, which was in agreement with results from a sandy aquifer from Denmark (52).

The mineralization [% of AR] of **IPU** in the **iron-reducing** aquifer **Mulder-Beilen** (modified groundwater) and applied as single substance was in the range of radiochemical purity (Table 53; page 106; 2.1.2), and therefore not significant. This is in accordance to results from various aquifer studies, who failed to detect the anaerobic mineralization of IPU (83;85;86). If IPU was applied in a mixture with IMI and DKN, the metabolisation of the active ingredient [% of AR] was between 10 and 20% higher, compared to the single replicates (Figure 35; page 137, table 55; page 107). This was independent from the groundwater treatment (Table 63; page 110). Like Krauthausen, the sandy aquifer Mulder-Beilen (2.2.2) had only a low adsorption potential with respect to Isoproturon and the non-extractable fraction was > 3% for the 10 µg $l^{-1}$ concentration after 310 days of incubation (Table 11; page 79).

The mineralization [% of AR] and metabolisation of **IPU** in the **sulphur-reducing** aquifer **Nature A** was comparable to the iron-reducing aquifer Mulder-Beilen. If IPU was applied in a mixture with modified, as well as unmodified groundwater, the recovery of the parent compound was lowest (about 60% of AR) with respect to all aquifers (Figure 35; page 137), whereas the amount of the non-extractable fraction was highest (about 11% of AR), probably due to the higher clay content of the aquifer Nature A (Table 12; page 80). During incubation time (310 days), the mineralization [% of AR] of **Imidacloprid (IMI)** in the **aerobic** aquifer **Krauthausen** (modified groundwater) was not detectable for the 0.1 µg $l^{-1}$ concentration. It was low, but steadily increased up to 0.5% of AR, and 0.02 µg $kg^{-1}$ aquifer material for the 10 µg $l^{-1}$ concentration (Tables 45, 46; pages 102, 103). After 310 days of incubation, more than 84% of IMI were extractable at a concentration of 0.1 and 10 µg $l^{-1}$, whereas the non-extractable fraction was about 5% of AR. The recovery of the active ingredient (AI) was comparable between the modified and unmodified groundwater samples (Table 51; page 105), and reached about 75%, independent whether applied as single substance (Figure 34; page 136, table 47; page 103) or in a mixture with IPU and DKN (Table 52; page 106).

The mineralization, extraction and combustion [% of AR] of **IMI** in the **iron-reducing** aquifer **Mulder-Beilen** showed also no significant differences between the unmodified and modified groundwater treatment (Table 62; page 110). The mineralization [µg $kg^{-1}$ aquifer material] reached about 0.02 µg for both concentrations, whereas the extractable organic $^{14}C$ fraction was about 20% less for the 10 µg $l^{-1}$ concentration. If IMI was applied as a single substance, the recovery of the active ingredient was constant (about 50%) after 170 days of incubation (Figure 34; page 136). Unlike the mixtures, where the recovery of the active ingredient was only about 20% of AR after 310 days of incubation (Table 63; page 110). Mulder-Beilen had a higher adsorption potential than Krauthausen, and about 30% of AR were bound to soil particles. **IMI** was strongly bound to sediment particles of the aquifer **Nature A**, probably due to the clay content (Table 12; page 80). After 310 days of incubation, about 90% of AR were measured in the non-extractable organic C-fraction (Table 67; page 112).

The mineralization [% of AR] was in the range of radiochemical impurity, and the recovery of the AI steadily decreased and was even not detectable at the end of the study (Table 69; page 112). Therefore, it is supposed that mainly the active ingredient was bound to the sediment particles (Figure 34; page 136). As a result, Imidacloprid was not available to microbial degradation, whether applied as a single substance or in the mixture (Table 74; page 114). At a concentration of 10 µg $l^{-1}$, **Diketonitrile (DKN)** was steadily mineralized up to 0.003 µg $kg^{-1}$ aquifer material in the aquifer **Krauthausen** (Table 49; page 104).

However, the mineralization was in the range of radiochemical impurity and, like for 0.1 µg $l^{-1}$, where the measured values fluctuated and ranged between 0.4 and 0.8% of AR (Table 48; page 104), was therefore not singnificant. More than 90% of AR remained solved in the groundwater, mainly the active ingredient (Figure 37; page139, table 50; page 105), and less than 2% of AR were bound to the sediment particles. The recovery of the active ingredient was flucuating between 68.7 and 92.7% at a concentration of 10 µg $l^{-1}$ (Table 50; page 105), and with respect to the results from the mixtures (Table 52; page 106), no clear trend could be assessed.

**DKN** was not mineralized in the aquifer **Mulder-Beilen** (Table 60; page 109). About 90% of AR remained solved in the groundwater, and less than 2% were bound to soil particles (Table 60; page 109). The recovery of the AI steadily decreased to about 60% after 310 days of incubation (Figure 37; page 139), showing no difference whether applied as a single substance or in a mixture, as well as between the groundwater treatments (Table 63; page 110).

The mineralization of **DKN** in the aquifer **Nature A** was in the range of radiochemical impurity and therefore not significant. About 90% of AR remained solved in the groundwater, composed to about 50% of the active ingredient (Table 72; page 113), whereas about 4% of AR were bound to sediment particles (Table 70; page 113). If DKN was applied in a mixture with IMI and IPU, the recovery of the active ingredient was somewhat less compared to the single substance (Figure 37; page139, table 74; page 114).

### 3.2.2 DYNAMIC INCUBATION SYSTEM

The first **DAISY incubation study - D4** was accomplished with unmodified groundwater and due to the limited space of the anaerobic chamber (TOEPFER Lab Systems, Göttingen) only one single system was operated for Mulder-Beilen and Nature A. For the anaerobic aquifers, the CPA were applied in a mixture at a concentration of 10 µg $l^{-1}$. This was in contrast to the aerobic aquifer, where 20 replicates were included, in twofold repetition, applied as single substance and in a mixture (Table 25; page 87). After the setup and again one day after the application of the CPA, the pH, redox potential, and oxygen content of the groundwater were measured. The groundwater pH values were strongly increased (Krauthausen between 8.4 and 10, Mulder-Beilen up to 9.1, Nature A up to 9.0) and the study was stopped seven days after the setup to determine the relevant processes.

The second **DAISY incubation study - D5** was set up and processed as the study **D4** with the exception of using modified groundwater (2.2.4), a flow volume of about ½ of the total groundwater volume, and a flow velocity of about 0.2 m $h^{-1}$.

The test substances were applied after nine days of stable operation at a constant pH and redox potential (Krauthausen 6.8, oxygen-, Mulder-Beilen 7.4, iron-, Nature A 7.2, sulphur-reducing conditions). The microbial degradation activity should be studied by the measurement of mineralization rates, determined by LSC. After the second sampling point, it got obvious that qualitative informations were necessary to study also the metabolisation of CPA, as the measured mineralization rates were to low to study the microbial degradation activity (Figures 28, 29, 30, 32; pages 130, 131, 132, 134). During incubation time, and starting with day 75, radio high performance liquid chromotography (HPLC) was used to distinguish between active ingredients and metabolites. However, the measurements were restricted to the 10 µg $l^{-1}$ concentration, as the radioactivity of all other replicates was below the detection limit of HPLC (Tables 28, 31, 34, 37, 39, 41; pages 89, 92, 95, 98, 99, 100). HPLC analysis started only with incubation day 75, and specific degradation rates [µg $kg^{-1}$ aquifer material] were determined for the single substances (Tables 29, 32, 35; pages 90, 93, 96). This was impossible for the mixture, as it was impossible to distinguish between the mineralization of the respective CPA. However, the test systems were set up with an equal amount of groundwater (450 ml) and aquifer material (150 g). Therefore, it was possible to compare degradation rates [µg $kg^{-1}$ aquifer material] between the 0.1, 1, and 10 µg $l^{-1}$ concentration of the aquifer Krauthausen (applied as single substance), as well as the mineralization [% of AR] and metabolisation [AI% of AR] between the selected aquifers of a single concentration. The mass balances (=recovery) of all replicates ranged between 88.98 and 109.35% of AR, and standard deviations of LSC measurements were acceptable (Tables 27, 30, 33, 36, 38, 40; pages 88, 91, 94, 97, 99, 100). This confirmed the good reproducibility of DAISY.

The mineralization [% of AR] of **IPU** in the aquifer **Krauthausen** steadily increased during incubation time (Figure 28; page 130), and was 8.3% for 0.1 µg $l^{-1}$, as well as 1.3% for 1 and 10 µg $l^{-1}$ at final processing. However, the mineralization [% of AR] did not allow any statement about degradation rates [µg $kg^{-1}$ aquifer material], which were 0.678 µg for 10 µg $l^{-1}$, 0.023 µg for 1 µg $l^{-1}$, and 0.015 µg for 0.1 µg $l^{-1}$ (Table 29; page 90). Degradation rate and concentration were probably positively correlated, as the degradation rate of 10 µg $l^{-1}$ was about 29 times as large compared to 1 µg $l^{-1}$, and about 45 times to 0.1 µg $l^{-1}$. This was most likely due to the availability of IPU at the reactive sites, as most of the microorganisms were supposed to be attached to sediment surfaces. The revovery of the active ingredient [% of AR] decreased from about 90% to < 5% for 10 µg $l^{-1}$ (Figure 28; page 130, table 28; page 89), which approved that the metabolisation of the parent compound was much faster than the mineralization of the entire molecule (Table 27; page 88).

This was in accordance to the mixture, where the recovery of the active ingredient in the groundwater was only 1.9 and 9.6% for 10 µg $l^{-1}$, as well as 3.1% for 1 µg $l^{-1}$ (Table 37; page 98), showing a strong decrease of the active ingredient between day 75 and 150. For all variants, between 5 and 7% of the AR were recovered in the extract (almost 100% AI). They were probably bound to sediment particles, and therefore not accessible to the microbial degradation (Figures 28, 29; pages 130; 131). However, only about 2% of the AR were measured as non-extractable, and more than 80% were dissolved in the groundwater, which confirmed the general low adsorption potential of the aquifer Krauthausen (Table 27; page 88).

The mineralization [% of AR], and the respective degradation rates [µg $kg^{-1}$ aquifer material] of **IMI** in the aquifer **Krauthausen** were 4.3% ⇔ 0.008 µg for 0.1 µg $l^{-1}$, 1.1% ⇔ 0.02 µg and 1.8% ⇔ 0.033 µg for 1 µg $l^{-1}$, as well as 0.35% ⇔ 0.063 µg and 0.36% ⇔ 0.064 µg for the 10 µg $l^{-1}$ concentration (Table 30, 32; pages 91, 93). Degradation rate and test substance concentration were correlated, as the degradation rate of 10 µg $l^{-1}$ was about 4 times as large compared to 1 µg $l^{-1}$, and about 8 times to 0.1 µg $l^{-1}$. The extractable organic $^{14}C$ fraction was between 6.5 and 8.3% of AR (almost 100% AI), and no significant differences were measured between the selected concentrations. The revovery of the active ingredient [% of AR] for 10 µg $l^{-1}$ decreased in the groundwater from about 80% to about 70% within 300 days of incubation (Figure 28; page 130, table 31; page 92). For the mixture, the metabolisation of the active ingredient was considerably faster (Figure 29; page 131), and the recovery of the active ingredient ranged between 49.8 and 57.2% at 10 µg $l^{-1}$, and 55.9% and 73.7% at 1 µg $l^{-1}$ (Table 37; page 98). Due to the absence of any metabolite > 10%, a primary attack of the parent molecule was supposed, which probably determined the aerobic degradation kinetic of IMI (103-105). The aquifer Krauthausen had low adsorption potential with respect to IMI and the non-extractable organic $^{14}C$ fraction increased, if the test substance concentration decreased (Table 30; page 91). This was in accordance with results from the literature, where low application rates showed highest sorption (111;112).

The mineralization [% of AR] and degradation rates [µg $kg^{-1}$ aquifer material] of **DKN** in the aquifer **Krauthausen** were 1.68% ⇔ 0.003 µg for 0.1 µg $l^{-1}$, 0.66% ⇔ 0.011 µg and 1.25% ⇔ 0.022 µg for 1 µg $l^{-1}$, as well as 0.33% ⇔ 0.060 µg for 10 µg $l^{-1}$ (Tables 33, 35; pages 94, 96). The degradation rate and concentration of DKN were also correlated, as the degradation rate at 10 µg $l^{-1}$ was about 3 times as large compared to 1 µg $l^{-1}$, and about 20 times to 0.1 µg $l^{-1}$ (Table 35; page 96). For all samples, more than 86% of AR remained as dissolved organic $^{14}C$ fraction in the groundwater, and only less than 2% were recovered as non-extractable organic $^{14}C$ (Table 33; page 94). This confirmed the general and DKN specific low adsorption potential of the aquifer Krauthausen.

DKN had the lowest specific radioactivity with respect to all selected test substances (2.1), and the radioactivity of liquid samples was often below the detection limit of radio HPLC (Table 34; page 95). However, the revovery of the active ingredient [% of AR] in the groundwater decreased from about 84% (day 75) to about 13% (day 300) for the 10 µg $l^{-1}$ concentration (Figure 28; page 130). The presence of IPU, and DKN probably increased the metabolisation of the parent compound, but standard deviations were high, and it was impossible to put a clear statement (Figure 29; page 131). The extractable organic $^{14}C$ fraction ranged between 6.6 and 7.8% of AR for all samples and concentrations, which was composed of almost 100% active ingredient for the 10 µg $l^{-1}$ (Table 34; page 95). After 300 days of incubation, the aquifer Krauthausen showed the strongest mineralization and metabolisation potential with respect to IPU, followed by DKN and IMI, applied as single substances (Figure 28; page 130). This was in contrast to the mixture, where the metabolisation of IMI strongly increased, whereas the metabolisation of IPU strongly decreased (Figure 29; page 131).

| Concentration [µg $l^{-1}$] | | IPU | IMI | DKN | |
|---|---|---|---|---|---|
| single substance | 0.1 | 8.3 | 4.3 | 1.7 | [$^{14}CO_2$% of AR] |
| | 1 | 1.3 | 1.1/ 1.8 | 0.7/ 1.3 | |
| | 10 | 1.3 | 0.4 | 0.3 | |
| Mixture | 10 | 3.3/ 4.3 | 68.2/ 72.5 | 13.3/ 14.0 | [AI% of AR] |
| | 10 | 57.2/ 49.8 | 2.0/ 9.6 | 4.9/ 10.1 | |

**Table 7: The DAISY incubation study - D5: Aquifer Krauthausen, mineralization [% of AR] and active ingredient [% of AR], test substances Isoproturon (IPU), Imidacloprid (IMI), and Diketonitrile (DKN), selected values of tables 27, 28, 30, 31, 33, 34, 36, 37**

The mineralization [% of AR] of IMI, IPU, and DKN, applied in a mixture (1:1:1), in the aquifer Mulder-Beilen was < 0.2% within 300 days of incubation (Table 38; page 99), in the range of radiochemical impurity (2.1) and therefore not significant. Dissolved organic $^{14}C$ was 73.5%, extractable organic $^{14}C$ was 10.9%, and non-extractable organic $^{14}C$ was 7.7%. Mulder-Beilen had a somewhat greater adsorption potential compared to the aquifer Krauthausen, probably due to the higher potential cation exchange capacity (Table 10, 11; pages 78, 79). The extractable organic $^{14}C$ fraction was analysed by radio HPLC to measure the metabolisation of CPA. After 300 days of incubation, the recovery of the active ingredients [% of AR] were only 0.4% for IPU, 6.2% for IMI, and 1.9% for DKN.

For Isoproturon, between 10.6% (day 75) and 4.5% (day 300) were recovered as active ingredient [% of AR] in the groundwater, which was in contrast to Krauthausen, where 49.8% and 57.2% were detected. The recovery of the IMI active ingredient [% of AR] was between 54.6% (day 75) and 34.9 (day 300) that was also in contrast to Krauthausen, where 1.9 and 9.6% were recovered. At iron-reducing redox conditions, the metabolisation of the IPU parent compound was increased, whereas it was decreased for IMI, and was unaltered for DKN (Figure 30; page 132).

| | | Active ingredient [% of AR] | | |
|---|---|---|---|---|
| **Aquifer** | **Fraction** | **IPU** | **IMI** | **DKN** |
| K | groundwater | 49.8/ 57.2 | 2.0/ 9.6 | 4.9/ 10.1 |
| MB | | 4.5 | 35.0 | 14.8 |
| NA | | n.d. | 29.9 | 13.1 |
| K | extract | 7.1/ 7.1 | 4.9/ 6.2 | 0.9/ 7.5 |
| MB | | 0.4 | 6.2 | 1.9 |
| NA | | n.d. | 19.1 | 1.8 |

**Table 8: The DAISY incubation study - D5: Krauthausen (K), Mulder-Beilen (MB), and Nature A (NA), active ingredient [% of AR] Isoproturon (IPU), Imidacloprid (IMI), and Diketonitrile (DKN), concentration 10 µg $l^{-1}$, selected values of tables 31, 34, 37, 39, 41**

The mineralization [% of AR] of the selected CPA in the aquifer **Nature A** and applied in the mixture was < 0.2% (Table 40, page 100), and like for Mulder-Beilen in the range of radiochemical impurity and therefore not significant. The adsorption potential was greatest for the aquifer Nature A and the non-extractable $^{14}C$ fraction reached 22.9% at final processing (Mulder-Beilen 11.0%; Krauthausen 0.8%). The dissolved organic $^{14}C$ fraction in the groundwater was 45.6% of AR, whereas the extractable organic $^{14}C$ was 23.5%. Like for Mulder-Beilen, it was supposed that primarily the parent compounds were adsorbed, as the extractable organic $^{14}C$ fraction was composed of 19.1% IMI and 1.8% DKN [% of AR]. In addition and also in accordance to Mulder-Beilen, IMI had the strongest adsorption potential with respect to the selected CPA, probably due to the highest potential cation exchange capacity of the aquifer (Table 12; page 80). After 75 days of incubation, only 0.4% of the IPU active ingredient [% of AR] were recovered in the groundwater (Figure 32; page 134). This confirmed that the metabolisation of IPU was correlated with the redox potential.

The recovery of the IMI active ingredient [% of AR] in the groundwater was somewhat lower compared to Mulder-Beilen (Figure 30; page 132), but confirmed the already supposed correlation of metabolisation rate and redox potential (Table 41; page 100).

## 3.3 THE INTRINSIC MICROBIAL DEGRADATION POTENTIAL – ENRICHMENT OF MICROBIAL AQUIFER COMMUNITIES IN BIOREACTORS

Microbial growth was supposed, as the bacterial cell number increased for all enrichment cultures from about $7.6 \times 10^5$ particles $ml^{-1}$ (in-situ groundwater) to about $8 \times 10^8$ (R1), $2 \times 10^9$ (R2), and $7.6 \times 10^7$ (R3) within two weeks (Figure 41; page 143). This assumption was confirmed by the subsequent accumulation of biofilms in R1 and R2, which inhibited further particle measurements. In addition, the comparison of DGGE fingerprints of R1, R2, and R3 with the in-situ groundwater population, showed a significant shift with respect to the pattern of bands (Figure 18; page 73). However, due to the objectives of the thesis (1.2) and limitations in time, the DNA fingerprints were not uploaded into a database to assess similarities, or to determine the microbial structural differences among the treatments. But as each band is theoretically representing a certain bacterial strain present in the community (145), a strong impact of the enrichment conditions, as well as available C-source on the in-situ groundwater population was assessed.

For R1, some single bands strongly increased, accociated with a decrease of the general population differentiation, which can be explained by the fact that some bacteria from the near-natural aquifer have profited more, or faster than others from the C-sources supplied. After day 50, the occurrence and accumulation of biofilms lead to a lower DNA concentration in the sampled aliquots of R1. The shift of the R2 fingerprint seemed to be somewhat comparable to the fingerprint of R1, but one single band was more dominant, and the influence of the biofilms on the DNA concentration in the sampled aliquots was additionally less. The enrichment of the microbial community was not really expected for R3, as IMI, IPU, and DKN were the only available C-sources in the medium. Therefore, it was a great success to determine an increase of the DNA concentration after day 50, as the appearance of DGGE bands must have been associated with the metabolism of one of the three CPA.

UV-HPLC was used to detect the metabolisation of CPA , and the in- and outflow concentration of CPA was quantified for R2 and R3. HPLC results did support the assumption of microbial growth. After 50 incubation days, only 40.8% of the initial IPU concentration was measured in the outflow of R2, as well as 94.3% in the outflow of R3 (Figure 40; page 142).

IPU was probably degraded as a single substrate in R3, as well as a co-metabolic substrate in R2, as IMI and DKN were not degraded, IPU was the only available C-source in R3, and the recovery of IPU in R2 was less compared to R3. Analysis by denaturing gradient gel electrophoresis revealed that the microbial community was responsible for the mineralization activity, as e.g. described for Linuron (146). The results showed that the bacterial community was disturbed after the start of treatment, continued to change for about 50 days and then formed a relatively stable community, different from the original community structure. The findings are in accordance with the results from topsoil studies (89), where a soil bacterium (designated strain SRS2) was isolated from a previously IPU-treated agricultural soil, able to metabolize the phenylurea herbicide Isoproturon 3-(4-isopropylphenyl)-1,1-dimethylurea (IPU). However, the results are even more significant, as the inoculum was derived from a near-natural aquifer.

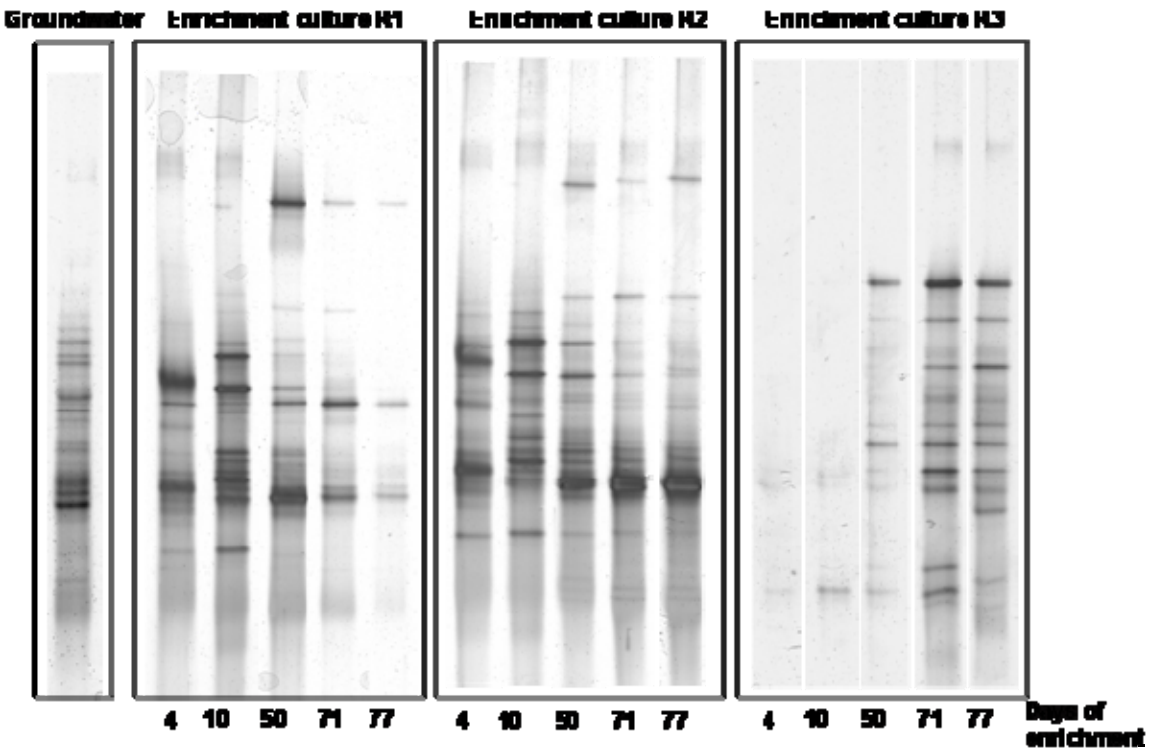



**Figure 18: DGGE Fingerprints: In-situ groundwater and microbial enrichment cultures**

# 4 GENERAL CONCLUSIONS AND OUTLOOK

The results of the static and dynamic degradation studies confirmed that near natural aquifers do have a general microbial degradation potential with respect to aromatic molecules at concentrations ≤ 10 µg $l^{-1}$ (3.2). It was shown that the storage of aquifer material at +4°C for about two years had no detectable effect on the microbial degradation potential of benzoic acid (3.1), and biological control samples proved that the degradation of selected CPA was a biological process (3.2.1). By developing a static and dynamic test system (2.5) and using comparative degradation studies with benzoic acid (BA), I showed that dynamic flow conditions generally increase the degradation kinetics of benzoic acid (3.1.3). This effect can also be assumed for the degradation of other aromatic substances. However, comparative degradation studies are only feasible when the test systems differ exclusively in the factor under investigation. In addition to the concentration of the test substance, the selected quantities of aquifer material and groundwater, and particularly their ratio, are crucial factors concerning the degradation kinetics and the comparability of the studies.

Microbial aquifer populations appear to have distinct capabilities with respect to the degradation of aromatic substances (16), although the degradation kinetics were generally slower decreased as compared to topsoils. One reason is probably the ambient temperature, as it was shown that the degradation kinetics of the aquifer microflora, which should be adapted to even and low temperatures, were enhanced at higher termperatures (3.1.3; 3.2.1). The microbial degradation activity was strongly dependent on the molecule structure, as e.g. BA was mineralized within hours, whereas Imidacloprid (IMI), Isoproturon (IPU) and Diketonitrile (DKN) were more recalcitrant and only noteworthy metabolized on the long-term scale (> 360 days). The mineralization [% of AR] was predominant small and it was more important to measure the metabolisation of CPA. However, the dissipitation of the active ingredient [% of applied radioactivity] has to be supplemented with information concerning the main metabolites, involved in the subsequent degradation pathways. This was shown for IMI at anerobic conditions, where an appreciable accumulation of metabolites was measured (3.2.1), as already described in the literature (103-105). The simulation of different aquifer conditions included oxygen, iron- and sulphur reducing conditions, as the redox-conditions are an important parameter to study the degradation of CPA (17).

As part of the work, I compiled important information on the experimental design of future groundwater studies. BA was introduced as a suitable model compound to assess the microbiological activity of shallow aquifers with respect to various aromatic substances. We used it to investigate processes relevant to degradation within the scope of developing a static and dynamic test system. It was shown that simple physical parameters, such as the dynamic solubility of carbon dioxide in water, were not taken into account in groundwater studies in the past (12). This led, for example, to an incorrect estimation of the existing degradation potential in the existing benzoic acid studies (3.1.2).

It was also determined that continuous absorption of $CO_2$, by e.g. sodium hydroxide that is widely used for the absorption of mineralized $^{14}C$-labelled test substances (53;140;141), interferes considerably with the chemical groundwater equilibrium. The reason for this is the unspecific absorption of $CO_2$, its supply from the groundwater bicarbonates, and the resulting enrichment of $OH^-$ ions (2.2.4). This ultimately gives rise to a strong increase in the pH value (3.1.2). As the pH value is an important factor for the degradation pathway of many aromatic molecules, it is astonishing that this fact has not been taken into account in the past. Future degradation studies should therefore completely dispense without continuous absorption of $CO_2$.

The test systems developed within the scope of this work (2.5) were also used to investigate the current intrinsic degradation potential of three shallow aquifers for IMI, IPU and DKN. In various long-term studies, a general potential was assessed under oxygen-, iron-, and sulfur-reducing conditions and at a concentration of 0.1, 1, and 10 µg $l^{-1}$. These were only comparable, if the measured mineralization was refered to the amount of aquifer material. Degradation rates and concentration of test substances were generally correlated, as e.g. shown for IPU in the DAISY incubation study – D5 (3.2.2). It was revealed, however, that the lower mineralization rates led to the accumulation of metabolites becoming the most important parameter for estimating the degradation potential at a concentration of ≤ 10 µg $l^{-1}$.

The combined application of test substances had a positive effect on the degradation of individual substances here, as e.g. shown for IPU in the microcosm incubation study – M4 (3.2.1). Furthermore, I demonstrated the degradation of IPU under anaerobic conditions for the first time. The absorption potential of the selected CPA was influenced by the physical and chemical characteristics of the selected aquifers (Tables 10, 11, 12 ; pages 78, 79, 80). The strongest absorption potential was measured for the sulphur-reducing aquifer Nature A, where most of IMI was bound to the sediment particles (3.2).

Not all of the findings from the BA studies could be taken into account when setting up and conducting the long-term studies (3.1.4). Moreover, the test systems were only developed in the final stages of this work and led to a patent application for the dynamic test system, as well as to a contract for another GLP study from the same sponsor. At the end of the thesis, I was able to operate DAISY without the continuous absorption of $CO_2$, which offered the possibility to accomplish aquifer degradation studies using in-situ groundwater. The test systems and methods developed enable a realistic estimate to be made of the intrinsic microbial degradation potential of shallow aquifers in the laboratory under GLP conditions. Further studies will be conducted to estimate the hazard potential associated with different test substances and to transfer the results to the field scale.

The intrinsic degradation potential of the aerobic shallow aquifer was analysed in enrichment cultures, for which a variety of carbon sources were available (2.4). The microorganisms metabolised IPU as both the only C-source as well as co-metabolically. This was accompanied by an increase in the microbial biomass. Furthermore, using molecular biological “fingerprint” techniques (DGGE) a strong displacement of the microbial population was confirmed. The results confirmed that it is necessary to distinguish between the current microbial degradation activity and the total microbial degradation potential. Further studies should focus on questions concerning the effect of substrate limitation, where DGGE analysis should be used to monitor changes in bacterial diversity. The approach (Figure 39; page 141) holds the potential to isolate bacterial strains with a specific degradation potential for individual pesticides in future enrichment experiments.

# 5 ANNEX

**Table 9: The aquifer Krauthausen (K), Mulder-Beilen (MB), and Nature A (NA): Redox potential, temperature, and pH**

| Aquifer | Date | Measurement | Time | Eh (mV) | pH | Temperature (°C) |
|---|---|---|---|---|---|---|
| **Krauthausen** | 03/16/2004 | flow-through | 14:00 | installation | | |
| | | | 14:20 | 308 | 6.79 | 11.5 |
| | | | 14:30 | 311 | 6.83 | 11.6 |
| **Mulder-Beilen** | 03/12/2004 | in-situ | 10:55 | installation | | |
| | | | 11:16 | 234 | | |
| | | | 12:05 | 230 | | |
| | | flow-through | 11:56 | 61 | | 8.9 |
| | | | 11:59 | 46 | 7.18 | |
| | | | 12:02 | 39 | | |
| | | | 12:03 | | 7.21 | |
| | 04/08/2004 | in-situ | 13:30 | installation | | |
| | | | 13:40 | 245 | | |
| | | | 14:25 | 229 | | |
| | | | 14:50 | 228 | | |
| | | flow-through | 11:59 | 95 | 7.09 | 8.3 |
| | | | 12:10 | 57 | 7.23 | |
| | | | 12:25 | 51 | 7.23 | |
| **Nature A** | 03/18/2004 | flow-through | 17:13 | 4 | 7.38 | |
| | | | 17:19 | -30 | | |
| | | | 17:25 | -58 | | |
| | | | 17:30 | -65 | 7.44 | 10.5 |
| | 04/07/2004 | in-situ | 13:30 | installation | | |
| | | | 13:45 | 173 | | |
| | | | 14:05 | 195 | | |
| | | | 14:30 | installation | | |
| | | | 14:40 | 180 | | |
| | | | 14:45 | 187 | | |
| | | flow-through | 12:35 | -5 | 7.29 | |
| | | | 12:50 | -27 | 7.40 | 9.6 |
| | | | 12:58 | -26 | 7.38 | |
| | | | 14:28 | -80 | 7.39 | |
| | | | 14:40 | -86 | 7.40 | |

**Table 10: The aquifer Krauthausen (K): Physical and chemical characteristics of sampled groundwater and aquifer material**

| Groundwater | Assay | Result | Unit |
|---|---|---|---|
| | pH | 6,90 | - |
| | electrical conductivity | 908,00 | µS $cm^{-1}$ |
| | nitrite-nitrogen | < 0,02 | mg $l^{-1}$ |
| | nitrate-nitrogen | 14,06 | mg $l^{-1}$ |
| | phosphate-phosphorous | < 0,50 | mg $l^{-1}$ |
| | fluoride | 0,37 | mg $l^{-1}$ |
| | cyanide | < 0,06 | mg $l^{-1}$ |
| | mercury | < 0,20 | µg $l^{-1}$ |
| | boron | 22,90 | µg $l^{-1}$ |
| | calcium | 124500,00 | µg $l^{-1}$ |
| | potassium | 65700,00 | µg $l^{-1}$ |
| | magnesium | 19000,00 | µg $l^{-1}$ |
| | sodium | 29200,00 | µg $l^{-1}$ |
| | total hardness of water according to German standards | 21,80 | °dH |
| | TOC (total organic carbon) | n.m. | mg $l^{-1}$ |
| | DOC (dissolved organic carbon) | 1,1 | mg $l^{-1}$ |

| Aquifer material | Assay | Result | Unit |
|---|---|---|---|
| | pH ($CaCl_2$) | 6,8 | - |
| | pH ($H_2O$) | 7,0 | - |
| | phosphorous (CAL) | < 1 | mg $100g^{-1}$ |
| | potassium ($K_2O$ in CAL) | 2 | mg $100g^{-1}$ |
| | magnesium (Mg) in $CaCl_2$ | 2 | mg $100g^{-1}$ |
| | sodium (Na) in $CaCl_2$ | 1,2 | mg $100g^{-1}$ |
| | iron (Fe) in EDTA | 13 | mg $kg^{-1}$ |
| | copper (Cu) in CAT | < 0,5 | mg $kg^{-1}$ |
| | manganese (Mn) in CAT | 9,6 | mg $kg^{-1}$ |
| | zinc (Zn) in CAT | 0,4 | mg $kg^{-1}$ |
| | boron (B) in CAT | < 0,10 | mg $kg^{-1}$ |
| | aluminum (Al) in $H_2O$ | 17 | mg $kg^{-1}$ |
| | calcium (Ca) in sodium formate solution | 176 | mg $kg^{-1}$ |
| | humus | < 0,70 | % |
| | total carbon (TC) | < 0,40 | % |
| | total nitrogen (TN) | < 0,10 | % |
| | clay (<0,002 mm) | 0,8 | % |
| | fine silt (0,002 - 0,0063 mm) | 1,4 | % |
| | medium silt (0,0063 - 0,02 mm) | 1,2 | % |
| | coarse silt (0,02 - 0,063 mm) | 1,5 | % |
| | fine sand (0,063 - 0,2 mm) | 4,0 | % |
| | medium sand (0,2 - 0,63 mm) | 57,4 | % |
| | coarse sand (0,63 - 2 mm) | 33,7 | % |
| | sulfur (S) | 13 | mg $kg^{-1}$ |
| | potential cation exchange capacity | 2,354 | cmol + $kg^{-1}$ |
| | exchangeable sodium | < 0,10 | cmol + $kg^{-1}$ |
| | exchangeable potassium | 1,77 | cmol + $kg^{-1}$ |
| | exchangeable calcium | 0,435 | cmol + $kg^{-1}$ |
| | exchangeable magnesium | 0,149 | cmol + $kg^{-1}$ |

**Table 11: The aquifer Mulder-Beilen (MB): Physical and chemical characteristics of sampled groundwater and aquifer material**

| Groundwater | Assay | Result | Unit |
|---|---|---|---|
| | pH | 7,40 | - |
| | electrical conductivity | 255,00 | µS $cm^{-1}$ |
| | nitrite-nitrogen | < 0,02 | mg $l^{-1}$ |
| | nitrate-nitrogen | < 0,23 | mg $l^{-1}$ |
| | phosphate-phosphorous | < 0,50 | mg $l^{-1}$ |
| | fluoride | 0,25 | mg $l^{-1}$ |
| | cyanide | < 0,06 | mg $l^{-1}$ |
| | mercury | < 0,20 | µg $l^{-1}$ |
| | calcium | 41250,00 | µg $l^{-1}$ |
| | iron | 218,50 | µg $l^{-1}$ |
| | potassium | 2460,00 | µg $l^{-1}$ |
| | magnesium | 14950,00 | µg $l^{-1}$ |
| | manganese | 171,50 | µg $l^{-1}$ |
| | sodium | 8020,00 | µg $l^{-1}$ |
| | TOC (total organic carbon) | 3 | mg $l^{-1}$ |
| | DOC (dissolved organic carbon) | 3,3 | mg $l^{-1}$ |

| Aquifer material | Assay | Result | Unit |
|---|---|---|---|
| | pH ($CaCl_2$) | 7,3 | - |
| | pH ($H_2O$) | 7,5 | - |
| | phosphorous (CAL) | < 1 | mg $100g^{-1}$ |
| | potassium ($K_2O$ in CAL) | 3 | mg $100g^{-1}$ |
| | magnesium (Mg) in $CaCl_2$ | 1 | mg $100g^{-1}$ |
| | sodium (Na) in $CaCl_2$ | 1,0 | mg $100g^{-1}$ |
| | iron (Fe) in EDTA | 79 | mg $kg^{-1}$ |
| | copper (Cu) in CAT | 0,8 | mg $kg^{-1}$ |
| | manganese (Mn) in CAT | 2,3 | mg $kg^{-1}$ |
| | zinc (Zn) in CAT | 0,8 | mg $kg^{-1}$ |
| | boron (B) in CAT | < 0,10 | mg $kg^{-1}$ |
| | aluminum (Al) in $H_2O$ | 101 | mg $kg^{-1}$ |
| | calcium (Ca) in sodium formate solution | 255 | mg $kg^{-1}$ |
| | humus | < 0,70 | % |
| | total carbon (TC) | < 0,40 | % |
| | total nitrogen (TN) | < 0,10 | % |
| | clay (<0,002 mm) | 2,2 | % |
| | fine silt (0,002 - 0,0063 mm) | 0,4 | % |
| | medium silt (0,0063 - 0,02 mm) | 0,7 | % |
| | coarse silt (0,02 - 0,063 mm) | 1,5 | % |
| | fine sand (0,063 - 0,2 mm) | 31,3 | % |
| | medium sand (0,2 - 0,63 mm) | 61,4 | % |
| | coarse sand (0,63 - 2 mm) | 2,5 | % |
| | sulfur (S) | 46 | mg $kg^{-1}$ |
| | potential cation exchange capacity | 2,667 | cmol + $kg^{-1}$ |
| | exchangeable sodium | < 0,10 | cmol + $kg^{-1}$ |
| | exchangeable potassium | 2,01 | cmol + $kg^{-1}$ |
| | exchangeable calcium | 0,524 | cmol + $kg^{-1}$ |
| | exchangeable magnesium | 0,133 | cmol + $kg^{-1}$ |

**Table 12: The aquifer Nature A (NA): Physical and chemical characteristics of sampled groundwater and aquifer material**

| Groundwater | Assay | Result | Unit |
|---|---|---|---|
| | pH | 7,40 | - |
| | electrical conductivity | 2410,00 | µS $cm^{-1}$ |
| | nitrite-nitrogen | < 0,02 | mg $l^{-1}$ |
| | nitrate-nitrogen | < 0,23 | mg $l^{-1}$ |
| | phosphate-phosphorous | < 0,50 | mg $l^{-1}$ |
| | fluoride | 0,63 | mg $l^{-1}$ |
| | cyanide | < 0,06 | mg $l^{-1}$ |
| | mercury | < 0,20 | µg $l^{-1}$ |
| | boron | 293,00 | µg $l^{-1}$ |
| | calcium | 127000,00 | µg $l^{-1}$ |
| | potassium | 48300,00 | µg $l^{-1}$ |
| | magnesium | 33100,00 | µg $l^{-1}$ |
| | manganese | 528,00 | µg $l^{-1}$ |
| | sodium | 333000,00 | µg $l^{-1}$ |
| | TOC (total organic carbon) | 14 | mg $l^{-1}$ |
| | DOC (dissolved organic carbon) | 14,0 | mg $l^{-1}$ |

| Aquifer material | Assay | Result | Unit |
|---|---|---|---|
| | pH ($CaCl_2$) | 5,7 | - |
| | pH ($H_2O$) | 7,7 | - |
| | phosphorous (CAL) | 3 | mg $100g^{-1}$ |
| | potassium ($K_2O$ in CAL) | 10 | mg $100g^{-1}$ |
| | magnesium (Mg) in $CaCl_2$ | 6 | mg $100g^{-1}$ |
| | sodium (Na) in $CaCl_2$ | 8,9 | mg $100g^{-1}$ |
| | iron (Fe) in EDTA | 190 | mg $kg^{-1}$ |
| | copper (Cu) in CAT | 0,5 | mg $kg^{-1}$ |
| | manganese (Mn) in CAT | 14 | mg $kg^{-1}$ |
| | zinc (Zn) in CAT | 3,3 | mg $kg^{-1}$ |
| | boron (B) in CAT | 0,42 | mg $kg^{-1}$ |
| | aluminum (Al) in $H_2O$ | 17 | mg $kg^{-1}$ |
| | calcium (Ca) in sodium formate solution | 15056 | mg $kg^{-1}$ |
| | humus | 0,99 | % |
| | total carbon (TC) | 0,58 | % |
| | total nitrogen (TN) | < 0,10 | % |
| | clay (<0,002 mm) | 2,7 | % |
| | fine silt (0,002 - 0,0063 mm) | 0,8 | % |
| | medium silt (0,0063 - 0,02 mm) | 0,9 | % |
| | coarse silt (0,02 - 0,063 mm) | 2,0 | % |
| | fine sand (0,063 - 0,2 mm) | 65,9 | % |
| | medium sand (0,2 - 0,63 mm) | 27,5 | % |
| | coarse sand (0,63 - 2 mm) | 0,1 | % |
| | sulfur (S) | 1008 | mg $kg^{-1}$ |
| | capacity | 10,37 | cmol + $kg^{-1}$ |
| | exchangeable sodium | < 0,10 | cmol + $kg^{-1}$ |
| | exchangeable potassium | 8,45 | cmol + $kg^{-1}$ |
| | exchangeable calcium | 1,61 | cmol + $kg^{-1}$ |
| | exchangeable magnesium | 0,313 | cmol + $kg^{-1}$ |

**Table 13: The aquifer Krauthausen (K), Mulder-Beilen (MB), and Nature A (NA): Groundwater chemistry (selected values unmodified, bicarbonate free, and modified groundwater)**

| | | Krauthausen | | | Mulder-Beilen | Nature A |
|---|---|---|---|---|---|---|
| year | 1995 | 2005 | 2005[1] | 2005[2] | 2005 | 2005 |
| | | | **cations mg/ l** | | | |
| $Na^+$ | 28.1 | 38.8 | 74.7 | 148.0 | 391.2 | 13.0 |
| $K^+$ | 3.3 | 4.7 | 8.7 | 72.6 | 96.2 | 7.8 |
| $Mg^{2+}$ | 21.9 | 20.1 | 20.1 | 24.0 | 50.8 | 4.2 |
| $Ca^{2+}$ | 135.0 | 130.5 | 133.3 | 125.4 | 180.5 | 33.8 |
| ∑ Fe | 0.1 | < 0.03 | 0.086 | 0.094 | 1.5 | 5.0 |
| ∑ Mn | n.d. | 0.007 | < 0.02 | < 0.02 | 1.8 | 0.068 |
| $Nh_4^+$ | 0.27 | - | - | - | - | - |
| ∑ cations | 188.67 | 194.14 | 236.9 | 370.15 | 722 | 63.8 |
| | | | **anions mg/ l** | | | |
| $HCO_3^-$ | 201 | 204 | - | - | 119 | 319 |
| $CL^-$ | 75.8 | 74.7 | 221.0 | 218.0 | 242 | 12.5 |
| $NO_3^-$ | 96.8 | 69.0 | 68.6 | 67.7 | 0.02 | 0.02 |
| $Br^{2-}$ | - | 0.15 | 0.15 | 0.15 | 2.91 | < 0.02 |
| $SO^{2-}_4$ | 139.0 | 138 | 136 | 136 | 243 | 22.6 |
| $PO^{4-}_3$ | - | < 0.05 | < 0.05 | 98.2 | 0.09 | < 0.05 |
| ∑ anions | 512.6 | 240.77 | 289.82 | 287.22 | 242.11 | 15.54 |

**Table 14: The aquifer Krauthausen (K): Increase of pH by atmospheric pressure change**

| Method | pH 1 | pH 2 |
|---|---|---|
| pH/Ion meter | 6.8 | 8.3 |
| litmus paper | < 7 | > 8 |

The pH 1 was measured directly after groundwater sampling (temperature 12°C), whereas pH 2 was measured after two weeks at atmospheric equilibrium (temperature 20°C)

**Table 15: The aquifer Krauthausen (K): Increase of groundwater pH by continuous $CO_2$ absorption**

| | | | incubation time (days) | | |
|---|---|---|---|---|---|
| **Matrix** | | **$CO_2$ sink** | **0** | **3** | **7** |
| Groundwater (GW) | 40 ml | Sodium hydroxide | 6.8 | 8.8 | 9.2 |
| Aquifer material (AM) | - | Soda lime | 6.8 | 8.9 | 9.3 |
| GW /AM | 40 ml/ 100 g | Sodium hydroxide | 6.8 | 8.8 | 8.9 |
| GW /AM | 40 ml/ 100 g | Soda lime | 6.8 | 8.7 | 8.7 |

**Table 16: The relative and absolute amount of test substance with respect to the aquifer material to groundwater ratio of the Microcosm, DAISY, and Column test system**

| | Microcosm<br>static | DAISY<br>dynamic | Column<br>static |
|---|---|---|---|
| **matrix** | | | |
| groundwater [ml] | 40 | 450 | 450 |
| aquifer material [g] | 100 | 150 | 150 |
| **test substance** | | | |
| [µg] | 0.4 | 4.5 | 4.5 |
| [µg $l^{-1}$] | 10 | 10 | 10 |
| [µg $g^{-1}$] | 0.004 | 0.03 | 0.03 |

**Table 17: Enrichment cultures: Inorganic media components (10 l)**

| **Components** | | | |
|---|---|---|---|
| $KH_2PO_4$[g] | 0.35 | **Stock solution A** | |
| $K_2HPO_4$ [g] | 2.19 | $MgSO_4$ x 7 $H_2O$ [g $l^{-1}$] | 10.0 |
| $(NH_4)_2SO_4$ [g] | 0.5 | $Na_2MoO_4$ x 2 $H_2O$ [g $l^{-1}$] | 1.0 |
| | | **Stock solution B** | |
| Stock solution A [ml] | 10.0 | NaCl [g $l^{-1}$] | 10.0 |
| Stock solution B [ml] | 10.0 | $CaCL_2$ x 2 $H_2O$ [g $l^{-1}$] | 26.0 |
| Trace element solution [ml] | 10.0 | $Na_2$(EDTA) x 2 $H_2O$ [g $l^{-1}$] | 2.8 |
| | | $FeCl_3$ x 6 $H_2O$ [g $l^{-1}$] | 2.0 |

**Table 18: The microcosm incubation study - M1: Distribution of applied radioactivity [%], aquifer Krauthausen, test substance benzoic acid**

| Sample | Mineralization [% of AR] | Extraction [% of AR] | Combustion [% of AR] | Recovery [% of AR] |
|---|---|---|---|---|
| 1 | 5.69 | 48.28 | 31.77 | 85.74 |
| 2 | 5.60 | 51.63 | 34.46 | 91.69 |
| 3 | 7.10 | 48.61 | 35.11 | 90.82 |
| 4 | 6.60 | 50.00 | 32.45 | 89.05 |
| 5 | 4.50 | 51.52 | 34.00 | 90.03 |
| 6 | 5.40 | 50.95 | 34.36 | 90.71 |
| 7 | 6.26 | 48.65 | 33.01 | 87.92 |
| 8 | 5.89 | 48.84 | 33.65 | 88.37 |
| 9 | 5.53 | 49.16 | 34.98 | 89.67 |
| 10 | 5.43 | 51.35 | 32.10 | 88.89 |
| 11 | 5.22 | 51.62 | 32.68 | 89.52 |
| 12 | 5.35 | 51.43 | 32.90 | 89.68 |
| mean value | 5.71 | 50.17 | 33.46 | 89.34 |
| standard deviation | 0.68 | 1.38 | 1.13 | 1.55 |

**Table 19: The microcosm incubation study - M2: Distribution of applied radioactivity [%], aquifer Krauthausen, test substance benzoic acid**

| Sample | Mineralization [% of AR] | Extraction [% of AR] | Combustion [% of AR] | Recovery [% of AR] |
|---|---|---|---|---|
| 1 | 14.87 | 52.00 | 32.90 | 99.77 |
| 2 | 11.16 | 52.59 | 37.71 | 101.45 |
| 3 | 11.37 | 54.69 | 34.88 | 100.95 |
| 4 | - | - | - | - |
| 5 | 12.45 | 52.56 | 34.89 | 99.90 |
| 6 | 11.96 | 51.15 | 35.29 | 98.40 |
| 7 | 12.83 | 47.11 | 33.60 | 93.54 |
| 8 | 12.06 | 54.19 | 34.22 | 100.46 |
| 9 | 12.77 | 52.44 | 32.71 | 97.92 |
| mean value | 12.43 | 52.09 | 34.53 | 99.05 |
| standard deviation | 1.15 | 2.31 | 1.60 | 2.53 |

**Table 20: The long-term DAISY incubation study - D1: Distribution of applied radioactivity [%], aquifer Krauthausen, test substance benzoic acid**

| concentration [µg $l^{-1}$] | incubation [days] | mineralization [% of AR] gas phase | dissolved | groundwater [% of AR] | combustion [% of AR] | recovery [% of AR] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | 8.20 | n.a. | 78.87 | | |
| | 3 | 17.56 | n.a. | 68.65 | | |
| | 7 | 22.72 | n.a. | 65.22 | | |
| | 11 | 30.03 | n.a. | 56.43 | | |
| | 15 | 33.99 | n.a. | 46.78 | | |
| | 21 | 39.78 | n.a. | 38.72 | | |
| | 35 | 48.03 | n.a. | 30.77 | | |
| | 56 | 58.05 | n.a. | 21.11 | | |
| | 84 | 63.38 | n.a. | 10.06 | 22.30 | 95.74 |
| 10 | 1 | 4.10 ± 0.34 | n.a. | 71.20 ± 0.50 | | |
| | 3 | 10.52 ± 0.37 | n.a. | 63.47 ± 0.27 | | |
| | 7 | 14.23 ± 0.38 | n.a. | 60.87 ± 0.63 | | |
| | 11 | 19.80 ± 0.49 | n.a. | 54.87 ± 0.84 | | |
| | 15 | 23.17 ± 0.54 | n.a. | 50.26 ± 1.45 | | |
| | 21 | 27.45 ± 0.61 | n.a. | 45.76 ± 0.83 | | |
| | 35 | 34.08 ± 0.79 | n.a. | 39.41 ± 0.51 | | |
| | 56 | 42.56 ± 0.45 | n.a. | 31.61 ± 0.29 | | |
| | 84 | 48.07 ± 0.66 | n.a. | 26.74 ± 0.51 | 21.13 ± 0.94 | 95.94 ± 0.22 |

**Table 21: The microcosm incubation study - M3: Variants**

| compound: | | IMI | | | IPU | | | DKN | | | Mixture (1:1:1) | | | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquifer: | temperature | K | MB | NA | K | MB | NA | K | MB | NA | K | MB | NA | flasks |
| **0.1 µg l⁻¹** | 10°C | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 3 | 3 | 3 | 72 |
| **1.0 µg l⁻¹** | 10°C | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | | | 27 |
| **10 µg l⁻¹** | 10°C | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 3 | 3 | 3 | 72 |
| **10 µg l⁻¹** | 20°C | | | | | | | | | | 3 | 3 | 3 | 9 |
| **10 µg l⁻¹ sterile** | 10°C | | | | | | | | | | 2 | 2 | 2 | 6 |
| **sum** | | **17** | **17** | **17** | **17** | **17** | **17** | **17** | **17** | **17** | **11** | **11** | **11** | **186** |

**Table 22: The microcosm incubation study - M3: Time schedule**

| compound: | incubation | processing | IMI | | | IPU | | | DKN | | | Mixture (1:1:1) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquifer | [days] | date | K | MB | NA | K | MB | NA | K | MB | NA | K | MB | NA |
| | 0* | May-05 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | 90 ± 10 | Aug-05 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | 180 ± 10 | Oct-05 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | 270 ± 10 | Jan-06 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | 360 ± 10 | Apr-06 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | 450 ± 10 | Jul-06 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | spare sample | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | | | |
| | 0* | May-05 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 |
| | 225 ± 10 | Dec-05 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 |
| | 450 ± 10 | Jul-06 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 4 |
| | spare sample | | | | | | | | | | | 1 | 1 | 1 |
| **sum** | | | **17** | **17** | **17** | **17** | **17** | **17** | **17** | **17** | **17** | **11** | **11** | **11** |

Number in cell: number of sampling intervals (incl. one spare sample per variant)

Test aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA)

Test substance Imidacloprid (IMI), Isoproturon (IPU), Diketonitrile (DKN)

**Table 23: The Microcosm incubation study - M4: Variants**

| compound: | | IMI | | | | IPU | | | | DKN | | | | Mixture (1:1:1) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquifer: | | K1 | K2 | MB | NA | K1 | K2 | MB | NA | K1 | K2 | MB | NA | K | | MB | | NA | |
| groundwater | temperature | M | M | M | M | M | M | M | M | M | M | M | M | IS | M | IS | M | IS | M |
| **0.1 µg l⁻¹** | 10°C | 5 | 5 | 3 | 3 | 5 | 5 | 3 | 3 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **1.0 µg l⁻¹** | 10°C | | | | | | | | | | | | | | | | | | |
| **10 µg l⁻¹** | 10°C | 5 | 5 | 3 | 3 | 5 | 5 | 3 | 3 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| **10 µg l⁻¹** | 20°C | | | | | | | | | | | | | | | | | | |
| **10 µg l⁻¹ sterile** | 10°C | | | | | | | | | | | | | | | | | | |
| **sum** | | **10** | **10** | **6** | **6** | **10** | **10** | **6** | **6** | **10** | **10** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** |

**Table 24: The microcosm incubation study - M4: Time schedule**

| compound: | incubation | processing | IMI | | | IPU | | | DKN | | | Mixture (1:1:1) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquifer: | [days] | date | K | MB | NA | K | MB | NA | K | MB | NA | K | | MB | | NA | |
| groundwater | | | M | M | M | M | M | M | M | M | M | IS | M | IS | M | IS | M |
| | 0* | Dec-05 | 4 | | | 4 | | | 4 | | | | | | | | |
| | 90 ± 10 | Mar-06 | 4 | | | 4 | | | 4 | | | | | | | | |
| | 180 ± 10 | Jun-06 | 4 | | | 4 | | | 4 | | | | | | | | |
| | 270 ± 10 | Sep-06 | 4 | | | 4 | | | 4 | | | | | | | | |
| | 330 ± 10 | Nov-06 | 4 | | | 4 | | | 4 | | | | | | | | |
| | 0* | Dec-05 | | 2 | 2 | | 2 | 2 | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 180 ± 10 | Jun-06 | | 2 | 2 | | 2 | 2 | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 330 ± 10 | Nov-06 | | 2 | 2 | | 2 | 2 | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| **sum** | | | **20** | **6** | **6** | **20** | **6** | **6** | **20** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | **6** |

Number in cell: number of sampling intervals (incl. one spare sample per variant)

Aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA), Groundwater: M = modified, IS = in situ

Test substance Imidacloprid (IMI), Isoproturon (IPU), Diketonitrile (DKN)

**Table 25: The DAISY incubation study - D4/ D5: Variants**

| compound: | IMI | | | IPU | | | DKN | | | Mixture (1:1:1) | | | total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| aquifer: | K | MB | NA | K | MB | NA | K | MB | NA | K | MB | NA | number |
| **0.1 µg l⁻¹** | 1 | | | 1 | | | 1 | | | 1 | | | 4 |
| **1.0 µg l⁻¹** | 2 | | | 2 | | | 2 | | | 2 | | | 8 |
| **10 µg l⁻¹** | 2 | | | 2 | | | 2 | | | 2 | 1 | 1 | 10 |
| **sum** | **5** | **0** | **0** | **5** | **0** | **0** | **5** | **0** | **0** | **5** | **1** | **1** | **22** |

Number in cell: number of test systems

Aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA)

Test substance Imidacloprid (IMI), Isoproturon (IPU), Diketonitrile (DKN)

**Table 26: Radio-HPLC gradients of test substances Imidacloprid (1), Isoproturon (1), and Diketonitrile (2)**

1.)

| time [min] | gradient (ACN, $H_2O$ ($H_3PO_4$ 0.1%)) |
|---|---|
| 0 | 40 : 60 |
| 3 | 40 : 60 |
| 6 | 100 : 0 |
| 9 | 100 : 0 |
| 10 | 40 : 60 |
| 20 | 40 : 60 |

2.)

| time [min] | gradient (ACN, $H_2O$ ($H_3PO_4$ 0.1%)) |
|---|---|
| 0 | 60 : 40 |
| 6 | 60 : 40 |
| 11 | 90 : 10 |
| 11.5 | 90 : 10 |
| 16 | 60 : 40 |
| 21 | 60 : 40 |

**Table 27: The DAISY incubation study - D5: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [%] | Groundwater [%] | Extraction [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | 1.12 | 91.72 | | | |
| | 45 | 4.29 | 91.31 | | | |
| | 75 | 5.04 | 90.74 | | | |
| | 150 | 6.38 | 89.44 | | | |
| | 200 | 7.41 | 87.16 | | | |
| | 300 | 8.30 | 87.92 | 7.25 | 5.89 | 109.35 |
| 1 | 1 | 0.07 | 87.51 | | | |
| | 45 | 0.38 | 85.95 | | | |
| | 75 | 0.51 | 86.81 | | | |
| | 150 | 0.77 | 83.18 | | | |
| | 200 | 1.01 | 82.47 | | | |
| | 300 | 1.30 | 81.09 | 7.12 | 1.35 | 90.87 |
| 1 | 1 | 0.06 | 88.50 | | | |
| | 45 | 0.36 | 87.43 | | | |
| | 75 | 0.50 | 88.66 | | | |
| | 150 | 0.79 | 85.70 | | | |
| | 200 | 1.06 | 84.68 | | | |
| | 300 | 1.29 | 83.19 | 7.14 | 1.20 | 92.82 |
| 10 | 1 | 0.10 | 91.32 | | | |
| | 45 | 0.39 | 90.59 | | | |
| | 75 | 0.52 | 90.39 | | | |
| | 150 | 0.82 | 89.19 | | | |
| | 200 | 1.09 | 88.25 | | | |
| | 300 | 1.29 | 86.20 | 8.07 | 1.07 | 96.62 |
| 10 | 1 | 0.12 | 92.43 | | | |
| | 45 | 0.40 | 90.84 | | | |
| | 75 | 0.54 | 90.39 | | | |
| | 150 | 0.81 | 89.09 | | | |
| | 200 | 1.01 | 89.19 | | | |
| | 300 | 1.28 | 86.85 | 7.51 | 0.98 | 96.62 |

**Table 28: The DAISY incubation study - D5: Active ingredient [AI] groundwater and extract [% of AR], aquifer Krauthausen (K), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Active ingredient [% of AR] IPU Groundwater | Extract |
|---|---|---|---|
| 0.1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | n.d. | n.d. |
| 1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | 4.94 | 7.12 |
| 1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | 1.59 | 7.14 |
| 10 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | 89.71 | |
| | 150 | 70.37 | |
| | 200 | 39.13 | |
| | 300 | 3.25 | 7.33 |
| 10 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | 89.29 | |
| | 150 | 68.14 | |
| | 200 | 47.77 | |
| | 300 | 4.27 | 7.03 |

**Table 29: The DAISY incubation study – D5: Aquifer Krauthausen (K), test substance Isoproturon [IPU], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [% of AR] | Mineralisation [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | 1.12 | 0.002 |
| | 45 | 4.29 | 0.008 |
| | 75 | 5.04 | 0.009 |
| | 150 | 6.38 | 0.011 |
| | 200 | 7.41 | 0.013 |
| | 300 | 8.30 | 0.015 |
| 1 | 1 | 0.07 | 0.002 |
| | 45 | 0.38 | 0.009 |
| | 75 | 0.51 | 0.011 |
| | 150 | 0.77 | 0.015 |
| | 200 | 1.01 | 0.019 |
| | 300 | 1.30 | 0.023 |
| 1 | 1 | 0.06 | 0.001 |
| | 45 | 0.36 | 0.008 |
| | 75 | 0.50 | 0.011 |
| | 150 | 0.79 | 0.016 |
| | 200 | 1.06 | 0.020 |
| | 300 | 1.29 | 0.023 |
| 10 | 1 | 0.10 | 0.052 |
| | 45 | 0.39 | 0.207 |
| | 75 | 0.52 | 0.275 |
| | 150 | 0.82 | 0.430 |
| | 200 | 1.09 | 0.572 |
| | 300 | 1.29 | 0.678 |
| 10 | 1 | 0.12 | 0.065 |
| | 45 | 0.40 | 0.209 |
| | 75 | 0.54 | 0.284 |
| | 150 | 0.81 | 0.424 |
| | 200 | 1.01 | 0.529 |
| | 300 | 1.28 | 0.675 |

**Table 30: The DAISY incubation study - D5: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [%] | Groundwater [%] | Extraction [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | 2.49 | 91.92 | | | |
| | 45 | 3.93 | 91.13 | | | |
| | 75 | 3.93 | 86.11 | | | |
| | 150 | 3.93 | 85.14 | | | |
| | 200 | 4.28 | 84.35 | | | |
| | 300 | 4.31 | 81.87 | 8.17 | 4.53 | 98.87 |
| 1 | 1 | 0.07 | 90.78 | | | |
| | 45 | 0.62 | 88.80 | | | |
| | 75 | 0.82 | 88.82 | | | |
| | 150 | 1.25 | 87.92 | | | |
| | 200 | 1.61 | 87.46 | | | |
| | 300 | 1.82 | 86.02 | 7.56 | 3.04 | 98.45 |
| 1 | 1 | 0.08 | 89.30 | | | |
| | 45 | 0.42 | 90.24 | | | |
| | 75 | 0.56 | 90.64 | | | |
| | 150 | 0.75 | 88.95 | | | |
| | 200 | 0.91 | 88.22 | | | |
| | 300 | 1.08 | 86.62 | 6.54 | 3.14 | 97.38 |
| 10 | 1 | 0.09 | 86.24 | | | |
| | 45 | 0.25 | 85.99 | | | |
| | 75 | 0.27 | 85.22 | | | |
| | 150 | 0.31 | 84.44 | | | |
| | 200 | 0.34 | 83.50 | | | |
| | 300 | 0.36 | 80.90 | 6.67 | 1.04 | 88.98 |
| 10 | 1 | 0.09 | 89.87 | | | |
| | 45 | 0.25 | 88.84 | | | |
| | 75 | 0.26 | 88.81 | | | |
| | 150 | 0.29 | 88.16 | | | |
| | 200 | 0.32 | 87.56 | | | |
| | 300 | 0.35 | 85.16 | 8.34 | 0.83 | 94.68 |

**Table 31: The DAISY incubation study - D5: Active ingredient [AI] groundwater and extract [% of AR], aquifer Krauthausen (K), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Active ingredient [% of AR] IMI Groundwater | IMI Extract |
|---|---|---|---|
| 0.1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | n.d. | n.d. |
| 1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | 62.70 | 7.56 |
| 1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | 46.80 | 6.54 |
| 10 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | 77.50 | |
| | 150 | 70.93 | |
| | 200 | - | |
| | 300 | 72.45 | 6.47 |
| 10 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | 81.90 | |
| | 150 | 69.64 | |
| | 200 | 71.87 | |
| | 300 | 68.17 | 8.26 |

**Table 32: The DAISY incubation study – D5: Aquifer Krauthausen (K), test substance Imidacloprid [IMI], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [% of AR] | Mineralisation [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | 2.49 | 0.004 |
| | 45 | 3.93 | 0.007 |
| | 75 | 3.93 | 0.007 |
| | 150 | 3.93 | 0.007 |
| | 200 | 4.28 | 0.008 |
| | 300 | 4.31 | 0.008 |
| 1 | 1 | 0.07 | 0.002 |
| | 45 | 0.62 | 0.014 |
| | 75 | 0.82 | 0.018 |
| | 150 | 1.25 | 0.025 |
| | 200 | 1.61 | 0.031 |
| | 300 | 1.82 | 0.033 |
| 1 | 1 | 0.08 | 0.002 |
| | 45 | 0.42 | 0.010 |
| | 75 | 0.54 | 0.012 |
| | 150 | 0.75 | 0.015 |
| | 200 | 0.91 | 0.017 |
| | 300 | 1.08 | 0.020 |
| 10 | 1 | 0.09 | 0.017 |
| | 45 | 0.25 | 0.044 |
| | 75 | 0.27 | 0.048 |
| | 150 | 0.31 | 0.055 |
| | 200 | 0.34 | 0.061 |
| | 300 | 0.36 | 0.064 |
| 10 | 1 | 0.09 | 0.017 |
| | 45 | 0.25 | 0.044 |
| | 75 | 0.26 | 0.044 |
| | 150 | 0.29 | 0.053 |
| | 200 | 0.32 | 0.058 |
| | 300 | 0.35 | 0.063 |

**Table 33: The DAISY incubation study - D5: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [%] | Groundwater [%] | Extraction [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | 0.89 | 89.64 | | | |
| | 45 | 1.63 | 90.75 | | | |
| | 75 | 1.63 | 89.80 | | | |
| | 150 | 1.63 | 90.28 | | | |
| | 200 | 1.66 | 87.81 | | | |
| | 300 | 1.68 | 87.70 | 7.81 | 1.66 | 98.80 |
| 1 | 1 | 0.15 | 89.67 | | | |
| | 45 | 0.36 | 90.27 | | | |
| | 75 | 0.38 | 91.09 | | | |
| | 150 | 0.46 | 88.21 | | | |
| | 200 | 0.55 | 87.80 | | | |
| | 300 | 0.66 | 87.73 | 7.39 | 1.08 | 96.85 |
| 1 | 1 | 0.18 | 91.58 | | | |
| | 45 | 0.50 | 91.82 | | | |
| | 75 | 0.60 | 91.99 | | | |
| | 150 | 0.84 | 89.96 | | | |
| | 200 | 1.07 | 89.41 | | | |
| | 300 | 1.25 | 88.18 | 6.60 | 1.46 | 97.49 |
| 10 | 1 | 0.10 | 87.75 | | | |
| | 45 | 0.23 | 87.26 | | | |
| | 75 | 0.25 | 86.03 | | | |
| | 150 | 0.27 | 83.35 | | | |
| | 200 | 0.30 | 84.63 | | | |
| | 300 | 0.33 | 82.87 | 7.10 | 0.41 | 90.71 |
| 10 | 1 | 0.13 | 90.19 | | | |
| | 45 | 0.24 | 89.74 | | | |
| | 75 | 0.25 | 89.98 | | | |
| | 150 | 0.28 | 86.01 | | | |
| | 200 | 0.30 | 87.95 | | | |
| | 300 | 0.33 | 86.34 | 7.68 | 0.41 | 94.75 |

**Table 34: The DAISY incubation study - D5: Active ingredient (AI) groundwater and extract [% of AR], aquifer Krauthausen (K), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Active ingredient [% of AR] DKN | |
|---|---|---|---|
| | | Groundwater | Extract |
| 0.1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | n.d. | n.d. |
| 1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | n.d. | n.d. |
| 1 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.a. | |
| | 150 | n.a. | |
| | 200 | n.a. | |
| | 300 | n.d. | n.d. |
| 10 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | n.d. | |
| | 150 | 63.86 | |
| | 200 | n.d. | |
| | 300 | 14.02 | 7.00 |
| 10 | 1 | n.a. | |
| | 45 | n.a. | |
| | 75 | 83.68 | |
| | 150 | n.d. | |
| | 200 | n.d. | |
| | 300 | 13.30 | 7.61 |

**Table 35: The DAISY incubation study – D5: Aquifer Krauthausen (K), test substance Diketonitrile [DKN], mineralization [% of AR ⇔ µg kg$^{-1}$ aquifer material]**

| Concentration [µg l$^{-1}$] | Incubation [days] | Mineralisation [% of AR] | Mineralisation [µg kg$^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | 0.98 | 0.002 |
| | 45 | 1.63 | 0.003 |
| | 75 | 1.63 | 0.003 |
| | 150 | 1.63 | 0.003 |
| | 200 | 1.66 | 0.003 |
| | 300 | 1.68 | 0.003 |
| 1 | 1 | 0.15 | 0.003 |
| | 45 | 0.36 | 0.008 |
| | 75 | 0.38 | 0.008 |
| | 150 | 0.46 | 0.009 |
| | 200 | 0.55 | 0.010 |
| | 300 | 0.66 | 0.011 |
| 1 | 1 | 0.18 | 0.004 |
| | 45 | 0.50 | 0.011 |
| | 75 | 0.60 | 0.013 |
| | 150 | 0.84 | 0.016 |
| | 200 | 1.07 | 0.020 |
| | 300 | 1.25 | 0.022 |
| 10 | 1 | 0.10 | 0.018 |
| | 45 | 0.23 | 0.043 |
| | 75 | 0.25 | 0.045 |
| | 150 | 0.27 | 0.050 |
| | 200 | 0.30 | 0.055 |
| | 300 | 0.33 | 0.060 |
| 10 | 1 | 0.13 | 0.024 |
| | 45 | 0.24 | 0.044 |
| | 75 | 0.25 | 0.046 |
| | 150 | 0.28 | 0.051 |
| | 200 | 0.30 | 0.054 |
| | 300 | 0.33 | 0.060 |

**Table 36: The DAISY incubation study - D5: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [%] | Groundwater [%] | Extraction [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | 0.85 | 92.70 | | | |
| | 45 | 2.26 | 88.20 | | | |
| | 75 | 2.39 | 89.10 | | | |
| | 150 | 2.64 | 86.13 | | | |
| | 200 | 2.86 | 86.27 | | | |
| | 300 | 3.23 | 82.95 | 10.44 | 1.93 | 98.54 |
| 1 | 1 | 0.08 | 90.05 | | | |
| | 45 | 0.30 | 88.05 | | | |
| | 75 | 0.38 | 88.76 | | | |
| | 150 | 0.56 | 85.95 | | | |
| | 200 | 0.72 | 84.96 | | | |
| | 300 | 0.95 | 83.40 | 6.68 | 1.00 | 92.03 |
| 1 | 1 | 0.08 | 91.02 | | | |
| | 45 | 0.30 | 90.59 | | | |
| | 75 | 0.37 | 90.73 | | | |
| | 150 | 0.54 | 87.96 | | | |
| | 200 | 0.70 | 87.41 | | | |
| | 300 | 0.81 | 85.98 | 7.38 | 1.16 | 95.32 |
| 10 | 1 | 0.10 | 88.61 | | | |
| | 45 | 0.35 | 88.34 | | | |
| | 75 | 0.44 | 87.72 | | | |
| | 150 | 0.81 | 85.90 | | | |
| | 200 | 0.77 | 85.80 | | | |
| | 300 | 0.93 | 83.04 | 7.20 | 0.86 | 92.04 |
| 10 | 1 | 0.15 | 92.22 | | | |
| | 45 | 0.33 | 91.30 | | | |
| | 75 | 0.42 | 90.69 | | | |
| | 150 | 0.62 | 89.16 | | | |
| | 200 | 0.78 | 89.10 | | | |
| | 300 | 0.96 | 86.66 | 6.83 | 0.83 | 95.28 |

**Table 37: The DAISY incubation study - D5: Active ingredient [AI] groundwater and extract [% of AR], aquifer Krauthausen (K), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| | | Active ingredient [% of AR] | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | Incubation | IPU | | IMI | | DKN | |
| [µg l$^{-1}$] | [days] | Groundwater | Extract | Groundwater | Extract | Groundwater | Extract |
| 0.1 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | n.a. | | n.a. | | n.a. | |
| | 150 | n.a. | | n.a. | | n.a. | |
| | 200 | n.a. | | n.a. | | n.a. | |
| | 300 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 1 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | n.a. | | n.a. | | n.a. | |
| | 150 | n.a. | | n.a. | | n.a. | |
| | 200 | n.a. | | n.a. | | n.a. | |
| | 300 | 55.97 | 7.79 | 3.11 | 0.19 | 13.20 | n.d. |
| 1 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | n.a. | | n.a. | | n.a. | |
| | 150 | n.a. | | n.a. | | n.a. | |
| | 200 | n.a. | | n.a. | | n.a. | |
| | 300 | 73.68 | n.d. | 3.08 | | n.d. | n.d. |
| 10 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | 79.40 | | 82.02 | | 31.69 | |
| | 150 | 77.71 | | 4.63 | | 30.95 | |
| | 200 | 79.67 | | 5.99 | | n.d. | |
| | 300 | 57.16 | 7.12 | 1.99 | 6.19 | 4.85 | 7.51 |
| 10 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | 85.26 | | 92.11 | | 7.47 | |
| | 150 | 90.97 | | 12.38 | | 5.90 | |
| | 200 | 83.05 | | 19.12 | | 1.95 | |
| | 300 | 49.76 | 7.12 | 9.57 | 4.89 | 10.14 | 0.94 |

**Table 38: The DAISY incubation study - D5: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [%] | Groundwater [%] | Extraction [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 10 | 1 | 0.13 | 91.72 | | | |
| | 45 | 0.14 | 86.69 | | | |
| | 75 | 0.14 | 85.85 | | | |
| | 150 | 0.16 | 80.80 | | | |
| | 200 | 0.16 | 77.72 | | | |
| | 300 | 0.17 | 73.53 | 10.97 | 7.67 | 92.34 |

**Table 39: The DAISY incubation study - D5: Active ingredient (AI) groundwater and extract [% of AR], aquifer Mulder-Beilen (MB), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| | | Active ingredient [% of AR] | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | Incubation | IPU | | IMI | | DKN | |
| [µg $l^{-1}$] | [days] | Groundwater | Extract | Groundwater | Extract | Groundwater | Extract |
| 10 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | 10.58 | | 54.64 | | 10.06 | |
| | 150 | 9.11 | | 38.05 | | 11.00 | |
| | 200 | 5.56 | | 35.02 | | 7.97 | |
| | 300 | 4.47 | 0.44 | 34.99 | 6.16 | 14.81 | 1.88 |

**Table 40: The DAISY incubation study - D5: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralisation [%] | Groundwater [%] | Extraction [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 10 | 1 | 0.09 | 72.61 | | | |
| | 45 | 0.14 | 56.69 | | | |
| | 75 | 0.15 | 55.17 | | | |
| | 150 | 0.17 | 51.27 | | | |
| | 200 | 0.19 | 50.25 | | | |
| | 300 | 0.19 | 45.56 | 23.52 | 22.94 | 92.21 |

**Table 41: The DAISY incubation study - D5: Active ingredient (AI) groundwater and extract [% of AR], aquifer Nature A (NA), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| | | Active ingredient [% of AR] | | | | | |
|---|---|---|---|---|---|---|---|
| Concentration | Incubation | IPU | | IMI | | DKN | |
| [µg $l^{-1}$] | [days] | Groundwater | Extract | Groundwater | Extract | Groundwater | Extract |
| 10 | 1 | n.a. | | n.a. | | n.a. | |
| | 45 | n.a. | | n.a. | | n.a. | |
| | 75 | 0.38 | | 37.10 | | 8.03 | |
| | 150 | n.d. | | 36.02 | | 3.93 | |
| | 200 | n.d. | | 21.73 | | 3.46 | |
| | 300 | n.d. | n.d. | 29.88 | 19.14 | 13.06 | 1.76 |

**Table 42: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Isoproturon [IPU], groundwater modified**

| Concentration [µg l$^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d | 97.78 ± 0.61 | n.d | n.c. |
| | 90 | 1.85 ± 0.05 | 94.77 ± 3.67 | n.d | n.c. |
| | 170 | 1.63 ± 0.01 | 93.10 ± 1.67 | n.d | n.c. |
| | 260 | 2.01 ± 0.17 | 86.19 ± 1.88 | n.d | n.c. |
| | 310 | 1.99 ± 0.39 | 85.94 ±10.30 | n.d | n.c. |
| 10 | 1 | n.d | 93.27 ± 0.78 | 1.97 ± 0.10 | 95.24 ± 0.87 |
| | 90 | 0.78 ± 0.04 | 92.01 ± 0.95 | 3,16 ± 0.40 | 95.95 ± 1.39 |
| | 170 | 1.22 ± 0.00 | 92.11 ± 0.17 | 2.98 ± 0.03 | 96.31 ± 0.20 |
| | 260 | 1.60 ± 0.01 | 89.95 ± 0.29 | 4.08 ± 0.14 | 95.64 ± 0.17 |
| | 310 | 1.73 ± 0.02 | 88.82 ± 0.60 | 4.59 ± 1.23 | 95.14 ± 1.82 |

**Table 43: The microcosm incubation study - M4: Aquifer Krauthausen (K), test substance Isoproturon [IPU], mineralization [% of AR ⇔ µg kg$^{-1}$ aquifer material], groundwater modified**

| Concentration [µg l$^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg kg$^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d | n.d |
| | 90 | 1.85 | 0.0008 |
| | 170 | 1.63 | 0.0007 |
| | 260 | 2.01 | 0.0008 |
| | 310 | 1.99 | 0.0008 |
| 10 | 1 | n.d | n.d |
| | 90 | 0.78 | 0.0326 |
| | 170 | 1.22 | 0.0511 |
| | 260 | 1.60 | 0.0671 |
| | 310 | 1.73 | 0.0725 |

**Table 44: The microcosm incubation study M4: Active ingredient (AI) extract [% of AR], aquifer Krauthausen (K), test substance Isoproturon [IPU], groundwater modified**

| Concentration [µg l$^{-1}$] | Incubation [days] | Active ingredient [% of AR] IPU |
|---|---|---|
| 10 | 1 | 90.47 ± 3.1 |
| | 90 | 73.51 ± 4.1 |
| | 170 | 83.16 ± 0.2 |
| | 260 | 84.72 ± 0.9 |
| | 310 | 83.74 ± 1.1 |

**Table 45: The microcosm incubation study M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Imidacloprid [IMI], groundwater modified**

| Concentration [µg l$^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d | 98.62 ± 0.18 | n.d | n.c. |
| | 90 | n.d | 96.95 ± 0.30 | n.d | n.c. |
| | 170 | n.d | 94.69 ± 2.18 | n.d | n.c. |
| | 260 | n.d | 86.30 ± 0.26 | n.d | n.c. |
| | 310 | n.d | 84.86 ± 9.22 | n.d | n.c. |
| 10 | 1 | n.d | 92.14 ± 0.38 | 2.23 ± 0.05 | 94.37 ± 0.42 |
| | 90 | 0.20 ± 0.00 | 91.78 ± 0.34 | 3.13 ± 0.00 | 95.11 ± 0.34 |
| | 170 | 0.30 ± 0.01 | 91.51 ± 1.97 | 3.37 ± 0.04 | 95.18 ± 2.01 |
| | 260 | 0.45 ± 0.02 | 90.18 ± 0.71 | 5.76 ± 0.18 | 96.40 ± 0.90 |
| | 310 | 0.51 ± 0.03 | 87.77 ± 2.70 | 4.85 ± 1.82 | 93.13 ± 0.91 |

**Table 46: The microcosm incubation study - M4: Aquifer Krauthausen (K), test substance Imidacloprid [IMI], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material], groundwater modified**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d | n.d |
| | 90 | n.d | n.d |
| | 170 | n.d | n.d |
| | 260 | n.d | n.d |
| | 310 | n.d | n.d |
| 10 | 1 | n.d | n.d |
| | 90 | 0.20 | 0.086 |
| | 170 | 0.30 | 0.0128 |
| | 260 | 0.45 | 0.0190 |
| | 310 | 0.51 | 0.0213 |

**Table 47: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Krauthausen (K), test substance Imidacloprid [IMI], groundwater modified**

| Concentration [µg $l^{-1}$] | Incubation [days] | Active ingredient [% of AR] IMI |
|---|---|---|
| 10 | 1 | - |
| | 90 | - |
| | 170 | 88.98 ± 1.9 |
| | 260 | 89.55 ± 0.1 |
| | 310 | 76.01 ± 13.8 |

**Table 48: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Diketonitrile [DKN], groundwater modified**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d | 96.41 ± 1.73 | n.d | n.c. |
| | 90 | 0.82 ± 0.06 | 94.06 ± 2.65 | n.d | n.c. |
| | 170 | 0.73 ± 0.22 | 92.95 ± 4.78 | n.d | n.c. |
| | 260 | 0.47 ± 0.14 | 90.88 ± 6.99 | n.d | n.c. |
| | 310 | 0.86 ± 0.57 | 95.86 ± 3.61 | n.d | n.c. |
| 10 | 1 | n.d | 94.87 ± 0.44 | 1.87 ± 0.07 | 96.75 ± 0.37 |
| | 90 | 0.12 ± 0.00 | 93.31 ± 0.29 | 1.74 ± 0.01 | 95.18 ± 0.28 |
| | 170 | 0.19 ± 0.01 | 95.23 ± 1.14 | 1.63 ± 0.09 | 97.04 ± 1.22 |
| | 260 | 0.24 ± 0.00 | 92.49 ± 0.81 | 1.81 ± 0.01 | 94.54 ± 0.82 |
| | 310 | 0.25 ± 0.02 | 91.74 ± 0.74 | 1.68 ± 0.25 | 93.68 ± 0.97 |

**Table 49: The microcosm incubation study - M4: Aquifer Krauthausen (K), test substance Diketonitrile [DKN], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material], groundwater modified**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d | n.d |
| | 90 | 0.82 | 0.0003 |
| | 170 | 0.73 | 0.0003 |
| | 260 | 0.47 | 0.0002 |
| | 310 | 0.86 | 0.0003 |
| 10 | 1 | n.d | n.d |
| | 90 | 0.12 | 0.0003 |
| | 170 | 0.19 | 0.0003 |
| | 260 | 0.24 | 0.0002 |
| | 310 | 0.25 | 0.0003 |

**Table 50: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Krauthausen (K), test substance Diketonitrile [DKN], groundwater modified**

| Concentration [µg $l^{-1}$] | Incubation [days] | Active ingredient [% of AR] DKN |
|---|---|---|
| 10 | 1 | 92.66 ± 0.0 |
| | 90 | 68.65 ± 5.5 |
| | 170 | 91.44 ± 2.2 |
| | 260 | 90.62 ± 2.1 |
| | 310 | 78.73 ± 12.8 |

**Table 51: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | phosphate buffered | n.d. | 93.90 | n.d. | n.c. |
| | 170 | [1 mM] | 0.82 | 92.42 | n.d. | n.c. |
| | 310 | | 1.94 | 91.40 | n.d. | n.c. |
| 0.1 | 1 | in-situ | n.d. | 93.67 | n.d. | n.c. |
| | 170 | | 0.87 | 92.82 | n.d. | n.c. |
| | 310 | | 1.58 | 90.23 | n.d. | n.c. |
| 10 | 1 | phosphate buffered | n.d. | 92.09 | 1.76 | 93.85 |
| | 170 | [1 mM] | 0.74 | 91.21 | 3.07 | 94.28 |
| | 310 | | 1.27 | 89.82 | 3.34 | 93.16 |
| 10 | 1 | in-situ | n.d. | 93.36 | 2.26 | 95.62 |
| | 170 | | 0.75 | 91.24 | 3.09 | 94.33 |
| | 310 | | 1.29 | 90.05 | 3.52 | 93.57 |

**Table 52: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Krauthausen (K), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] IMI | IPU | DKN |
|---|---|---|---|---|---|
| 10 | 1 | phosphate buffered | 68.35 | - | n.d. |
| | 170 | [1 mM] | 84.85 | 77.85 | 85.30 |
| | 310 | | 65.02 | 77.55 | 65.46 |
| 10 | 1 | in-situ | 76.40 | 90.99 | n.d. |
| | 170 | | 75.17 | 77.25 | 89.65 |
| | 310 | | 64.02 | 71.52 | 82.09 |

**Table 53: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d. | 86.58 | n.d. | n.c. |
| | 170 | n.d. | 87.60 | n.d. | n.c. |
| | 310 | 6.23 | 52.93 | n.d. | n.c. |
| 10 | 1 | n.d. | 94.46 | 1.21 | 95.67 |
| | 170 | 0.07 | 91.61 | 2.81 | 94.44 |
| | 310 | 0.11 | 86.92 | 2.72 | 89.69 |

**Table 54: The microcosm incubation study - M4: Aquifer Mulder-Beilen (MB), test substance Isoproturon [IPU], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d. | n.d. |
| | 170 | n.d. | n.d. |
| | 310 | 6.23 | 0.0623 |
| 10 | 1 | n.d. | n.d. |
| | 170 | 0.07 | 0.0003 |
| | 310 | 0.11 | 0.0005 |

**Table 55: The microcosm incubation study - M4: Active ingredient (AI) extract and groundwater [% of AR], aquifer Mulder-Beilen (MB), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] IPU |
|---|---|---|---|
| 10 | 1 | phosphate buffered [1 mM] | 94.30 |
| | 170 | | 91.28 |
| | 310 | | 85.33 |

**Table 56: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d. | 96.69 | n.d. | n.c. |
| | 170 | n.d. | 100.08 | n.d. | n.c. |
| | 310 | 1.91 | 89.82 | n.d. | n.c. |
| 10 | 1 | n.d. | 88.94 | 5.53 | 94.47 |
| | 170 | 0.06 | 67.58 | 33.78 | 101.38 |
| | 310 | 4.51 | 63.23 | 26.49 | 91.62 |

**Table 57: The microcosm incubation study - M4: Aquifer Mulder-Beilen (MB), test substance Imidacloprid [IMI], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d. | n.d. |
| | 170 | n.d. | n.d. |
| | 310 | 1.91 | 0.0191 |
| 10 | 1 | n.d. | n.d. |
| | 170 | 0.06 | 0.0003 |
| | 310 | 4.51 | 0.0190 |

**Table 58: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Mulder-Beilen (MB), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] IMI |
|---|---|---|---|
| 10 | 1 | phosphate buffered [1 mM] | n.a. |
| | 170 | | 53.80 |
| | 310 | | 50.33 |

**Table 59: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d. | 87.30 | n.d. | n.c. |
| | 170 | 2.52 | 85.79 | n.d. | n.c. |
| | 310 | n.d. | 137.84 | n.d. | n.c. |
| 10 | 1 | n.d. | 91.18 | 0.63 | 91.82 |
| | 170 | n.d. | 89.88 | 1.57 | 91.46 |
| | 310 | n.d. | 89.09 | 1.39 | 90.48 |

**Table 60: The microcosm incubation study - M4: Aquifer Mulder-Beilen (MB), test substance Diketonitrile [DKN], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d. | n.d. |
| | 170 | 2.52 | 0.0025 |
| | 310 | n.d. | n.d. |
| 10 | 1 | n.d. | n.d. |
| | 170 | n.d. | n.d. |
| | 310 | n.d. | n.d. |

**Table 61: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Mulder-Beilen (MB), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] DKN |
|---|---|---|---|
| 10 | 1 | phosphate buffered [1 mM] | 90.66 |
| | 170 | | 74.82 |
| | 310 | | 60.38 |

**Table 62: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | phosphate buffered [1 mM] | n.d. | 92.67 | n.d | n.c. |
| | 170 | | n.d | 91.96 | n.d | n.c. |
| | 310 | | 0.36 | 75.68 | n.d | n.c. |
| 0.1 | 1 | in-situ | n.d | 91.36 | n.d | n.c. |
| | 170 | | n.d | 91.05 | n.d | n.c. |
| | 310 | | 2.66 | 66.71 | n.d | n.c. |
| 10 | 1 | phosphate buffered [1 mM] | n.d | 95.92 | 1.57 | 97.50 |
| | 170 | | 0.04 | 84.82 | 10.03 | 94.86 |
| | 310 | | 0.08 | 76.88 | 14.64 | 91.53 |
| 10 | 1 | in-situ | n.d | 95.63 | 1.42 | 97.05 |
| | 170 | | 0.04 | 84.19 | 12.16 | 96.35 |
| | 310 | | 0.06 | 78.28 | 14.22 | 92.50 |

**Table 63: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Mulder-Beilen (MB), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] | | |
|---|---|---|---|---|---|
| | | | IMI | IPU | DKN |
| 10 | 1 | phosphate buffered [1 mM] | 62.19 | 95.77 | - |
| | 170 | | 49.64 | 89.90 | 79.04 |
| | 310 | | 18.62 | 66.97 | 71.22 |
| 10 | 1 | in-situ | 59.29 | 97.04 | - |
| | 170 | | 37.51 | 86.01 | 68.95 |
| | 310 | | 20.08 | 76.82 | 61.22 |

**Table 64: The microcosm incubation study – M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d. | 90.24 | n.d. | n.c. |
| | 170 | n.d. | 85.91 | n.d. | n.c. |
| | 310 | n.d. | 33.77 | n.d. | n.c. |
| 10 | 1 | n.d. | 94.52 | 1.83 | 96.35 |
| | 170 | 0.07 | 88.62 | 6.11 | 94.78 |
| | 310 | 0.08 | 82.03 | 11.03 | 93.12 |

**Table 65: The microcosm incubation study - M4: Aquifer Nature A (NA), test substance Isoproturon [IPU], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d. | n.d. |
| | 170 | n.d. | n.d. |
| | 310 | n.d. | n.d. |
| 10 | 1 | n.d. | n.d. |
| | 170 | 0.07 | 0.0005 |
| | 310 | 0.08 | 0.0005 |

**Table 66: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Nature A (NA), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] IPU |
|---|---|---|---|
| 10 | 1 | phosphate buffered [1 mM] | 93.29 |
| | 170 | | - |
| | 310 | | 81.35 |

**Table 67: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d. | 93.10 | n.d. | n.c. |
| | 170 | n.d. | 95.76 | n.d. | n.c. |
| | 310 | n.d. | 12.77 | n.d. | n.c. |
| 10 | 1 | n.d. | 87.25 | 3.47 | 90.73 |
| | 170 | 0.93 | 29.87 | 73.42 | 103.92 |
| | 310 | 0.06 | 9.63 | 87.01 | 96.68 |

**Table 68: The microcosm incubation study - M4: Aquifer Nature A (NA), test substance Imidacloprid [IMI], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d. | n.d. |
| | 170 | n.d. | n.d. |
| | 310 | n.d. | n.d. |
| 10 | 1 | n.d. | n.d. |
| | 170 | 0.93 | 0.0063 |
| | 310 | 0.06 | 0.0004 |

**Table 69: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Nature A (NA), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] IMI |
|---|---|---|---|
| 10 | 1 | phosphate buffered [1 mM] | 82.45 |
| | 170 | | 14.66 |
| | 310 | | n.d. |

**Table 70: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|
| 0.1 | 1 | n.d. | 90.99 | n.d. | n.c. |
| | 170 | n.d. | 90.16 | n.d. | n.c. |
| | 310 | n.d. | - | n.d. | n.c. |
| 10 | 1 | n.d. | 91.11 | 1.96 | 93.08 |
| | 170 | 0.02 | 91.29 | 4.24 | 95.55 |
| | 310 | 0.09 | 88.68 | 4.03 | 92.77 |

**Table 71: The microcosm incubation study - M4: Aquifer Nature A (NA), test substance Diketonitrile [DKN], mineralization [% of AR ⇔ µg $kg^{-1}$ aquifer material]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Mineralization [%] | Mineralization [µg $kg^{-1}$ aquifer material] |
|---|---|---|---|
| 0.1 | 1 | n.d. | n.d. |
| | 170 | n.d. | n.d. |
| | 310 | n.d. | n.d. |
| 10 | 1 | n.d. | n.d. |
| | 170 | 0.02 | 0.0002 |
| | 310 | 0.09 | 0.0002 |

**Table 72: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Nature A (NA), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] DKN |
|---|---|---|---|
| 10 | 1 | phosphate buffered [1 mM] | 89.45 |
| | 170 | | 85.63 |
| | 310 | | 51.36 |

**Table 73: The microcosm incubation study - M4: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | phosphate buffered | n.d. | 88.90 | n.d. | n.c. |
| | 170 | [1 mM] | 0.29 | 72.31 | n.d. | n.c. |
| | 310 | | n.d. | 70.19 | n.d. | n.c. |
| 0.1 | 1 | in-situ | n.d. | 87.98 | n.d. | n.c. |
| | 170 | | 1.63 | 73.80 | n.d. | n.c. |
| | 310 | | 0.26 | 64.89 | n.d. | n.c. |
| 10 | 1 | phosphate buffered | n.d. | 90.29 | 2.46 | 92.75 |
| | 170 | [1 mM] | 0.05 | 71.01 | 23.90 | 94.90 |
| | 310 | | 0.07 | 67.93 | 26.43 | 94.36 |
| 10 | 1 | in-situ | n.d. | 89.45 | 2.83 | 92.28 |
| | 170 | | 0.04 | 73.00 | 23.84 | 96.84 |
| | 310 | | 0.08 | 71.02 | 26.27 | 97.29 |

**Table 74: The microcosm incubation study - M4: Active ingredient (AI) extract [% of AR], aquifer Nature A (NA), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | Groundwater | Active ingredient [% of AR] | | |
|---|---|---|---|---|---|
| | | | IMI | IPU | DKN |
| 10 | 1 | phosphate buffered | 91.39 | 87.10 | 80.56 |
| | 170 | [1 mM] | 22.01 | 59.77 | 66.54 |
| | 310 | | n.d. | 59.79 | 87.06 |
| 10 | 1 | in-situ | 93.94 | 85.14 | 89.26 |
| | 170 | | 20.47 | 78.27 | 64.02 |
| | 310 | | n.d. | 63.87 | 59.03 |

**Table 75: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 87.48 | n.d. | n.c. |
| | 90 | - | 1.28 | 85.12 | n.d. | n.c. |
| | 180 | 8.0 | 1.30 | 72.21 | n.d. | n.c. |
| | 270 | 8.0 | 2.51 | 86.22 | n.d. | n.c. |
| | 360 | 7.6 | 4.96 | 79.39 | n.d. | n.c. |
| | 450 | 7.9 | 4.56 | 80.21 | n.d. | n.c. |
| 1 | 1 | - | - | 90.14 | 4.37 | 94.51 |
| | 245 | 8.0 | 1.28 | 86.73 | 9.71 | 97.73 |
| | 450 | 8.7 | 4.53 | 79.17 | 11.16 | 94.86 |
| 10 | 1 | - | - | 97.05 | 3.10 | 100.15 |
| | 90 | - | 0.55 | 89.85 | 2.30 | 92.69 |
| | 180 | 8.0 | 1.05 | 88.24 | 4.08 | 93.37 |
| | 270 | 8.3 | 8.77 | 32.60 | 32.85 | 74.23 |
| | 360 | 8.1 | 1.04 | 88.24 | 6.03 | 95.32 |
| | 450 | 8.0 | 2.06 | 77.84 | 7.33 | 87.23 |

**Table 76: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 87.27 | n.d. | n.c. |
| | 90 | - | 2.76 | 90.16 | n.d. | n.c. |
| | 180 | 7.2 | 4.59 | 86.78 | n.d. | n.c. |
| | 270 | 7.7 | 4.75 | 83.47 | n.d. | n.c. |
| | 360 | 6.9 | 7.10 | 93.22 | n.d. | n.c. |
| | 450 | 6.9 | 2.72 | 89.47 | n.d. | n.c. |
| 1 | 1 | - | - | 90.95 | 5.03 | 95.98 |
| | 245 | 8.3 | 0.42 | 81.73 | 20.45 | 102.60 |
| | 450 | 7.8 | 0.41 | 72.34 | 30.03 | 102.77 |
| 10 | 1 | - | - | 89.42 | 4.66 | 94.08 |
| | 90 | - | 0.22 | 94.82 | 3.76 | 98.80 |
| | 180 | 8.0 | 0.16 | 90.36 | 4.62 | 95.14 |
| | 270 | 8.1 | 0.24 | 87.76 | 4.88 | 92.89 |
| | 360 | 7.4 | 0.54 | 88.47 | 5.10 | 94.12 |
| | 450 | 8.0 | 0.54 | 87.68 | 5.14 | 93.35 |

**Table 77: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 80.38 | n.d. | n.c. |
| | 90 | - | 2.24 | 82.87 | n.d. | n.c. |
| | 180 | 8.2 | 4.51 | 82.24 | n.d. | n.c. |
| | 270 | 8.6 | 15.06 | 86.15 | n.d. | n.c. |
| | 360 | 7.8 | 1.25 | 83.64 | n.d. | n.c. |
| | 450 | 7.2 | 11.90 | 84.42 | n.d. | n.c. |
| 1 | 1 | - | - | 93.58 | 2.65 | 96.23 |
| | 245 | 8.4 | 0.15 | 91.87 | 4.45 | 96.47 |
| | 450 | 8.6 | 0.48 | 88.70 | 6.11 | 95.29 |
| 10 | 1 | - | - | 90.07 | 2.50 | 92.57 |
| | 90 | - | 0.03 | 95.46 | 1.84 | 97.34 |
| | 180 | 8.2 | 0.07 | 91.70 | 3.25 | 95.01 |
| | 270 | 8.6 | 0.09 | 56.30 | 2.03 | 58.41 |
| | 360 | 8.2 | 0.05 | 92.36 | 1.90 | 94.30 |
| | 450 | 8.3 | 0.18 | 90.51 | 2.77 | 93.46 |

**Table 78: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Krauthausen (K), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Temperature [°C] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|---|
| 0.1 | 10 | 1 | - | - | 94.51 | 8.61 | 103.12 |
| | | 245 | 7.0 | 2.76 | 79.84 | 15.45 | 98.05 |
| | | 450 | 7.0 | 3.25 | 77.02 | 21.52 | 101.79 |
| 10 | 10 | 1 | - | - | 91.01 | 1.86 | 92.89 |
| | | 245 | 7.9 | 0.84 | 88.32 | 5.00 | 94.16 |
| | | 450 | 8.4 | 2.13 | 83.79 | 6.74 | 92.65 |
| 10 | 20 | 1 | - | - | 89.70 | 1.37 | 91.07 |
| | | 245 | 8.5 | 3.60 | 79.91 | 7.47 | 90.98 |
| | | 450 | 8.7 | 4.01 | 87.08 | 6.12 | 97.21 |

**Table 79: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 81.11 | n.d. | n.c. |
| | 90 | - | 1.06 | 77.37 | n.d. | n.c. |
| | 180 | 7.2 | 7.86 | 43.08 | n.d. | n.c. |
| | 270 | 6.0 | 7.10 | 36.69 | n.d. | n.c. |
| | 360 | 6.7 | - | 42.36 | n.d. | n.c. |
| | 450 | 6.9 | 12.93 | 37.83 | n.d. | n.c. |
| 1 | 1 | - | - | 72.47 | 8.08 | 80.55 |
| | 245 | 7.2 | 11.94 | 47.52 | 28.56 | 88.02 |
| | 450 | 8.5 | 13.48 | 40.11 | 36.32 | 89.91 |
| 10 | 1 | - | - | 91.85 | 2.14 | 93.99 |
| | 90 | - | 0.26 | 89.85 | 2.58 | 92.69 |
| | 180 | 8.5 | 4.28 | 72.98 | 15.29 | 92.55 |
| | 270 | 7.2 | 8.08 | 51.61 | 29.59 | 89.29 |
| | 360 | 6.8 | 9.07 | 46.66 | 33.49 | 89.22 |
| | 450 | 7.9 | 10.28 | 45.26 | 32.04 | 87.58 |

**Table 80: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), test substance Imidacloprid [IMI]**

| Concentration [µg l$^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 81.91 | n.d. | n.c. |
| | 90 | - | 2.16 | 76.16 | n.d. | n.c. |
| | 180 | 7.0 | 11.73 | 68.27 | n.d. | n.c. |
| | 270 | 8.0 | - | 65.67 | n.d. | n.c. |
| | 360 | 7.2 | 5.65 | 62.30 | n.d. | n.c. |
| | 450 | 7.1 | 7.29 | 58.05 | n.d. | n.c. |
| 1 | 1 | - | - | 82.77 | 4.73 | 87.49 |
| | 245 | 8.2 | 1.67 | 67.01 | 38.35 | 107.03 |
| | 450 | 7.1 | 2.94 | 61.94 | 39.83 | 104.71 |
| 10 | 1 | - | - | 84.87 | 1.36 | 86.22 |
| | 90 | - | 0.52 | 84.05 | 6.41 | 90.97 |
| | 180 | 7.6 | 1.25 | 84.06 | 12.86 | 98.17 |
| | 270 | 8.4 | 1.83 | 44.12 | 13.23 | 59.18 |
| | 360 | 5.6 | 0.82 | 74.12 | 15.12 | 90.05 |
| | 450 | 4.6 | 10.24 | 94.25 | - | n.c. |

**Table 81: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 75.21 | n.d. | n.c. |
| | 90 | - | 3.30 | 75.36 | n.d. | n.c. |
| | 180 | 7.2 | 7.09 | 67.98 | n.d. | n.c. |
| | 270 | 7.5 | 25.14 | 76.66 | n.d. | n.c. |
| | 360 | 7.0 | 4.95 | 78.21 | n.d. | n.c. |
| | 450 | 7.0 | 5.54 | 78.68 | n.d. | n.c. |
| 1 | 1 | - | - | 89.34 | 2.46 | 91.80 |
| | 245 | 8.1 | 0.26 | 90.92 | 4.60 | 95.78 |
| | 450 | 8.5 | 0.96 | 89.76 | 2.69 | 93.41 |
| 10 | 1 | - | - | 92.33 | 1.81 | 94.14 |
| | 90 | - | 0.12 | 90.73 | 1.46 | 92.31 |
| | 180 | 7.4 | 0.15 | 92.57 | 2.29 | 95.05 |
| | 270 | 8.4 | 0.18 | 58.87 | 2.98 | 62.03 |
| | 360 | 7.2 | 0.29 | 90.56 | 1.85 | 92.71 |
| | 450 | 8.0 | 0.46 | 76.42 | 3.14 | 80.02 |

**Table 82: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Temperature [°C] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|---|
| 0.1 | 10 | 1 | - | - | 77.87 | 18.67 | 96.54 |
| | | 245 | 8.5 | 5.17 | 62.87 | 36.44 | 104.48 |
| | | 450 | 8.1 | 5.29 | 40.38 | 43.45 | 89.12 |
| 10 | 10 | 1 | - | - | 85.30 | 1.47 | 86.81 |
| | | 245 | 7.6 | 3.16 | 73.27 | 12.78 | 89.21 |
| | | 450 | 8.4 | 6.45 | 57.76 | 23.22 | 87.44 |
| 10 | 20 | 1 | - | - | 86.49 | 1.44 | 87.93 |
| | | 245 | 7.9 | 6.07 | 64.92 | 18.13 | 89.13 |
| | | 450 | 8.6 | 10.36 | 50.33 | 30.31 | 91.00 |

**Table 83: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), test substance Isoproturon [IPU]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 81.21 | n.d. | n.c. |
| | 90 | - | 7.43 | 32.44 | n.d. | n.c. |
| | 180 | 8.6 | 9.86 | 27.14 | n.d. | n.c. |
| | 270 | 8.8 | 13.82 | 14.38 | n.d. | n.c. |
| | 360 | 7.8 | 11.32 | 16.18 | n.d. | n.c. |
| | 450 | 8.0 | 14.28 | 7.63 | n.d. | n.c. |
| 1 | 1 | - | - | 87.12 | 5.32 | 92.44 |
| | 245 | 8.3 | 14.86 | 20.86 | 56.99 | 92.71 |
| | 450 | 8.6 | 15.44 | 9.29 | 71.13 | 95.86 |
| 10 | 1 | - | - | 89.29 | 3.57 | 92.86 |
| | 90 | - | 3.29 | 41.90 | 38.58 | 83.77 |
| | 180 | 8.0 | 8.69 | 36.16 | 44.68 | 89.53 |
| | 270 | 8.0 | 9.97 | 16.73 | 57.67 | 84.37 |
| | 360 | 8.7 | 16.08 | 7.73 | 70.76 | 94.57 |
| | 450 | 7.9 | 5.39 | 4.60 | 72.48 | 82.47 |

**Table 84: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), test substance Imidacloprid [IMI]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 82.23 | n.d. | n.c. |
| | 90 | - | 3.02 | 68.97 | n.d. | n.c. |
| | 180 | 8.6 | 3.30 | 66.76 | n.d. | n.c. |
| | 270 | 9.0 | 2.71 | 40.22 | n.d. | n.c. |
| | 360 | 8.8 | 2.64 | 40.16 | n.d. | n.c. |
| | 450 | 7.0 | 3.44 | 37.14 | n.d. | n.c. |
| 1 | 1 | - | - | 85.83 | 3.31 | 89.14 |
| | 245 | 8.9 | 0.66 | 66.51 | 23.14 | 90.31 |
| | 450 | - | - | - | - | - |
| 10 | 1 | - | - | 87.86 | 3.67 | 91.53 |
| | 90 | - | 0.32 | 65.98 | 25.98 | 92.28 |
| | 180 | 7.8 | 1.69 | 16.82 | 68.65 | 87.16 |
| | 270 | 8.8 | 1.25 | 78.50 | 13.85 | 93.60 |
| | 360 | 7.7 | 0.61 | 63.87 | 24.69 | 89.16 |
| | 450 | 8.6 | 3.60 | 12.76 | 80.78 | 97.13 |

**Table 85: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), test substance Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 0.1 | 1 | - | - | 95.74 | n.d. | n.c. |
| | 90 | - | 1.30 | 64.86 | n.d. | n.c. |
| | 180 | 8.7 | 2.15 | 55.56 | n.d. | n.c. |
| | 270 | 8.2 | 9.04 | 28.74 | n.d. | n.c. |
| | 360 | 7.8 | 14.61 | 18.59 | n.d. | n.c. |
| | 450 | 7.4 | 21.15 | 12.59 | n.d. | n.c. |
| 1 | 1 | - | - | 82.36 | 24.83 | 107.19 |
| | 245 | 8.0 | 4.26 | 43.46 | 40.12 | 87.84 |
| | 450 | 9.0 | 11.38 | 18.78 | 56.12 | 86.27 |
| 10 | 1 | - | - | 84.69 | 9.48 | 94.17 |
| | 90 | - | 0.23 | 77.07 | 17.12 | 94.41 |
| | 180 | 8.6 | 1.00 | - | 29.17 | n.c. |
| | 270 | 8.6 | 2.21 | 45.88 | 24.57 | 72.66 |
| | 360 | 8.6 | 3.60 | 48.29 | 42.61 | 94.50 |
| | 450 | 8.7 | 8.91 | 42.10 | 37.88 | 88.88 |

**Table 86: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], aquifer Nature A (NA), mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Temperature [°C] | Incubation [days] | pH | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|---|
| 0.1 | 10 | 1 | - | - | 83.43 | 7.94 | 91.37 |
| | | 245 | 7.9 | 4.18 | 35.82 | 58.89 | 98.88 |
| | | 450 | 7.5 | 10.58 | 22.67 | 63.21 | 96.47 |
| 10 | 10 | 1 | - | - | 88.79 | 2.92 | 91.74 |
| | | 245 | 8.8 | 7.43 | 35.86 | 47.40 | 90.69 |
| | | 450 | 8.7 | 15.16 | 22.63 | 55.19 | 92.98 |
| 10 | 20 | 1 | - | - | 87.87 | 3.54 | 91.41 |
| | | 245 | 8.8 | 10.75 | 33.37 | 47.27 | 91.38 |
| | | 450 | 8.6 | 18.82 | 13.44 | 60.15 | 92.43 |

**Table 87: The microcosm incubation study - M3: Radioactive balance and distribution of applied radioactivity [%], biological control, mixture (1:1:1) of test substances Isoproturon [IPU], Imidacloprid [IMI], Diketonitrile [DKN]**

| Concentration [µg $l^{-1}$] | Aquifer | Incubation [days] | Mineralization [%] | Extraktion [%] | Combustion [%] | Recovery [%] |
|---|---|---|---|---|---|---|
| 10 | K | 477 | 0.39 | 97.08 | 2.90 | 100.37 |
| 10 | MB | 477 | 0.14 | 84.58 | 4.75 | 89.47 |
| 10 | NA | 477 | 0.19 | 80.44 | 16.77 | 97.39 |

**Figure 19: The DAISY test system: Online control (Screen shot)**


**Figure 20: The aquifer Krauthausen: Generalized cross-section of the uppermost aquifer, derived from field studies (137;148)**

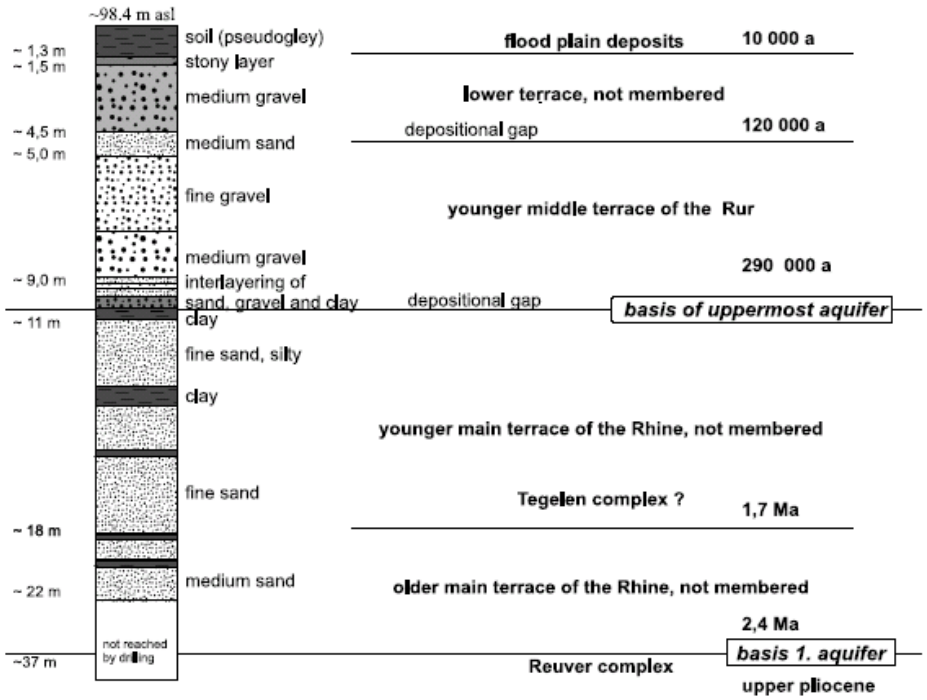


**Figure 21: The aquifer Krauthausen: Installed observation wells and groundwater flow direction (137;148)**

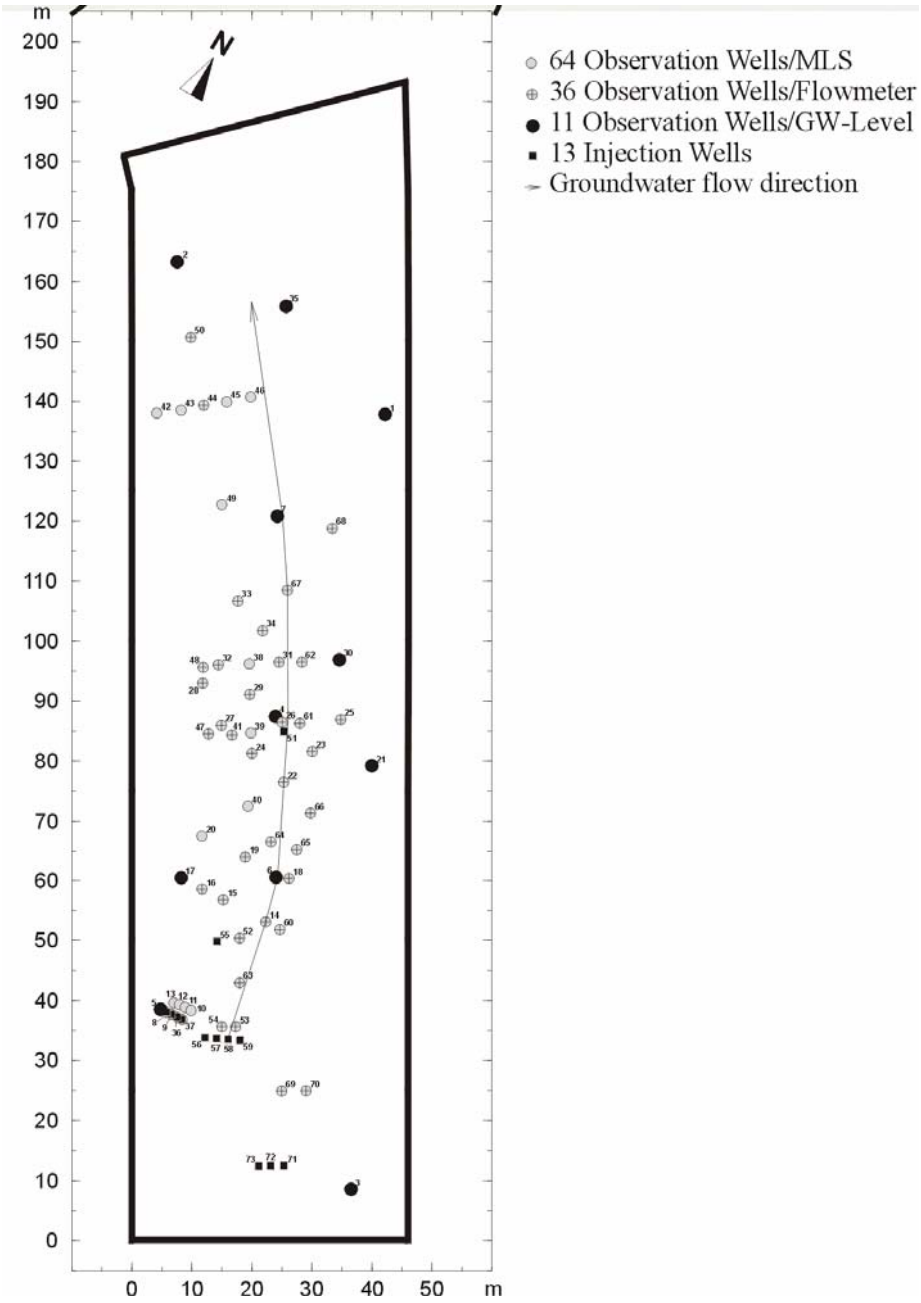


**Figure 22: The aquifer Nature A (NA): Drawing of position of sampling spot**

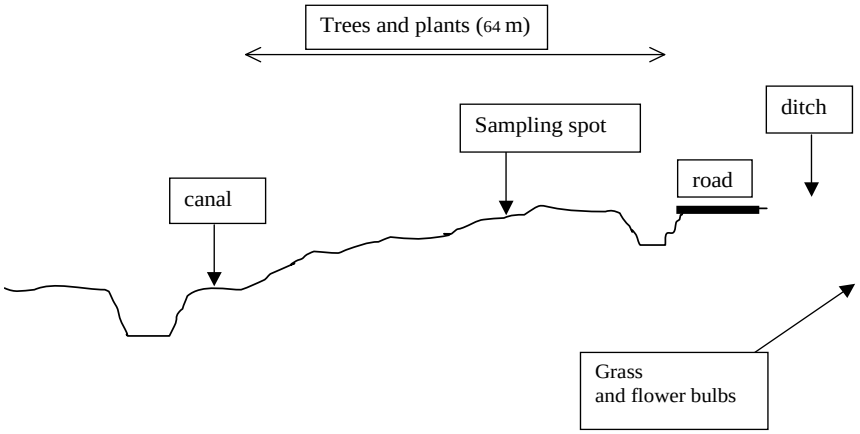
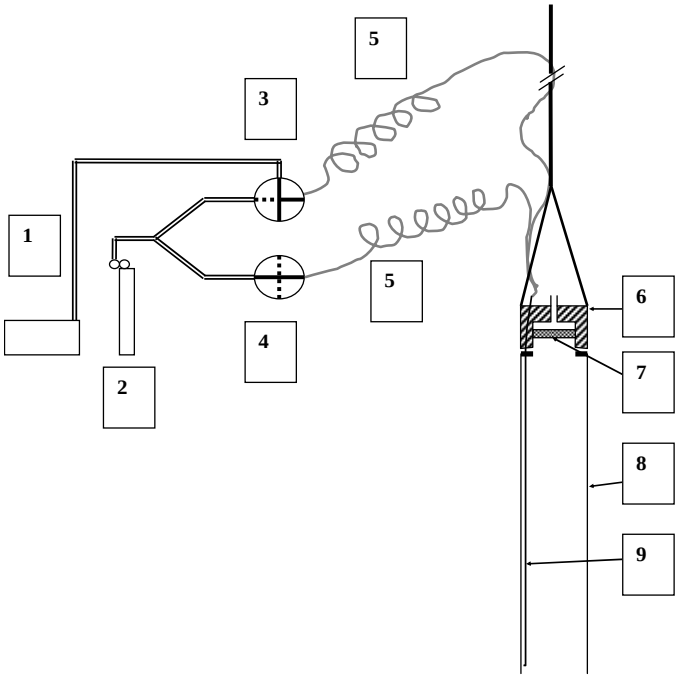
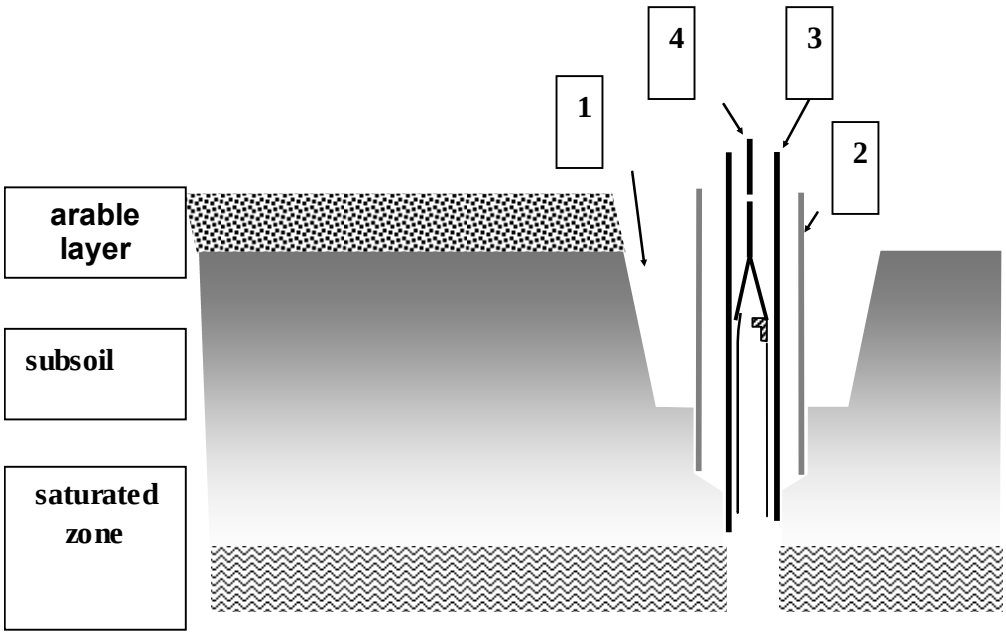



**Figure 23: Scheme of the anaerobic sampler to collect water-saturated subsoil**




1.Vacuum pump with pressure regulator 2. Nitrogen cylinder with pressure regulator 3. Three-way valve to apply under pressure or N2 pressure to head of sampler 4. On-off switching valve to apply N2 to tube (9) to overcome under pressure with pulling up sampler 5. Flexible plastic tubing 6. Head of sampler, connected to 1m extension rods with marks 7. Glass filter, mounted in head of sampler 8. Sampling tube (50 cm length, 6 cm effective diameter), airtight connected to head 9. 1/8 inch steel tube inside sampling tube through which N2 is blown while pulling up sampler to compensate

**Figure 24: Principle of the anaerobic sampling method**




1.Dug trench, 50-70 cm deep 2. Wide casing tube, protection against collapsing of topsoil into deeper layers 3. Casing tube, 1 m sections (to max. 5 m) connected with screw thread 4. Sampler with extension rods

**Figure 25: Principle of the aerobic sample system (aqua lock sampler)**

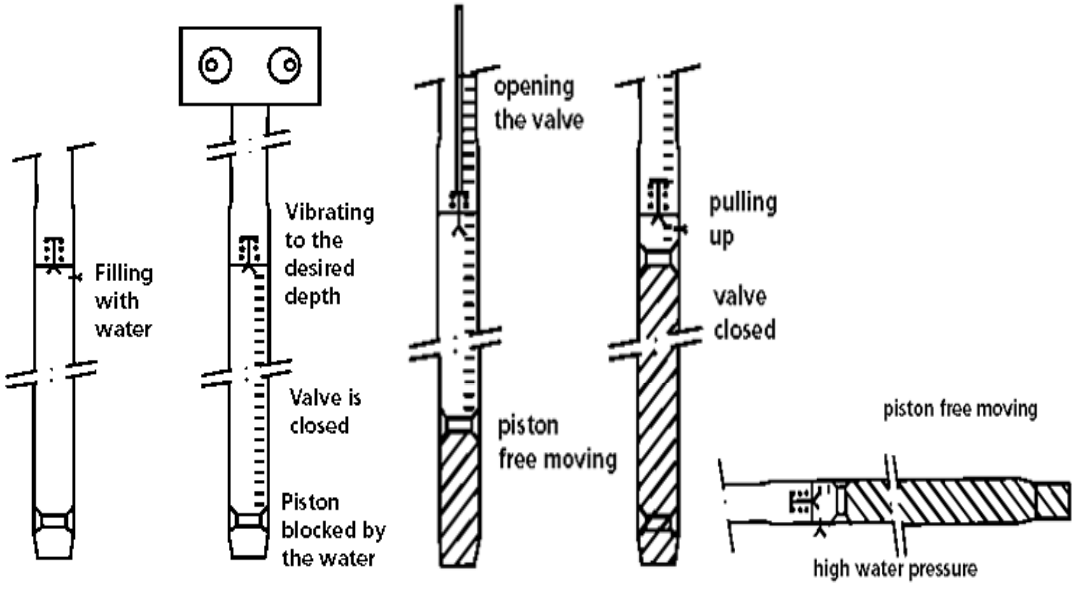


**Figure 26: Ground temperature: Mean monthly isotherms (56)**

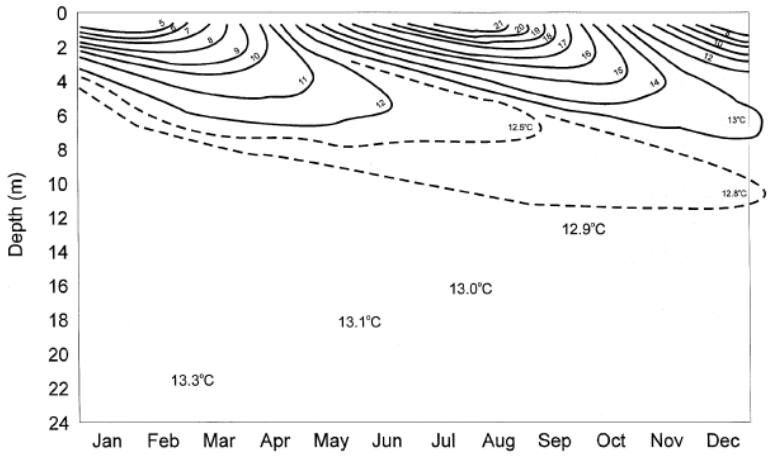
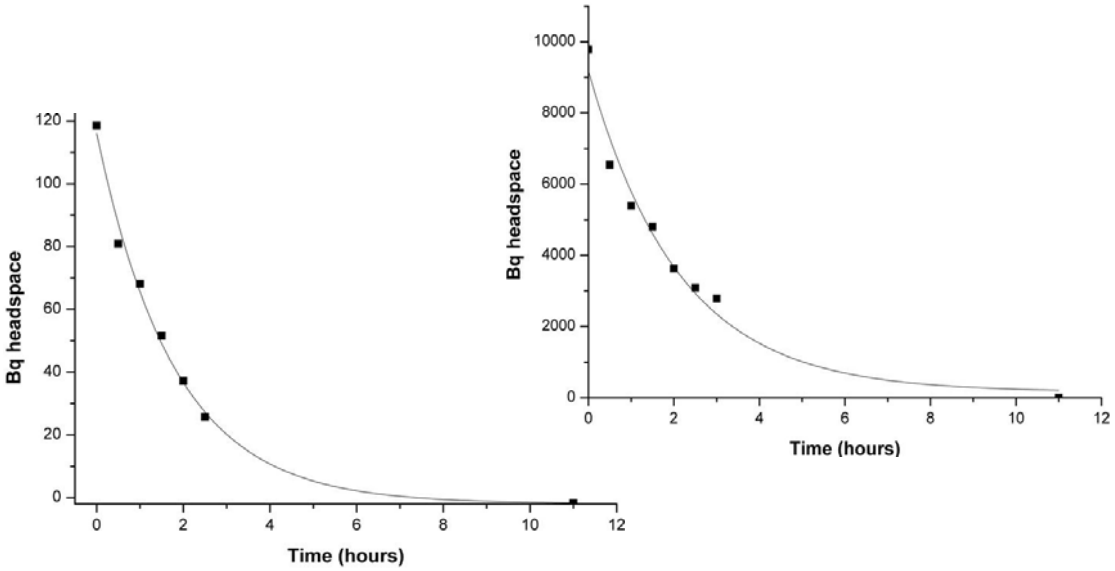



**Figure 27: Absorber traps: Kinetic (flask, V = 500 ml)**




x axis = remaining Bq in the headspace of the flasks

y axis = time in hrs after the addition of HCL to the $NaH^{14}CO_3$ and the release of $^{14}CO_2$

**Figure 28: The DAISY incubation study - D5: Distribution of applied radioactivity [%], aquifer Krauthausen (K), active ingredient [AI] - Imidacloprid [IMI], Isoproturon [IPU], and Diketonitrile [DKN], concentration 10 µg $l^{-1}$**

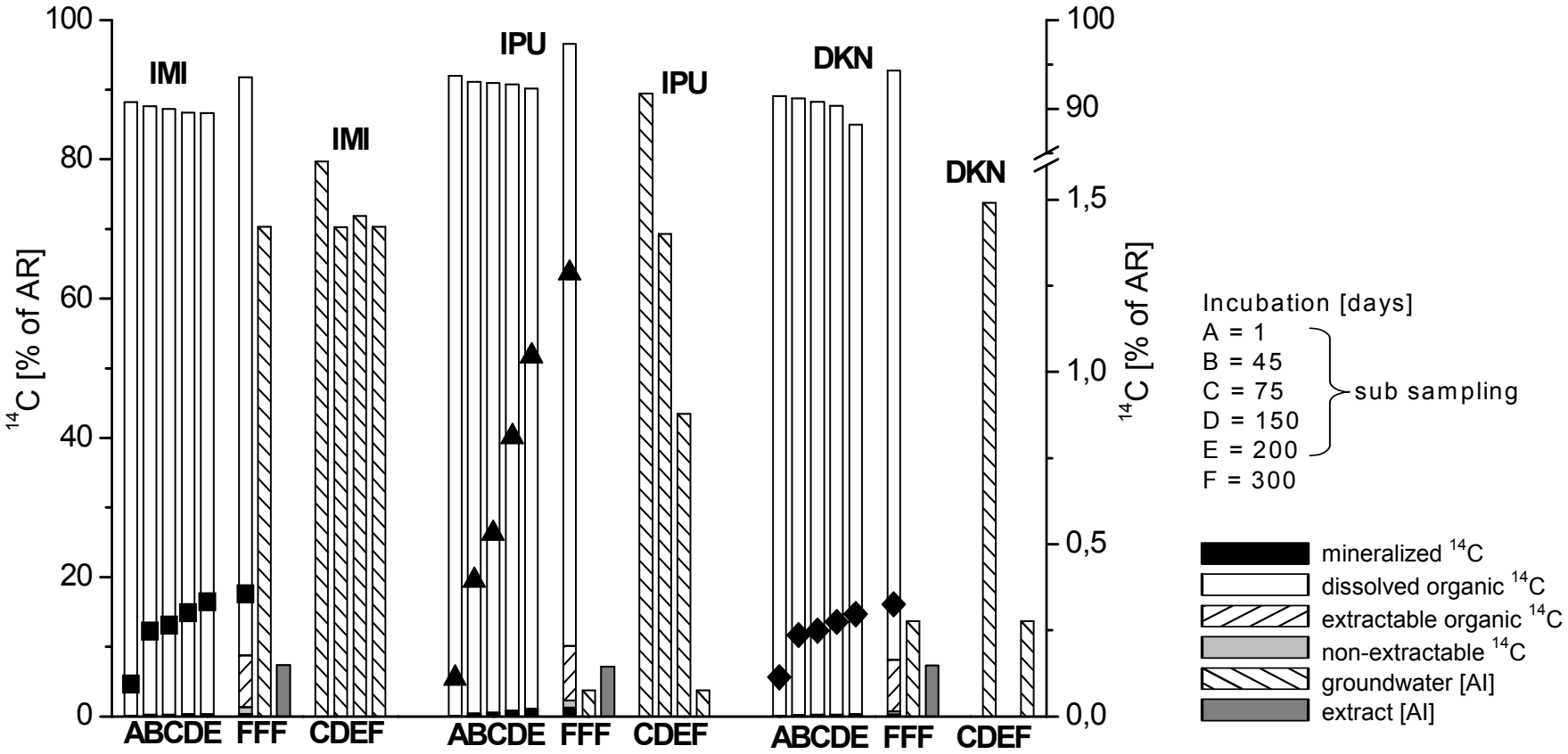


**Figure 29: The DAISY incubation study - D5: Distribution of applied radioactivity [%], aquifer Krauthausen (K), active ingredient [AI] – mixture (1:1:1) of test substances Imidacloprid [IMI], Isoproturon [IPU], and Diketonitrile [DKN], concentration 10 µg $l^{-1}$**

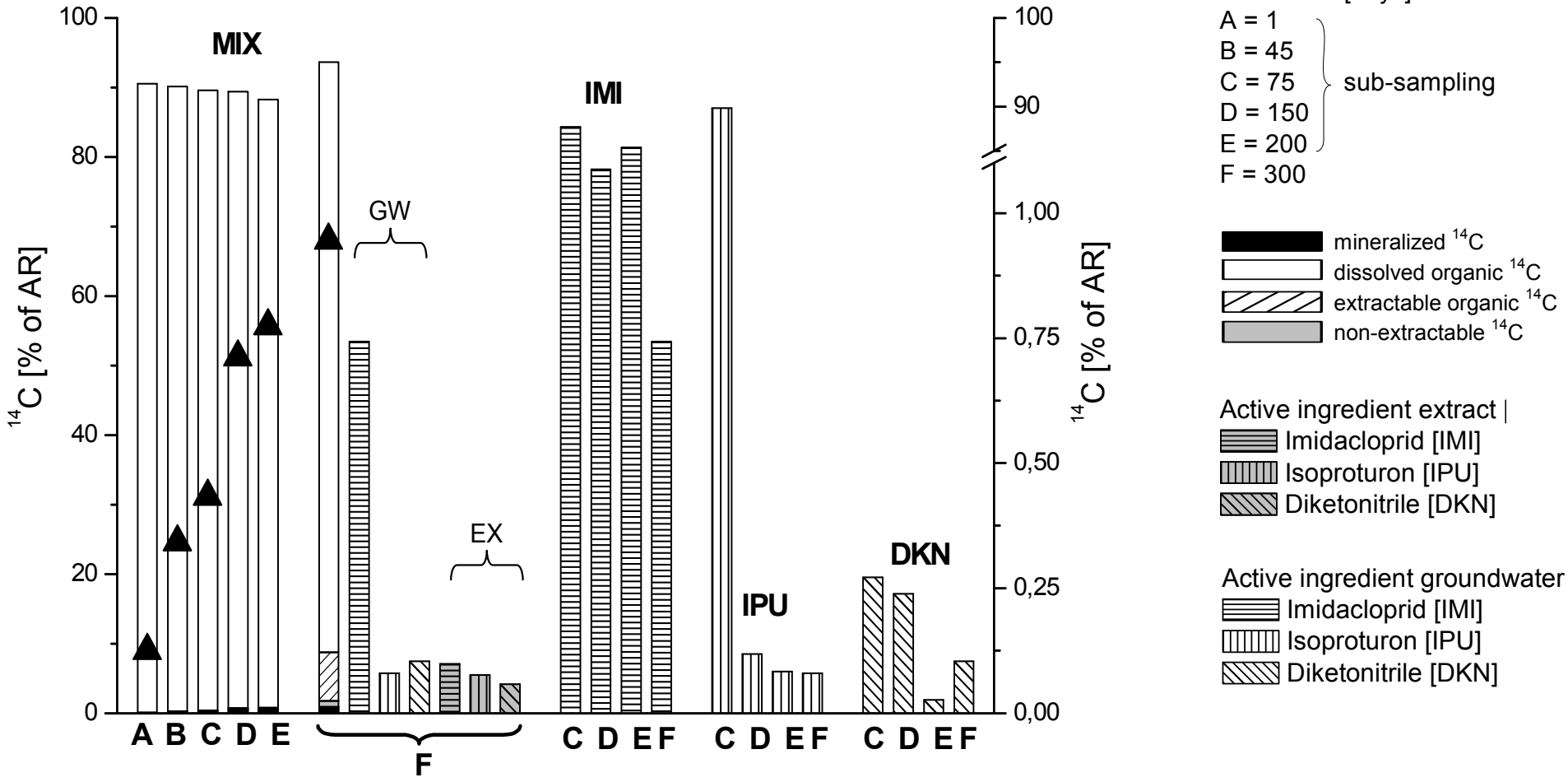


**Figure 30: The DAISY incubation study - D5: Distribution of applied radioactivity [%], aquifer Mulder-Beilen (MB), active ingredient [AI] – mixture (1:1:1) of Imidacloprid [IMI], Isoproturon [IPU], and Diketonitrile [DKN], concentration 10 µg $l^{-1}$**

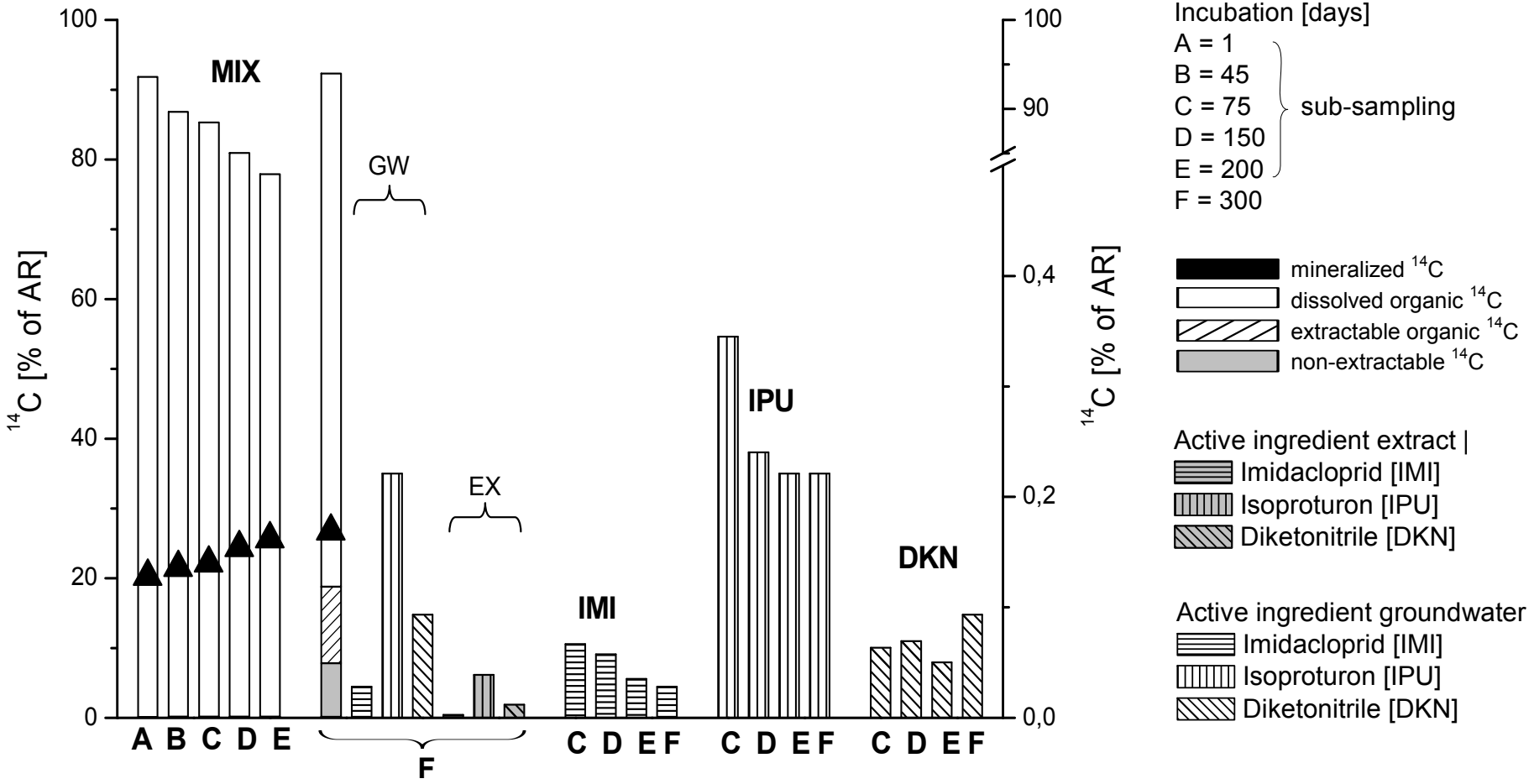
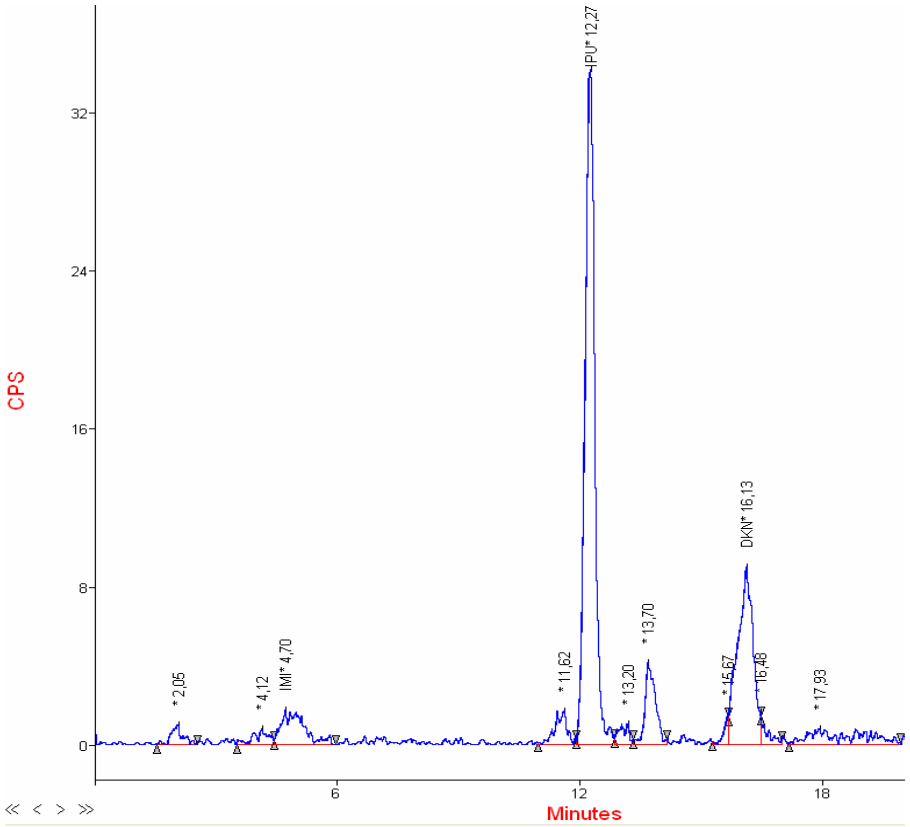


**Figure 31: The DAISY incubation study - D5: HPLC chromatograms, aquifer Mulder-Beilen (MB), day 300, active ingredient [AI] – mixture (1:1:1) of Imidacloprid [IMI], Isoproturon [IPU], and Diketonitrile [DKN], concentration 10 µg $l^{-1}$**

**Groundwater**




**Extract**

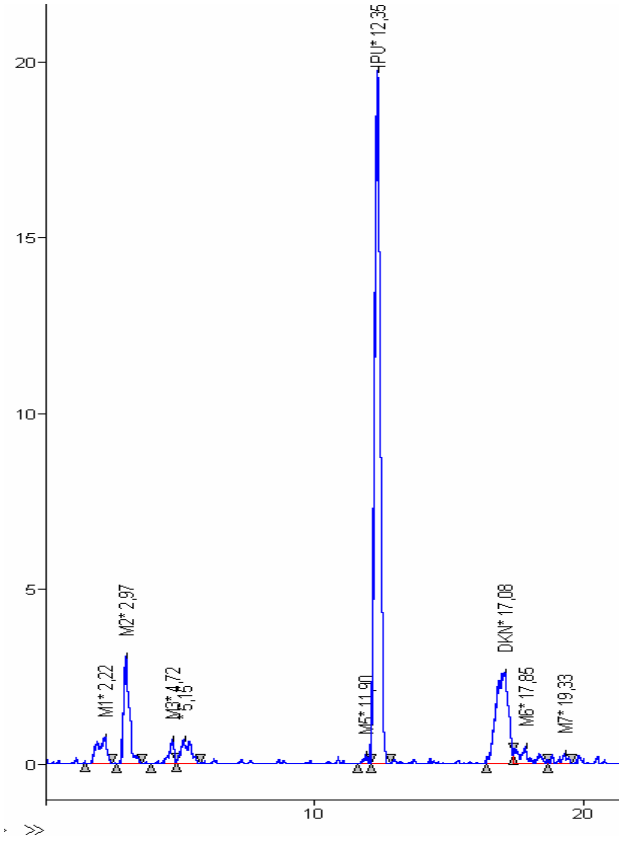


**Figure 32: The DAISY incubation study - D5: Distribution of applied radioactivity [%], aquifer Nature A (NA), active ingredient [AI] – mixture (1:1:1) of Imidacloprid [IMI], Isoproturon [IPU], and Diketonitrile [DKN], concentration 10 µg $l^{-1}$**

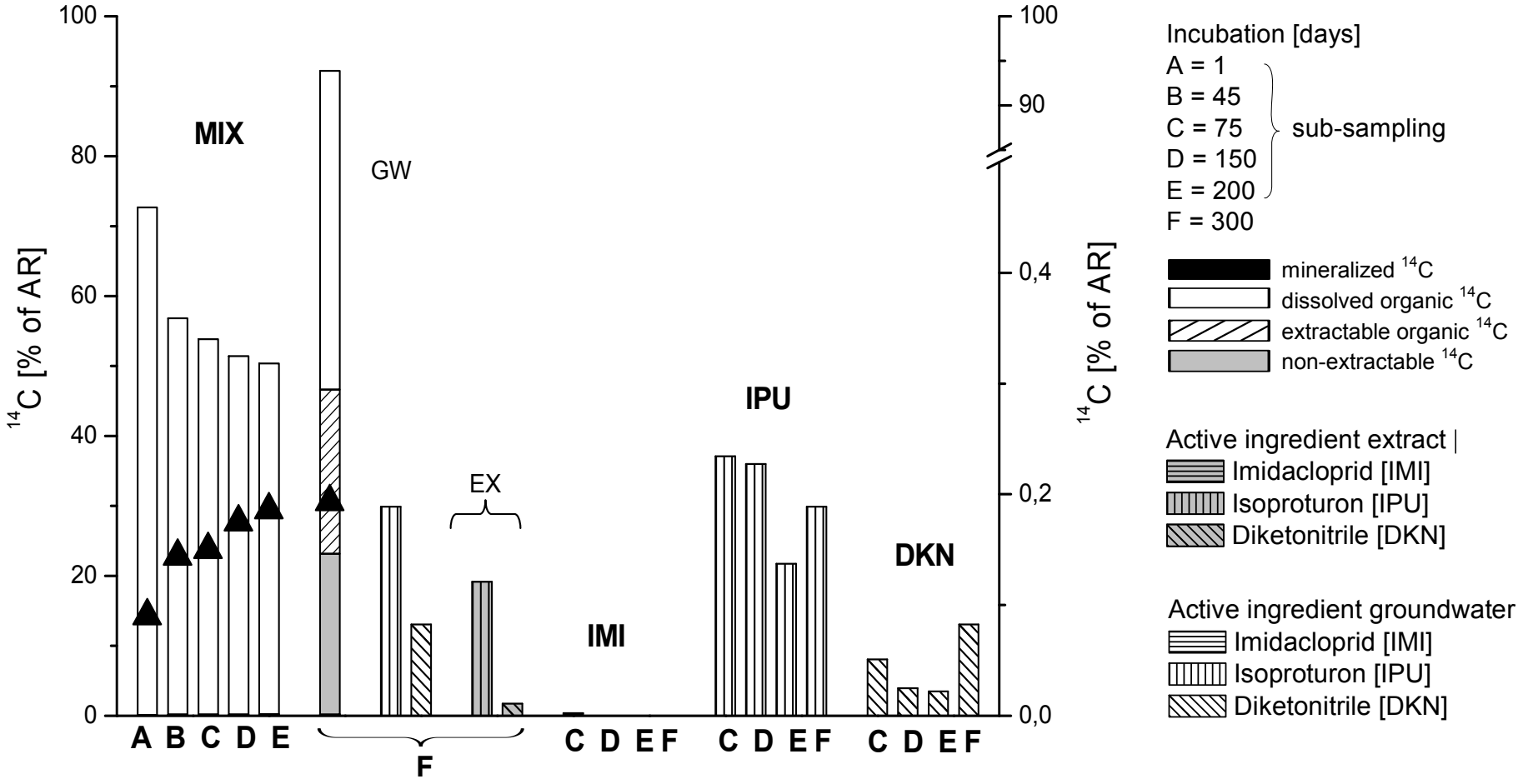
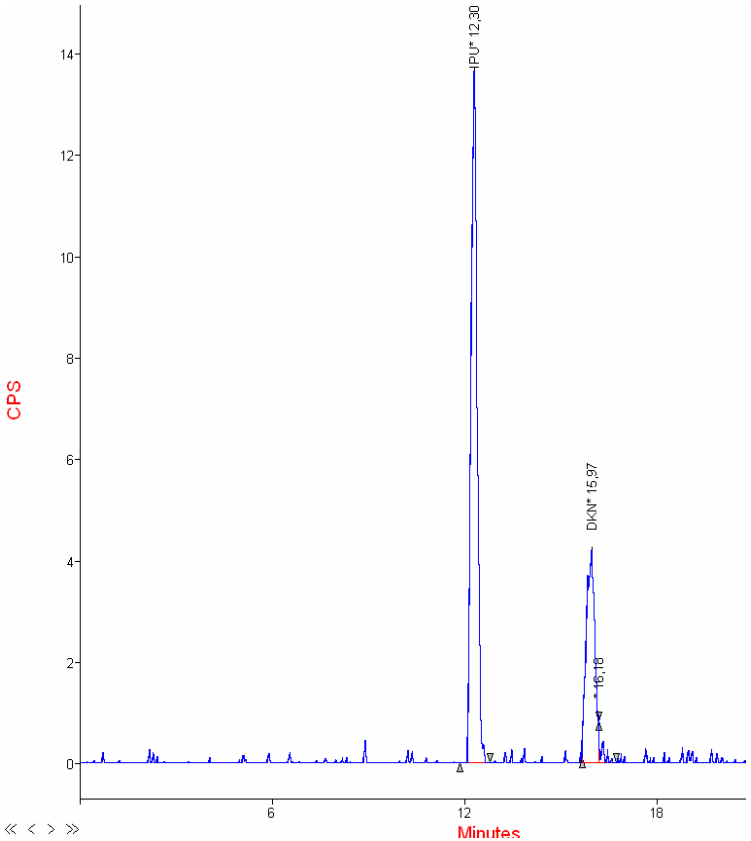


**Figure 33: The DAISY incubation study - D5: HPLC chromatograms, aquifer Nature A (NA), day 300, active ingredient [AI] - mixture (1:1:1) of Imidacloprid [IMI], Isoproturon [IPU] and Diketonitrile [DKN], concentration 10 µg $l^{-1}$**

**Groundwater**




**Extract**

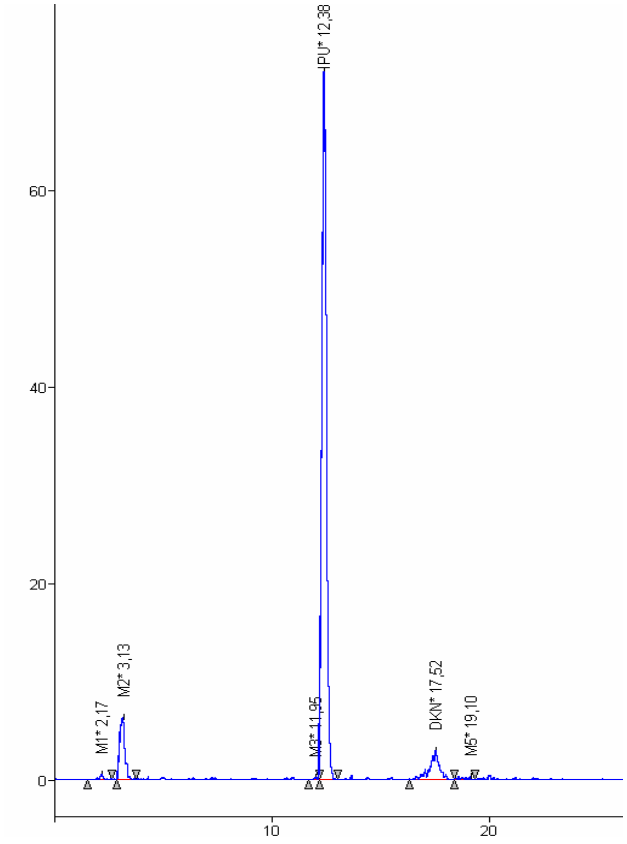


**Figure 34: The microcosm incubation study - M4: Distribution of applied radioactivity [%], active ingredient [AI] – Imidacloprid [IMI], aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA), concentration 10 µg $l^{-1}$**

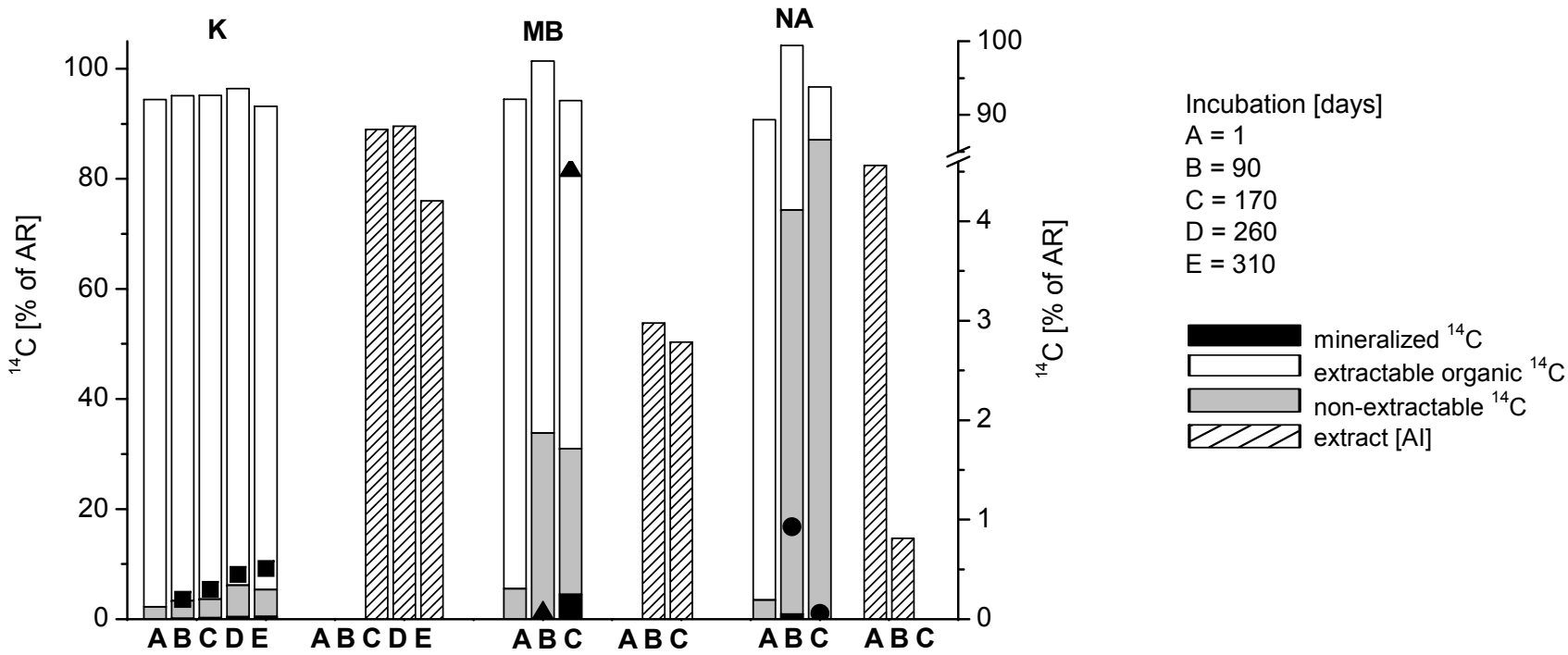


**Figure 35: The microcosm incubation study - M4: Distribution of applied radioactivity [%], active ingredient [AI] – Isoproturon [IPU], aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA), concentration 10 µg $l^{-1}$**

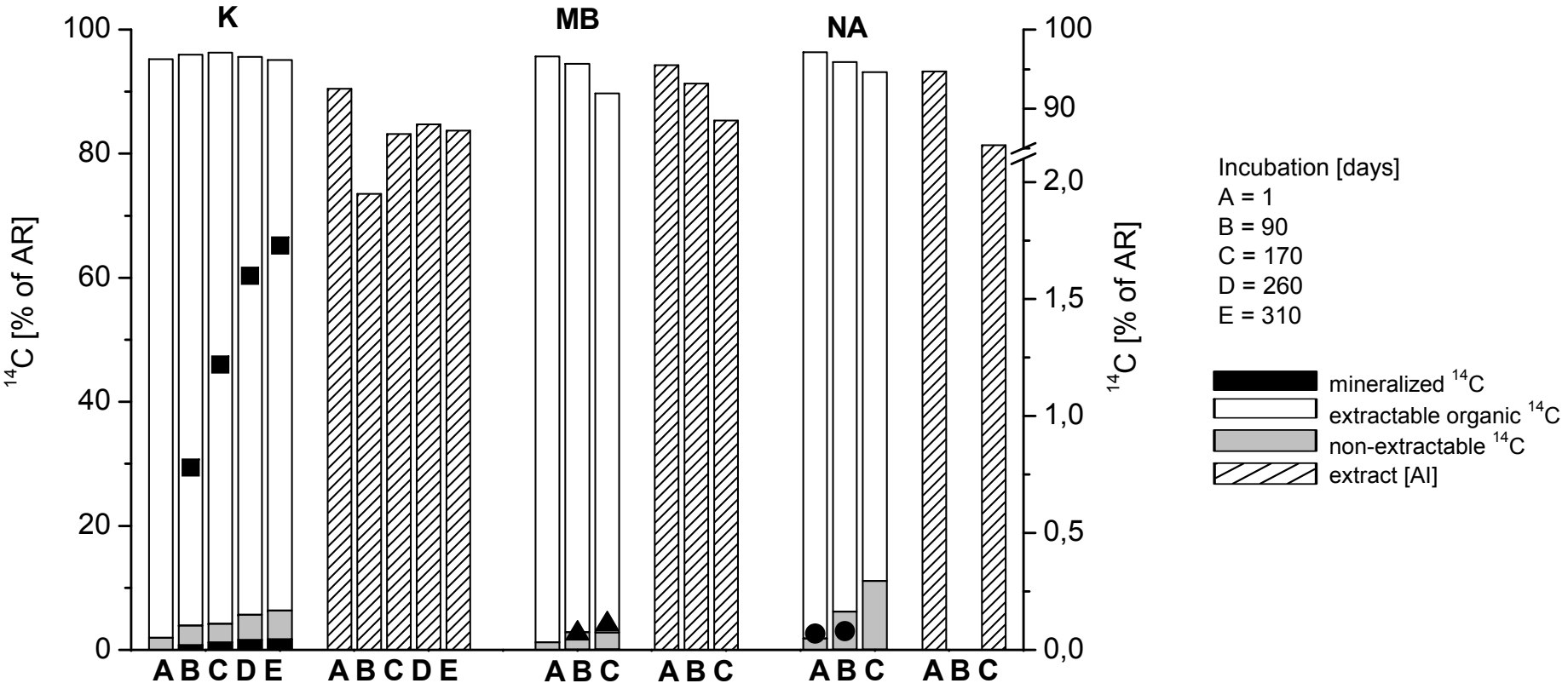


**Figure 36: The microcosm incubation study - M4: Selected HPLC chromatograms day 300, active ingredient [AI] – Isoproturon [IPU], aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA), concentration 10 µg $l^{-1}$**

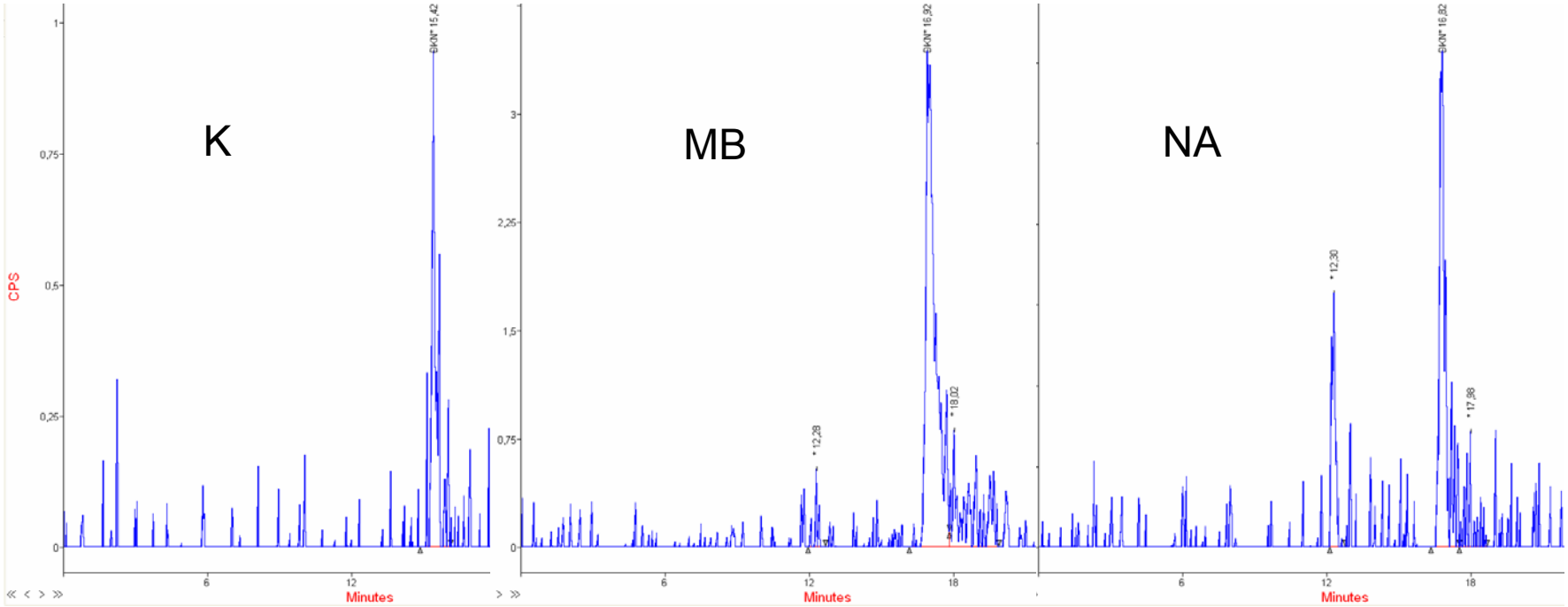


**Figure 37: The microcosm incubation study - M4: Distribution of applied radioactivity [%], active ingredient [AI] – Diketonitrile [DKN], aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA), concentration 10 µg $l^{-1}$**

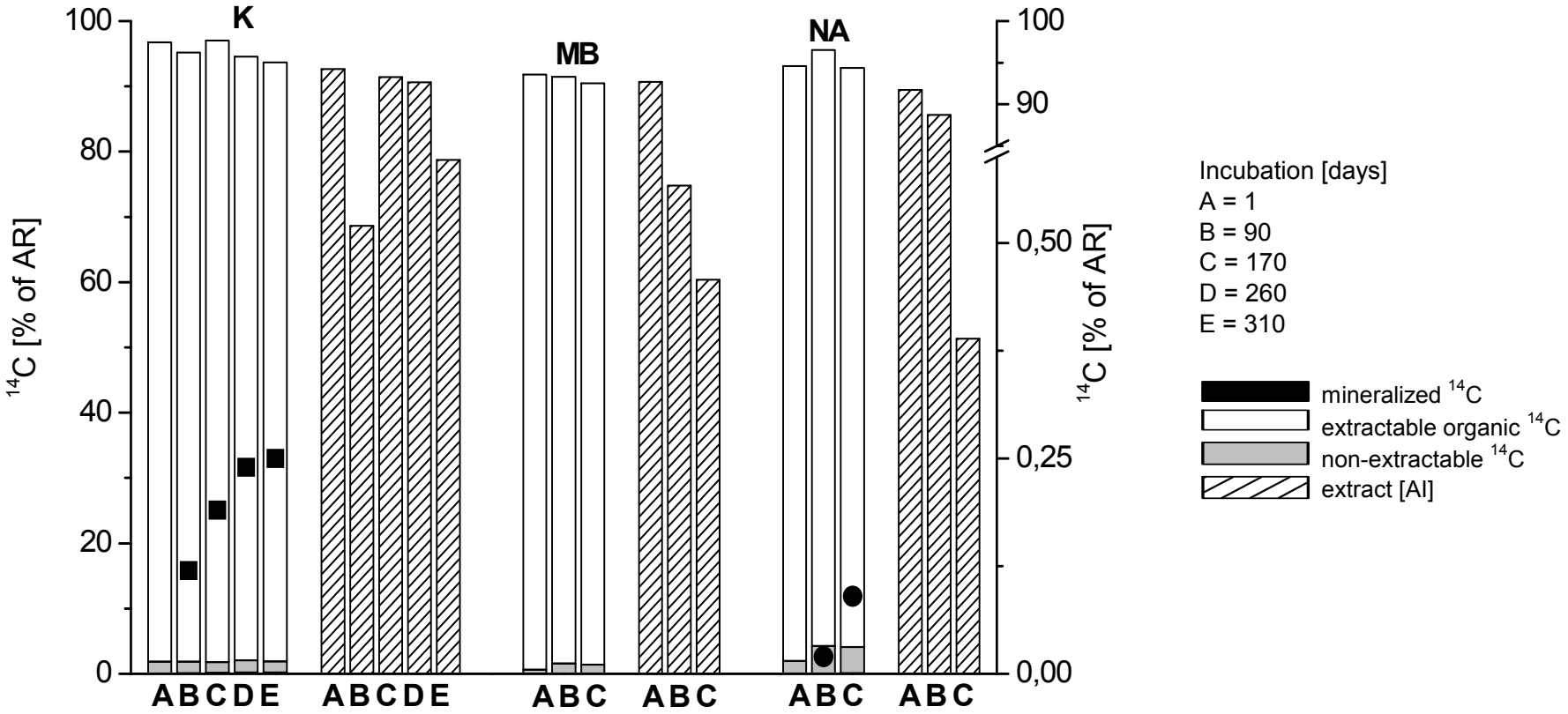


**Figure 38: The microcosm incubation study - M4: Selected HPLC chromatograms day 300, active ingredient [AI] – Isoproturon [IPU], aquifer Krauthausen (K), Mulder-Beilen (MB), Nature A (NA), concentration 10 µg $l^{-1}$**

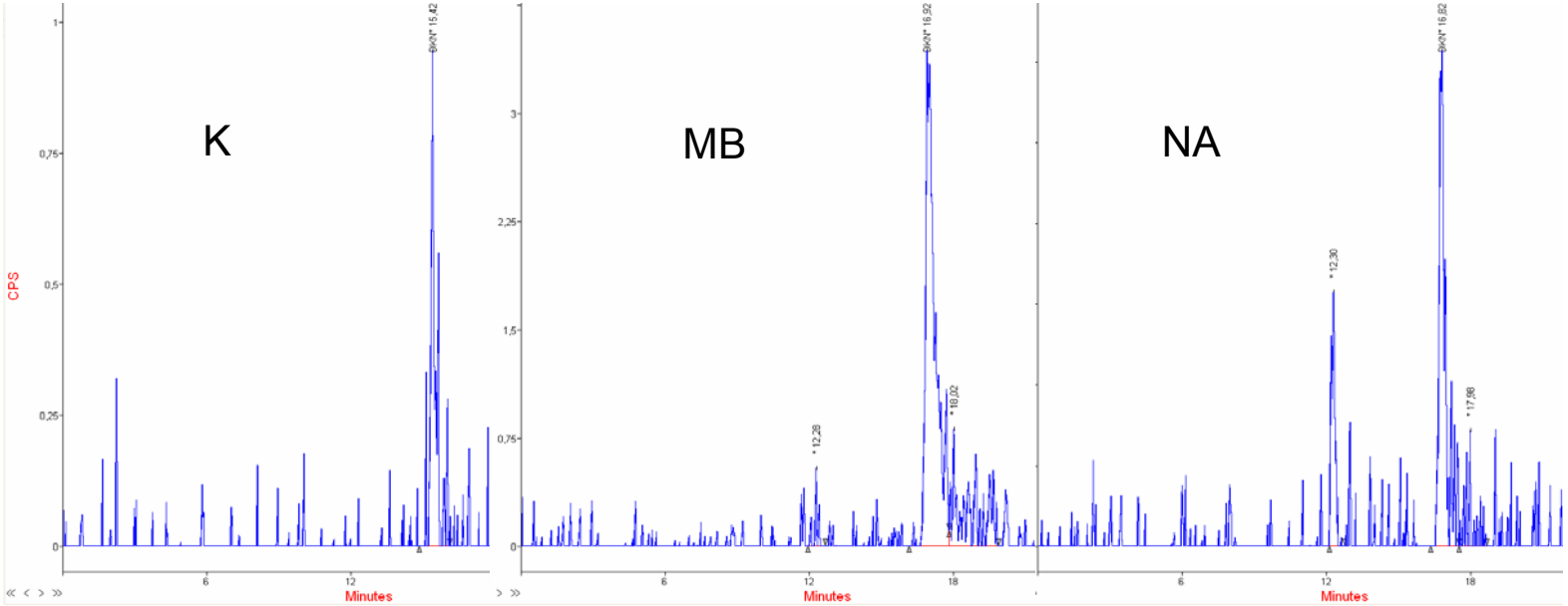
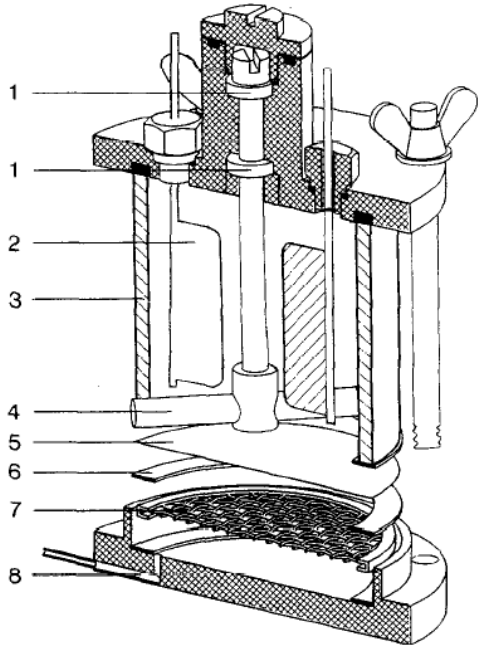
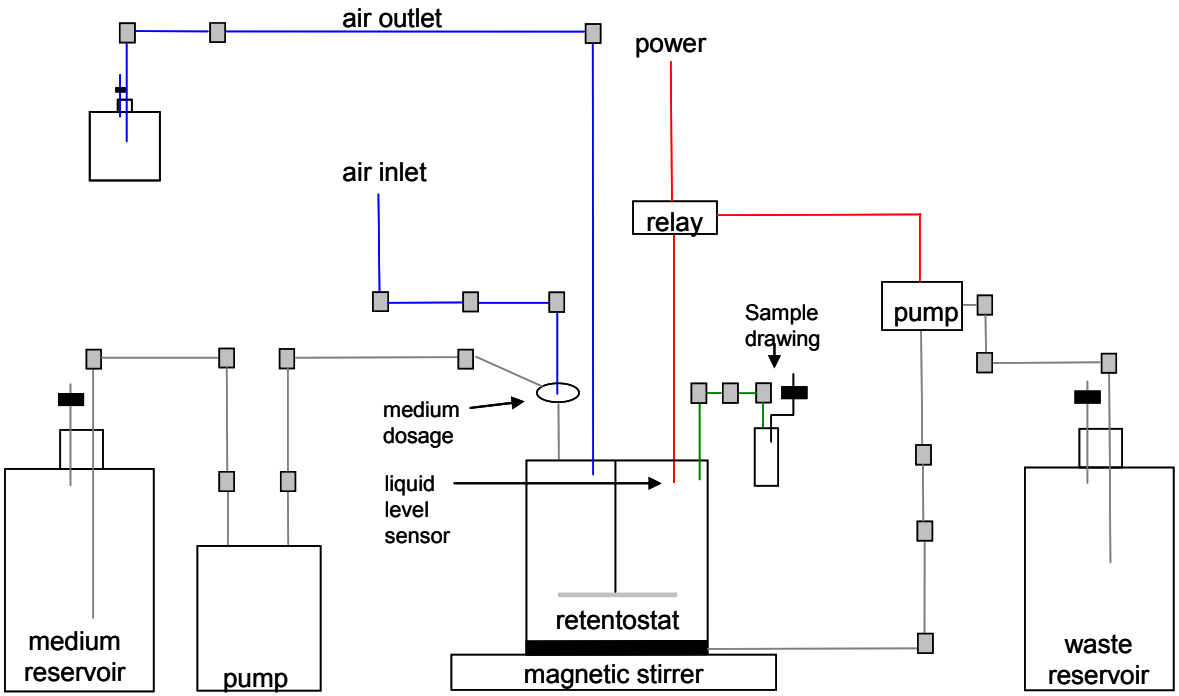


**Figure 39: Enrichment cultures: Experimental setup and technical draft of the Retentostat (55;143)**




1 ball bearing

2 baffle and in- or outlet

3 glass cylinder

4 stirrer

5 filter membrane

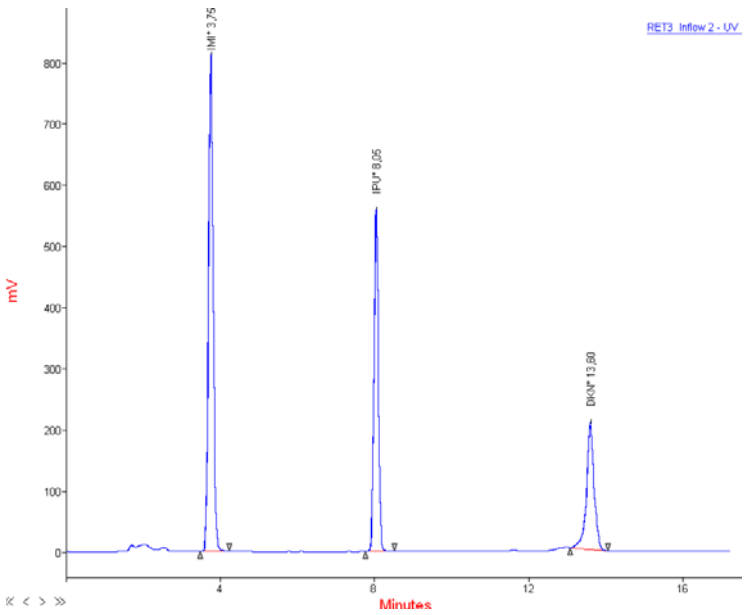
6 silicon rubber gasket;

7 steel grid

8 filtrate outlet

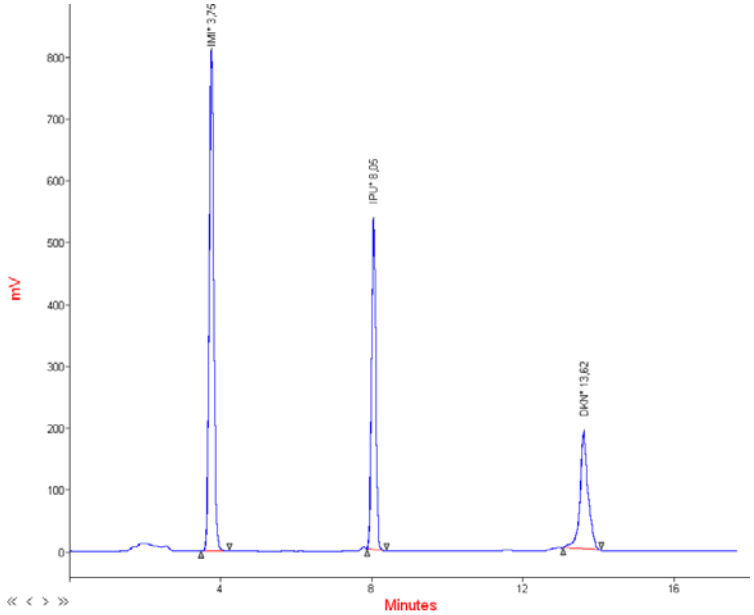
**Figure 40: Enrichment cultures: Retentostat 3 - HPLC measurements (UV, 02.03.2006) of the in- and out flow concentration of Imidacloprid [IMI], Isoproturon [IPU], and Diketonitrile [DKN]**

**Inflow**




| #BMP(379) | Peak name | Rt. | **Area** |
|---|---|---|---|
| 1 | IMI | 3,75 | **6806,6** |
| 2 | IPU | 8,05 | **4413,89** |
| 3 | DKN | 13,6 | **2783,58** |
| SUM | | | 14004,07 |

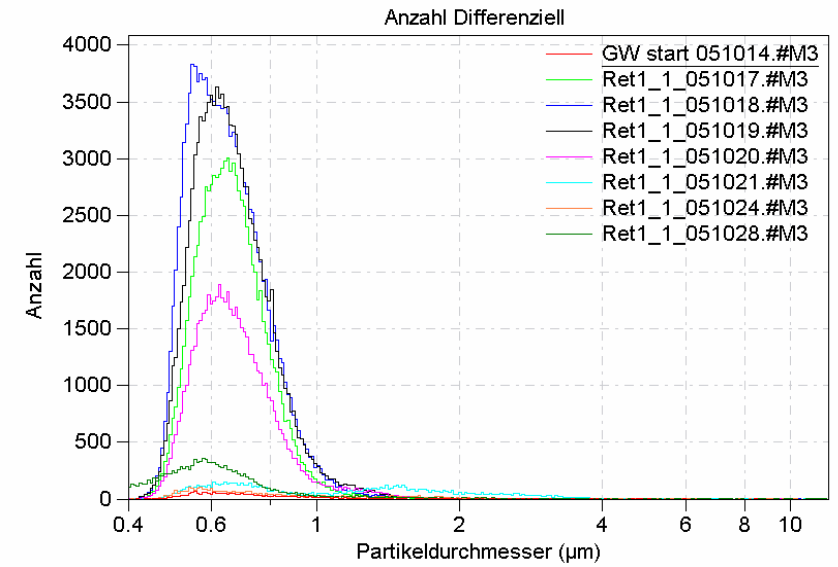
**Outflow**




| #BMP(379) | Peak name | Rt. | **Area** |
|---|---|---|---|
| 1 | IMI | 3,75 | **6801,94** |
| 2 | IPU | 8,05 | **4162,04** |
| 3 | DKN | 13,62 | **2785,86** |
| SUM | | | 13749,83 |

**Figure 41: Enrichment cultures: Particle concentration**

**Retentostat 1**




**Retentostat 2**

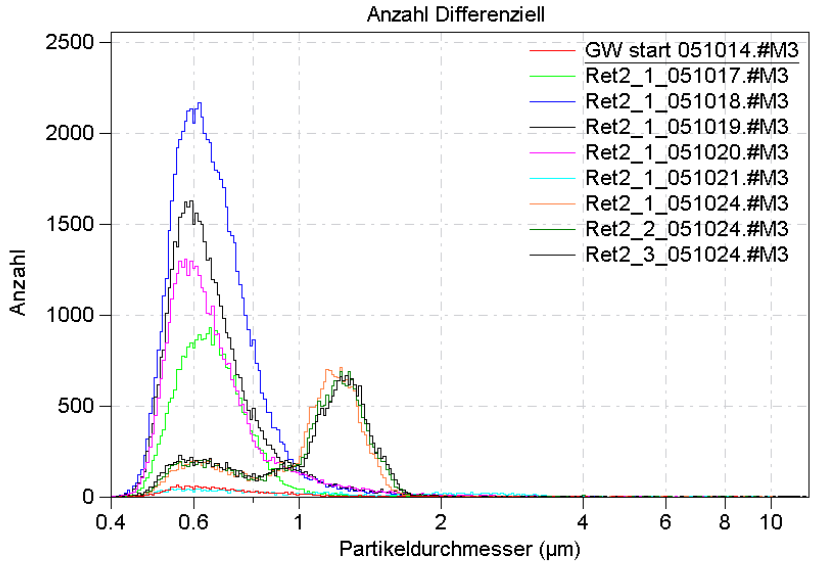


**Figure 42: Equilibria pH/ $CO_2$**

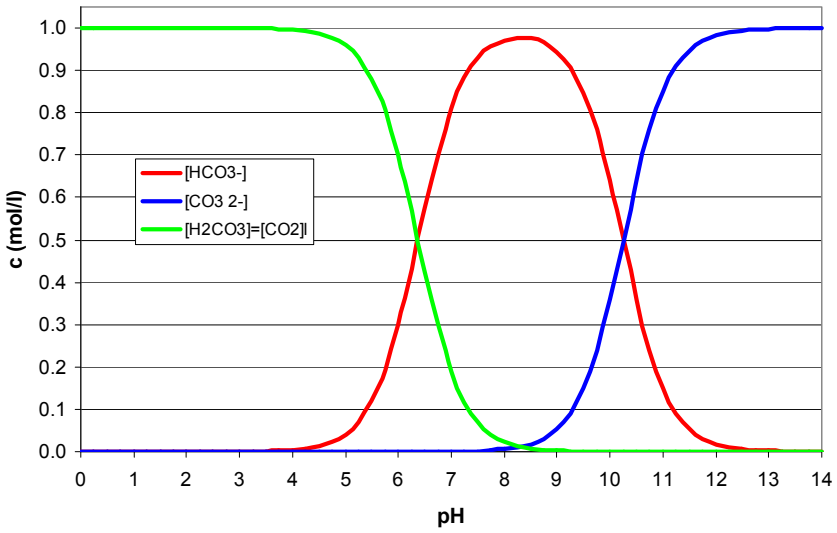
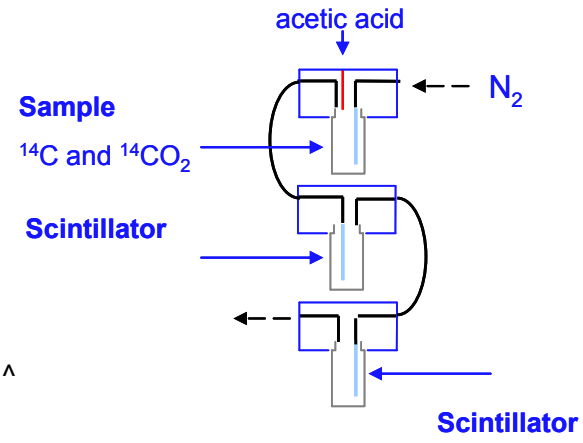



**Figure 43: Separation of dissolved $^{14}C$ and dissolved $^{14}CO_2$**

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