Electronic Supplementary Material (ESI) for Environmental Science: Advances.

Silylation: A Reproducible Method for Characterization of Non-Extractable Residues (NER) of REACH Chemicals, Pharmaceuticals and Pesticides in Soil and Sediment

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Supplementary Information

Tables:

Table S1: Characteristics of the used soils

soil	according to DIN			according to USDA							
	Sand	Silt	Clay	Sand	Silt	clay	TOC	total N	pH ($CaCl_2$)	CECeff	WHC
	%	%	%	%	%	%	%	g/ kg		mmol /kg	g/kg
IME 03-G	20.3	55.0	24.6	22.1	52.8	25.1	3.02	4.42	6.03	112.8	697
Lufa 2.4	26.9	47.1	29.9	32.0	42.0	25.9	1.95	2.2	7.4	212	458

Table S2: Extraction conditions to derive soils containing only NER. All extracts were stored in closed bottles below -18°C in the dark until chemical analysis.

Step	Solvent	Treatment	
Isoproturon			
1.	70 ml MeOH/H_2O (80:20/v:v)	10 min sonication	60 min horizontal shaking
2.	45 ml MeOH/H_2O (80:20/v:v)	10 min sonication	60 min horizontal shaking
3.	45 ml MeOH/H_2O (80:20/v:v)	10 min sonication	60 min horizontal shaking
Bromoxynil			
1.	70 ml Acetonitrile		20 min horizontal shaking
2.	45 ml Acetonitrile		20 min horizontal shaking
3.	45 ml Acetonitrile		60 min horizontal shaking
Sulfadiazine			
1.	70 ml Methanol (MeOH)		20 min horizontal shaking
2.	45 ml Methanol (MeOH)		20 min horizontal shaking
3.	45 ml Methanol (MeOH)		60 min horizontal shaking
Centrifugation between extraction steps: 10 min at 2000 rpm (1000 g)			

Table S3: PLE (pressurized liquid extraction) as the last extraction step to derive soils containing only NER

Instrumentation	ASE 350 (Dionex).
Extraction vessel	100 ml, stainless steel
Extraction	100°C, 3 cycles 10 min per cycle
Rinse volume	60%
Solvents used	
Isoproturon	Methanol / Water (80:20 / v:v)
Bromoxynil	Acetonitrile
Sulfadiazine	Methanol

Table S4: TLC-analysis to identify type I NER residues released by silylation

Sampler	Automatic TLC Sampler 4 (CAMAG)
TLC plates	TLC Silica gel 60 RP18 F254S 20x20 cm (Merck)
Software	VisionCats (CAMAG)
Applied activity	>2,5 Bq per sample
TLC separation method:	
Isoproturon	Chloroform : Ethanol (90 : 10 / v:v), Rf = 0.61
Bromoxynil	Chloroform : Diethylether : Acetic acid (80 : 18 : 2 / v:v:v), Rf = 0.60
Sulfadiazine	Chloroform : Methanol (90 : 10 / v:v), Rf = 0.47
Plate reading after 1 week exposure time:	
Reader	Amersham Typhoon, Software Version 2.0.0.6, Firmware Version 303, FPGA version 10, Serial Number 86110330
Evaluation software	AIDA (Advanced Image Data Analyzer) Version 3.44.035

Table S5: Instrumentation and conditions for LC-MS analysis of silylation extracts (^{12}C-substances: given mass -1)

Chromatographic System	Agilent (1290 HSP, Multisampler, MCT)		
Analytical Column*	Phenomenex Luna C18(2) 150 x 3.0 mm, 3.0 µm		
Column Temperature	30°C		
Injection Volume	10 µl		
Mobile Phase A Mobile Phase B	Water : Acetonitrile:, 950:50:, v/v + 0.2 % formic acid Acetonitrile + 0.2 % formic acid		
Flow Rate	1.0 mL/min		
Gradient	Time [min]	Phase A [%]	Phase B [%]
	0.0 1.0 2.0 5.0 5.1 8.0	90 90 5 5 90 90	10 10 95 95 10 10
Divert Valve	no		
Detection System**	Sciex Q TRAP 5500		
Ionisation	Electro Spray (ESI)		
Analyte	**Transitions**	**Polarity**	**Retention Time**
13C- Bromoxynil	281.8 → 80.9 281.8 → 78.9	negative	2.6 min
13C- Sulfadiazin	255.9 → 97.1	positive	2.1 min
13C- Isoproturon	213.2 → 72.0 213.2 → 46.0	positive	2.6 min

Table S6: Parent substance release from NER by silylation of ^{13}C and ^{14}C-test samples release of parent by silylation of NER in % of initially applied parent substance

sample	**Bromoxynil**		**Sulfadiazine**		**Isoproturon**	
	^{13}C silylation extract, LC-MS analysis	**^{14}C silylation extract, radio-TLC analysis**	**^{13}C silylation extract, LC-MS analysis**	**^{14}C silylation extract, radio-TLC analysis**	**^{13}C silylation extract, LC-MS analysis**	**^{14}C silylation extract, radio-TLC analysis**
7d	2.0	1.3	1.4	1.2	0.1	0.5
14d	2.4	0.9	1.2	1.2	0.1	0.5
28d	1.3	0.3	1.7	1.2	0.2	0.6
60d	1.5	0.2	2.1	1.8	0.2	0.3
120d	1.5	0.1	4.6	1.2	0.1	0.0
14d sterile	1.4	4.3	2.2	1.5	--	--
120d sterile	1.7	4.7	2.1	3.5	--	--

Figures

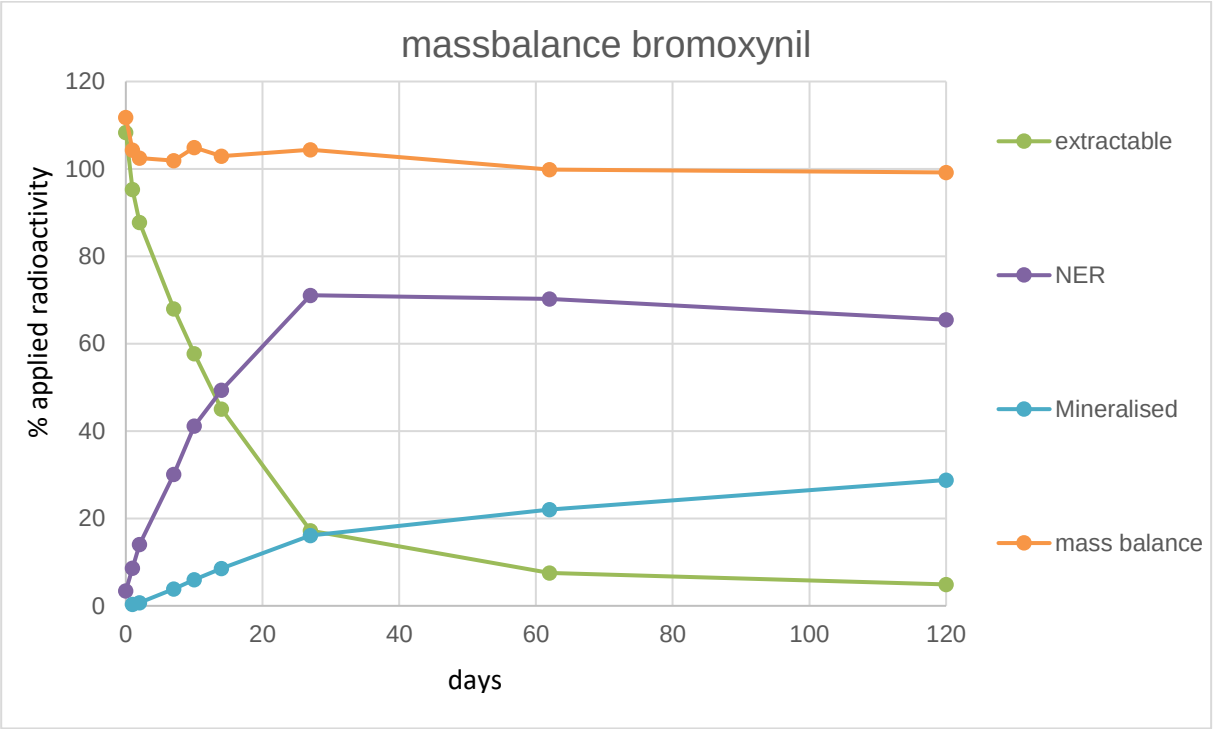


Figure S1: Mass balance bromoxynil in soil degradation (data see table 1 in the manuscript)

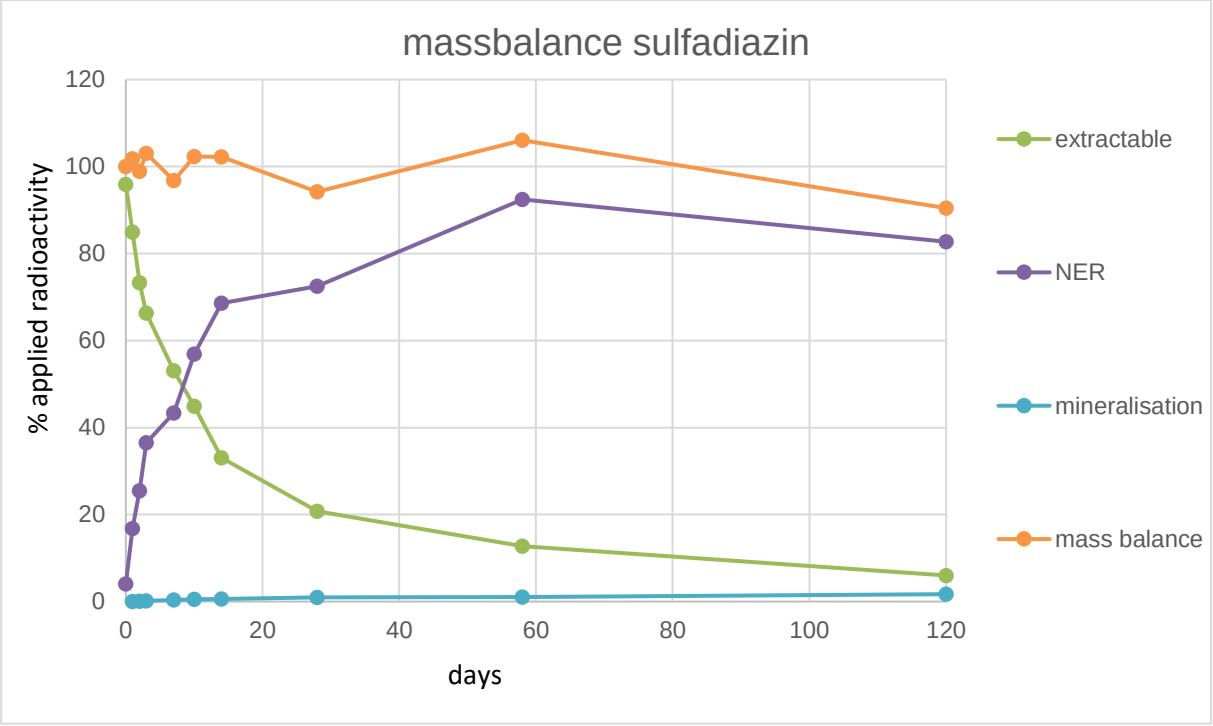


Figure S2: Mass balance sulfadiazin in soil degradation (data see table 1 in the manuscript)

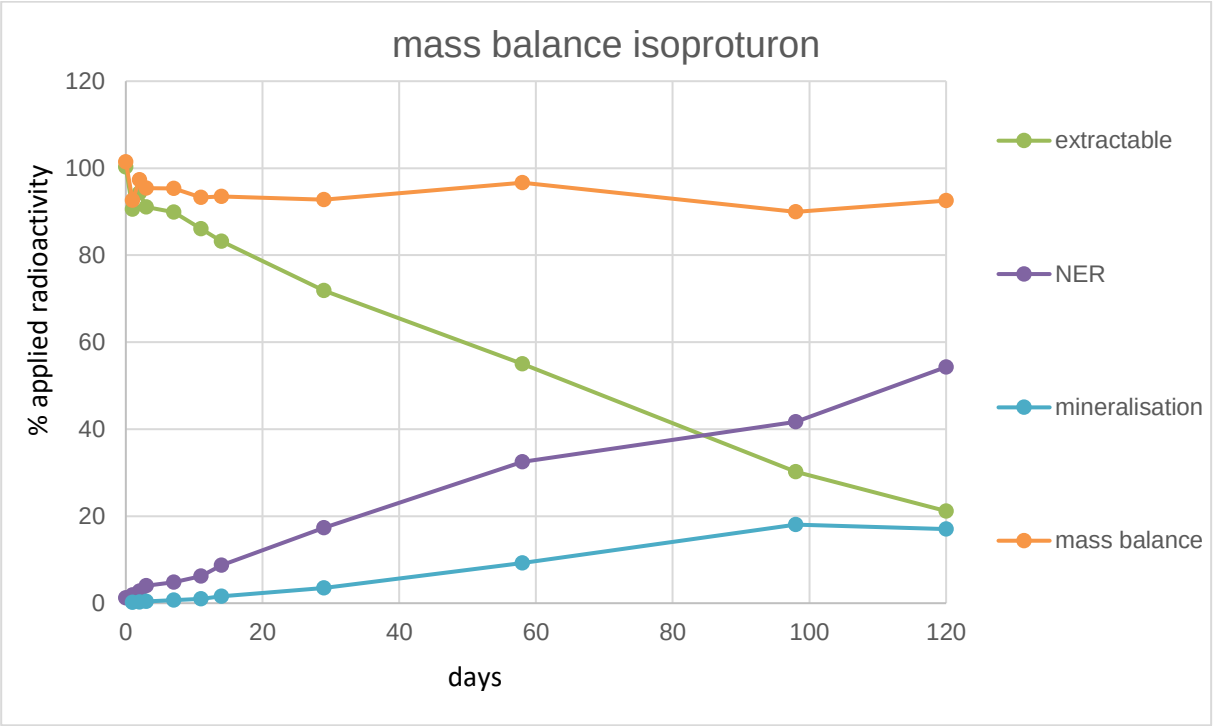


Figure S3: Mass balance isoproturon in soil degradation (data see table 1 in the manuscript)

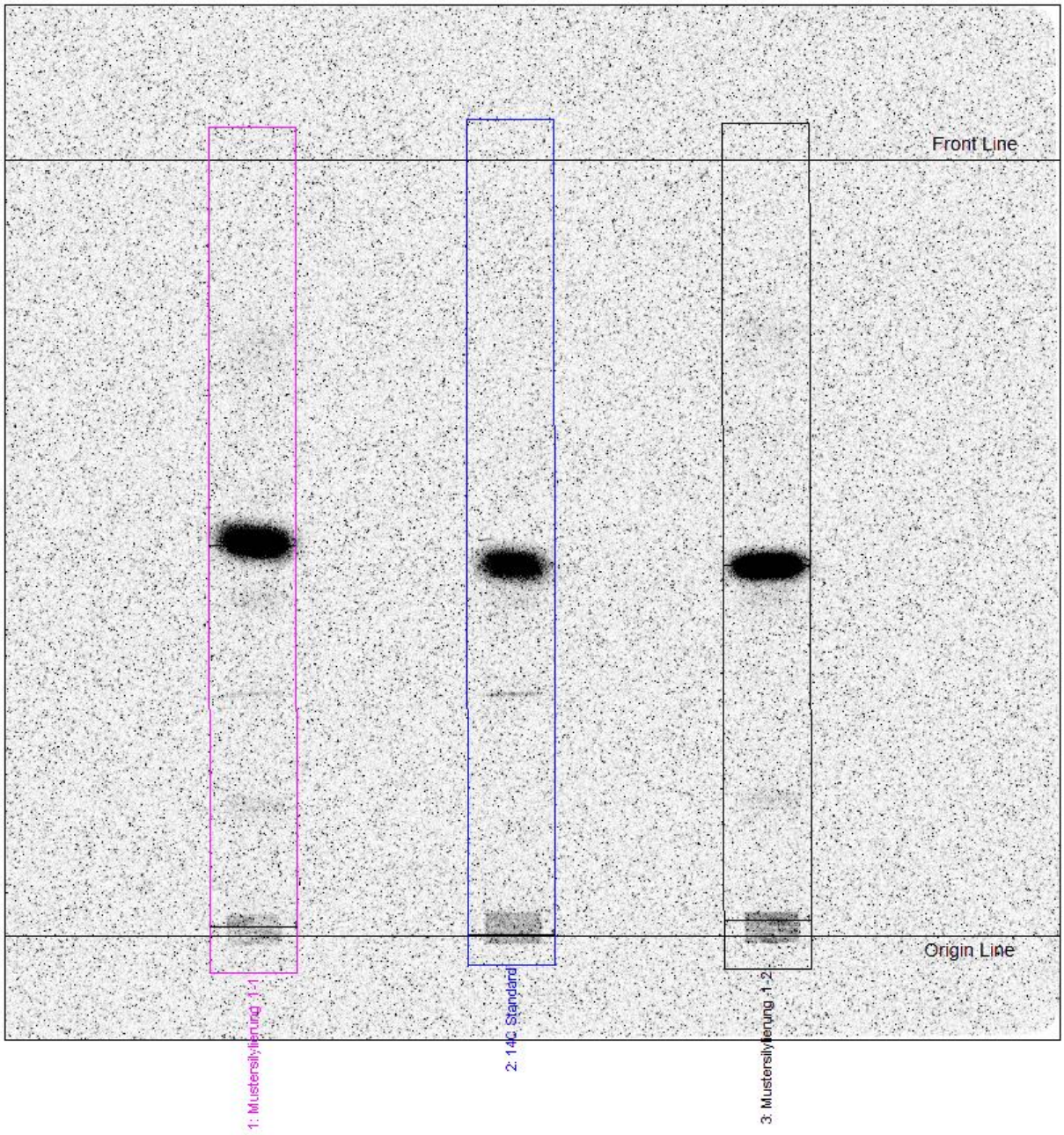


Figure S4: TLC-analysis of silylation extract in bromoxynil spiking experiment

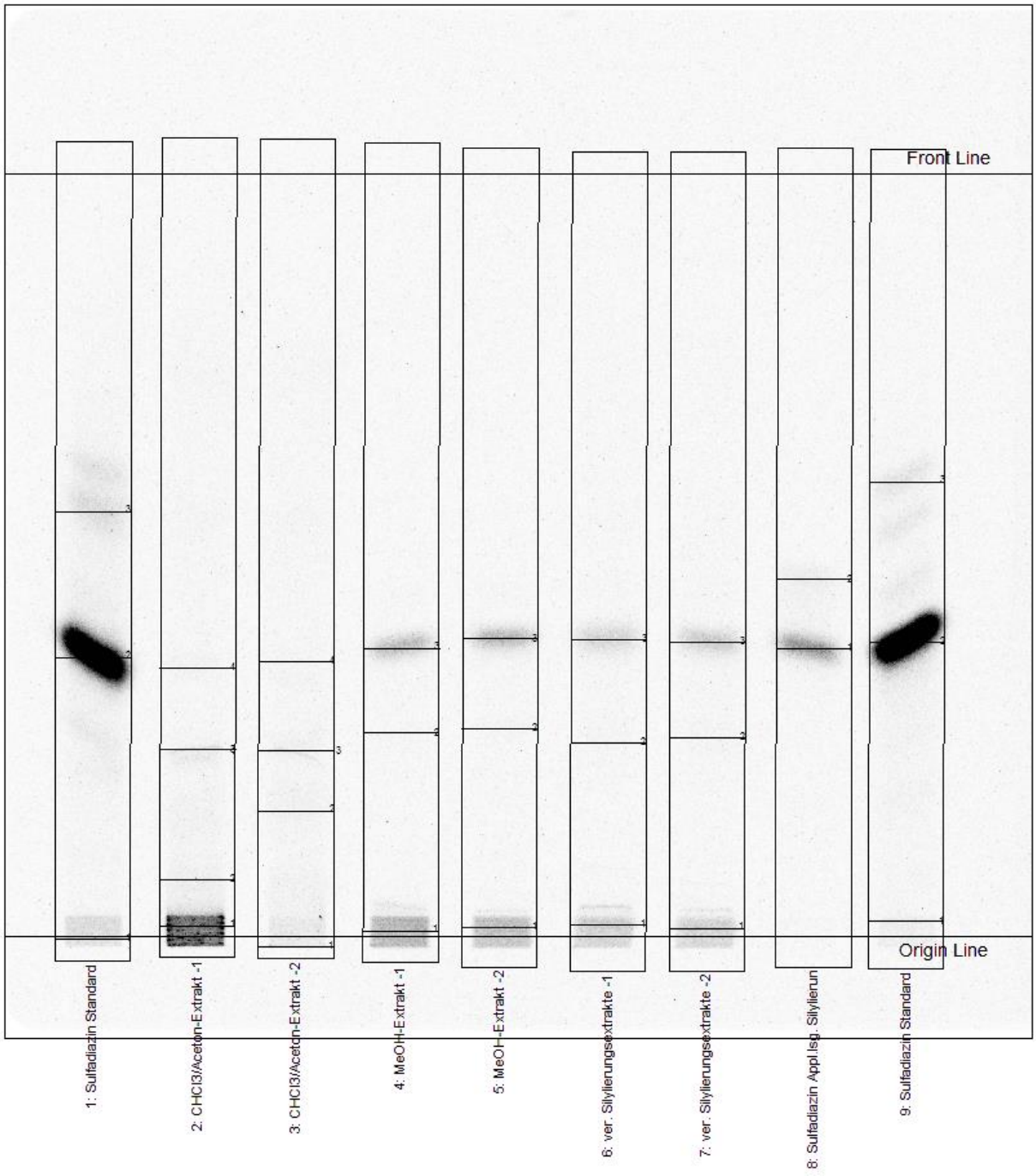


Figure S5: TLC-analysis of silylation extract in sulfadiazine spiking experiment

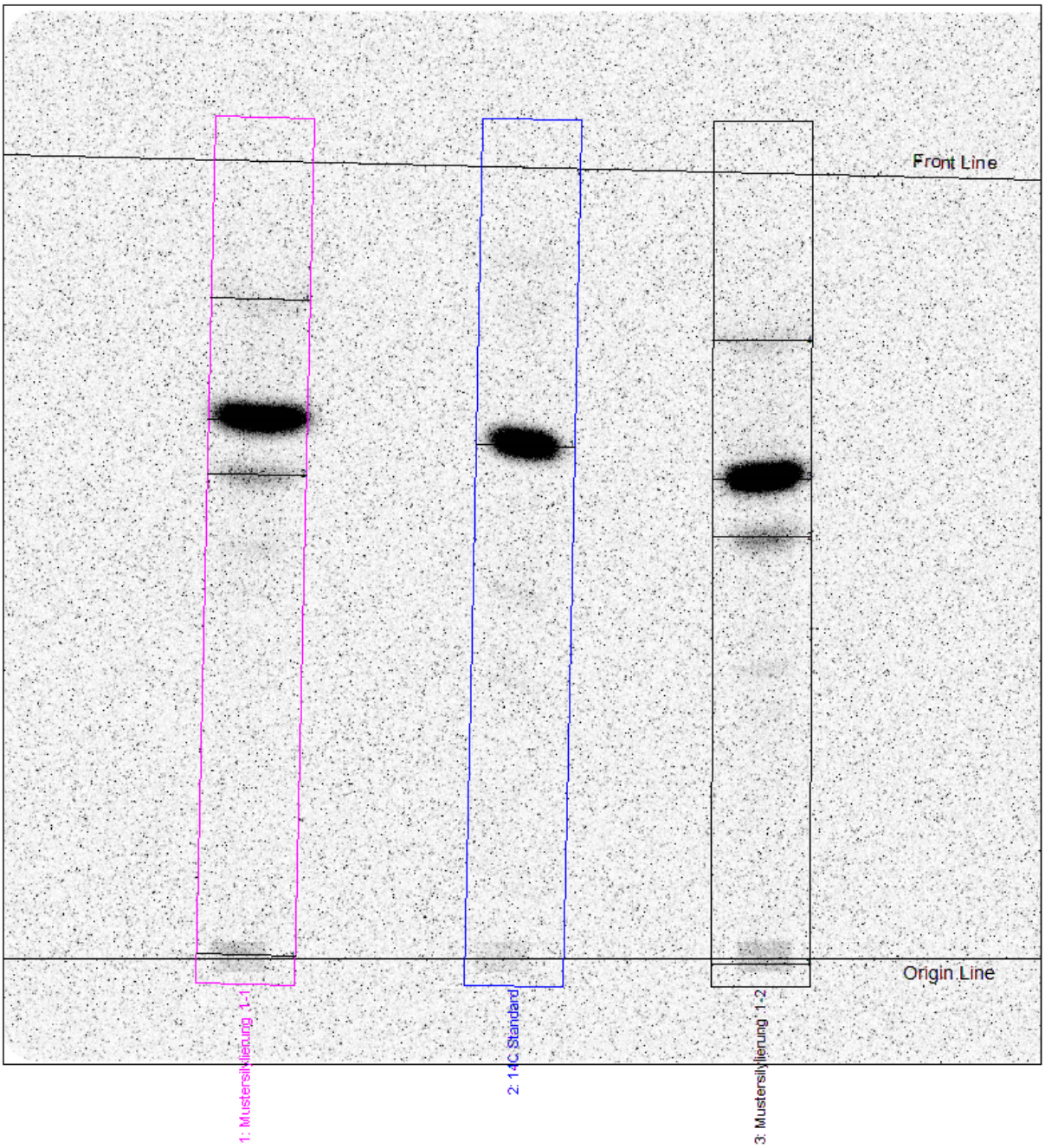


Figure S6: TLC-analysis of silylation extract in isoproturon spiking experiment

Statistical evaluation

Table S7: Data set for analysis of method reproducibility

Sulfadiazin

Sample	7d-1	7d-2	14d-1	14d-2	14ds-1	14ds-2	28d-1	28d-2	58d-1	58d-2	120ds-1	120ds-2	121d-1	121d-2
replicate 1	30.0	30.0	24.4	29.7	29.3	27.8	31.3	29.7	28.3	27.5	31.4	29.0	28.0	27.5
replicate 2	28.9	32.8	25.9	38.3	29.0	27.6	31.9	33.2	28.3	28.3	29.9	29.6	27.1	26.8

Isoproturon

Sample	7d-1	7d-2	14d-1	14d-2	15ds-1	15ds-2	29d-1	29d-2	59d-1	59d-2	120ds-1	120ds-2	120d-1	120d-2
replicate 1	30.9	35.8	26.7	20.4	43.4	27.4	23.2	23.0	30.1	28.0	24.3	23.8	44.7	54.4
replicate 2	35.6	28.9	28.1	22.4	29.5	30.8	23.0	20.5	29.0	28.3	23.8	24.6	34.3	52.7

Bromoxynil

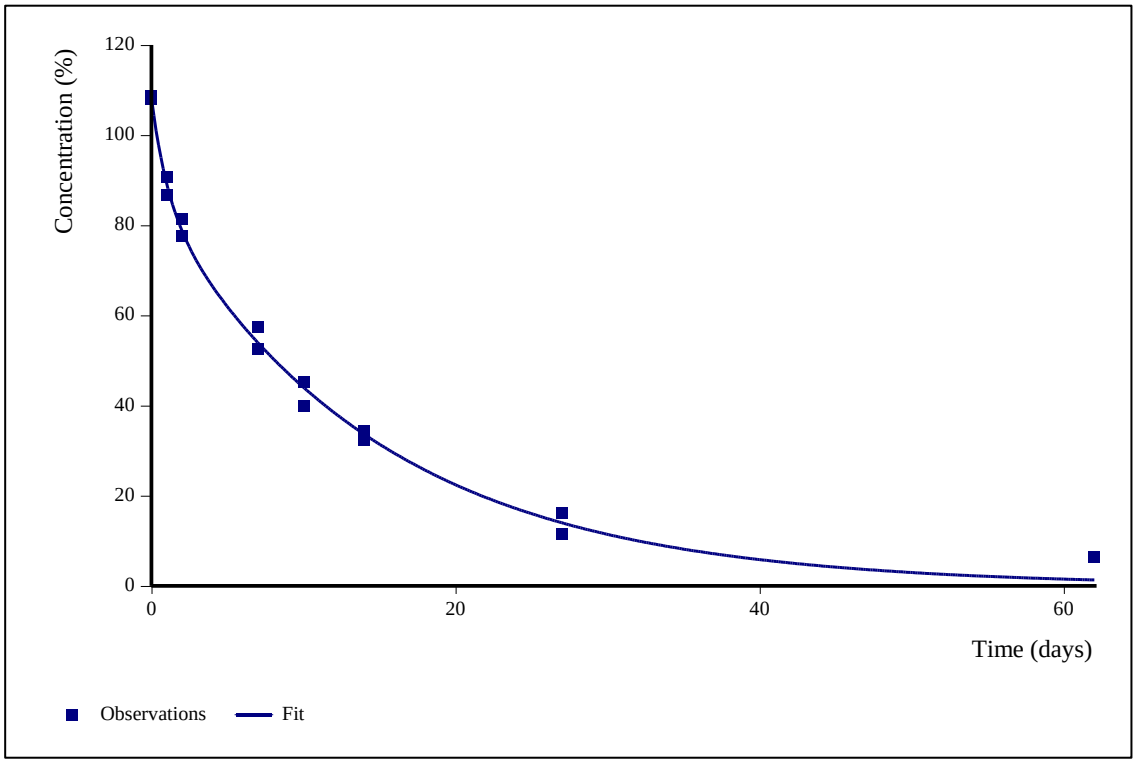
Sample	7d-1	7d-2	14d-1	14d-2	14ds-1	14ds-2	27d-1	27d-2	62d-1	62d-2	119ds-1	119ds-2	120d-1	120d-2
replicate 1	31.0	45.4	21.9	27.3	74.0	81.1	20.5	18.0	16.9	16.6	78.1	26.0	15.3	16.8
replicate 2	36.7	42.3	22.2	28.1	76.8	82.0	20.8	16.8	16.3	15.6	98.7	33.2	15.7	16.5

Table S8: Result of statistical functions LeveneTest and Wilcox.test (R output)

```
Levene's Test for Homogeneity of Variance (center = median)
      Df F value Pr(>F)
group  1  0.0142 0.9055
      82
```

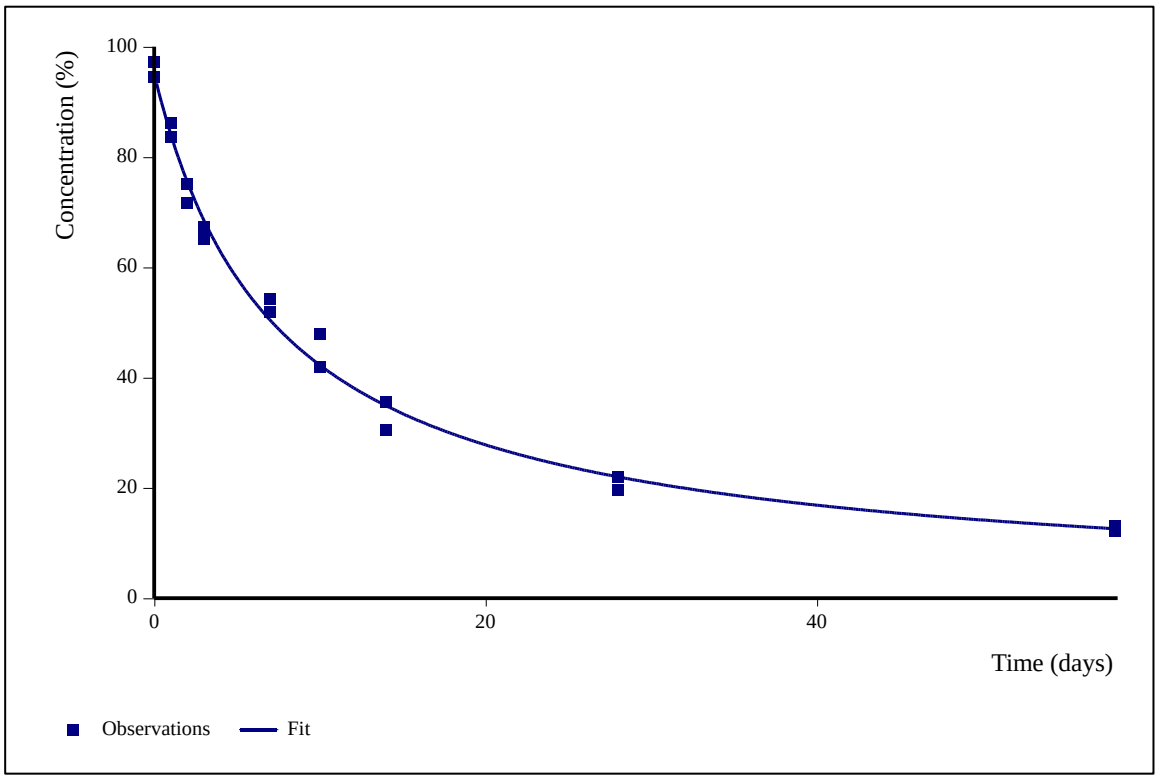
```
        Wilcoxon rank sum test

data:  data2$Value by data2$Replicate
W = 851.5, p-value = 0.7849
alternative hypothesis: true location shift is not equal to 0
```



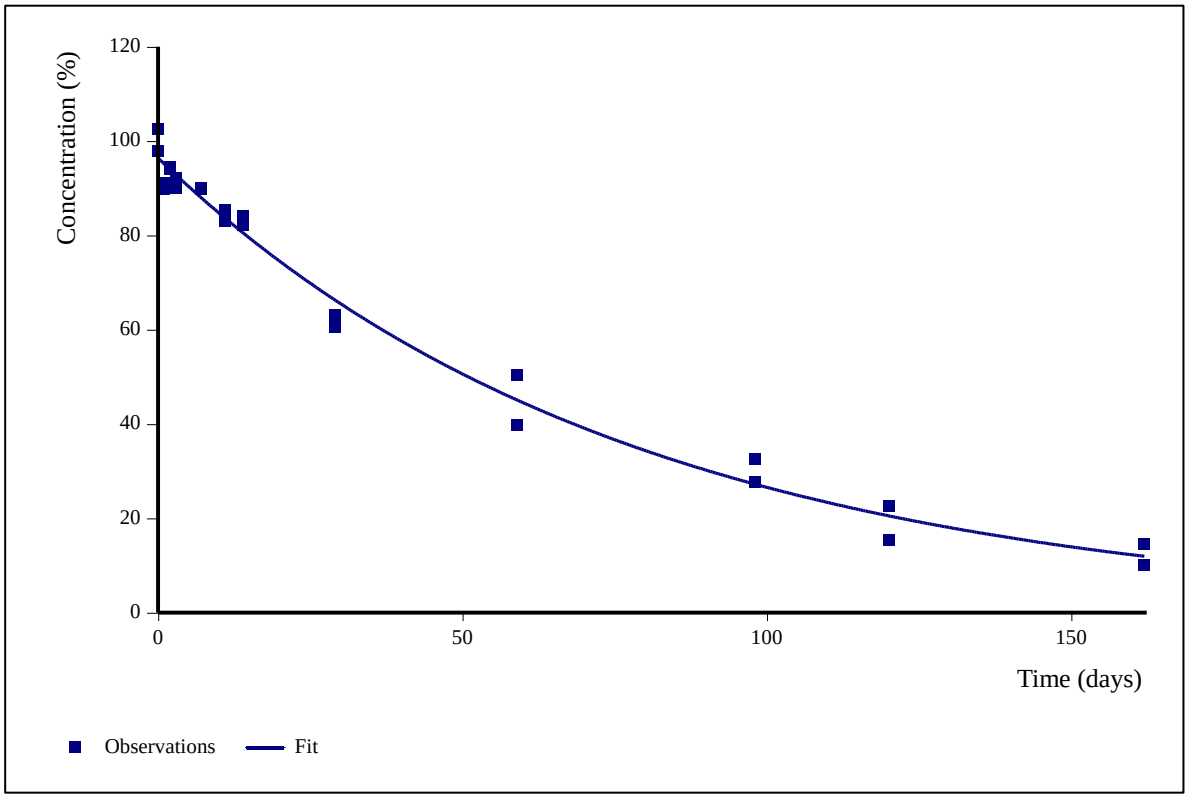
	chi² (%)	r² (-)	Prob. > t k_deg	DT50 (d)
SFO	6.63	0.9829	$1.8\ 10^{-10}$	8.29
DFOP	**3.36**	**0.9946**	**$1.03\ 10^{-7}$***	**6.95**
HS	6.35	0.9853	$1.33\ 10^{-9}$*	8.02
FOMC	4.47	0.9902	-	6.93

Figure S7: Plot of degradation kinetic of ^{14}C- Bromoxynil calculated using CAKE



	chi² (%)	r² (-)	Prob. > t k_deg	DT50 (d)
SFO	8.44	0.9635	4.3E-09	10.5
DFOP	3.77	0.9891	0.000371	7.94
HS	5.71	0.9804	0.0000359	7.67
FOMC	**2.88**	**0.9915**	**-**	**7.93**

Figure S8: Plot of degradation kinetic of ^{14}C- Sulfadiazin calculated using CAKE



	chi² (%)	r² (-)	Prob. > t k_deg	DT50 (d)
SFO	**3.18**	**0.9871**	**-**	**53.8**
DFOP	3.42	0.9873	-	52.8
HS	3.44	0.9872	-	54
FOMC	3.28	0.9872	-	52.8

Figure S9: Plot of degradation kinetic of ^{14}C- Isoproturon calculated using CAKE