TEXTE
149/2025

Final report

Determination of log Koc values according to OECD TG 106

for Melamine (CAS: 108-78-1)

by:

Michel Hubert, Hans Peter H. Arp
Norwegian Geotechnical Institute, Oslo

Lars Eschlbeck, Stephan Wagner
Hochschule Fresenius: University of Applied Sciences, Idstein

publisher:
German Environment Agency

TEXTE 149/2025

Project No. 187916
FB001851/ENG

Final report

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

for Melamine (CAS: 108-78-1)

by

Michel Hubert, Hans Peter H. Arp
Norwegian Geotechnical Institute, Oslo

Lars Eschlbeck, Stephan Wagner
Hochschule Fresenius: University of Applied Sciences, Idstein

On behalf of the German Environment Agency

Imprint

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

Report performed by:
Norwegian Geotechnical Institute
P.O. Box. 3930 Ullevål Stadion
0806 Oslo
Norway

Report completed in:
July 2025

Edited by:
Section IV 2.3 Chemicals
Name Ivo Schliebner & Oliver Machate

DOI:
https://doi.org/10.60810/openumwelt-7998

ISSN 1862-4804

Dessau-Roßlau, November 2025

Abstract: Determination of log Koc 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 melamine (CAS: 108-78-1) 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.

The derived log K_{oc} values from the five individual soils applied in the test range for melamine from 1.57 to 3.07. The average log K_{oc} of the soil with the lowest adsorption is 1.66±0.09 (n=8) (lowest value 1.57); this soil was reanalysed using a second internal standard, and a similar result is obtained of 1.59 ± 0.03 (n = 6) (lowest value 1.53). In this study, there is no clear indication that the log K_{oc} of melamine is affected by soil pH, which ranges from 4.6 to 7.8. Melamine should be considered to be very mobile (vM) using the lowest derived log K_{oc} values.

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 den Stoff Melamin (CAS: 108-78-1) gemäß der OECD TG 106 Testmethode ermittelt, welche in der Leitlinie zur Anwendung der CLP-Kriterien vorgeschlagen wird.

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

Die abgeleiteten log K_{oc} Werte aller 5 Böden liegen für Melamin zwischen 1,57 und 3,07. Der durchschnittliche log K_{oc} des Bodens mit der geringsten Adsorption beträgt 1,71±0,13 (n = 8) (niedrigster Wert 1,57). Dieser Boden wurde mit einem zweiten internen Standard erneut analysiert, wobei ein ähnliches Ergebnis von 1,59±0,03 (n = 6) (niedrigster Wert 1,53) erzielt wurde. 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 liegt. Basierend auf den in dieser Studie abgeleiteten, niedrigsten log K_{oc}-Werten, sollten alle vier Testsubstanzen als sehr mobil (vM) angesehen werden.

Table of content

List of figures

List of tables

List of abbreviations

Abbreviation	Explanation
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
m_0	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 melamine (CAS: 108-78-1) 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 melamine and to 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. Due to melamine's polar molecular properties the separation of melamine was performed using Hydrophilic Interaction Liquid Chromatography (HILIC). 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 melamine the lowest derived log K_{oc} values from the five individual soils applied in the test ranges from 1.57 to 3.07. For 3 out of 5 soils the fraction of melamine adsorbed to the soil remains constant over all experimental time steps, indicating fast adsorption kinetics (≤6 h). The average log K_{oc} of the soil with the lowest adsorption is 1.66 ± 0.09 (n=8) (minimum 1.57); this soil was reanalysed using a second internal standard, and a similar result is obtained of 1.59 ± 0.03 (n = 6) (minimum 1.53).

The highest log K_{oc} values were observed in soils with high TOC content or high silt/clay content. Additionally, there is some indication that log K_{oc} is influenced by soil pH, with increased sorption at lower pH levels. Based on the lowest log K_{oc} values measured, melamine should be considered to be very mobile (vM).

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 den Stoff Melamin (CAS: 108-78-1) 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 Melamin 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 Trennung von Melamin erfolgt aufgrund der polaren Moleküleigenschaften mittels Hydrophilic Interaction Liquid Chromatography (HILIC), bei der die stationäre Phase polarer ist als die mobile Phase. 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 Melamin liegen die niedrigsten abgeleiteten log K_{oc} Werte aller 5 Böden (L/Kg) zwischen 1.57 und 3.07. Der Anteil des an die Böden adsorbierten Melamins bleibt für 3 der 5 getesteten Böden über alle experimentellen Zeitpunkte hinweg konstant, was auf eine schnelle Adsorptionskinetik (≤6 Stunden) hinweist. Der durchschnittliche log K_{oc} des Bodens mit der geringsten Adsorption beträgt 1,71±0,13 (n = 8) (Minimum 1,57). Dieser Boden wurde mit einem zweiten internen Standard erneut analysiert, wobei ein ähnliches Ergebnis von 1,59±0,03 (n = 6) (Minimum 1,53) erzielt wurde.

Die höchsten log K_{oc} Werte wurden in Böden mit hohem TOC-Gehalt oder hohem Schluff-/Tonanteil beobachtet. Es gibt zudem Hinweise darauf, dass der log K_{oc} Wert vom Boden-pH beeinflusst wird, wobei bei niedrigeren pH-Werten eine erhöhte Sorption auftritt. Basierend auf den in dieser Studie abgeleiteten minimalen log K_{oc}-Werten, sollte Melamin als sehr mobil (vM) 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, introduced 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 compromises 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 melamine (CAS: 108-78-1, EC: 203-615-4), according to OECD TG 106. In this study Tier 1 and Tier 2 are carried out.

2 Materials and Methods

2.1 Substance properties and spiking procedure

The test substance melamine (purity: 99%) (CAS 108-78-1) was obtained from Sigma-Aldrich (Seelze, Germany). Melamine's physico-chemical properties are shown in Table 1.

Table 1: Chemical and physiochemical parameters for melamine

Substance	Molecular weight [g/mol]	Solubility in water [g/L]	Vapour pressure at 25°C [mmHg]	Henry's law constant (atm-m³/mol)	Log K_{ow} [-]	pK_a [-]
Melamine	126.12$^{a)}$	3.48$^{c)1)}$	0.0±1.5$^{b)2}$	1.84E-14 $^{1)}$ 1.89E-13 $^{2)}$	1.51 $^{c)2)}$	5.0$^{d)1}$

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

a) ChemSpider database

b) ACD/Labs Percepta Platform – PhysChem Module

c) SVHC Support Document- Melamine 2022 – using KOCWIN v.2.00 for log Kow

d) Jang et al. (2009)

Based on Hofman-Caris and Claßen (2020), the biodegradability for melamine is (very) low in the environment (Hofman-Caris & Claßen, 2020). Abiotic hydrolysis was not expected to be a relevant degradation pathway for melamine under environmentally relevant conditions (Gong et al., 2016). Direct photolysis and therefore possible photodegradation in aqueous media of melamine was also not expected to be a relevant degradation pathway at ambient conditions for the test conditions of OECD TG 106 (Bianco Prevot et al., 2020).

Melamine, received from Sigma-Aldrich, was in powder/crystal form. An individual substance spike was prepared using a 0.01M $CaCl_2$ solution (Milli-Q water). Melamine was directly dissolved in 0.01 M $CaCl_2$ solution at a concentration of 50 µg/mL. After preparation, the standard was stored in a closed box at 4 °C until further use. The spiking solution was 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 (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 are 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 melamine. Details are provided in section 2.4.

2.3.1 Tier 1

The selection of the appropriate L/S ratio was determined in a preliminary study (OECD TG 106 - Tier 1) and depended on the expected soil-water distribution coefficient (K_d) and the requested relative degree of adsorption, which was based on the limit of detection (LOD) and limit of quantification (LOQ) of the analytical method used. Following the requirements of OECD TG 106 the percentage of a substance adsorbed to the soil should be at least >20%. As a starting point for identifying the optimal L/S ratio, expected log K_{oc} values were taken from previous studies. For melamine expected log K_{oc} values was 1.1 (Arp et al., 2023). The Tier 1 study was conducted on two of the five soils to be tested for a single time period. The time period used was 48 h, during which achievement of equilibrium conditions was expected for melamine, and which is the longest time step recommended by OECD TG 106. The soil selection was based on the OECD TG 106 recommendations to use one soil with a high total organic carbon (TOC) content and low clay content, and another soil with low TOC and high clay content. 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 was taken and heated at 105 °C until no significant change in weight could be observed anymore (approx. 12h). 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 $CaCl_2$. Before melamine was added to the vials, the dried and sieved soil was shaken with $CaCl_2$ 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, melamine 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.). The intended spike concentrations was 5 µg/mL of melamine, 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 0.1M $CaCl_2$ solution), 2:1 (10 g of soil and 20 mL of 0.1M $CaCl_2$ solution), and 5:1 (4g of soil and 20 mL of 0.1 M$CaCl_2$ 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 for all five soils following the same protocol as described above at one concentration and the selected L/S ratio 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 melamine concentration 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 melamine was performed using Hydrophilic Interaction Liquid Chromatography (HILIC), due to its polar molecular properties. An ACQUITY UPLC BEH Amide column (130 Å, 1.7 µm, 2.1 mm × 100 mm, Waters, Eschborn) is used for HILIC. The measuring device is an LC-MS/MS system (LC: Nexera HPLC (Shimadzu), MS: 3200 QTrap® (Sciex)). Prior to measurement external calibration was conducted, and quality control standards were measured alongside the samples to ensure consistency in the results.

For separation the following method parameters are used: Eluent A consists of 95% MilliQ-H_2O and 5% acetonitrile with 5 mmol NH_4-formate buffer, and Eluent B consists of 5% MilliQ-H_2O and 95% acetonitrile with 5 mmol NH_4-formate buffer. Both eluents were adjusted to pH 3 using formic acid. Two transitions were measured and evaluated, one served as quantifier and the other one served as qualifier to confirm the results. Quantification was performed via an external calibration for each target analyte. To ensure accuracy and precision, a 7-point calibration curve with increasing concentrations (0.03 to 1.00 µg/ml) was used. If needed, samples were diluted to ensure that all concentrations fell within the range of the calibration curve.

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:

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

Where $C_{spiked\ sample}$ 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 ($C_8H_9D_5ClN_5$), a structural analogue, was used as internal standard for melamine due to its similar structure rendering it likely to behave in a similar way, giving similar retention time, peak shape and response. Carbamazepine-D10 was additionally spiked into all samples and considered a structural analogue IS, but it was not used for quantification because of the better response of atrazine-d5. Due to high matrix effects selected water samples from one soil were reanalysed with the stabled isotope labelled Analog IS Melamine-13C3-15N3 as an additional quality assurance step.

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, respectively were mixed with Mili-Q water (Type 1, electrical resistance of 18.2 MΩcm at 25 °C) and fortified with 10 µL of internal standard (atrazine-d5, 5 µg/mL or Melamine-13C3-15N3). Atrazine-d5 ($C_8H_9D_5ClN_5$) was used due to its similar structure rendering it likely to behave in a similar way, giving similar retention time, peak shape and response. Ideally, a deuterated form of the target analyte would be used as the internal standard; however, this was not feasible due to procurement challenges and time constraints.

The standard injection volume was 20 µl but was adjusted in Tier 2 due to witnessed poor peak shapes and high matrix effects (ion enhancement) and hence excessively high analytical recovery rates. For the spiked water samples (used for recovery calculations) additionally 15 µL of spiking solution (10 µg/mL) 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. This was conducted using 3 mL of MeOH with 1%NH_3. The soil-solution was then vortexed for 2 minutes 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 internal standard to correct for errors and some of the samples were spiked with the analyte to calculate analytical recovery rates.

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 $CaCl_2$ solution) and control samples (substance + 0.01 M $CaCl_2$ solution) were included in the experimental set up. Blank samples were used to exclude any background concentration of the test substance and to check for possible matrix effects caused by the soil. Control samples were hereby used to validate the stability of the test substance in the 0.1 M $CaCl_2$ solution and to 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 including shaking, centrifuging and all samples preparation steps for further analysis.

To verify the mass balance, soil samples from selected batch tests were additionally solvent-extracted to quantify the solid-phase concentration in the soil. The concentrations of melamine 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.

2.6 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):

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

C^{ads}_{aq} (eq.) is hereby measured, whereas C^{ads}_{s} (eq) is 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, for deriving K_d and finally K_{oc} values only batch test data was used where equilibrium conditions were 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):

$$K_{OC} = K_d \ x \ f_{OC} \qquad \text{eq.3}$$

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):

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

Where m_s^{ads} [µg] is the mass of the test substance adsorbed on the soil 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.

To establish a mass balance (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):

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

Where V_{rec} [mL] is the mass of water recovered after centrifugation, $C^{ads}_{wet\ soil}$ [µg/g w.w] is the substance concentration in the wet soil after centrifugation, C^{ads}_{aq} (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. $C^{ads}_{wet\ soil}$ 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 mass balance is carried out on selected batch test samples.

Boxplots and statistical analysis were conducted using Office 365. Reported values are the minimum log K_{oc} and the average log K_{oc} ± the standard derivation for each soil tested. The reporting of the results follows the requirements of OECD TG 106, Annex 6 (parallel method).

Selected chromatograms for each test substance in Tier 1 and Tier 2 can be found in the appendix (A.1.1 and A.2.4).

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 melamine.

3.1.1 L/S ratios

For melamine, the fraction adsorbed to the TS soil is between 14% (L/S 1:5) and 61% (L/S 1:1). For the N25 soil, the adsorbed fraction ranges from 97% (1:5) to >99% (1:1). It should be noted that the concentration of melamine in the water samples after shaking and phase separation for both duplicates of the N25 soil using an L/S ratio of 1 are below the LOD, indicating high sorption to this soil.

To have more than 20% of the initial mass of melamine adsorbed to the soil, as recommend by OECD TG 106, and while remaining above the LOD in the water phase, a L/S ratio of 1:5 was used for the batch tests with soil N25 and an L/S ratio of 1:1 for the soils TS, N15, L_2.1 and L_2.4, due to lower TOC contents and hence expected lower sorption.

3.1.2 Quality Assurance

. The results of blank samples and control samples are summarized in Appendix A.1. All 0.1 M $CaCl_2$ solution blanks and soil-water blanks are below LOD for melamine indicating that the soils used are not contaminated with the test substance and that there is no measurable cross-contamination in both laboratories.

To ensure accuracy and precision, a 7-point calibration curve with increasing concentration ranging from 0.03 to 1.00 µg/ml for melamine was used. Obtained R^2 values demonstrate linear response ($R^2>0.99$). The LOD for melamine was 0.01 µg/ml and the LOQ 0.03 µg/ml.

To verify the accuracy of the spiking procedure, the 0.1 M $CaCl_2$ spiking solutions used in the batch tests were analysed. The measured concentrations are 52.8 µg/mL for melamine using atrazine-d5 as IS. The respective control samples (1 mL spiking solution + 9 mL 0.1M $CaCl_2$ solution) have measured concentrations of 4.99 to 5.01, which corresponds to an average error of 5%. These values lie within the acceptable error range according to the OECD TG 106 guideline, and the concentrations in the control samples indicate no instability of the test substance in the time scale of the experiment

Analytical recovery rates in the water samples (water RR) vary depending on the soil type used in the batch test. The observed recoveries range from 95.3% to 106.6% for melamine (Table A 2 - Analytical recovery rates in the water phase in Tier 1Table A 2).

The analytical recovery rates for the soil extracts are high and ranged from 345 to 529% (Table A 3) using atrazine-d5 as IS. A reason for these high recovery rates in the soil extracts can be matrix effects from dissolved ions and dissolved organic carbon interacting with the analyte in ways that either dampen (reduce; ion suppression) or increase (ion enhancement) the signal of the analyte in the chromatogram. To assess possible matrix effects, two selected soil samples (nr. 2 and nr. 22) were reanalysed with a reduced injection volume (5 µl instead of 20µl), which yield recovery rates between 64% and 80%—a decrease from previous rates over 400%, suggesting that matrix effects are particularly strong in the soil extracts. Furthermore, the chromatogram peaks improved with a lower injection volume (Appendix A1.1).

It should be noted that even though the analytical recovery rates in the soil extracts vary a lot, the measured concentrations in the soil ($C^{ads}_{wet\ soil}$) show less variation. For soil sample nr.2 the concentration is measured at 0.95 µg/g w.w. (soil RR =529%) compared to 1.05 µg/g w.w. (soil RR =64%). For soil sample nr 22 the concentrations measured is 1.22 µg/g w.w. (soil RR =457%) compared to 2.57 µg/g w.w. (soil RR =80%). All measured aqueous phase and soil concentrations fall in the range of the calibration curve and thus should allow for an accurate quantification.

3.1.3 Mass balance (MB)

The mass balance was calculated for selected batch tests samples (Table 4) based on concentrations of the substance detected in the liquid (C^{ads}_{aq}, directly measured) and solid phase ($C^{ads}_{wet\ soil}$, after extraction of the wet soil). For melamine the mass analytically recovered MB range from 11.5 to 23.5% for N25 and from 50.8 to 56.5% for TS using atrazine-d5 as IS. In comparison no substantial loss has occurred for the control samples, nr 32 and 34 (0.01 M $CaCl_2$ solution and substance spike) Table 4. All samples hereby followed identical sample preparation steps described in paragraph 2.4 and 2.5.

The low melamine recovery in the batch tests can be attributed to several factors. One likely cause is insufficient extraction, where melamine that is adsorbed to the soil is not fully recovered by the used extraction protocol. The analytical recovered mass of melamine is especially low for the soil N25, which has the highest TOC content of all soils tested. Stronger sorption of charged organic compounds has hereby been reported by studies before (Hubert et al., 2023; Sigmund et al., 2022; Ukalska-Jaruga et al., 2023). For melamine, (Wang et al. ., (2014) found in their study a strong correlation between K_f values (Freundlich adsorption coefficient) and TOC in the soil.

Another potential cause is loss during filtration of water samples, particularly from low-density colloidal particles (>0.045 µm) that are not removed by centrifugation and thus excluded, as the internal standard (IS) was added post-filtration. Notably, the N25 aqueous phase, which exhibited especially low recovery, was still visibly turbid after centrifugation. The control samples, consisting solely of melamine and 0.01M $CaCl_2$, were not affected similarly by filtration, as they lacked soil colloids.

Beyond physical losses, matrix effects from dissolved ions and organic carbon may also influence the analyte signal in the chromatogram, thus impacting the mass balance to a certain extent (the analytical recovery rates for Tier 1 are summarised in the Appendix (A.1). As mentioned before, the analytical recovery rates for the soil samples are by far higher.

Further, the results of the quality assurance (3.1.2) do not indicate an instability of the test substance. Given the non-linearity of potential matrix effects (Müller et al., 2020) and the inability to correct for incomplete extraction of the substance, no adjustments are made for these mass losses and the data from the aqueous phase is used for further calculations.

Table 4: Mass balance (MB) for melamine in Tier 1 (shaking time = 48h)

Soil (Water) Sample Nr.	Soil	L/S [-]	C^{ads}_{aq} [µg/mL]	$C^{ads}_{wet\ soil}$ [ug/g w.w]	V_{rec} [mL]	$M_{wet,soil}$ [g w-w-]	m_0 [µg]	MB [%]	Water RR in Samples L/S = 2 [%]	Soil RR [%]
1 (39)a)	TS	1	2.18	1.20	5.92	14.08	52.80	56.5	95.3 to 103.3 (n=2)	345%
2b)(40) a)	TS	1	2.08	0.95	5.89	14.11	53.33	50.8		64%
21(59)a)	N25	5	0.15	2.90	16.29	7.71	105.6	23.5	98.0 to 106.6 (n=2)	351%
22c)(60)a)	N25	5	0.15	1.22	15.93	8.07	106.1	11.5		80%
Control Samples (0.01M $CaCl_2$ Solution)										
32	-	-	5.01	-	10.01	0	52.8	95%	Not available	-
34	-	-	4.99	-	10.01	0	52.8	95%	Not available	-

Explanation of Abbreviations used:

C^{ads}_{aq} = Concentration in the water phase	$C^{ads}_{wet\ soil}$ = Soil concentration (porewater + soil)
V_{rec}= Volume recovered after centrifugation	$M_{wet,soil}$ = Mass of wet soil after centrifugation
M_0= Mass of substance spiked to the batch test	MB = Mass balance
Water RR = Recovery rater in water samples	Soil RR = Recovery rate in soil samples

Explanation of used Indexes

a) indicating duplicates

b) 5 µl of injection volume used. Soil RR reduced from 529% to 64%.

c) 5 µl of injection volume used. Soil RR reduced from 457% to 80%.

3.1.4 Test results for all water samples for melamine in Tier 1 using atrazine-d5 as IS

Sample Nr.	Soil	Equilibration. time [h]	L/S [-]	Wet soil [g]	m_{soil} [g d.w.]	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 [%]
39	TS	48	1	10.00	9.91	0.09	9.03	1.00	10.12	5.22	52.80	2.18	3.10	1.42	2.55	58%
40	TS	48	1	10.00	9.91	0.09	9.02	1.01	10.12	5.27	53.33	2.08	3.26	1.57	2.59	61%
43	TS	48	2	10.00	9.91	0.09	18.02	2.03	20.14	5.32	107.18	3.61	3.48	0.96	2.38	32%
44	TS	48	2	10.00	9.91	0.09	18.00	2.02	20.11	5.30	106.66	3.6	3.46	0.96	2.38	32%
47	TS	48	5	4.00	3.96	0.04	18.00	2.00	20.04	5.27	105.60	4.52	3.79	0.84	2.32	14%
48	TS	48	5	4.00	3.96	0.04	18.01	1.99	20.04	5.24	105.07	4.51	3.71	0.82	2.31	14%
51	N25	48	1	10.00	9.62	0.38	9.02	1.01	10.41	5.12	53.33	<LOD	$m_w > m_0$	-	-	<0.1%
52	N25	48	1	10.00	9.62	0.38	9.00	1.00	10.38	5.09	52.80	<LOD	$m_w > m_0$	-	-	<0.1%
55	N25	48	2	10.00	9.62	0.38	18.12	2.00	20.50	5.15	105.60	0.03	10.93	437.0	3.33	100%
56	N25	48	2	10.00	9.62	0.38	18.00	2.00	20.38	5.18	105.60	0.04	10.89	272.4	3.13	99%
59	N25	48	5	4.00	3.85	0.15	18.00	2.00	20.15	5.24	105.60	0.15	26.66	177.7	2.94	97%
60	N25	48	5	4.00	3.85	0.15	18.02	2.01	20.18	5.26	106.13	0.15	26.80	178.7	2.94	97%

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 batch tube, V_0= Total volume of water in the batch tube, C_0= Initial spiked concentration in the water phase, M_0= 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 are considered: the fraction of mass adsorbed to the soil must be greater than 20%, and sorption equilibrium must be reached. The fraction of melamine sorbed to the five soils is plotted in Figure 1 shown as the average of analysed duplicates ± standard deviation, based on the aqueous phase concentration using the indirect method. Data points with too high (>300%) analytical recovery rates, for which adsorption processes have not reach equilibrium in the timestep and for which the water concentration after conducting the batch test is above the spiked concentrations of the substance are excluded. The data excluded from further calculations are 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 Quality Assurance

As in Tier 1 all 0.1 M $CaCl_2$ solution blanks and soil-water blanks are below LOD for melamine indicating that the test samples of the soil used are not contaminated with the test substance and that there is no measurable cross-contamination in both laboratories (Table A 4). Recovery rates for all water samples, where the analogue internal standard (atrazine-d5) was used, can be found in section 3.2.4. Recovery rates are all above 100% ranging from 120 to 340% and unexpectedly higher than in Tier 1 (section 3.1.2).

A stable-labelled analogue internal standard (Melamine-13C_3-15N_3) was used, to verify the results of the analytical method using the analogue internal standard (atrazine-d5). Therefore, the samples that yield the lowest K_{oc} values (those using soil L_2.4) and for additional control the spiking solution used were reanalysed as well. This reanalysis improved the recovery rates, which range from 96.7% to 104.6% (Table A 6) and align with OECD TG 106, which suggests a suitable analytical method should have recovery rates between 90% and 110%.

Overall, differences in measured water concentrations for the selected water samples (using soil L2.4) are minimal. The variation in melamine concentration in the spiking solution is <8%. Water concentrations measured using Melamine-13C3-15N3 are between 5% lower and 23% higher compared to those obtained using atrazine-d5 for selected water samples (Table A 8). Chromatograms with the stable-labelled analogue IS are provided in Appendix A.2.4.

3.2.2 Kinetic study – fraction of adsorbed mass

The fraction of adsorbed mass of melamine to the soil, A [%], is summarized Figure 1.

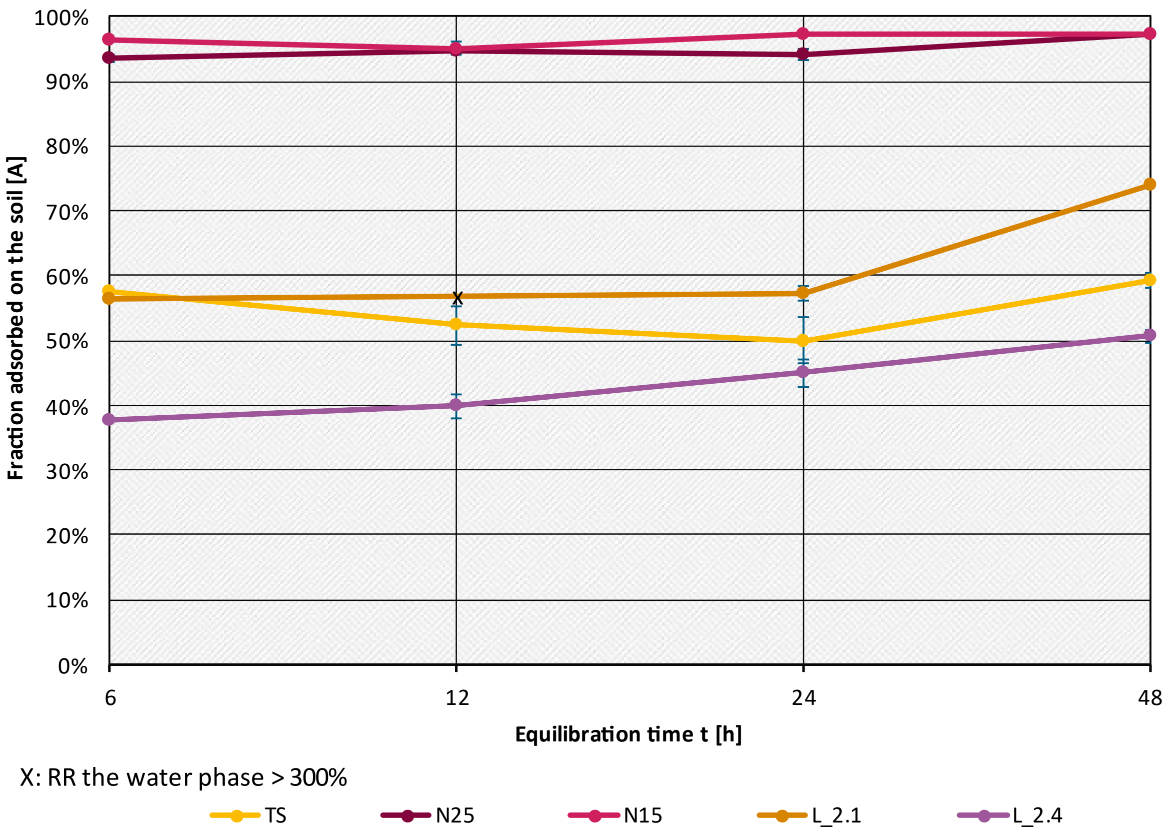
In all batch tests, the fraction of mass adsorbed to the soil is greater than 20%. For the soils TS (54.8±3.8%), N25 (94.9±1.3%), and N15 (96.5±0.9%) the fraction of melamine adsorbed to the soil remains constant over all experimental times, which indicates fast adsorption kinetics (<6 h) and that equilibrium is reached in all tested times steps.

For the soil L_2.4, sorption slightly increases with time steps, ranging from 37.8±0.2% after 6 h up to 50.6±1.0% after 48 h. Since the changes in the fraction sorbed on the soil were minimal, all data points are considered in further calculations.

For the soil L_2.1, both duplicate samples (sample nr. 287 and 288) for a shaking time of 12 h (83.5±1.7%) are identified as outliers. Both samples hereby indicated a very high recovery rate (>300%) which can lead to underestimation of the melamine concentration in the water phase. Hence the duplicate values are not used for further calculations.

To calculate the log K_{oc} values for melamine all data indicating equilibrium conditions are used.

Figure 1 – Fraction adsorbed on the test soils at different time steps for melamine



X: RR the water phase > 300%

Source: Original Figure

3.2.3 Derived log K_{oc} values

The calculated log K_{oc} using atrazine-d5 as IS for each soil are summarized in (Figure 2), using all representative data points as described before. All derived data from the Tier 2 batch tests for melamine are compiled in section 3.2.4.

According to OECD TG 106, the following minimum log K_{oc} for melamine are derived for each soil: 1.57 (L_2.4), 2.29 (L_2.1), 2.34 (TS), 2.54 (N25) and 3.07 (N15).

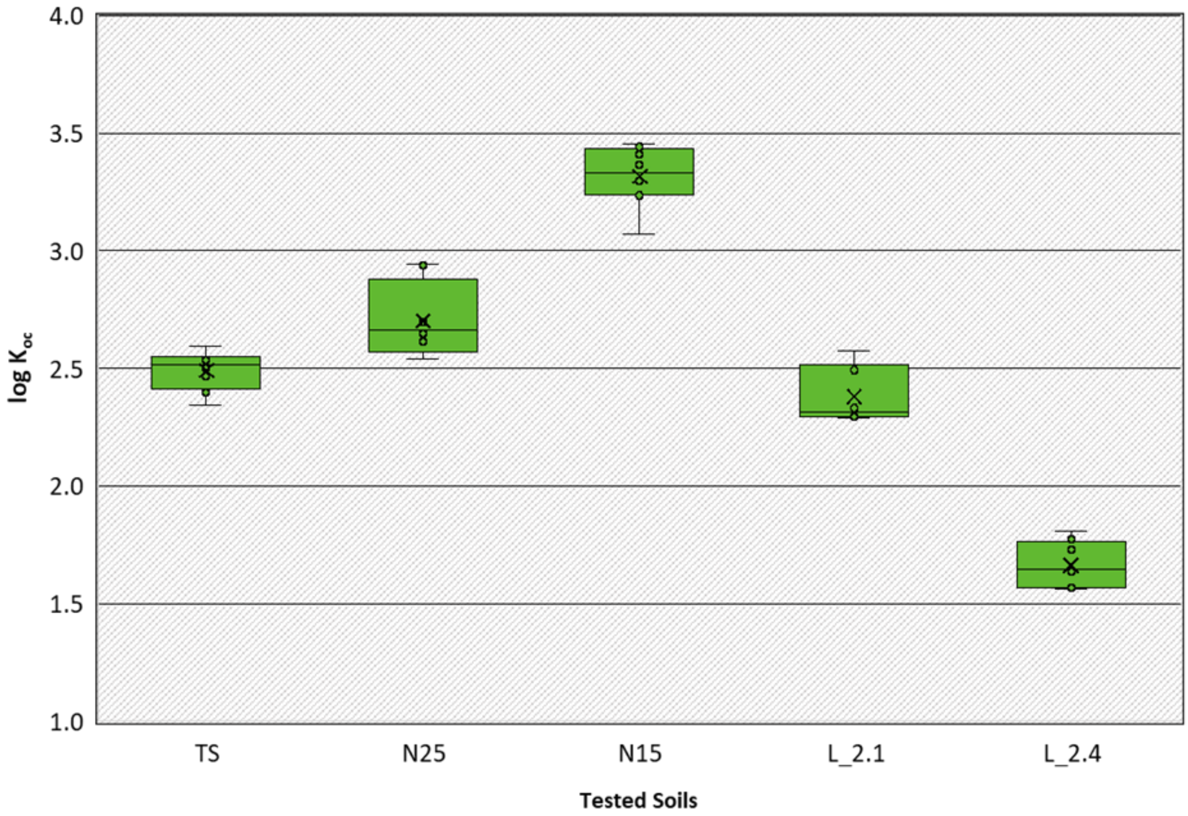
The average log K_{oc} values for all soils are 3.32±012 (n=8) for N15, 2.70±0.15 for N25 (n=8), 2.49±0.08 for TS (n=8). 2.38±0.11 (n=6) for L_2.1 and 1.66±0.09 for L_2.4 (n=8).

The sorption of melamine to the different soils is influenced by soil properties. The highest log K_{oc} values are observed in soils with high total organic carbon (TOC) content (N25) or high clay content (TS and N15). Furthermore, there is some indication that log K_{oc} is affected by soil pH. The soils used in this study have pH values ranging from 4.6 (L_2.1) to 7.8 (TS), covering a range both below and above the pKb1 (7.3) of melamine (ECHA, 2022). For example, L_2.1, which has lower silt, clay, and organic carbon content, shows a higher log K_{oc} than L_2.4, which could be explained by differences in soil pH. At the lower pH of 4.6 in L_2.1, melamine is predominantly positively charged, which increases sorption to negatively charged soil particles (Droge & Goss, 2013; Sigmund et al., 2022). In contrast, at the higher pH of 7.5 in L_2.4, melamine is mostly uncharged and therefore exhibits reduced sorption to the soil.

As an additional quality assurance step all available samples for soil L2.4 were reanalysed using a stable-labelled analogue internal standard, Melamine13C_3-15N_3. The log K_{oc} values range from 1.53 to 1.80, with an average log K_{oc} of 1.59±0.03 (n = 6) and are summarized in Table A 6. These values are consistent with the average log K_{oc} derived using the structural analogue internal standard atrazine-d5, confirming the reliability of the results.

According to criteria as set out in the CLP regulation (EC No. 1272/2008 - CLP, 2008), melamine is a very mobile substance as the lowest log K_{oc} values is below 2.

Figure 2 – log K_{oc} values for melamine (n_{total}=38) using atrazine-d5 as IS.



Source: Original Figure

3.2.4 Test results for all water samples for melamine in Tier 2 using atrazine-d5 as IS.

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	$M_{Wet\ soil}$ [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 [%]
239	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.00	5.28	52.80	2.23	3.05	1.4	2.53	58%	177.1%
240	TS	6	1	10.00	9.91	0.09	9.00	1.00	10.00	5.28	52.80	2.21	3.07	1.4	2.54	58%	177.1%
247	L_2.1	6	1	10.00	9.98	0.02	9.00	1.00	10.00	5.28	52.80	2.29	2.99	1.3	2.29	57%	165.9%
248	L_2.1	6	1	10.00	9.98	0.02	9.00	1.00	10.00	5.28	52.80	2.29	2.99	1.3	2.30	57%	165.9%
255	L_2.4	6	1	10.00	9.89	0.11	9.00	1.00	10.00	5.28	52.80	3.26	2.02	0.6	1.56	38%	156.3%
256	L_2.4	6	1	10.00	9.89	0.11	9.00	1.00	10.00	5.28	52.80	3.24	2.04	0.6	1.57	39%	156.3%
263	N15	6	1	10.00	9.91	0.09	9.00	1.00	10.00	5.28	52.80	0.20	5.08	25.1	3.25	96%	184.4%
264	N15	6	1	10.00	9.91	0.09	9.00	1.00	10.00	5.28	52.80	0.18	5.10	27.8	3.30	97%	184.4%
271	N25	6	5	4.00	3.85	0.15	18.01	2.00	20.01	5.28	105.6	0.31	24.85	80.4	2.61	94%	177.3%
272	N25	6	5	4.00	3.85	0.15	18.02	2.00	20.02	5.27	105.6	0.36	24.60	68.2	2.54	93%	177.3%
279	TS	12	1	10.00	9.91	0.09	9.02	1.01	10.03	5.32	53.33	2.35	2.93	1.2	2.50	55%	134.3%
280	TS	12	1	10.00	9.91	0.09	9.01	1.00	10.01	5.27	52.80	2.64	2.64	1.0	2.40	50%	134.3%
287	L_2.1	12	1	10.00	9.98	0.02	9.02	1.00	10.02	5.27	52.80	0.95	4.33	4.5	2.84	82%	340.4%
288	L_2.1	12	1	10.00	9.98	0.02	9.01	1.01	10.02	5.32	53.33	0.78	4.50	5.7	2.94	85%	340.4%
295	L_2.4	12	1	10.00	9.89	0.11	9.03	1.01	10.04	5.31	53.33	3.26	2.02	0.6	1.57	38%	138.3%
296	L_2.4	12	1	10.00	9.89	0.11	9.03	1.01	10.04	5.31	53.33	3.06	2.22	0.7	1.64	42%	138.3%
303	N15	12	1	10.00	9.92	0.09	9.00	1.00	10.00	5.28	52.80	0.21	5.07	24.1	3.24	96%	131.4%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	$M_{Wet\ soil}$ [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 [%]
304	N15	12	1	10.00	9.91	0.09	9.02	1.02	10.04	5.36	53.86	0.31	4.97	16.1	3.07	94%	131.4%
311	N25	12	5	4.00	3.85	0.15	18	2.00	20.00	5.28	105.6	0.29	24.97	87.5	2.65	95%	168.3%
312	N25	12	5	4.00	3.85	0.15	18	2.01	20.01	5.30	106.1	0.27	25.05	93.1	2.68	95%	168.3%
319	TS	24	1	10.00	9.91	0.09	9.02	1.00	10.02	5.27	52.80	2.80	2.48	0.9	2.34	47%	125.1%
320	TS	24	1	10.00	9.91	0.09	9.01	1.00	10.01	5.27	52.80	2.43	2.85	1.2	2.47	54%	125.1%
327	L_2.1	24	1	10.00	9.98	0.02	9.01	1.00	10.01	5.27	52.80	2.31	2.97	1.3	2.29	56%	138.7%
328	L_2.1	24	1	10.00	9.98	0.02	9.02	1.00	10.02	5.27	52.80	2.18	3.10	1.4	2.33	59%	138.7%
335	L_2.4	24	1	10.00	9.89	0.11	9.01	1.00	10.01	5.27	52.80	2.98	2.30	0.8	1.65	43%	150.8%
336	L_2.4	24	1	10.00	9.89	0.11	9.03	1.00	10.03	5.26	52.80	2.76	2.52	0.9	1.73	48%	150.8%
343	N15	24	1	10.00	9.91	0.09	9.02	1.00	10.02	5.27	52.80	0.13	5.15	39.7	3.45	98%	170.2%
344	N15	24	1	10.00	9.91	0.09	9.02	1.00	10.02	5.27	52.80	0.16	5.12	32.6	3.37	97%	170.2%
351	N25	24	5	4.00	3.85	0.15	18.07	2.00	20.07	5.26	105.6	0.35	24.66	70.7	2.56	93%	149.4%
352	N25	24	5	4.00	3.85	0.15	18.00	2.00	20.00	5.28	105.6	0.26	25.11	97.6	2.70	95%	149.4%
363	L_2.1	48	1	10.00	9.98	0.02	9.00	1.00	10.00	5.28	52.80	1.52	3.76	2.5	2.63	71%	126.0%
364	L_2.1	48	1	10.00	9.98	0.02	9.01	1.00	10.01	5.27	52.80	1.74	3.54	2.0	2.64	67%	126.0%
371	L_2.4	48	1	10.00	9.89	0.11	9.02	1.00	10.02	5.27	52.80	2.63	2.65	1.0	1.77	50%	119.5%
372	L_2.4	48	1	10.00	9.89	0.11	9.01	1.00	10.01	5.27	52.80	2.52	2.76	1.1	1.81	52%	119.5%
379	N15	48	1	10.00	9.91	0.09	9.01	1.00	10.01	5.27	52.80	0.14	5.14	35.8	3.41	97%	213.5%

Sample Nr.	Soil	Equilibration time [h]	L/S [-]	$M_{Wet\ soil}$ [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 [%]
380	N15	48	1	10.00	9.91	0.09	9.01	1.00	10.01	5.27	52.80	0.13	5.15	38.7	3.44	97%	203.5%

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, V_0= Total volume of water in the batch tube, C_0= Initial spiked concentration in the water phase, M_0= 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

4 Conclusion

This report presents the determination of log K_{oc} values for melamine (CAS: 108-78-1) 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 performed using LC-MS/MS.

The derived log K_{oc} values for melamine across the five individual test soils range from 1.57 to 3.07. The soil with the lowest adsorption shows an average log K_{oc} of 1.66±0.09 (n = 8), with the lowest individual value being 1.57. This soil was reanalysed using a second internal standard, yielding a similar result of 1.59±0.03 (n = 6), with the lowest value being 1.53.

The log K_{oc} values are found to depend on soil parameters. The highest values were observed in soils with high TOC content (N25) or high silt/clay content (TS and N15). Additionally, there is some indication that log K_{oc} is influenced by soil pH, with increased sorption at lower pH levels. The soils used in this study have pH values ranging from 4.6 (L_2.1) to 7.8 (TS), thus covering a range both below and above melamine's pK_{b1} (7.3).

Based on the lowest derived log K_{oc} values observed in this study, and according to the criteria outlined in the CLP Regulation (EC No. 1272/2008), melamine is classified as very mobile (vM).

5 List of references

Arp, H. P. H., & Hale, S. E. (2022). Assessing the Persistence and Mobility of Organic Substances to Protect Freshwater Resources. ACS Environmental Au, 2(6), 482–509. https://doi.org/10.1021/acsenvironau.2c00024

Arp, H. P. H., Hale, S. E., Borchers, U., Valkov V., Wiegand, L., Zahn, D., Neuwald, I., Nödler, K., & Scheurer, M. (2023). A prioritization framework for PMT/vPvM Substances under REACH for registrants, regulators, researchers and the water sector. https://www.umweltbundesamt.de/publikationen/a-prioritization-framework-for-pmtvpvm-substances

Arp, H. P. H., Hale, S. E., Schliebner, I., & Neumann, M. (2023). Prioritised PMT/vPvM substances in the REACH registration database. https://www.umweltbundesamt.de/publikationen/prioritised-pmtvpvm-substances-in-the-reach

Bianco Prevot, A., Maurino, V., Fabbri, D., Braun, A. M., & Gonzalez, M. C. (2020). Degradation of melamine in aqueous systems by vacuum UV-(VUV-) photolysis. An alternative to photocatalysis. Catalysis Today, 340, 286–293. https://doi.org/10.1016/j.cattod.2018.11.045

Droge, S. T. J., & Goss, K.-U. (2013). Development and Evaluation of a New Sorption Model for Organic Cations in Soil: Contributions from Organic Matter and Clay Minerals. Environmental Science & Technology, 47(24), 14233–14241. https://doi.org/10.1021/es4031886

EC No. 1272/2008 - CLP (2008).

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). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32023R0707

Gong, H., Tang, S., & Zhang, T. (2016). Catalytic hydrolysis of waste residue from the melamine process and the kinetics of melamine hydrolysis in NaOH solution. Reaction Kinetics, Mechanisms and Catalysis, 118(2), 377–391. https://doi.org/10.1007/s11144-016-1014-9

Hofman-Caris, R., & Claßen, D. (2020). Persistence of Gabapentin, 1Hbenzotriazole, Diglyme, DTPA, 1,4-Dioxane, Melamine and Urotropin in Surface Water: Testing of Chemicals According to the OECD 309 Guideline.

Hubert, M., Arp, H. P. H., Hansen, M. C., Castro, G., Meyn, T., Asimakopoulos, A. G., & Hale, S. E. (2023). Influence of grain size, organic carbon and organic matter residue content on the sorption of per- and polyfluoroalkyl substances in aqueous film forming foam contaminated soils - Implications for remediation using soil washing. Science of The Total Environment, 875, 162668. https://doi.org/10.1016/j.scitotenv.2023.162668

Jang, Y. H., Hwang, S., Chang, S. B., Ku, J., & Chung, D. S. (2009). Acid Dissociation Constants of Melamine Derivatives from Density Functional Theory Calculations. The Journal of Physical Chemistry A, 113(46), 13036–13040. https://doi.org/10.1021/jp9053583

Müller, K., Zahn, D., Frömel, T., & Knepper, T. P. (2020). Matrix effects in the analysis of polar organic water contaminants with HILIC-ESI-MS. Analytical and Bioanalytical Chemistry, 412(20), 4867–4879. https://doi.org/10.1007/s00216-020-02548-1

OECD. (2000). Test No. 106: Adsorption -- Desorption Using a Batch Equilibrium Method. OECD. https://doi.org/10.1787/9789264069602-en

Sigmund, G., Arp, H. P. H., Aumeier, B. M., Bucheli, T. D., Chefetz, B., Chen, W., Droge, S. T. J., Endo, S., Escher, B. I., Hale, S. E., Hofmann, T., Pignatello, J., Reemtsma, T., Schmidt, T. C., Schönsee, C. D., & Scheringer, M. (2022). Sorption and Mobility of Charged Organic Compounds: How to Confront and Overcome Limitations in

Their Assessment. Environmental Science & Technology, 56(8), 4702–4710. https://doi.org/10.1021/acs.est.2c00570

Ukalska-Jaruga, A., Bejger, R., Smreczak, B., & Podlasiński, M. (2023). Sorption of Organic Contaminants by Stable Organic Matter Fraction in Soil. Molecules, 28(1), 429. https://doi.org/10.3390/molecules28010429

Wang, H.-Y., Wang, H., Tang, L.-L., Dong, Y.-H., Zhao, L., & Toor, G. (2014). Sorption characteristics of cyromazine and its metabolite melamine in typical agricultural soils of China. Environmental Science and Pollution Research, 21(2), 979–985. https://doi.org/10.1007/s11356-013-1956-7

A.1 QA for Tier 1

Table A 1 - Test results blank-, control samples and spiking solutions Tier 1 using atrazine-d5 as IS

Sample Nr.	Sample Type	Soil used	Equilibration time [h]	L/S	Melamine [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
32	Control sample (5.28 ug/mL Melamine)	-	48	-	5.01
34	Control sample (5.28 ug/mL Melamine)	-	48	-	4.99
35	Spiking Solution Melamine	-	-	-	52.8

Table A 2 - Analytical recovery rates in the water phase in Tier 1 using atrazine-d5 as IS

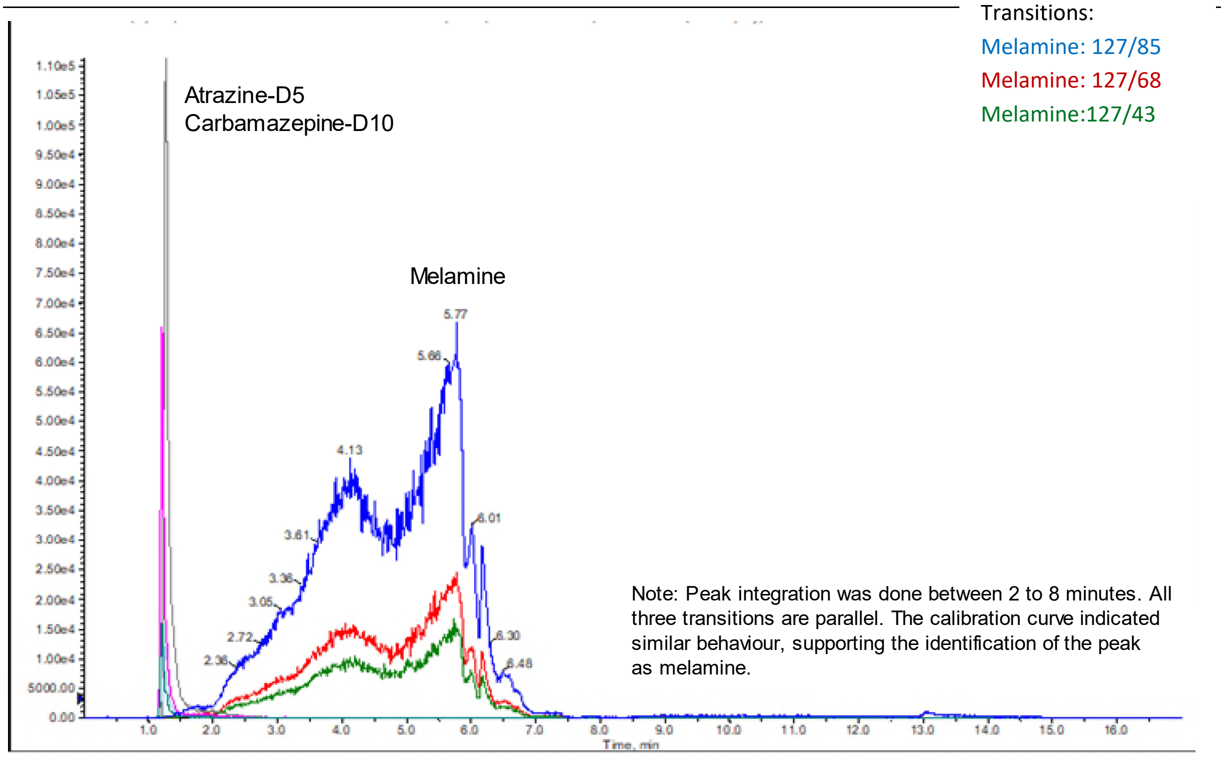
Soil used	Equilibration time [h]	L/S	Melamine [%]
TS	48	2	95.3 to 103.3 (n=2)
N25	48	2	98.0 to 106.6 (n=2)

Table A 3 - Analytical recovery rates in selected soil samples in Tier 1 using atrazine-d5 as IS

Soil used	Equilibration time [h]	L/S	Melamine [%] V_{inj} = 20 µl	Melamine [%] V_{inj} = 5 µl (V_{inj} = 20 µl)
TS	48	1	345 to 529 (n=2)	64 (529)
N25	48	1	351 to 457 (n=2)	80 (457)

A.1.1 Selected Chromatograms from Tier 1

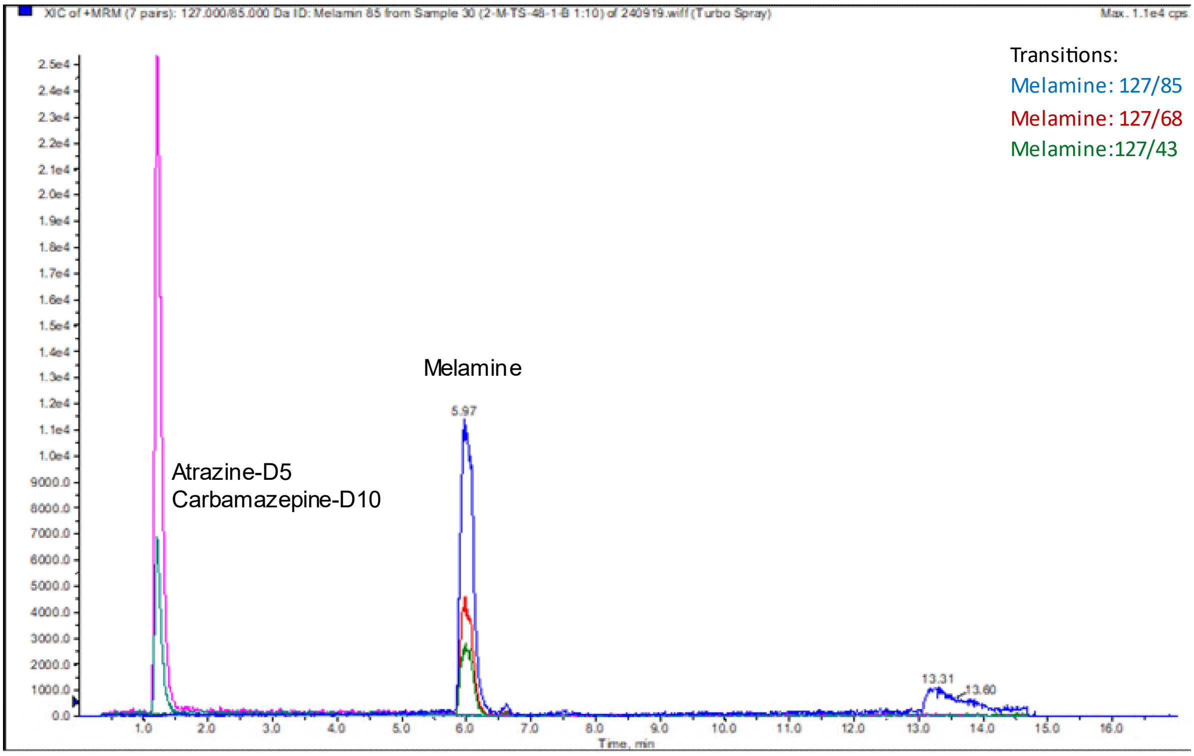
Figure A 1 – Chromatogram for soil sample nr. 1: M-TS-48-1_A - 20µL injection volume using atrazine-d5 as IS



Note: Peak integration was done between 2 to 8 minutes. All three transitions are parallel. The calibration curve indicated similar behaviour, supporting the identification of the peak as melamine.

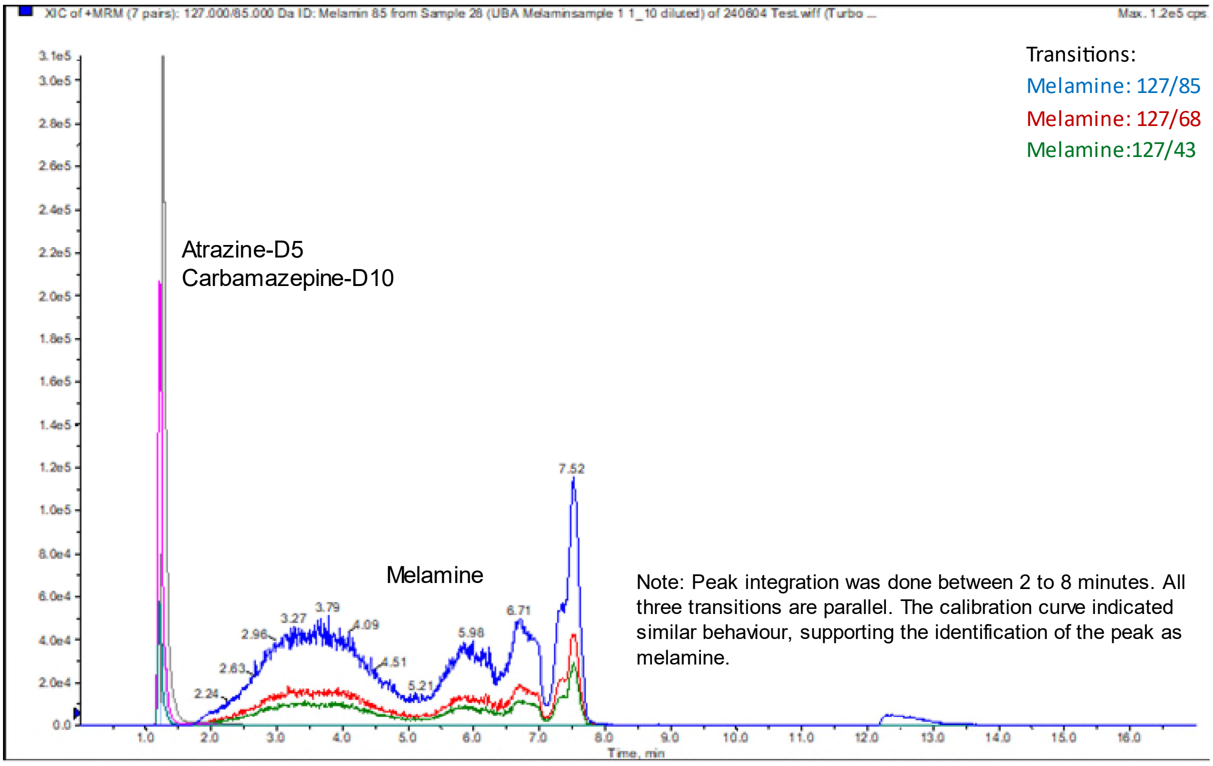
Source: Original Figure

Figure A 2 - Chromatogram for soil sample nr. 2: M-TS-48-1_B - 5µL injection volume using atrazine-d5 as IS



Source: Original Figure

Figure A 3 – Chromatogram for water sample nr. 39: M-TS-48-1_A – 20 µL injection volume using atrazine-d5 as IS

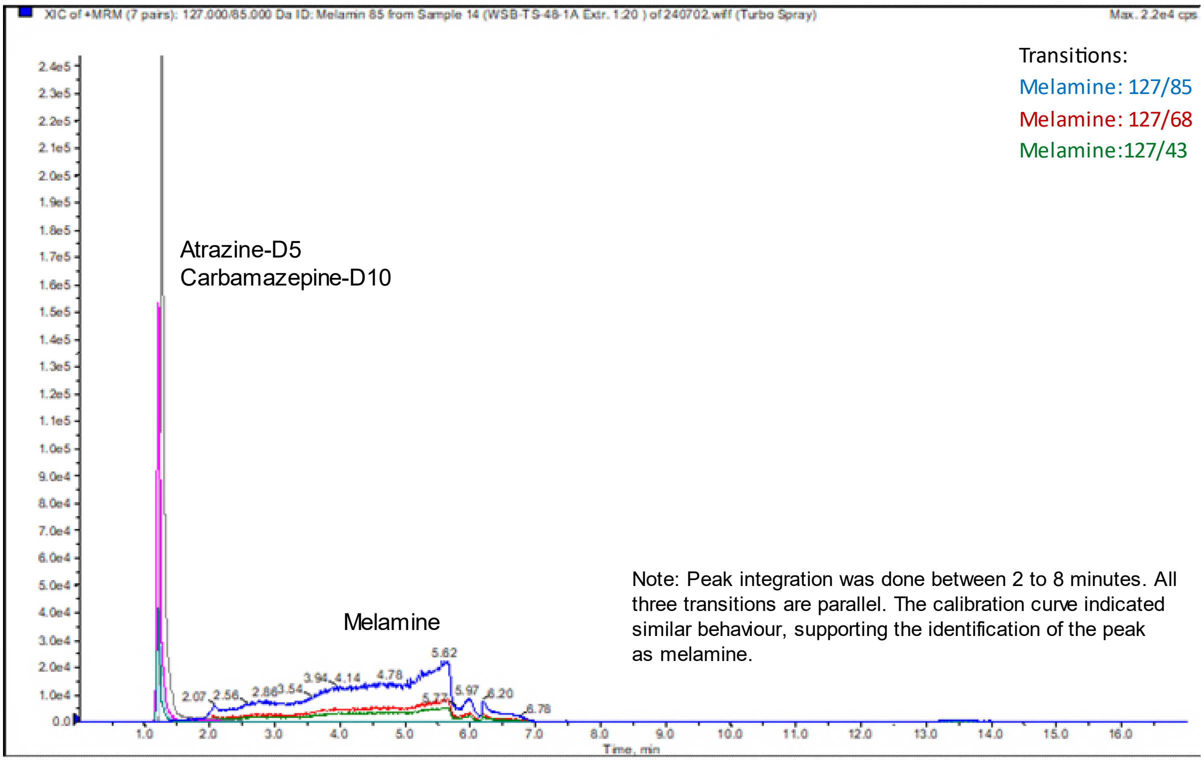


Transitions:
Melamine: 127/85
Melamine: 127/68
Melamine:127/43

Note: Peak integration was done between 2 to 8 minutes. All three transitions are parallel. The calibration curve indicated similar behaviour, supporting the identification of the peak as melamine.

Source: Original Figure

Figure A 4– Chromatogram for water sample nr. 27: WSB-TS-48-1_A-Soil-Water Blank - 20µL injection volume using atrazine-d5 as IS



Transitions:
Melamine: 127/85
Melamine: 127/68
Melamine:127/43

Note: Peak integration was done between 2 to 8 minutes. All three transitions are parallel. The calibration curve indicated similar behaviour, supporting the identification of the peak as melamine.

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 using atrazine-d5 as IS

Sample Nr.	Sample Typ	Soil used	Shaking time [h]	L/S	Melamine [ug/L]
391	Soil-Water blank	TS	48	-	<LOD
393	Soil-Water blank	L_2.1	48	-	<LOD
395	Soil-Water blank	L_2.4	48	1	<LOD
397	Soil-Water blank	N15	48	1	<LOD
399	Soil-Water blank	N25	48	1	Not measured
411	Spiking Solution Melamine (IS used: Melamin-$^{13}C_3$-$^{15}N_3$)	-	-	-	49.11

A.2.2 Analytical recovery rates

Table A 5 - Analytical recovery rates in the water phase for each timestep Tier 2 using atrazine-d5 as IS

Soil used	L/S	Equilibration time [t]	Melamine [%]
TS	1	6h	177%
L-2.1	1	6h	166%
L-2.4	1	6h	156%
N15	1	6h	184%
N25	5	6h	177%
TS	1	12h	134%
L-2.1	1	12h	340%
L-2.4	1	12h	138%
N15	1	12h	131%
N25	5	12h	168%
TS	1	24h	125%
L-2.1	1	24h	139%
L-2.4	1	24h	151%
N15	1	24h	170%

Soil used	L/S	Equilibration time [t]	Melamine [%]
N25	5	24h	149%
TS	1	48h	-
L-2.1	1	48h	126%
L-2.4	1	48h	119%
N15	1	48h	209%
N25	5	48h	-

A.2.3 Analysis using the stable-labelled analogue IS (Melamine-13C_3-15N_3).

Table A 6 – Reanalysed water samples for L.S 2.4 (L/S=1) using Melamine-13C3-15N3 as IS.

Sample Nr.	Equilibration time [h]	C_w [ug/mL]	C_s [µg/g]	K_d [L/Kg]	log K_{oc} [L/Kg]	A [%]	RR Water [%]
255	6	3.70	1.56	0.4	1.80	29%	95%
256	6	3.34	1.92	0.6	1.53	36%	98%
295	12	3.11	2.20	0.7	1.62	41%	105%
296	12	3.13	2.18	0.7	1.61	40%	104%
335	24	3.26	2.00	0.6	1.56	37%	98%
336	24	3.34	1.92	0.6	1.53	36%	97%
371	48	3.10	2.17	0.7	1.61	41%	98%
372	48	3.11	2.16	0.7	1.61	40%	97%

Table A 7 - Test results spiking solutions Tier 2 using Melamine-13C3-15N3 as IS.

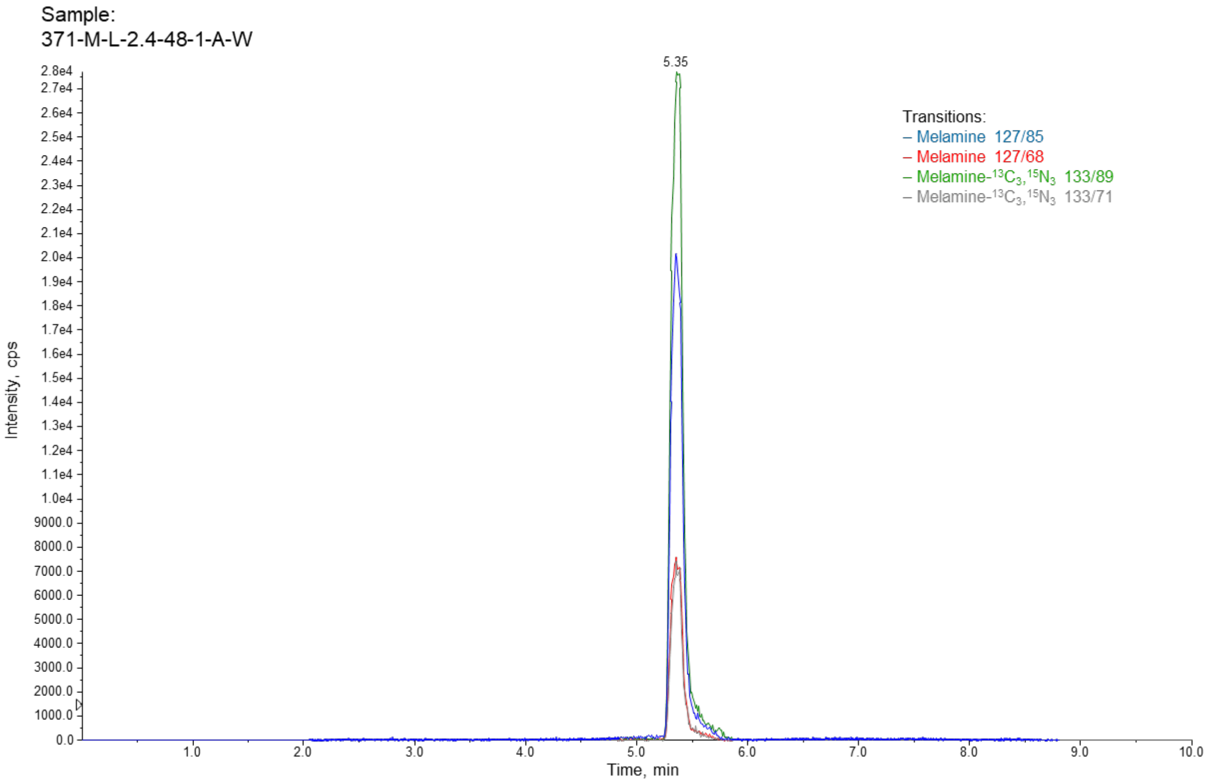
Sample Nr.	Sample Typ	Soil used	Shaking time [h]	L/S	Melamine [ug/L]
411	Spiking Solution Melamine (IS used: Melamin-13C_3-15N_3)	-	-	-	49.11

Table A 8 –Comparison of Melamine concentration in the water phase and derived logK_{oc} values for L.S 2.4 (L/S=1) using Melamine-13C3-15N3 and atrazine-d5 as IS.

Sample Nr.	C_w Melamine-13C3-15N3 [ug/mL]	C_w atrazine-d5	Factor C_w Melamine 13C3-15N3 / C_w atrazine-d5	log K_{oc} [L/Kg] Melamine-13C3-15N3	Log K_{oc} atrazine-d5	Difference log K_{oc} Melamine-13C3-15N3 and log K_{oc} atrazine-d5
255	3.70	3.26	1.13	1.80	1.97	-0.17
256	3.34	3.24	1.03	1.53	1.57	-0.04
295	3.11	3.26	0.95	1.62	1.57	0.05
296	3.13	3.06	1.02	1.61	1.64	-0.03
335	3.26	2.98	1.09	1.56	1.65	-0.10
336	3.34	2.76	1.21	1.53	1.73	-0.20
371	3.10	2.63	1.18	1.61	1.77	-0.16
372	3.11	2.52	1.23	1.61	1.81	-0.20

A.2.4 Selected Chromatograms from Tier 2

Figure A 5 – Chromatogram for water sample Nr. 371-L2.4 (48h, L/S =1), 5 µL injection volume using Melamine-13C3-15N3.



Source: Original Figure