

TEXTE

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Final report

Determination of log Koc values according to OECD TG 106

For 1H-benzotriazole (CAS: 95-14-7), 4-methyl- (CAS: 29878-31-7) and 5-methyl-benzotriazole (CAS: 136-85-6)

by:

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On behalf of the German Environment Agency

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Abstract: Determination of log K_{oc} values according to OECD TG 106

Since April 20, 2023, the CLP Regulation (EC No 1272/2008) has introduced the new hazard classes PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile), to label substances that can cause (very) long-lasting and diffuse contamination of water resources. As part of the PMT/vPvM criteria, the determination of the mobile (M) and very mobile (vM) properties is based on the adsorption coefficient between the substance and the organic fraction of the soil (log K_{oc}). In this study log K_{oc} values for 1H-benzotriazole (CAS: 95-14-7), 4-methyl-benzotriazole (CAS: 29878-31-7) and 5-methyl-benzotriazole (CAS: 136-85-6) were derived following the OECD TG 106 test method. This method is designed to determine the soil adsorption coefficients of a substance for soils with a varying range of organic carbon content, clay content, soil texture, and soil pH. In this study Tier 1 ("Preliminary Study") and Tier 2 ("Screening Test") of the OECD TG 106 test protocol were conducted.

Among the five tested soils, the lowest derived log K_{oc} values ranged from 1.51 to 2.85 for 1H-benzotriazole, 1.39 to 2.52 for 4-methyl-benzotriazole and 1.91 to 2.72 for 5-methyl-benzotriazole. The average log K_{oc} for the soil with the lowest adsorption was 1.55±0.03 (lowest value 1.51), 1.48±0.07 (lowest value 1.39) and 1.96±0.03 (lowest value 1.91) for 1H-benzotriazole, 4-methyl-benzotriazole and 5-methyl-benzotriazole, respectively. Due to high matrix effects leading to low analytical recovery rates it was decided to reanalyse selected soil samples under the use of a substance specific internal standard. Slightly higher results for the log K_{oc} of the soil with the lowest adsorption were obtained at 2.12±0.09 (lowest value 2.01), 2.21±0.05 (lowest value 2.15) and 2.36±0.06 (lowest value 2.30), respectively.

In this study, there is no clear indication that the log K_{oc} of all three substances is affected by soil pH, which ranged from 4.6 to 7.8. Therefore, based on the lowest derived log K_{oc} values determined using the substance specific ISs, all three substances should be classified as mobile (M).

Kurzbeschreibung: Bestimmung des Log K_{oc} nach OECD TG 106

Seit dem 20. April 2023 werden nun Stoffe mit PMT/vPvM-Eigenschaften systematisch im regulatorischen Rahmen der Verordnung (EG) Nr. 1272/2008) – kurz CLP-Verordnung – adressiert. Die Bestimmung der Mobilität der getesteten organischen Stoffe (M und vM Kriterium) basiert hierbei auf dem Verteilungskoeffizient zwischen Stoff und dem organischen Anteil des Bodens (log K_{oc}). In dieser Studie wurden die log K_{oc}-Werte für die Stoffe 1H-Benzotriazol (CAS: 95-14-7), 4-Methyl-Benzotriazol (CAS: 29878-31-7) und 5-Methyl-Benzotriazol (CAS: 136-85-6) gemäß der OECD TG 106 Testmethode ermittelt, welche in der Leitlinie zur Anwendung der CLP-Kriterien vorgeschlagen wird.

Die Testprozedur sieht die Bestimmung der Bodenadsorptionskoeffizienten einer Testsubstanz auf unterschiedlichen Bodentypen mit variierendem organischem Kohlenstoffanteil, Tongehalt, Bodentextur sowie Boden-pH-Wert vor. In dieser Studie wurden Tier 1 („Vorstudie“) und Tier 2 („Screening-Test“) des Testprotokolls durchgeführt.

Der jeweils niedrigste abgeleitete log K_{oc}-Wert der fünf untersuchten Böden lag für 1H-Benzotriazol zwischen 1,51 und 2,85, für 4-Methyl-Benzotriazol zwischen 1,39 und 2,52 und für 5-Methyl-Benzotriazol zwischen 1,91 und 2,72.. Der durchschnittliche und reproduzierbare log K_{oc} für den Boden mit der niedrigsten Adsorption betrug jeweils 1,55±0,03 (niedrigster Wert 1,51), 1,48±0,07 (niedrigster Wert 1,39) und 1,96±0,03 (niedrigster Wert 1,91) für 1H-Benzotriazol, 4-Methyl-Benzotriazol und 5-Methyl-Benzotriazol.

Aufgrund starker Matrixeffekte, die zu niedrigen Wiederfindungsraten führten, wurde entschieden, den Boden mit der geringsten Sorption unter Verwendung eines isotope markierten internen Standards erneut zu analysieren. Hierbei wurden höhere

Wiederfindungsraten sowie leicht höhere log K_{oc} Ergebnisse von jeweils $2,12 \pm 0,09$ (niedrigster Wert 2,01), $2,21 \pm 0,05$ (niedrigster Wert 2,15) und $2,36 \pm 0,06$ (niedrigster Wert 2,30) ermittelt.

Für alle drei Substanzen in dieser Studie gibt es keine eindeutigen Hinweise darauf, dass der Log K_{oc} durch den Boden-pH-Wert beeinflusst wird, der zwischen 4,6 und 7,8 lag. Basierend auf den in dieser Studie abgeleiteten, niedrigsten log K_{oc}-Werten unter Verwendung des isotonen-markierten internen Standards, sollten alle drei Testsubstanzen als mobil (M) angesehen werden.

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List of abbreviations

Abbreviation	Explanation
4-MB	4-methyl-benzotriazole
5-MB	5-methyl-benzotriazole
BTZ	1H-benzotriazole
C^{ads}_{aq} (eq.)	Concentration in the water phase at equilibrium conditions
C^{ads}_s (eq.)	Concentration in the soil phase at equilibrium conditions, calculated based on C ^{ads} _{aq} (eq.)
C^{ads}_s measured	Concentration in the soil phase at equilibrium conditions, measured.
C_w	Concentration measured in the water phase
C_s	Concentration in the soil phase calculated based on C _w
CEC	Effective Cation Exchange Capacity []
CLP	Classification, labelling and packaging of substances
f_{oc}	Fraction of the total organic carbon content in the soil [-]
IS	Internal Standard
K_d	Soil-water partitioning coefficient [L/Kg]
K_{oc}	Organic carbon-water partitioning coefficient [L/Kg]
L/S ratio	Liquid to Solid ratio
L_2.1	Standard Soil LUFA 2.1 (soil sample used for testing)
L_2.4	Standard Soil LUFA 2.4 (soil sample used for testing)
LOD	Limit of Detection
LOQ	Limit of Quantification
m₀	Mass of the test substance at the beginning of each batch test
m_E	Total mass of test substance extracted from the soil
m_s^{ads}	Mass of the test substance adsorbed on the soil
n	Sample size
N15	Stiff clay (soil sample used for testing)
N25	Silty intermediate clay, Humus (soil sample used for testing)
OECD	Organisation for Economic Co-operation and Development
RR	Analytical Recovery Rate
TOC	Total Organic Carbon Content [%]
TS	Dry clust clay (soil sample used for testing)
V_{rec}	Mass of water recovered after centrifugation
V_{inj}	Injection volume into the LC-MS-MS

Summary

Since April 20, 2023, the CLP Regulation (EC No 1272/2008) has introduced the new hazard classes PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile), to label substances that can cause (very) long-lasting and diffuse contamination of water resources. As part of the PMT/vPvM criteria, the determination whether a substance is mobile (M) or very mobile (vM) is based on the adsorption coefficient between the substance and the organic fraction of the soil (K_{oc}). Substances are considered M when the lowest derived log K_{oc} value for pH between 4 and 9 is less than 3, and vM when it is less than 2 (CLP - Sections 4.4.2.1.2 and 4.4.2.2.2)

In this study log K_{oc} values for 1H-benzotriazole (CAS: 95-14-7), 4-methyl- (CAS: 29878-31-7) and 5-methyl-benzotriazole (CAS: 136-85-6) were derived following the OECD TG 106 test method. This method is designed to determine the soil adsorption coefficients of a substance for soils with a varying range of organic carbon content, clay content, soil texture, and soil pH. In this study Tier 1 (“Preliminary Study”) and Tier 2 (“Screening Test”) of the OECD test protocol were conducted for the test substances.

In Tier 1 three different (1:1, 2:1 and 5:1) liquid to solid ratios (L/S ratios) were tested on 2 selected soils to determine optimal batch test conditions and to verify that the analytical approach can detect the substances in both the water and soil phases. In Tier 2 the test substances were applied at one concentration on five different soils and sampled at 4 different time steps (6, 12, 24 and 48 h) to evaluate the adsorption kinetics. The time steps were chosen based on the expected low sorption behaviour of the test substances and ensure that sorption processes reach equilibrium in the batch test setup.

The soils tested, were selected based on their variable total organic carbon content (TOC), soil pH and clay content according to the recommendation in OECD TG 106. Hereby, the analysis of the samples was carried out using LC-MS/MS measurement. The determination of 4-methyl-benzotriazole, 5-methyl-benzotriazole, and 1H-benzotriazole was achieved using reverse phase chromatography (RP). For quality assurance, blank samples and control samples were analysed to rule out any cross contamination in the test procedure. Furthermore, analytical recovery rates were calculated to identify matrix effects.

For 1H-benzotriazole the lowest derived log K_{oc} values for all test soils range from 1.51 to 2.85. The sorbed fraction varies widely between the different soils and equilibration times, which could be explained by high matrix effects in combination with low analytical recovery rates using atrazine-d5 as an internal standard. For 4-methyl-benzotriazole the lowest derived log K_{oc} values for all test soils range from 1.39 to 2.52. Adsorption equilibrium conditions are reached for all soils after ≥12 h. For 5-methyl-benzotriazole the minimal derived log K_{oc} for all test soils range from 1.91 to 2.72. Adsorption equilibrium conditions are reached for all soils after ≥24 h. The average log K_{oc} for the soil with the lowest adsorption is 1.80±0.16 (lowest value 1.61), 1.48±0.07 (lowest value 1.39) and 1.96±0.03 (lowest value 1.91) for 1H-benzotriazole, 4-methyl-benzotriazole and 5-methyl benzotriazole, respectively using atrazine-d5 as internal standard. As an additional quality assurance step, the soil with the lowest average log K_{oc} value using atrazine-d5 were reanalysed using a different, substance-specific internal standard. Due to higher recovery rates and normative less matrix effects this data set was used for the mobility classification. The lowest derived log K_{oc} values were hereby 2.01, 2.15 and 2.30 for 1H-benzotriazole, 4-methyl-benzotriazole and 5-methyl benzotriazole, respectively.

For all three test substances there is no clear indication that the log K_{oc} is affected by soil pH in this study, which ranged from 4.6 to 7.8. When considering the lowest log K_{oc} value measured using the more accurate substance-specific internal standards, according to the criteria set out

in the CLP regulation (EC No. 1272/2008 - CLP, 2008), all three test substances should be considered to be mobile (M).

Zusammenfassung

Seit dem 20. April 2023 werden nun Stoffe mit PMT/vPvM-Eigenschaften systematisch im regulatorischen Rahmen der Verordnung (EG) Nr. 1272/2008) – kurz CLP-Verordnung – adressiert. Die Bestimmung der Mobilität der getesteten organischen Stoffe (M und vM Kriterium) basiert hierbei auf dem Verteilungskoeffizient zwischen Stoff und dem organischen Anteil des Bodens (log K_{oc}). Hierbei werden alle ionisierbaren Stoffe als M (mobil) eingestuft, wenn der niedrigste abgeleitete Log K_{oc}-Wert für pH-Werte zwischen 4 und 9 weniger als 3 beträgt und als vM (sehr mobil) wenn dieser weniger als 2 beträgt (CLP - Abschnitt 4.4.2.1.2 und 4.4.2.2.2).

In dieser Studie wurden die log K_{oc}-Werte für die Stoffe 1H-benzotriazol (CAS: 95-14-7), 4-Methyl-Benzotriazol (CAS: 29878-31-7) und 5-Methyl-Benzotriazol (CAS: 136-85-6) gemäß der OECD TG 106 Testmethode ermittelt, welche in den Leitlinien zur Anwendung der CLP-Kriterien vorgeschlagen wird.

Die Testmethode dient der Bestimmung der Bodenadsorptionskoeffizienten von Substanzen anhand von Böden mit unterschiedlichen organischen Kohlenstoffgehalten, Tongehalten, Bodenstrukturen und pH-Werten. In dieser Studie wurden Tier 1 („Voruntersuchung“) und Tier 2 („Screening-Test“) des Testprotokolls für die Testsubstanzen durchgeführt.

In Tier 1 wurden drei verschiedene (1:1, 2:1 and 5:1) Flüssig-zu-Feststoff-Verhältnisse (L/S-Verhältnisse) an zwei ausgewählten Böden getestet, um optimale Batch-Testbedingungen zu ermitteln und zu überprüfen, ob das ausgewählte analytische Verfahren alle vier Testsubstanzen sowohl in der Wasser- als auch in der Bodenphase nachweisen kann. In Tier 2 wurden die Testsubstanzen mit konstanter Konzentration an fünf verschiedenen Böden und zu vier unterschiedlichen Zeitintervallen (6, 12, 24 und 48 Stunden) getestet, um die Adsorptionskinetik zu validieren und sicherzustellen, dass die Sorptionsprozesse im Batchsystem Gleichgewichtsbedingungen erreichen. Die getesteten Böden wurden aufgrund ihres variablen Gesamtgehalts an organischem Kohlenstoff (TOC), pH-Wert und Tongehalt gemäß den Empfehlungen der OECD TG 106 ausgewählt. Die Analyse der Proben erfolgte mittels LC-MS/MS-Messung. Die Bestimmung von 4-Methyl-Benzotriazol, 5-Methyl-Benzotriazol und 1H-Benzotriazol wurde mittels Umkehrphasen-Chromatographie (reverse phase, RP) erreicht. Zur Qualitätssicherung wurden Blindproben und Kontrollproben analysiert, um Kreuzkontaminationen im Testverfahren auszuschließen. Darüber hinaus wurden die Wiederfindungsraten aller Testsubstanzen dokumentiert, um ggf. auftretende Matrixeffekte zu identifizieren.

Für 1H-Benzotriazol liegen die niedrigsten, abgeleitete log K_{oc} (L/Kg) für alle getesteten Böden zwischen 1,51 und 2,85. Der sorbierte Anteil variiert stark zwischen den Böden und den Testzeitpunkten. Eine Erklärung hierfür könnten hohe Matrixeffekte in einigen Boden-Wasser-Gemischen in Verbindung mit niedrigen Wiederfindungsraten des internen Standards sein. Für 4-Methyl-Benzotriazol liegt der niedrigste abgeleitete log K_{oc} (L/Kg) aller Testböden zwischen 1,39 und 2,52. Das Sorptionsgleichgewicht wurde für alle Böden nach ≥12 Stunden erreicht. Für 5-Methyl-Benzotriazol liegen die niedrigsten abgeleiteten log K_{oc} (L/Kg) für alle Testböden zwischen 1,91 und 2,72. Das Sorptionsgleichgewicht wurde für alle Böden nach ≥24 Stunden erreicht. Der durchschnittliche log K_{oc} für den Boden mit der niedrigsten Adsorption beträgt jeweils 1,80 ±0,16 (niedrigster Wert 1,61), 1,48±0,07 (niedrigster Wert 1,39) und 1,96±0,03 (niedrigster Wert 1,91) für 1H-Benzotriazol, 4-Methyl-Benzotriazol und 5-Methyl-Benzotriazol. Als zusätzliche Qualitätssicherungsmaßnahme wurde der Boden unter Verwendung eines anderen, substanzspezifischen internen Standards erneut analysiert. Aufgrund höherer Wiederfindungsraten und normativ geringerer Matrixeffekte wurde dieser Datensatz für die

Mobilitätsklassifizierung verwendet. Die niedrigsten abgeleiteten log K_{oc}-Werte betrugen hierbei 2,01 für 1H-Benzotriazol, 2,15 für 4-Methyl-Benzotriazol und 2,30 für 5-Methyl-Benzotriazol.

Die Ergebnisse für alle drei Substanzen legen den Schluss nahe, dass eine Veränderung des Boden-pH-Wertes (4,6 bis 7,8) das Sorptionsverhalten und damit auch die ermittelten log K_{oc} Werte in dieser Studie nicht stark beeinflusst haben. Basierend auf den in dieser Studie abgeleiteten minimalen log K_{oc}-Werten unter Verwendung der substanzspezifischen internen Standards, sollten alle drei Testsubstanzen als mobil (M) eingestuft werden.

1 Introduction

In recent years, research on water pollution has indicated that there is an increasing threat from persistent, mobile, and toxic substances (PMT), as well as very persistent and very mobile substances (vPvM) (Arp, Hale, Schliebner, et al., 2023; Arp & Hale, 2022). These substances persist in the environment and are polar enough to exhibit high mobility in water. This enables them to spread through the water cycle, posing a threat to both the environment and human health, particularly if they are emitted in substantial enough volumes to contaminate drinking water (Arp, Hale, Borchers, et al., 2023)

Since April 20, 2023, the European Union's regulation on the Classification, Labelling, and Packaging of Substances and Mixtures (Regulation (EC) No 1272/2008), commonly referred to as the CLP Regulation, includes the new hazard classes PMT (persistent, mobile, toxic) and vPvM (very persistent, very mobile). Substances meeting the criteria for classification in this hazard class (PMT and vPvM properties) shall be labelled accordingly, so that it is clear to manufacturers, importers and downstream users that these substances can cause (very) long-lasting and diffuse contamination of water resources (European Commission (2022) Commission Delegated Regulation (EU) 2023/707 of 19 December 2022 Amending Regulation (EC) No. 1272/2008 as Regards Hazard Classes and Criteria for the Classification, Labelling and Packaging of Substances and Mixtures, 2022). According to the CLP criteria, whether a substance is considered mobile (M) or very mobile (vM) is based on the distribution coefficient between the substance and the organic fraction of the soil (K_{oc}). Substances are considered M when the lowest derived log K_{oc} value for a pH between 4 and 9 is less than 3, and vM when it is less than 2 (CLP - Sections 4.4.2.1.2 and 4.4.2.2.2)

The OECD Test Guideline 106 (OECD TG 106) is a standardized method for assessing the adsorption and desorption behaviour of chemicals in soil. The test is designed to determine the soil adsorption coefficients (e.g. K_d and K_{oc}) of a chemical on different soil types with a varying range of organic carbon content, clay content, soil texture and soil pH (OECD, 2000).

The OECD TG 106 Guideline comprises hereby of the following three tiers.

- ▶ Tier 1 ("Preliminary Study"): Batch tests are conducted to determine the optimal soil/solution ratio and the mass of each substance needed to ensure that the analytical setup can detect the substances in both the water and soil phases.
- ▶ Tier 2 ("Screening Test"): The test substance is tested at one concentration on different soils. Batch tests are conducted at different time points to validate adsorption kinetics and ensure that the adsorption and desorption processes reach equilibrium in the batch test setup.
- ▶ Tier 3 ("Determination of Freundlich adsorption isotherms"): Batch tests with different concentrations of the substance under investigation are conducted to determine the influence of the water concentration on the extent of adsorption onto the soil.

This report presents the determination of the log K_{oc} values for the following three substances, according to OECD TG 106:

- ▶ 1H-benzotriazole (CAS: 95-14-7, EC: 202-394-1)
- ▶ 4-methyl-benzotriazole (CAS: 29878-31-7, EC: 249-921-1)
- ▶ 5-methyl-benzotriazole (CAS: 136-85-6, EC: 205-265-8)

In this study Tier 1 and Tier 2 are carried out.

2 Materials and Methods

2.1 Substance properties and spiking procedure

The three test substances, 4-methyl-benzotriazole (4-MB) (purity: 90%) (CAS 29878-31-7), 5-methyl-benzotriazole (5-MB) (purity: 98%) (CAS 136-85-6) and 1H-benzotriazole (BTZ) (purity: 99 %) (CAS 95-14-7) were obtained from Sigma-Aldrich (Seelze, Germany). Their physico-chemical properties are shown in Table 1.

Table 1: Chemical and physiochemical parameters for each test substance

Substance	Molecular weight [g/mol]	Solubility in water [g/L]	Vapour pressure at 25°C [mmHg]	Henry's law constant (atm·m ³ /mol)	Log K _{ow} [-]	pK _a [-]
BTZ	119.13 ^{a)}	1 to 5 ^{e)}	0.0±0.8 ^{b)2)}	1.47E-07 ^{c)2)}	1.44 ^{c)1)}	8.37 ^{e)3)}
4-MB	133.15 ^{a)}	3.1 ^{c)2)}	0.0±0.8 ^{b)2)}	1.62E-07 ^{c)2)}	1.71 ^{c)2)}	8.85 ^{d)2)}
5-MB	133.15 ^{a)}	3.1 ^{c)2)}	0.0±0.8 ^{b)2)}	1.62E-07 ^{c)2)}	1.71 ^{c)2)}	9.15 ^{d)2)}

1) experimental values, 2) modelled/calculated values 3) handbook data

a) ChemSpider database

b) ACD/Labs Percepta Platform – PhysChem Module

c) EPI Suite – using WSKOW 1.41 for solubility in water and KOWWIN 1.67 for log K_{ow}

d) ECHA CHEM Database (predicted by SPARC)

e) PubChem database

Based on Hofman-Caris and Claßen (2020), the biodegradability for 1H-benzotriazole is (very) low in the environment (Hofman-Caris & Claßen, 2020). Based on a study investigating the degradation of Tolyltriazole (which is a technical mixture of 4-MB and 5-MB) in surface water (OECD 309) it can be shown that neither 4-MB nor 5-MB was degraded in water during 62 days of incubation (ECHA, 2024). Abiotic hydrolysis and direct photolysis are not expected to be a relevant degradation pathway for 1H-benzotriazole and structurally similar substances under environmentally relevant conditions in the laboratory (Janssen et al., 2015).

The three substances received from Sigma-Aldrich were in powder/crystal form. Individual substance spikes were prepared using a 0.01M CaCl₂ solution (Milli-Q water). All substances were first dissolved in methanol (MeOH) to create individual spiking solutions, which were then used to spike the 0.01M CaCl₂ solutions at concentrations of 10 µg/mL for direct use in the latter batch test set up. The final concentration of MeOH in these spiking solutions was maintained at less than 0.1%. After preparation, the standards were stored in a closed box at 4 °C until further use. The spiking solutions were analysed alongside the water samples to verify the concentrations used in the batch tests and to confirm the stability in the spiking solution.

2.2 Soil characteristics and preparation

The soils used in this study were collected from two regions: 1) The southern part of Norway and; 2) Lower Saxony in Germany. Sample locations and land use patterns for each soil are summarized in Table 2. Except for the dry crust clay (TS), which was collected in a commercial area by sampling below the topsoil layer (0.5 to 3 m), the rest of the soil samples were taken at agricultural areas from the topsoil layer (depth of 0 to 0.2 m).

Table 2: Selected soils for the planned sorption tests

Acronym	Name	Soil type	Sample location (ETRS 89)	Date of sampling	Use pattern / Depth [m]
TS	dry crust clay	clay	59°55'29.08"N 10°48'44.48"E	05.2023	commercial area/ 0.5-3
N25	silty intermediate clay, humus	clay with high organic content	59° 39' 38.7" N 10° 44' 57.4" E	2013	agricultural/ 0-0.2
N15	stiff clay	clay	59° 19' 11.7" N 11° 02' 09.7" E	2005	agricultural/ 0-0.2
L_2.1	standard soil LUFA 2.1	silty sand	49°19'6.5136"N 8°23'0.6504"E	04.2022	agricultural/ 0-0.2
L_2.4	standard soil LUFA 2.4	silt	49°7'53.1768"N 8°19'55.5636"E	03.2022	agricultural/ 0-0.2

According to the recommendation in Table 1 from OECD TG 106 the soils were selected based on their variable total organic carbon content (TOC), soil pH and clay content. The characteristic of each soil is summarized in Table 3.

Table 3: Soil parameters for the selected soils

Acronym	Sand Content [%]	Silt Content [%]	Clay Content [%]	SOM ^{a)} [%]	TOC ^{b)} [%]	Soil pH ^{c)} [-]	CEC [cmol/100g]
TS	1.0	54.0	45.0	3.15	0.40	7.8	<0.050 ^{d)}
N25	17.0	57.0	26.0	36.0	20.40	5.6	0.967 ^{d)}
N15	11.0	47.0	42.0	4.4	1.41	6.1	0.238 ^{d)}
L_2.1	87.8	8.60	3.60	-	0.66	4.6	<0.050 ^{d)} / 2.9±0.2 ^{e)}
L_2.4	32.8	43.4	23.8	-	1.70	7.5	17.2±0.5 ^{e)}

a) Determination of Loss on Ignition by weight difference at 550°C (DIN EN 15934)

b) Determination of TOC by combustion method using IR (ISO 10694)

c) Determination of soil pH electrochemically in soil - 0.01 M CaCl₂ suspension (DIN EN 15933 or VDLUFA MB 1, A5). The soil pH of the 1:1/5:1 L:S system after 12 h of equilibration time was checked with the pH measured using the named methods, and the values agreed for all soils except L2.1 which had a higher pH of 0.6.

d) Determination of Effective CEC and Base Saturation Level Using Barium Chloride Solution (ISO 11260) measuring Al.

e) Determination of Effective CEC following DIN 19684:1973-1

Before being used in the batch tests, the soils were oven-dried at a temperature slightly higher than the ambient temperature (<40 °C) to ensure an effective drying process for the soils with high water content. Changes of soil parameters were not to be expected at these low temperatures.

In case aggregates formed during the drying process they were gently crushed before sieving in order to not change the original texture of the soil. The soil was then sieved through a 2 mm mesh and collected in a polypropylene (PP)-bucket, which was swirled several times for soil homogenization. The soils were stored at 4 °C prior further usage.

2.3 Adsorption test

All batch adsorption tests were performed according to OECD TG 106 at the Norwegian Geotechnical Institute AS in Oslo, Norway. Water and soil samples generated from these tests were sent in cooling boxes overnight to Hochschule Fresenius, the University of Applied Sciences, Idstein Germany. There the samples were analysed for the different test substances. Details are provided in section 2.4.

2.3.1 Tier 1

The preliminary study (OECD TG 106 - Tier 1), conducted to determine the optimal liquid-to-solid ratio (L/S ratio) and spiked mass needed to ensure reliable analytical detection, was performed using BTZ. The selection of the appropriate L/S ratio depends on the expected soil-water distribution coefficient (K_d) and the requested relative degree of adsorption, which is based on the limit of detection (LOD) and limit of quantification (LOQ) of the analytical method used. BTZ was used as it is the most mobile of the three benzotriazole substances investigated, and therefore low sorption to soil was expected. For BTZ the expected log K_{oc} value was 1.5 (Arp et al., 2023). The maximum expected log K_{oc} for 5-MB was 2.0 (Hart et al., 2004), which was also expected to be in a similar range for 4-MB. Following the requirements of OECD TG 106 the percentage of a substance adsorbed to the soil should be at least >20%. The Tier 1 study was conducted on two of the five test soils for a single time period. The time period used was 48 h, during which an achievement of equilibrium conditions was expected for BTZ, and which is also the longest time step recommended by OECD TG 106. The soil selection was based on the OECD TG 106 recommendations. Accordingly, one soil with a high total organic carbon (TOC) content and low clay content, and another soil with low TOC and high clay content were selected. The soil moisture content was determined for each of the stored soils shortly before usage in the batch tests. In short, 10 g of soil were taken and heated at 105 °C until no significant change in weight could be observed anymore (approx. 12 h). For all calculations the mass of soil refers to oven dry mass, i.e. the weight of soil corrected for moisture content.

The batch tests were set up using 50 mL Falcon® conical centrifuge tubes and Milli-Q water containing 0.01 M CaCl₂. Before the test substance was added to the vials, the dried and sieved soil was shaken with CaCl₂ solution overnight (>12 h) to ensure soil-water equilibration. In addition to the soil pH measurements following DIN EN 15933/VDLUFA MB 1, A5 the soil pH of each soil was measured in duplicates after 12 h of soil-water equilibration using an Ino Lab pH Level 2 system from WTW (Table 3). Afterwards, each substance was added to the vials using the individual produced spiking solutions. The added volume did not exceed 10% of the final volume of the aqueous phase as stated in OECD TG 106 (paragraph 34.). Each vial was spiked with only one substance. The intended spike concentrations ranged from 1 to 5 µg/mL depending on the substance tested, to ensure clear identification in the chromatogram. As required by OECD TG 106, three different liquid-to-solid ratios (L/S) were tested in duplicates. Given the expected low sorption of the tested substances, the batch tests were set up at normalized L/S ratios of 1:1 (10 g of soil and 10 mL of 0.1 M CaCl₂ solution), 2:1 (10 g of soil and 20 mL of 0.1 M CaCl₂ solution), and 5:1 (4 g of soil and 20 mL of 0.1 M CaCl₂ solution). To reach sorption equilibrium between the water and solid phase, the vials as duplicates were placed on

an overhead shaker for 48 h This was followed by centrifugation (30 min, 4,800 rpm) and the removal of the supernatant water phase. All experiments were conducted at a laboratory temperature of 22.5±0.5 °C.

2.3.2 Tier 2

For the kinetic tests at one concentration (Tier 2), batch tests in duplicates with different contact times (6, 12, 24, and 48 h in parallel mode) were conducted. This was done for each of the three test substances and five soils, thereby following the same protocol as described above at one concentration. The L/S ratio was selected based on the Tier 1 results (see paragraph 2.3.1 Tier 1). Batch tests already performed in Tier 1 were not repeated. The distribution coefficients were determined based on the concentration of the substances in the water phase (indirect method).

2.4 Sample Preparation and LC-MS/MS Analysis

The analysis of the samples was carried out using liquid chromatography tandem mass spectrometry (LC-MS/MS) measurement. The separation of 4-MB, 5-MB, and BTZ was achieved using reversed-phase chromatography (RP). An ACQUITY UPLC T3 column (100 Å, 1.8 µm, 2.1 mm × 50 mm, Waters, Eschborn) was used for RP. The measuring devices were LC-MS/MS systems (LC: Nexera UPLC (Shimadzu), MS: QTrap® 5500 (Sciex) and LC: Nexera HPLC (Shimadzu), MS: 3200 QTrap® (Sciex)).

For separation the following method parameters were used: For the determination of 4-MB, 5-MB, and BTZ, Eluent A consisted of 95% MilliQ-H₂O and 5% acetonitrile with 5 mmol NH₄-formate buffer, and Eluent B consisted of 5% MilliQ-H₂O and 95% acetonitrile with 5 mmol NH₄-formate buffer. Both eluents were adjusted to a pH of 3 using formic acid. Two transitions per substance were measured and evaluated, one serving as a quantifier and the other one serving as a qualifier to confirm the results. For both, the MS QTrap® 5500 and the MS 3200 QTrap® quantification was performed via an external calibration for each target analyte. To ensure accuracy and precision, a 7-point calibration curve with increasing concentrations (0.002 to 0.05 µg/ml) was used. If needed, samples were diluted to ensure that all concentrations fell within the range of the calibration curve. In addition, quality control standards were measured alongside the samples to ensure consistency in the results for BTZ, 4-MB and 5-MB.

Thereby, quantification was verified by spiking every fourth sample with the respective analyte to determine the recovery rate. Furthermore, an internal standard (IS) was added to every sample to correct for systematic and random errors that might be introduced in further sample processing steps or during the measurement. Hereby the signal of the analyte was divided by the signal of the IS. The spiking solution as well as the IS were added after filtration of the sample. The analytical recovery rate (RR) in water samples (water RR) and in the soil extracts (soil RR) for each substance was calculated using the following equation:

$$RR = \frac{C_{\text{spiked sample}} - C_{\text{sample}}}{C_{\text{spike}}} \quad \text{eq.1}$$

Where $C_{\text{spiked sample}}$ equals the concentration of the analyte in the spiked sample, C_{sample} is the concentration of the analyte in the non-spiked sample and C_{spike} is the concentration of the spike added. A high analytical recovery rate can result from either a lower signal from the IS, a higher signal from the analyte, or both. Conversely, the same applies to low analytical recovery rates. Atrazine-d₅ (C₈H₉D₅ClN₅), a structural analogue, was used as IS for all three test substances. It was chosen due to its similar structure, which renders it likely to behave in a similar way, with a

similar retention time, peak shape and response. Additionally, carbamazepine-D10 was spiked into all samples and considered a structural analogue IS, but it was not used for quantification.

The sample preparation steps were different for water and soil samples. Water samples were first vortexed for 5 sec, followed by filtration using ROTILABO® regenerated cellulose 0.2 µm syringe filters (Carl Roth, Germany). Samples were diluted based on estimated concentrations (dilution factor 10 to 100). Hereby, 10 to 100 µL of the samples were mixed with Mili-Q water (Type 1, electrical resistance of 18.2 MΩcm at 25 °C) and fortified with 10 µL of IS (atrazine-d5, 5 µg/mL).

For the spiked water samples (used for recovery calculations) additionally 10 µL of spiking solution (1 µg/mL) for all three substances were added to the sample prior to analysis.

Soil samples were first dried for 48 h at 80 °C. In a second step 0.50±0.01 g of dried soil was extracted using 3 mL of MeOH. The soil-solution was then vortexed for 2 min and centrifuged at 3,000 rpm for 4 min. Following, the solution was then treated equal to the water samples described before. Each analysed soil sample was fortified with IS to correct for errors and some of the samples were spiked with the analytes to calculate analytical recovery rates.

Selected chromatograms for each test substance in Tier 1 and Tier 2 can be found in the appendix (A.1.1 and A.2.3 and A.2.5).

2.5 Quality Assurance

Analytical QA/QC protocols were implemented during all experimental and analytical steps. The validation of the analytical results was based on the matrix-dependent LOD and LOQ, as well as the recovery rate of the respective substances.

Following OECD TG 106 recommendation, blank samples (soil and 0.01 M CaCl₂ solution) and control samples (substance + 0.01 M CaCl₂ solution) were included in the experimental set up. Blank samples were used to exclude any background concentration of the test substances and to check for possible matrix effects caused by the soil. Control samples were used to validate the stability of the test substance in the 0.1 M CaCl₂ solution and check for possible adsorption on the surfaces of the 50 mL Falcon® Tubes. All blank and control samples went through the same experimental set up as all regular samples, including shaking, centrifuging and all other sample preparation steps necessary for further analysis.

To verify the mass balance (MB), soil samples from selected batch tests were additionally solvent-extracted at HSF to quantify the solid-phase concentration in the soil. The concentrations of the three substances adsorbed to the soil were then analytically determined in the soil extracts. All water and soil samples were stored at 4 °C prior to analysis.

2.5.1 Quality improvement measures

In Tier 2, unexpectedly low recovery rates for the IS atrazine-d5 were observed. It was hypothesized that this is due to strong matrix effects from the soils used. As a first approach for BTZ in Tier 2, a lower injection volume (5 µL) was tested because of the observed high matrix effects and consequently low analytical recovery rates. This adjustment led to slightly improved recovery rates. In cases where the recovery was better with the 5 µL injection, those results were used.

In a second step, selected water samples were reanalysed using more specific ISs as an additional quality assurance measure (1H-benzotriazole-D4 for BTZ, 4-methyl-1H-benzotriazole-D3 for 4-MB, and 5-methyl-1H-benzotriazole-D6 for 5-MB). Due to budget and

time constraints, only selected water samples, and no soil samples, were reanalysed during this additional quality control step. For the selected samples reanalysed using the more specific ISs, which were less affected by matrix effects, the standard injection volume of 20 µL was used.

2.6 Data and Reporting

For each batch test the soil-water-distribution coefficient (K_d value [L/Kg]) was calculated as the ratio of the concentration of the test substance in the soil phase ($C_{ads_s}^{(eq)}$ [ug/g]) and the concentration in the water phase ($C_{ads_{aq}}^{(eq)}$ [ug/mL]) at equilibrium conditions (eq.2):

$$K_d = \frac{C_s^{ads} (eq)}{C_{aq}^{ads} (eq)} \quad \text{eq.2}$$

$C_{ads_{aq}}^{(eq)}$ was hereby measured, whereas $C_{ads_s}^{(eq)}$ was calculated as the difference between the amount of test substance initially present in solution and the amount remaining at the end of the experiment in the liquid phase (indirect method described in OECD TG 106).

Based on the recommendations of OECD TG 106 on how to derive K_d and finally K_{oc} values only batch test data were used where equilibrium conditions are met and the percentage of substance mass sorbed to the soil is above 20%.

Based on the K_d values the organic carbon-water partitioning coefficient (K_{oc} [L/Kg]) was calculated by normalizing the K_d values with the fraction of the total organic carbon content in the soil (f_{oc} [-]), as following (eq. 3):

$$K_{oc} = K_d \times f_{oc} \quad \text{eq.3}$$

To verify how much of the test substance was adsorbed to the soil, the adsorption rate A [%] was calculated for each batch test, using the following equation (eq. 4):

$$A = \frac{m_s^{ads} \times 100}{m_0} \quad \text{eq.4}$$

Where m_s^{ads} [µg] is the mass of the test substance adsorbed on the soil and m_0 [µg] equals to the mass of the test substance spiked into the water phase of each batch test at the start of the experiment.

To establish a MB, for selected batch test samples additional soil samples were analysed. Hereby, the MB is defined as the percentage of substance which can be analytically recovered after an adsorption test versus the nominal amount of substance at the beginning of the test. MB was calculated using the following equation (eq. 5):

$$MB = 100\% * \left(\frac{V_{rec} \times C_{aq}^{ads} (eq) + C_{wet\ soil}^{ads} \times M_{wet\ soil}}{m_0} \right) \quad \text{eq. 5}$$

Where V_{rec} [mL] is the mass of water recovered after centrifugation, $C_{wet\ soil}^{ads}$ [µg/g w.w] is the substance concentration in the wet soil after centrifugation, $C_{aq}^{ads} (eq)$ (ug/mL] is the concentration in the water phase at equilibrium conditions, $M_{wet, soil}$ is the mass of the wet soil [g] and m_0 [µg] equals the mass of the test substance spiked into the water phase of each batch test at the start of the experiment. $C_{wet\ soil}^{ads}$ includes the substance mass extracted from both the soil particles and the porewater, which was evaporated during the preparation of the soil sample for further extraction (section 2.4). The MB evaluation was carried out on selected batch test samples. Accordingly, the soils with the lowest and highest calculated log K_{oc} values were prioritized, and one selected soil was always analysed in duplicate. The reporting of the results is following the requirements of OECD TG 106, Annex 6 (parallel method). Boxplots and statistical analyses were conducted using Office 365. Reported values are the minimum log K_{oc} and the average log $K_{oc} \pm$ the standard derivation for each soil tested.

3 Results

3.1 Tier 1 - Preliminary study

In Tier 1 batch tests were conducted over 48 h, where the soils TS and N25 were spiked with BTZ – 4-MBT and 5-MBT were not tested during tier 1.

3.1.1 1H-benzotriazole (BTZ)

3.1.1.1 L/S ratios

For the TS soil, the adsorbed mass of BTZ to the soil increases from 32% (at a L/S of 5:1) to 76% (at a L/S of 1:1). This is similar to soil N25, which has a high organic carbon content. At an L/S of 5:1 88% are adsorbed to the soil while only 98% are adsorbed to soil at an L/S of 1:1.

The results show, that in order to achieve the goal of having more than 20% of the initial mass of BTZ adsorbed to the soil - as recommended by OECD TG 106 - and while remaining above the LOD in the water phase, a L/S ratio of 5:1 should be used for soil N25 in the batch tests of Tier 2 and a L/S ratio of 1:1 for soil TS. As the remaining soils (N15, L_2.1 and L_2.4) are relatively similar to soil TS in regard of their TOC content and hence their sorption ability, they should be tested at the same L/S ration.

3.1.1.2 Quality Assurance

For quality assurance, blank samples and control samples were analysed. The results are summarized in Appendix A.1. All 0.1 M CaCl₂ solution blanks and soil-water blanks are below LOD for BTZ. This indicates that the soils used are not contaminated with the test substances and that there is no measurable cross-contamination in both laboratories.

To verify the accuracy of the spiking procedure, the 0.1 M CaCl₂ spiking solutions used in the batch tests were analysed. The measured concentrations are 11.7 µg/mL for BTZ. The respective control samples for BTZ (1 mL spiking solution + 9 mL 0.1M CaCl₂ solution) have measured concentrations of 1.03 to 1.10 µg/mL, which corresponds to an average error of 9%. These values lie in the acceptable error range according to OECD TG 106 .

The calculated and planned spiking concentration was 20 µg/mL, meaning the analysed concentrations are approximately 40% lower than expected. The measured concentration was used for further calculations. Since a lower initial water concentration results in a lower log K_{oc}, this approach provides a conservative estimate for classifying the three substances. Additionally, the spiking solution was remeasured in Tier 2.

Obtained R² values for the 7-point calibration curve shows a linear response (R²>0.99). The LOD for BTZ was 0.00007 µg/ml and the LOQ 0.002 µg/ml.

Recovery rates of the IS (atrazine-D5) in the water samples varies depending on the soil type used in the batch test. The observed recoveries for BTZ range from 97.4% to 116% for the samples with an L/S of 2. All measured aqueous phase concentrations fall in the range of the calibration curve and thus should allow for an accurate quantification.

The analytical recovery rates for the soil extracts (soil RR) are lower compared to the RR water. For soil TS and the analysed duplicates for N25 the soil RR are 44% (sample nr. 3), 73% (sample nr.62) and 84% (sample nr.61), respectively.

3.1.1.3 Mass balance (MB)

The MB was calculated for selected batch test samples (Table 4). **Error! Reference source not found.** based on concentrations of the substance detected in the liquid (C_{aq}^{ads} , directly measured) and solid phase ($C_{wet\ soil}^{ads}$, after extraction of the wet soil). For BTZ, the mass analytically recovered ranges from 54.8±4.4% (N25) to 96.2% (TS), with a lower recovery being observed in the soil with higher total organic carbon (TOC).

Breedveld et al. (2003) identified that sorption of BTZ to soil is positively correlated with a higher soil organic carbon content. The lower analytical recovery from N25 may be due to unextracted mass of BTZ that sorbed to the organic matter in the soil, possible losses to the filter and matrix effects. The results hereby do not indicate an instability of the test substance. Due to the various potential sources of error, no corrections are made for the observed mass losses, and the data from the aqueous phase is used for further calculations.

Table 4: Mass balance (MB) for BTZ in Tier 1 using atrazine-d5 as IS (shaking time = 48h)

Soil (Water) Sample Nr.*	Soil	L/S [-]	C_{aq}^{ads} [µg/mL]	$C_{wet\ soil}^{ads}$ [µg/g w.w.]	V_{rec} [mL]	$M_{wet\ soil}$ [g w.w.]	m_0 [µg]	MB [%]	Water RR [%] in L/S = 2 samples	Soil RR [%]
3 (41)	TS	1	0.26	0.58	5.85	14.15	11.70	83.7	97.4 to 116 (n=2)	44%
23 (61) ^a	N25	5	0.13	1.17	16.26	7.74	23.40	47.8	102.0 to 110.7 (n=2)	84%
24 (62) ^{a*}	N25	5	0.12	1.27	16.02	7.98	23.39	51.6		73%
Control Samples (0.01M CaCl₂ Solution)										
31	-	-	1.03	-	10	0	11.7	88.0	Not available	-
33	-	-	1.10	-	10	0	11.7	94.0	Not available	-

Explanation of Abbreviations used:

C_{aq}^{ads} = Concentration in the water phase

V_{rec} = Volume recovered after centrifugation

M_0 = Mass of substance spiked to the batch test

Water RR = Recovery rate in water samples

Explanation of used Indexes

^a indicating duplicates

^b 5 µl of injection volume used. Soil RR increased from 36% to 73%.

$C_{wet\ soil}^{ads}$ = Soil concentration (porewater + soil)

$M_{wet\ soil}$ = Mass of wet soil after centrifugation

MB = Mass balance

Soil RR = Recovery rate in soil samples

3.1.1.4 Test results for all water samples using atrazine-d5 as IS for BTZ in Tier 1

Sample Nr.	Soil	Equilibration. time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [ug/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]
41	TS	48	1	10.00	9.91	0.09	9.01	1.00	10.10	1.01	10.18	0.26	0.74	2.9	2.85	74%
42	TS	48	1	10.00	9.91	0.09	9.02	1.00	10.11	1.01	10.18	0.23	0.77	3.3	2.91	77%
45	TS	48	2	10.00	9.91	0.09	18.01	2.00	20.10	1.01	20.36	0.40	0.61	1.54	2.59	61%
46	TS	48	2	10.00	9.91	0.09	18.00	2.00	20.09	1.01	20.36	0.41	0.59	1.43	2.55	59%
49	TS	48	5	4.00	3.96	0.04	18.01	2.01	20.06	1.02	20.46	0.68	0.33	0.5	2.09	33%
50	TS	48	5	4.00	3.96	0.04	18.00	2.00	20.04	1.02	20.36	0.72	0.30	0.4	2.02	30%
53	N25	48	1	10.00	9.62	0.38	9.01	1.01	10.40	0.99	10.28	0.02	0.98	48.5	2.38	98%
54	N25	48	1	10.00	9.62	0.38	9.00	1.00	10.38	0.98	10.18	0.02	0.98	44.9	2.34	98%
57	N25	48	2	10.00	9.62	0.38	18.01	2.00	20.39	1.00	20.36	0.05	0.95	20.5	2.00	95%
58	N25	48	2	10.00	9.62	0.38	18.00	2.00	20.38	1.00	20.36	0.05	0.95	18.2	1.95	95%
61	N25	48	5	4.00	3.85	0.15	18.02	2.00	20.17	1.01	20.36	0.13	0.87	6.6	1.51	87%
62	N25	48	5	4.00	3.85	0.15	18.01	2.00	20.16	1.01	20.36	0.12	0.88	7.5	1.56	88%

Explanation of Abbreviations used:

M_{soil} = dry weight of soil added to the batch tube, V_{ws} = Amount of water in the soil sample added to the batch tube, Vol. stock solution = Volume of stock solution added to the batch tube, V₀= Total volume of water in the batch tube, C₀= Initial spiked concentration in the water phase, M₀= Initial spiked mass in the batch tube, C_w= Measured water concentration after phase separation, C_s= Calculated soil concentration after phase separation based on the measured water concentration and the initial mass spiked, K_d= soil- water partitioning coefficient, log K_{oc}= log value of the organic carbon-water partitioning coefficient, A= Adsorption rate

3.2 Tier 2 – Screening test

To confirm that the kinetic study results for one test concentration in Tier 2 meet the OECD TG 106 recommendation, two criteria were considered: the fraction of mass adsorbed to the soil must be greater than 20%, and the sorption equilibrium must be reached. The fraction of each test substance sorbed to the five soils was plotted and displays the average of the analysed duplicates \pm standard deviation. Thereby, the calculations were performed based on the aqueous phase concentration and hence by using the indirect method to quantify the sorbed fraction. Some data points were excluded: 1) Those that revealed too low (<35%, see Figure A 4) analytical recovery rates, 2) when the adsorption processes did not reach equilibrium at the timestep and 3) when the water concentration after conducting the batch test was above the spiked concentrations of the substance. The data excluded from further calculations were hereby marked in each figure. Log K_{oc} values were derived using the representative data from Tier 1 and Tier 2 for overlapping L/S ratios. According to the CLP regulation, the lowest log K_{oc} value for each soil in the pH range between 2 and 9 is stated.

3.2.1 1H-benzotriazole (BTZ)

3.2.1.1 Quality Assurance Tier 2

For quality assurance, blank samples and control samples were analysed and the results are summarized in appendix **Error! Reference source not found.** All 0.1 M CaCl₂ solution blanks and soil-water blanks were, as in Tier 1, below the LOD for BTZ. The obtained R² values between analyte concentrations and instrument response (e.g. peak area) demonstrated a linear response (R²>0.99). The LOD and LOQ for BTZ were identical at Tier 1 (see section 3.1).

Analytical recovery rates in the water samples varied strongly depended on the soil type used in the batch test. Analytical recovery rates for BTZ ranged from 24% to 105% (with an average value of 58.7 \pm 26.3%). These values are lower than those observed during Tier 1. This could be explained by the different L/S ratio that was used in Tier 1 to derive analytical recovery rates (L/S =2). This is higher compared to the L/S ratio used in Tier 2 and thus might lead to higher matrix effects as discussed before. Sample individual analytical recovery rates for the water phase are listed in 3.2.1.5.

The spiking solution originally used in Tier 1 was re-measured at 12.36 μ g/L (sample nr. 38 - Table A 4), which was similar to the result of the first analysis (11.7 μ g/L). An additional subsample of the same spiking solution used for the log K_{oc} calculations in Tier 2 indicated a similar concentration (sample nr. 412 - Table A 4 – 11.68 μ g/L). These concentrations were much lower than expected from the spiking 20 μ g/mL solution and are not in line with latter results using the more accurate IS (1H benzotriazole-D4). For the consecutive calculations of fraction sorbed to the soil and the log K_{oc} values the measured spiking solution concentrations were used.

When using the standard injection volume of 20 μ L this caused analytical recovery rates for BTZ sometimes to be (<35%) (Table A6). This introduced a concentration bias (3.2.1.5) and hence causes over- or underestimation of the analyte concentrations. One suspected cause of this issue were matrix effects derived from the initial soils as discussed before. To mitigate these effects, a smaller injection volume of 5 μ L was tested. However, this adaptation only resulted in a slight improvement of the recovery in most cases (A.2.2) that still for many samples remained below the 90% suggested by the OECD TG 106. To avoid a concentration bias at low recovery rates

samples with analytical recovery rates below 35% were excluded from subsequent calculations and graphical presentations (Figure A 4).

As another way to try and improve the analytical recovery rates another, more accurate IS was used (1H-benzotriazole-D4). However, due to time and budget constraints in this project, this could only be done for a subset of measurements. To avoid a wrong mobility classification following the CLP criteria, it was decided to reassess those two soils that showed the lowest log K_{oc} values. The reanalysed water samples showed higher analytical recovery rates, ranging from 50% to 111%, with an average of 91% (Table A 7). The reanalysed BTZ spiking solution had a concentration of 26.12 µg/L, which was closer to the expected spiking concentration of 20 µg/L (Table A 8). These results render the dataset relying on 1H-benzotriazole-D4 as an IS more reliable. Due to this difference in bias, and because only a minority of data was obtained with 1H-benzotriazole-D4 as an IS, the hereby obtained data were processed and discussed separately. The mobility classification was based on the more analytically reliable log K_{oc} values obtained using 1H-benzotriazole-D4 as IS.

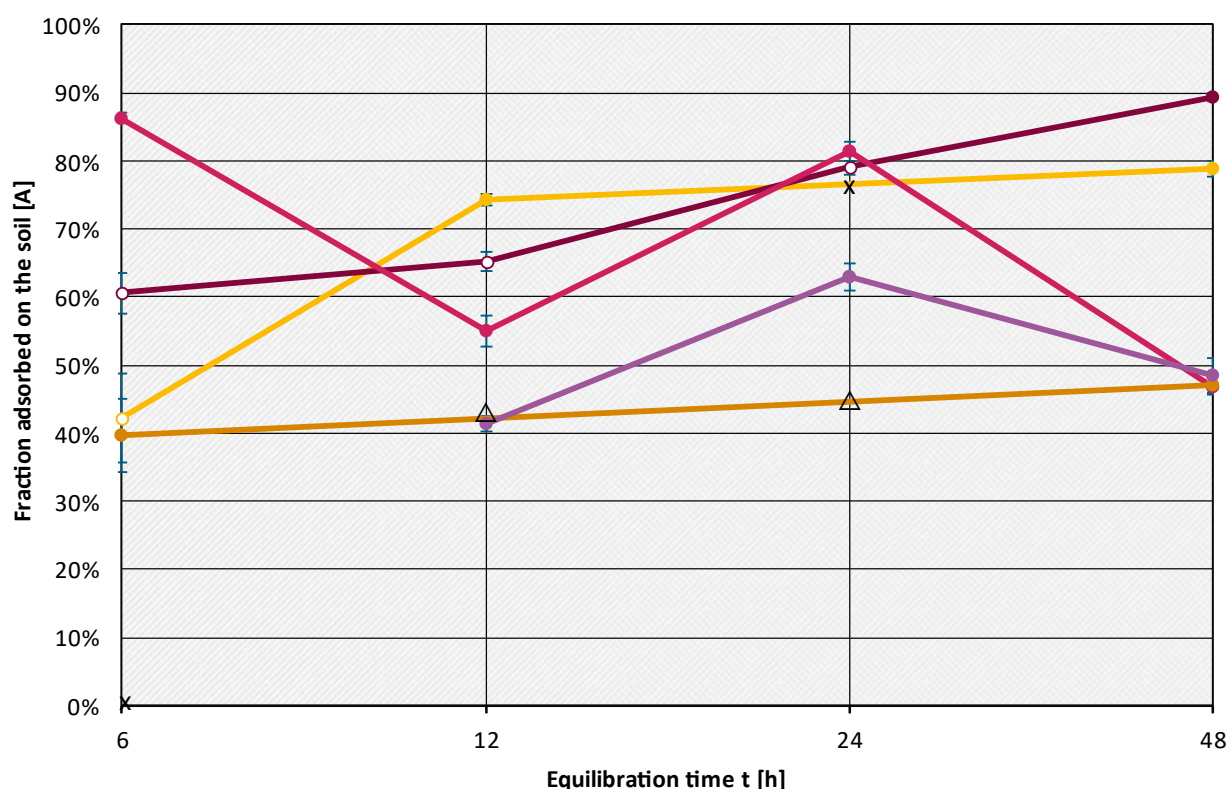
3.2.1.2 Kinetic tests and fraction adsorbed on the soil

3.2.1.2.1 Atrazin-D5 based results

The adsorbed mass fraction of BTZ on the soil (A) [%], is summarized in Figure 1. Due to the analytical challenges described before, samples with recovery rates <35% were excluded. Specifically, this applied to the 24-hour sample from the TS soil (RR=29%) and the 6-hour sample from the L_2.4 soil (23%), which are marked with an “x” in Figure 1.

The fraction absorbed to N15 ranged from 46.7±0.9% (48 h) up to 86.4±0.6% (6 h). In regard of L_2.1 the fraction adsorbed ranged from 39.7±5.3 (6 h) up to 47.2±1.4 (48 h). The two samples taken at 12 and 24 h showed water concentrations slightly above the spiked values with relative concentrations of 115.6±7.7% and 125.0±21.2%. this indicates that there is no significant sorption to the soil. Therefore, these two datapoints were not included in the derivation of log K_{oc} values for BTZ. For the soil TS the adsorbed fraction was lowest at 6 h (42.2±6.6%) and later increased up to 78.9 ±1.1%. This indicates that ≥12 h equilibrium was reached. The fraction adsorbed to the L_2.4 soil were in average 49.1±6.5 % for ≥12 h and indicated sorption equilibrium. In regard of soil N25 the adsorbed fraction increases over all time steps, ranging from 61±2.9% (6 h) up to 89±0.6% (48 h). Hence only the last time step for soil N25 was included in the derivation of log K_{oc} values for BTZ.

Figure 1 – Fraction adsorbed on the test soils at different time steps for BTZ using atrazine-d5 as IS



△: $C_{aq}^{ads} > \text{spiked concentrations}$

X: $RR < 35\%$

O: Equilibrium not reached

—●— TS —●— N25 —●— N15 —●— L_2.1 —●— L_2.4

Source: Original Figure

3.2.1.3 Derived log K_{oc} values

3.2.1.3.1 Atrazine-d5 based result

The calculated log K_{oc} for each soil are summarized in Figure 2. To calculate the log K_{oc} values for BTZ all data indicating equilibrium conditions were used. A detailed description of all datapoints from the Tier 2 batch tests that were used to calculate the log K_{oc} for BTZ can be found in paragraph 3.2.1.5.

According to OECD TG 106, the following minimum log K_{oc} for BTZ was derived for each soil using atrazine-d5 as IS: 1.51 (N25), 1.61 (L_2.4), 1.90 (L_2.1), 1.92 (N15) and 2.85 (TS).

The average log K_{oc} values, ranked from highest to lowest, are 2.89 ± 0.03 (n=4) for TS, 2.36 ± 0.32 (n=6) for N15, 2.07 ± 0.1 (n=4) for L_2.1, 1.80 ± 0.16 (n=6) for L_2.4 and 1.55 ± 0.03 (n=2) for N25. Only 22 samples were available for deriving the log K_{oc} (using atrazine-d5 as IS) for BTZ. In comparison to the other substances that were tested in this report, this are relatively few datapoints, which makes a comparison with other scientific studies essential. Breedveld et al. (2003) also determined K_f values for six different soils under the use of the OECD TG 106. For two soils, a peat soil (carbon content = 0.47%, pH = 3.7) and a medium sand (carbon content = 0.2%, pH = 6.3), the sorption isotherms were nearly linear. In such cases, the K_f values equal the K_d values, which ranged from 0.86 L/kg for the medium sand to 119 L/kg for the peat. These correspond to log K_{oc} values of approximately 2.1 and 2.6, respectively. When comparing these results towards the results obtained here it can be seen that they are falling within a similar range.

The higher sorption observed for the soils TS and N15 could be explained by their high silt and clay content. There was no clear indication that this might be caused by soil pH. Compared to the other soils under investigation The soil pH of TS (7.8) and L_2.4 (7.5) were closer to the pK_a value of BTZ (8.2), yet no uniform pH-dependent sorption behaviour was observed.

3.2.1.3.2 1H-benzotriazole-D4 based results

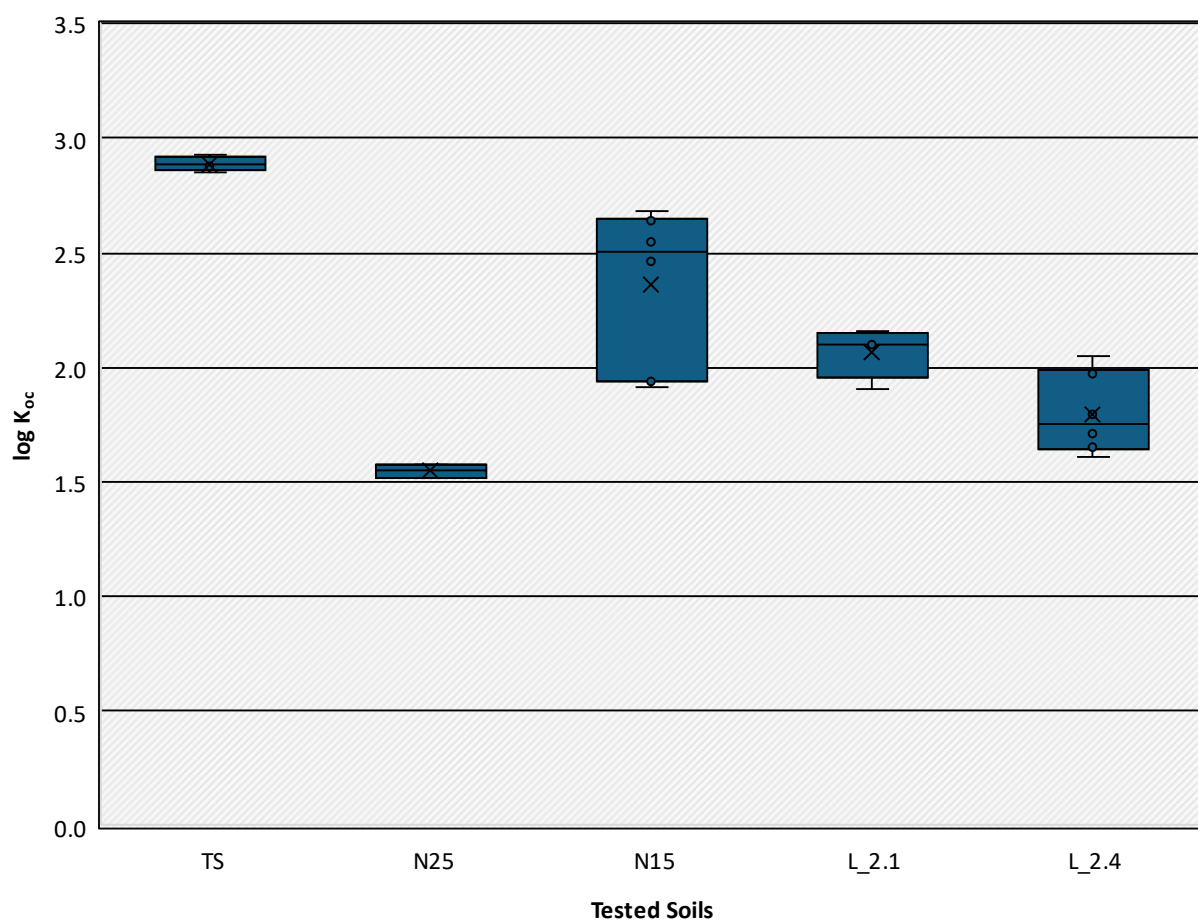
All water samples from the soils L_2.4 and N25, which showed the lowest log K_{oc} values when using atrazine-d5, were reanalysed using the stable isotope-labelled analogue IS 1H-benzotriazole-D4. The results are presented in Table A 7. For soil L_2.4, the average log K_{oc} was 2.12±0.09 (n=4), with a minimum log K_{oc} of 2.01. In regard of soil N25, the average log K_{oc} was 2.17±0.10 (n=6), with a minimum of 2.05. A detailed comparison of the results can be found in Table A 9.

3.2.1.3.3 Overall conclusion on the log K_{oc} for BTZ

Based on these data, the lowest log K_{oc} – which is according to the CLP regulation the one that should be used to assess the mobility criterium – can be found in soil L_2.4. The values observed amount to a log K_{oc} of 1.80±0.16 (lowest value 1.61) and 2.12±0.09 (lowest value 2.01) based on atrazine-d5 and 1H-benzotriazole-D4 as an IS, respectively.

According to the criteria set out in the CLP regulation (EC No. 1272/2008 - CLP, 2008), and considering the more accurate 1H-benzotriazole-D4 based results, BTZ qualifies as a mobile (M) substance.

Figure 2 – log K_{oc} values for BTZ (n total=22) using atrazine-d5 as IS



Source: Original Figure

3.2.1.4 Mass balance

The mass balance for Tier 2 was calculated for selected batch test samples of soils that were not part of the investigation in Tier 1 (Table 5) and used atrazine-d5 as IS. Overall, the mass analytically recovered in the liquid phase and the soil after extraction was higher compared to Tier 1 and was for all samples above 100%. The mass balance (MB) in Tier 2 ranged for BTZ from 105.3% (L_2.4) to 159.5% (L_2.1). These values above 100% can mostly be explained by the fact that the concentrations of BTZ in the spiking solution was found to be lower than intended (as described before). Therefore, it was impossible to correct the results for these mass losses. Accordingly, the obtained data had to be used as they were to perform the further calculations.

Table 5: Mass balance for BTZ in Tier 2 using atrazine-d5 as IS

Soil (Water) Sample Nr.	Soil	L/S	C ^{ads} _{aq} [µg/mL]	C ^{ads} _{wet soil} [µg/g w.w.]	V _{rec} [mL]	M _{wet soil} [g w.w.]	m ₀ [µg]	MB [%]	Water Soil [%]	RR Soil [%]
193 (365)	L_2.1	1	1.12	0.65	7.16	12.84	11.84	138.4	96%	38%
201 (373)	L_2.4	1	0.63	0.50	5.72	14.28	11.65	92.0	42%	47%

Explanation of Abbreviations used:

C^{ads}_{aq} = Concentration in the water phase

V_{rec} = Volume recovered after centrifugation

M₀ = Mass of substance spiked to the batch test

Water RR = Recovery rater in water samples

C^{ads}_{wet soil} = Soil concentration (porewater + soil)

M_{wet, soil} = Mass of wet soil after centrifugation

MB = Mass balance

Soil RR = Recovery rate in soi samples

3.2.1.5 Test results for all water samples for BTZ using atrazine-d5 in Tier 2

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-eq. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [μg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
241	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.60	0.58	1.0	2.38	49%	57.0%
242	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.75	0.42	0.56	2.15	36%	57.0%
249	L_2.1	6	1	10.00	9.98	0.02	9.01	1.00	10.03	1.01	10.18	0.64	0.53	0.8	2.10	45%	103%
250	L_2.1	6	1	10.00	9.98	0.02	9.00	1.00	10.02	1.02	10.18	0.77	0.40	0.5	1.90	34%	103%
257	L_2.4	6	1	10.00	9.89	0.11	9.01	1.00	10.12	1.01	10.18	1.79	m _w > m ₀	-	-	<0%	24.0%
258	L_2.4	6	1	10.00	9.89	0.11	9.01	1.00	10.12	1.01	10.18	1.79	m _w > m ₀	-	-	<0%	24.0%
265	N15	6	1	10.00	9.91	0.09	9.02	1.00	10.11	1.01	10.18	0.15	1.04	6.8	2.68	87%	N.A
266	N15	6	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.17	1.02	6.1	2.64	86%	N.A
273	N25	6	5	4.00	3.85	0.15	18.00	2.00	20.15	1.01	20.36	0.43	3.89	9.1	1.65	64%	38.0%
274	N25	6	5	4.00	3.85	0.15	18.01	2.00	20.16	1.01	20.36	0.49	3.53	7.1	1.54	58%	38.0%
281	TS	12	1	10.00	9.91	0.09	9.01	1.01	10.11	1.02	10.28	0.31	0.88	2.8	2.85	74%	105%
282	TS	12	1	10.00	9.91	0.09	9.02	1.00	10.11	1.01	10.18	0.29	0.90	3.1	2.89	75%	105%
289	L_2.1	12	1	10.00	9.98	0.02	9.00	1.00	10.02	1.02	10.18	1.26	m _w > m ₀	-	-	<0%	62.0%
290	L_2.1	12	1	10.00	9.98	0.02	9.02	1.00	10.04	1.01	10.18	1.44	m _w > m ₀	-	-	<0%	62.0%
297	L_2.4	12	1	10.00	9.89	0.11	9.02	1.00	10.13	1.01	10.18	0.67	0.51	0.76	1.65	43%	51.0%
298	L_2.4	12	1	10.00	9.89	0.11	9.00	1.01	10.12	1.02	10.28	0.70	0.48	0.69	1.61	40%	51.0%
305	N15	12	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.50	0.68	1.36	1.98	57%	54.0%
306	N15	12	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.55	0.63	1.14	1.91	53%	54.0%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-eq. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [ug/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
313	N25	12	5	4.00	3.85	0.15	18.01	2.00	20.16	1.01	20.36	0.39	4.08	10.5	1.71	67%	31.0%
314	N25	12	5	4.00	3.85	0.15	18.00	2.00	20.15	1.01	20.36	0.42	3.90	9.2	1.66	64%	31.0%
321	TS	24	1	10.00	9.91	0.09	9.03	1.02	10.14	1.02	10.38	0.90	0.27	0.30	1.88	23%	29.0%
322	TS	24	1	10.00	9.91	0.09	9.01	1.00	10.10	1.01	10.18	0.98	0.19	0.20	1.69	16%	29.0%
329	L_2.1	24	1	10.00	9.98	0.02	9.02	1.00	10.04	1.01	10.18	1.71	m _w > m ₀	-	-	<0%	40.0%
330	L_2.1	24	1	10.00	9.98	0.02	9.01	1.01	10.04	1.02	10.28	1.21	m _w > m ₀	-	-	<0%	40.0%
337*	L_2.4	24	1	10.00	9.89	0.11	9.00	1.01	10.12	1.02	10.28	0.41	0.78	1.9	2.05	65%	66.0%
338*	L_2.4	24	1	10.00	9.89	0.11	9.02	1.01	10.14	1.01	10.28	0.46	0.73	1.6	1.97	61%	66.0%
345	N15	24	1	10.00	9.91	0.09	9.01	1.01	10.11	1.02	10.28	0.20	0.99	5.0	2.55	83%	103%
346	N15	24	1	10.00	9.91	0.09	9.02	1.00	10.11	1.01	10.18	0.23	0.95	4.1	2.46	80%	103%
353	N25	24	5	4.00	3.85	0.15	18.00	2.00	20.15	1.01	20.36	0.23	4.89	20.9	2.01	80%	53.0%
354	N25	24	5	4.00	3.85	0.15	18.00	2.00	20.15	1.01	20.36	0.26	4.78	18.7	1.96	78%	53.0%
365	L_2.1	48	1	10.00	9.98	0.02	9.00	1.01	10.03	1.02	10.28	0.60	0.57	0.95	2.16	49%	96.0%
366	L_2.1	48	1	10.00	9.98	0.02	9.00	1.00	10.02	1.02	10.18	0.63	0.54	0.85	2.11	46%	96.0%
373	L_2.4	48	1	10.00	9.89	0.11	9.02	1.00	10.13	1.01	10.18	0.63	0.55	0.9	1.71	46%	42.0%
374	L_2.4	48	1	10.00	9.89	0.11	9.00	1.00	10.11	1.01	10.18	0.57	0.61	1.1	1.80	51%	53.0%
381*	N15	48	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.53	0.65	1.2	1.94	55%	39.0%
382*	N15	48	1	10.00	9.91	0.09	9.00	1.00	10.09	1.01	10.18	0.55	0.63	1.2	1.92	53%	39.0%

* due to higher analytical recovery rates results were used for 20ul injection volume.

3.2.2 4-methyl-benzotriazole (4-MB)

3.2.2.1 Quality assurance

For quality assurance, blank samples and control samples were analysed. The results are summarized in Appendix **Error! Reference source not found.** All 0.1 M CaCl₂ solution blanks and soil-water blanks were below LOD for 4-MB.

The 0.1 M CaCl₂ spiking solution used in the batch tests contained 8.71 µg/mL of 4-MB. The respective control samples (1 mL spiking solution + 9 mL 0.1M CaCl₂ solution) had measured concentrations of 0.76 µg/mL using atrazine-d5 as IS, which corresponds to an error of 13%. Similar to the BTZ measurements, the spiking solution was significantly below the planned and calculated spiking concentration of 20 µg/L.

The obtained R² values of the used calibration curve demonstrated a linear response (R²>0.99). The LOD and LOQ for 4-MB were 0.0007 µg/ml and 0.002 µg/ml, respectively. All analytical recovery rates for 4-MB were above the earlier defined threshold of ≥35% (3.2.1.1). Overall, the recovery in the water samples ranged from 35% to 62%. Sample individual analytical recovery rates for the water phase are listed in 3.2.2.5.

Due to time and budget constraints in this project, only a subset of measurements could be verified using the stable isotope-labelled IS 4-methyl-1H-benzotriazole-D3. The reanalysed water samples showed higher analytical recovery rates, which ranged from 92% to 119%, with an average of 111% (Table A 10). The reanalysed 4MB spiking solution had a concentration of 22.8 µg/L, which was closer to the intended spiking concentration (Table A 11). When comparing the 4-MB concentrations obtained via the two different IS it should be noted that the difference in the water samples was smaller than those in the spiking solution, (Table A 12). Furthermore, the control samples were consistent with these concentrations (Table A 11). Hence, the dataset derived using 4-methyl-1H-benzotriazole-D3 as an IS appears to be more reliable.

Due to this difference in bias, and because only a minority of data was obtained using the IS 4-methyl-1H-benzotriazole-D3, these data are discussed separately from the rest of the data. Those data that were generated under the use of atrazine-D5 as an IS offer a more complete data set and are more conservative due to the lower spiking concentrations used for calculating log K_{oc}. The mobility classification was based on the more analytically reliable log K_{oc} values obtained using 4-methyl-1H-benzotriazole-D4 as IS.

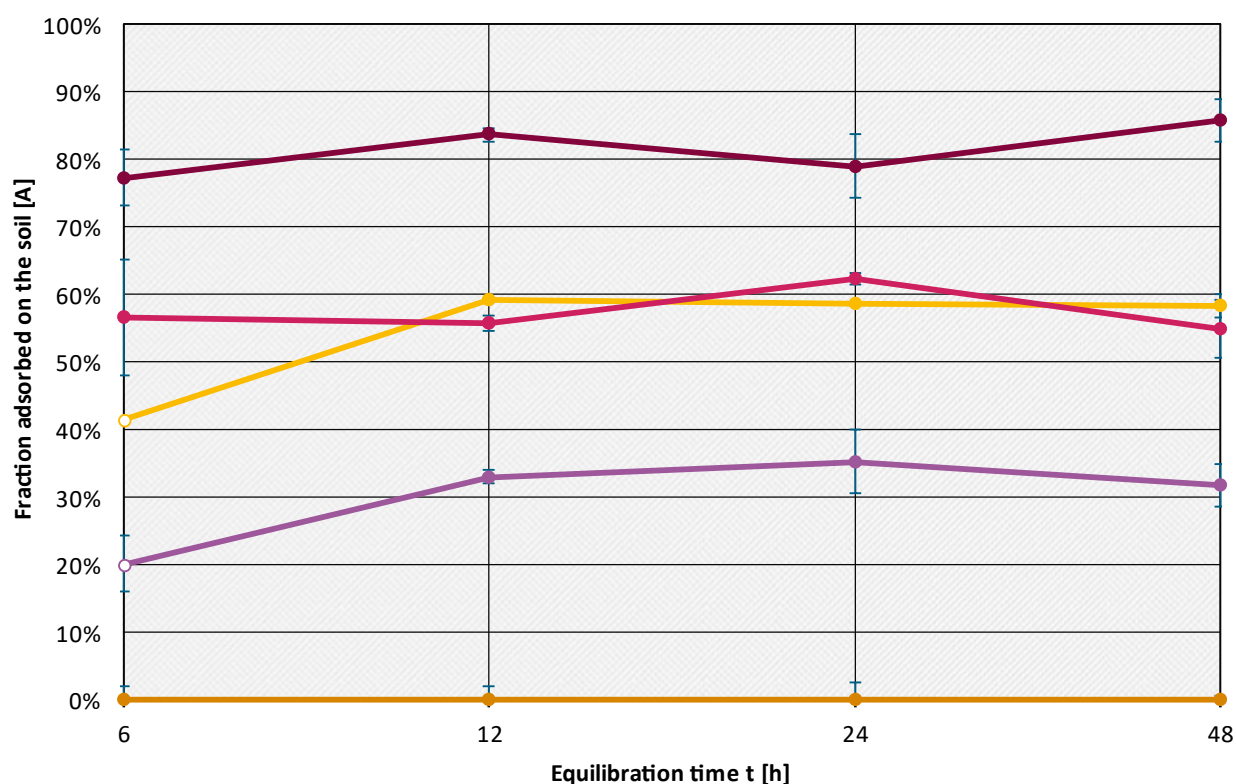
3.2.2.2 Kinetic tests and fraction adsorbed on the soil

The fraction of adsorbed mass (A[%]) of 4-MB on the soil, using atrazine-d5 as IS, is summarized in Figure 3.

For the soils N15 (56.7±2.6%) and N25 (81.5±3.1%), the fraction of 4-MB adsorbed to the soils remained nearly constant over all experimental times. This indicates fast adsorption kinetics (<6 h) and that a state of equilibrium was reached quickly. In contrast, for the soil TS and L_2.4, sorption processes seem to reach equilibrium only after 12 h. Thereby, the sorbed fraction for TS was 58.8±0.3% and for L_2.4 33.4±1.3%. The batch test results for soil L_2.1 indicate that there was no detectable sorption to the soil.

To calculate the log K_{oc} values for 4-MB all data indicating equilibrium conditions were used. Accordingly, it was impossible to calculate log K_{oc} values soil L_2.1 and it could not be considered then assessing the mobility of 4-MB.

Figure 3 – Fraction adsorbed on the test soils at different time steps for 4-MB



O: Equilibrium not reached

● TS ● N25 ● N15 ● L_2.1 ● L_2.4

Source: Original Figure

3.2.2.3 Derived log K_{oc} values

3.2.2.3.1 Atrazine-d5 based result

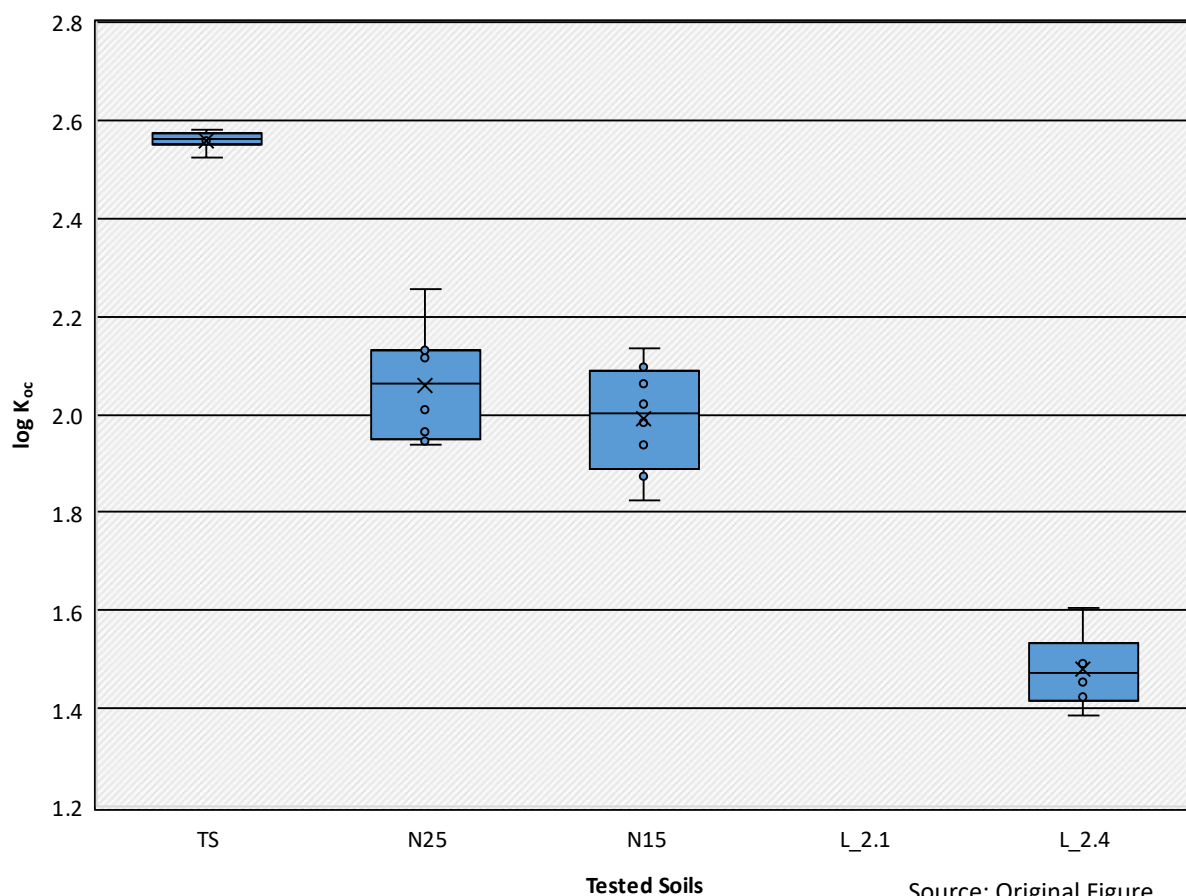
The calculated log K_{oc} values for each soil are summarized in Figure 4. For this all representative data points using atrazine-d5 as an IS were used. The derived data from the batch tests conducted under Tier 2 for 4-MB can be found in section 3.2.2.5.

According to OECD TG 106, the following minimum log K_{oc} for 4-MB were derived for each soil: 1.39 (L_2.4), 1.82 (N15), 1.94 (N25) and 2.52 (TS). For soil L_2.1 no log K_{oc} values could be calculated due to no analytical measurable sorption to the soil.

The average log K_{oc} values, ranked from highest to lowest, are 2.56±0.02 (n=6) for TS, 2.06±0.11 (n=8) for N25, 1.99±0.10 (n=8) for N15, 1.48±0.07 (n=6) for L_2.4.

The soil pH of different soils investigated in this study was well below the expected pK_a (9.15) of 4-MB. Therefore, sorption changes due to protonation or deprotonation processes were not expected.

Figure 4 – log K_{oc} values for 4-MB using atrazine-d5 as IS



3.2.2.3.2 4-methyl-1H-benzotriazole-D3 based results

All water samples from soil L_2.4 were reanalysed using the stable isotope-labelled analogue IS 4-methyl-1H-benzotriazole-D3. The results are presented in Table A 10. For L_2.4, the average log K_{oc} was 2.21 ± 0.05 (n=6), with a minimum log K_{oc} of 2.15. A detailed comparison can be found in Table A 12.

3.2.2.3.3 Overall conclusion on the log K_{oc} for 4-MB

Based on these data, the lowest log K_{oc} – which is according to the CLP regulation the one that should be used to assess the mobility criterium - can be found in soil L_2.4. The values observed amount to 1.48 ± 0.07 (lowest value 1.39) and 2.21 ± 0.05 (lowest value 2.15) based on atrazine-d5 and 4-methyl-1H-benzotriazole-D3 as an IS, respectively.

According to the criteria set out in the CLP regulation (EC No. 1272/2008 - CLP, 2008), and considering the more accurate 4-methyl-1H-benzotriazole-D3 based results, 4-MB qualifies as a mobile (M) substance.

3.2.2.4 Mass balance (MB)

The MB for Tier 2, something that was not investigated in Tier 1, was calculated for 4-MB under the use of atrazine-d5 as an IS. The results are summarized in Table 6. Overall, MB was calculated based on the liquid ($C_{ads,ag}$, directly measured) and solid phase after extraction ($C_{ads,wet\ soil}$). The MB was found to range from 103.3% for N25 (sample nr. 216 (388)) up to 163.5% for L_2.4 (sample nr. 204(376)) and was therefore, above 100%. for all samples.

These high analytical recoveries >100% can likely be explained by the fact that the concentration of 4-MB in the spiking solution was found to be lower than intended (as described before). Therefore, it was not possible to correct the results for these mass losses. Accordingly, the obtained data had to be used as they were to perform the further calculations.

Table 6: Mass balance (MB) for 4-MB in Tier 2 (shaking time = 48h) using atrazine-d5 as IS

Soil (Water) Sample Nr.	Soil	L/S	C ^{ads} _{aq} [µg/mL]	C ^{ads} _{wet soil} [µg/g w.w.]	V _{rec} [mL]	M _{wet soil} [g w.w.]	m ₀ [µg]	MB [%]	Water RR [%]	Soil RR [%]
187 (359)	TS	1	0.37	0.80	6.04	13.96	8.71	154.6	38%	50%
204 (376)	L_2.4	1	0.61	0.75	5.74	14.26	8.71	163.5	44%	55%
215 ^a (387)	N25	5	0.11	2.92	16.02	7.98	17.50	143.0	45%	62%
216 ^a (388)	N25	5	0.14	1.99	16.14	7.86	17.33	103.3	45%	80%

Control Samples (0.01M CaCl₂ Solution)

(225)	-	-	0.76	-	10.0	0	8.7	87.4%	Not available	-
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Explanation of Abbreviations used:

C^{ads}_{aq} = Concentration in the water phase

V_{rec} = Volume recovered after centrifugation

M₀ = Mass of substance spiked to the batch test

Water RR = Recovery rater in water samples

Explanation of used Indexes

^a indicating duplicates

C^{ads}_{wet soil} = Soil concentration (porewater + soil)

M_{wet,soil} = Mass of wet soil after centrifugation

MB = Mass balance

Soil RR = Recovery rate in soi samples

3.2.2.5 Test results for 4-MB (Tier 2) using atrazine-d5 as IS

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [µg/mL]	m ₀ [µg]	C _w [µg/mL]	C _s [µg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
243	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.09	0.86	8.71	0.51	0.36	0.7	2.25	41%	40%
244	TS	6	1	10.00	9.91	0.09	9.00	1.01	10.10	0.87	8.80	0.51	0.37	0.7	2.26	42%	40%
251	L_2.1	6	1	10.00	9.98	0.02	9.00	1.00	10.02	0.87	8.71	1.04	m _w > m ₀	-	-	<0.1%	41%
252	L_2.1	6	1	10.00	9.98	0.02	9.01	1.00	10.03	0.87	8.71	1.08	m _w > m ₀	-	-	<0.1%	41%
259	L_2.4	6	1	10.00	9.89	0.11	9.01	1.00	10.12	0.86	8.71	0.65	0.21	0.33	1.29	24%	42%
260	L_2.4	6	1	10.00	9.89	0.11	9.02	1.00	10.13	0.86	8.71	0.72	0.14	0.20	1.06	16%	42%
267	N15	6	1	10.00	9.91	0.09	9.00	1.00	10.09	0.86	8.71	0.45	0.42	0.9	1.82	48%	62%
268	N15	6	1	10.00	9.91	0.09	9.01	1.00	10.10	0.86	8.71	0.30	0.57	1.9	2.13	65%	62%
275	N25	6	5	4.00	3.85	0.15	18.03	2.00	20.18	0.86	17.42	0.20	3.49	17.7	1.94	77%	40%
276	N25	6	5	4.00	3.85	0.15	18.01	2.00	20.16	0.86	17.42	0.20	3.50	18.0	1.94	77%	40%
283	TS	12	1	10.00	9.91	0.09	9.00	1.00	10.09	0.86	8.71	0.35	0.52	1.5	2.57	59%	42%
284	TS	12	1	10.00	9.91	0.09	9.00	1.00	10.09	0.86	8.71	0.35	0.52	1.5	2.56	59%	42%
291	L_2.1	12	1	10.00	9.98	0.02	9.00	1.00	10.02	0.87	8.71	0.96	m _w > m ₀	-	-	<0.1%	40%
292	L_2.1	12	1	10.00	9.98	0.02	9.01	1.00	10.03	0.87	8.71	0.93	m _w > m ₀	-	-	<0.1%	40%
299	L_2.4	12	1	10.00	9.89	0.11	9.01	1.00	10.12	0.86	8.71	0.57	0.30	0.53	1.49	34%	39%
300	L_2.4	12	1	10.00	9.89	0.11	9.01	1.00	10.12	0.86	8.71	0.58	0.28	0.48	1.45	32%	39%
307	N15	12	1	10.00	9.91	0.09	9.01	1.01	10.11	0.87	8.80	0.37	0.51	1.4	1.98	57%	36%
308	N15	12	1	10.00	9.91	0.09	9.01	1.00	10.10	0.86	8.71	0.39	0.48	1.2	1.94	55%	36%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [μg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
315	N25	12	5	4.00	3.85	0.15	18.00	2.00	20.15	0.86	17.42	0.14	3.80	27.3	2.13	84%	40%
316	N25	12	5	4.00	3.85	0.15	18.00	2.00	20.15	0.86	17.42	0.14	3.78	26.6	2.11	84%	40%
323	TS	24	1	10.00	9.91	0.09	9.00	1.01	10.10	0.87	8.80	0.36	0.52	1.45	2.56	59%	43%
324	TS	24	1	10.00	9.91	0.09	9.02	1.00	10.11	0.86	8.71	0.36	0.51	1.44	2.56	59%	43%
331	L_2.1	24	1	10.00	9.98	0.02	9.03	1.02	10.07	0.88	8.88	1.08	m _w > m ₀	-	-	<0.1%	39%
332	L_2.1	24	1	10.00	9.98	0.02	9.01	1.01	10.04	0.88	8.80	1.03	m _w > m ₀	-	-	<0.1%	39%
339	L_2.4	24	1	10.00	9.89	0.11	9.03	1.00	10.14	0.86	8.71	0.51	0.35	0.7	1.61	40%	46%
340	L_2.4	24	1	10.00	9.89	0.11	9.02	1.00	10.13	0.86	8.71	0.60	0.27	0.5	1.42	31%	46%
347	N15	24	1	10.00	9.91	0.09	9.03	1.00	10.12	0.86	8.71	0.32	0.56	1.8	2.10	63%	40%
348	N15	24	1	10.00	9.91	0.09	9.03	1.00	10.12	0.86	8.71	0.33	0.54	1.6	2.06	62%	40%
355	N25	24	5	4.00	3.85	0.15	18.00	2.00	20.15	0.86	17.42	0.17	3.62	20.8	2.01	80%	40%
356	N25	24	5	4.00	3.85	0.15	18.01	2.00	20.16	0.86	17.42	0.19	3.54	18.9	1.97	78%	40%
359	TS	48	1	10.00	9.91	0.09	9.01	1.00	10.10	0.86	8.71	0.37	0.50	1.3	2.52	57%	38%
360	TS	48	1	10.00	9.91	0.09	8.99	1.00	10.08	0.86	8.71	0.34	0.53	1.5	2.58	60%	38%
367	L_2.1	48	1	10.00	9.98	0.02	9.01	1.01	10.04	0.88	8.80	1.13	m _w > m ₀	-	-	<0.1%	41%
368	L_2.1	48	1	10.00	9.98	0.02	9.00	1.00	10.02	0.87	8.71	1.14	m _w > m ₀	-	-	<0.1%	41%
375	L_2.4	48	1	10.00	9.89	0.11	9.00	1.00	10.11	0.86	8.71	0.56	0.31	0.5	1.51	35%	44%
376	L_2.4	48	1	10.00	9.89	0.11	9.01	1.00	10.12	0.86	8.71	0.61	0.25	0.41	1.39	29%	44%
383	N15	48	1	10	9.91	0.09	9.00	1.01	10.11	0.87	8.80	0.36	0.52	1.5	2.02	59%	35%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [µg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
384	N15	48	1	10	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.43	0.45	1.1	1.87	51%	35%
387	N25	48	5	4	3.85	0.15	18.00	2.01	20.16	0.87	17.50	0.11	3.98	36.8	2.26	88%	45%
388	N25	48	5	4	3.85	0.15	18.00	2.00	20.15	0.86	17.42	0.14	3.81	27.6	2.13	84%	45%

3.2.3 5-methyl-benzotriazole (5-MB)

3.2.3.1 Quality assurance

For quality assurance, blank samples and control samples were analysed and the results are summarized in Appendix A.2. All 0.1 M CaCl₂ solution blanks and soil-water blanks were below LOD for 5-MB.

The 0.1 M CaCl₂ spiking solution used in the batch tests contained 11.6 µg/mL of 5-MB. The respective control samples (1 mL spiking solution + 9 mL 0.1M CaCl₂ solution) had measured concentrations of 1.05 µg/L using atrazine-d5 as IS, which is corresponding to an error of 9%. Similar to the BTZ and 4-MB measurements, the spiking solution was significantly below the planned and calculated spiking concentration of 20 µg/L.

The obtained R² values of the used calibration curve demonstrated linear response (R²>0.99). The LOD and LOQ for 5-MB were 0.0007 µg/ml and 0.002 µg/ml, respectively. All analytical recovery rates for 5-MB in the water samples were above the earlier defined threshold of ≥35% (Table A 6). Overall, the recovery in the water samples ranged from 35% to 62%. Sample individual analytical recovery rates for the water phase are listed in 3.2.3.5.

Due to time and budget constraints in this project, only a subset of measurements could be verified using the stable isotope-labelled IS 5-methyl-1H-benzotriazole-D6. The reanalysed water samples showed higher analytical recovery rates, which ranged from 89% to 110%, with an average of 101% (Table A 13Table A 10). The reanalysed 5-MB spiking solution had a concentration of 25.85 µg/L, which was closer to the intended spiking concentration (Table A 14Table A 11). When comparing the 5-MB concentrations obtained via the two different IS it should be noted that the difference in the water samples was smaller than in the spiking solution (Table A 15). Hence, the dataset derived using 5-methyl-1H-benzotriazole-D6 as an IS appears to be more reliable.

Due to this difference in bias, and because only a minority of data was obtained using 5-methyl-1H-benzotriazole-D6 as an IS, these data are discussed separately from the rest of the data. Those data that were generated under the use of atrazine-d5 as an IS offer a more complete data set and are more conservative, due to the lower spiking concentrations used for calculating log K_{oc}. The mobility classification was based on the more analytically reliable log K_{oc} values obtained using 5-methyl-1H-benzotriazole-D6 as IS.

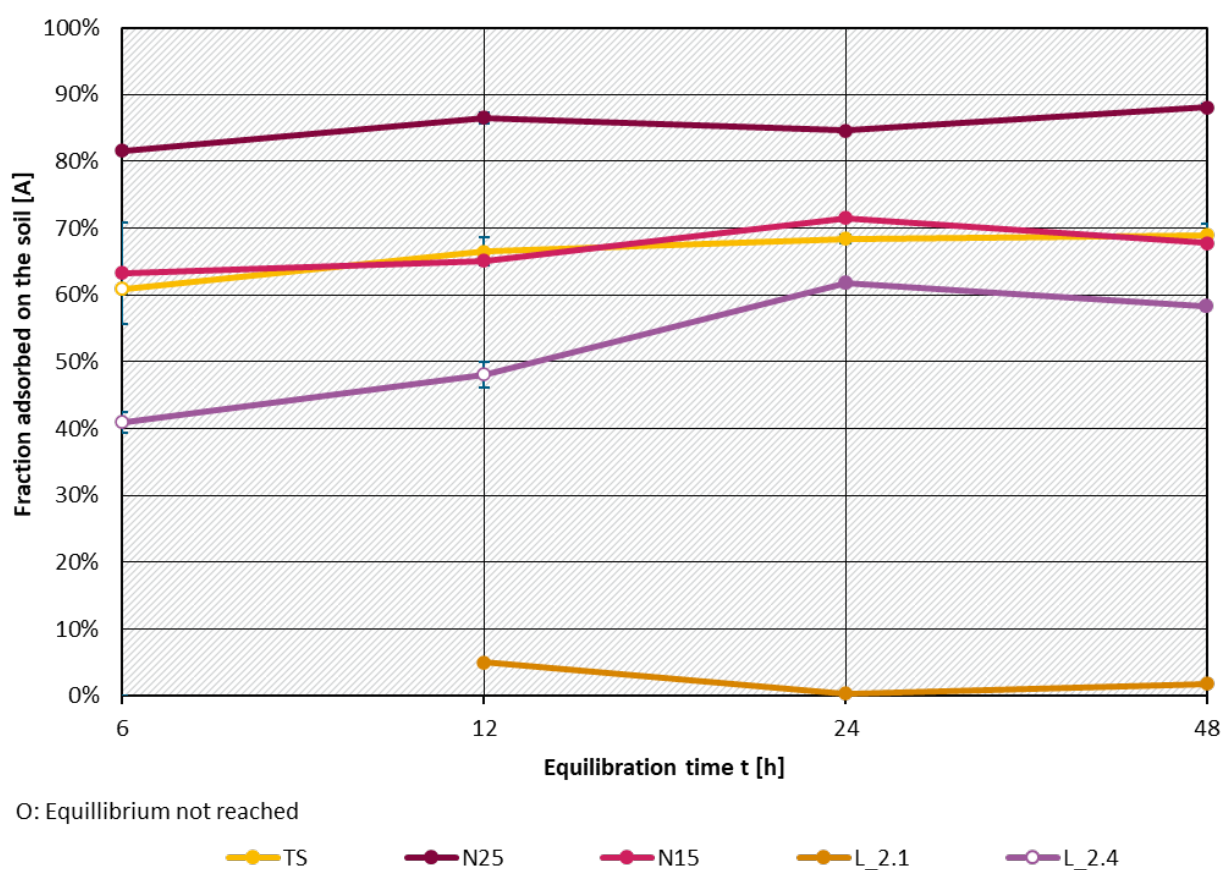
3.2.3.2 Kinetic tests and fraction adsorbed on the soil

The fraction of adsorbed mass (A[%]) of 5-MB on the soil, using atrazine-d5 as IS, is summarized in Figure 5.

For the soils N15 (66.9±3.1%), and N25 (85.2±2.4%), the fraction of 5-MB adsorbed to the soils remained nearly constant over all equilibration times. This indicates fast adsorption kinetics (<6 h) and that a state of equilibrium was reached quickly. In contrast, for the soil TS, sorption processes seem to have reach equilibrium only after ≥12-h. Thereby, the sorbet fraction amounted on average to 59.1±0.3%. For soil L_2.4 sorption processes seem to reach equilibrium after ≥24-h. The average sorbet fraction at equilibrium amounts to 52.3±8.2%. The batch test results for the soil L_2.1 indicate that over all tested time steps there was only little sorption (≤5.0%) to the soil.

To calculate the log K_{oc} values for 5-MB all data indicating equilibrium conditions were used. However, the data obtained for soil L_2.1 were excluded as they did not fulfil the criteria of OECD TG 106 (at least 20% of the substances should be sorbed to the soil).

Figure 5 – Fraction adsorbed on the test soils at different time steps for 5-MB



Source: Original Figure

3.2.3.3 log K_{oc} values

3.2.3.3.1 Atrazine-d5 based result

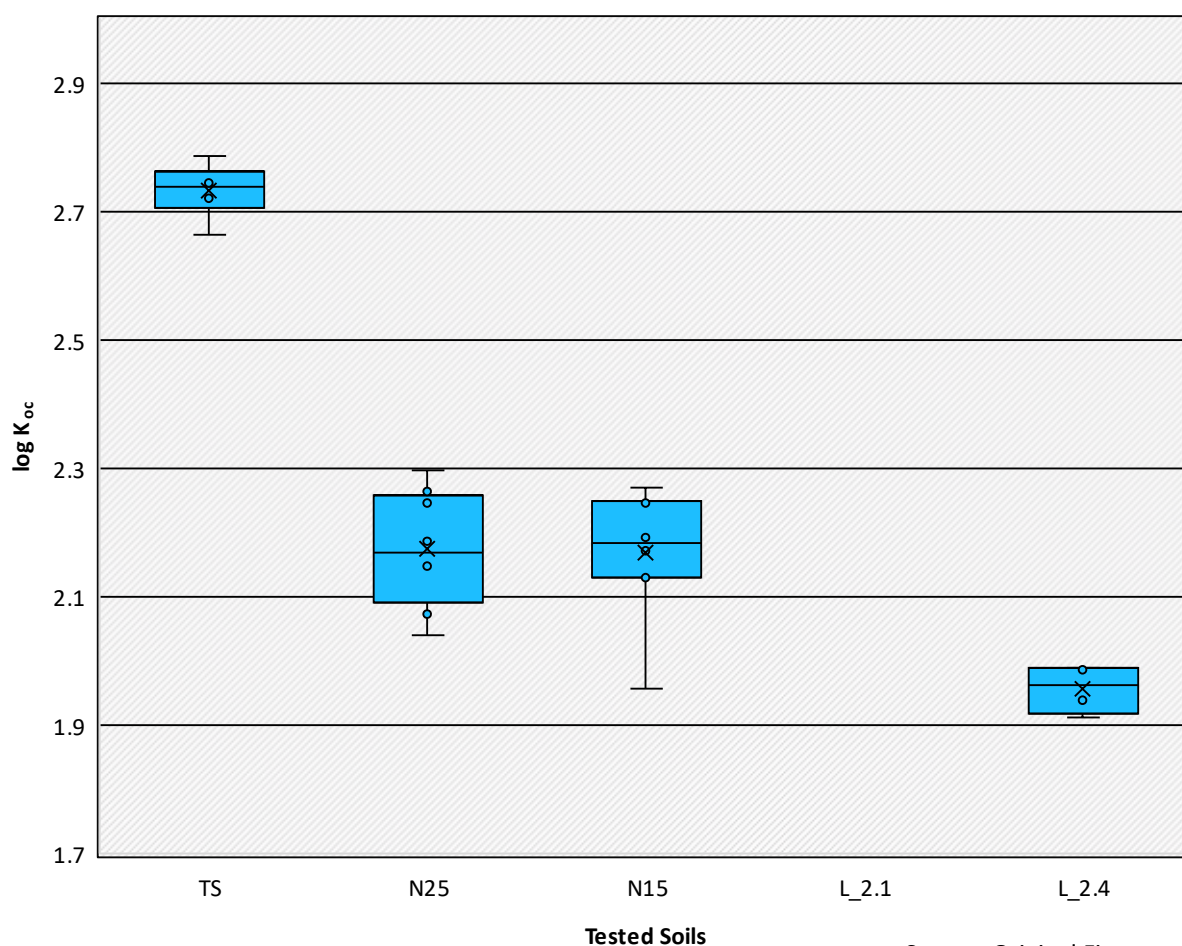
The calculated log K_{oc} values for each soil using atrazine-d5 as IS are summarized in Figure 6. For this all representative data points were used (described in section 3.2). All derived data from the batch test conducted under Tier 2 for 5-MB can be found in paragraph 3.2.3.5.

According to OECD TG 106, the following minimum log K_{oc} values for 5-MB were derived for each soil: 1.91 (L_2.4), 1.96 (N15), 2.04 (N25) and 2.72 (TS). For soil L_2.1 no log K_{oc} values could be calculated as the observed sorption to the soil was too low (<20%).

The average log K_{oc} values, ranked from highest to lowest, are 2.73±0.04 (n=6) for TS, 2.18±0.08 (n=8) for N25, 2.17±0.09 (n=8) for N15, 1.96±0.03 (n=4) for L_2.4.

The soil pH of the different soils investigated in this study was well below the expected pK_a value (9.15) of 5-MB. Therefore, sorption changes due to protonation or deprotonation processes were not expected.

Figure 6 – log K_{oc} for 5-MB using atrazine-d5 as IS



3.2.3.3.2 5-methyl-1H-benzotriazole-D6 based results

All water samples from soil L_2.4 were reanalysed using the stable isotope-labelled analogue IS 5-methyl-1H-benzotriazole-D6. The results are presented in Table A 13. For L_2.4, the average

log K_{oc} was 2.36±0.06 (n=6), with a minimum log K_{oc} of 2.30. A detailed comparison can be found in Table A 15.

3.2.3.3.3 Overall conclusion on the log K_{oc} for 5-MB

Based on the presented data the lowest log K_{oc} - which is according to the CLP regulation the one that should be used to assess the mobility criterium – can be found in soil L_2.4. The values observed amount to 1.96±0.03 (lowest value 1.91) and 2.36±0.06 (lowest value 2.30) based on atrazine-d5 and 5-methyl-1H-benzotriazole-D6 as an IS, respectively.

According to the criteria set out in the CLP regulation (EC No. 1272/2008 - CLP, 2008) and considering the more accurate 5-methyl-1H-benzotriazole-D6 based results, 5-MB qualifies as a mobile (M) substance.

3.2.3.4 Mass balance (MB)

The MB for Tier 2, something that was not tested in Tier 1, was calculated for selected batch test samples for 5-MB using atrazine-d5 as IS. The results are summarized in Table 7. Overall, the MB based on concentrations of the substance in liquid (C^{ads}_{aq}, directly measured) and solid phase after extraction (C^{ads}_{wet soil}) ranged for 5-MB from 130.2% (N25) up to 164.7% (L_2.2).

A MB above 100% can likely be explained by the low concentrations found in the spiking solution (as described in detail before). Due to the described concentrations bias using atrazine-d5 it was not possible to correct for any mass losses, and the data from the aqueous phase was used for further calculations.

Table 7: Mass balance for 5-MB in Tier 2 (shaking timer = 48h) using atrazine-d5 as IS

Soil (Water) Sample Nr	Soil	L/S	C ^{ads} _{aq} [µg/mL]	C ^{ads} _{wet soil} [µg/g w.w.]	V _{rec} [mL]	M _{wet soil} [g w.w.]	m ₀ [µg]	MB [%]	Water RR [%]	Soil RR [%]
189 (361) ^{a)}	TS	1	0.38	1.18	6.04	13.96	11.60	161.4	38%	46%
190 (362) ^{a)}	TS	1	0.34	0.60	6.02	13.98	11.60	90.0	38%	50%
197 (369)	L_2.1	1	1.13	0.85	7.20	12.80	11.60	164.7	41%	49%
217 (389)	N25	5	0.14	3.55	16.09	7.91	23.32	130.2	45%	68%

Control Samples (0.01M CaCl₂ Solution)

(227)	-	-	1.05	-	10.0		11.6	90.5		
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Explanation of Abbreviations used:

C^{ads}_{aq} = Concentration in the water phase

V_{rec} = Volume recovered after centrifugation

M₀ = Mass of substance spiked to the batch test

Water RR = Recovery rater in water samples

Explanation of used Indexes

^{a)} indicating duplicates

C^{ads}_{wet soil} = Soil concentration (porewater + soil)

M_{wet,soil} = Mass of wet soil after centrifugation

MB = Mass balance

Soil RR = Recovery rate in soi samples

3.2.3.5 Test results for 5-MB (Tier 2) using atrazine-d5 as IS

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [μg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
245	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.09	1.15	11.60	0.46	0.71	1.5	2.59	60%	40%
246	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.09	1.15	11.60	0.44	0.72	1.6	2.61	61%	40%
253	L_2.1	6	1	10.00	9.98	0.02	9.03	1.00	10.05	1.15	11.60	1.29	m _w > m ₀	-	-	<0.1%	41%
254	L_2.1	6	1	10.00	9.98	0.02	9.00	1.00	10.02	1.16	11.60	1.59	m _w > m ₀	-	-	<0.1%	41%
261	L_2.4	6	1	10.00	9.89	0.11	9.00	1.00	10.11	1.15	11.60	0.70	0.46	0.66	1.59	39%	42%
262	L_2.4	6	1	10.00	9.89	0.11	9.02	1.00	10.13	1.15	11.60	0.66	0.50	0.76	1.65	43%	42%
269	N15	6	1	10.00	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.33	0.83	2.5	2.25	71%	62%
270	N15	6	1	10.00	9.91	0.09	9.00	1.00	10.09	1.15	11.60	0.51	0.65	1.3	1.96	56%	62%
277	N25	6	5	4.00	3.85	0.15	18.00	2.00	20.15	1.15	23.20	0.21	4.96	24.1	2.07	82%	40%
278	N25	6	5	4.00	3.85	0.15	18.01	2.01	20.17	1.16	23.32	0.22	4.91	22.5	2.04	81%	40%
285	TS	12	1	10.00	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.41	0.75	1.8	2.66	64%	40%
286	TS	12	1	10.00	9.91	0.09	9.00	1.00	10.09	1.15	11.60	0.36	0.80	2.2	2.74	69%	42%
293	L_2.1	12	1	10.00	9.98	0.02	9.01	1.00	10.03	1.16	11.60	1.17	m _w > m ₀	-	-	<0.1%	42%
294	L_2.1	12	1	10.00	9.98	0.02	9.03	1.00	10.05	1.15	11.60	1.10	0.06	0.05	0.91	5%	40%
301	L_2.4	12	1	10.00	9.89	0.11	9.01	1.01	10.13	1.16	11.72	0.62	0.55	0.88	1.71	46%	40%
302	L_2.4	12	1	10.00	9.89	0.11	9.00	1.00	10.11	1.15	11.60	0.57	0.59	1.02	1.78	50%	39%
309	N15	12	1	10.00	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.40	0.76	1.9	2.13	65%	39%
310	N15	12	1	10.00	9.91	0.09	9.03	1.00	10.12	1.15	11.60	0.40	0.76	1.9	2.13	65%	36%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2} solution Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [µg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
317	N25	12	5	4.00	3.85	0.15	18.00	2.01	20.16	1.16	23.32	0.15	5.29	35.9	2.25	87%	36%
318	N25	12	5	4.01	3.86	0.15	18.00	2.00	20.15	1.15	23.20	0.16	5.16	31.4	2.19	86%	40%
325	TS	24	1	10.00	9.91	0.09	9.02	1.00	10.11	1.15	11.60	0.35	0.81	2.3	2.76	69%	43%
326	TS	24	1	10.00	9.91	0.09	9.01	1.01	10.11	1.16	11.72	0.37	0.80	2.2	2.73	68%	43%
333	L_2.1	24	1	10.00	9.98	0.02	9.01	1.00	10.03	1.16	11.60	1.19	m _w > m ₀	-	-	<0.1%	39%
334	L_2.1	24	1	10.00	9.98	0.02	9.02	1.00	10.04	1.15	11.60	1.15	0.00	0.00	-0.31	0%	39%
341	L_2.4	24	1	10.00	9.89	0.11	9.03	1.00	10.14	1.14	11.60	0.44	0.72	1.7	1.99	62%	46%
342	L_2.4	24	1	10.00	9.89	0.11	9.03	1.00	10.14	1.14	11.60	0.44	0.72	1.7	1.99	62%	46%
349	N15	24	1	10.00	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.32	0.84	2.6	2.27	72%	40%
350	N15	24	1	10.00	9.91	0.09	9.00	1.00	10.09	1.15	11.60	0.33	0.83	2.5	2.25	71%	40%
357	N25	24	5	4.00	3.85	0.15	18.01	2.00	20.16	1.15	23.20	0.18	5.10	28.8	2.15	85%	40%
358	N25	24	5	4.00	3.85	0.15	18.01	2.00	20.16	1.15	23.20	0.18	5.10	28.6	2.15	85%	40%
361	TS	48	1	10.00	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.38	0.79	2.1	2.72	67%	38%
362	TS	48	1	10.00	9.91	0.09	9.02	1.00	10.11	1.15	11.60	0.34	0.83	2.5	2.79	71%	38%
369	L_2.1	48	1	10.00	9.98	0.02	9.00	1.00	10.02	1.16	11.60	1.13	0.02	0.02	0.50	2%	41%
370	L_2.1	48	1	10.00	9.98	0.02	9.02	1.00	10.04	1.15	11.60	1.14	0.02	0.02	0.41	2%	41%
377	L_2.4	48	1	10.00	9.89	0.11	9.00	1.01	10.12	1.16	11.72	0.49	0.68	1.4	1.91	58%	44%
378	L_2.4	48	1	10.00	9.89	0.11	9.01	1.00	10.12	1.15	11.60	0.47	0.69	1.5	1.94	59%	44%
385	N15	48	1	10.00	9.91	0.09	9.01	1.00	10.10	1.15	11.60	0.36	0.80	2.2	2.19	68%	35%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	Wet soil [g]	m _{soil} [g]	V _{ws} [g]	V _{CaCl2 solution} Pre-equ. [mL]	Vol. stock solution [mL]	V ₀ [mL]	C ₀ [ug/mL]	m ₀ [ug]	C _w [ug/mL]	C _s [μg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR water [%]
386	N15	48	1	10.00	9.91	0.09	9.00	1.00	10.09	1.15	11.60	0.38	0.79	2.1	2.17	67%	35%
389	N25	48	5	4.00	3.85	0.15	18.03	2.01	20.19	1.15	23.32	0.14	5.32	37.5	2.26	88%	45%
390	N25	48	5	4.00	3.85	0.15	18.00	2.00	20.15	1.15	23.20	0.13	5.34	40.3	2.30	88%	45%

4 Conclusion

This report presents the determination of log K_{oc} values for three substances: 1H-benzotriazole (CAS: 95-14-7, EC: 202-394-1), 4-methyl-benzotriazole (CAS: 29878-31-7, EC: 249-921-1), and 5-methyl-benzotriazole (CAS: 136-85-6, EC: 205-265-8), in accordance with OECD Test Guideline 106. The soils selected for testing varied in total organic carbon (TOC) content, pH, and clay content, as recommended by the guideline. Sample analysis was conducted using measurement via LC-MS/MS.

The lowest derived log K_{oc} values for 1H-benzotriazole range from 1.51 to 2.85, for 4-methyl-benzotriazole from 1.39 to 2.52, and for 5-methyl-benzotriazole from 1.91 to 2.72 across all test soils. The average log K_{oc} values for the soils with the lowest adsorption were 1.55±0.03 (lowest value 1.51), 1.48±0.07 (lowest value 1.39) and 1.96±0.03 (lowest value 1.91) for 1H-benzotriazole, 4-methyl-benzotriazole, and 5-methyl-benzotriazole, respectively, using atrazine-d5 as the IS. As an additional quality assurance step, the soils with the lowest indicated log K_{oc} were reanalyzed using substance-specific ISs. The results were overall more reliable based on analytical recovery and yielded slightly higher log K_{oc} values.

Therefore, based on the lowest derived log K_{oc} values (2.01 for 1H-benzotriazole, 2.15 for 4-methyl-benzotriazole, and 2.30 for 5-methyl-benzotriazole) determined using the substance specific ISs, and according to the criteria outlined in the CLP Regulation (EC No. 1272/2008), all three substances are classified as mobile (M).

5 List of references

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A.1 QA for Tier 1

Table A 1 - Test results blank-, control samples and spiking solutions Tier 1

Sample Nr.	Sample Type	Soil used	Equilibration time [h]	L/S	BTZ [ug/L]
25	0.1 M CaCl ₂ Blank	-	48	-	<LOD
26	0.1 M CaCl ₂ Blank	-	48	-	<LOD
63	Soil-Water blank	TS	48	1	<LOD
64	Soil-Water blank	TS	48	1	<LOD
65	Soil-Water blank	N25	48	1	<LOD
66	Soil-Water blank	N25	48	1	<LOD
31	Control sample (1.17 ug/mL BTZ)	-	48	-	1.03
33	Control sample (1.17 ug/mL BTZ)	-	48	-	1.10
38	Spiking Solution BTZ	-	-	-	11.70

Table A 2 - Analytical recovery rates in the water phase in Tier 1

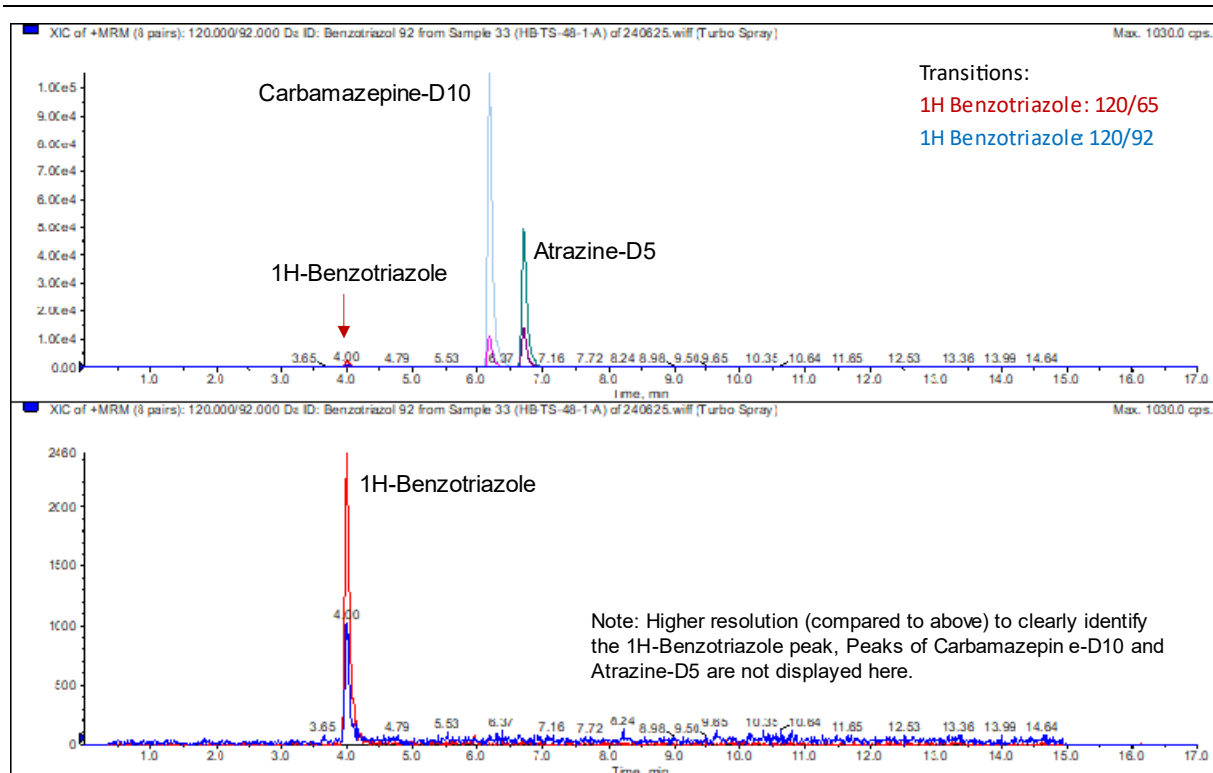
Soil used	Equilibration time [h]	L/S	BTZ [%]
TS	48	2	97.4 to 116 (n=2)
N25	48	2	102.0 to 110.7 (n=2)

Table A 3 - Analytical recovery rates in selected soil samples in Tier 1

Soil used	Equilibration time [h]	L/S	BTZ [%] V _{inj} = 20 µl	BTZ [%] V _{inj} = 5 µl (V _{inj} = 20 µl)
TS	48	1	43.9	-
N25	48	1	36.0 to 84.0 (n=2)	73 (36)

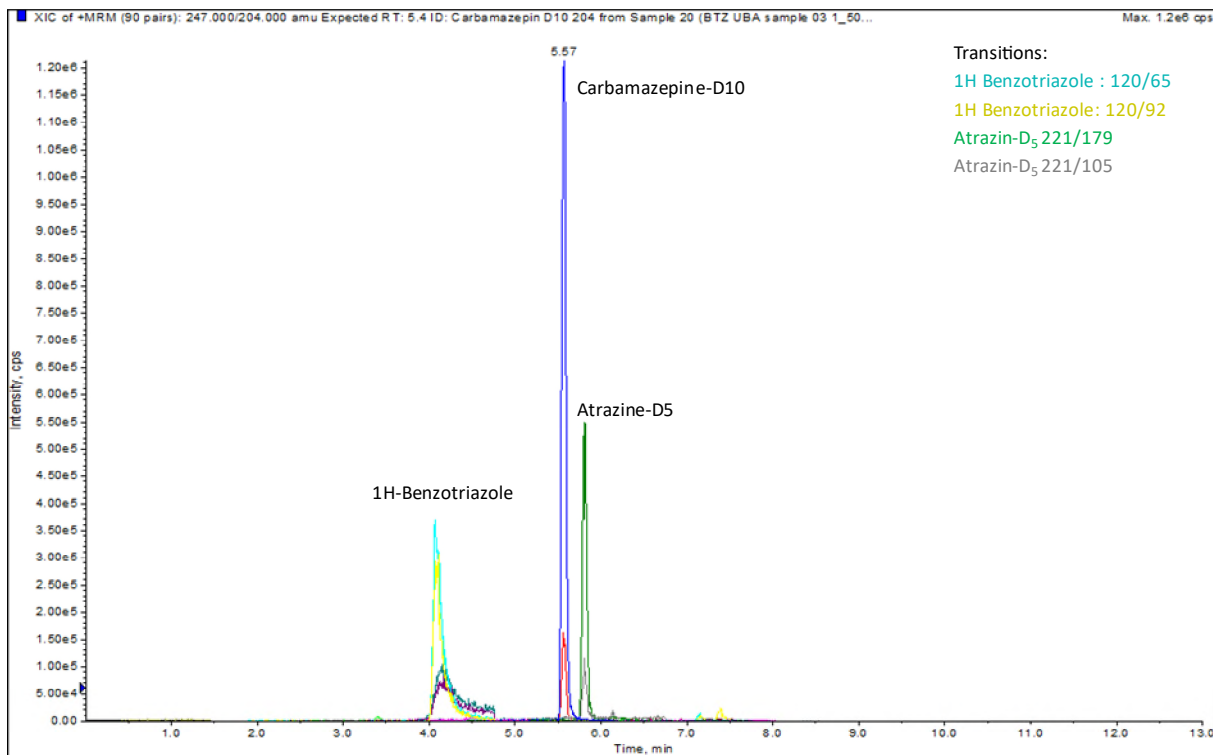
A.1.1 Selected Chromatograms from Tier 1

Figure A 1 – Chromatogram for soil sample nr. 3: BTZ-TS-48-1-A- 20µL injection volume using atrazine-d5 as IS.



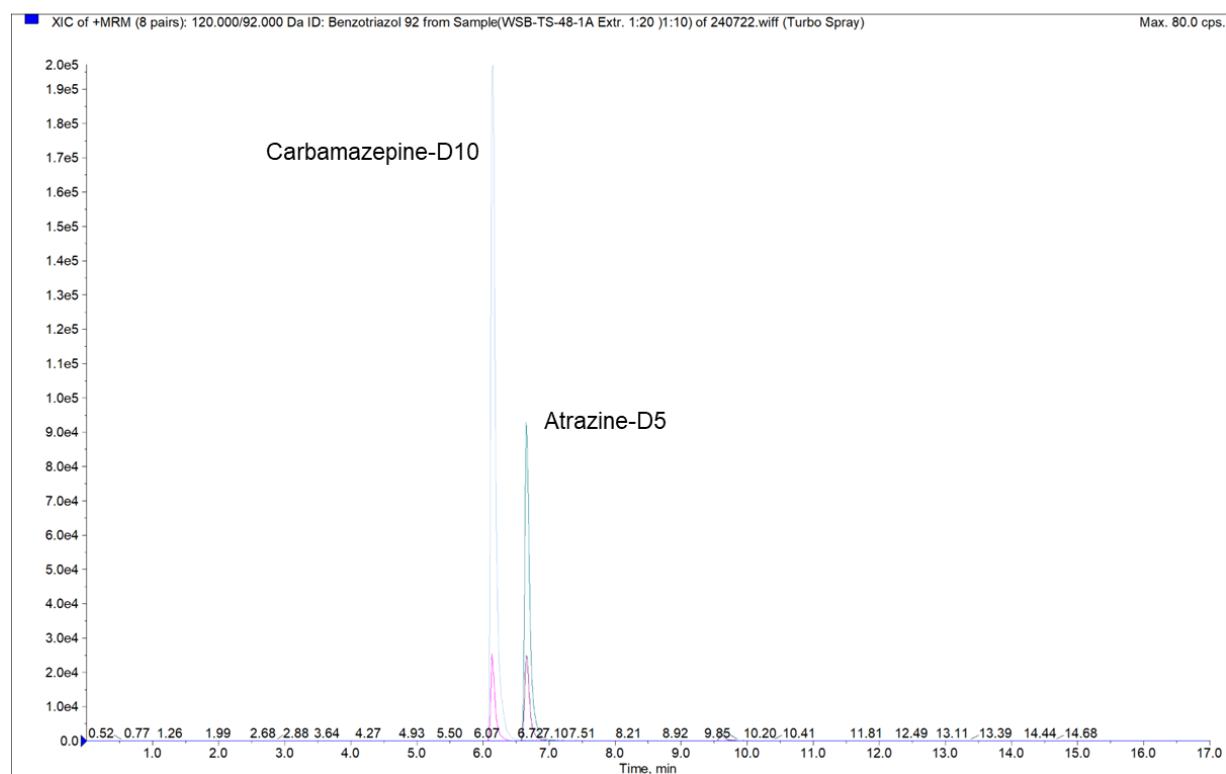
Source: Original Figure

Figure A 2 – Chromatogram for water sample nr. 41: BTZ-TS-48-1-A- 20µL injection volume using atrazine-d5 as IS.



Source: Original Figure

Figure A 3 - Chromatogram for water sample nr.27 WSB-TS-48-1_A – Soil-Water Blank- 20µL injection volume using atrazine-d5 as IS.



Source: Original Figure

A.2 QA for Tier 2

A.2.1 Analysis using the analogue IS (atrazine-d5)

Table A 4 - Test results blank-, control samples and spiking solutions Tier 2

Sample Nr.	Sample Typ	Soil used	Shaking time [h]	L/S	BTZ [ug/L]	4-MB [ug/L]	5-MB [ug/L]
391	Soil-Water blank	TS	48	-	N.A*	<LOD	<LOD
393	Soil-Water blank	L_2.1	48	-	<LOD	<LOD	<LOD
395	Soil-Water blank	L_2.4	48	1	<LOD	<LOD	<LOD
397	Soil-Water blank	N15	48	1	<LOD	<LOD	<LOD
399	Soil-Water blank	N25	48	1	N.A*	<LOD	<LOD
225	Control sample (0.87 ug/mL 4-MB)	-	48	-	N.A.	0.76	N.A
227	Control sample (1.16 ug/mL 5-MB)	-	48	-	N.A.	N.A.	1.05
37	Spiking solution 5-MB	-	48	-	N.A	N.A	11.6
413	Spiking solution 4-MB	-	48	-	N.A	8.71	N.A
38	Spiking solution (BTZ) – Tier 1	-	-	-	12.36	N.A	N.A
412	Spiking solution (BTZ) (5 µl injection)	-	-	-	11.68	N.A	N.A

*Analysed in Tier 1

A.2.2 Analytical recovery rates

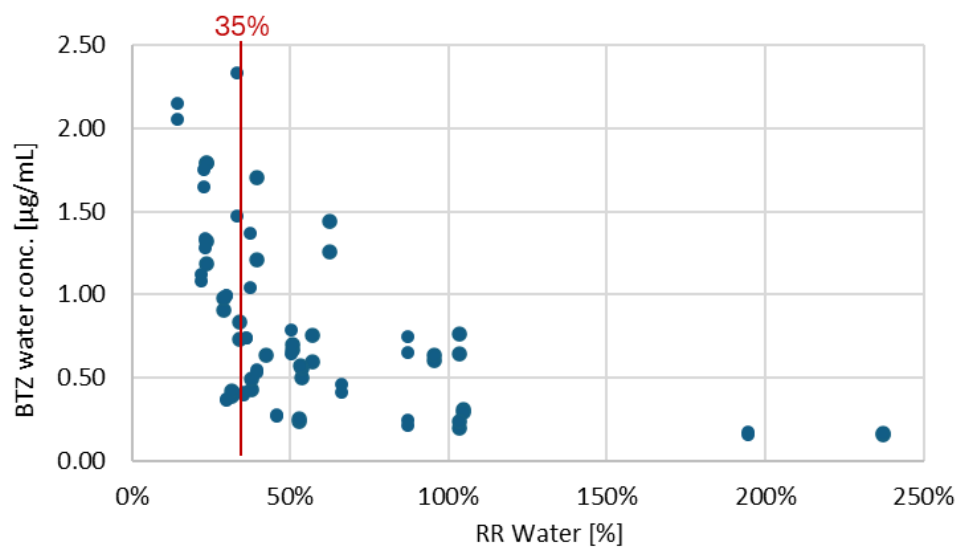
Table A 5 - Average analytical recovery rates in soil samples in Tier 2

Soil used	L/S	BTZ [%] V _{inj} = 20 ul	4-MB [%] V _{inj} = 20 ul	4-MB [%] V _{inj} = 5 ul	5-MB [%] V _{inj} = 20 ul
TS	1	-	50 (n=1)		50 (60) (n=1)
N15	1	-	-	-	-
N25	5	-	62 to 65 (n=2)	80 (65) (n=1)	-
L_2.1	1	38 (n=1)	45 (n=1)	-	-
L_2.4	1	47 (n=1)	55 (n=1)	-	-

Table A 6 - Analytical recovery rates in the water phase for each timestep Tier 2

Soil used	L/S	Equilibration time [t]	BTZ [%] V _{inj} = 20 ul	BTZ [%] V _{inj} = 5 ul	4-MB, 5-MB [%]
TS	1	6h	50%	57%	40%
L-2.1	1	6h	87%	103%	41%
L-2.4	1	6h	23%	24%	42%
N15	1	6h	195%	237%	62%
N25	5	6h	35%	38%	40%
TS	1	12h	73%	105%	42%
L-2.1	1	12h	30%	62%	40%
L-2.4	1	12h	14%	31%	39%
N15	1	12h	22%	54%	36%
N25	5	12h	30%	31%	40%
TS	1	24h	23%	29%	43%
L-2.1	1	24h	33%	40%	39%
L-2.4	1	24h	66%	24%	46%
N15	1	24h	87%	103%	40%
N25	5	24h	46%	53%	40%
TS	1	48h	42%	-	38%
L-2.1	1	48h	37%	96%	41%
L-2.4	1	48h	36%	48%	44%
N15	1	48h	39%	34%	35%
N25	5	48h	42%	-	45%

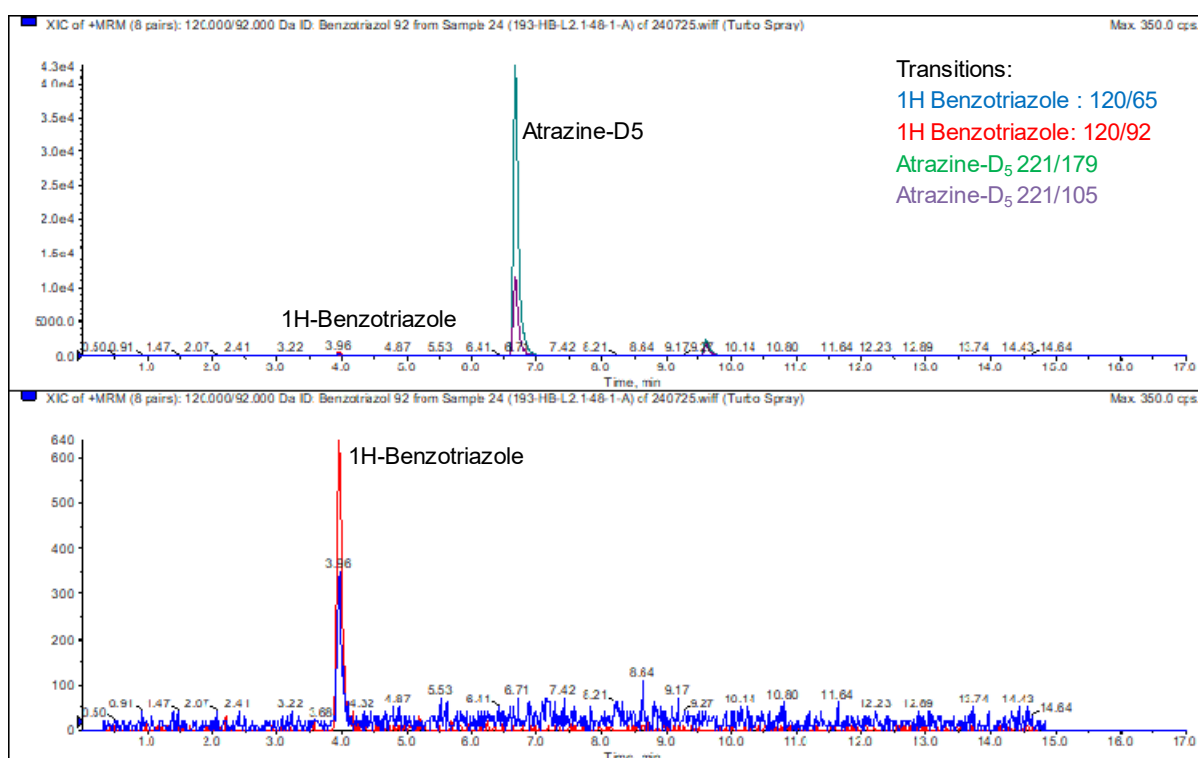
Figure A 4 – Plotted recovery rates (RR) in water samples and the measured water concentration for all soils for BTZ using 5 and 20 µl of injection volume.



Note: For each soil, the same number of samples was analyzed for BTZ. Therefore, if the recovery rate [%] in the water phase does not influence the measured concentrations, the displayed data should be grouped. The scattering of the data points indicates that the recovery rate tends to decrease with increasing concentration. A cutoff of 35% was chosen as a compromise of not removing too much data (particularly at high concentrations) vs biases caused by decreasing recovery rates. A higher cut off would have led to a selection bias in choosing data with lower water concentrations.

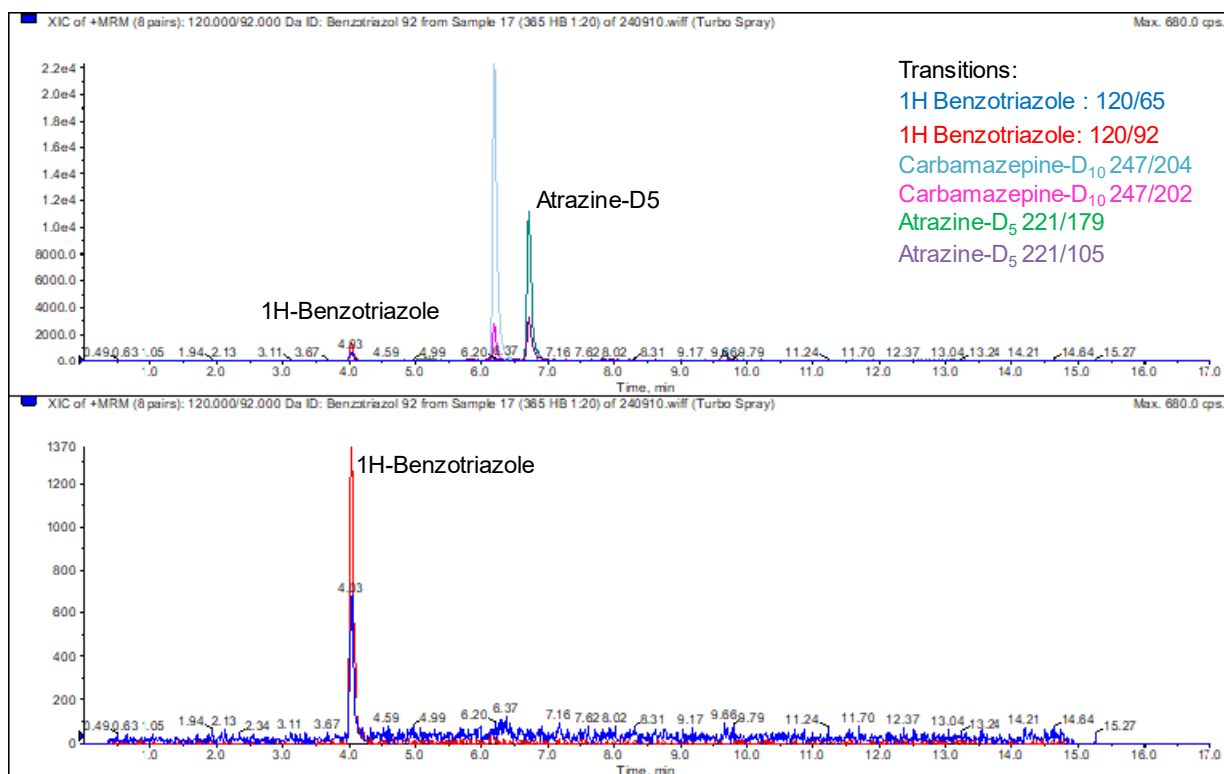
A.2.3 Selected Chromatograms from Tier 2

Figure A 5 – Chromatogram for soil sample nr. 193: BTZ-L2.1-48-1-A- 20µL injection volume using atrazine-d5 as IS.



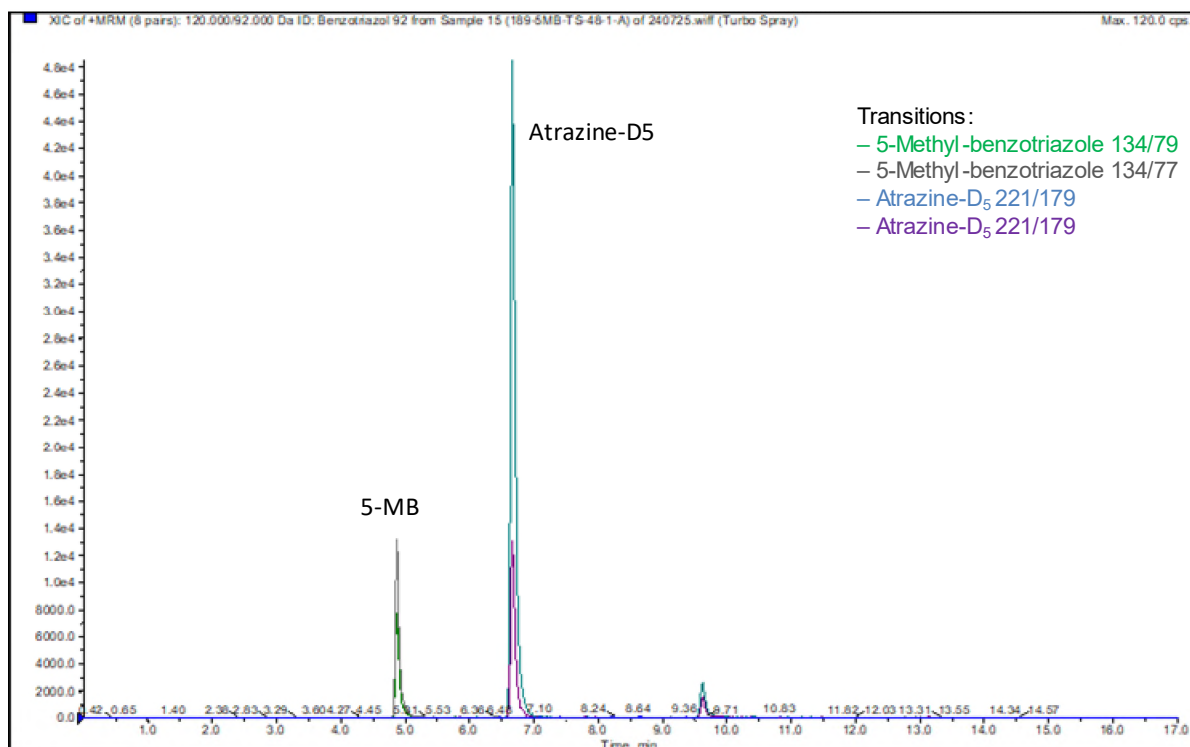
Source: Original Figure

Figure A 6 – Chromatogram for water sample nr. 365: BTZ-L2.1-48-1-A- 20µL injection volume using atrazine-d5 as IS.



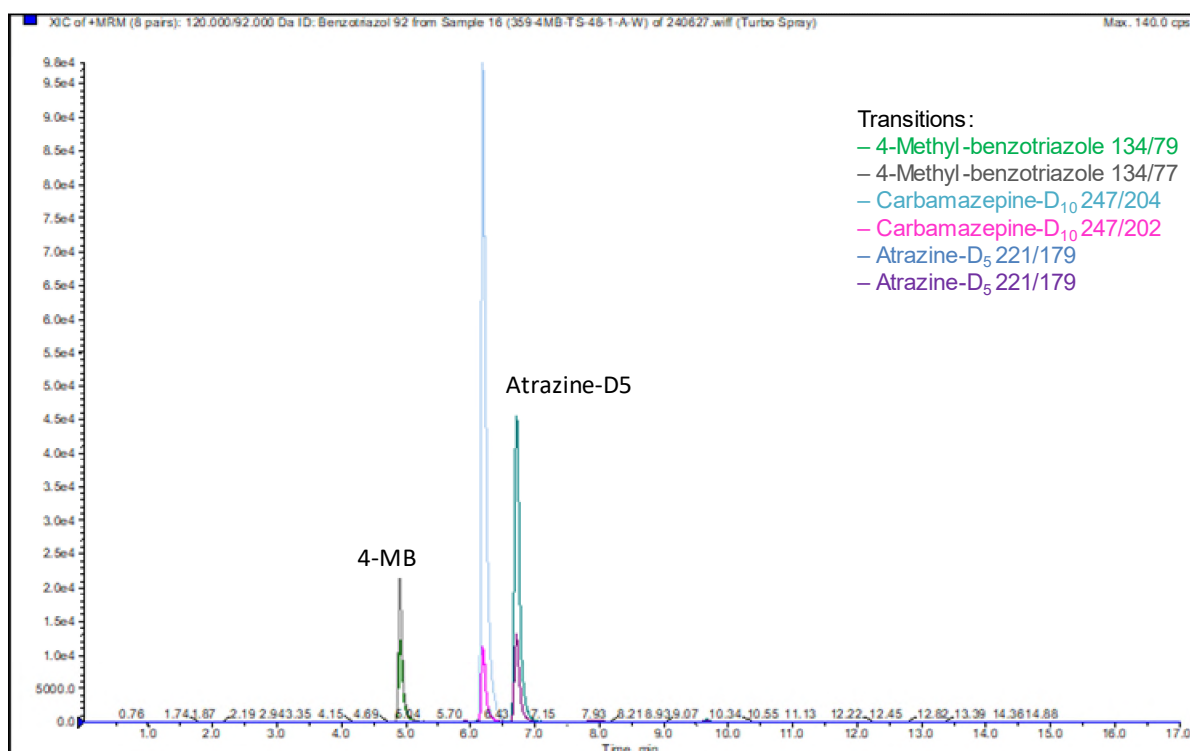
Source: Original Figure

Figure A 7- Chromatogram for soil sample nr.189-5MB-TS-48-1_A – 20µL injection volume using atrazine-d5 as IS.



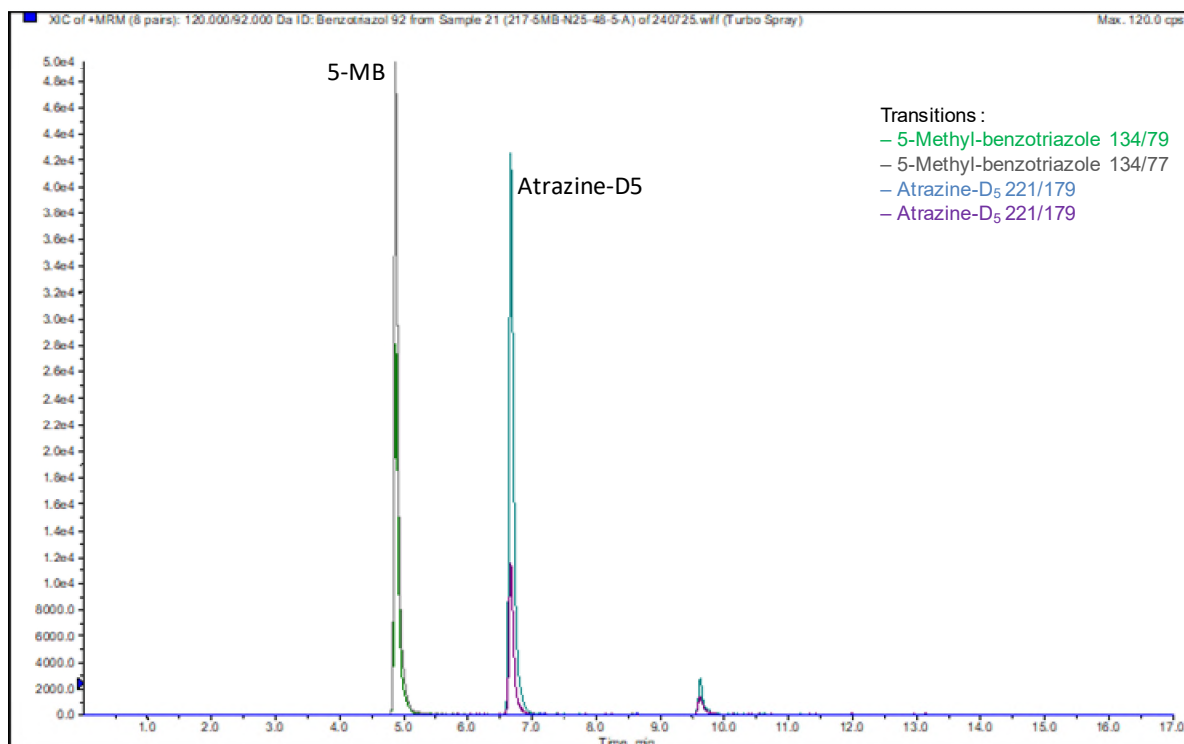
Source: Original Figure

Figure A 8 - Chromatogram for water sample nr.359-4MB-TS-48-1_A – 20µL injection volume using atrazine-d5 as IS.



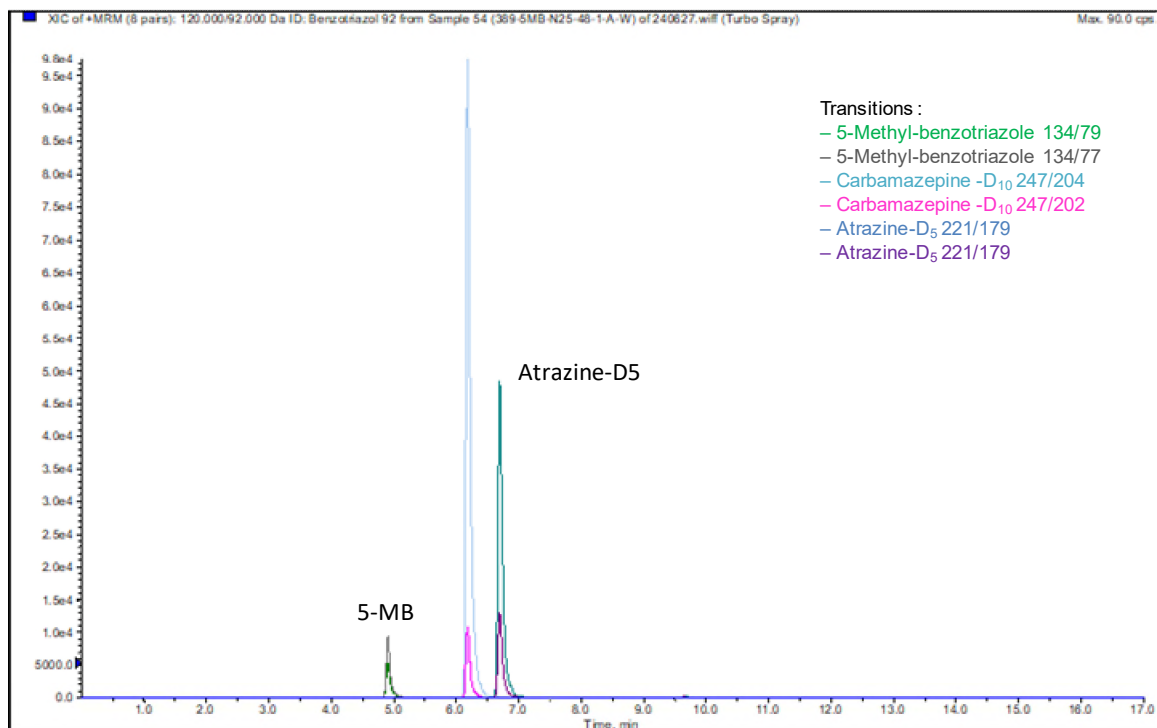
Source: Original Figure

Figure A 9 - Chromatogram for soil sample nr.217-5MB-N25-48-5_A – 20µL injection volume using atrazine-d5 as IS.



Source: Original Figure

Figure A 10 - Chromatogram for water sample nr.389-5MB-N25-48-5_A – 20µL injection volume using atrazine-d5 as IS.



Source: Original Figure

A.2.4 Analysis using the stable-labelled analogue IS

1H-benzotriazole

Table A 7 – Reanalysed water samples for BTZ using 1H-benzotriazole-D4 as IS.

Sample Nr.	Soil type	Equilibration time [h]	C _w [µg/mL]	C _s [µg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR Water [%]
257	L_2.4	6	2.13	0.46	0.21	1.10	17%*	50.4%
258	L_2.4	6	1.27	1.34	1.06	1.79	51%*	89.2%
297	L_2.4	12	1.14	1.48	1.30	1.88	56%*	86.0%
298	L_2.4	12	1.36	1.28	0.94	1.74	48%*	73.6%
337	L_2.4	24	0.73	1.92	2.6	2.19	72%	110.6%
338	L_2.4	24	0.91	1.73	1.9	2.05	65%	87.4%
373	L_2.4	48	0.95	1.67	1.8	2.01	63%	66.8%
374	L_2.4	48	0.70	1.93	2.8	2.21	73%	102.6%
273**	N25	6	0.40	11.46	28.4	2.14	84%	109.3%
274**	N25	6	0.43	11.31	26.1	2.11	83%	107.8%
313	N25	12	0.48	11.08	23.3	2.06	82%	92.0%
314	N25	12	0.43	11.33	26.4	2.11	83%	96.8%
353	N25	24	0.48	11.07	23.1	2.05	82%	87.6%
354	N25	24	0.42	11.37	27.0	2.12	84%	91.2%
61	N25	48	0.30	12.01	40.3	2.30	88%	102.2%
62	N25	48	0.30	12.02	40.6	2.30	89%	98.5%

*Samples were not included in log K_{oc} calculations due to the fact that for at least one duplicate the fraction sorbed to the soil [A] was <50%, the minimum threshold recommended by OECD TG 106.

**Samples were excluded from the log K_{oc} calculations to ensure conformity with the data included in the CLP assessment.

Table A 8 - Test results spiking solutions Tier 2 using 1H-benzotriazole-D4 as IS.

Sample Nr.	Sample Typ	Soil used	Shaking time [h]	L/S	BTZ [µg/L]
412	Spiking Solution BTZ	-	-	-	26.12

Table A 9 –Comparison of BTZ concentration in the water phase and derived log K_{oc} values using 1H-benzotriazole-D4 and atrazine-d5 as IS.

Sample Nr.	C _w 1H-benzotriazole-D4 [ug/mL]	C _w atrazine-d5	Factor C _w 1H-benzotriazole-D4 / C _w atrazine-d5	log K _{oc} [L/Kg] 1H-benzotriazole-D4	Log K _{oc} atrazine-d5	Difference log K _{oc} 1H-benzotriazole-D4 and log K _{oc} atrazine-d5
257	2.13	1.79	1.19	1.10	-0.48	1.58
258	1.27	1.79	0.71	1.79	-0.48	2.28
297	1.14	0.67	1.70	1.88	1.65	0.23
298	1.36	0.70	1.95	1.74	1.61	0.14
337	0.73	0.41	1.80	2.19	2.05	0.14
338	0.91	0.46	2.00	2.05	1.97	0.07
373	0.95	0.63	1.51	2.01	1.71	0.30
374	0.70	0.57	1.22	2.21	1.80	0.41
273	0.40	0.43	0.95	2.14	1.55	0.60
274	0.43	0.49	0.88	2.11	1.43	0.68
313	0.48	0.39	1.22	2.06	1.61	0.45
314	0.43	0.42	1.01	2.11	1.55	0.56
353	0.48	0.23	2.05	2.05	1.93	0.12
354	0.42	0.26	1.65	2.12	1.88	0.24
61	0.30	0.13	2.27	1.35	2.30	0.95
62	0.30	0.12	2.51	1.52	2.30	0.77

4-methyl-benzotriazole (4-MB)

Table A 10 – Reanalysed water samples for 4-MB using 4-methyl-1H-benzotriazole-D3 as IS.

Sample Nr.	Soil type	Equilibration time [h]	C _w [ug/mL]	C _s [μg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR Water [%]
259*	L_2.4	6	0.67	1.62	2.43	2.16	70%	114.7%
260*	L_2.4	6	0.87	1.42	1.64	1.98	62%	91.5%
299	L_2.4	12	0.65	1.64	2.53	2.17	71%	110.6%
300	L_2.4	12	0.64	1.65	2.59	2.18	72%	116.6%
339	L_2.4	24	0.59	1.70	2.9	2.23	74%	119.0%
340	L_2.4	24	0.67	1.62	2.4	2.15	70%	103.9%
375	L_2.4	48	0.57	1.72	3.0	2.25	75%	113.4%
376	L_2.4	48	0.53	1.76	3.29	2.29	76%	119.3%

*Samples were not included in log K_{oc} because, for at least one duplicate, the fraction sorbed to the soil [A] had not reached equilibrium compared to later time points.

Table A 11 - Test results spiking solutions and control samples Tier 2 using 4-methyl-1H-benzotriazole-D3 as IS.

Sample Nr.	Sample Typ	Soil used	Shaking time [h]	L/S	BTZ [ug/L]
413	Spiking Solution 4-MB	-	-	-	22.8
225	Control sample 4-MB	-	48	-	2.49
226	Control sample 4-MB	-	48	-	2.27

Table A 12 –Comparison of 4-MB concentration in the water phase and derived log K_{oc} values using 4-methyl-1H-benzotriazole-D3 and atrazine-d5 as IS for the soil L_2.4.

Sample Nr.	C _w 4-methyl-1H-benzotriazole-D3 [ug/mL]	C _w atrazine-d5	Factor C _w 4-methyl-1H-benzotriazole-D3 / C _w atrazine-d5	log K _{oc} [L/Kg] 4-methyl-1H-benzotriazole-D3	Log K _{oc} atrazine-d5	Difference log K _{oc} 4-methyl-1H-benzotriazole-D3 and log K _{oc} atrazine-d5*
259	0.67	0.65	1.02	2.16	1.29	0.87
260	0.87	0.72	1.20	1.98	1.06	0.92
299	0.65	0.57	1.15	2.17	1.49	0.68
300	0.64	0.58	1.09	2.18	1.45	0.73
339	0.59	0.51	1.14	2.23	1.61	0.63
340	0.67	0.60	1.12	2.15	1.42	0.73
375	0.57	0.56	1.02	2.25	1.51	0.74
376	0.53	0.61	0.87	2.29	1.39	0.90

*It should be noted that the difference between the calculated log K_{oc} values using 4-methyl-1H-benzotriazole-D3 and atrazine-d5 is primarily driven by the concentration differences in the spiking solution used for the calculations and, to a lesser extent, by variations in C_w in the water samples.

5-methyl-benzotriazole (5-MB)

Table A 13 – Reanalysed water samples for 5-MB using 5-methyl-1H-benzotriazole-D6 as IS.

Sample Nr.	Soil type	Equilibration time [h]	C _w [ug/mL]	C _s [μg/g]	K _d [L/Kg]	log K _{oc} [L/Kg]	A [%]	RR Water [%]
261*	L_2.4	6	0.68	1.92	2.81	2.22	73%	94.6%
262*	L_2.4	6	0.55	2.05	3.77	2.35	79%	109.8%
301	L_2.4	12	0.60	2.02	3.37	2.30	77%	100.1%
302	L_2.4	12	0.58	2.02	3.50	2.31	77%	106.0%
341	L_2.4	24	0.53	2.07	3.9	2.37	79%	96.5%
342	L_2.4	24	0.59	2.01	3.4	2.30	77%	88.7%
377	L_2.4	48	0.46	2.17	4.7	2.44	82%	107.5%
378	L_2.4	48	0.47	2.13	4.5	2.42	82%	99.4%
269	N15	6	0.43	2.17	5.0	2.55	83%	106.3%
270	N15	6	0.46	2.14	4.6	2.52	82%	102.7%

*Samples were not included in log K_{oc} because, for at least one duplicate, the fraction sorbed to the soil [A] had not reached equilibrium compared to later time points.

Table A 14 - Test results spiking solutions Tier 2 using 5-methyl-1H-benzotriazole-D6 as IS.

Sample Nr.	Sample Typ	Soil used	Shaking time [h]	L/S	BTZ [ug/L]
414	Spiking Solution 5-MB	-	-	-	25.85

Table A 15 –Comparison of 5-MB concentration in the water phase and derived log K_{oc} values using 5-methyl-1H-benzotriazole-D6 and atrazine-d5 as IS for the soil L_2.4 and N15 (nr. 269 and 270).

Sample Nr.	C _w 5-methyl-1H-benzotriazole-D6 [ug/mL]	C _w atrazine-d5	Factor C _w 5-methyl-1H-benzotriazole-D6 / C _w atrazine-d5	log K _{oc} [L/Kg] 5-methyl-1H-benzotriazole-D6	Log K _{oc} atrazine-d5	Difference log K _{oc} 5-methyl-1H-benzotriazole-D6 and log K _{oc} atrazine-d5*
261	0.68	0.70	0.98	2.22	1.59	0.63
262	0.55	0.66	0.83	2.35	1.65	0.70
301	0.60	0.62	0.96	2.30	1.71	0.58
302	0.58	0.57	1.01	2.31	1.78	0.53
341	0.53	0.44	1.20	2.37	1.99	0.38
342	0.59	0.44	1.34	2.30	1.99	0.32
377	0.46	0.49	0.94	2.44	1.91	0.53
378	0.47	0.47	1.01	2.42	1.94	0.48
269	0.43	0.33	1.30	2.55	2.25	0.30
270	0.46	0.51	0.90	2.52	1.96	0.56

*It should be noted that the difference between the calculated log K_{oc} values using 4-methyl-1H-benzotriazole-D3 and atrazine-d5 is primarily driven by the concentration differences in the spiking solution used for the calculations and, to a lesser extent, by variations in C_w in the water samples.

A.2.5 Selected Chromatograms from Tier 2 using stable isotope-labelled analogue ISs

1H-benzotriazole

Figure A 11 – Chromatogram for water sample nr. 373: BTZ-L-2.4-48-1-A- 20µL injection volume using 1H-benzotriazole-D₄ as IS.

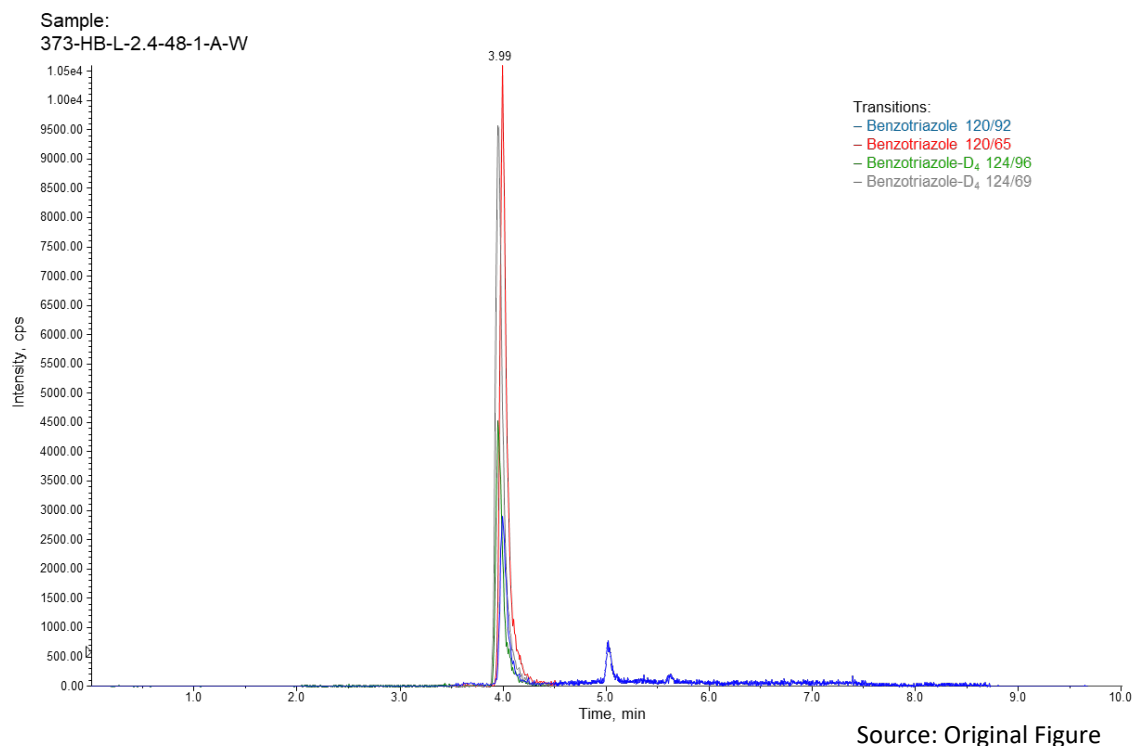
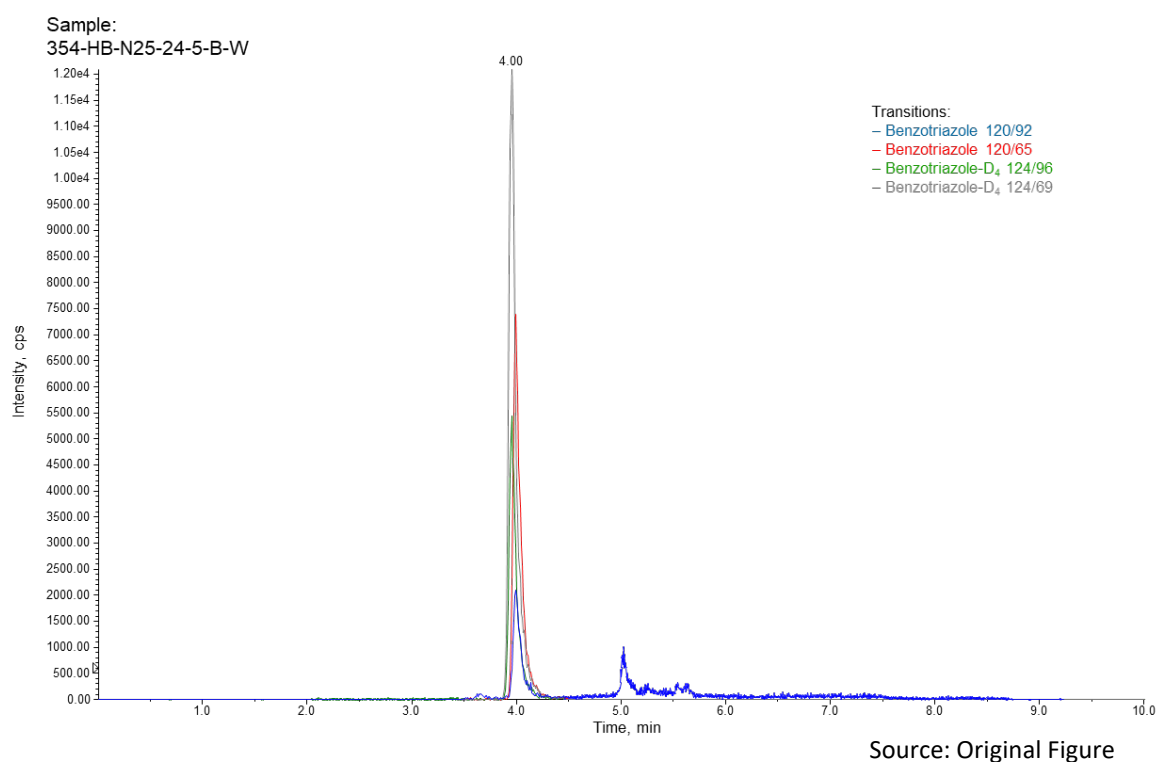
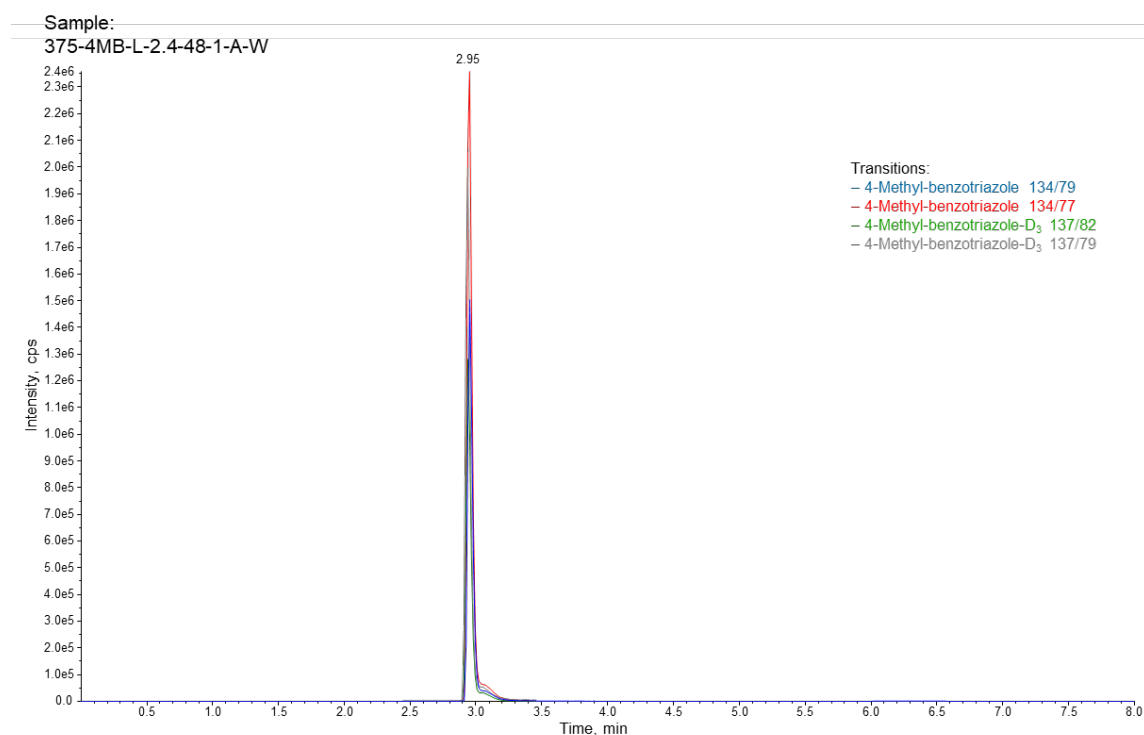


Figure A 12 – Chromatogram for water sample nr. 354: BTZ-N25-24-5-B- 20µL injection volume using 1H-benzotriazole-D₄ as IS.



4-methyl-benzotriazole (4-MB)

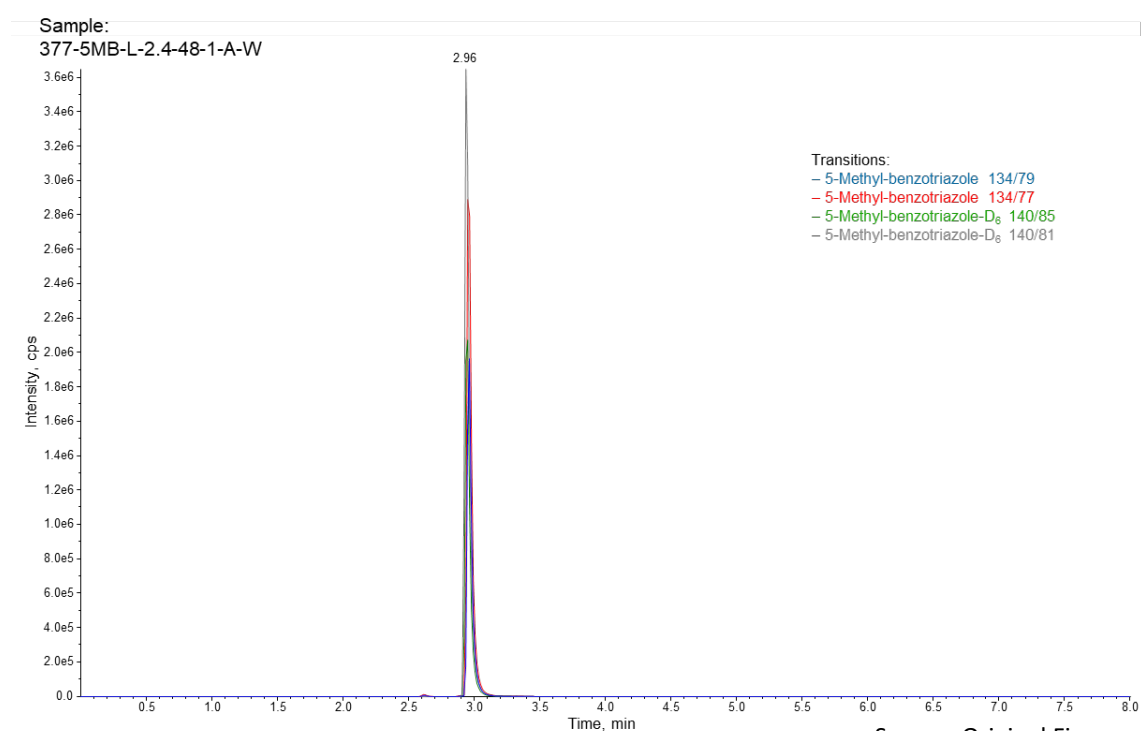
Figure A 13 – Chromatogram for water sample nr. 375: 4-MB-L2.4-48-1-A- 20µL injection volume using 4-methyl-1H-benzotriazole-D3 as IS.



Source: Original Figure

5-methyl-benzotriazole (5-MB)

Figure A 14 – Chromatogram for water sample nr. 377: 5-MB-L2.4-48-1-A- 20µL injection volume using 5-methyl-1H-benzotriazole-D6 as IS.



Source: Original Figure